Chen Han (Orcid ID: 0000-0001-9477-5541)
Chu Chengjin (Orcid ID: 0000-0002-0606-449X)
Ma Zilong (Orcid ID: 0000-0002-4621-5766)

Author Manuscript

Legacy effects control root elemental composition and stoichiometry in subtropical forests: empirical support for the biogeochemical niche hypothesis

Author: Mingyan Hu[1], Yang Chen[2], Jordi Sardans[3,4], Josep Peñuelas[3,4] , Han Y.H.Chen[5,6], Chengjin Chu[1] and Zilong Ma[1]*

Affiliations:

[1]State Key Laboratory of Biocontrol, School of Ecology, Sun Yat-sen University, Guangzhou, 510275, China

[2]State Key Laboratory of Biocontrol, School of Life Science, Sun Yat-sen University, Guangzhou, 510275, China

[3]CSIC, Global Ecology Unit CREAF-CSIC-UAB, Bellaterra, Spain

[4]CREAF, Cerdanyola del Vallès, Spain

[5]Faculty of Natural Resources Management, Lakehead University, 955 Oliver Road, Thunder Bay, Ontario P7B5E1, Canada

[6]Institute for Global Change Biology, and School for Environment and Sustainability, University of Michigan, Ann Arbor, MI 48104

***Corresponding author:** Zilong Ma; E-mail: mazlong@mail.sysu.edu.cn

This is the author manuscript accepted for publication and has undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/1365-2745.70031

This article is protected by copyright. All rights reserved.

Author Manuscript

Abstract

1. Under biogeochemical niche (BN) theory, plant allocation of elements to organs to maintain fundamental biological processes varies with species, leading to the formation of species-specific BNs. However, empirical support for the BN theory is largely restricted to plant leaf elemental composition and stoichiometry, with a lack of clarity of the contribution of fine root element content.

2. Here, we analyzed fine root concentrations and stoichiometry of 9 elements including carbon (C), nitrogen (N), phosphorus (P), potassium(K), calcium (Ca), magnesium (Mg), iron (Fe), aluminum (Al), and manganese (Mn) in 137 tree species of a subtropical forest, to quantify BN and test the theoretical framework of BN hypothesis.

3. Our study showed phylogenetic signals of fine root elemental composition and stoichiometry. Fine root elemental composition of the 21 most abundant co-existing species tends to be unique and primarily driven by root N content, as indicated by canonical discriminant analysis. Legacy effects (phylogeny and species) explained 23.3–70.7% of the variation across the different variables used to characterize fine root elemental composition, stoichiometry and BNs, whereas combined effects of soil property, mycorrhizal association type, and topography factors explained 3.9–17.7%.

4. Synthesis: These results indicate that phylogenetic and taxonomic distance (calculated as distance metrics) represents a proxy for species-specific evolution and achievement of optimal

function linked to bio-element use. Thus, our study provides new empirical evidence in support of the BN hypothesis, based on plant fine root elemental composition and stoichiometry, and improves our mechanistic understanding of species coexistence dynamics.

Keywords

Biogeochemical niche; phylogeny; fine root elemental composition; soil property; stoichiometry; topography; tropical forest

Author Manuscript

Author Manuscript

Author Manuscript

摘要（TRANSLATED ABSTRACT）

1.生物地球化学生态位假说认为物种因具有独特的生活史策略和生理代谢过程，对化学元素的需求、分配和利用存在差异，进而形成特异性生态位。然而，现有研究主要基于叶片的元素组成量化和表征物种特异性生态位，细根元素组成变化是否能支持并验证生物地球化学生态位假说仍缺乏局域尺度证据。

2.本研究依托广东省黑石顶自然保护区森林动态监测样地，通过测定137个树种细根的碳（C）、氮（N）、磷（P）、钾（K）、钙（Ca）、镁（Mg）、铁（Fe）、铝（Al）和锰（Mn）含量，结合土壤理化性质等环境因子以检验生物地球化学生态位假说的理论框架。

3.研究发现，细根元素组成及其C:N:P化学计量具有显著的系统发育信号。典型判别分析表明，森林群落中21个优势树种的细根元素组成具有独特性，并主要受细根N含量的驱动。遗传效应（系统发育和物种）解释了细根元素组成和C:N:P化学计量以及生物地球化学生态位23.3-70.7%的变异，而土壤性质、菌根真菌类型和地形共同解释了细根元素组成和C:N:P化学计量以及生物地球化学生态位3.9-17.7%的变异。

4.综合：研究结果表明，细根的元素组成和化学计量比也遵循生物地球化学生态位假说。细根的元素组成和C:N:P化学计量以及生物地球化学生态位是遗传效应、菌根共生类型以及局域尺度资源异质性共同作用的结果。

Author Manuscript

1. Introduction

Ecological theory states that stable coexistence among competing species requires occupancy of different ecological niches (Gause, 1934); however, the quantification of species niches has long been a challenge in community ecology (Hutchinson, 1957, Silvertown, 2004). Organisms comprise chemical elements, including carbon (C), nitrogen (N), and phosphorus (P), that are closely linked to life history strategy, such as reproduction and survival (Wright et al., 2004, Sardans et al., 2021, Furey and Tilman, 2023). The biogeochemical niche (BN) hypothesis, where BN is defined as the species-specific occupation of an n-dimensional bio-element space (Peñuelas et al., 2008, González et al., 2017, Peñuelas et al., 2019), has been proposed as an approach to quantify ecological niches and explain community assembly (Zhao et al., 2017, Fernandez-Martinez et al., 2021, Sardans et al., 2021). Specifically, the BN is founded on three fundamental complementary rules (Peñuelas et al., 2019, Sardans et al., 2021). First, distinct taxonomic groups display different elemental composition and stoichiometry, with greater differences observed as taxonomic distance and evolutionary time increase. Second, at equilibrium, coexisting species typically utilize distinct elemental composition and stoichiometry to minimize competitive pressures. Third, trade-offs between adaptation to stable environments for competitive advantage and success in more fluctuating conditions result in variations in homeostasis and plasticity among species, reflecting a continuum of strategies. To date, empirical studies have tested the theoretical framework of the BN based on foliar elemental composition and stoichiometry across a range of spatial scales (Sardans and Penuelas, 2014, Sardans et al.,

Author Manuscript

2016, Peñuelas et al., 2019, Sardans et al., 2021). However, these studies of the BN hypothesis regard leaf elemental composition and stoichiometry as a proxy for whole-plant element status, which ignores whether the regulation mechanism of elemental composition and stoichiometry in other organs (i.e., fine roots) is similar to that in leaves, and thus further attention needs to be paid to other plant organs such as roots involved in biological processes.

There are contrasts in the function of plant organs in the support of fundamental biological processes, such as growth, secondary metabolism, and reproduction, where organs tend to be characterized by unique chemical characteristics under long-term singular evolution (Zhang et al., 2017, Zhao et al., 2019). For example, ratios of C:N and N:P in leaf organs of terrestrial plants have been shown to be lower and higher, respectively, than in roots (Yuan et al., 2011, Zhang et al., 2017, Zhang et al., 2021). Compared to leaves, however, the quantization of BN from roots has been limited due to a lack of sufficient data (Wang et al., 2024a). Since roots and leaves of the same species could maintain a certain level of functional coordination (Reich, 2014, Li et al., 2021), we predicted that each species with specific fine root elemental composition and stoichiometry from different taxonomic groups could occupy a specific position in the multidimensional space. In fact, species BN is the result of a trade-off between stoichiometric homeostasis and plasticity under the control of evolutionary changes and environmental shifts (Peñuelas et al., 2019, Sardans et al., 2021). The homeostatic component of BN reflects each species tends to maintain its own identity relative to the BNs of other species, while the plastic component of BN represents the position of each species in the multi-

Author Manuscript

dimensional space can shift with time and changing environmental conditions (Peñuelas et al., 2019, Sardans et al., 2021). Significantly, recent studies have found that the stoichiometric sensitivity to environmental change is greater in roots, as indicated by higher levels of phenotypic plasticity than in leaf organs where stoichiometric homeostasis (element regulation) is greater (Zhang et al., 2017). However, it is unclear whether species BN based on fine root elemental composition and stoichiometry is mainly controlled by homeostatic components or plastic components.

The fundamental basis for the BN hypothesis is that unique species gene pools are the product of long-term specific evolutionary processes (Kay et al., 2005, Peñuelas et al., 2019). Specifically, genes can encode transport proteins, thereby driving roots to acquire specific nutrients from the soil solution according to the plant's nutrient status (Chen et al., 2008, Mitra, 2017). Significantly, abiotic stress (e.g., nutrient availability and soil moisture) can result in epigenetic variation in plants without altering the DNA sequence (Mohanty et al., 2023), which can be inherited by progeny for multiple generations (Henderson and Jacobsen, 2007), finally resulting in interspecific and intraspecific variation of plant elemental composition and stoichiometry. Each species is characterized by specific morphological structures and functionality, from gene expression to physiological and metabolic processes. For example, rates of fundamental biological processes, such as growth, secondary metabolism, reproduction, and storage of bio-elements, vary among species, depending on selection pressure, such as topographic resource heterogeneity (Körner, 2007, Wang et al., 2018), leading to species-specific

Author Manuscript

allocation of elements to tissues and organs, and an associated plant elemental composition and stoichiometry (Zhang et al., 2017, Zhang et al., 2021). Since a plant's identity at a specific time results from the combined influences of phylogeny and biogeographic history, both phylogeny and species are typically considered legacy effects when quantifying the homeostatic component of BN, where phylogeny represents the long-term consequences of evolutionary history, while species reflect taxonomic differences independent of evolutionary history, such as epigenetic inheritance and convergent or divergent evolution (Sardans et al., 2021, Gong et al., 2024, Wang et al., 2024a). Given that organisms tend to establish and persist in environments where they are already well adapted (i.e., niche conservatism) for improving individual fitness (Ackerly, 2003), we predicted that legacy effects primarily drove species-specific fine root element use in the subtropical forest.

Phenotypic plasticity for adaptation to environmental shifts and resource competition is reflected by changes in elemental composition and stoichiometry (Peñuelas et al., 2019, Sardans et al., 2021, Trethowan et al., 2021) likely derived from prevailing soil physicochemical properties, such as level of pH and content of N, P, and potassium (K) (Zhang et al., 2017, Wang et al., 2019, Wang et al., 2020, Zhang et al., 2021, Hartemink and Barrow, 2023); for example, soil pH and moisture affect soil nutrient availability (Cavagnaro, 2016, Hartemink and Barrow, 2023), root growth (Haling et al., 2011), and plant nutrient status (Zhao et al., 2017, Wang et al., 2019). Some plants have evolved to recruit beneficial mycorrhizal fungi to uptake key nutrients, such as N and P (Johnson, 2009, Phillips et al., 2013, Lu and Hedin, 2019, Genre et al., 2020),

Author Manuscript

where species associated with arbuscular mycorrhizal (AM) fungi scavenge inorganic nutrients decomposed by microorganisms, while species symbiotic with ectomycorrhizal (EM) fungi tend to easily mine organic-bound nutrients (Phillips et al., 2013, Lu and Hedin, 2019), likely leading to contrasting nutrient status of AM- and EM-associated plant species. Considering that plants can regulate nutrient absorption, transportation, and storage in their root systems according to surrounding environmental conditions to mitigate competition for belowground resources (Reichardt and Timm, 2020), we hypothesized that the root elemental composition, stoichiometry and BNs of subtropical tree species are influenced by legacy effects, the type of mycorrhizal associations, and local-scale resource availability.

Given the links between leaf and root chemical traits (Reich, 2014, Li et al., 2021) and local-scale species coexistence through BN differentiation (Zhao et al., 2017), we quantified fine root elemental composition and stoichiometry of 137 subtropical forest woody species in Southern China to test the hypotheses that: (1) each species with specific fine root elemental composition and stoichiometry from different taxonomic group occupy a specific position in the multidimensional space; (2) legacy effects (phylogenetic and species) primarily drive species-specific element use; and, (3) fine root elemental composition, stoichiometry and BNs of subtropical tree species are a product of legacy effects, type of mycorrhizal association, and local-scale variability in resources.

2. Materials and methods

Author Manuscript

2.1 Study area

The study was conducted across a 50-ha plot located within a subtropical evergreen broadleaf monsoon forest in the Heishiding Provincial Natural Reserve, Guangdong Province, southern China (23°27′N, 111°52′E), on ferrasols developed from granite and where elevation ranges from 430 to 700 m asl, with slopes ranging from 10 to 70°. The prevailing climate is a typical subtropical monsoon, with mean annual temperature of 19.6 ℃ and mean annual precipitation of 1740 mm that accumulates predominantly during April to September (Jiang et al., 2020).

The 500 × 1000 m plot was established in 2011 and all living trees with a diameter at breast height (DBH) ≥1 cm were identified to species and measured (DBH) in 2016. In total, 213,629 individuals, belonging to 256 species from 138 genera and 61 families were recorded, with dominant species including *Cryptocarya concinna* (Lauraceae), *Litsea lancilimba* (Lauraceae), *Neolitsea phanerophlebia* (Lauraceae), *Altingia chinensis* (Altingiaceae), *Castanopsis carlesii* (Fagaceae), and *Artocarpus styracifolius* (Moraceae).

2.2 Root sampling and analysis

In 2020, we selected at least three replicates of 137 commonly occurring species (Figure 1), comprising 118 and 19 AM and EM species, respectively, amounting to 708 individuals (Figure S1). We used a shovel to loosen soil within a 2-m radius around each selected tree stem, from which we traced and removed terminal root branch orders (1–5) that were subsequently washed gently using water to remove soil and other adherent material. We selected fine roots (diameter ≤ 2 mm) from the collected samples for subsequent analysis. Then, fine roots were removed and

oven-dried at 65 ℃ for 48 h to a constant weight prior to analysis. We did not need permission for our fieldwork.

We determined total C and N contents using an elemental analyzer (Vario ELcube, Elemental analysis, Germany), and total P content was determined using the sodium hydrogen carbonate solution-Mo-Sb anti-spectrophotometric method (Wang et al., 2024b). Total potassium (K), calcium (Ca), magnesium (Mg), iron (Fe), aluminum (Al), and manganese (Mn) content was determined using the HNO_3- $HClO_4$ (5:1) digestion method and inductively coupled plasma atomic emission spectrometry (iCAP-7200) (Hu et al., 2023, Wang et al., 2024b).

2.3 Soil sampling and physicochemical analysis

In 2016, we collected 625 soil cores (10 cm depth) across the study plot and from which we measured the content of total N, P, and K, pH, electrical conductivity, and water content. Using elevation and slope topographical measurements of the study plot collected in 2011, we created Kriging maps to interpolate soil physicochemical properties and topography (He et al., 2018) for locations of the 708 sampled plants.

2.4 Data analysis

All analyses were conducted with R 4.3.2 (R Core Team, 2023). We used the R package 'Taxonstand' (Cayuela et al., 2012) to match species with accepted names in 'The Plant List' (http://www.theplantlist.org/) and then phylogeny was constructed using the V.PhyloMaker2' package in R (Jin and Qian, 2022). As in a previous study (Sardans et al., 2021), we tested phylogenetic signals of fine root elemental composition and stoichiometry based on Pagel's λ (P

Author Manuscript

Author Manuscript

≤ 0.05), calculated using the 'phytools' package in R (Revell, 2012). Pagel's λ indicates phylogenetic dependence of traits, based on the assumption that trait evolution follows the Brownian motion (BM) model (Pagel, 1997), and, compared with other methods, it does not vary with increasing number of phylogenetic species and is more suitable for large phylogenies of >50 species (Felsenstein, 1985, Sardans et al., 2021). We assumed phylogenetic independence of traits when $\lambda = 0$, weak phylogenetic dependence when $0 < \lambda < 1$, and trait dependence when $\lambda = 1$.

Similar to previous studies (Sardans et al., 2021, Sardans et al., 2023), we compared the range of scores in the axes of principal component analysis (PCA) or canonical discriminant analysis (CDA) as a measure of the size of the 'niche space' of each species. We quantified BN space size for 137 study species based on a PCA of log-transformed fine root elemental composition and C:N:P stoichiometry as independent variables (Sardans et al., 2016), using the 'FactoMineR' package in R (Lê et al., 2008). We tested for variation in BN size between 21 of the most abundant species (Table S1), which are the top 15% tree species with the highest abundance among 137 tree species, using CDA of log-transformed root element content, based on squared Mahalanobis distance of CDA scores between species pairs; CDA derives the optimal inter-group separation by maximizing inter-group variance, while minimizing intra-group variance (Cacoullos, 2014).

As in a previous study (Sardans et al., 2023), we tested the importance of species in root elemental composition and C:N:P stoichiometry performing permutational multivariate analyses

of variance (PERMANOVA) using the 'vegan' package in R, based on Euclidean distance (Anderson et al., 2008), with soil physicochemical property, type of mycorrhizal association, topography, and species as independent factors.

The relative contributions of evolutionary history, soil physicochemical property, mycorrhizal association type, and topography to root elemental composition, stoichiometry, and BN size, as estimated by the PCA, were estimated by Bayesian phylogenetic general linear mixed models (GLMM) using the 'MCMCglmm' package in R (Hadfield, 2010), with soil property, mycorrhizal association type, and topography as fixed factors, and phylogeny and species as random factors. The analysis showed that phylogeny accounted for variability in shared ancestry, while species accounted for species-specific traits independent of shared ancestry (Sardans et al., 2021); therefore, phylogeny and species together accounted for variance explained by heritability. Additionally, we also used the generalized linear model (GLM) without considering legacy effects to explore the effects of soil property, mycorrhizal association type, and topography on root elemental composition and stoichiometry.

We tested for spatial autocorrelation of fine root elemental composition, stoichiometry in the 708 individual study trees, and environmental (soil physicochemical and topography) variables at the tree locations using Moran's I correlograms in the sp. correlogram function of the 'spdep' package in R (Bivand and Piras, 2015). While there was no evidence of spatial autocorrelation of root elements (Figure S2), there was strong spatial autocorrelation of the spatially interpolated soil and topography variables (Figure S3). Therefore, we implemented

Author Manuscript

Author Manuscript

pairwise Pearson's correlation for the environmental variables to avoid collinearity, excluding pairwise variables with correlation coefficients >0.75 from the analysis (Jiang et al., 2020); as a result, eight environmental factors (Figure S4) were then included in two multi-response phylogenetic mixed models (MR-PMM) to explore the effects of soil and topography, and mycorrhizal association type on fine root elemental composition and C:N:P stoichiometry using the 'brms' package in R (Bürkner, 2018, Westoby et al., 2023). In the first model, we used root C:N, C:P, and N:P ratios as response variables, with eight environmental factors and mycorrhizal type as explanatory variables. The second model included log-transformed fine root contents of C, N, P, K, Ca, Mg, Fe, Al, and Mn as response variables, with the same predictors described in the first model. Following the approach of Fernandez-Martinez et al. (2021), both models were fitted using the Gaussian distribution for the response variables, with species treated as a random factor and phylogenetic effects controlled using the consensus phylogenetic tree. All explanatory variables were log-transformed and subsequently standardized (mean = 0, SD = 1). The probability of direction (PD) and Bayes factors (BFs) were estimated for all predictors as a measure of the relevance of the explanatory variables, utilizing the 'bayestestR' package in R (Makowski et al., 2019). Conditional effects plots were employed to visualize the relationships between the response variables and explanatory variables, employing the conditional_effects function from the 'brms' package in R.

3. Results

Author Manuscript

3.1 Phylogenetic signal and biogeochemical niche size of fine root element traits

Phylogenetic signals in fine root elemental composition and stoichiometry were observed for all measured elements across the 137 tree species and, with the exception of Mn, for AM species; for EM species, there were phylogenetic signals for fine root content of P and Ca and ratio of C:P (Table 1).

Axes 1 and 2 (PCs 1 and 2) of the PCA accounted for 52.1% of the variation in fine root elemental composition and stoichiometry in the 137 tree species (Figure 2), while PCs 1–3 accounted for 66.7% of the variation; therefore, we selected scores along PCs 1–3 to represent the BNs of the study species. There were phylogenetic signals in BN for each PC (Table 1; Figure S5) and BNs were most similar in closely-related species and by mycorrhizal type (Figure 1; Figure S5).

Elemental composition of the 21 most abundant co-existing species tended to be distinct (Table S2), and as indicated by separation of CDA scores along axes 1 and 2 (Can1 and 2) that jointly explained 61.3% of variation in root elemental composition, principally driven by root N and Mg contents (Figure 3, Table S3).

3.2 Species, phylogeny, mycorrhizal association type, and environmental conditions as drivers of root elemental composition and stoichiometry

The combined effects of soil physicochemical properties, mycorrhizal association type, and topography accounted for little variation in fine root elemental composition and stoichiometry ($R^2 = 0.068$), whereas species accounted for greater proportions when included in the mixed

PERMANOVA model (R^2 = 0.592) or as a single independent factor (R^2 = 0.638) (Table 2).

Bayesian phylogenetic GLMM estimates showed legacy effects of phylogeny and species explained 23.4–70.7% of variation in fine root elemental composition and stoichiometry, and BNs, as indicated by PCA scores, while combined effects of soil physicochemical property, mycorrhizal association type, and topography explained 3.9–17.7% of variation (Figure 4; Table S4). Estimates of the phylogenetic GLMM accounted for similar levels of variation in legacy effects root elemental composition and stoichiometry (average: 54%) to PERMANOVA estimates (59.2%) (Table 2).

3.3 Relationships between root elemental composition and stoichiometry and abiotic factors.

The variance (R^2) explained by the models was high for N (0.66), K (0.61), Ca (0.60), Mg (0.69), and C:N (0.61), while it was lower for C (0.37) and Fe (0.27) (Table S5). We identified several relationships between root elemental composition, stoichiometry and environmental factors (Table S6 & S7; Figure 5). Specifically, fine root P was negatively correlated with elevation, whereas the N:P ratio was positively correlated with elevation. Additionally, fine root Ca and Mn contents were negatively related to soil total N content (Figure 5). We observed that root Al and Fe contents decreased with increasing soil electrical conductivity (Figure 5). Furthermore, fine root N, P, K, and Mn contents increased, while fine root Fe and Al contents decreased with rising soil total K contents. Soil pH exhibited a positive correlation with fine root Ca, Fe, and Al contents, but was negatively related to fine root C and K contents. Additionally, we found no

Author Manuscript

difference in fine root elemental composition and stoichiometry with mycorrhizal association type (Table S6 & S7).

4. Discussion

We found contrasting BNs between co-occurring species, based on analysis of fine root elemental composition and stoichiometry, supporting our first hypothesis, where the positive and negative values for *Lauraceae* and *Altingiaceae* spp., respectively, on Can1 of the CDA plot (Figure 3) indicate contrasting BN position and size in multi-dimensional space. These findings are supported by previous, smaller-scale studies of trees and herbs in subtropical forest and wetland ecosystems that report the separation of root traits in coexisting species (Zhao et al., 2017, Hu et al., 2018). A key determinant of the contrasts in BNs of co-existing species was found to be root N content, inferring that phylogenetic variation in chemical traits may drive niche differentiation through differences in plant metabolic and physiological function and reduced nutrient competition, given the essential role of N in plant growth and reproduction (Güsewell, 2004). For example, the root N content of *Lauraceae* spp. (*C. concinna*, *L. lancilimba*, *N. phanerophlebia, Lindera chunii*) was greater than in phylogenetically distant species of other families (*A. chinensis*, Altingiaceae; *Myrsine seguinii*, Primulaceae; *Schima superba*, Theaceae) that occupy different BNs in the hypervolume (Figure 3).

Our study showed greater differences in fine root elemental composition and stoichiometry in phylogenetically distant species, such as *Rhodomyrtus tomentosa* and

Author Manuscript

Author Manuscript

Dendropanax dentiger (Figure S5), consistent with studies based on leaf element traits that show a positive correlation between phylogenetic distance and species-specific BNs (Sardans et al., 2016, Sardans et al., 2021). Although our results indicated that the elemental composition and stoichiometry of plant organs depended to a high degree on phylogeny, there were contributions from other legacy effect factors (e.g., epigenetic inheritance and convergent or divergent evolution). For example, distantly related species that have recently been subject to analogous environmental conditions, such as those caused by orogenic processes, may be subject to convergent evolution (Sardans et al., 2021), whereas evolutionary paths in other species may diverge from those of close relatives under environments with intense inter-specific competition, resulting in accelerated divergent evolution. Thus, we suggest phylogeny did not account for 100% of legacy effect variation in BNs among species, due to ancestry and evolution factors, and we recommend that the inclusion of species as a factor will lead to more robust analyses of BN legacy effects.

Our results indicated that legacy effects (phylogeny + species) directly explained 23.4–70.7% of the variation in fine root elemental composition, stoichiometry, and BN, as indicated by PCA scores, supporting our second hypothesis. The results may be attributed to the involvement of genes in encoding proteins related to element absorption, transportation, and utilization, which enables plants to acquire specific nutrients from the soil solution and transport them across the plasma membranes of root hair cells (Mitra, 2017). Furthermore, plants tend to retain the traits and environmental tolerances of their ancestors to persist in communities where they are already

Author Manuscript

well-adapted, resulting in stabilizing selection and trait conservatism (Ackerly, 2003, Cavender-Bares et al., 2016). Consistent with our findings, a recent study demonstrated that legacy effects accounted for 6.0–43.0% of the variation in root element content across Chinese grasslands (Wang et al., 2024a), while another study of semi-pristine South American lowland wet tropical forests reported that legacy effects accounted for 28.6–75.4% of the variation in leaf element content (Sardans et al., 2023). Thus, our results agree with one of the basic postulates of BN, that coexisting species maintain a degree of divergent evolution to avoid direct competition for resources.

Combined effects of soil property, mycorrhizal association type, and topography explained 3.9–11% of the variation in root elemental composition and stoichiometry, as supported by the PERMANOVA analysis (6.8%), where soil property explained 4.9% of the variation in root element content. One possible explanation was that root nutrient acquisition (e.g., root proliferation, transporter function and the diffusion of nutrient ions to the root surface) were determined by soil properties (e.g., nutrient availability and pH) (Chapman et al., 2012), which could directly influence the accumulation of nutrients in the root system. Furthermore, compared to root elemental composition and stoichiometry, soil property explained a lower proportion of previously reported variation in the element content of tree leaves (0.1–2%) (Sardans et al., 2021). These contrasting impacts of soil properties on leaf and root element content may be due to regulation of plant nutrient use strategies, and greater sensitivity of root than leaf chemical traits to environmental change (Zhang et al., 2017). Our results showed fine

Author Manuscript

root macronutrients (e.g., N, P) were less correlated with soil pH and soil electrical conductivity than micronutrients (e.g., Ca, Fe and Al), suggesting a stronger homeostatic control for macro- than for micronutrients (Fernandez-Martinez et al., 2021).

Plant-mycorrhizal symbiotic associations benefit plants, through greater root uptake of nutrients, such as N, P, K, Al, Zn, Cu, and Mn (Clark and Zeto, 2000, Kariman et al., 2012), that then drive plant population and community dynamics, through effects of individual plant competitive ability (Tedersoo and Bahram, 2019, Tedersoo et al., 2020). However, our results unexpectedly showed that mycorrhizal association type explained just 1.2% of the variation in root elemental composition, stoichiometry and BNs (PCA scores), possibly due to the small number of EM species included in our study (19 out of 137). Indeed, when using the same number (19) of AM and EM tree species or further analysis, we found that mycorrhizal association type explained a larger proportion of the variation in root elemental composition, stoichiometry and BNs (3.8%) (Table S8).

We found that topography explained only 0.6% of the variation in fine root elemental composition and stoichiometry, supporting findings from a study that showed topography and geography accounted for 3.7% of variation in leaf elemental composition of tropical trees (Sardans et al., 2023), indicating that topography, being closely associated with soil temperature and resource availability (Körner, 2007), may be regarded as a driver of subtropical species diversity (species coexistence) (Medvigy et al., 2019, van Breugel et al., 2019). Our results showed a decrease in root P content and an increase in root N:P stoichiometry with increasing

Author Manuscript

elevation, supporting a previous study that reports similar elevational decreases in fine root P content and N:P ratios in *Quercus aquifolioides* (Wang et al., 2018), possibly due to low soil P availability at high elevations (Tsui et al., 2004, Wang et al., 2018) caused by reduced microbial activity and associated declines in P mineralization rates under low temperatures (temperature-biogeochemistry hypothesis) (Reich and Oleksyn, 2004).

Generalized linear model (GLM) analysis, which considered soil property, mycorrhizal association type, and topography as fixed factors and excluded phylogeny and species as random factors, explained 7.8–25.5% of the variation in root elemental composition and stoichiometry (Table S9), while MCMCglmm with phylogeny and species as random factors shows that soil physicochemical properties, mycorrhizal types, and topography collectively explain 3.9–11.0% of the composition of root elements. The higher (1.5–16.2%) explanatory power of fixed factor in GLM suggests that variance explained by differences in environment are partially explained by legacy effects (phylogeny + species). These results are consistent with those reported by Sardans et al. (2021) for a Bayesian analysis, who found a decrease in variance explained by climatic variables when legacy effects were added as a random factor. Thus, variation in plant species-specific responses to environmental conditions reflects genetic adaptations in function, physiology, metabolism, and morphology and specific use of the contrasting bio-elements.

We found that legacy effects (phylogeny and species) accounted for 65.2, 68.8, and 23.4% of variation in PC1, PC2, and PC3 scores, respectively, whereas combined effects of soil property, mycorrhizal association type, and topography explained only 6.1, 4.1, and 17.7%,

Author Manuscript

respectively, supporting our third hypothesis (i.e., the fundamental basis of the BN hypothesis). Similar to our results, a recent study has shown that legacy effects and environmental variables accounted for 30.0–44.0% and 6.0–9.0% of the variance root elemental composition and stoichiometry across Chinese grasslands, respectively (Wang et al., 2024a). These results suggest that plants tend to be characterized by an optimal elemental composition and stoichiometry that coincides with their optimal niche function and occupation, representing the homeostatic component of BN (Peñuelas et al., 2019); concurrently, plants require a degree of flexibility in elemental composition and stoichiometry, to meet the demands of phenotypic plasticity during adaptation to fluctuating environmental conditions (Han et al., 2011, Sardans and Penuelas, 2014, Sardans et al., 2016, Sardans et al., 2017, Sardans et al., 2021). However, species display varying capacities to tolerate competition and stressors that lead to the occupation of distinct BN positions within the n-dimensional space around their associated homeostatic component. Positioning of multiple individuals of a species occurs within a given volume, due to inherent intra-specific variation in BN, which then facilitates community-level responses to fluctuating environmental conditions (Liu et al., 2024). For example, species growing in environments with low levels of competitive pressure and high abundance of resources exhibit higher levels of BN plasticity than those growing in environments with high levels of competitive pressure and poor resource availability and are characterized by greater homeostatic life histories and lower capacities to respond to environmental time-space fluctuations in resource-rich environments (Yu et al., 2011, Peñuelas et al., 2019). The position of species in the hyper-dimensional volume may

shift with time and changing environmental conditions, depending on the degree of species-specific stoichiometric plasticity; however, there should be a maintenance (homeostasis) of relative species BN (Peñuelas et al., 2019, Liu et al., 2024).

5. Conclusion

Our study provides empirical evidence for the quantification of local-scale species BN using fine root elemental composition and stoichiometry, in support of the BN hypothesis. We found phylogenetic signals in fine root elemental composition, stoichiometry and BNs of the 137 coexisting subtropical tree species, largely driven by legacy (phylogeny + species) effects, with smaller contributions from soil property, mycorrhizal association type, and topography factors. While quantification of species BN based on root chemical traits improves understanding of species coexistence, it remains unclear whether root chemical traits better represent and account for BN than other plant organs with contrasting functions and associated elemental composition and stoichiometry. Nevertheless, this study shows similar levels of support for the BN hypothesis using plant root elemental composition and stoichiometry as previous studies based on leaf elements. We suggest further studies of whole-plant chemical traits to quantify species BN to improve understanding of community assembly dynamics.

Author Manuscript

Author Manuscript

Acknowledgments: This research was supported by Guangdong Basic and Applied Basic Research Foundation (2023A1515010643), Shenzhen Science and Technology Program (JCYJ20230807111116034), National Natural Science Foundation of China (32101272) and Fundamental Research Funds for the Central Universities, Sun Yat-sen University (23lgbj009).

Author contributions: Zilong Ma designed the study. Mingyan Hu and Yang Chen collected samples and conducted experiments. Mingyan Hu performed the data analysis and wrote the first draft of the manuscript with assistance from Zilong Ma. Jordi Sardans, Josep Peñuelas, and Han Y.H.Chen contributed to the manuscript revision. Chengjin Chu provided the soil and topography data.

Data Availability Statement

All data needed to evaluate the conclusions in the paper are present in the paper and/or the Supplementary Materials. All data analyzed in the study have been deposited in Figshare: https://doi.org/10.6084/m9.figshare.26392993.v2 (Hu et al., 2025).

Conflict of interest: The authors declare no competing interests.

Author Manuscript

References

Ackerly, David D. (2003) Community Assembly, Niche Conservatism, and Adaptive Evolution in Changing Environments. *International Journal of Plant Sciences,* **164,** S165-S184. https://doi.org/10.1086/368401

Anderson, M. J., Gorley, R. N., Clarke, K. S., Anderson, M. S., Gorley, R. N., Clarke, K. R. & Andersom, M. (2008) PERMANOVA+ for PRIMER. Guide to software and statistical methods.

Bivand, R. & Piras, G. (2015) Comparing Implementations of Estimation Methods for Spatial Econometrics. *Journal of Statistical Software,* **63,** 1-36. https://doi.org/10.18637/jss.v063.i18

Bürkner, P.-C. (2018) Advanced bayesian multilevel modeling with the R Package brms. *The R Journal,* **10,** 395-411.

Cacoullos, T. (2014) *Discriminant analysis and applications.* Academic Press. https://doi.org/https://doi.org/10.1016/C2013-0-10453-9

Cavagnaro, T. R. (2016) Soil moisture legacy effects: Impacts on soil nutrients, plants and mycorrhizal responsiveness. *Soil Biology and Biochemistry,* **95,** 173-179. https://doi.org/https://doi.org/10.1016/j.soilbio.2015.12.016

Cavender-Bares, J., Ackerly, D. D., Hobbie, S. E. & Townsend, P. A. (2016) Evolutionary Legacy Effects on Ecosystems: Biogeographic Origins, Plant Traits, and Implications for Management in the Era of Global Change. *Annual Review of Ecology, Evolution, and Systematics,* **47,** 433-462. https://doi.org/10.1146/annurev-ecolsys-121415-032229

Cayuela, L., Granzow-de la Cerda, Í., Albuquerque, F. S. & Golicher, D. J. (2012) taxonstand: An r package for species names standardisation in vegetation databases. *Methods in Ecology and Evolution,* **3,** 1078-1083. https://doi.org/https://doi.org/10.1111/j.2041-

Author Manuscript

210X.2012.00232.x

Chapman, N., Miller, A. J., Lindsey, K. & Whalley, W. R. (2012) Roots, water, and nutrient acquisition: let's get physical. *Trends in Plant Science,* **17,** 701-710. https://doi.org/10.1016/j.tplants.2012.08.001

Chen, Y. F., Wang, Y. & Wu, W. H. (2008) Membrane Transporters for Nitrogen, Phosphate and Potassium Uptake in Plants. *Journal of Integrative Plant Biology,* **50,** 835-848. https://doi.org/10.1111/j.1744-7909.2008.00707.x

Clark, R. B. & Zeto, S. K. (2000) Mineral acquisition by arbuscular mycorrhizal plants. *Journal of Plant Nutrition,* **23,** 867-902. https://doi.org/10.1080/01904160009382068

Felsenstein, J. (1985) Phylogenies and the Comparative Method. *The American Naturalist,* **125,** 1-15. https://doi.org/10.1086/284325

Fernandez-Martinez, M., Preece, C., Corbera, J., Cano, O., Garcia-Porta, J., Sardans, J., Janssens, I. A., Sabater, F. & Penuelas, J. (2021) Bryophyte C:N:P stoichiometry, biogeochemical niches and elementome plasticity driven by environment and coexistence. *Ecology Letters,* **24,** 1375-1386. https://doi.org/10.1111/ele.13752

Furey, G. N. & Tilman, D. (2023) Plant chemical traits define functional and phylogenetic axes of plant biodiversity. *Ecology Letters,* **26,** 1394-1406. https://doi.org/10.1111/ele.14262

Gause, G. F. (1934) Experimental Analysis of Vito Volterra's Mathematical Theory of the Struggle for Existence. *Science,* **79,** 16-17. https://doi.org/10.1126/science.79.2036.16.b

Genre, A., Lanfranco, L., Perotto, S. & Bonfante, P. (2020) Unique and common traits in mycorrhizal symbioses. *Annals of Botany,* **18,** 649-660. https://doi.org/https://doi.org/10.1038/s41579-020-0402-3

Gong, H., Sardans, J., Huang, H., Yan, Z., Wang, Z. & Peñuelas, J. (2024) Global patterns and controlling factors of tree bark C : N : P stoichiometry in forest ecosystems consistent with biogeochemical niche hypothesis. *New Phytologist,* **244,** 1303-1314.

Author Manuscript

https://doi.org/10.1111/nph.20119

González, A. L., Dézerald, O., Marquet, P. A., Romero, G. Q. & Srivastava, D. S. (2017) The Multidimensional Stoichiometric Niche. *Frontiers in Ecology and Evolution,* **5**. https://doi.org/10.3389/fevo.2017.00110

Güsewell, S. (2004) N : P ratios in terrestrial plants: variation and functional significance. *New Phytologist,* **164,** 243-266. https://doi.org/10.1111/j.1469-8137.2004.01192.x

Hadfield, J. D. (2010) MCMC Methods for Multi-Response Generalized Linear Mixed Models: The MCMCglmm R Package. *Journal of Statistical Software,* **33,** 1-22. https://doi.org/10.18637/jss.v033.i02

Haling, R. E., Simpson, R. J., Culvenor, R. A., Lambers, H. & Richardson, A. E. (2011) Effect of soil acidity, soil strength and macropores on root growth and morphology of perennial grass species differing in acid-soil resistance. *Plant, Cell & Environment,* **34,** 444-456. https://doi.org/https://doi.org/10.1111/j.1365-3040.2010.02254.x

Han, W. X., Fang, J. Y., Reich, P. B., Ian Woodward, F. & Wang, Z. H. (2011) Biogeography and variability of eleven mineral elements in plant leaves across gradients of climate, soil and plant functional type in China. *Ecology Letters,* **14,** 788-96. https://doi.org/10.1111/j.1461-0248.2011.01641.x

Hartemink, A. E. & Barrow, N. J. (2023) Soil pH - nutrient relationships: the diagram. *Plant and Soil,* **486,** 209-215. https://doi.org/10.1007/s11104-022-05861-z

He, D., Chen, Y., Zhao, K., Cornelissen, J. H. C. & Chu, C. (2018) Intra- and interspecific trait variations reveal functional relationships between specific leaf area and soil niche within a subtropical forest. *Annals of Botany,* **121,** 1173-1182. https://doi.org/10.1093/aob/mcx222

Henderson, I. R. & Jacobsen, S. E. (2007) Epigenetic inheritance in plants. *Nature,* **447,** 418-424. https://doi.org/10.1038/nature05917

Author Manuscript

Hu, M., Chen, Y., Sardans, J., Peñuelas, J., Chen, H., Chu, C. & Ma, Z. (2025) Legacy effects control root elemental composition and stoichiometry in subtropical forests: empirical support for the biogeochemical niche hypothesis. figshare. https://doi.org/10.6084/m9.figshare.26392993.v2

Hu, M., Ma, Z. & Chen, H. Y. H. (2023) Effects of forest conversion and recovery age on fine root multi-element stoichiometry in subtropical forests. *Plant and Soil,* **492,** 1-12. https://doi.org/10.1007/s11104-023-06208-y

Hu, M., Peñuelas, J., Sardans, J., Sun, Z., Wilson, B. J., Huang, J., Zhu, Q. & Tong, C. (2018) Stoichiometry patterns of plant organ N and P in coastal herbaceous wetlands along the East China Sea: implications for biogeochemical niche. *Plant and Soil,* **431,** 273-288. https://doi.org/10.1007/s11104-018-3759-6

Hutchinson, G. (1957) Concluding remarks. cold spring harb. Symp. Quant. Biol 22, 415-427.

Jiang, Y., Zhang, B., Wang, W., Li, B., Wu, Z. & Chu, C. (2020) Topography and plant community structure contribute to spatial heterogeneity of soil respiration in a subtropical forest. *Science of The Total Environment,* **733**. https://doi.org/10.1016/j.scitotenv.2020.139287

Jin, Y. & Qian, H. (2022) V.PhyloMaker2: An updated and enlarged R package that can generate very large phylogenies for vascular plants. *Plant Diversity,* **44,** 335-339. https://doi.org/https://doi.org/10.1016/j.pld.2022.05.005

Johnson, N. C. (2009) Resource stoichiometry elucidates the structure and function of arbuscular mycorrhizas across scales. *New Phytologist,* **185,** 631-47. https://doi.org/https://10.1111/j.1469-8137.2009.03110.x

Kariman, K., Barker, S. J., Finnegan, P. M. & Tibbett, M. (2012) Dual mycorrhizal associations of jarrah (Eucalyptus marginata) in a nurse-pot system. *Australian Journal of Botany,* **60**. https://doi.org/10.1071/bt12152

Author Manuscript

Kay, A. D., Ashton, I. W., Gorokhova, E., Kerkhoff, A. J., Liess, A. & Litchman, E. (2005) Toward a stoichiometric framework for evolutionary biology. *Oikos,* **109,** 6-17. https://doi.org/https://doi.org/10.1111/j.0030-1299.2005.14048.x

Körner, C. (2007) The use of 'altitude' in ecological research. *Trends in Ecology & Evolution,* **22,** 569-574. https://doi.org/https://doi.org/10.1016/j.tree.2007.09.006

Lê, S., Josse, J. & Husson, F. (2008) FactoMineR: An R Package for Multivariate Analysis. *Journal of Statistical Software,* **25,** 1-18. https://doi.org/10.18637/jss.v025.i01

Li, J., Chen, X., Niklas, K. J., 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. https://doi.org/10.1111/1365-2745.13800

Liu, R., Yang, X., Gao, R., Jiao, B., Huang, Z. & Cornelissen, J. H. C. (2024) Testing the biogeochemical niche hypothesis using leaves, stems and roots of 62 Artemisia species across China. *Journal of Ecology*. https://doi.org/10.1111/1365-2745.14469

Lu, M. & Hedin, L. O. (2019) Global plant-symbiont organization and emergence of biogeochemical cycles resolved by evolution-based trait modelling. *Nature Ecology & Evolution,* **3,** 239-250. https://doi.org/https://doi.org/10.1038/s41559-018-0759-0

Makowski, D., Ben-Shachar, M. S. & Lüdecke, D. (2019) bayestestR: Describing effects and their uncertainty, existence and significance within the Bayesian framework. *Journal of Open Source Software,* **4,** 1541. https://doi.org/doi.org/10.21105/joss.01541

Medvigy, D., Wang, G., Zhu, Q., Riley, W. J., Trierweiler, A. M., Waring, Bonnie G., Xu, X. & Powers, J. S. (2019) Observed variation in soil properties can drive large variation in modelled forest functioning and composition during tropical forest secondary succession. *New Phytologist,* **223,** 1820-1833. https://doi.org/https://doi.org/10.1111/nph.15848

Mitra, G. (2017) Essential Plant Nutrients and Recent Concepts about their Uptake. *Essential*

Author Manuscript

Plant Nutrients: Uptake, Use Efficiency, and Management (eds M. Naeem, A. A. Ansari & S. S. Gill), pp. 3-36. Springer International Publishing, Cham. https://doi.org/10.1007/978-3-319-58841-4_1

Mohanty, S., Mahapatra, S. K., Dasgupta, M., Panigrahy, M., Swain, D. & Rout, G. R. (2023) Regulation of gene expression in plant growth and metabolism: A review. *Trends in Horticulture,* **6**. https://doi.org/10.24294/th.v6i2.3023

Pagel, M. (1997) Inferring evolutionary processes from phylogenies. *Zoologica Scripta,* **26,** 331-348. https://doi.org/https://doi.org/10.1111/j.1463-6409.1997.tb00423.x

Peñuelas, J., Fernández-Martínez, M., Ciais, P., Jou, D., Piao, S., Obersteiner, M., Vicca, S., Janssens, I. A. & Sardans, J. (2019) The bioelements, the elementome, and the biogeochemical niche. *Ecology,* **100,** e02652. https://doi.org/https://doi.org/10.1002/ecy.2652

Peñuelas, J., Sardans, J., Ogaya, R. & Estiarte, M. (2008) Nutrient stoichiometric relations and biogeochemical niche in coexisting plant species: effect of simulated climate change. *Polish Journal of Ecology,* **56,** 613-622.

Phillips, R. P., Brzostek, E. & Midgley, M. G. (2013) The mycorrhizal-associated nutrient economy: a new framework for predicting carbon-nutrient couplings in temperate forests. *New Phytologist,* **199,** 41-51. https://doi.org/https://doi.org/10.1111/nph.12221

R Core Team (2023) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Reich, P. B. (2014) The world-wide 'fast-slow' plant economics spectrum: a traits manifesto. *Journal of Ecology,* **102,** 275-301. https://doi.org/https://doi.org/10.1111/1365-2745.12211

Reich, P. B. & 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*

Author Manuscript

America, **101,** 11001. https://doi.org/10.1073/pnas.0403588101

Reichardt, K. & Timm, L. C. (2020) How Plants Absorb Nutrients from the Soil. *Soil, Plant and Atmosphere: Concepts, Processes and Applications* (eds K. Reichardt & L. C. Timm), pp. 313-330. Springer International Publishing, Cham. https://doi.org/10.1007/978-3-030-19322-5_16

Revell, L. J. (2012) phytools: an R package for phylogenetic comparative biology (and other things). *Methods in Ecology and Evolution,* **3,** 217-223. https://doi.org/https://doi.org/10.1111/j.2041-210X.2011.00169.x

Sardans, J., Alonso, R., Carnicer, J., Fernández-Martínez, M., Vivanco, M. G. & Peñuelas, J. (2016) Factors influencing the foliar elemental composition and stoichiometry in forest trees in Spain. *Perspectives in Plant Ecology, Evolution and Systematics,* **18,** 52-69. https://doi.org/10.1016/j.ppees.2016.01.001

Sardans, J., Grau, O., Chen, H. Y. H., Janssens, I. A., Ciais, P., Piao, S. & Peñuelas, J. (2017) Changes in nutrient concentrations of leaves and roots in response to global change factors. *Global Change Biology,* **23,** 3849-3856. https://doi.org/https://doi.org/10.1111/gcb.13721

Sardans, J., Llusià, J., Ogaya, R., Vallicrosa, H., Filella, I., Gargallo‐Garriga, A., Peguero, G., Van Langenhove, L., Verryckt, L. T., Stahl, C., Courtois, E. A., Bréchet, L. M., Tariq, A., Zeng, F., Alrefaei, A. F., Wang, W., Janssens, I. A. & Peñuelas, J. (2023) Foliar elementome and functional traits relationships identify tree species niche in French Guiana rainforests. *Ecology,* **104**. https://doi.org/10.1002/ecy.4118

Sardans, J. & Penuelas, J. (2014) Climate and taxonomy underlie different elemental concentrations and stoichiometries of forest species: the optimum "biogeochemical niche". *Plant Ecology,* **215,** 441-455. https://doi.org/10.1007/s11258-014-0314-2

Sardans, J., Vallicrosa, H., Zuccarini, P., Farré-Armengol, G., Fernández-Martínez, M., Peguero,

Author Manuscript

G., Gargallo-Garriga, A., Ciais, P., Janssens, I. A., Obersteiner, M., Richter, A. & Peñuelas, J. (2021) Empirical support for the biogeochemical niche hypothesis in forest trees. *Nature Ecology & Evolution,* **5,** 184-194. https://doi.org/10.1038/s41559-020-01348-1

Silvertown, J. (2004) Plant coexistence and the niche. *Trends in Ecology & Evolution,* **19,** 605-611. https://doi.org/10.1016/j.tree.2004.09.003

Tedersoo, L. & Bahram, M. (2019) Mycorrhizal types differ in ecophysiology and alter plant nutrition and soil processes. *Biological Reviews,* **94,** 1857-1880. https://doi.org/10.1111/brv.12538

Tedersoo, L., Bahram, M. & Zobel, M. (2020) How mycorrhizal associations drive plant population and community biology. *Science,* **367,** eaba1223. https://doi.org/10.1126/science.aba1223

Trethowan, L. A., Blonder, B., Kintamani, E., Girmansyah, D., Utteridge, T. M. A. & Brearley, F. Q. (2021) Metal‐rich soils increase tropical tree stoichiometric distinctiveness. *Plant and Soil,* **461,** 579-589. https://doi.org/10.1007/s11104-021-04839-7

Tsui, C.-C., Chen, Z.-S. & Hsieh, C.-F. (2004) Relationships between soil properties and slope position in a lowland rain forest of southern Taiwan. *Geoderma,* **123,** 131-142. https://doi.org/https://doi.org/10.1016/j.geoderma.2004.01.031

van Breugel, M., Craven, D., Lai, H. R., Baillon, M., Turner, B. L. & Hall, J. S. (2019) Soil nutrients and dispersal limitation shape compositional variation in secondary tropical forests across multiple scales. *Journal of Ecology,* **107,** 566-581. https://doi.org/https://doi.org/10.1111/1365-2745.13126

Wang, A., Wang, X., Tognetti, R., Lei, J.-P., Pan, H.-L., Liu, X.-L., Jiang, Y., Wang, X.-Y., He, P., Yu, F.-H. & Li, M.-H. (2018) Elevation alters carbon and nutrient concentrations and stoichiometry in Quercus aquifolioides in southwestern China. *Science of The Total*

Author Manuscript

Environment, **622-623,** 1463-1475. https://doi.org/10.1016/j.scitotenv.2017.12.070

Wang, C., Geng, Y., Sardans, J., Peñuelas, J. & He, J. S. (2024a) Coordinated variation in elemental composition and morphology in leaves, but independence in roots across Chinese grasslands. *Journal of Ecology*. https://doi.org/10.1111/1365-2745.14464

Wang, H., Liu, H., Li, J., Chen, S., uz Zaman, Q., Sultan, K., Rehman, M., Saud, S., El-Kahtany, K., Fahad, S., Deng, G. & Chen, A. (2024b) Combined passivators regulate physiological, antioxidant potential and metals accumulation in potato grown in metals contaminated soil. *Science of The Total Environment,* **912,** 168956. https://doi.org/https://doi.org/10.1016/j.scitotenv.2023.168956

Wang, Z., Lv, S., Song, H., Wang, M., Zhao, Q., Huang, H. & Niklas, K. J. (2020) Plant type dominates fine root C:N:P stoichiometry across China: A meta‐analysis. *Journal of Biogeography,* **47,** 1019-1029. https://doi.org/10.1111/jbi.13791

Wang, Z., Yu, K., Lv, S., Niklas, K. J., Mipam, T. D., Crowther, T. W., Umaña, M. N., Zhao, Q., Huang, H. & Reich, P. B. (2019) The scaling of fine root nitrogen versus phosphorus in terrestrial plants: A global synthesis. *Functional Ecology,* **33,** 2081-2094. https://doi.org/https://doi.org/10.1111/1365-2435.13434

Westoby, M., Yates, L., Holland, B. & Halliwell, B. (2023) Phylogenetically conservative trait correlation: Quantification and interpretation. *Journal of Ecology,* **111,** 2105-2117. https://doi.org/https://doi.org/10.1111/1365-2745.14150

Wright, I. J., Reich, P. B., Westoby, M., Ackerly, D. D., Baruch, Z., Bongers, F., Cavender-Bares, J., Chapin, T., Cornelissen, J. H. & Diemer, M. (2004) The worldwide leaf economics spectrum. *Nature,* **428,** 821-827. https://doi.org/https://doi.org/10.1038/nature02403

Yu, Q., Elser, J., He, N., Wu, H., Quansheng, C., Zhang, G. & Han, X.-G. (2011) Stoichiometry homeostasis of vascular plants in the Inner Mongolia grassland. *Oecologia,* **166,** 1-10. https://doi.org/10.1007/s00442-010-1902-z

Author Manuscript

Yuan, Z. Y., Chen, H. Y. & Reich, P. B. (2011) Global-scale latitudinal patterns of plant fine-root nitrogen and phosphorus. *Nature Communications,* **2,** 1-6. https://doi.org/10.1038/ncomms1346

Zhang, J., Li, M., Xu, L., Zhu, J., Dai, G. & He, N. (2021) C:N:P stoichiometry in terrestrial ecosystems in China. *Science of The Total Environment,* **795**. https://doi.org/10.1016/j.scitotenv.2021.148849

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. https://doi.org/10.1111/1365-2435.12979

Zhao, N., Liu, H., Wang, Q., Wang, R., Xu, Z., Jiao, C., Zhu, J., Yu, G., He, N. & Niu, S. (2017) Root elemental composition in Chinese forests: Implications for biogeochemical niche differentiation. *Functional Ecology,* **32,** 40-49. https://doi.org/10.1111/1365-2435.12938

Zhao, N., Yu, G., Wang, Q., Wang, R., Zhang, J., Liu, C., He, N. & Aerts, R. (2019) Conservative allocation strategy of multiple nutrients among major plant organs: From species to community. *Journal of Ecology,* **108,** 267-278. https://doi.org/10.1111/1365-2745.13256

Author Manuscript

Table 1. Pagel's lambda estimates of phylogenetic signals for fine root elemental composition, stoichiometry, and biogeochemical niche size, based on PCA scores, in all 137 tree species and species associated with arbuscular mycorrhizal (AM) or ectomycorrhizal (EM) fungi.

Attributes	All plants		AM plants		EM plants	
	Pagel's λ	P	Pagel's λ	P	Pagel's λ	P
Root Carbon (mg/g)	0.50	**<0.001**	0.51	**<0.001**	0.00	1.000
Root Nitrogen (mg/g)	0.71	**<0.001**	0.70	**<0.001**	0.28	0.892
Root Phosphorus (mg/g)	0.64	**<0.001**	0.55	**<0.001**	0.85	**<0.001**
Root Potassium (mg/g)	0.71	**<0.001**	0.63	**<0.001**	0.99	0.076
Root Calcium (mg/g)	0.76	**<0.001**	0.66	**<0.001**	1.00	**<0.001**
Root Magnesium (mg/g)	0.86	**<0.001**	0.85	**<0.001**	0.00	1.000
Root Iron (mg/g)	0.33	**0.002**	0.32	**0.010**	0.00	1.000
Root Aluminum (mg/g)	0.58	**<0.001**	0.57	**<0.001**	0.00	1.000
Root Manganese (mg/g)	0.47	**0.001**	0.33	0.089	0.00	1.000
Root Carbon: nitrogen	0.70	**<0.001**	0.70	**<0.001**	0.00	1.000
Root Carbon: phosphorus	0.72	**<0.001**	0.69	**<0.001**	1.00	**0.010**
Root Nitrogen: phosphorus	0.67	**<0.001**	0.67	**<0.001**	0.00	1.000
PC1	0.81	**<0.001**	–	–	–	–
PC2	0.75	**<0.001**	–	–	–	–
PC3	0.35	**<0.001**	–	–	–	–

Values in bold are $P < 0.05$.

Author Manuscript

Table 2. PERMANOVA of effects of soil physicochemical property, topography, mycorrhizal association type, and species on fine root elemental composition and stoichiometry.

Variables	Df	Sum Of Squares	F	R^2	Pr (>F)
Model including the effects of biotic and abiotic factors and species					
TN	1	27.52	27.69	0.017	**0.001**
TP	1	9.44	9.50	0.006	**0.001**
TK	1	26.57	26.73	0.016	**0.001**
SEC	1	5.06	5.09	0.003	**0.001**
SWC	1	5.62	5.66	0.003	**0.002**
pH	1	7.07	7.12	0.004	**0.001**
Elevation	1	9.62	9.68	0.006	**0.001**
Slope	1	0.73	0.73	0.000	0.573
Mycorrhizal type	1	20.16	20.28	0.012	**0.001**
Species	135	975.51	7.27	0.592	**0.001**
Residual	563	559.63		0.340	
Total	707	1646.94		1	
Model including the main effect of species					
Species	136	1051.11	7.41	0.638	**0.001**
Residual	571	595.83		0.362	
Total	707	1646.94		1	

TN: soil total nitrogen concentration; TP: soil phosphorus concentration; TK: soil potassium concentration; SEC: soil electrical conductivity; SWC: soil water content.

Author Manuscript

Figure legends

Figure 1. Principal component (PC) analysis scores from PCs 1–3 of fine root elemental composition and stoichiometry in 137 tree species with arbuscular mycorrhizal and ectomycorrhizal fungal associations. AM: arbuscular mycorrhizal (blue); EM: ectomycorrhizal (yellow).

Figure 2. Principal component analysis scores (means ± SE) (a) and loadings (b) of fine root elemental composition and stoichiometry in the 137 study tree species. C: carbon; N: nitrogen; P: phosphorus; K: potassium; Ca: calcium; Mg: magnesium; Fe: iron; Al: aluminum; Mn: manganese; CP: carbon: phosphorus ratio; CN: carbon: nitrogen ratio; NP: nitrogen: phosphorus ratio.

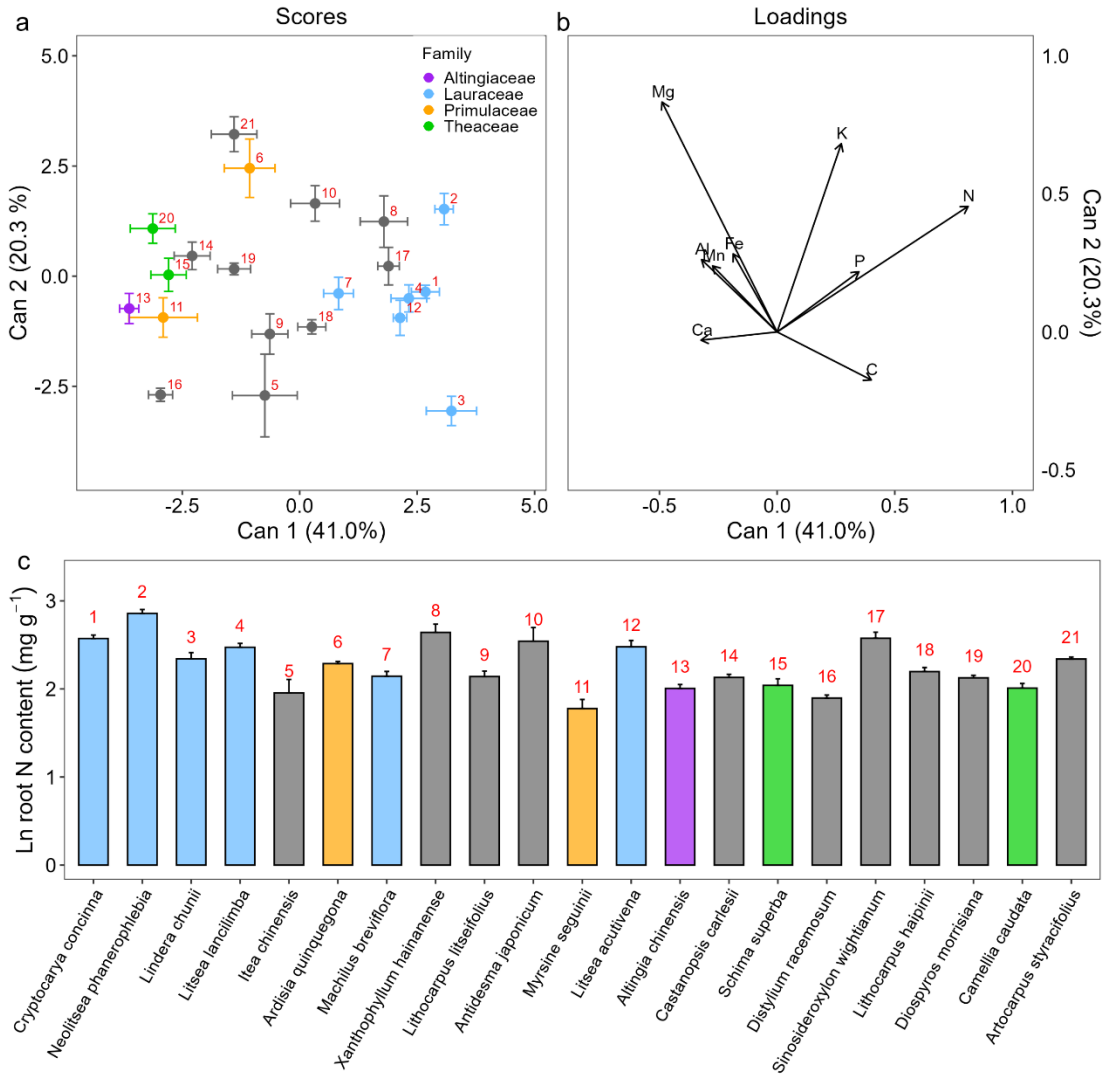
Figure 3. Scores (a) and loadings (b) of the Canonical discriminant analysis biplots, with the fine root C, N, P, K, Ca, Mg, Fe, Al, and Mn concentrations of 21 dominant species as dependent variables and tree species as independent grouping factors in the 50-ha forest plot. The mean values and corresponding SEs of the Root 1 and Root 2 scores for each species are presented in panel (a). The mean nitrogen content and corresponding SEs in roots of each tree species are presented in panel (c), where the red number is consistent with panel (a). Numbers in the figure refer to species listed in Table S1. Black solid circles represent species from other families,

Author Manuscript

which can be identified by numbers, as shown in Table S1.

Figure 4. Bayesian phylogenetic general linear mixed model estimates of variation in fine root elemental composition and stoichiometry, and biogeochemical niche, as indicated by PC1-3 scores, explained by biotic and abiotic factors and legacy effects (phylogeny + species). R^2m: nine biotic and abiotic factors; R^2p: phylogeny; R^2s: species; R^2c: biotic and abiotic factors and legacy effects.

Figure 5. Conditional effect plots of the estimated relationships between fine root elemental composition and stoichiometry with soil physicochemical and topography factors. Shaded bands correspond to the 2.5-97.5% posterior credible intervals of the slopes. Green and blue lines indicate Bayes factors (BFs, against slope = 0) <1 and >1, respectively. The probability of direction (PD) is >0.98 for all cases (see Table S6 and S7). C: carbon; N: nitrogen; P: phosphorus; K: potassium; Ca: calcium; Fe: iron; Al: aluminum; Mn: manganese.

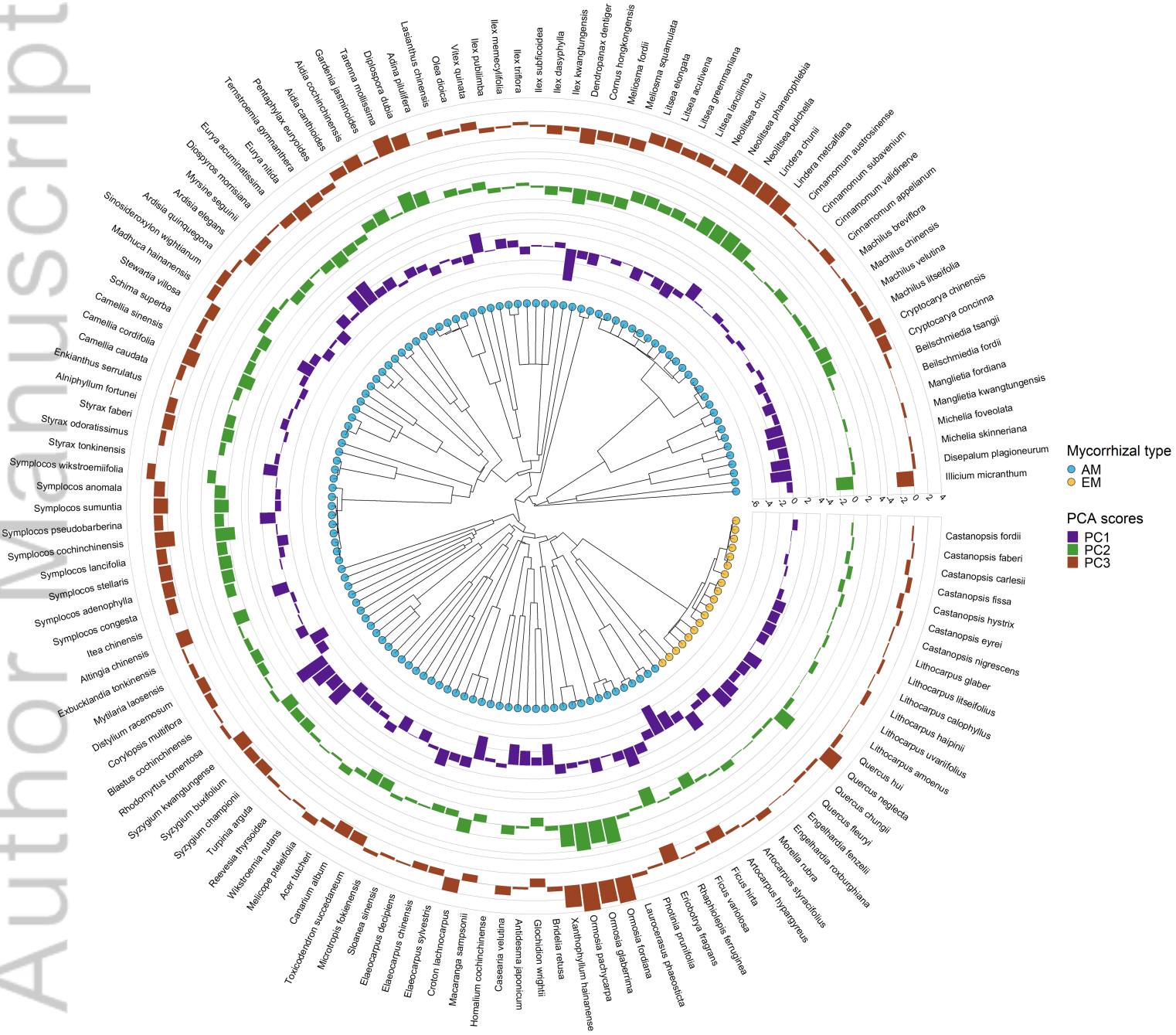


Graphical abstract:

Fine root elemental composition of the 21 most abundant co-existing species in subtropical forests tends to be unique and primarily driven by root N content, as indicated by canonical discriminant analysis. Our study provides new empirical evidence in support of the biogeochemical niche hypothesis, based on plant fine root elemental composition.

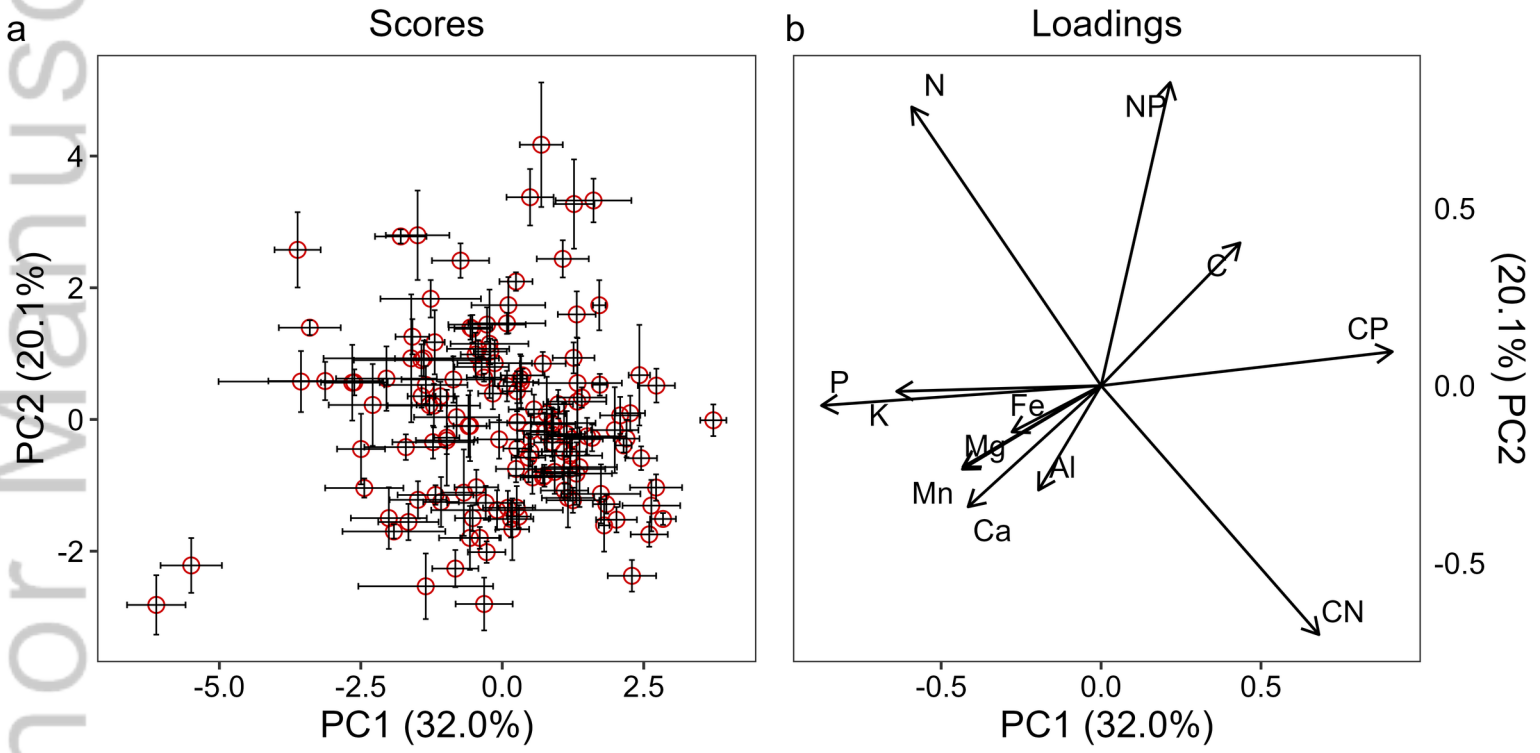
Author Manuscript

Author Manuscript

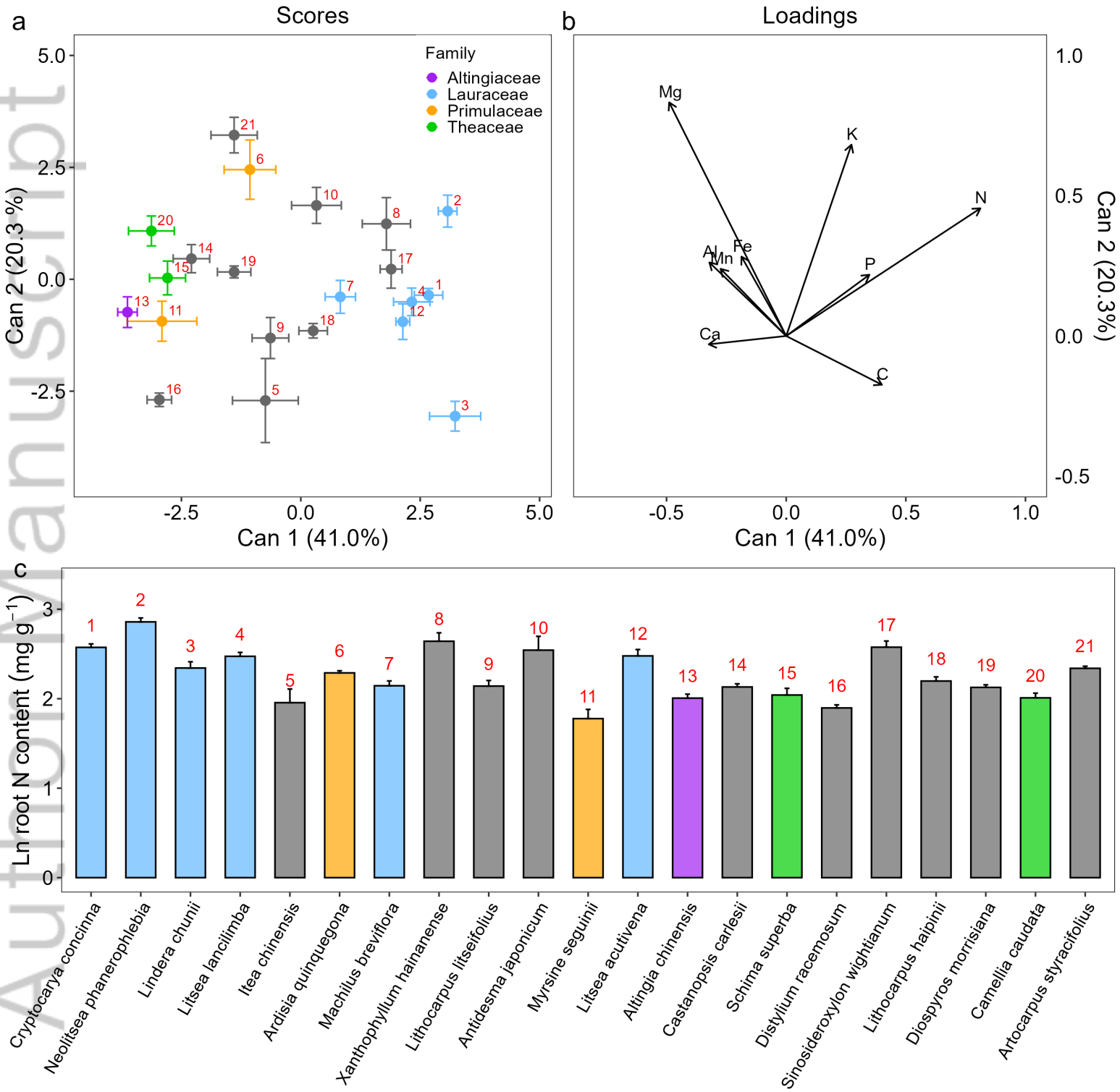


JEC_70031_f1_Figure_1.tiff

Author Manuscript

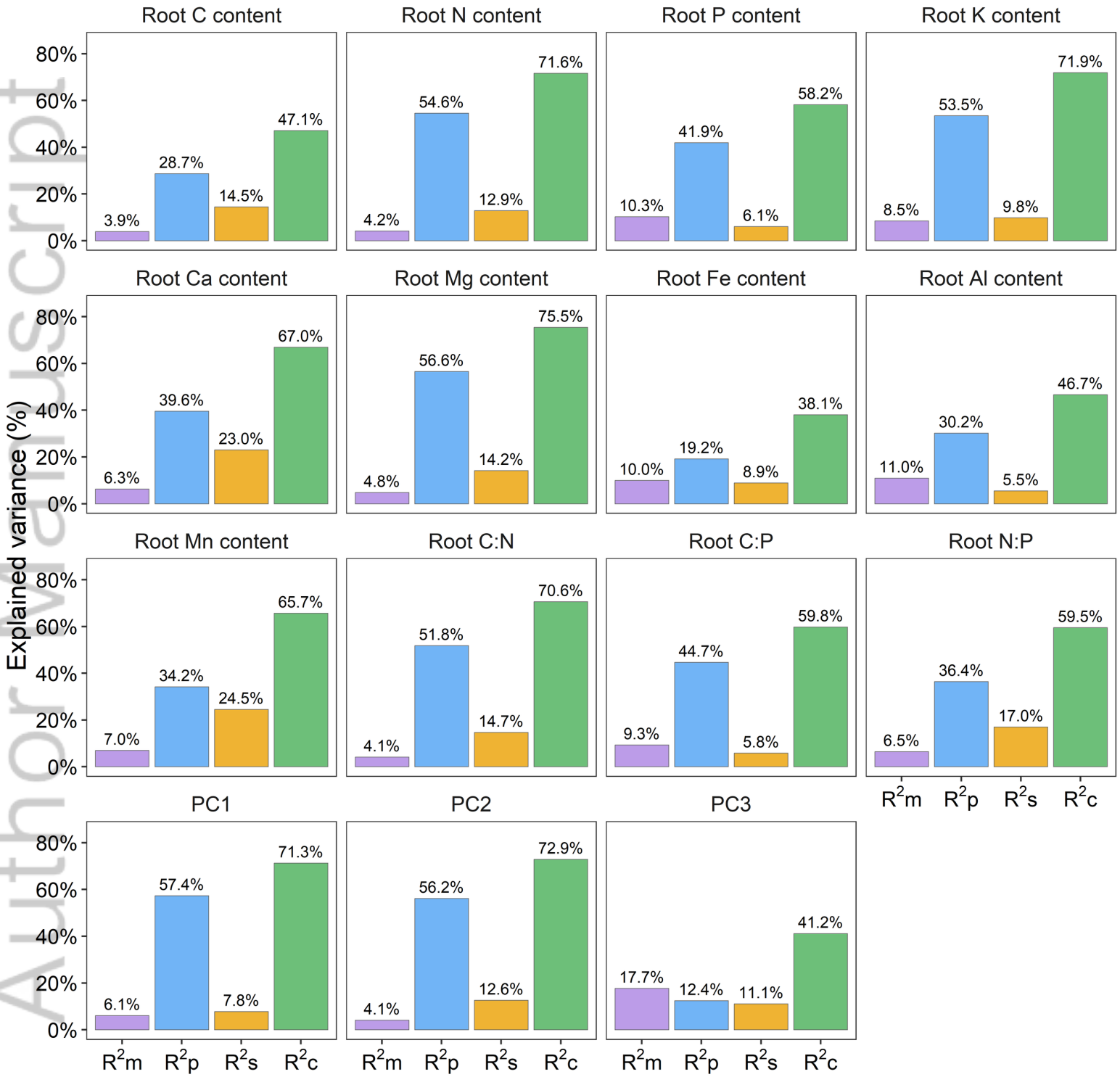


JEC_70031_f2_Figure_2.tiff



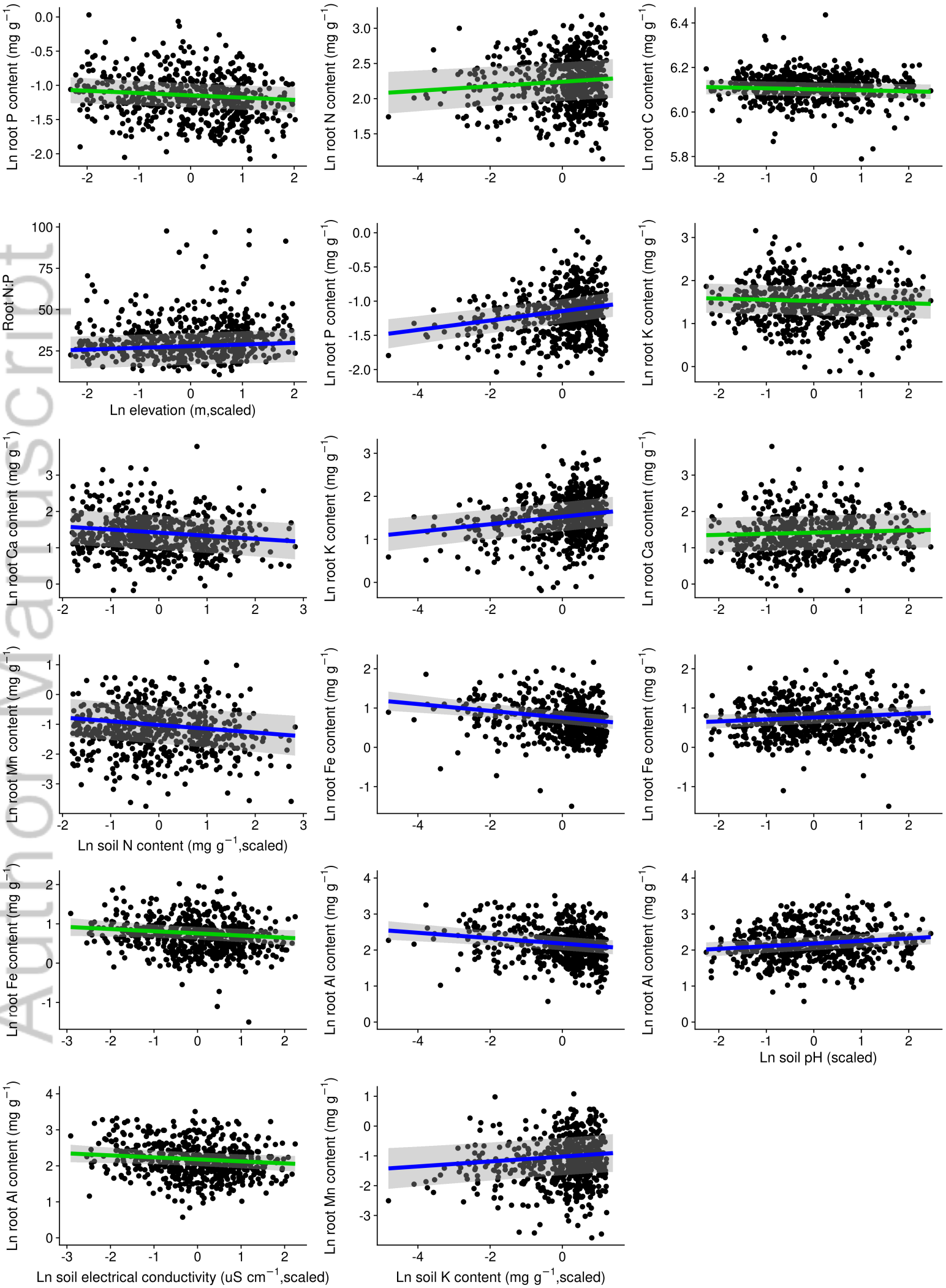
JEC_70031_f3_Figure_3.tiff

Author Manuscript



JEC_70031_f4_Figure_4.tiff

Author Manuscript



JEC_70031_f5_Figure_5.tiff

Author Manuscript