Full paper

Climate and phylogenetic history structure morphological and architectural trait variation among fine-root orders

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Summary

• Fine roots mediate below-ground resource acquisition, yet understanding of how fine-root functional traits vary along environmental gradients, within branching orders and across phylogenetic scales remains limited.

• Morphological and architectural fine-root traits were measured on individual root orders of 20 oak species (genus *Quercus*) from divergent climates of origin that were harvested after three growing seasons in a glasshouse. These were then compared with similar measurements obtained from a phylogenetically diverse dataset of woody species from the Fine-Root Ecology Database (FRED).

• For the oaks, only precipitation seasonality and growing season moisture availability were correlated to aspects of root diameter and branching. Strong correlations among root diameters and architecture of different branch orders were common, while correlations between diameter and length were weakly negative. By contrast, the FRED dataset showed strong positive correlations between diameter and length and fewer correlations between root diameter and architectural traits.

• Our findings suggest that seasonal patterns of water availability are more important drivers of root adaptation in oaks than annual averages in precipitation and temperature. Furthermore, contrasting patterns of trait relationships between the oak and FRED datasets suggest that branching patterns are differentially constrained at narrow vs broad phylogenetic scales.

Introduction

Trait coordination and variance, both within and across species, determine the range of functional strategies exhibited by organisms across diverse environments (Reich, 2014; Díaz *et al.*, 2016). While the majority of plant trait assessments have focused on leaves and stems, the traits of fine roots are increasingly recognized as important codeterminants of plant functional strategies (Comas *et al.*, 2013; Eissenstat *et al.*, 2015). Despite this growing recognition, relatively little is known about fine-root trait variation across species and along environmental gradients compared to variation in the traits of above-ground plant structures (McCormack *et al.*, 2017). Given the importance of fine roots for both soil resource acquisition and total plant resource allocation (Eshel & Beeckman, 2013), it is critical to understand how fine-root traits differ among species and in relation to varied environmental conditions.

Recent analyses suggest a pivotal role for climate and plant phylogeny in determining patterns of fine-root trait variation, especially root diameter, across species at large spatial scales. For example, multiple studies have observed smaller average fine-root diameters among species growing in colder, higher latitude biomes compared to those found in more tropical systems (Chen *et al.*, 2013; Freschet *et al.*, 2017). Ma *et al.* (2018) also observed similar differences across biomes, but further noted the importance of plant evolutionary history as a codeterminant of climate-related patterns. In addition, previous studies have reported strong differences in root morphology when comparing earlier diverging lineages of angiosperms (e.g. Magnoliids) to more recently derived groups (Baylis, 1975; Comas *et al.*, 2012; Chen *et al.*, 2013; Valverde-Barrantes *et al.*, 2016). Together, these results suggest that the reduced prominence of evolutionarily earlier diverging plant families (e.g. Magnoliaceae and Lauraceae) at higher latitudes may be the proximate driver of decreased root diameter in woody species under colder climates.

Given the aforementioned effects of phylogeny on key fine-root traits, efforts to identify linkages between root trait variation and broader climate variables may also benefit from case studies within a narrower phylogenetic range (Ackerly, 2004; Mason & Donovan, 2015). For example, working within the genus

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Rhododendron, Medeiros *et al.* (2017) found climate of origin, and most often mean annual temperature (MAT), of an individual species' origin to be significantly correlated to several root traits including root diameter and specific root length. Similarly, recent observations within the single species, Scots pine (*Pinus sylvestris*) have also identified climate-driven patterns of fine-root trait variation and resource allocation within fine roots (Zadworny *et al.*, 2016, 2017). Intriguingly, the results of these genus- and species-level studies suggest an increase in root diameter in cooler climates, whereas the phylogenetically diverse, cross-biome datasets indicate that diameter tends to decrease from warmer to cooler climates (Freschet *et al.*, 2017; Ma *et al.*, 2018). These together suggest that root trait organization and variation along environmental gradients may differ at relatively narrow vs broad phylogenetic scales, although this has not been tested.

Complicating assessments of root trait relationships to climate, most previous comparative fine-root studies have assessed traits measured for many roots pooled together (e.g. all roots < 2 mm in diameter), which can mask variation among species. Researchers have more recently emphasized an order-based approach to facilitate more direct comparisons of functionally equivalent roots among species (Pregitzer *et al.*, 2002; Guo *et al.*, 2008; Chen *et al.*, 2013; Kong *et al.*, 2014). Order-based comparisons are usually made at the level of first-order roots, the most distal and metabolically active roots in the fine-root system (Chen *et al.*, 2013; McCormack & Iversen, 2019). Although this facilitates a robust basis for comparison across species, it also excludes higher order roots (i.e. second- and third-order) from analysis despite their importance as structural components of the absorptive root system. As a result, there has been little direct assessment of how morphological and architectural traits are correlated, not just within species, but also within the branching hierarchy (Pregitzer *et al.*, 2002; Guo *et al.*, 2008; Sun *et al.*, 2016). In particular, it is unknown whether variation in root morphology and branching are predictable within the branched fine-root system based on individual root orders and if trait organization differs among lineages or phylogenetic scales. Put simply, it is not known if having relatively thin or highly branched roots in one portion of the fine-root system (e.g. first-order roots) means that we can broadly characterize the entire fine-root system similarly as being thin or highly branched. Furthermore, there has been no previous test to determine if trait variation in higher order roots follows similar trends to those of first-order roots along climate gradients.

In the present study, our goals were two-fold. First, in light of the contradictory findings of previous studies, we aimed to better understand how fine-root traits vary across climate gradients. Our second goal was to determine how patterns of fine-root trait variation are organized at different phylogenetic scales. To address these goals, we measured fine-root traits on a functionally and climatically diverse woody plant clade, the genus *Quercus* L. (oaks), primarily focusing on North American species. Oaks were chosen as a focus due to their global ecological importance and the growing momentum among researchers of the Fagaceae as a powerful model system to understand the evolutionary ecology of plant traits (Cavender-Bares, 2019; Kremer & Hipp, 2020). We

began by testing whether morphological and architectural fine-root traits of oaks were correlated to MAT, mean annual precipitation (MAP), precipitation seasonality and an index of moisture in the growing season (I_mGS; Kaproth & Cavender-Bares, 2016) relating to each species' climate of origin. We specifically measured the diameter and length of individual root orders within the branching hierarchy as well as the branching ratios of lower order roots to higher order roots. These traits were selected due to their connection to root construction costs and resource acquisition (McCormack & Iversen, 2019; Bergmann *et al.*, 2020; G. T. Freschet *et al.,* unpublished). We hypothesized that root diameter and length would be lower but that branching ratios would be higher in species from cooler, drier climates compared to species from warmer, wetter climates. Next, we tested whether morphological and architectural traits were coordinated within the fine-root branch hierarchy across the 20 oak species, indicating that traits of higher order roots could be inferred from lower-order roots. We then contrasted these observations with a phylogenetically diverse suite of 60 woody plant species representing all major forest biomes using the Fine-Root Ecology Database (FRED; Iversen *et al.*, 2017) to determine whether patterns of within-branch trait coordination were consistent at relatively narrow (i.e. within *Quercus*) vs relatively broad phylogenetic scales. For both datasets, we hypothesized that root length and root diameter would be positively correlated within and across fine-root branch orders. By contrast, we hypothesized that root branching ratio would be negatively correlated to root diameter but still positively correlated to root length.

Materials and Methods

The oak root trait data (20 species) were collected from individual plants grown in a glasshouse under common garden conditions using seed collected from native habitats while additional fine-root trait data (60 species, 29 families) were obtained using the FRED Database. All measurements and trait comparisons were conducted on a root-order basis and emphasize the most absorptive roots of the fine-root systems (McCormack *et al.*, 2015). Experimental and methodological details for each are described separately below.

Glasshouse study

To compare the morphology of oaks from a wide range of environments and clades, acorns were collected in the autumn of 2012 from the USA, Mexico and Costa Rica from over 35 species and planted as a common garden in February 2013 in paired glasshouses at the University of Minnesota (44°59′19″N, 93°10′52″W). For full details of this common garden experiment see Kaproth & Cavender-Bares (2016). Briefly, glasshouses were maintained at a growing season temperature of 22–32°C (8–16°C in the winter). To limit root binding and stunted growth over three growing seasons, plants were transplanted twice in the first year; the final transplant was into 0.75-m-deep pots (two 12.5-l T616 pots stacked; Stuewe & Sons, Tangent, OR, USA). The top, original pot had its bottom removed to produce a

continuous soil column with the pot below. The pots contained 1 : 1 by volume mix of coarse sand and Sunshine LC8 potting mix which is a blend of peat moss (75%), coarse-grade perlite (20%) and coarse-grade vermiculite (5%) with gypsum, dolomitic limestone, a wetting agent, and major and minor nutrients. Peter's Professional Acid Fertilizer (N : P : K of 21 : 7 : 7; ICL Specialty Fertilizers, Dublin, OH, USA) was then used to maintain fertility during the study (Kaproth & Cavender-Bares, 2016). Soil moisture in the pots was allowed to drop to 14–21% volumetric water content (VWC) and then watered to saturation to simulate mesic to hydric soil conditions.

From the full glasshouse study, we selected 20 oak species to harvest and sample fine-root traits. Each species was represented by one to three populations (35 mothers in total). Species were selected to encompass a gradient of climate origin ranging from 7.8 to 24.2°C MAT and 650 to 1851 mm MAP (Table 1). Additionally, species were selected to encompass broad phylogenetic diversity within the genus, emphasizing the four major representative sections of the Americas including white oaks (section *Quercus*, seven species), red oaks (section *Lobatae*, eight species), live oaks (section *Virentes*, two species) and golden cup oaks (section *Protobalanus*, one species), as well as two species representing the Eurasian oak clades (*Q. ilex* of section *Ilex*; and *Q. acutissima* of section *Cerris*; Fig. 1).

Oak root sample processing

Plants were destructively harvested and fine roots were sampled after three full growing seasons in September 2015. For each species, three or four individual plants were harvested by cutting away the plastic pots and carefully rinsing the majority of adhering soil from the roots. Once the soil was removed, intact branches of fine roots were cut off from the coarse roots (roots > 2 mm in diameter). Root samples were then put in zippered plastic bags, placed inside a rigid box to prevent damage to the roots during storage and then placed in a freezer (−20°C).

For trait assessments, an individual bag containing roots of an individual plant was taken from the freezer and allowed to thaw before roots were handled, to prevent breakage of the intact roots that may occur when processing frozen roots. Once thawed, intact root branches were given a final cleaning with tap water to remove adhering soil. Each selected branch contained at least three intact root orders. One exception was made in the case of *Q. acutissima*, which often only produced one or two orders of fine roots from parent coarse roots. Additionally, colonization of fine roots by ectomycorrhizal fungi in the glasshouse-grown seedlings was generally low and highly variable among individuals (data not shown). As such, we focused our analyses on uncolonized roots to ensure that measurements were as comparable as possible across the study.

Intact root branches were observed under a dissecting scope and the diameter and length of individual roots was measured manually using a reticule. The root order was noted for each root measurement according to the morphometric ordering approach, where the most distal root is a first-order root, its parent root is a second-order root and so on (Pregitzer *et al.*, 2002). At least three

Table 1 Species names, location of seed origin and relevant mean range climate details for the 20 oak (*Quercus*) species used in the common garden glasshouse study.

Species name	Seed origin	MAT	MAP	Precipitation seasonality	$I_m GS$
Q. acutissima	USA: Tennessee	14.5	1641	33.0	26.1
Q. alba	USA: Minnesota	12.6	1167	18.9	−32.1
Q. aristata	Mexico: Oaxaca	21.8	1203	76.7	27.9
Q. castanea	Mexico: Jalisco, Michoacán, Oaxaca	17.7	1040	96.5	20.5
Q. chrysolepis	USA: California	11.5	928	76.7	−87.9
Q. crassipes	Mexico: Michoacán	15.2	997	94.7	23.6
Q. grahamii	Mexico: Oaxaca	18.3	1127	92.6	25.7
Q. hemisphaerica	USA: Florida	18.8	1409	26.1	−15.3
Q. ilex	USA: California	13.0	650	39.0	−71.7
Q. imbricaria	USA: Illinois	11.8	1020	26.1	−33.8
Q. lobata	USA: California	14.3	681	86.4	−95.8
Q. lyrata	USA: Illinois	17.3	1339	18.9	−30.5
Q. macrocarpa	USA: Illinois, Minnesota	7.8	817	41.5	−37.9
Q. margarettae	USA: Florida	18.1	1320	23.4	−21.2
Q. montana	USA: Pennsylvania	11.2	1164	12.5	−33.4
Q. oleoides	Costa Rica: Guanacaste	24.2	1851	76.7	45.9
Q. rubra	USA: Minnesota, Pennsylvania	9.4	1042	22.6	−33.8
Q. rugosa	Mexico: Oaxaca; USA: Arizona	13.7	752	82.6	−19.6
Q. virginiana	USA: Florida, Pennsylvania	19.7	1190	34.7	−20.2
Q. wislizeni	USA: California	13.6	745	82.8	−94.2

Mean annual temperature (MAT, °C), mean annual precipitation (MAP, mm) and seasonality of precipitation were taken from the WorldClim database from variables BIO_1, BIO_{12} and BIO_{15}. The index of moisture in the growing season ($I_m GS$) was calculated for this study with more negative values representing lower moisture availability. The seed origin is indicated by the country and the local state or province.

root branches were measured for each plant. From each branch we measured length and diameter for at least 10 randomly selected first- and second-order roots, and then diameter was measured for all third-order roots within a given branch (generally 1–3 per branch). Three plants of each species were collected from the mesic soil condition (for details see Kaproth & Cavender-Bares, 2016) with the exceptions of *Q. montana*, *Q. hemisphaerica* and *Q. chrysolepis*, for each of which only two individuals were processed. Additionally, four individuals of

Fig. 1 Phylogenetic tree along with summary information of fine-root trait variation among 20 species of oak (*Quercus*). Tree phylogeny was derived from Hipp *et al.* (2020) and the names of the major oak sections included are given at their respective branches. Range of variation is displayed for each fine-root trait with relatively larger values indicated in black and smaller values indicated progressively from grey to white. The coefficient of variation calculated across the 20 species is provided at the bottom of each trait column. Traits for which no data are available are indicated with striped boxes for *Q. accutissima* and *Q. macrocarpa*.
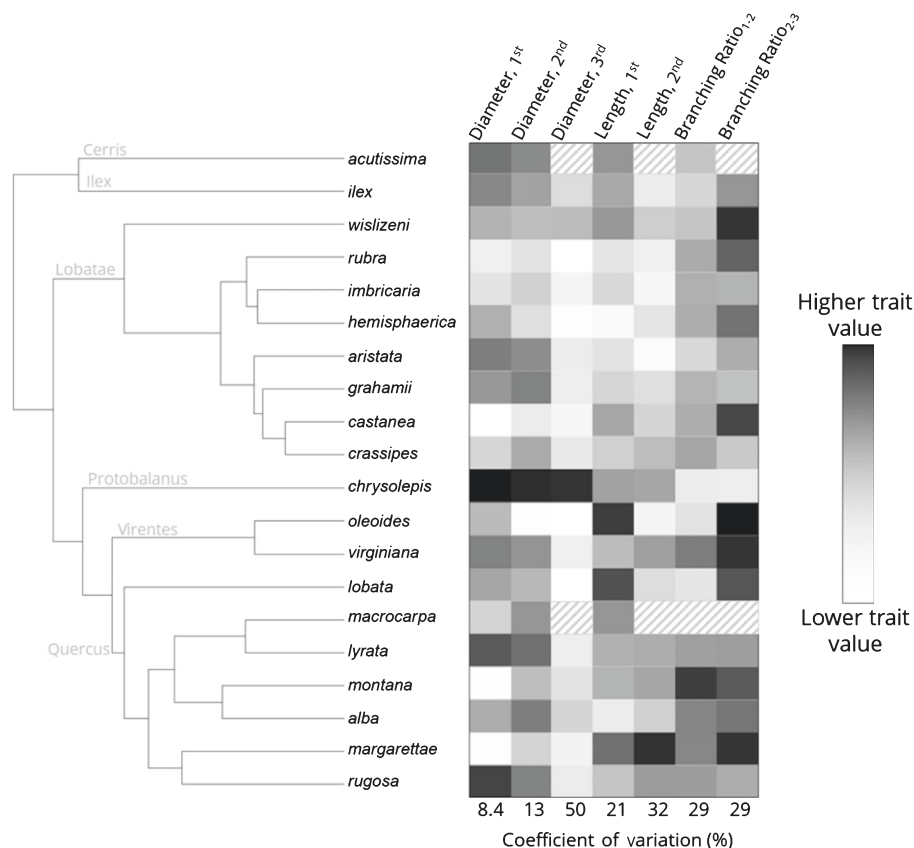
Q. alba were used (two of which were grown under hydric soil conditions). We observed no consistent differences in fine-root traits among individuals from mesic and hydric conditions and, as such, all replicates were combined for analyses. Length measurements of second-order roots and diameter measurements on third-order roots were not made for *Q. acutissima* or *Q. macrocarpa* due to sample constraints. In total, 3655 and 3069 roots were measured for diameter and length, respectively.

Branching ratio, defined as the number of lower order roots per higher order root, was measured for the number of first-to-second order roots (BR_{1-2}) and the number of second-to-third order roots (BR_{2-3}). The same branches used in the above morphological assessments were also used here with the exception that no assessments of branching ratio were made for *Q. macrocarpa* and only BR_{1-2} could be measured for *Q. acutissima* due to sample constraints. For each branch, BR_{1-2} was measured for between 10 and 30 second-order roots and BR_{2-3} was measured for all available third-order roots. In total, 2836 and 208 assessments of branching ratio were collected from second- and third-order roots, respectively. Before analysis, measurements of individual root morphology (diameter and length) and architecture (branching ratio) were first averaged at the branch level, then the individual plant level and finally to the species level. We used species as the unit of replication and average trait values by species for all subsequent comparisons of root length, diameter and branching ratio during our analyses. All data generated on glasshouse-grown oaks and used in this analysis are available in the Supporting Information (Dataset S1).

Accessing woody root traits in FRED

Data collected for the broader woody plant trait dataset were obtained from FRED v.2.0 (Iversen *et al.*, 2018). We limited our analysis to data that were collected on woody species, collected and reported on an individual root order basis following or adapted to the morphometric ordering approach (Pregitzer *et al.*, 2002), collected from field-grown plants, and accompanied by information concerning the root traits of interest for this study (diameter, length, and branching ratio for first-, second- and third-order roots). In many cases, species were represented by more than one observation and in these instances all observations for a given trait, root order and species were averaged to yield a single species average. Finally, we limited our species list to only those which included a full matrix (i.e. no missing data) for the traits of interest. In total, we obtained a phylogenetically diverse dataset of 60 woody species from 29 families (Fig. S1). All data obtained from FRED and used in this analysis are available in Dataset S1.

Statistical analysis

To assess the relationships between oak fine-root traits and their species climate of origin, we examined a number of different climate factors, including MAT, MAP, I_mGS and precipitation seasonality. Estimates of MAT, MAP and precipitation seasonality were obtained directly from WorldClim (i.e. BIO1, BIO12 and BIO15, respectively; year 1950–2000 averages; Hijmans *et al.*,

2005), while I_mGS was calculated for each location as $100 \times$ (precipitation $\times$ PET)/(PET) for May to August (Kaproth & Cavender-Bares, 2016). Potential evapotranspiration (i.e. PET) was derived from WorldClim based on mean monthly air temperature and day length for each location (Kaproth & Cavender-Bares, 2016). Climate variables were averaged within each species range and used for analysis (Hipp *et al.*, 2018). We tested for phylogenetic shifts in bioclimatic variables and root morphospace by evaluating the relative support for alternative Ornstein–Uhlenbeck (O-U) models, which model transitions in trait values as responses to shifting selective regimes (Hansen, 1997; Martins & Hansen, 1997; Butler & King, 2004). This analysis was performed using an Expectation Maximization (EM) search algorithm (Bastide *et al.*, 2018) over the space of $K = 1$–10 transitions, allowing for covariance among traits for multivariate analyses (using the scalar O-U process model) or independent individual traits (using the O-U model for independent traits). Finally, we assessed trait–trait relationships between root orders to determine the organization of morphological and architectural trait relationships among woody plants.

Linear regression was used to test relationships between root traits and climate variables and to test relationships among fine-root traits. Analyses were initially conducted using Pearson's correlations followed by phylogenetic generalized least squares (PGLS) with a phylogenetic covariance matrix, simultaneously estimating a parameter for phylogenetic heritability of the regression residuals (Pagel's λ; Pagel, 1999). Results are presented from both approaches to facilitate comparison with previous studies; however, we place greater emphasis on results that account for phylogenetic covariance throughout the discussion. We similarly report Pearson's r to enable comparisons with previous studies. For reporting relationships (r) of models accounting for nonindependence and unequal variances expected in phylogenetically structured data, we used phylogenetic independent contrasts (PICs) among traits based on λ-rescaled phylogenies pruned from Hipp *et al.* (2020) (Felsenstein, 1985; Freckleton *et al.*, 2002). All rescaling was truncated to a Pagel's λ between 0 and 1.

After assessing the morphological and architectural relationships among fine-roots traits within the phylogenetically constrained oak root dataset, we then repeated the same analyses in the FRED-based dataset. Phylogenetic analysis of the FRED dataset was carried out as described above for the oaks, but using a phylogeny pruned from Zanne *et al.* (2014). All analyses were conducted in R version $\geq 3.3.2$ (R Core Development Team, 2019) with packages APE v.5.1 (Paradis & Schliep, 2018), GEIGER v.2.0.6 (Harmon *et al.*, 2015), MAGRITTR v.1.5 (Bache & Wickham, 2014), NLME v.3.1-137 (Pinheiro *et al.*, 2020), PEZ v.1.1-1 (Pearse *et al.*, 2015), PHYLOGENETICEM v.1.4.0 (Bastide *et al.*, 2017) and PHYTOOLS v.0.6-44 (Revell, 2012).

Results

Root trait variation among oaks

Morphological and architectural traits demonstrated notable variation among oak species. The lowest variation was observed for first- and second-order root diameter (CV < 15%) and the highest variation in third-order root diameter (CV = 49.5%), while all other traits expressed more moderate variation (CV from 21% to 32%; Fig. 1). The range of trait values observed across all 20 species was well represented within each of the major North American clades. As such the mean, minimum and maximum values of various traits were qualitatively similar in most cases when comparing across red oaks (section *Lobatae*), white oaks (section *Quercus*) and the live oaks (section *Virentes*). However, a few species stood out as having more peripheral trait values relative to other species in the study (Fig. 1). For example, *Q. chrysolepis* (section *Protobalanus*) expressed the largest first-, second- and third-order root diameters as well as the lowest branching ratios for both first-to-second- and second-to-third-order roots. By contrast, *Q. rubra* (section *Lobatae*) displayed consistently small root diameters and root lengths with moderate to high branching ratios.

The general absence of strong differences among the major oak clades in morphological and architectural traits was supported by the O-U models, which recovered no significant shifts among major clades. The one exception was our modelling of I_mGS, which identified nine shifts with strong support. This exceptionally high number of shifts relative to the total number of taxa in the tree (20) suggests that there is more heterogeneity within than among clades. However, even in this scenario the selection strength was relatively low ($\alpha = 0.539$ on a tree of height = 51.5 m) and the variance relatively high ($\sigma^2 = 3.94$), suggesting low explanatory power of the model and that its significance may derive from overfitting of the data.

On average, the oak species produced relatively thin, but somewhat long and well-branched roots compared to averages observed across the phylogenetically diverse FRED root-trait dataset. First-, second- and third-order root diameters were roughly 76%, 72% and 40% thinner than the averages contained in the FRED dataset, while branching ratios of first-to-second-order roots were 14% higher and over an order of magnitude higher for second-to-third-order oak roots. Despite smaller diameters, oaks also tended to produce longer average root lengths than the averages across the FRED dataset. Specifically, the mean first- and second-order roots were 0.78 and 5.0 mm in length among the oaks, respectively, compared with mean lengths of 0.63 and 1.5 mm for first- and second-order roots on average within the FRED dataset.

Trait relationships with climate of origin

Among the four climate variables considered, seasonality of precipitation and I_mGS were the most apparent drivers of variation in oak fine-root traits (Table 2; Figs 2, S2). Increasing seasonality of precipitation was negatively correlated to branching ratio of first-to-second-order roots with ($r = -0.56$, $P = 0.02$) and without accounting for phylogeny ($r = -0.54$, $P = 0.02$), while it was positively correlated to first-order root length ($r = 0.45$, $P < 0.10$), but only after accounting for phylogenetic covariance. I_mGS was negatively correlated to both first-order root diameter ($r = -0.20$, $P = 0.06$) and third-order

Table 2 Correlation coefficients for Pearson's r and phylogenetic generalized least squares (PGLS) analyses of relationships between fine-root traits and climate of origin for 20 oak species.

	I_mGS		MAT		MAP		Precipitation seasonality	
	r	PGLS	r	PGLS	r	PGLS	r	PGLS
Root diameter								
1st order	−0.17	−0.20*	0.13	0.09	−0.06	−0.13	0.19	0.19
2nd order	−0.24	−0.29	−0.24	−0.31	−0.26	−0.36	0.05	−0.01
3rd order	−0.48**	−0.62	−0.35	−0.44	−0.29	−0.04	0.17	−0.22
Root length								
1st order	−0.15	−0.12	0.16	0.19	0.09	0.03	0.24	0.45*
2nd order	−0.14	0.04	−0.05	0.04	0.01	0.18	−0.19	−0.36
Branching ratio								
1st to 2nd	0.12	0.15	−0.18	−0.23	0.09	0.06	−0.54**	−0.56**
2nd to 3rd	0.01	0.13	0.28	0.29	0.31	0.26	−0.22	−0.15

The significance of each test is indicated: *, $P \leq 0.10$; **, $P \leq 0.05$. Sample size (n) equals 20 (df = 18) for all contrasts except correlations with branching ratio 1st to 2nd ($n = 19$, df = 17) as well as branching ratio 2nd to 3rd and 3rd order root diameter ($n = 18$, df = 16).

root diameter ($r = 0.48$, $P < 0.05$). However, the relationship with first-order roots was only apparent after accounting for phylogenetic covariance, while the relationship with third-order roots was only significant without considering phylogeny. Neither MAT nor MAP showed any significant relationship with the root traits measured in this study (Table 2; Fig. S2).

Trait relationships within and across fine-root orders

The diameters of first-, second- and third-order roots were consistently and positively correlated to one another among the oaks as well as across the FRED dataset ($P < 0.05$ in all cases; Fig. 3; Tables S1–S3). Within the oak dataset, the diameters of first-, second- and third-order roots were negatively correlated to branching ratios of second-to-third-order roots ($P < 0.05$; Fig. 3), while first-order diameter was also weakly correlated to branching of first-to-second-order roots ($P = 0.06$, $r = -0.44$; Table S1). The lengths of second-order roots were positively correlated to branching ratio of first-to-second-order roots ($P < 0.05$) while the lengths of first-order roots were weakly but negatively correlated to branching of first-to-second- and positively correlated branching of second-to-third-order roots ($P < 0.10$). By contrast, there were few relationships between root morphology and root branching ratios in the FRED dataset, which only revealed a positive relationship between second-order length and branching of second-to-third-order roots ($P = 0.02$; Fig. 3) as well as a weak, negative relationship between third-order root diameter and branching of first-to-second order roots ($P < 0.10$; Table S2). While the lengths of first-order roots and second-order roots were not correlated among the oaks, they were strongly and positively correlated in the FRED dataset ($P < 0.001$ in all cases, correlation coefficients > 0.7). Additionally, larger diameter roots were associated with shorter first-order roots among the oaks, while the opposite was true within the FRED dataset ($P < 0.05$, Fig. 3; Tables S1, S2).

Discussion

Connections between fine-root traits and climate of origin

The observed shifts in oak fine-root traits were best captured by climate variables related to water availability, with precipitation seasonality and I_mGS being correlated to aspects of root morphology and branching. This highlights the importance of water as a critical resource dictating plant adaptation and growth as well as the importance of changing water availability at time scales of weeks to months rather than annual averages. However, the links between fine-root variation and water availability were limited overall and we found no significant relationships between measured fine-root traits and either MAP or MAT. Collectively, these findings suggest that, in contrast to previous studies, the relative importance of MAT and MAP in driving fine-root variation are low within the genus *Quercus*.

The unique evolutionary history of oaks within North America makes this study an interesting contrast to previous assessments of root trait–climate interactions. Most previous comparisons across populations and species correspond with evolutionary radiations of plant species moving from warmer, lower latitude environments to cooler, higher latitude environments. By contrast, oaks in North America originated from a common, high-latitude ancestor *c.* 45 million years ago and radiated southward into warmer climates (Hipp *et al.*, 2018). We speculate that this temperate-toward-tropical pattern of radiation may account for the lack of covariation of root traits with MAT in this study. Plant radiations from the tropics toward higher latitudes often correspond to significant reductions in species' average root diameter. This shift may be driven by environmental pressures to evolve thinner roots with reduced risk of hydraulic failure associated with freezing and drought (*sensu* Zanne *et al.*, 2014) or the need for more competitive strategies in areas with reduced, and often pulsed nutrient availability in colder climates. In either case, the predominant constraints driving changes in root traits seem to occur moving from low to high latitudes. Due to their common
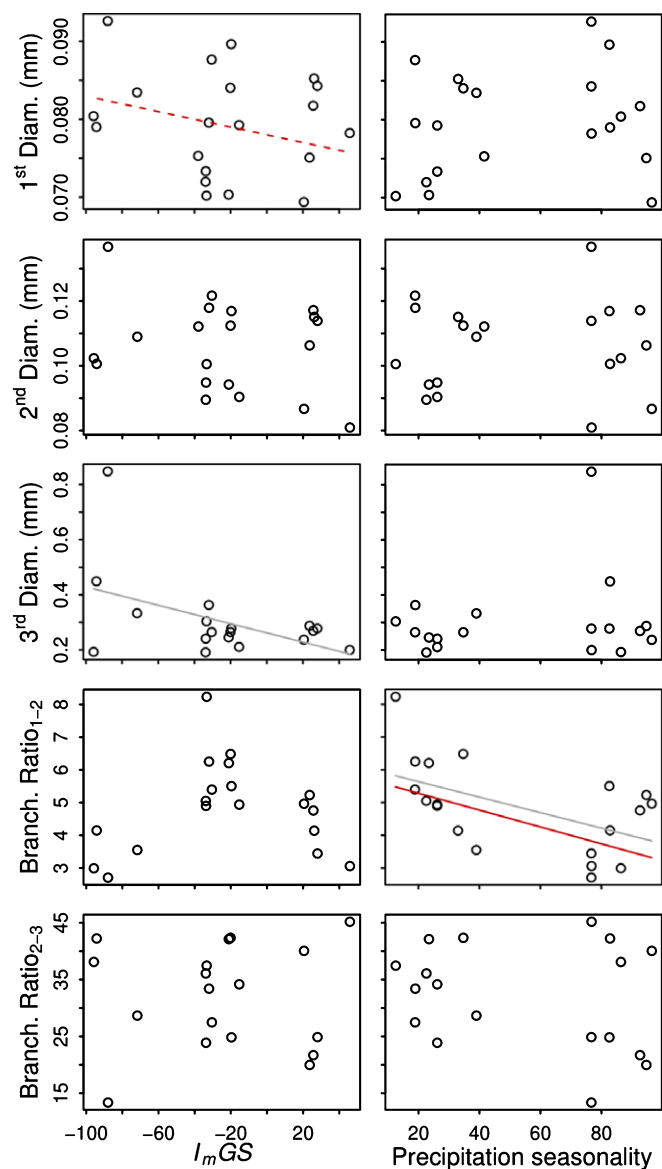
Fig. 2 Relationships between oak root traits and climate identified by Pearson's *r* and phylogenetic generalized least squares (PGLS) shown in grey and red lines, respectively. Relationships significant at $P \leq 0.05$ are shown with solid lines and $P \leq 0.10$ with dashed lines. Abbreviations: 1st, 2nd and 3rd represent first-, second- and third-order fine roots; Diam. (diameter), Branch. ratio (branching ratio); $I_m GS$ (index of moisture during growing season), and Precipitation seasonality (seasonality of precipitation).

It is also possible that the limited fine-root trait variation observed in this study was due to the experimental approach of growing all individuals under common glasshouse conditions (i.e. removed from climate of origin). However, previous studies indicate that substantial variation in fine-root traits is under genetic control and suggest that observed differences in root traits measured across different environments are also likely to be present when populations or species are grown in common garden settings. For example, Zadworny *et al.* (2016) showed that climate-driven variation in allocation to absorptive fine roots identified across populations of *P. sylvestris* along a regional transect was similarly present among individuals sourced from these populations when grown together in a common garden. Similarly, Medeiros *et al.* (2017) identified notable plasticity in first-order root diameter, root link length, specific root length and specific root tip abundance when species were grown in relatively cooler vs warmer common garden locations, but also detected a broader signal associated with the climate of origin among species of *Rhododendron*. Given that the oak species used in our study originated from locations that were over 3500 km apart and encompassed a climate range of 16.5°C in MAT and 1200 mm in MAP, we expect that genetically determined relationships between climate of origin and species-level variation in the fine-root traits assessed here should have been detectable where they existed. However, we also expect that trends observed in our study could shift, either strengthening or weakening, if measured across individuals growing in different environments.

Patterns of within-branch trait variation at different phylogenetic scales

Patterns of fine-root trait variation among the distal root orders were largely consistent with our expectations, but with some notable exceptions. In both the phylogenetically constrained oak root dataset and the diverse species dataset from FRED, there were strong, positive correlations in root diameter among all root orders, demonstrating that relatively larger first-order roots tend to arise from relatively larger second-order roots and so on across higher root orders. Larger diameters were further associated with longer roots in both first- and second-order roots within the FRED dataset, but not in the oak dataset. At the broader phylogenetic scale captured within FRED, there appears to be some physical constraint such that larger diameter roots are associated with longer roots. This has not been previously shown to apply broadly across species or among fine-root orders; yet, this finding is intuitively satisfying as longer roots with greater potential to absorb resources should also be connected to larger diameter roots that effectively represent larger 'pipes' with lower resistances to conduct those resources (Tyree & Ewers, 1991). The absence of this relationship in the oak dataset suggests that at narrow phylogenetic scales, and within smaller trait ranges, there is sufficient flexibility among these traits to allow for independent adaptations to external environmental factors.

Contrasting trait–trait relationships observed in the oak and FRED datasets may also be due to the fact that oaks have repeatedly adapted to a variety of habitats within clades, both in the
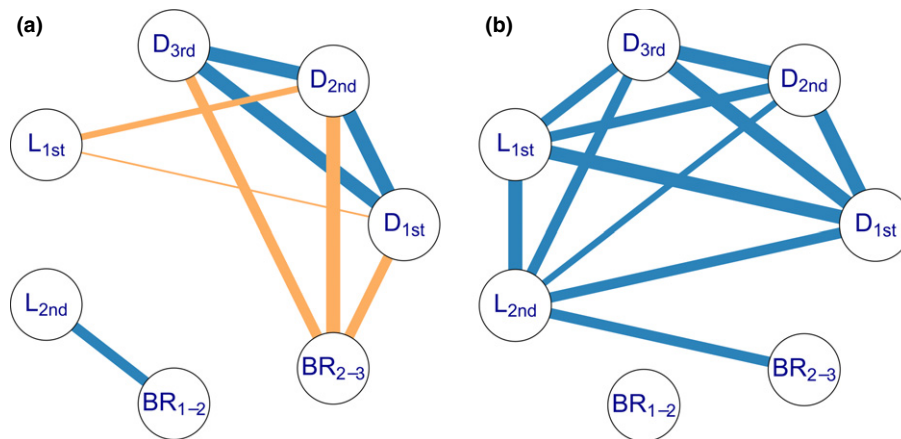
origin from a high-latitude ancestor, we suspect that the North American oaks already possessed rooting strategies that were competitive and tolerant of cool, high-latitude systems before radiating into lower latitudes. For example, all oak species commonly construct first-order roots that are thinner than 80–90% of other woody plants currently represented in the global FRED database (data not shown). Therefore, we suggest that limited changes in root traits observed in this study with the major climate variables, especially MAT, may be due to lack of a strong constraint on viable root morphology for the North American oak species moving from cooler to warmer climates.

Fig. 3 Correlation network for fine-root traits among 20 oak (*Quercus*) species (a) and 60 phylogenetically diverse woody plant species (b) based on phylogenetic generalized least squares (PGLS). The morphological traits diameter (D) and length (L) are shown for first-, second- and third- (diameter only) order roots and the architectural trait branching ratio (BR) for the number for first-order roots per second-order root (1–2) and second-order roots per third-order root (2–3). Positive correlations are shown in blue and negative correlations are shown in red while the strength of the correlation is represented by the width of the line. Only lines associated with $P \leq 0.05$ and correlation coefficient ≥ 0.10 or ≤ -0.10 are shown. Detailed correlation information for both PGLS and Pearson's *r* is available in Supporting Information Tables S1 and S2.

Americas (Cavender-Bares, 2019) and globally (Hipp *et al.*, 2020). If fine-root allometry is partly a function of phylogenetic heritage, the rapid rate of evolution within oak clades may break down any apparent allometric relationships. Stated another way, the broad-scale correlations among root traits observed in woody plants globally may not be due solely to functional or environmental constraints, but also to historical contingency. It is telling that the variation in root traits among oak clades is no greater than expected under a random walk (Brownian motion) model of trait evolution, but that there is still a significant relationship between fine-root branching architecture and I_mGS, which exhibits high variation within clades. This finding provides strong evidence for the role of drought adaptation in the evolutionary diversification of fine-root branching architecture in oaks. The high rate of adaptation observed within oak clades in leaf traits (Pearse & Hipp, 2012) is probably reflected in fine-root traits as well, resulting in high within-clade root diversity and low among-clade trait diversity.

Fine-root branching architecture has long been considered an important part of how root systems adapt to their environment (Fitter, 1987), and recent studies have further highlighted the important role that branching architecture may play in defining plant resource acquisition strategies below ground (Beidler *et al.*, 2015; Li *et al.*, 2017; Liese *et al.*, 2017). Previous work relating branching ratios to root diameters have noted substantial independence between these traits and suggested that architecture and morphology represent separate axes of trait variation (Kong *et al.*, 2014; Liese *et al.*, 2017). Using the broader FRED dataset, we also found substantial independence between branching ratios at both the first-to-second-order level and the second-to-third-order level from most length and diameter measures among different root orders. These results further support the suggestion that architectural and morphological traits can vary independently from one another and probably enable multiple strategies for woody species to adapt in different environments. Surprisingly, we observed several significant relationships within the oak

dataset that suggest some connection to root morphology and phylogenetic limitations in branching architecture. In particular, the branching ratio of second-to-third order roots was negatively correlated with root diameters of first-, second- and third-order roots, which may indicate a trade-off among oak species to either build relatively large diameter fine roots or thinner but more highly branched fine-root systems. At the same time, the positive relationship between the length of second-order roots and first-to-second-order branching ratio may simply be a physical relationship, as a larger number of branches is more easily accommodated on longer roots.

While the patterns observed in this study among root traits and climate of origin should be robust, it is important to note that the absolute trait values expressed by our oak plants grown under glasshouse conditions are probably somewhat different from those observed in the field due to different edaphic and environmental conditions (Poorter *et al.*, 2016). Additionally, the limited connections between root traits and climate observed in this study do not preclude the possibility that oak roots may still express significant plasticity to different environments via shifts in other fine-root traits (e.g. tissue density, chemistry, anatomy), by associations with local microbial communities (Pickles *et al.*, 2015; Chen *et al.*, 2018), and especially shifts in colonization by mycorrhizal fungi (McCormack *et al.*, 2017; Ostonen *et al.*, 2017). Finally, plants may also significantly alter their below-ground strategies for resource acquisition beyond those measured at the level of individual fine roots by changes in total biomass allocation to the fine-root system (Freschet *et al.*, 2015) and by shifting coarse root architecture and rootitng depth distributions.

Conclusion

Utilizing a common garden study of an emerging model plant clade (i.e. American oaks), we evaluated potential drivers of interspecific variation in morphological and architectural fine-root

traits related to climate of origin. While the most commonly used climate variables, MAT and MAP, demonstrated no connection to oak fine-root variation, there was evidence that measures of water availability during the growing season may relate to shifts in fine-root traits as oaks adapt to different environments. We suggest that the evolutionary history of the American oak clade, which radiated from cooler, high-latitude environments to warmer, low-latitude environments, may be responsible for the weak connections to MAT and provides an informative contrast to previous root trait studies focused on radiations from warmer to cooler environments. Furthermore, this study provides a novel assessment of correlations among core fine-root traits within the branching hierarchy and shows that patterns of fine-root morphology at the level of individual roots can be used to infer probable variation among other fine-root orders. Finally, our assessments of within-branch trait variation also revealed important differences regarding correlations between morphological and architectural traits when evaluated within the phylogenetically constrained oak root dataset vs within the more diverse FRED dataset. The distinct relationships observed between these two datasets highlight the importance of considering both the ecological and the phylogenetic breadth among study species when interpreting patterns of fine-root trait variation and their relationships with external environmental factors.

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Author contributions

MLM and MAK designed the study with input from JCB and PGK. EC, YH and MLM conducted measurements while MAK conducted analyses with ALH. MLM wrote the manuscript with MAK. All authors provided critical input to subsequent manuscript drafts. MLM and MAK contributed equally to this work.

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Supporting Information

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

Dataset S1 Data generated on glasshouse-grown oaks and data obtained from FRED.

Fig. S1 Phylogenetic tree for 60 species contained in a woody plant dataset obtained from the Fine-Root Ecology Database (FRED).

Fig. S2 Relationships between root traits and climate identified by Pearson's *r* and PGLS.

Table S1 Pairwise correlation coefficients among morphological and architectural fine-root traits within the oak dataset.

Table S2 Pairwise correlation coefficients among seven morphological and architectural fine-root traits within a woody plant dataset obtained from FRED.

Table S3 Pagel's lambda (λ)* for fine-root trait-by-trait relationships estimated through phylogenetic Generalized Least Squares (PGLS) regression on the oak and FRED datasets.

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