Evolutionary history resolves global organization of root functional traits

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Abstract

Plant roots have diversified greatly in both form and function since the first land plants emerged[1,2], but the global organization of root functional traits remains poorly understood[3,4]. We analyzed a new global dataset of ten functionally important traits, compiled from metabolically active first-order roots collected from 369 species distributed across natural plant communities of seven biomes globally. Our results identify a high degree of organization of root traits across species and biomes, but also that the observed pattern differs from expectations based on studies of leaf traits. Root diameter exerted the strongest influence on root trait variation across plant species, growth forms, and biomes. Our analysis suggests that plants have evolved thinner roots since they first emerged in land ecosystems, which, in turn, has allowed them to dramatically improve the efficiency of soil exploration per unit of carbon invested and reduce dependence on symbiotic mycorrhizal fungi. We also found that diversity in root morphological traits is greatest in the tropics where plant diversity is highest and many ancestral phylogenetic groups are preserved, but declines sharply from the tropics to temperate regions to desert biomes, presumably due to changes in resource supply caused by seasonally inhospitable abiotic conditions. Our results suggest that root traits have evolved along a spectrum bounded by two contrasting strategies of root life: from an ancestral 'conservative' strategy in which plants with thick roots depend on symbiosis with mycorrhizal fungi for soil resources, to a derived 'opportunistic' strategy in which thin roots allow

plants to more efficiently leverage photosynthetic carbon for soil exploration. These findings imply that innovations of belowground traits have been key for preparing plants to colonize new habitats, and for generating biodiversity within and across biomes.

69 Recent efforts to understand how functional traits are organized across land
70 plants have revealed striking patterns across the leaf economic spectrum[5,6], but
71 whether such high degree of organization is reflected also in root traits remains
72 controversial[4,7]. A key factor limiting progress has been the paucity of data on root
73 traits across plant species and biomes, as roots are difficult to sample and
74 characterize[8,9]. Yet, roots are vital for the ability of plants to acquire nutrients and
75 water – two functions of fundamental importance to whole-plant performance and for
76 predicting how plants respond to elevated CO_2 and climate change[10-12].
77 Roots face ecological and physiological challenges that differ fundamentally
78 from leaves. Roots must compete for, and acquire, nutrients and water in
79 environments that vary greatly across the world's biomes, with biophysical conditions
80 ranging from relatively stable (*e.g.*, tropical rainforests) to highly seasonal (*e.g.*,
81 deserts or boreal forests). The high diversity that exists in root form and function, and
82 in the degree of association with symbiotic mycorrhizal fungi, raises a fundamental
83 question: How are root traits organized across the diverse taxa that inhabit different
84 ecological conditions worldwide?
85 Here we propose a new model of root trait organization that is functionally
86 decoupled from the leaf economic spectrum, and that derives from the phylogenetic
87 history of root diameter and its evolutionary consequences for plant resource
88 acquisition.
89 Specifically, we evaluated an unprecedented species- and biome-specific dataset
90 of 10 root traits in three major categories[3,13] (morphology, physiology, and

91 mycorrhizal association, *SI* note 1), from >1,200 individual plants distributed across
92 369 species (210 genera, and 79 families), seven major biomes, and three continents
93 of the world (Extended Data Table 1). This dataset is unique in that our observations:
94 (i) derive solely from native plant communities with natural soil and nutrient
95 conditions; (ii) focus on first-order roots (the most distal and absorptive roots of the
96 branching system), which are subject to strong selection by the local
97 environment[8,9,14]; (iii) accurately identify species and root order (*i.e.*, measure of
98 branching hierarchy[8]) in mixed-species ecosystems, by tracing roots to parent trees[15];
99 and (iv) apply consistent analytical methods to trait measures across all species and
100 biomes (94% of observations were collected by ourselves; Methods).
101 We first evaluated whether the first-order root traits are globally organized in a
102 manner analogous to the leaf economic spectrum[5,6], a composite axis of trait variation
103 that ranges from nitrogen-rich leaves with high specific leaf area and short leaf
104 lifespan, to nitrogen-poor leaves with low specific leaf area and long leaf lifespan. In
105 roots, nitrogen supports metabolic activity including nutrient and water transport,
106 enzyme functioning, and mycorrhizal symbiosis[16]. As a result, nitrogen has been
107 proposed to serve a similarly central role in the trait organization of roots, with high
108 root nitrogen occurring in species with high leaf nitrogen, rapid growth, and short root
109 lifespan[4,17].
110 Our results do not support the idea of an analogous organizing role of nitrogen in
111 a global root economic spectrum (*c.f.*, similar conclusion from taxonomically and
112 geographically smaller datasets; refs.4,18,19). First, a principal component analysis

failed to identify root nitrogen (analogous to leaf nitrogen) as a significant contributor to the primary axis of trait variation (Extended Data Fig.1 and Table 2). Instead, root traits were most strongly (46%) explained by root diameter and a group of traits associated with root construction and mycorrhizal association (axis1 in Extended Data Fig.1).

Second, root nitrogen was not correlated to specific root length in a manner analogous to the relationship between specific leaf area and leaf nitrogen (Extended Data Fig.2). Third, *in situ* (n=73) and hydroponic-based (n=119) measures showed no systematic relationship between root nitrogen uptake (analogous to leaf photosynthetic capacity) and root diameter, specific root length, or plant growth form (Extended Data Figs.3b,d and 5). Combined, these results suggest that nitrogen is less important in belowground nutrient foraging than in aboveground light and CO_2 capture (*SI* note 3). Moreover, root lifespan (analogous to leaf lifespan) was correlated with root diameter and specific root length, but explained only 14% and 17% of the respective variance (P<0.01 for both, linear model; Extended Data Fig.3a,c).

We next analyzed the organizing role of root diameter in determining trait variation across plants. We found that the length of root per unit biomass invested – or specific root length (*SRL*) – increases non-linearly with decreasing root diameter (D) following the allometric relationship $SRL = \frac{16.8}{\pi D^2}$ (Fig.1a, red line; *SI* note 1). This relationship indicates that as roots get thinner, plants can explore dramatically greater volumes of soil per unit carbon invested. Albeit with some overlap, we also

found that woody and herbaceous plants occupy different parts of the specific root length vs. root diameter relationship: woody plants (Fig.1a, brown points) occur in a region where differences in root diameter have limited effect on specific root length, while herbaceous plants (green points) reside in a region where even small diameter variations cause large changes in specific root length.

We further found that – in thin-rooted species – even a modest evolutionary change in tissue density of first-order roots can greatly alter the soil length explored per unit carbon invested. The dashed red lines in Fig.1a indicate the sensitivity of the *SRL*-diameter relationship to changes in root tissue density across a physiologically relevant range (0.1 to 1g cm^{-3}). For example, low root density allows the grass *Agropyron cristatum* to explore ~350m more soil per gram biomass than the shrub *Rhaphiolepis indica*, despite similar (~0.2mm) root diameter (Fig.1a, red arrows and cross section photos). We infer that, over evolutionary time, plants can use both root diameter (Fig.1a, x-axis) and tissue density (Fig.1a, dashed lines) to influence specific root length: thin and soft first-order roots have the advantage of efficient soil exploration, but incur the cost tradeoff of sacrificing water conduction, tissue permanence, and the ability to penetrate the soil matrix.

We next examined the role of evolutionary history in structuring the differences in root diameter across all major vascular plant families in our dataset. We found that (Fig.1b), on average, thick roots are associated with evolutionarily ancient taxa (*e.g.*, *Magnoliaceae*) while thin roots are increasingly common in taxa that have recently diverged from their ancestral lineage (*e.g.*, *Betulaceae*)(weighted linear regression:

157 r^2=0.54; P<0.001). Herbaceous plants evolved more recently (Fig.1b, green circles)
158 and – with the exception of *Amaryllidaceae* and *Boraginaceae* – are thus
159 characterized by thin roots and specific root lengths that exceed woody plants (Fig.
160 1a). Together, these patterns broadly characterize an evolutionary transition from
161 ancient tree taxa, defined by thick first-order roots, to more recently radiated[20,21]
162 woody and herbaceous plants with thin roots that can explore dramatically greater
163 lengths of soil per carbon invested.

164 The trend towards thinner roots has had major consequences for the symbiosis
165 between plant roots and mycorrhizal fungi. We found that mycorrhizal colonization
166 (*i.e.*, % root length colonized) declines as roots get thinner (Fig.1c), but also that
167 herbaceous roots have ~30% less colonization than woody plants at the same root
168 diameter (linear model; r^2=0.63 with difference between herbaceous and woody
169 plants at P<0.001). In addition, herbaceous plants have on average 33% lower root
170 tissue density than woody plants (Fig.1d; unequal variance t-test; P<0.001), though
171 considerable unexplained variation exists across taxa. These differences suggest that
172 first-order roots have become less dependent on mycorrhizae as they have evolved
173 thinner diameter, but also that the innovation of the short-lived herbaceous growth
174 form has fundamentally changed the relationship between root diameter and
175 mycorrhizal colonization.

176 A phylogenetic independence contrasts (PICs) analysis[17] confirmed that variation
177 in root diameter, specific root length, and mycorrhizal colonization are strongly
178 influenced by evolutionary history (Blomberg's K value in Extended Data Table1).

179 In contrast, root chemical traits did not display a clear phylogenetic signal, indicating
180 that, for these traits ecological variation overshadows evolutionary constraints[22].
181 When combined, our results identify a general evolutionary trend from thick
182 roots that rely on mycorrhizal fungi for resource acquisition, to thin roots that can
183 explore the soil at high carbon use efficiency but with less reliance on mycorrhizae.
184 The observed root trait combinations imply selection for two contrasting plant
185 strategies: (i) a '*conservative'* strategy, in which carbon allocation to mycorrhizae
186 enhances the ability of plants to compete in environments with stable resources and
187 intense plant-plant competition; and (ii) an '*opportunistic'* strategy, in which thin
188 roots benefit plants in less predictable environments (*e.g.*, seasonal drought or cold),
189 where rapid root growth response to fluctuating resource supply is rewarded.
190 It is less clear, however, why herbaceous plants have lower mycorrhizal
191 colonization than woody plants at similar diameter (Fig.1c), although softer tissue
192 may cause roots to be less permanent (Extended Data Fig.2), and therefore less able to
193 maintain stable mycorrhizal relationships.
194 We next evaluated whether the distribution of root diameter changed across
195 biomes that may differ in the pattern and stability of resource supply. First, we found
196 an overall trend of decreasing variance in root diameter of woody plants from the
197 more stable conditions of tropical and sub-tropical forests, to the highly seasonal
198 boreal and desert biomes (Fig.2, Levene's test, Extended Data Table 3). Second,
199 while woody plants were limited to thin-rooted species in the most seasonal biomes,

200 the diameter of herbaceous plant roots did not differ systematically across biomes
201 (Extended Data Fig.4a,b).
202 These patterns are consistent with biome-specific differences in both evolutionary
203 history and stability of resource supply and abiotic conditions. The tropical forest
204 biome is ancient[23], characterized by seasonally stable supplies of soil resources, and
205 holds species that range from ancestral thick-rooted to more derived thin-rooted taxa.
206 In contrast, boreal and desert biomes are evolutionarily young[24], and have been
207 colonized mainly by thin-rooted species that, in theory, can rapidly respond[25] to
208 fluctuating soil resources and seasonally inhospitable conditions. The coexistence of
209 thin-rooted plants with more ancient thick-rooted strategies suggests that
210 heterogeneity within the tropical biome is sufficient to maintain a range of niche
211 conditions for plant belowground strategies.
212 Our findings suggest that – at the timescale of plant evolution – innovations of
213 belowground traits have been key for preparing plants to colonize new habitats, and
214 for the rich generation of biodiversity within and across biomes. The dominant
215 dimension of trait evolution for first-order roots has been a decrease in diameter,
216 which, in turn, has reduced the dependence on mycorrhizal fungi, increased the
217 efficiency of root growth, and thus elevated the ability of plants to leverage
218 photosynthetic carbon for soil exploration. An improved functional understanding of
219 root traits is critical for comprehending the history and distribution of plant life, and
220 may help to predict the risk of species extinction and to conserve biodiversity in the
221 face of environmental change.

METHODS SUMMARY

We collected roots from natural plant communities across seven major biomes and three continents (Asia, Europe and North America) during 2004 to 2016. We adopted the root-order based methodology (established in 2002; ref.8). At each sampling site, we selected common indigenous species that are representative of the local plant community. For each species, we sampled at least three individual plants to derive mean trait values for each species. We analyzed four morphological traits (diameter, specific root length, root tissue density, and root length), three physiological-chemical traits (root nitrogen content, root carbon content, and root carbon to nitrogen ratio), and the extent of mycorrhizal colonization (Detailed in Methods). We calculated percent mycorrhizal colonization by sampling first-order roots and determining by microscope the presence of either arbuscular mycorrhizal or ectomycorrhizal fungal structures within an individual root segment. For each species, percent colonization was calculated across 20-150 first-order root segments as detailed in Methods. We enhanced coverage of some biomes by including literature data (~5% of final dataset), but only if methodologies were consistent with our methods. In total, we gathered traits from 369 species (281 woody, 88 herbaceous, Extended Data Table 1, *SI* Fig.S3).

We collected data on plant root lifespan for 40 species using *in situ* minirhizotrons across boreal and temperate forests, with individual measures spanning at least one year. We acquired additional lifespan data from published literature identified via Web of Science and Google Scholar. We measured per-

biomass root nitrogen uptake rates in 36 plant species and acquired data for an additional 101 species through published literature (Extended Data Table 4).

Principal component analysis (PCA) and variance partitioned among traits in our database were analyzed by the FactorR package of the R platform. Linear models, linear mixed effects models (package lme4) and Levene's test were also conducted in R. Data were log transformed to correct for deviations from normality. We followed the APG III phylogenetic system in all analyses[26] and used PHYLOCOM[27] to construct phylogenetic trees (*SI* Fig.S4). Following Wikstrom, et al.[28], we defined the divergence time of a plant family using the earliest diverging genus within that family.

Full Methods and any associated references are available in the online version of the paper at www.nature.com/nature.

Methods

Sampling approach. We collected roots from natural plant communities across seven major biomes and three continents (Asia, Europe, and North America) during 2004 to 2016. Our sampling sites range from –1.4°C to 22.4°C in mean annual temperature, and from 35mm to 2651mm in mean annual precipitation. At each site, we selected common indigenous species that are representative of local plant communities. We sampled multiple root branches or segments from at least three individual plants from each species to derive the mean species trait value. For species

that occupied more than one sample location, we merged the local means into one species trait value. Eleven species occurred in more than a single biome; for these we calculated a mean value for each biome.

We identified roots to species level in mixed-species ecosystems by tracing a root to its parent tree. During the growing season, we selected mature individuals and excavated the surface soil (0–20cm) around the plant stem to expose lateral roots. We then sampled multiple intact root branches and gently cleared the attached soil. Sampled roots were bagged and immediately placed in a cooler, and then either transferred to a refrigerator for processing within the next few days or kept frozen until later laboratory analyses.

Laboratory analyses of root functional traits. We adopted a root branching-order based approach, where absorptive fine roots are sorted based on their position in the branching architecture (established in 2002, details in *SI* note 2; ref. 8). We dissected root branches according to standard methodology[15] and determined root diameter, root length, specific root length, root tissue density, root nitrogen and carbon concentration, and mycorrhizal colonization (*SI* note 1).

Root diameter and length were measured using stereomicroscope with an ocular micrometer. Specific root length was determined by dividing root length by the dry biomass weight. We calculated the volume of root segments from root diameter and length, assuming segments are cylinders. Root tissue density was then calculated using dry mass and volume. Sampled roots were oven-dried at 60°C for 48h, ground

287 to fine powder with a ball-mill for subsequent measurements of carbon and nitrogen
288 on an elemental analyzer (Vario EL Cube; Elementar, Hanau, Germany).
289 We measured the length of root colonized by mycorrhizal fungi in 137 species
290 from sub-tropical[19] forests, temperate forests[15], and temperate grasslands[29]. We
291 calculated percent mycorrhizal colonization by sampling first-order roots and
292 determining by microscope the presence of either arbuscular mycorrhizal or
293 ectomycorrhizal fungal structures within an individual root segment. For arbuscular
294 mycorrhizal fungi we used a standard staining technique to identify coils and
295 arbuscules[19,29]; no stain was needed to identify ectomycorrhzal fungal sheaths[19]. For
296 each individual plant, we selected at least 10 root branches (containing multiple order
297 of roots). We next randomly selected 20-150 first-order root segments[19,29] for each
298 species, ensuring that each segment length was consistent across all roots sampled.
299 We then calculated the species-specific percent length colonization as the ratio of the
300 sum of infected root segments over all root segments examined. We used two
301 different techniques: one based on cross-sectional analysis (MC_1; n=110 species) and
302 one based on scanning the root surface (MC_2; n=27 species); both allowed us to
303 quantify fungal association within a standardized root area. We kept the effective
304 area examined per root segment approximately the same for both methods (173 vs.
305 169 mm^2), such that the results are equivalent ($MC_2 = 1.02 \times MC_1 - 0.02$; r^2=0.988).
306 Since distal fine roots (*i.e.*, first-order roots) are primarily responsible for plant
307 nutrient acquisition[8,9], we focused our analyses on first-order root traits. We
308 accumulated 480 species-specific observations, of which 187 are unpublished and 256

published by our groups[14,15,19,25,29]. To cover a broader range of biomes (*e.g.*, boreal and Mediterranean), we added 37 observations from the literature[8,30-32] into our dataset, taking care to only include studies of first-order roots and consistent methods. In total, we compiled 480 species-specific observations, covering 210 genera and 79 families (Extended Data Table 1).

Root lifespan. We collected multi-year root lifespan data of 40 species using *in situ* minirhizotrons across subtropical forest, tropical forest and temperate forest of Europe[33], Asia[34,35] and North America[36,37]. We further added data from the published literature to develop a global dataset. We carefully selected observations only from studies of first-order roots or distal roots, using *in situ* minirhizotrons or root windows[38-59]. When corresponding root traits (*e.g.*, diameter, *SRL*) were not available, we used species-specific observations from our own dataset to match the life span data. In total, we obtained 70 species-specific observations and 13 community-observations across 5 biomes.

Root nitrogen uptake rates. We measured root biomass-specific nitrogen uptake rates using two standard approaches: (i) by isolating an intact living root branch and exposing it to a hydroponic solution labeled with isotopically labeled ammonium nitrate (intrusive approach, elevated nitrogen concentration; see ref. 60); and (ii) by applying nutrient solution to soil and allowing plant roots to take up nutrients *in situ* (non-intrusive, low nitrogen concentration; see ref. 61). The first approach allows an estimation of the maximum uptake rate of absorptive roots, while the second approach more accurately reflects the uptake rate of roots in natural conditions. We then

supplemented our dataset with 43 published measurements (keyword search for "root nitrogen uptake" using the Web of Science and Google Scholar). This dataset included 210 species-specific and 34 community-specific observations across major five biomes (Extended Data Table 4).

Species and phylogeny. Our root trait dataset represented a wide range of taxa, covering 210 genera and 79 families, with species names confirmed in The Plant List (http://www.theplantlist.org). We constructed the plant phylogenetic relationship using PHYLOCOM [27] (http://phylodiversity.net/phylomatic) and determined the divergence time of plant families based on the earliest diverging genus within that family[28]. We calculated Blomberg's *K*-statistic[62] using the "Picante" package in R and evaluated the strength of the phylogenetic signal for each traits; a large Blomberg's K value is thought to indicate phylogenetic conservatism. We performed phylogenic independent contrasts analyses (PICs) to correct for shared evolutionary histories among traits and look for the pure effect of environmental influences (Extended Data Table 5).

Statistical analyses. Shapiro-Wilk tests revealed that all of our traits were significantly non-normal ($P<0.05$), which we corrected by log10 transforming our data. We performed the Principal component analysis (PCA) using R package FactorR. The linear regression between root diameter vs. divergence time was weighted by the number of species within each family. We used linear regression to test the effect of root diameter and growth form (woody vs. herbaceous) on root mycorrhizal colonization, and tested equality of variance in root diameter among

biomes using Levene's test. We used linear mixed effects model to compare the difference of root diameter across biomes. All statistical analyses were performed using the R software, version 2.15.0.

Code availability. The R scripts used in Fig.1 and Fig.2 are available from the corresponding author upon reasonable request.

Data availability. The data that support the findings of this study are available from the corresponding authors upon request.

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640

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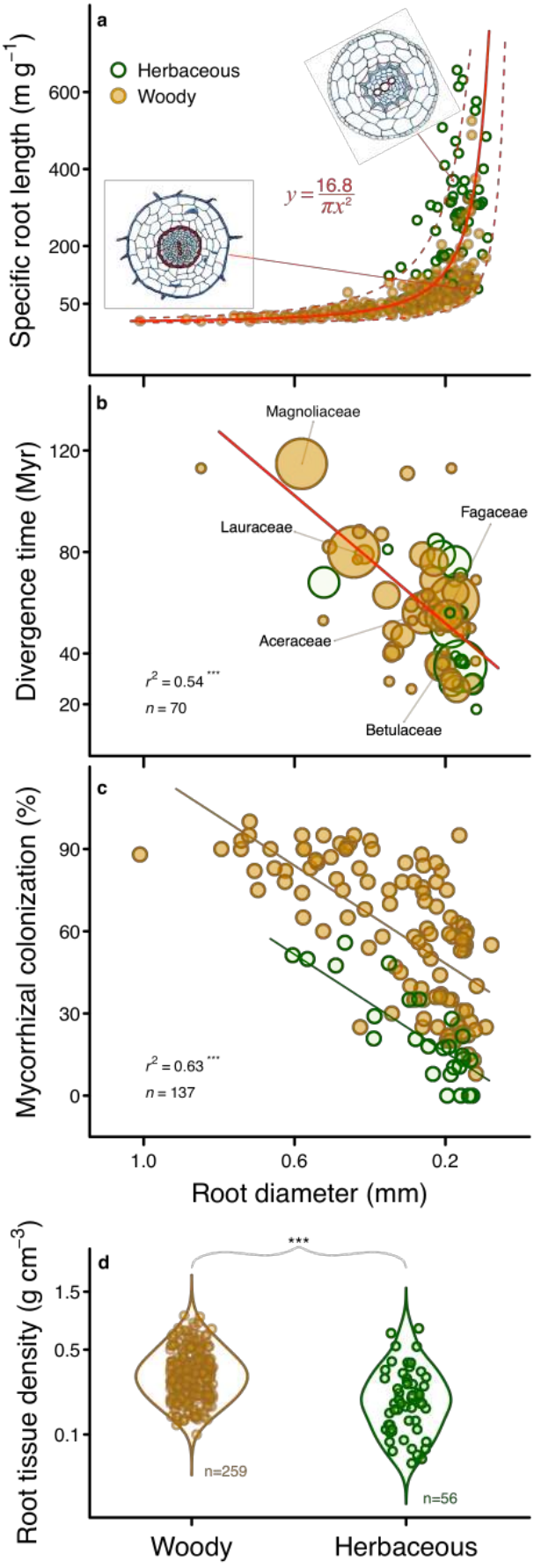
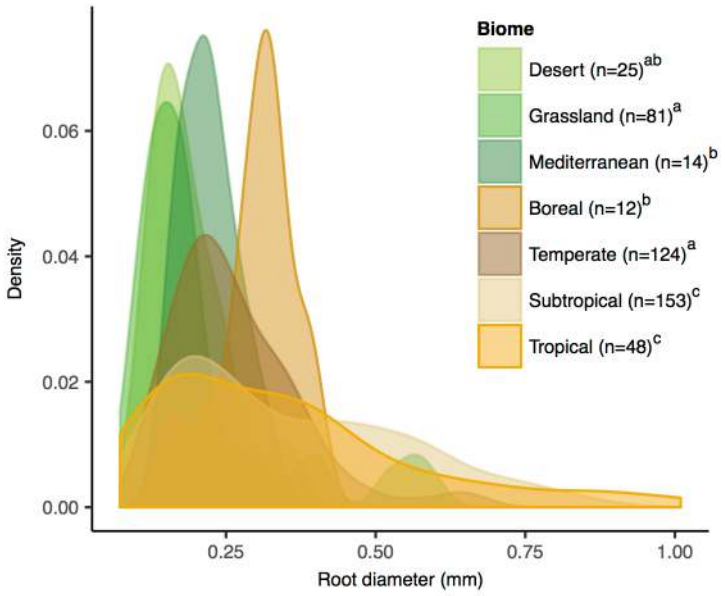
Author Information Reprints and permissions information is available at www.nature.com/reprints. The authors declare no competing financial interests. Readers are welcome to comment on the online version of the paper. Correspondence and requests for materials should be addressed to D.G. (guodl@igsnrr.ac.cn) or L.O.H. (lhedin@princeton).


a
Herbaceous
Woody
Specific root length (m g^{-1})
600
400
200
50
$y = \frac{16.8}{\pi x^2}$
b
Magnoliaceae
Lauraceae
Fagaceae
Aceraceae
Betulaceae
Divergence time (Myr)
120
80
40
20
$r^2 = 0.54$ ***
$n = 70$
c
Mycorrhizal colonization (%)
90
60
30
0
$r^2 = 0.63$ ***
$n = 137$
1.0
0.6
0.2
Root diameter (mm)
d
***
Root tissue density (g cm^{-3})
1.5
0.5
0.1
n=259
n=56
Woody
Herbaceous

659

660 **Figure 1 | Root trait dimensions organized by root diameter and growth form.**
661 At the species level, the diameter of first-order roots is inversely correlated with
662 specific root length (*SRL*) (**a**), positively related to the evolutionary time of
663 divergence of major taxonomic groups (**b**), and positively related to the length of root
664 (in %) colonized by mycorrhizal fungi (**c**). Moreover, root tissue density (RTD)
665 differs across plant growth form (**d**), with herbaceous plants (green points) displaying
666 more constrained variation than woody plants (brown points)(F-test, $P<0.001$; note
667 logarithmic scale on Y-axis). The solid red line in (**a**) identifies the relationship (*SI*
668 note 1) between specific root length and root diameter assuming a root tissue density
669 of 0.25g cm^{-3}; dashed red lines identify tissue densities of 0.1 and 1.0g cm^{-3} (upper vs.
670 lower line, respectively). We used a linear regression weighted by number of species
671 in panel (**b**) and a linear regression with woody and non-woody growth forms as
672 categorical variables in panel (**c**). Root cross-section images in (**a**) are from the low-
673 density grass *Agropyron cristatum* (upper right) and the high-density woody shrub
674 *Rhaphiolepis indica* (lower left), as discussed in main text.
675



676

677 **Figure 2 | Density distributions of first-order root diameter across seven biomes.**

678 The variance in root diameter declines from biomes with equable conditions (*e.g.*,

679 tropical forests) to biomes with pronounced seasonality in soil resource supplies (*e.g.*,

680 deserts). Numbers in brackets identify species-specific observations, and letters

681 identify significant pairwise differences in a Levene's variance test (Extended Data

682 Table 3). Woody biomes are identified as shades of tan to yellow and non-woody

683 biomes as shades of green.