You, C., Li, J., Yang, K., Tan, B., **Yin, R.**, Li, H., Zhang, L., Cui, X., Liu, S., Wang, L., Liu, Y., Chen, L., Yuan, Y., Li, J., Sardans, J., Zhang, J., Xu, Z., Peñuelas, J. (2023):
Variations and patterns of C and N stoichiometry in the first five root branch orders across 218 woody plant species
New Phytol. **238** (5), 1838 - 1848

http://dx.doi.org/10.1111/nph.18870

Variations and patterns of C and N stoichiometry in the first five root branch orders across 218 woody plant species

Chengming You[1], Jihong Li[1], Kaijun Yang[1,2], Bo Tan[1], Rui Yin[3], Han Li[1], Li Zhang[1], Xinglei Cui[1], Sining Liu[1], Lixia Wang[1], Yang Liu[1], Lianghua Chen[1], Yaling Yuan[1], Jiao Li[1], Jordi Sardans[2,4], Jian Zhang[1], Zhenfeng Xu[1]*, Josep Peñuelas[2,4]

[1]Forestry Ecological Engineering in the Upper Reaches of the Yangtze River Key Laboratory of Sichuan Province & National Forestry and Grassland Administration Key Laboratory of Forest Resources Conservation and Ecological Safety on the Upper Reaches of the Yangtze River & Institute of Ecology and Forestry, Sichuan Agricultural University, Chengdu 611130, China

[2]CSIC, Global Ecology Unit CREAF-CSIC-UAB, CSIC, Bellaterra 08193, Barcelona, Catalonia, Spain

[3]Helmholtz-Centre for Environmental Research-UFZ, Department of Community Ecology, Theodor-Lieser-Strasse 4, 06110 Halle (Saale), Germany

[4]CREAF, Cerdanyola del Vallès 08193, Barcelona, Catalonia, Spain

*Corresponding author at: Institute of Ecology and Forestry, Sichuan Agricultural University, Chengdu 611130, China. Dr. Zhenfeng Xu, Email: xuzf@sicau.edu.cn

Received: *8 April 2022*

Accepted: *27 February 2023*

ORCID：

Chengming You: 0000-0002-7033-4773

Xinglei Cui: 0000-0002-0853-8986

Jordi Sardans:0000-0003-2478-0219

Zhenfeng Xu:0000-0002-9777-017X

Josep Peñuelas:0000-0002-7215-0150

Summary

- Despite the vital role in carbon (C) sequestration and nutrient retention, variations and patterns in root C and nitrogen (N) stoichiometry of the first five root orders across woody plant species remains unclear.
- We compiled a dataset to explore variations and patterns of root C and N stoichiometry in the first five orders of 218 woody plant species.
- Across the five orders, root N concentrations were greater in deciduous, broadleaf, and arbuscular mycorrhizal species than in evergreen, coniferous species, and ectomycorrhizal associations species, respectively. Contrasting trends were found for root C:N ratios. Most root branch orders showed clear latitudinal and altitudinal trends in root C and N stoichiometry. There were opposite patterns in N concentrations between latitude and altitude. Such variations were mainly driven by plant species, and climatic factors together.
- Our results indicate divergent C and N use strategies among plant types and convergence and divergence in the patterns of C and N stoichiometry between latitude and altitude across the first five root orders. These findings provide important data on the root economics spectrum and biogeochemical models to improve understanding and prediction of climate change effects on C and nutrient dynamics in terrestrial

ecosystems.

Key words: woody plants, fine roots, carbon and nitrogen stoichiometry, root branch order, biogeography, plant functional groups.

Introduction

Carbon (C) and nitrogen (N) are two basic elements of life that are essential for plant growth and ecosystem function (Zhang *et al.*, 2020). Plant root networks modulate a broad spectrum of ecological processes, from soil formation to the cycling of nutrients, including C and N, through their acquisition of water and nutrients (Jackson *et al.*, 1997; McCormack *et al.*, 2015; Adamczyk *et al.*, 2019; Ding *et al.*, 2020). Root C and N stoichiometry largely reflect physiological constraints, evolutionary, and ecological adaptation strategies of plants, as well as their responses to climate change (Ågren, 2004; Zhang *et al.*, 2020). Root C and N concentrations usually represent the assimilation investment and metabolic activity of plants, respectively (Zadworny *et al.*, 2015; Razaq *et al.*, 2017; Zadworny *et al.*, 2017). Thus, a more complete knowledge of the biogeographical patterns of root C and N stoichiometry can improve our understanding of the evolutionary history and environmental adaptations of woody plant species.

Fine roots (≤ 2 mm in diameter) have tended to be regarded as a homogenous mass compartment (Jackson *et al.*, 1997; Strand *et al.*, 2008); however, this classification does not account for the diversity of form and function of root systems, and ignores the related roles in structure and function (Pregitzer *et al.*, 1997; Pregitzer *et al*., 2002). An increasing number of observations have indicated that plant roots are highly branched, structurally and functionally heterogeneous systems, and have also demonstrated associations between root trait function and branch order, rather than the diameter of fine roots (Goebel *et al.*, 2011; Mucha *et al.*, 2019), based on separation of roots into shorter-lived absorptive (orders 1 and 2) and longer-lived transportive (orders 3 to 5) roots (Guo *et al*., 2008; McCormack *et al.*, 2015; Kou *et al.*, 2018). Divergences in root traits between absorptive and transport roots, such as morphology, physiology and structure, may mediate root C and N accumulation and use efficiency (McCormack *et al.*, 2015; Kou *et al.*, 2018). Therefore, a better understanding of variations and patterns of root C and N stoichiometry across branch orders in woody plants is critical for accurately modeling and predicting belowground ecological processes in terrestrial ecosystems.

Plant growth forms have been considered as part of the plant economic spectrum, and their traits could mediate the concentrations of C and N and their ratios (C:N) in plants (Freschet *et al*., 2013; Roumet *et al*., 2016; Valverde-Barrantes & Blackwood, 2016). The leaf economic spectrum showed that resource-acquisitive species often exhibited high leaf N concentrations relative to resource-conservative species (Wright *et al*., 2004). The results from previous studies indicate that broadleaf plants and deciduous plants tend to show trait syndromes related to a resource-acquisition strategy as compared to coniferous and evergreen plants, respectively (Vergutz *et al.*, 2012; Tian *et al*., 2018; Zhang *et al*., 2020; Li *et al*., 2021). Thus, leaf N concentrations are greater in broadleaf and/or deciduous plants than in coniferous and/or evergreen plants, but the opposite is true for C:N ratios (Vergutz *et al.*, 2012; Tian *et al*., 2018). Plant roots are likely to exhibit similar patterns observed in plant leaves (Zhao *et al.*, 2016) because they are also at the plant-environment interface of resource exchange (Reich & Cornelissen, 2014).

According to the root economic spectrum, root N concentrations may be higher in acquisitive species than conservative species (Reich & Cornelissen, 2014; Weemstra *et al.*, 2016), indicating mycorrhizal association is an important factor shaping root C and N stoichiometry (Bergmann *et al.*, 2020). Plants with arbuscular mycorrhizal associations are more likely to show trait syndromes associated with a resource-acquisition strategy relative to plants with ectomycorrhizal associations (Phillips *et al.*, 2013). Therefore, plants with arbuscular mycorrhizal associations should bear higher N concentrations and lower C:N ratios than that with ectomycorrhizal associations (Reich & Cornelissen, 2014). However, current accounts of root C and N stoichiometry variation in plant types at a wide scale are most often limited to diameter-based, focusing between woody and herbaceous species (Yuan *et al.*, 2011; Wang *et al.*, 2020), and few studies have examined differences among contrasting woody functional groups. A recent global analysis shows variation in diameter-based root decomposition between contrasting plant functional groups, indicating divergence in root chemistry, such as ratios of C:N, between plant types (See *et al.*, 2019); however, how plant functional group and root branch order modulate root C and N stoichiometry across large-scale for woody plant species remains unclear.

Both latitude and altitude may affect root stoichiometry via changes in climate conditions (e.g., temperature and precipitation) and plant species. Previous studies have reported the geographical patterns of diameter-based root C, N, and P stoichiometry in terrestrial plants (Yuan *et al.*, 2011; Zhang *et al.*, 2017; Wang *et al.*, 2020; Zhang *et al.*, 2020). Two possible hypotheses have been proposed to explain these variations (Reich & Oleksyn, 2004). Wang *et al*. (2020) observed that the fine-root (diameters < 2 mm) N concentrations increased with increasing latitude, supporting the temperature-plant physiological hypothesis, which states that plant N concentrations increase with latitude to compensate for decreased rates of metabolic reactions (Reich & Oleksyn, 2004). On the contrary, the temperature-biogeochemistry hypothesis (Reich & Oleksyn, 2004) indicates that N uptake is inhibited at lower temperatures due to reduced microbial activity and decomposition of soil organic matter, which is supported by the results that N concentrations of fine-root diameters < 2 mm are negatively correlated to altitude (He *et al*., 2016). However, these hypotheses have not been comprehensively tested using order-based data. Besides, global change studies have demonstrated that root nutrient concentrations in lower-order roots are more sensitive to environmental changes (e.g., warming) as compared to higher-order roots (Yin *et al*., 2014; Pu *et al*., 2016), pointing at potential divergences in geographical patterns of root C and N stoichiometry between lower- and higher-order roots. To our knowledge, however, an analysis of the order-based geographical patterns of C and N stoichiometry across woody plant species at a large scale is lacking.

In this study, we compiled a dataset, comprising 218 woody plant species distributed on a broad spatial scale, to compare root C and N stoichiometry of the first five orders between plant functional group types (taxonomical grouping, leaf habit, type of mycorrhizal association) and among geographic location and climate conditions. The questions addressed are the following: (1) Are there differences in root C and N stoichiometry of the first five branch orders between contrasting plant functional types? (2) What are the dominant factors regulating geographical patterns of order-based C and N stoichiometry across woody plant species? (3) Do lower-

(orders 1 and 2) and higher-order (3 - 5 orders) roots respond differently to geographical factors (e.g., latitude and altitude)? We hypothesized that, for woody plant species, (1) Root C concentration, and C:N ratio would increase and N concentration would decrease with increasing root branch order, and root N concentration would be higher and C:N ratio would be lower in broadleaf, deciduous, and arbuscular mycorrhizal species than in coniferous, evergreen, and ectomycorrhizal species, respectively; and (2) the order-based geographical patterns would be mainly driven by plant species and/or climate factors (e.g., mean annual temperature, mean annual precipitation, aridity index, and solar radiation); and (3) root C and N stoichiometry of lower-order roots would be more responsive to geographical factors as compared to higher-order roots.

Materials and Methods

Data compilation

We assembled a meta-dataset of root C and N stoichiometry of the first five branch orders across woody plant species based on the Fine-Root Ecology Database (Iversen *et al.*, 2017), and using published site-referenced observations available via the Web of Science and Chinese CNKI up to 2021. Overall, our database contained 4180 records for 218 woody plant species distributed across 85 study sites worldwide, comprising 1170 root C, 1685 root N, and 1325 root C:N ratio observations (Notes S1).

To ensure consistency and robustness in the inclusion of data, records were selected based on the following criteria: as conducted in previous individual studies mainly focused on the C and N stoichiometry across the first five root branch orders, we focused on species data comprising observations for all individual branches of the first five root orders to quantify the differences between different root orders, especially absorptive (orders 1 and 2) and transport roots (orders 4 and 5), and also to ensure sufficient sample size. The designation of root branch order in the original studies followed the topological nomenclature developed in previous studies (Pregitzer *et al*.,1997; Pregitzer *et al*., 2002), namely, root tips as first-order branches, the root from which two first-order roots branched was classified as second-order, and so on; data derived from natural conditions; and, where appropriate, seasonal data were averaged across seasons. Original data were extracted directly from tables of published articles and from figures using the Get Data Graph Digitizer (Version 2.24, Russian Federation).

Geographic location (latitude; longitude; altitude) and climate (mean annual temperature, MAT; mean annual precipitation, MAP; aridity index; solar radiation) factors for each study site were extracted from the original literature, where possible. When geographical information was not provided, we extrapolated relevant data for the same study site reported in another publication, and when climate data were not reported, we extracted MAT, MAP, and solar radiation data from the WorldClim Version 2.0 (http://worldclim.org/version2) and aridity index data from CGIAR-CSI (http://www.cgiar-csi.org) based on study site geographical coordinates. The final database represented sites distributed across wide geographical (18.50–69.63°N; 84.48°W–128.57°E), altitudinal (40−4000 m a.s.l.), and climate (MAT: -1.69−24.50 °C; MAP: 35−2651 mm) ranges (Fig. S1; Notes S1).

In our database, all species names were confirmed using The Plant List (http://www.theplantlist.org), and based on their leaf traits (Kong *et al*., 2019; See *et al*., 2019), the woody plant species were categorized into two functional groups, comprising taxonomical grouping (196 broadleaf species and 22 coniferous species), leaf habit (80 deciduous species and 138 evergreen species). Meanwhile, the type of mycorrhizal association of the tree species was obtained from the primary studies as well as from mycorrhizal classification databases (Maherali *et al*., 2016; Brundrett, 2017), and the mycorrhizal association included arbuscular mycorrhiza (98 species) and ectomycorrhiza (36 species) in this study (Notes S1).

Statistical analysis

Data were log-transformed before analysis to improve the homogeneity of variances. To assess the mean differences in C and N stoichiometry among root orders and each individual root order differences in C and N concentrations and C:N ratios between plant functional groups, we first ran linear mixed models by fitting each predictor variable as a fixed effects factor, and the identity of original studies and species were treated as random effects to enhance data independence.

The linear mixed model also was performed to assess the responses of C and N stoichiometry to root branch orders between contrasting plant functional groups by comparing the 95% confidence intervals of the regression slope. The slope indicates the change in direction and rate of functional group C and N stoichiometry with increasing root branch order, and the difference between contrasting plant functional groups was considered insignificant if the bounds of the 95% confidence intervals of the slope overlapped each other (Zhang *et al.*, 2020). The linear mixed models were fitted by the restricted maximum likelihood estimation using the R package *lme4* version 1.1-29 (Zhang *et al.*, 2020).

In order to estimate the effect of phylogenetic relationships on root C and N stoichiometry of the first five root orders across woody plant species, we constructed an updated phylogeny for all recorded species. We extracted the phylogeny of the 218 species contained in our study from the mega-tree GBOTB.extended to construct a phylogenetic tree by using the R package V. phylomaker version 0.1.0 (Jin & Qian, 2019). The *phylosignal* function in the R package picante version 1.8.2 was performed to analysis the phylogenetic signal, and the parametric Pagel's λ indices were used to assess the strength of phylogenetic signals for root C and N stoichiometry (Valverde-barrantes *et al*., 2017). To reduce the bias from the phylogenetic auto-correlation, we performed a phylogenetic generalized least squares (PGLS) model to estimate the strength and direction of geographical (latitude; altitude) and climate (MAT; MAP; aridity index; solar radiation) patterns of C and N stoichiometry among the first five branch orders by using the R package Caper version l.0.1 (Holdaway *et al*., 2011). Due to the limited data at high latitudes (only two points) and altitudes (only five points) (Notes S1), the PGLS models were conducted at given latitudinal (< 52 °) and altitudinal (≤ 1000 m) ranges to reduce the uncertainty of the results.

Relative effects of species, plant functional group, and geographical and climate factors on C and N concentrations and C:N ratio of the first five branch orders were tested by hierarchical partitioning method with redundancy analysis using the R package rdacca.hp version 1.0.8, where the total variance of each factor explained is composed of independent and interaction effects of each factor (Lai *et al*., 2022).

In our database, 218 species were used to quantify the differences of root C and N stoichiometry in general among plant functional groups, and 215 and 198 species were used to analyse the latitudinal and altitudinal patterns of root C and N stoichiometry, respectively. Detailed information on how many replicates were used in the analyses for each species is provided in Notes S2. All statistical analyses were performed using R software (version 4.2.0; R Development Core Team, Vienna, Austria), and graphs were constructed by using Origin 2019 (OriginLab Co) and R package ggplot2 version 3.3.6.

Results

Functional group divergence in root C and N stoichiometry

There was a unimodal frequency distribution of root C and N stoichiometry across the first five branch root orders of 218 woody plant species (Fig. 1). Across all the observations, the mean C and N concentrations and C:N ratios in the five branch orders were 462.90 g/kg and 14.76 g/kg and 41.19, respectively (Fig. 1a-c). Their coefficient of variation (CV %) was 10.3%, 41.4%, and 44.9%, respectively (Fig. 1a-c). There were increasing trends from the first- to fifth-order roots in mean C concentration (439.89 to 478.04 g/kg) and C:N ratio (26.34 to 57.50). However, root N concentrations were approximately 1.9 times greater in first-order than in fifth-order roots (Fig. 1a–c). Regardless of branch order, root N concentrations in deciduous plants, broadleaf plants, and those with arbuscular mycorrhizal associations were greater than in evergreen plants, coniferous plants, and plants with ectomycorrhizal associations, respectively (Fig. 1e, i, l). The opposite trends were observed for C:N ratios (Fig. 1f, j, m). Additionally, the slope sign and magnitude of root C and N stoichiometry with increasing root branch order were similar between contrasting functional groups (Table 1).

Latitudinal and altitudinal patterns in root C and N stoichiometry

There were clear latitudinal and altitudinal trends in woody plant root C and N stoichiometry across most of the root branch orders (Fig. 2). Both root C concentrations of the five branch orders and N concentrations of the first two orders were positively related to latitude ($p < 0.05$; Fig. 2a, b). Irrespective of branch orders, root C concentrations and C:N ratios increased but root N concentrations decreased with increasing altitude ($p < 0.05$; Fig. 2d−f). In general, the change rates of root C and N stoichiometry with increasing latitude or altitude did not differ between the first five root orders (Fig. 2; Table S1).

Climate and phylogenetic effects on root C and N stoichiometry

The PGLS models showed that both the C concentrations of the first five root orders and the N concentrations of the first two orders were negatively related to MAT ($p < 0.05$; Fig. S2a, b; Table S2). Root C concentrations of the three to five orders also negatively correlated with MAP ($p < 0.05$; Fig. S2d; Table S2). Across the five branch orders, aridity index was positively associated with C concentrations (except for the first two orders) and C:N ratios, but was a negative correlation with N concentrations ($p < 0.05$; Fig. S3a-c; Table S3). Solar radiation was positively related to root C concentrations of the fourth-order roots only ($p < 0.05$; Fig. S3d; Table S3).

Regardless of branch order, the parametric indices based on evolutionary models (Pagel's λ) showed intermediate phylogenetic signals in root C and N concentrations and C:N ratios (Table S4), indicating that the root C and N stoichiometry were strongly constrained by phylogeny.

Relative effects of species, geography, climate, and plant types

The hierarchical partitioning analysis showed that the combined effects of species, geographic and climate factors, and plant functional groups explained 18.6−55.8%, 16.2−34.1%, and 0−39.7% of the variations in root C and N concentrations and C:N ratios across woody plants, respectively (Fig. 3). Species, geographic and climate factors, and plant functional groups individual effects accounted for 8.0−36.8%, 2.5−6.7%, 6.6−10.9%, and 1.2−3.2% of the variations in C concentrations explained by models (Fig. 3a), 0−21.1%, 0−1.7%, 8.0−10.3%, and 4.7−6.2% of the variations in N concentrations explained by models (Fig. 3b), and 0−14.6%, 1.0−3.2%, 4.3−16.1%, and 1.8−6.3% of the variations in C:N ratios explained by models (Fig. 3c). In general, the effects of species on root C and N concentrations were the strongest of all, and climate factors tended to explain variation in C and N stoichiometry either independently or through the interactions with plant species (Fig. 3).

Discussion

Root C and N stoichiometry in woody plant functional groups

Previous studies have showed that root nutrient concentrations of woody plant species vary with branch orders (Pregitzer *et al.*, 2002; Guo *et al.*, 2004). Similarly, our results demonstrated that, irrespective of plant functional group, both root C concentrations and C:N ratio increased but N concentrations decreased with increasing root branch order (Fig. 1). Our analyses show that the aggregation of plant species into functional groups is a useful approach to improve understanding of C and N stoichiometry across the first five root branch orders. In this case, we found greater root N concentrations in deciduous plants than in evergreen plants, regardless of branch order (Fig. 1e). This may be because deciduous plants often have more rapid rates of nutrient return, photosynthesis, and growth as compared to evergreen species (Reich & Cornelissen, 2014; Augusto *et al.*, 2015; Huang *et al.*, 2018; See *et al.*, 2019). We also found that N concentrations were lower in coniferous than broadleaf plants (Fig. 1i). The lower specific root length of conifers reflects their lower nutrient absorption capacity (Wang *et al.*, 2018) and lower decomposition rate, which may increase soil acidification and aluminum toxicity and hinder meristem differentiation and root growth (Zadworny *et al.*, 2017). In addition, we found that root N concentrations were greater in woody plants with arbuscular mycorrhizal associations than in those associated with ectomycorrhiza associations (Fig. 1l). A possible explanation is that the maintenance of plants with ectomycorrhiza associations usually requires higher C investment relative to arbuscular mycorrhizal species, which could lead to lower root biomass, inorganic N acquisition rates, and nutrient absorption capacity, but higher root tissue density and more conservative root trait syndromes in ectomycorrhizal associations species compared to arbuscular mycorrhizal species (Chen *et al.*, 2016; Liese *et al.*, 2018; Valverde-Barrantes *et al.*, 2018). However, the trends in root C:N ratios were opposite to N concentrations between contrasting plant functional groups (Fig. 1f, j, m), indicating that evergreen plants, coniferous plants, and plants with ectomycorrhizal associations may manifest resource-conservative strategies and higher N use efficiencies as compared to deciduous plants, broadleaf plants, and plants with arbuscular mycorrhizal (Devi & Garkoti, 2013; Phillips *et al.*, 2013; Zhang *et al.*, 2020; Li *et al.*, 2021).

The function-dominant hypothesis states that plant functional traits in organs may converge in change direction to ensure the cooperation among organs, but may diverge in change rates to perform their specific functions (Zhang *et al.*, 2020). A recent study confirmed this hypothesis through the C:N ratios of different organs of 2139 species (Zhang *et al.*, 2020), but whether this phenomenon exists in different parts of the same organ remains poorly understood. Previous studies have suggested that there are significant differences in root traits, such as structure and function, between lower-order roots (orders 1 and 2) and higher-order roots (orders 3 to 5) (Guo *et al.*, 2008; McCormack *et al.*, 2015; Kong *et al.*, 2021). However, we found that the change in the direction and rate of root C and N stoichiometry along root order is similar between contrasting plant functional groups (Table 1). Such findings do not support the function-dominant hypothesis in the same organ. This is mainly because plants may transfer nutrients between lower-order and higher-order roots to maintain basic physiological activities, structural support, and defense (Zadworny *et al.*, 2015; Aubrey & Teskey, 2018; Zadworny *et al.*, 2017). Thus, taken together, these results support our first hypothesis, that both plant functional group and root branch order are two determinants regulating root C and N stoichiometry across woody plant species.

Geographical patterns of root C and N stoichiometry in the first five root branch orders

Similar to the geographical patterns observed in both plant leaves (Reich & Oleksyn, 2004; Hu *et al.*, 2021) and diameter-based roots (Wang *et al.*, 2020; Zhang *et al.*, 2020), we found that most root branch orders in woody plant species showed clear latitudinal and altitudinal trends in C and N stoichiometry (Fig. 2). For example, root N concentrations in the first two orders were positively associated with latitude (Fig. 2b), which is consistent with the results noted in the diameter-based fine-roots (diameter < 2 mm) (Wang *et al.*, 2020). This is mainly because high N concentrations can enhance plant metabolism to compensate for decreased rates of metabolic reactions at low temperatures, which is in agreement with the temperature-plant physiological hypothesis (Reich & Oleksyn, 2004). Additionally, a global analysis of diameter-based root traits also showed that plant species from high-latitude areas have higher specific root length, N concentrations, and thinner diameters as compared to those from tropical zones, representing traits of resource-acquisitive (Freschet *et al.*, 2017). However, we found that N concentrations were negatively correlated with altitude in the present case (Fig. 2e), which may to some extent support the temperature-biogeochemistry hypothesis (Reich & Oleksyn, 2004). Similar results are found in tropical montane forests (Pierick *et al*., 2022) and subtropical forests (He *et al*., 2016). A current study has suggested that root diameter and tissue density generally increased, but specific root length and nutrient concentrations decreased with increasing altitude, implying that plants exhibit a conservative resource-use strategy to adapt to the nutrient limitation at high altitude biome (Pierick *et al*., 2022). Thus, the contrasting patterns of root N concentrations between latitude and altitude may be attributed to the specific traits (e.g., root morphology and physiological activities) and complexity of species diversity (Wu *et al*., 2016; Freschet *et al*., 2017; Pierick *et al*., 2022). In order to adapt to the complex and diverse micro-habitats in the ecosystems, woody plants may form special and diverse ecological adaptation strategies, but additional evidence is needed. We also found that C concentrations were positively correlated with latitude and altitude (Fig. 2a, d), which is similar to an existing study that non-structural carbohydrate concentrations increased with increasing latitude (Li *et al*., 2016). Similar to prior findings (He *et al*., 2016; Zadworny *et al*., 2017), C:N ratios were positively related to altitude (Fig. 2f), potentially because plants can improve N use efficiency to adapt to adverse environmental conditions, such as low temperatures (Zhang *et al*., 2020).

Plant functional traits generally do not vary independently in changing environments (Watson & Szathmáry, 2016) because the traits share genetic controls, or they have linked roles in functions (Fajardo & Piper, 2011). Contrary to what we expected, the change direction and rates of C and N stoichiometry along increasing latitude or altitude did not differ between root orders (Fig. 2; Table S1). A previous study has indicated that root C:N ratios decreased with increasing MAT among both absorptive and transport roots and exhibited similar R-squares (Zadworny *et al*., 2017), supported the results in our study. Further, warming experiments also found that the effects of warming on root C and N concentrations (Yin *et al.*, 2014) and root respiration (Jia *et al.*, 2011) were consistent among root branch orders.

Drivers of root C and N stoichiometry in the first five root branch orders

Plant functional traits are basically controlled by internal and external variables (Valverde-barrantes *et al.*, 2017; Wang *et al.*, 2020). In this study, plant species and climatic factors generally accounted for more variation in C and N stoichiometry across the first five root branch orders (except for the explanation of C:N ratios by plant species) than either geographical factors or plant functional groups (Fig. 3). In line with the species composition hypothesis (Reich & Oleksyn, 2004), plant species was one of the most important factors regulating the variations of root C and N stoichiometry. Firstly, woody plants may adapt to heterogeneous resources and environments by altering root traits (Kong *et al.*, 2014). The large coefficients of variation of root C and N stoichiometry noted in this study could to some degree reflect this process (Fig. 1; Notes S1). Secondly, there were significant phylogenetic signals of root C and N stoichiometry across the five branch orders of 218 woody plant species (Table S4). This result is agrees with the findings by Valverde-barrantes *et al*. (2017), who reported that phylogeny was the primary factor structuring the distribution of root traits among species. Notably, climatic factors tended to explain variation in C and N stoichiometry either independently or through interactions with plant species (Fig. 3). This is probably because climate can drive plant species composition and distributions (Hou *et al.*, 2021), and alters soil nutrient pools (Reich & Oleksyn, 2004) that then impact fine-root C and N stoichiometry (Wang *et al.*, 2020). However, due to the limitation of our analysis, there are potential confounding effects between species and other factors that call for further in-depth investigation.

The PGLS models also showed that MAT and MAP often negatively affected root C concentrations (except MAP pattern for the first two orders) across the five branch orders (Fig. S2a, d), which is similar to the results observed in non-structural carbohydrates concentrations (Li *et al.*, 2016). The N concentrations of the first two orders were negatively related to MAT (Fig. S2b), which is in agreement with the observed latitudinal pattern in our study. Similar results were also found in a previous study (Zhao *et al.*, 2019). Aridity index exerted negative effects on root N concentrations (Fig. S3b), implying that plant root growth and N uptake are inhibited by drought conditions (Foyer & Shigeoka, 2011; Shi *et al.*, 2017).

Conclusions

This study, for the first time, explores the variations and patterns of root C and N stoichiometry in the first five root branch orders across woody plant species at a large scale. Both plant functional group and root branch order are two determinants regulating root C and N stoichiometry across woody plant species, reflecting divergent root ecological strategies for plants adapting to environmental changes. The convergence and divergence in the patterns of C and N stoichiometry between latitude and altitude across the first five root orders are similar to those observed in diameter-based datasets. The variations in root C and N stoichiometry are mainly driven by plant species and climate factors; however, the influencing mechanisms and research on a larger scale require further in-depth investigation. These findings have important implications for the root economics spectrum and provide critical information for improving biogeochemical models to understand and predict the response of belowground processes in terrestrial ecosystems to climate change.

Acknowledgements

We are very grateful to all the researchers whose data were compiled in this study. This work was supported by the National Natural Science Foundation of China (32071745, 31870602, 31901295 and 32001165), the Program of Sichuan Excellent Youth Sci-Tech Foundation (2020JDJQ0052), the Program of Sichuan Applied Basic Research Foundation (2021YJ0340) and the Chinese Postdoctoral Science Foundation (2022M722297). Josep Penuelas and Jordi Sardans were funded by the European Research Council Synergy project SyG-2013-610028 IMBALANCE-P, the Spanish Government grants PID2019-110521GB-I00 and TED2021-132627B-I00, the Fundación Ramon Areces grant ELEMENTAL-CLIMATE, and the Catalan Government grant SGR 2017-1005.

Data availability

The data used to support the findings of this study are available in Supporting Information.

Author contributions

C.Y., Z.X. and J.P. designed the study, C.Y., Z.X., H.L., L.Z., S.L., L.W., Y.L., L.C. and K.Y. collected the data, C.Y., B.T., J.L., X.C., and R.Y. and J.S. performed the meta-analysis, C.Y., B.T. and R.Y. wrote the first draft of the manuscript, Y.Y., J.L., and J.Z. provided great help in the process of the writing and all authors annotated and approved the final version of the manuscript.

Competing interests

None declared.

References

Adamczyk B, Sietio OM, Strakova P, Prommer J, Wild B, Hagner M, Pihlatie M, Fritze H, Richter A, Heinonsalo J. 2019. Plant roots increase both decomposition and stable organic matter formation in boreal forest soil. *Nature Communications* 10: 3982.

Ågren GI. 2004. The C:N:P stoichiometry of autotrophs-theory and observations. *Ecology Letters* 7: 185-191.

Aubrey DP, Teskey RO. 2018. Stored root carbohydrates can maintain root respiration for extended periods. *New Phytologist* 218: 142-152.

Augusto L, De Schrijver A, Vesterdal L, Smolander A, Prescott C, Ranger J. 2015. Influences of evergreen gymnosperm and deciduous angiosperm tree species on the functioning of temperate and boreal forests. *Biological Reviews* 90: 444-66.

Bergmann J, Weigelt A, Plas F, Laughlin DC, Kuyper TW, Guerrero-Ramirez N, Valverde-Barrantes OJ, Bruelheide H, Freschet GT, Iversen CM. *et al*. 2020. The fungal collaboration gradient dominates the root economics space in plants. *Science Advances* 6:eaba3756.

Brundrett M C. 2017. Global diversity and importance of mycorrhizal and nonmycorrhizal plants. In: biogeography of mycorrhizal symbiosis (eds Tedersoo L.). *Springer International Publishing*, Cham, Switzerland.

Chen W, Koide RT, Adams TS, DeForest JL, Cheng L, Eissenstat DM. 2016. Root morphology and mycorrhizal symbioses together shape nutrient foraging strategies of temperate trees. *Proceedings of the National Academy of Sciences of the United States of America* 113: 8741-6.

Devi AF, Garkoti SC. 2013. Variation in evergreen and deciduous species leaf phenology in Assam, India. *Trees* 27: 985-997.

Ding J, Kong D, Zhang Z, Cai Q, Xiao J, Liu Q, Yin H, Wardle D. 2020. Climate and soil nutrients differentially drive multidimensional fine root traits in ectomycorrhizal-dominated alpine coniferous forests. *Journal of Ecology* 108: 2544-2556.

Fajardo A, Piper FI. 2011. Intraspecific trait variation and covariation in a widespread tree species (*Nothofagus pumilio*) in southern Chile. *New Phytologist* 189: 259-271.

Freschet GT, Cornwell WK, Wardle DA, Elumeeva TG, Liu W, Jackson BG, Onipchenko VG, Soudzilovskaia NA, Tao J, Cornelissen JH. 2013. Linking litter decomposition of above and belowground organs to plant-soil feedbacks worldwide. *Journal of Ecology* 101: 943-952.

Freschet GT, Valverde-Barrantes OJ, Tucker CM, Craine JM, McCormack ML, Violle C, Fort F, Blackwood CB, Urban-Mead KR, Iversen CM *et al*. 2017. Climate, soil and plant functional types as drivers of global fine-root trait variation. *Journal of Ecology* 105: 1182-1196.

Goebel M, Hobbie SE, Bulaj B, Zadworny M, Archibald DD, Oleksyn J, Eissenstat RDM. 2011. Decomposition of the finest root branching orders: linking belowground dynamics to fine-root function and structure. *Ecological. Monographs* 81: 89-102.

Guo D, Xia M, Wei X, Chang W, Liu Y, Wang Z. 2008. Anatomical traits associated with absorption and mycorrhizal colonization are linked to root branch order in twenty-three Chinese temperate tree species. *New Phytologist* 180: 673-83.

Guo D, Mitchell RJ, Hendricks JJ. 2004. Fine root branch orders respond differentially to carbon source-sink manipulations in a longleaf pine forest. *Oecologia* 140: 450-7.

He X, Hou E, Liu Y, Wen D. 2016. Altitudinal patterns and controls of plant and soil nutrient concentrations and stoichiometry in subtropical China. *Scientific Reports* 6: 24261.

Hou E, Wen D, Jiang L, Luo X, Kuang Y, Lu X, Chen C, Allen KT, He X, Huang X, Luo Y. 2021. Latitudinal patterns of terrestrial phosphorus limitation over the globe. *Ecology Letters* 00: 1-12.

Holdaway RJ, Richardson SJ, Dickie IA, Peltzer DA, Coomes DA. 2011. Species- and community-level patterns in fine root traits along a 120000-year soil chronosequence in temperate rain forest. *Journal of Ecology* 99: 954-963.

Hu Y, Liu X, He N, Pan X, Long S, Li W, Zhang M, Cui L, Kerkhoff A. 2021. Global patterns in leaf stoichiometry across coastal wetlands. *Global Ecology and Biogeography* 00: 1-18.

Huang Y, Zhang X, Zang R, Fu S, Ai X, Yao L, Ding Y, Huang J, Lu X. 2018. Functional recovery of a subtropical evergreen-deciduous broadleaved mixed forest following clear cutting in central China. *Scientific Reports* 8: 16458.

Iversen CM, Powell AS, McCormack ML, Blackwood CB, Freschet GT. 2017. Fine-Root Ecology Database (FRED): A global collection of root trait data with coincident site, vegetation, edaphic, and climatic data, *Version* 1.

Jackson RB, Mooney HA, Schulze ED, 1997. A global budget for fine root biomass, surface area, and nutrientcontents. *Proceedings of the National Academy of Sciences of the United States of America* 94: 7362-7366.

Jia S, Wang Z, Li X, Zhang X, McLaughlin NB. 2011. Effect of nitrogen fertilizer, root branch order and temperature on respiration and tissue N concentration of fine roots in *Larix gmelinii* and *Fraxinus mandshurica*. *Tree Physiology* 31: 718-726.

Jin Y, Qian H. 2019. V.PhyloMaker: an R package that can generate very large phylogenies for vascular plants. *Ecography* 42: 1353-1359.

Kong D, Ma C, Zhang Q, Li L, Chen X, Zeng H, Guo D. 2014. Leading dimensions in absorptive root trait variation across 96 subtropical forest species. *New Phytologist* 203: 863-872.

Kong D, Wang J, Valverde-Barrantes O, Kardol P. 2021. A framework to assess the carbon supply-consumption balance in plant roots. *New Phytologist* 229: 659-664.

Kong D, Wang J, Wu H, Valverde-Barrantes OJ, Wang R, Zeng H, Kardol P, Zhang H, Feng Y. 2019. Nonlinearity of root trait relationships and the root economics spectrum. *Nature Communications* 10: 2203.

Kou L, Jiang L, Fu X, Dai X, Wang H, Li S. 2018. Nitrogen deposition increases root production and turnover but slows root decomposition in *Pinus elliottii* plantations. *New Phytologist* 218: 1450-1461.

Lai J, Zou Y, Zhang J, Peres-Neto PR. 2022. Generalizing hierarchical and variation partitioning in multiple regression and canonical analyses using the rdacca.hp R package. *Methods in Ecology and Evolution* 13: 782-788.

Li J, Chen X, Niklas KJ, Sun J, Wang Z, Zhong Q, Hu D, Cheng D. 2021. A whole-plant economics spectrum including bark functional traits for 59 subtropical woody plant species. *Journal of Ecology* 110: 248-261.

Li N, He N, Yu G, Wang Q, Sun J. 2016. Leaf non-structural carbohydrates regulated by plant functional groups and climate: Evidences from a tropical to cold-temperate forest transect. *Ecological Indicators* 62: 22-31.

Liese R, Lubbe T, Albers NW, Meier IC. 2018. The mycorrhizal type governs root exudation and nitrogen uptake of temperate tree species. *Tree Physiology* 38: 83-95.

Maherali H , Oberle B , Stevens PF, Cornwell WK. McGlinn DJ. 2016. Mutualism persistence and abandonment during the evolution of the mycorrhizal symbiosis. *American Naturalist* 188 E113-E125.

McCormack ML, Dickie IA, Eissenstat DM, Fahey TJ, Fernandez CW, Guo D, Helmisaari HS, Hobbie EA, Iversen CM, Jackson RB *et al*. 2015. Redefining fine roots improves understanding of below-ground contributions to terrestrial biosphere processes. *New Phytologist* 207: 505-18.

Mucha J, Zadworny M, Helmisaari HS, Nihlgård B, Repo T, Żytkowiak M, Małek S, Reich PB, Oleksyn J. 2019. Fine root classification matters: nutrient levels in different functional categories, orders and diameters of roots in boreal *Pinus sylvestris* across a latitudinal gradient. *Plant and Soil* 447: 507-520.

Phillips RP, Brzostek E, Midgley MG. 2013. The mycorrhizal-associated nutrient economy: a new framework for predicting carbon-nutrient couplings in temperate forests. *New Phytologist* 199: 41-51.

Pierick K, Link RM, Leuschner C, Homeier J. 2022. Elevational trends of tree fine root traits in species-rich tropical Andean forests. *Oikos* e08975.

Pu X, Yin C, Xiao Q, Qiao M, Liu Q. 2016. Fine roots branch orders of *Abies faxoniana* respond differentially to warming in a subalpine coniferous forest ecosystem. *Agroforestry Systems* 91: 955-966.

Pregitzer KS, Kubiske ME, Yu CK, Hendrick RL. 1997. Relationships among root branch order, carbon, and nitrogen in four temperate species. *Oecologia* 111: 302-308

Pregitzer KS, DeForest JL, Burton AJ, Allen MF, Ruess RW, Hendrick RL. 2002. Fine root architecture of nine north American trees. *Ecological Monographs* 72: 293-309.

Razaq M, Salahuddin Shen HL, Sher H, Zhang P. 2017. Influence of biochar and nitrogen on fine root morphology, physiology, and chemistry of *Acer mono*. *Scientific Reports* 7: 5367.

Reich PB, Oleksyn J. 2004. Global patterns of plant leaf N and P in relation to temperature and latitude. *Proceedings of the National Academy of Sciences of the United States of America* 101: 11001-11006.

Reich PB, Cornelissen H. 2014. The world-wide 'fast-slow' plant economics spectrum: a traits manifesto. *Journal of Ecology* 102: 275-301.

Roumet C, Birouste M, Picon-Cochard C, Ghestem M, Osman N, Vignon-Brenas S, Cao KF, Stokes A. 2016. Root structure-function relationships in 74 species: evidence of a root economic spectrum related to carbon economy. *New Phytologist* 210: 815-826.

See CR, Luke McCormack M, Hobbie SE, Flores-Moreno H, Silver WL, Kennedy PG. 2019. Global patterns in fine root decomposition: climate, chemistry, mycorrhizal association and woodiness. *Ecology Letters* 22: 946-953.

Shi H, Ma W, Song J, Lu M, Rahman SU, Bui TTX, Vu DD, Zheng H, Wang J, Zhang Y. 2017. Physiological and transcriptional responses of *Catalpa bungei* to drought stress under sufficient-and deficient-nitrogen conditions. *Tree Physiology* 37: 1457-1468.

Strand AE, Pritchard SG, Mccormack ML, Davis MA, Oren R. 2008. Irreconcilable differences: fine-root life spans and soil carbon persistence. *Science* 319: 456-458.

Tian D, Yan Z, Niklas KJ, Han W, Kattge J, Reich PB, Luo Y, Chen Y, Tang Z, Hu H *et al*. 2018. Global leaf nitrogen and phosphorus stoichiometry and their scaling exponent. *National Science Review* 5: 728-739.

Valverde-Barrantes OJ, Blackwood CB. 2016. Root traits are multidimensional: specific root length is independent from root tissue density and the plant economic spectrum: Commentary on Kramer-Walter *et al*. *Journal of Ecology* 104: 1311-1313.

Valverde-Barrantes OJ, Freschetet GT, Roumetal C, Blackwood CB. 2017. A worldview of root traits: the influence of ancestry, growth form, climate and mycorrhizal association on the functional trait variation of fine-root tissues in seed plants. *New Phytologist* 215: 1562–1573.

Valverde-Barrantes OJ, Smemo KA, Feinstein LM, Kershner MW, Blackwood CB, 2018. Patterns in spatial distribution and root trait syndromes for ecto and arbuscular mycorrhizal temperate trees in a mixed broadleaf forest. *Oecologia* 186: 731-741.

Vergutz L, Manzoni S, Porporato A, Novais RF, Jackson RB, 2012. Global resorption efficiencies and concentrations of carbon and nutrients in leaves of terrestrial plants. *Ecological Monographs* 82: 205-220.

Wang R, Wang Q, Zhao N, Xu Z, Zhu X, Jiao C, Yu G, He N. 2018. Different phylogenetic and environmental controls of first-order root morphological and nutrient traits: Evidence of multidimensional root traits. *Functional Ecology* 32: 29-39.

Wang Z, Lv S, Song H, Wang M, Zhao Q, Huang H, Niklas KJ. 2020. Plant type dominates fine-root C:N:P stoichiometry across China: A meta-analysis. *Journal of Biogeography* 47: 1019-1029.

Watson RA, Szathmáry E, 2016. How can evolution learn? *Trends in Ecology Evolution* 31: 147-157.

Weemstra M, Mommer L, Visser EJW, van Ruijven J, Kuyper TW, Mohren GMJ, Sterck FJ. 2016. Towards a multidimensional root trait framework: a tree root review. *New Phytologist* 211: 1159-1169.

Wright IJ, Reich PB, Westoby M, Ackerly DD, Baruch Z, Bongers F, Cavender-Bares J, Chapin T, Cornelissen JH, Diemer M *et al*. 2004. The worldwide leaf economics spectrum. *Nature* 428: 821-827.

Wu Q, Pagès L, Wu J. 2016. Relationships between root diameter, root length and root branching along lateral roots in adult, field-grown maize. *Annals of Botany* 117: 379-390.

Yin C, Pu X, Xiao Q, Zhao C, Liu Q. 2014. Effects of night warming on spruce root around non-growing season vary with branch order and month. *Plant and Soil* 380: 249-263.

Yuan Z, Chen HY, Reich PB. 2011. Global-scale latitudinal patterns of plant fine-root nitrogen and phosphorus. *Nature Communications* 2: 344.

Zadworny, McCormack ML, Rawlik, Jagodzinski AM. 2015. Seasonal variation in chemistry, but not morphology, in roots of *Quercus robur* growing in different soil types. *Tree Physiology* 35: 644-652.

Zadworny M, McCormack ML, Zytkowiak R, Karolewski P, Mucha J, Oleksyn J. 2017. Patterns of structural and defense investments in fine roots of Scots pine

(*Pinus sylvestris L.*) across a strong temperature and latitudinal gradient in Europe. *Global Change Biology* 23: 1218-1231.

Zhang J, Zhao N, Liu C, Yang H, Li M, Yu G, Wilcox K, Yu Q, He N, Niu S. 2017. C:N:P stoichiometry in China's forests: From organs to ecosystems. *Functional Ecology* 32: 50-60.

Zhang J, He N, Liu C, Xu L, Chen Z, Li Y, Wang R, Yu G, Sun W, Xiao C *et al*. 2020. Variation and evolution of C:N ratio among different organs enable plants to adapt to N-limited environments. *Global Change Biology* 26: 2534-2543.

Zhao H, He N, Xu L, Zhang X, Wang Q, Wang B, Yu G. 2019. Variation in the nitrogen concentration of the leaf, branch, trunk, and root in vegetation in China. *Ecological Indicators* 96: 496-504.

Zhao N , Yu G, He N, Wang Q, Guo D, Zhang X, Wang R, Xu Z, Jiao C, Li N *et al*. 2016. Coordinated pattern of multi-element variability in leaves and roots across Chinese forest biomes. *Global Ecology and Biogeography* 25: 359-367.

Supporting Information

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

Fig. S1 Large-scale distribution of fine-root carbon (C) and nitrogen (N) stoichiometry of the first five orders for woody plants included in this study.

Fig. S2 Phylogenetic generalized least squares (PGLS) model analysis of relationships between climatic factors (Mean annual temperature, MAT and Mean annual precipitation, MAP) and fine-root carbon (C) and nitrogen (N) stoichiometry.

Fig. S3 Phylogenetic generalized least squares (PGLS) model analysis of relationships between climatic factors (Aridity index and Solar radiation) and fine-root carbon (C) and nitrogen (N) stoichiometry.

Table S1. Phylogenetic generalized least squares (PGLS) model analysis of the relationships between fine-root carbon (C) and nitrogen (N) stoichiometry and latitude, and altitude.

Table S2. Phylogenetic generalized least squares (PGLS) model analysis of the relationships between fine-root carbon (C) and nitrogen (N) stoichiometry and mean annual temperature (MAT), and mean annual precipitation (MAP).

Table S3. Phylogenetic generalized least squares (PGLS) model analysis of the relationships between fine-root carbon (C) and nitrogen (N) stoichiometry and aridity index, and solar radiation.

Table S4. Phylogenetic conservatism indices for root carbon (C) and nitrogen (N) stoichiometry across branch orders based on evolutionary models (Pagel's λ).

Notes S1. Dataset of carbon (C) and nitrogen (N) concentrations and C:N ratios of the first five root branch orders of woody plant species, based on more than 4100 observations at 85 study sites from 51 published articles.

Notes S2. Detailed information on which species and how many samples were presented by each species was used for our analysis.

Notes S3. A list of 51 articles from which data were extracted for the meta-analysis.

Table 1. Analysis of relationships between fine-root carbon (C) and nitrogen (N) concentrations, and C:N ratios of contrasting plant functional groups (e.g., deciduous species and evergreen species; broadleaf species and coniferous species; arbuscular mycorrhiza species (AM) and ectomycorrhiza species (EM)) and root branch orders based on the linear mixed model.

Variable	Plant functional group	n	Intercept	Slope	Lower CI	Upper CI	Marginal R^2	Conditional R^2	*p*
	Leaf habit								
C	Deciduous	425	2.632	0.009a	0.006	0.012	0.019	0.815	<0.001
	Evergreen	745	2.615	0.010a	0.009	0.011	0.082	0.813	<0.001
N	Deciduous	700	1.386	-0.075a	-0.081	-0.069	0.230	0.757	<0.001
	Evergreen	985	1.337	-0.080a	-0.083	-0.077	0.202	0.918	<0.001
C:N	Deciduous	430	1.250	0.072a	0.064	0.081	0.209	0.674	<0.001
	Evergreen	895	1.294	0.085a	0.081	0.089	0.226	0.890	<0.001
	Taxonomical grouping								
C	Broadleaved	1010	2.623	0.009b	0.008	0.010	0.025	0.858	<0.001
	Coniferous	160	2.615	0.015a	0.012	0.018	0.176	0.706	<0.001
N	Broadleaved	1420	1.366	-0.077a	-0.081	-0.074	0.222	0.836	<0.001
	Coniferous	265	1.271	-0.081a	-0.086	-0.075	0.284	0.916	<0.001
C:N	Broadleaved	1015	1.257	0.081a	0.076	0.085	0.228	0.812	<0.001
	Coniferous	310	1.385	0.081a	0.074	0.089	0.291	0.815	<0.001
	Mycorrhizal association type								
C	AM	560	2.620	0.011a	0.010	0.013	0.068	0.779	<0.001
	EM	235	2.635	0.010a	0.008	0.013	0.103	0.719	<0.001
N	AM	900	1.357	-0.073a	-0.078	-0.068	0.205	0.792	<0.001
	EM	335	1.370	-0.082a	-0.087	-0.076	0.238	0.901	<0.001
C:N	AM	565	1.265	0.076a	0.069	0.082	0.225	0.739	<0.001
	EM	385	1.301	0.079a	0.072	0.086	0.235	0.818	<0.001

Note: The C and N stoichiometry was transformed into a log-scale before analysis. n, sample size; CI, 95% confidence intervals of the regression slope; Marginal R^2, the model variation explained by the fixed effects; Conditional R^2, the model variation explained by both the fixed and random effects; *p*, the significance of the linear equations. Different lowercase letters indicate that the difference in root C and N stoichiometry between contrasting plant functional groups was dependent on root branch orders at the 0.05 level based on the likelihood ratio test.

Figure captions

Fig. 1 Raincloud plots show the variation in root carbon (C) and nitrogen (N) stoichiometry of the first five root order branches across woody plant functional groups (e.g., deciduous species and evergreen species; broadleaf species and coniferous species; arbuscular mycorrhiza species (AM) and ectomycorrhiza species (EM)). The lower and upper half of boxplots represent the 25th and 75th percentile, respectively. The whisker means min and max, the solid lines across each box present the median, and the hollow circle displays the mean. The distribution and the density of raw data are shown in half-violin. Different lowercase letters indicate among (a−c) and within (d−m) root branch order differences at $p < 0.05$ based on the maximum likelihood ratio test.

Fig. 2 Phylogenetic generalized least squares (PGLS) model analysis of relationships between geographical factors (latitude (a−c) and altitude (d−f)) and fine-root carbon (C) and nitrogen (N) stoichiometry. Brown lines represent linear fitting with PGLS analysis. Numbers (1-5), colours and symbols represent root order. R^2 and S are coefficients of determination of the equation and slope, respectively; ns denotes no significant difference between the slope of equation of the first five root orders at $p < 0.05$ based on the maximum likelihood ratio test.

Fig. 3 Venn diagrams illustrating the relative contribution of climate factors (C: mean annual temperature, MAT; mean annual precipitation, MAP; aridity index; solar radiation), plant functional groups (P), species identity (S), and geographical factors

(G: latitude; altitude) to the variation in root carbon (C) (a) and nitrogen (N) (b), and C:N ratios (c) of the first five root branch orders for woody plans based on the hierarchical partitioning method with redundancy analysis. The numbers in each subset figure show the percentage of total variation explained by the model and variations explained by the independent effect of (a) species (yellow), (b) climate (pink), (c) plant functional groups (wathet) and (d) geographical (green), and the joint effect of (e) species and climate, (f) species and plant functional groups, (g) species and geographical, (h) plant functional groups and climate, (i) climate and geographical (j) geographical and plant functional groups (k) species, plant functional groups and climate, (l) species, climate and geographical, (m) species, geographical and plant functional groups, (n) plant functional groups, geographical and climate (o) all four factors.

Figure captions

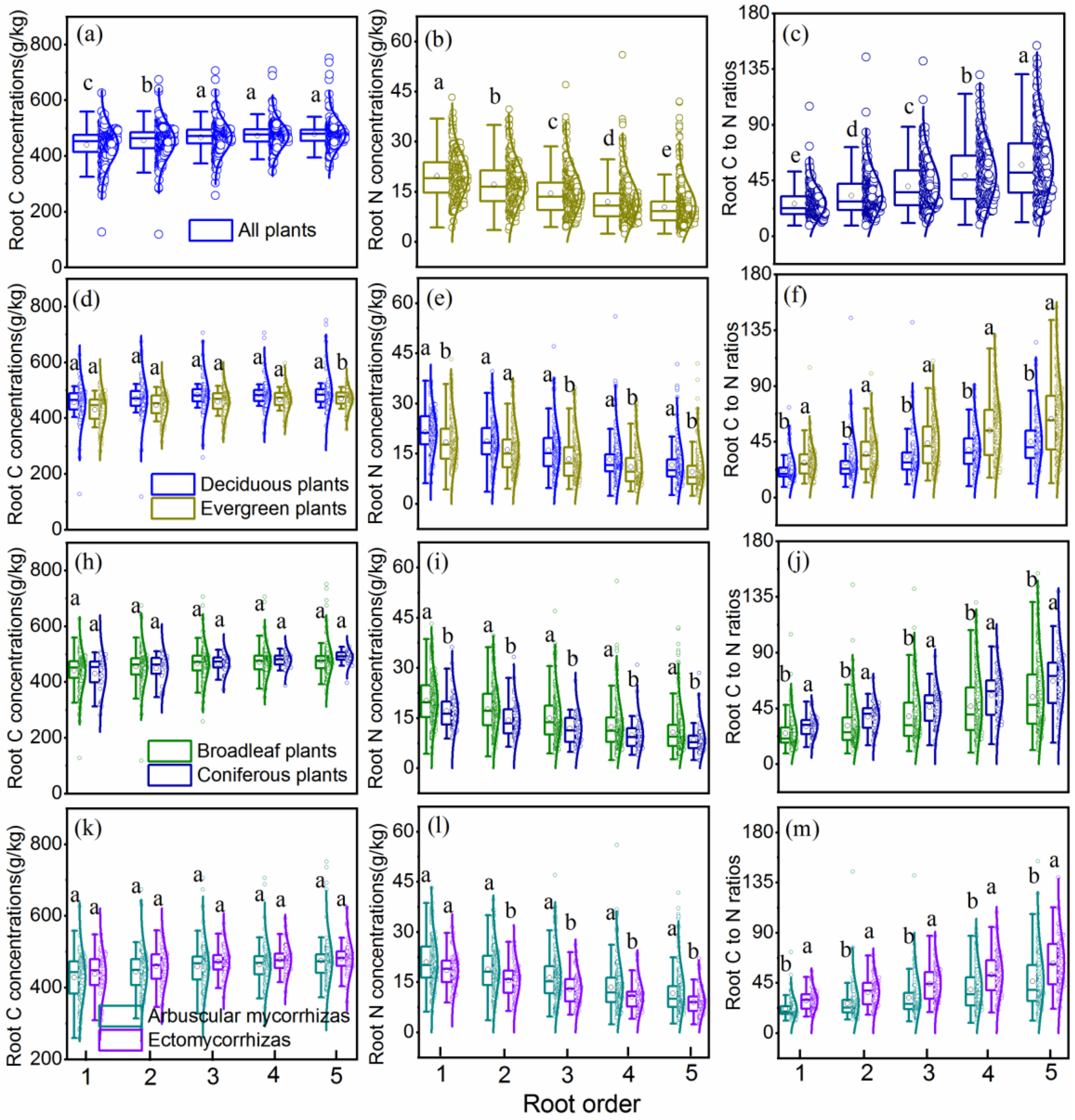

Fig. 1 Raincloud plots show the variation in root carbon (C) and nitrogen (N) stoichiometry of the first five root order branches across woody plant functional groups (e.g., deciduous species and evergreen species; broadleaf species and coniferous species; arbuscular mycorrhiza species (AM) and ectomycorrhiza species (EM)). The lower and upper half of boxplots represent the 25th and 75th percentile, respectively. The whisker means min and max, the solid lines across each box present the median, and the hollow circle displays the mean. The distribution and the density of raw data are shown in half-violin. Different lowercase letters indicate among (a−c)

and within (d−m) root branch order differences at $p < 0.05$ based on the maximum likelihood ratio test.

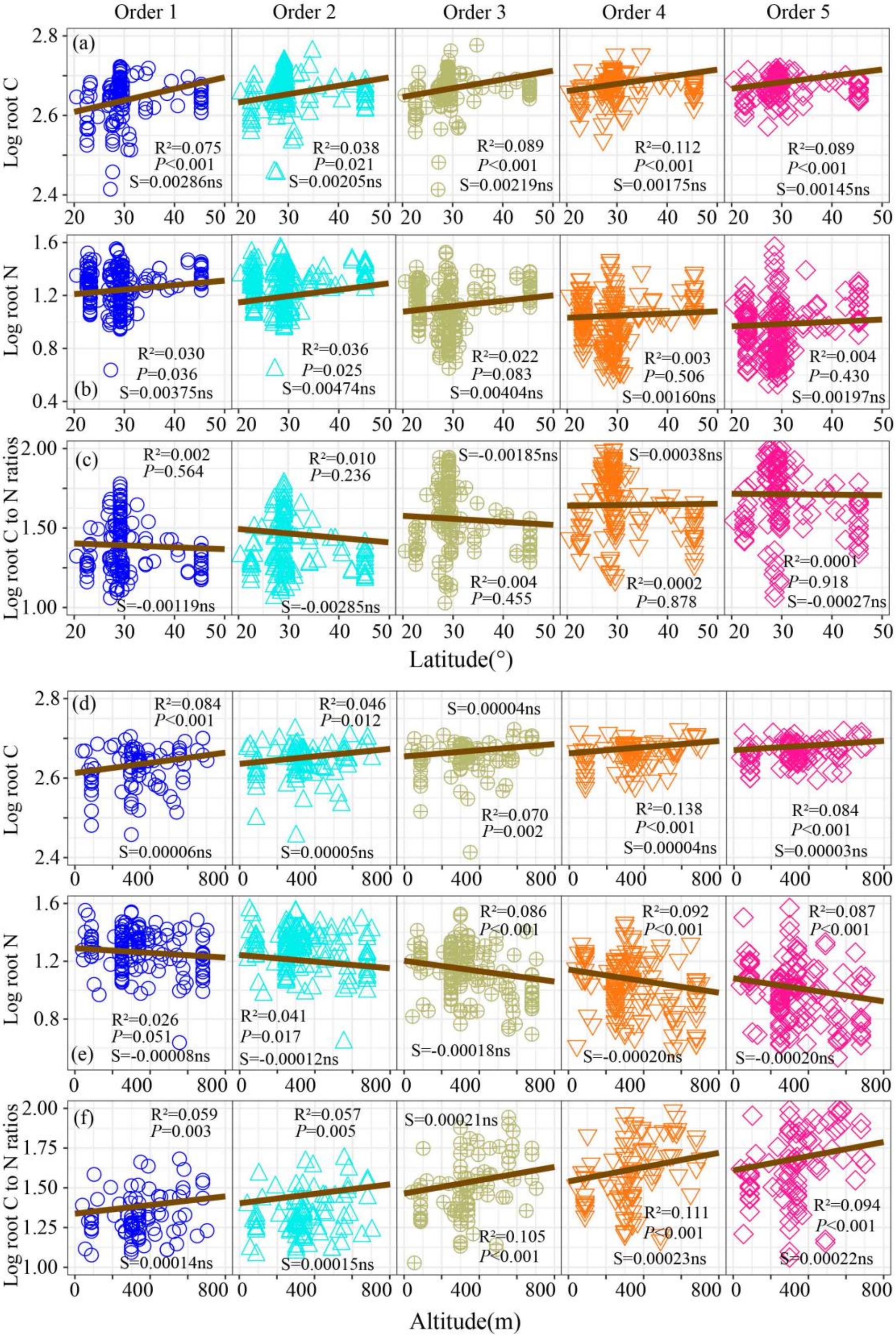

Fig. 2 Phylogenetic generalized least squares (PGLS) model analysis of relationships between geographical factors (latitude (a−c) and altitude (d−f)) and fine-root carbon (C) and nitrogen (N) stoichiometry. Brown lines represent linear fitting with PGLS

analysis. Numbers (1-5), colours and symbols represent root order. R^2 and S are coefficients of determination of the equation and slope, respectively; ns denotes no significant difference between the slope of equation of the first five root orders at $p < 0.05$ based on the maximum likelihood ratio test.

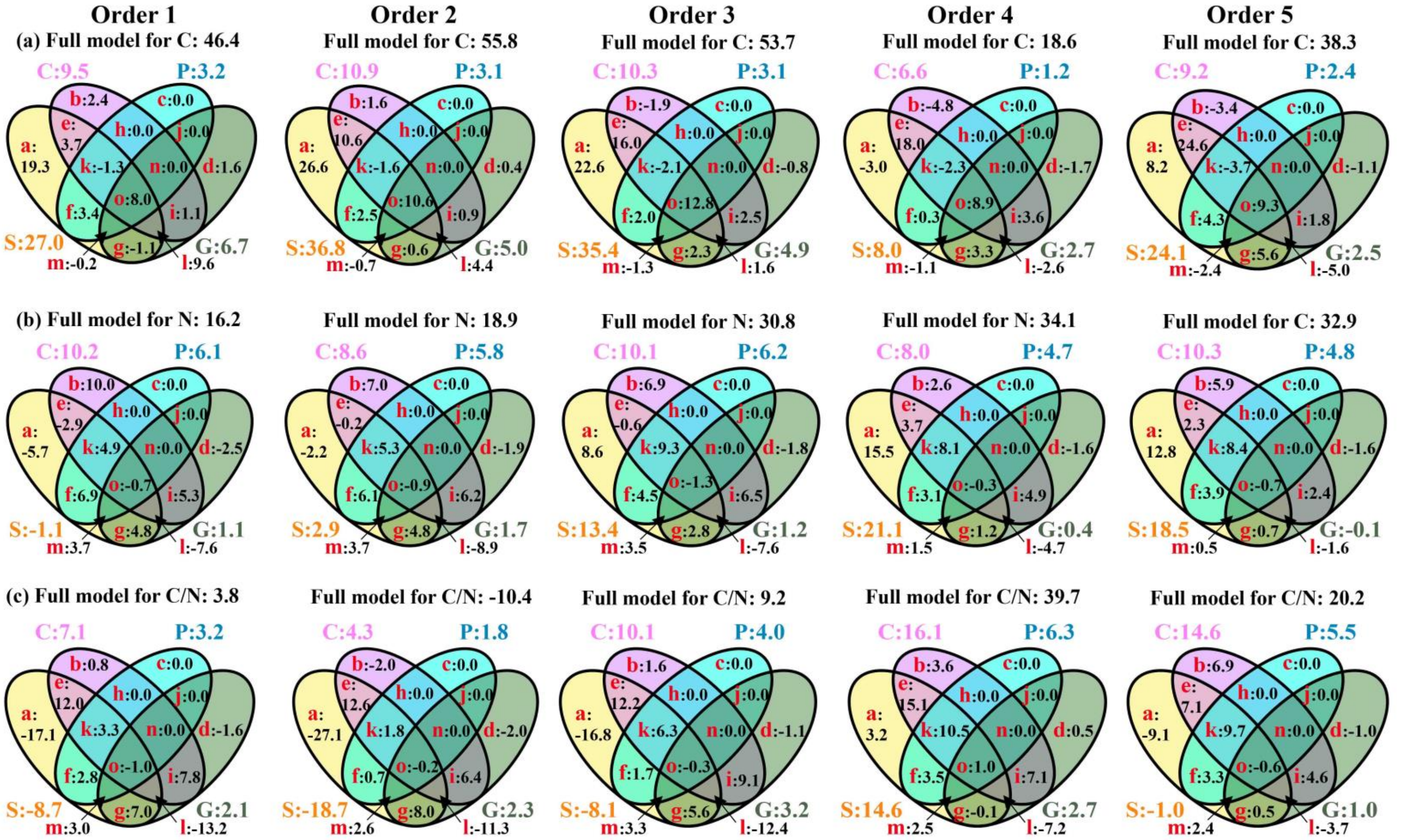

Fig. 3 Venn diagrams illustrating the relative contribution of climate factors (C: mean annual temperature, MAT; mean annual precipitation, MAP; aridity index; solar radiation), plant functional groups (P), species identity (S), and geographical factors (G: latitude; altitude) to the

variation in root carbon (C) (a) and nitrogen (N) (b), and C:N ratios (c) of the first five root branch orders for woody plans based on the hierarchical partitioning method with redundancy analysis. The numbers in each subset figure show the percentage of total variation explained by the model and variations explained by the independent effect of (a) species (yellow), (b) climate (pink), (c) plant functional groups (wathet) and (d) geographical (green), and the joint effect of (e) species and climate, (f) species and plant functional groups, (g) species and geographical, (h) plant functional groups and climate, (i) climate and geographical (j) geographical and plant functional groups (k) species, plant functional groups and climate, (l) species, climate and geographical, (m) species, geographical and plant functional groups, (n) plant functional groups, geographical and climate (o) all four factors.