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RESEARCH ARTICLE

Gymnosperms demonstrate patterns of fine-root trait coordination consistent with the global root economics space

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Abstract

1. Gymnosperms encompass a diverse group of mostly woody plants with high ecological and economic value, yet little is known about the scope and organization of fine-root trait diversity among gymnosperms due to the undersampling of most gymnosperm families and the dominance of angiosperm groups in recent syntheses.

2. New and existing data were compiled for morphological traits (root diameter, length, tissue density, specific root length [SRL] and specific root area [SRA]), the architectural trait branching ratio, root nitrogen content [N] and mycorrhizal colonization. We used phylogenetic least squares regression and principal component analysis to determine trait–trait relationships and coordination across 66 species, representing 11 of the 12 extant gymnosperm families from boreal, temperate, subtropical and tropical biomes. Finally, we compared the relationship between family divergence time and mean trait values to determine whether evolutionary history structured variation in fine-root traits within the gymnosperm phylogeny.

3. Wide variation in gymnosperm root traits could be largely captured by two primary axes of variation defined by SRL and diameter, and root tissue density and root nitrogen, respectively. However, individual root length and SRA each had significant correlations with traits defining both main axes of variation. Neither mycorrhizal colonization nor root branching ratio were closely related to other traits. We did not observe a directional evolution of mean trait values from older to more recently diverged gymnosperm families.

4. *Synthesis.* Despite their unique evolutionary history, gymnosperms display a root economic space similar to that identified in angiosperms, likely reflecting common constraints on plants adapting to diverse environments in both groups.

These findings provide greater confidence that patterns observed in broad syntheses justly capture patterns of trait diversity among multiple, distinct lineages. Additionally, independence between root architecture and other traits may support greater diversity in below-ground resource acquisition strategies. Unlike angiosperms, there were no clear trends towards increasingly thin roots over evolutionary time, possibly because of lower diversification rates or unique biogeographic history among gymnosperms, though additional observations are needed to more richly test evolutionary trends among gymnosperms.

KEYWORDS

collaboration gradient, conservation gradient, fine root, gymnosperm, plant functional traits, plant strategies, root economics

1 | INTRODUCTION

Fine roots are responsible for the acquisition of below-ground water and nutrients and, upon decomposition, return nutrients and plant-derived carbon to the soil. Consequently, these ephemeral organs directly influence individual plant health, interspecific competition and ecosystem nutrient cycling (Freschet et al., 2021). To better understand the relationships between fine roots and key plant and ecosystem processes, research has focused on identifying resource acquisition strategies across diverse plant species using trait-based approaches. Building on strategies and trait coordination observed in leaves above-ground (e.g. Wright et al., 2004), recent studies have organized suites of root traits into a defined 'root economics space' (Bergmann et al., 2020). In this framework, key root functional traits are aligned on two orthogonal axes. The first is a traditional conservation axis formed by root tissue density (RTD) and root nitrogen concentration (N) that mirrors patterns observed in leaf economic traits (i.e. leaf tissue density or leaf mass per area and leaf N; Weigelt et al., 2021). This axis represents a spectrum that ranges from highly metabolically active tissues with high root N but short lifespan to less active, but longer-lasting tissues with high tissue density, representing a slower return on investment (Reich, 2014; Wright et al., 2004). The second, collaboration axis, is shaped by trade-offs in root diameter and specific root length (SRL), which partly define the functional relationship between roots and mycorrhizal fungi as colonization rates tend to increase in larger diameter roots, especially among species that associate with arbuscular mycorrhizal fungi (Bergmann et al., 2020; Han et al., 2022; McCormack & Iversen, 2019).

Understanding how resource acquisition strategies have shifted among plant clades and across evolutionary time provides context for determining the responses of individual species and ecosystems to environmental change (Comas et al., 2012; Valverde-Barrantes et al., 2017). Some fine-root traits have been shown to be strongly linked to phylogeny (Comas et al., 2012), with anatomical and morphological traits having intermediate to strong phylogenetic signals (Kong et al., 2014; Liese et al., 2017; Valverde-Barrantes et al., 2017).

Further, first-order root diameter was shown to decrease with decreasing divergence time (i.e. more recently diverged), though this has only been well tested among angiosperm groups (Chen et al., 2013; Comas et al., 2012; Ma et al., 2018). In contrast, several studies have noted little to no phylogenetic conservatism in traits related to branching architecture (Kong et al., 2014; Liese et al., 2017).

Despite growing appreciation of large-scale root trait variation, we lack understanding of how consistent patterns of trait variation may be among major plant clades. Variation among modern, extant gymnosperms (i.e. acrogymnospermae)—an ecologically diverse and economically important plant group—has been largely unexplored as most recent syntheses have been heavily or entirely biased towards the angiosperm plant group. While it is possible that patterns of root trait coordination identified among angiosperms will accurately capture trait variation among gymnosperms, the unique evolutionary history, biogeography and functional traits of gymnosperms may result in a weakening of coordination among root traits, or possibly an entirely unique arrangement specific to gymnosperms (Wang & Ran, 2014). Indeed, the distinct wood anatomy and leaf morphologies found within the gymnosperm group often result in offsets or unique relationships among these traits in gymnosperms compared to angiosperms (Anderegg, 2015; Klein & Ramon, 2019; Stahl et al., 2013; Zhang et al., 2017). Further, these two major plant groups may express fundamentally different strategies in response to their environment. For example, Wang et al. (2019) found that angiosperms tended to increase root tip abundance and root tip density with increasing aridity, while gymnosperms decreased tip abundance and density. Given that angiosperms and gymnosperms are known to differ in ecologically important ways across many plant tissues, and the fact that gymnosperms have been poorly captured in previous root trait syntheses, it is clear that targeted efforts to sample and analyse functional variation in fine-root traits within the gymnosperm phylogeny are needed.

Relationships among the core traits that underlie the root economics space have been consistent and strong across most studies (Kong et al., 2014; Ma et al., 2018; McCormack & Iversen, 2019); yet, correlations between these traits and other aspects of functional root trait variation are less clear. For example, correlations

between root morphological and architectural traits, such as root system branchiness, have not been included in recent large-scale syntheses (e.g. McCormack & Iversen, 2019; Weigelt et al., 2021), because root architecture had historically been less frequently measured. Fine-root branching, which facilitates more intense exploration of patchy soil resources, seems to be weakly related and fully decoupled from root morphology (Kong et al., 2014; Liese et al., 2017). Furthermore, Li et al. (2017) noted high intraspecific variation in root branching architecture along a moisture gradient that enabled better adaption to changing environmental conditions. Architectural traits may therefore represent plastic plant responses to local soil conditions, rather than the conserved result of evolution towards unique resource acquisition strategies. Similarly, individual root length and specific root area (SRA) are important but understudied morphological traits whose coordination with other functional traits is not well known. SRA is, in theory, the most direct representation of the potential contact area between the soil and root surface per unit mass invested, while individual root length helps define the total surface area per root. Strong, positive correlations between root length and diameter have been reported when tested at broad phylogenetic scales (Chen et al., 2013; Kong et al., 2014), while negative correlations have been reported in studies with a narrower phylogenetic scope (i.e. within the genus *Quercus*; McCormack et al., 2020).

In this study, we aimed to quantify trait–trait relationships and variation in gymnosperm fine-roots and determine whether trait relationships among gymnosperm species are consistent with emerging global trait frameworks. We collected new samples for fine roots from 38 phylogenetically diverse species representing 11 of the 12 extant gymnosperm families, and combined our new observations with those in the Fine-Root Ecology Database (FRED; Iversen et al., 2017) so that our total dataset encompasses 66 gymnosperm species. Given that gymnosperms have evolved to occupy many of the same ecologically diverse habitats as angiosperms, we expected that gymnosperm fine-root traits would show substantial variation across species, similar to that observed among woody angiosperms. We hypothesized that: (1) the morphological traits diameter, SRL, SRA and individual root length will strongly covary among gymnosperm species as well as within a single trait measured across multiple root orders. Additionally, diameter and SRL would be positively and negatively related to mycorrhizal colonization rate, respectively. In contrast, RTD would be only weakly related to other morphological traits but would be negatively related to root N. (2) The architectural trait branching ratio will not be well correlated with fine-root morphological traits or root N, reflecting additional strategies for fine-root adaptation for resource foraging among gymnosperms. Based on our first two hypotheses, we expected that morphological traits, root N and mycorrhizal colonization rate will covary to form trait syndromes consistent with the global root economics space (sensu Bergmann et al., 2020), but will not align with the architectural trait branching ratio. (3) Traits previously observed to be more phylogenetically conserved (i.e. diameter and SRL) will demonstrate distinct patterns of evolution; specifically, families with older

divergence times will have larger diameter fine roots than more recently diverged families within the gymnosperm clade.

2 | MATERIALS AND METHODS

2.1 | Sampling and processing of new root trait data

Roots were sampled in the summer of 2019 from The Morton Arboretum in Lisle, Illinois, USA (41°48′50″ N, 88°4′6″ W), the summer of 2020 from the Kórnik Arboretum in western Poland (52°14′35″ N, 17°5′31″ E) and spring of 2022 from the Montgomery Botanical Center in Coral Gables, Florida, USA (25°39′47″ N, 80°16′55″ W). We obtained all necessary permits and permissions for sampling at each location. The Morton Arboretum has a warm to hot summer and humid continental climate (climate zone Dfa; Kottek et al., 2006), with a mean annual temperature and precipitation of 9.3°C and 977 mm, respectively. The climate at the Kórnik Arboretum is temperate (Cedro & Iszkuło, 2011), with a warm, humid continental climate (climate zone Dfb) with a mean annual temperature and precipitation of 8.3°C and 544 mm, respectively. The Montgomery Botanical Center has a tropical monsoon/savanna climate (climate zone Am/Aw) with a mean annual temperature and precipitation of 24.4°C and 1510 mm, respectively. For detailed site descriptions of The Morton Arboretum, Kórnik Arboretum, and Montgomery Botanical Center, see Midgley and Sims (2020), Kowalkowski and Prusinkiewicz (1959), Griffith et al. (2017), respectively.

We sampled mature trees of 38 species selected to broadly represent the diversity of extant gymnosperms (Forest et al., 2018; Lu et al., 2014), including 11 of 12 extant gymnosperm families (Figure 1). The site(s) where each species was measured is detailed in Figure S1. For each species at a given site, roots were sampled at three different locations, each within a few metres of mature individuals of the target species and at least 10 m from one another. At each location, the top 20 cm of soil was loosened, allowing intact fine-root branches (including first-order to at least fourth-order roots) to be excavated with minimal damage. These roots were gently removed and placed in zippered plastic bags and transported to the lab. The collected roots were then transferred to a refrigerator and processed within 3 days. In a few sampling plots with relatively low rooting densities and/or where the soils were difficult to remove from root branches, larger samples of bulk soil were collected (ca. 10 L of soil), placed into a bucket and allowed to soak in water overnight prior to careful removal of intact root branches.

When possible, we sampled from monoculture plots so roots of known identity could be obtained without the need to trace roots back to parent trees. When such monocultures were unavailable, we selected mixed-species sites where the target species was among species of different genera whose roots would be easily distinguishable from those of the target species based on visual, textural or olfactory cues. In cases of uncertainty, roots were traced to the target plant's base to confirm its origin. When possible, our sampling
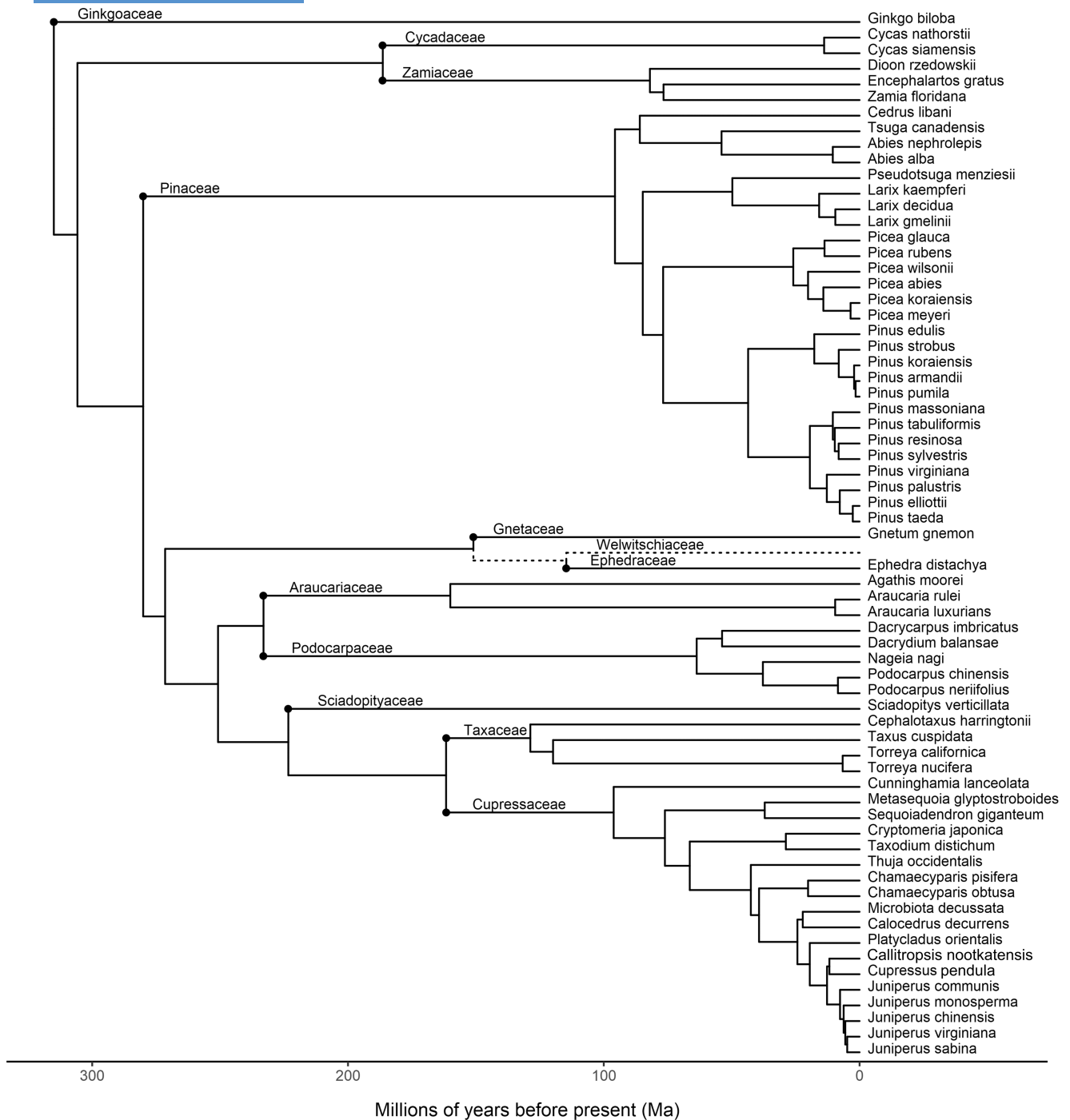
FIGURE 1 Phylogenetic tree of the 66 gymnosperm species included in this study. Family clades are labelled, with the dashed branch representing the single gymnosperm family not represented in our species set (Welwitschiaceae). Circles on the branches to the left of each family name identify the estimated divergence time for that family, following Forest et al. (2018).

included multiple plantings within sites, and often multiple sites. However, for the species *Sciadopitys verticillata* and *Sequoiadendron giganteum*, only one individual each was present at a single site, from which we collected only two samples.

Collected roots were used to measure five morphological traits (root diameter [mm], individual root length [mm], SRL [m/g], SRA [cm^2/g], RTD [g/cm^3]), the architectural trait (branching ratio: the ratio of lower order roots per higher order root; e.g. number of first-order

roots per single second-order root), the chemical trait root N (%) and mycorrhizal colonization (%). Morphological traits and root N were measured on individual root orders because they are the most comparable units of fine roots across species and play critical roles in soil resource acquisition (Guo et al., 2008; McCormack et al., 2015). In this context, the most distal root segments of a root branch are classified as first-order roots, and same-order root segments converge to produce a segment of the next highest order (Fitter, 1987; Pregitzer et al., 2002).

Mycorrhizal colonization was measured using intact root branches of first- to third-order roots. The full suite of traits considered here are commonly measured to characterize fine roots and their resource acquisition strategies (Bergmann et al., 2020; Liese et al., 2017). Notably, root diameter and RTD are both mathematically related to SRL and SRA and should correlate strongly (Ostonen et al., 2007). All four are included in this study to better understand how each relates independently to the other traits, such as mycorrhizal colonization.

We carefully washed intact root branches and then randomly selected five branches that were then used to measure individual root length, diameter and branching ratio under a dissecting microscope. Length and diameter were recorded as the length and diameter of individual roots for a given order. These root branches were then dried in an oven at 60°C for ca. 2 days and then weighed. Five additional branches were randomly selected and preserved in 60% ethanol in preparation for later assessments of mycorrhizal colonization. The remaining root branches were dissected in order until ca. 15 mg of dry mass was obtained. Grouped by order, these root segments were scanned at 800 dpi using WinRHIZO, dried in an oven and weighed. Using the scanned length, surface area, calculated volume together with the final dry weights, SRL, SRA and RTD were calculated for each root order. The dried samples for each individual root order were then ground to a fine powder and analysed for N and carbon concentrations (Vario El III, Elementar, Lengenselbold, Germany).

Using the samples preserved in ethanol, we assessed the mycorrhizal colonization rate of the AM-associating species following a process of clearing, staining and visual inspection (modified from Vierheilig et al., 1998). Roots were first submerged in 10% KOH for 6 days at room temperature to extract most tannins and phenolics. Roots were then autoclaved on a liquid cycle at 121°C for 20 min, rinsed with 2.5% HCl, and acidified with 2.5% HCl for 15 min. Once acidified, HCl was poured off, and the roots were lightly rinsed with distilled water. Roots were then immersed in Schaeffer black solution (5% Schaeffer black ink and 95% distilled vinegar v/v) for 30 min, then rinsed well with distilled water. Finally, we used the grid-line intersect method to quantify mycorrhizal colonization rates as the percentage of root length with observable mycorrhizal structures (McGonigle et al., 1990). All traits were measured for samples from all sites, with the exception of length of second-order roots, branching ratio of second-order roots per third-order root, which were not measured at the Kórnik Arboretum, and mycorrhizal colonization, which was not measured at the Kórnik Arboretum or Montgomery Botanical Center.

2.2 | Integrating root trait data from FRED

The full, unfiltered FRED database (version 3.0, Iversen et al., 2021) was downloaded on 13 April 2021 (https://roots.ornl.gov/public-release). We extracted species-specific data for each root trait that we measured in our samples. We used mycorrhizal colonization measurements from both species colonized by arbuscular mycorrhizal fungi and species associated with ectomycorrhizal fungi. In the case

of arbuscular mycorrhizal fungi, colonization was usually determined in a manner similar to that described above, while colonization by ectomycorrhizal fungi was estimated by counting the number of colonized versus uncolonized root tips.

We limited the root trait data to absorptive fine roots (typically just the first-to-third-order roots) by (1) removing any data listed as including coarse roots, rhizomes, below-ground stems, total root systems and total below-ground systems; (2) removing roots that were reported to be >2 mm in diameter; and (3) removing trait data not associated with roots listed as first-, second- or third-order roots as defined by a centripetal/morphometric ordering scheme (Fitter, 1982; Pregitzer et al., 2002). Mycorrhizal colonization data from FRED underwent the same filtering steps, except for final filtering based on root type (e.g. fine vs. coarse root) and individual root order because colonization is most often measured and reported on whole branches.

Additional filtering steps were used to ensure that the data were most comparable to our dataset. This included removing roots labelled as dead roots or as pioneer/framework roots (Zadworny & Eissenstat, 2011), removing roots of young plants (plant or stand age less than 5 years old, or marked as 'immature' or 'seedling') and removing roots associated with treatments (e.g. fertilization and burning) except in one case when control and fertilization treatments were averaged and no isolated control data were available. Only data collected from outdoor, in situ conditions were retained. Duplicate data were also removed.

2.3 | Phylogeny

Species' binomial names and taxonomic groups were standardized using the accepted names and groupings provided by The Plant List Version 1.1 and later updated according to accepted names in Plants of the World Online (powo.science.kew.org). The phylogenetic tree used in our analyses was modified from a dated phylogeny of the gymnosperm group that was built from DNA sequence data of 923 gymnosperm species (Forest et al., 2018). All but four of the 66 species in our final dataset matched species on the tree; the remaining four species were added manually by either substituting the species name in for a synonym species names (according to The Plant List), substituting the species for another species of the same genus (when we only had one species of that genus) or manually attaching to sister species using the function 'add.tips' from the package phangorn (Schliep, 2011). The four species that were manually added to the phylogenetic tree were *Cupressus funebris*, *Cycas nathorstii*, *Picea glauca* and *Zamia lucayana*. Once all 66 species were assigned to a tip of the tree, the remaining unassigned tips were dropped.

2.4 | Data analysis

Prior to formal analyses, we prepared a single data set with an average value for each trait and species. This was done by first averaging

the data within a site (i.e. Morton, Kórnik, Montgomery) or study (i.e. usually the name of the publication associated with that trait data in FRED). These were then merged together, and this combined data set was then averaged to a single species value per trait per root order. As with most large synthesis, the combination trait data from multiple data sources results in an unbalanced design where some species are represented by single measurements and other, more common species are represented by multiple measurements. Trait measurements were generally similar within a species, so we believe that the inclusion of multiple data sources/sites for some species did not qualitatively alter our results. However, future work specifically investigating broad-scale patterns of intraspecific variation, as has been done for individual species (e.g. Defrenne et al., 2019; Li et al., 2019; Ostonen et al., 2011; Zadworny et al., 2016), would be an appropriate step for future work. Here, means for each trait and species, along with the standard error when more than one site or study was available for a given species and trait, are presented in Figure S1. This final species average dataset contained 66 species, 37 genera and 11 families, and 901 total observations among the eight traits measured separately for first-to-third root orders. Of the 66 species in this dataset, 39 are associated predominately with arbuscular mycorrhizal fungi, while the remaining 27 are ectomycorrhizal (all in family Pinaceae).

All statistical analyses were conducted in R version 4.1.1 (R Core Team, 2021). To better meet statistical assumptions of normality and homoscedasticity, data for diameter, length, branching ratio, RTD, SRL and SRA were log transformed, while root N and mycorrhizal colonization were logit transformed. To address our first two hypotheses, we conducted bivariate analyses using phylogenetic generalized least squares (PGLS) regression to assess root trait correlations. PGLS analysis was conducted with the 'pgls' function of the 'caper' package (Orme et al., 2018). Phylogenetically corrected correlation coefficients (r-values) were calculated based on the adjusted r-square values of each model (by taking the square root and, if the coefficient was negative, multiplying with −1). Also, in cases where the model-adjusted r-square was negative, we assigned an r coefficient of 0. We also conducted a non-phylogenetically corrected analysis using Pearson's r correlation. The results here were generally consistent with those determined using PGLS, and we emphasized only the PGLS results for simplicity but provided results from both in Supporting Information. For all analyses, results were considered significant at $p \leq 0.05$ or marginally significant at $p < 0.10$.

We used principal component analysis (PCA) to assess the multidimensional correlation structure among traits across all species. This was done using both standard and phylogenetically informed PCA. The phylogenetically informed PCA was conducted using only the four traits that represent the root economic space and for which the most data are available: diameter, SRL, RTD and root N concentration. This required using only species for which data from all four traits were available (i.e. no missing values). Following confirmation of homogeneity of dispersion using the function '*betadisper*', we then used permutational multivariate ANOVA (PERMANOVA), using the *pairwise.adonis* function of the R package 'pairwiseAdonis', to

identify significant differences between subsets of species that associate with arbuscular mycorrhizal fungi and with ectomycorrhizal fungi (only represented by species in the Pinaceae) (Arbizu, 2017). Next, we conducted a standard PCA that would allow us to consider all traits while utilizing our full dataset. This was done separately using only the first-order root traits and then again using all traits and root orders.

Finally, we addressed our third hypothesis, relating variation in root traits to family divergence time within the gymnosperm phylogeny. Trait values were averaged at the family level, resulting in single mean estimates for each trait. Here we emphasized only first-order roots for the morphological traits and root N in addition to the branching ratio of first-to-second-order roots and mycorrhizal colonization. Divergence time for each gymnosperm family was extracted from the dated phylogeny by Forest et al. (2018), with divergence time defined as the date of the family's parent node (i.e. the age of the most recent common ancestor shared between the family and the adjacent group from which the family diverged; Figure 1). We then used linear regression to assess relationships between family divergence time and mean trait value for each trait.

3 | RESULTS

3.1 | Trait variation

Substantial trait variation was observed in all measured traits across the gymnosperm phylogeny, with roughly 5-, 20- and 40-fold variation observed for diameter, SRL and individual length of first-order roots (Figure 2; Table 1). However, in some cases, there were also notable similarities in traits, even among species from different families. For example, the mean RTD of the first-order roots of *Ephedra distachya* and *Taxodium distichum* were both ca. $0.14\,g/cm^3$ (Figure 2). Across all traits, the lowest and highest observed coefficients of variation (CV) in first-order roots were 30.5% and 77.4% for root N and individual root length, respectively (Table 1). The CV was generally similar across orders for all traits except for branching ratio, where the CV decreased from 74.9% in the branching ratio of first-to-second-order roots to 33.4% in second-to-third-order roots.

For each trait, mean trait values generally followed a consistent trend with increasing root order. For most species, root N, SRA and SRL decreased while diameter, RTD and individual root length increased with increasing root order (Figure 2; Figure S1). However, the range of trait variation across root orders was inconsistent across species. For example, the range of mean length between first- and second-order roots was over 20 mm in *Cephalotaxus harringtonii*, but only 2.7 mm in *Picea abies* (Figure S1).

3.2 | Trait–trait correlations

There were several strong correlations as predicted across most morphological traits (Figure 3; Figures S2–S4). For example, diameter
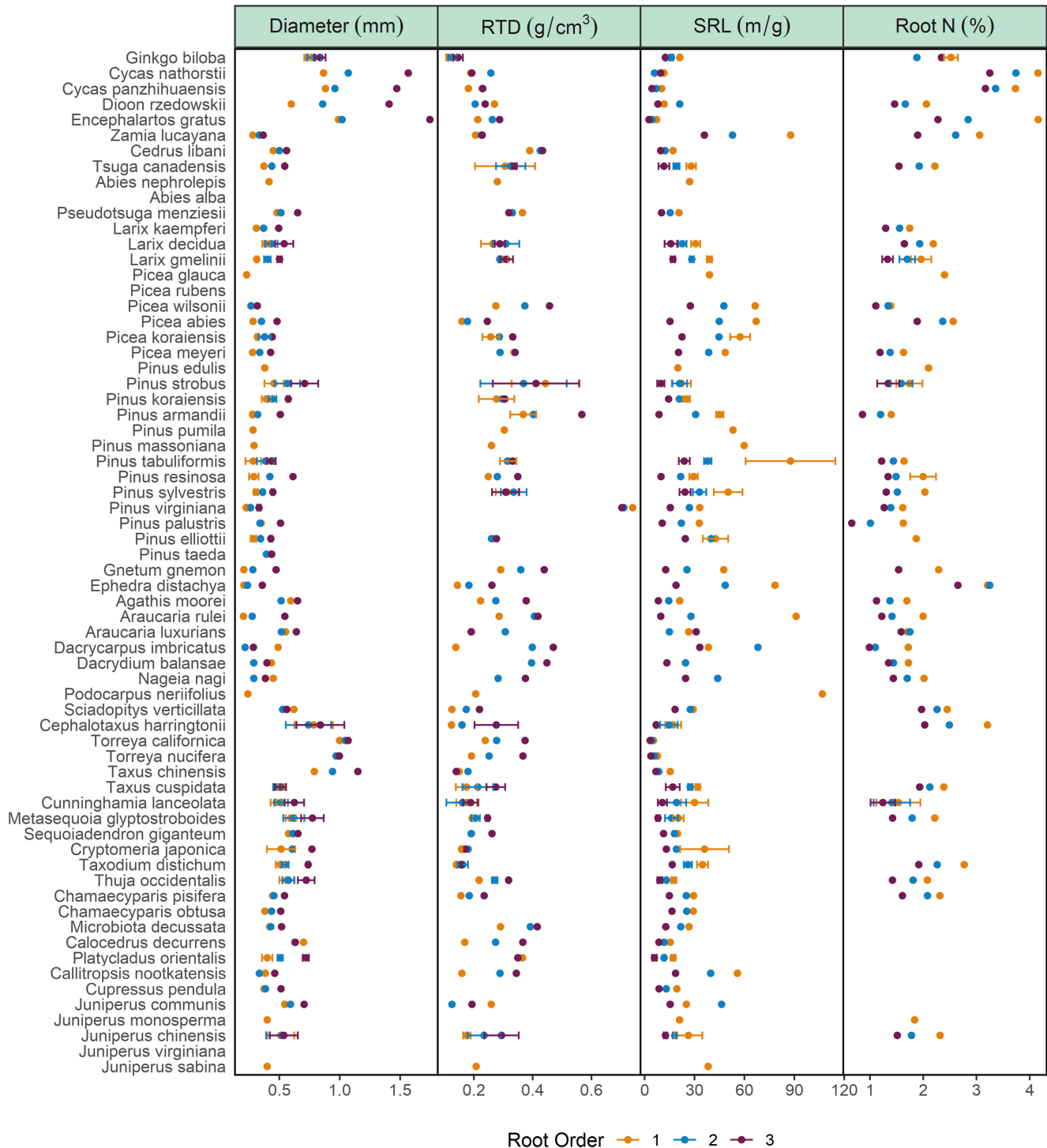
FIGURE 2 Mean (±SE) root diameter, root tissue density (RTD), specific root length (SRL) and root nitrogen concentration (Root N) of first-, second- and third-order roots. Species are ordered based on their position in the gymnosperm phylogeny (Figure 1). Trait values for specific root area, individual root length, branching ratio and mycorrhizal colonization, as well as the data sources where each species' data were collected, are provided in Figure S1.

was strongly and negatively related to SRL across all root orders (p < 0.05; all cases; Figure 3; Figure S2). Similarly, diameter and SRA were negatively related in first-order roots (p < 0.05; Figure 3; Figure S3), though this relationship weakened in higher orders. RTD was unrelated to SRL, but it was consistently and negatively related

to SRA in all root orders and, to a lesser extent, with root diameter for a few root orders (Figure 3; Figur S2). The length of first-order roots was negatively related to first-order SRL and SRA and positively related to the diameter of all root orders (p < 0.05; all cases, Figure S2).

TABLE 1 Summary values for each trait, by root order, along with coefficient of variation (CV, %) and numbers of species (n) available for each trait.

Trait1	Root order	Mean	Median	Minimum	Maximum	CV	n
Diameter	1	0.46	0.42	0.20	1.00	43.5	63
	2	0.51	0.44	0.22	1.07	43.4	55
	3	0.65	0.54	0.29	1.75	48.0	55
Length	1	5.14	3.80	0.39	16.54	77.4	47
	2	12.62	10.12	0.53	55.71	84.9	36
SRL	1	35.11	29.29	5.37	106.90	63.9	59
	2	24.83	21.76	4.38	68.14	56.0	53
	3	14.25	12.67	2.62	35.94	52.7	53
SRA	1	418.3	396.2	168.5	829.0	38.5	37
	2	342.5	313.4	140.1	829.4	41.3	39
	3	257.1	219.1	110.2	750.4	46.9	39
Root N	1	2.23	2.06	1.39	4.16	30.5	41
	2	1.89	1.73	1.01	3.74	33.8	36
	3	1.60	1.44	0.66	3.25	35.3	37
RTD	1	0.25	0.22	0.12	0.74	42.0	53
	2	0.28	0.28	0.13	0.71	36.1	50
	3	0.32	0.31	0.14	0.70	34.4	50
BR	1	5.1	4.1	1.0	19.3	74.9	45
	2	3.8	3.6	1.8	8.8	33.4	35
MC	Fine roots	67.3	72.9	21.2	100.0	31.0	18

Note: Diameter (mm); Length (mm).

Abbreviations: BR, branching ratio; MC, mycorrhizal colonization (%); Root N, root nitrogen concentration (%); RTD, root tissue density (g/cm^3); SRA, specific root area (cm^2/g); SRL, specific root length (m/g).

Relationships involving traits outside of morphology were mixed. Root N was mostly independent of other root traits but had consistent and negative correlations with RTD across all root orders ($p < 0.05$; all cases; Figure 3; Figure S2). Mycorrhizal colonization was not strongly correlated with any other traits and only demonstrated weak, positive trends ($p < 0.10$) with length and diameter of second- and third-order roots, respectively (Figure 3; Figure S3). Finally, branching ratios were largely independent of all other traits, with the exception that high branching ratios of first-to-second-order roots were associated with higher length and SRL of second-order roots and negatively related to first- and second-order root N ($r < -0.5$, $p < 0.05$; all cases; Figure 3). Within-trait correlations among first-second, second-third and first-third-order values were all significantly and strongly positively correlated ($r > 0.50$, $p < 0.05$, Figures S2 and S4).

3.3 | Multidimensional trait space

Our PCA revealed a two-dimensional arrangement of the four core economic traits, as root diameter and SRL formed an approximate axis while root N and RTD formed a separate, orthogonal axis (Figure 4; Table 2). This pattern remained consistent when all eight traits were included and phylogenetic relatedness was not accounted for (Figure 5). In this latter case, individual root length was closely aligned with root diameter. Mycorrhizal colonization was similarly aligned with diameter, though to a more limited extent (Figure 5; Table 3). Unlike SRL, SRA was roughly split between PCs 1 and 2, though it was more closely aligned with SRL than Root N. Finally, the branching ratio was not well aligned with any other root traits.

Most traits were well captured by principal components 1 and 2 in both PCAs focused on first-order roots (Figures 4 and 5; Tables 2 and 3), and these two components together accounted for 84% and 65% of the total variation in the 4- and 8-trait PCAs, respectively. The main exceptions were branching ratio and mycorrhizal colonization, which were poorly loaded onto the first and second principal components (Figure 5). Instead, both were best captured by PC3 (Table 3), which explained an additional 18.7% of variation in the eight trait PCA. Finally, our PCA that included all traits and all root orders (Figure S5; Table S1) showed patterns that were broadly consistent with those presented in the first-order roots only PCAs.

Results of the permutational multivariate analysis indicated significant differences between species that associate with arbuscular mycorrhizal fungi and species that associate with ectomycorrhizal fungi (i.e. Pinaceae; $p < 0.001$). Specifically, species in the Pinaceae tended to cluster together and were generally associated with relatively high RTD, lower root N and smaller root diameters while species that associate with arbuscular mycorrhizal fungi tended to be more dispersed in the trait space (Figure 4).
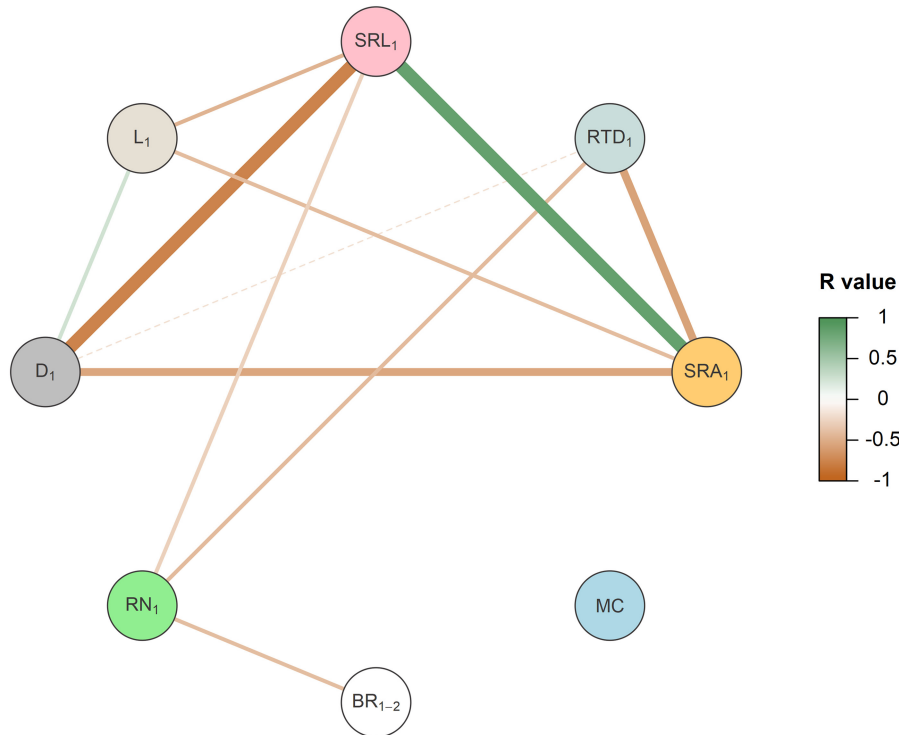
FIGURE 3 Trait–trait correlations among gymnosperm species based on phylogenetic least squares regression. Positive and negative correlations are indicated with green and orange lines, respectively. Line thickness and opacity indicate the strength of a correlation: thin and light ($|r| < 0.5$), medium ($0.5 < |r| < 0.75$) and thick and dark ($|r| > 0.75$). Solid lines indicate a significant relationship ($p \leq 0.05$), while dashed lines indicate a marginally significant trend ($0.05 < p < 0.10$). Trait abbreviations are as follows: BR, branching ratio; D, diameter; L, length; MC, mycorrhizal colonization; RN, root nitrogen concentration; RTD, root tissue density; SRA, specific root area; SRL, specific root length. Each trait is reported for first-order roots, or as the ratio first-to-second-order roots (BR_{1-2}), or for pooled absorptive fine roots in the case of mycorrhizal colonization (MC). Specific p-values, r-values and n values are described in Figures S3 and S4.

3.4 | Trait variation with family divergence time

Testing relationships between mean root trait values at the family level and the estimated time of evolutionary divergence of each family within the gymnosperm group revealed no significant relationships ($p > 0.10$; Figure 6). Because many root traits are expected to vary in unique ways based on their mycorrhizal association, we repeated the analysis, but with the only ectomycorrhizal-associating family, Pinaceae, removed. This again revealed no consistent directional evolution of fine-root functional traits associated with family divergence in the gymnosperm group.

4 | DISCUSSION

Our study was motivated to address gaps in data and knowledge of the traits of gymnosperm roots. Despite having fewer species and observations in our study compared to those incorporating angiosperms, we observed similar ranges of root trait variation among gymnosperm species compared to those reported elsewhere for woody angiosperms (Kong et al., 2014). For example, the trait range encompassed by gymnosperms was often similar to that reported in other large-scale analyses (e.g. diameter ranged from 0.18 to 1.0 mm observed in first-order roots across 66 species in this study

compared with ca. 0.1–1.0 mm observed across 369 species by Ma et al., 2018). The distribution of gymnosperms across the globe and through geologic history spans a wide range of climates and soil types, providing ample opportunity for diversification of root traits over time and space. The substantial variation observed in gymnosperm root traits may therefore reflect different resource acquisition strategies that have evolved as gymnosperms have adapted to compete in and often dominate many terrestrial ecosystems.

Individual traits and the covariance among multiple interacting traits indicate a species' unique resource acquisition strategy (Reich, 2014). Diameter, RTD, SRL and root N are considered core economic traits and represent plant investment in fine-root construction and the potential amount of resources the plants may acquire in return for that investment. Following our analysis of correlations among these traits, we found partial support for our hypotheses concerning how trait covariance limits the range of root functional strategies displayed by plants. From our first hypothesis, we expected morphological traits to covary among root orders and across species. We found strong support for consistent and positive relationships across multiple root orders for a given trait, suggesting consistent strategies throughout a branching hierarchy (e.g. thicker first-order roots were generally associated with thicker second- and third-order roots). This result is consistent with findings within the genus *Quercus* as well as across a more taxonomically diverse suite
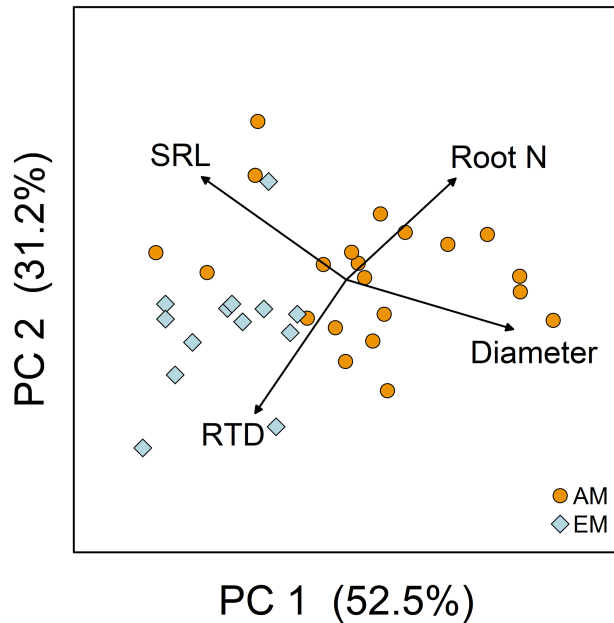
FIGURE 4 Phylogenetically informed principal component analysis (PCA) on first-order roots for four core traits associated with root economics space ($n=34$). Each plotted point represents a species, with each colour coded to reflect their mycorrhizal association as either arbuscular mycorrhizal (AM; orange circles) or ectomycorrhizal (EM; light blue diamonds, i.e. only species in Pinaceae). The variation explained by each principal component is shown with the component label. Loadings for each trait and principal component are given in Table 2. Diameter, root diameter; Root N, root nitrogen concentration; RTD, root tissue density; SRL, specific root length. Permutational multivariate analysis indicated significant differences between the AM-associating species and EM-associating species (all within the Pinaceae; $p<0.001$).

TABLE 2 Trait loadings for phylogenetically informed principal component analysis (Figure 4) for the four core first-order fine-root traits (shown in Figure 4) using species trait means for all species ($n=34$). The proportion of variance explained is listed next to each component name.

	PC 1	PC 2	PC 3
Eigenvalue	2.100	1.247	0.573
Variance	52.5%	31.2%	14.3%
Diameter	0.921	−0.273	−0.200
SRL	−0.798	0.567	−0.080
RTD	−0.502	−0.735	0.446
Root N	0.601	0.557	0.572

Abbreviations: Diameter, root diameter; RTD, root tissue density; Root N, root nitrogen concentration; SRL, specific root length.

of temperate tree species (Guo et al., 2008; McCormack et al., 2020; Pregitzer et al., 2002). The bivariate correlations we observed among most morphological traits in gymnosperms are also consistent with previous studies dominated by angiosperms (Bergmann et al., 2020; Kong et al., 2014; Ma et al., 2018). We observed consistent negative correlations between root N and RTD, which further
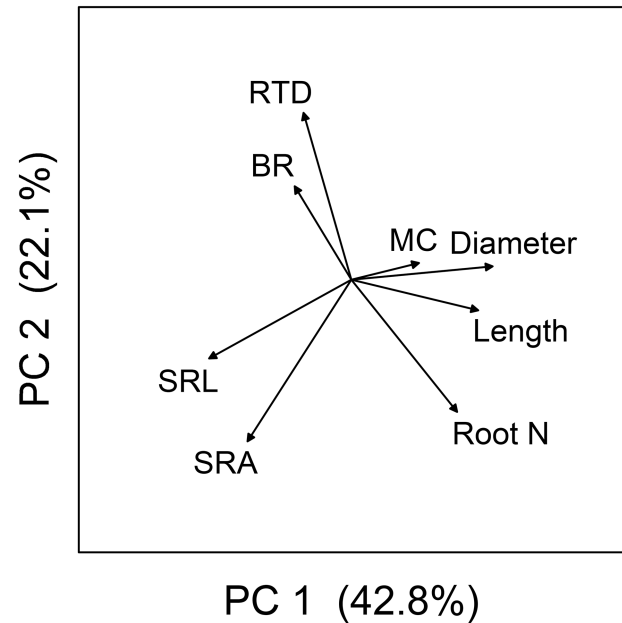
FIGURE 5 Correlational principal component analysis (PCA) conducted for all 8 traits and species measured in this study ($n=63$). The variation explained by each principal component is shown with the component label. Loadings for each trait and principal component are given in Table 3. Figure S4 includes PCA with all traits and all orders. BR, branching ratio of first-order roots per second-order root; Diameter, root diameter; MC, mycorrhizal colonization; Root N, root nitrogen concentration; RTD, root tissue density; SRA, specific root area; SRL, specific root length.

TABLE 3 Trait loadings for correlational principal component analysis (Figure 5) for first-order fine-root traits and mycorrhizal colonization (shown in Figure 5) using species trait means for all species ($n=63$). The proportion of variance explained is listed next to each component name.

Trait	PC 1	PC 2	PC 3
Eigenvalue	3.471	1.789	1.601
Variance	42.8%	22.1%	19.8%
Diameter	0.475	0.045	0.266
Length	0.426	−0.103	−0.323
BR	−0.189	0.312	0.547
RTD	−0.162	0.564	−0.437
SRL	−0.480	−0.266	−0.073
SRA	−0.351	−0.547	0.156
Root N	0.357	−0.446	−0.151
MC	0.222	0.055	0.531

Abbreviations: BR, branching ratio of first-order roots per second-order root; Diameter, root diameter; MC, mycorrhizal colonization; Root N, root nitrogen concentration; RTD, root tissue density; SRA, specific root area; SRL, specific root length.

supports observations by others (Bergmann et al., 2020). Finally, in line with our second hypothesis, the architectural trait branching ratio showed very few strong correlations to morphological traits.

This further supports the idea that architectural traits are largely independent of root morphology (Kong et al., 2014; Liese et al., 2017), and may instead be more plastic and allow root systems to alter branching in response to local environmental conditions (e.g. soil properties, depth in soil and season).

We found mixed results regarding expected relationships between the four most commonly measured root traits (diameter, SRL, RTD and Root N) and the less commonly measured traits of root length and SRA. First, consistent with our expectations and previous studies, length of first-order roots was positively related to diameter and negatively related to SRL of first-order roots (Chen et al., 2013; Ma et al., 2018). However, both of these previous studies noted a significant negative relationship between root length and the RTD of first-order roots. We did not observe this negative relationship and instead found a positive relationship between first-order root length and second-order RTD. Further, Chen et al. (2013) noted a significant negative relationship between first-order root length and branching ratio; we instead found that longer second-order roots were associated with higher numbers of first-order root branches. Given the dominance of angiosperms in both of the previous studies, our somewhat different results may indicate differences between angiosperm and gymnosperm root construction and architecture. It is also important to note that root length is, to some degree, related to all core economic traits. Our PCA clearly shows a stronger loading with the 'collaboration' axis defined by SRL and diameter. However, root length, especially of second-order roots, was also closely related to root N and RTD. Similarly, we observed that, while SRA was most closely related to SRL and diameter, it also had consistent negative relationships with RTD. These together highlight the fact that despite the orthogonal nature of the two main axes of the root economics space, there are still construction-based trade-offs that will impact all aspects of fine-root structure and function.

Despite a well-defined axis of variation characterized by roots with high SRL opposing roots with large diameters, we did not observe a clear trade-off with mycorrhizal colonization that denotes the ecological significance of the 'collaboration' gradient. Specifically, while we expected to observe a positive relationship between mycorrhizal colonization and root diameter and a negative relationship with SRL (Bergmann et al., 2020; Chen et al., 2013; Ma et al., 2018; McCormack & Iversen, 2019), we found only weak trends connecting colonization to first-order root diameter (Figure 5). The lack of correlation remained even when analysed separately for AM versus EM associates (*data not shown*). It is possible that this represents a weakening of the interactions expected between morphology and mycorrhizal fungi (Bergmann et al., 2020). However, this may have been the result of our limited sample size, as our coverage of colonization as a trait was the weakest within our dataset (*n* = 18 species). Furthermore, visual inspection of the weak positive trend between first-order root diameter and mycorrhizal colonization (Figure S3) shows a pattern that is generally consistent with other studies, as species with larger diameters and those associated with arbuscular mycorrhizal fungi invariably have moderate to high colonization rates, while there is much greater flexibility for species with thinner

roots to maintain either high or low colonization by mycorrhizal fungi (Liang et al., 2023; Ma et al., 2018; McCormack & Iversen, 2019). In this case, the trade-off with mycorrhizal colonization is likely unidirectional (i.e. constrained at only one end of the relationship) instead of bidirectional as is implicitly assumed with bivariate analysis (Laughlin et al., 2021). Finally, it is also interesting to note that in our dataset, most colonization rates were greater than 40%. This is in contrast to studies incorporating angiosperms, where species commonly have colonization rates below 40%, including many species at or near 0% colonization (Ma et al., 2018; McCormack & Iversen, 2019). This again may be due to the relatively limited number of observation in our study, but it may also reflect an important ecological distinction whereby gymnosperms as a group are more closely associated, and possibly more reliant on their mycorrhizal partners than angiosperms.

While early attempts to describe root trait organization mainly considered a single axis of root trait variation logically following from the discovery of the leaf economics spectrum (Wright et al., 2004), recent work has clearly highlighted the multidimensionality of root economics trait space. Building on our first two hypotheses, we tested whether fine-root functional traits within the gymnosperm phylogeny would align in a manner consistent with root economic space that has been observed in recent syntheses largely dominated by angiosperms (Bergmann et al., 2020; Ma et al., 2018). Consistent with our expectation, core root economics traits among gymnosperms aligned along two primary axes. First was an apparent gradient from roots with high SRL that can efficiently explore the soil to thick roots that are expected to be more reliant on mycorrhizal partners or alternative strategies for nutrient uptake. This is consistent with the collaboration axis of root economic space described in Bergmann et al. (2020). Accordingly, mycorrhizal colonization was also aligned with root diameter along the collaboration axis, despite its relatively weak bivariate relationship with diameter. We then observed a separate, orthogonal axis formed by root N and RTD consistent with the conservation axis. This suggests that despite their distinct evolutionary history, and despite known differences in many key functional traits (e.g. unique vasculature, leaf morphology and seed development), root traits of gymnosperms are coordinated in a similar manner to those of angiosperms at a global scale. This strongly suggests that fundamental biophysical and environmental constraints have helped to shape root trait evolution in similar ways across both angiosperms and gymnosperms, and perhaps indicates that continuing evolution of root traits may manifest in similar ways across these distinct plant groups, though the specific trends in past evolution may still differ.

Considering our third hypothesis, we found no clear indication that root traits have evolved consistently and directionally according to family divergence time among gymnosperms. This is in contrast to observations among angiosperms, which show a clear trend transitioning from thicker to thinner root diameters, moving from older to more recently diverged families (Chen et al., 2013; Comas et al., 2012; Ma et al., 2018). Furthermore, Chen et al. (2013) also provided evidence for directional evolutionary trends in additional
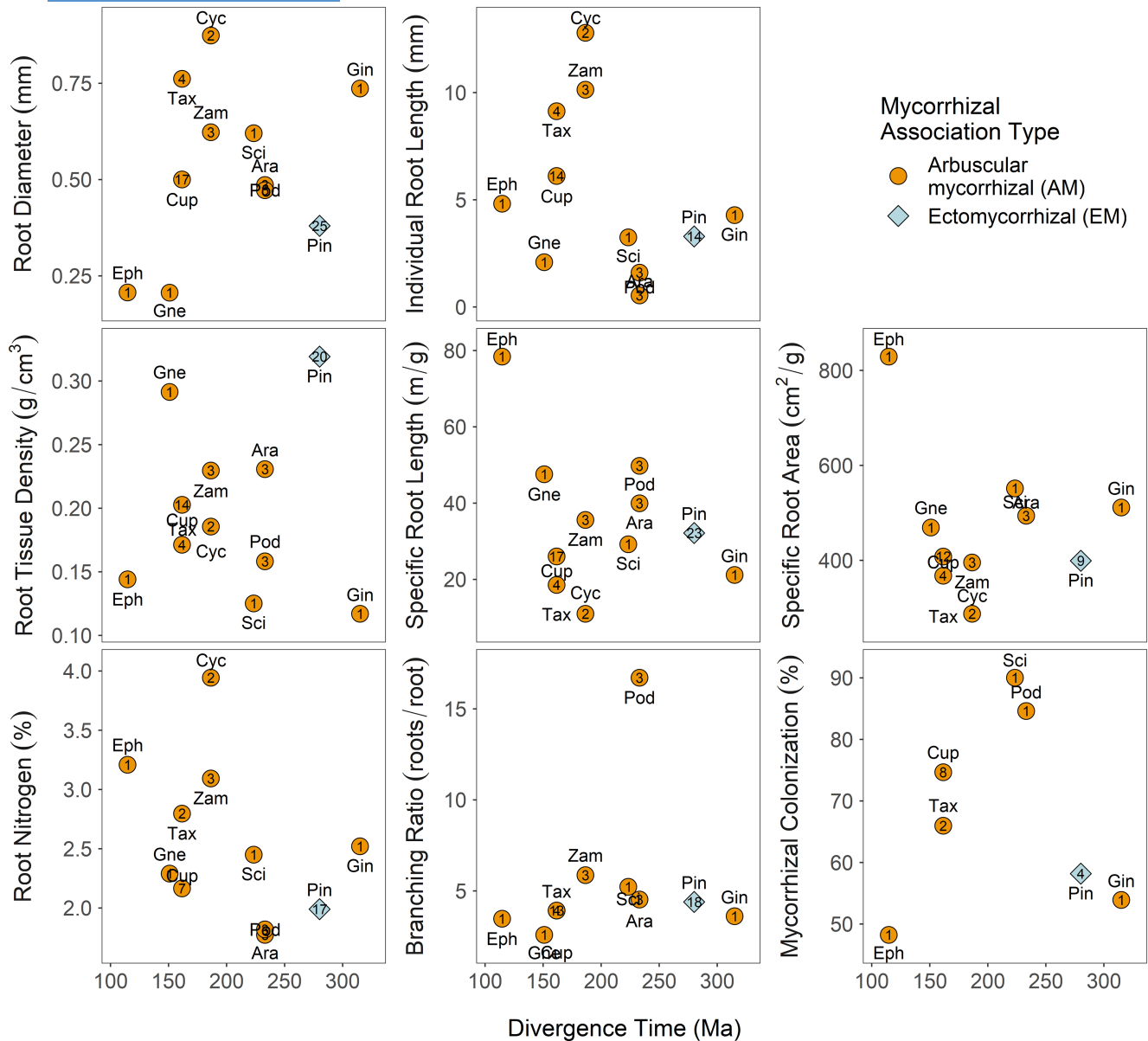
FIGURE 6 Relationships between mean first-order root traits at a family level and the estimated divergence time of each gymnosperm family. Standard linear models were fit using all families as well as separately using only the families that associate with arbuscular mycorrhizal fungi (i.e. removing Pinaceae). No models indicated significant relationships ($p > 0.10$). The family identity of each point is indicated by the first three letters of the family (full names in Figure 1), and the number of measured species used in the analysis for each family is noted inside each point (n). Ma, million years ago.

fine-root traits, including SRL and branching ratio. The lack of correlation between root traits and family divergence time among gymnosperms may be due to the limited numbers of families and overall data available for conducting the tests. However, it is also possible that unique biogeography and conditions experienced by gymnosperms as each family evolved may have led to these differences (Wang & Ran, 2014). For example, the divergence times of all extant gymnosperm families are roughly between 115 and 315 million years ago (Ma) (Forest et al., 2018), while all angiosperm families considered in previous analyses of root trait evolution diverged around or much more recently than 115 Ma. As a result, most gymnosperm families diverged under different climatic conditions of the Palaeozoic and early Mesozoic periods, while more recently diverged families within angiosperms came about under conditions that were cooler on average and with lower atmospheric concentrations of CO_2 (Wong et al., 2021). Cooler and drier conditions in more recent geologic history have since been linked with the development of thinner-diameter hydraulic conduits (Zanne et al., 2014) and increasingly thin root diameters in angiosperms (Comas et al., 2012), while gymnosperms have seen low diversification rates and increased extinction during the same period (Condamine et al., 2020). Finally, it must be acknowledged that relationships and divergence times among many major gymnosperm clades are still unresolved, and continued updates to

our understanding of gymnosperm evolution and relationships among families will undoubtedly continue to improve our understanding of trait evolution within and among these groups (Stull et al., 2021; Wang & Ran, 2014; Yang et al., 2022). Similarly, the inclusion of trait data collected across more species, gymnosperms and angiosperms alike, are needed to more confidently interpret evolutionary patterns over time.

An improved appreciation of the different below-ground resource acquisition strategies employed by diverse plant groups will lead to a better understanding of plant performance across environments and changing climates. We are only beginning to understand the nature and scope of fine-root functional trait variation among gymnosperms and how they may be similar or different from angiosperms. Root trait measurements beyond the Pinaceae and Cupressaceae are still limited, especially from species and families that are more common in tropical regions and in temperate regions of the southern hemisphere. In addition, to properly understand below-ground strategies, future studies should go beyond the most commonly measured traits (e.g. diameter, SRL, RTD and root N) by including other traits that are indicative of the costs and benefits of building and maintaining fine roots, such as anatomical adaptations, exudation, respiration and lifespan. Further, a better understanding of combined root and mycorrhizal resource uptake rates is clearly needed. Building this knowledge will allow for improved models to predict and explain plant health, species distribution and nutrient cycling in our changing world.

AUTHOR CONTRIBUTIONS

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CONFLICT OF INTEREST STATEMENT

PEER REVIEW

DATA AVAILABILITY STATEMENT

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Data S1. Full dataset with study averages and species averages across all species for the traits reported in this study.

Appendix S1. Supplementary Material.