

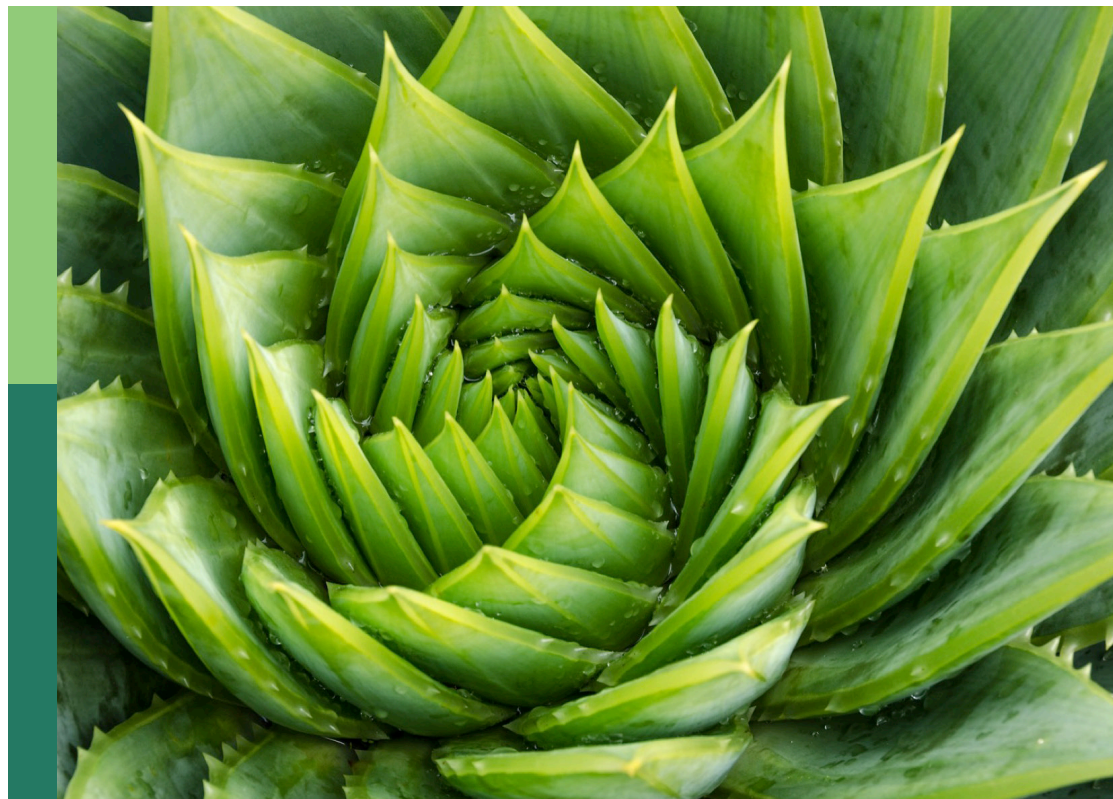
# Root functional traits: From fine root to community-level variation

**Edited by**

Iván Prieto, Patompong Johns Saengwilai, Ivika Ostonen,  
Kira A. Borden and Enrique García De La Riva

**Published in**

Frontiers in Plant Science



## FRONTIERS EBOOK COPYRIGHT STATEMENT

The copyright in the text of individual articles in this ebook is the property of their respective authors or their respective institutions or funders. The copyright in graphics and images within each article may be subject to copyright of other parties. In both cases this is subject to a license granted to Frontiers.

The compilation of articles constituting this ebook is the property of Frontiers.

Each article within this ebook, and the ebook itself, are published under the most recent version of the Creative Commons CC-BY licence. The version current at the date of publication of this ebook is CC-BY 4.0. If the CC-BY licence is updated, the licence granted by Frontiers is automatically updated to the new version.

When exercising any right under the CC-BY licence, Frontiers must be attributed as the original publisher of the article or ebook, as applicable.

Authors have the responsibility of ensuring that any graphics or other materials which are the property of others may be included in the CC-BY licence, but this should be checked before relying on the CC-BY licence to reproduce those materials. Any copyright notices relating to those materials must be complied with.

Copyright and source acknowledgement notices may not be removed and must be displayed in any copy, derivative work or partial copy which includes the elements in question.

All copyright, and all rights therein, are protected by national and international copyright laws. The above represents a summary only. For further information please read Frontiers' Conditions for Website Use and Copyright Statement, and the applicable CC-BY licence.

ISSN 1664-8714  
ISBN 978-2-83251-792-5  
DOI 10.3389/978-2-83251-792-5

## About Frontiers

Frontiers is more than just an open access publisher of scholarly articles: it is a pioneering approach to the world of academia, radically improving the way scholarly research is managed. The grand vision of Frontiers is a world where all people have an equal opportunity to seek, share and generate knowledge. Frontiers provides immediate and permanent online open access to all its publications, but this alone is not enough to realize our grand goals.

## Frontiers journal series

The Frontiers journal series is a multi-tier and interdisciplinary set of open-access, online journals, promising a paradigm shift from the current review, selection and dissemination processes in academic publishing. All Frontiers journals are driven by researchers for researchers; therefore, they constitute a service to the scholarly community. At the same time, the *Frontiers journal series* operates on a revolutionary invention, the tiered publishing system, initially addressing specific communities of scholars, and gradually climbing up to broader public understanding, thus serving the interests of the lay society, too.

## Dedication to quality

Each Frontiers article is a landmark of the highest quality, thanks to genuinely collaborative interactions between authors and review editors, who include some of the world's best academicians. Research must be certified by peers before entering a stream of knowledge that may eventually reach the public - and shape society; therefore, Frontiers only applies the most rigorous and unbiased reviews. Frontiers revolutionizes research publishing by freely delivering the most outstanding research, evaluated with no bias from both the academic and social point of view. By applying the most advanced information technologies, Frontiers is catapulting scholarly publishing into a new generation.

## What are Frontiers Research Topics?

Frontiers Research Topics are very popular trademarks of the *Frontiers journals series*: they are collections of at least ten articles, all centered on a particular subject. With their unique mix of varied contributions from Original Research to Review Articles, Frontiers Research Topics unify the most influential researchers, the latest key findings and historical advances in a hot research area.

Find out more on how to host your own Frontiers Research Topic or contribute to one as an author by contacting the Frontiers editorial office: [frontiersin.org/about/contact](https://frontiersin.org/about/contact)



# Root functional traits: From fine root to community-level variation

## Topic editors

Iván Prieto — Universidad de León, Spain

Patompong Johns Saengwilai — Mahidol University, Thailand

Ivika Ostonen — University of Tartu, Estonia

Kira A. Borden — Trent University, Canada

Enrique García De La Riva — Universidad de León, Spain

## Citation

Prieto, I., Saengwilai, P. J., Ostonen, I., Borden, K. A., De La Riva, E. G., eds. (2023).

*Root functional traits: From fine root to community-level variation.*

Lausanne: Frontiers Media SA. doi: 10.3389/978-2-83251-792-5

## Table of contents

- 05 **Editorial: Root functional traits: From fine root to community-level variation**  
Enrique G. de la Riva, Kira Borden, Ivika Ostonen, Patompong Saengwilai and Iván Prieto
- 08 **Root Functional Trait and Soil Microbial Coordination: Implications for Soil Respiration in Riparian Agroecosystems**  
Kira A. Borden, Tolulope G. Mafa-Attoye, Kari E. Dunfield, Naresh V. Thevathasan, Andrew M. Gordon and Marney E. Isaac
- 20 **Root Community Traits: Scaling-Up and Incorporating Roots Into Ecosystem Functional Analyses**  
Ruili Wang, Guirui Yu and Nianpeng He
- 25 **Plant Nutrient Contents Rather Than Physical Traits Are Coordinated Between Leaves and Roots in a Desert Shrubland**  
Xiaoyan Jiang, Xin Jia, Shengjie Gao, Yan Jiang, Ningning Wei, Cong Han, Tianshan Zha, Peng Liu, Yun Tian and Shugao Qin
- 36 **The Economics Spectrum Drives Root Trait Strategies in Mediterranean Vegetation**  
Enrique G. de la Riva, José Ignacio Querejeta, Rafael Villar, Ignacio M. Pérez-Ramos, Teodoro Marañón, Javier Galán Díaz, Sergio de Tomás Marín and Iván Prieto
- 46 **The Right-Skewed Distribution of Fine-Root Size in Three Temperate Forests in Northeastern China**  
Cunguo Wang, Ivano Brunner, Junni Wang, Wei Guo, Zhenzhen Geng, Xiuyun Yang, Zhijie Chen, Shijie Han and Mai-He Li
- 57 **Evidence of Differences in Covariation Among Root Traits Across Plant Growth Forms, Mycorrhizal Types, and Biomes**  
Nannan An, Nan Lu, Bojie Fu, Weiliang Chen, Maierdang Keyimu and Mengyu Wang
- 70 **Magnitude and determinants of plant root hydraulic redistribution: A global synthesis analysis**  
Guisei Yang, Lei Huang and Yafei Shi
- 83 **Root tragedy of the commons: Revisiting the mechanisms of a misunderstood theory**  
Ciro Cabal
- 92 **Impact on plant productivity under low-fertility sandy soil in arid environment by revitalization of lentil roots**  
Mohamed A. Abd El-hady, Yasser M. Abd-Elkrem, Mohamed O. A. Rady, Elsayed Mansour, Khaled A. El-Tarabily, Synan F. AbuQamar and Mohamed E. El-temsah

- 107 **Differential influence of cortex and stele components on root tip diameter in different types of tropical climbing plants**  
Haiwu Xu, Siyuan Wang, Liang Tang, Yan Wang, Zhongyue Li and Wenna Wang
- 119 **Precipitation predictability affects intra- and trans-generational plasticity and causes differential selection on root traits of *Papaver rhoeas***  
Martí March-Salas, J. F. Scheepens, Mark van Kleunen and Patrick S. Fitze
- 135 **Contrasting distribution of enzyme activities in the rhizosphere of European beech and Norway spruce**  
Bin Song, Bahar S. Razavi and Rodica Pena
- 151 **Mulching in lowland hay meadows drives an adaptive convergence of above- and below-ground traits reducing plasticity and improving biomass: A possible tool for enhancing phytoremediation**  
Michele Dalle Fratte, Antonio Montagnoli, Simone Anelli, Stefano Armiraglio, Peter Beatrice, Alex Ceriani, Elia Lipreri, Alessio Miali, Paolo Nastasio and Bruno Enrico Leone Cerabolini



## OPEN ACCESS

EDITED AND REVIEWED BY  
Catherine Picon-Cochard,  
Institut National de recherche pour  
l'agriculture, l'alimentation et  
l'environnement (INRAE), France

\*CORRESPONDENCE  
Enrique G. de la Riva  
✉ engar70@gmail.com; egarr@unileon.es

SPECIALTY SECTION  
This article was submitted to  
Functional Plant Ecology,  
a section of the journal  
Frontiers in Plant Science

RECEIVED 27 January 2023  
ACCEPTED 06 February 2023  
PUBLISHED 15 February 2023

CITATION  
de la Riva EG, Borden K, Ostonen I,  
Saengwilai P and Prieto I (2023) Editorial:  
Root functional traits: From fine root to  
community-level variation.  
*Front. Plant Sci.* 14:1152174.  
doi: 10.3389/fpls.2023.1152174

COPYRIGHT  
© 2023 de la Riva, Borden, Ostonen,  
Saengwilai and Prieto. This is an open-access  
article distributed under the terms of the  
[Creative Commons Attribution License](#)  
(CC BY). The use, distribution or  
reproduction in other forums is permitted,  
provided the original author(s) and the  
copyright owner(s) are credited and that  
the original publication in this journal is  
cited, in accordance with accepted  
academic practice. No use, distribution or  
reproduction is permitted which does not  
comply with these terms.

# Editorial: Root functional traits: From fine root to community-level variation

Enrique G. de la Riva<sup>1\*</sup>, Kira Borden<sup>2</sup>, Ivika Ostonen<sup>3</sup>,  
Patompong Saengwilai<sup>4</sup> and Iván Prieto<sup>1</sup>

<sup>1</sup>Ecology Department, Faculty of Biology and Environmental Sciences, Universidad de León, León, Spain, <sup>2</sup>School of the Environment, Trent University, Peterborough, ON, Canada, <sup>3</sup>Institute of Ecology and Earth Sciences, University of Tartu, Tartu, Estonia, <sup>4</sup>Department of Biology, Faculty of Science, Mahidol University, Bangkok, Thailand

## KEYWORDS

mycorrhizal, economic spectrum, root plasticity, specific root length, ecosystem service

## Editorial on the Research Topic

Root functional traits: from fine root to community-level variation

## Introduction

Plant roots perform multiple essential functions defining plant ecological success and ecosystem functioning. For instance, roots are vital for plant nutrient and water uptake, thus regulating net primary production and nutrient cycling (Freschet et al., 2021). In the last decade, the adoption and advancement of a functional trait approach has greatly improved our understanding of root ecology, evidenced by the recent increase of global syntheses on root trait research (e.g., Freschet et al., 2018; Bergmann et al., 2020; Carmona et al., 2021; Freschet et al., 2021). However, there are still gaps and controversy in our understanding of root trait–functioning relationships (Freschet et al., 2021). Roots display a wide diversity of morphologies and symbiotic associations (i.e., with mycorrhizal fungi and rhizobium), which has made it challenging to seek general patterns across the diverse taxa that inhabit different ecological conditions worldwide (Ma et al., 2018). In this special issue, we bring together studies on root ecology that tackle important unresolved questions and emerging topics, which collectively highlight new knowledge and critical knowledge gaps in belowground ecology.

## Root hydraulic redistribution

Water relations are key to understanding the ecology of terrestrial plant communities, and one determining component of water balance is the process of hydraulic redistribution (HR; Caldwell et al., 1998). The term refers to the passive movement of water through plant roots from moist to dry soil layers following a water potential gradient (Prieto et al., 2012). Many factors, such as plant transpiration (Howard et al., 2009), root architecture (Scholz et al., 2008) and soil conditions, e.g. soil humidity and texture (Prieto et al., 2010) affect the direction and magnitude of HR that has been found to be highly variable across species and



ecosystems worldwide (Neumann and Cardon, 2012). In a comprehensive review in this special issue, Yang et al. put forward that plant characteristics such as plant transpiration and root length were the main determinants of HR magnitude whereas soil factors such as water table depth or soil texture were also important yet indirect drivers.

## Root morphological traits and resource uptake strategies

The ability of plants to acquire soil water and nutrients determines their competitive success and productivity (Erktan et al., 2018a). In this regard, the variation in root traits in terms of resource acquisition has been gaining research attention (Freschet et al., 2021). Previous efforts to understand how functional traits are organized across terrestrial plants have revealed the existence of an acquisition – conservation trade-off, known as the leaf economic spectrum (Wright et al., 2004), but whether such a high degree of organization is also seen in root traits remains controversial and may depend on the type of mycorrhizal association (e.g., Bergmann et al., 2020; de la Riva et al., 2021). In this issue, An et al. and de la Riva et al. demonstrate that there is a main trend of variation in the multidimensional root space in line with expectations of the root economics spectrum in over 300 species in China and Spain, whereas Jiang et al. found weak or no correlation between fine-root traits in 48 species from a single semiarid ecosystem. Further, Wang et al. provide insights into the existence of a trade-off between the number and the size of fine roots of temperate tree species. Collectively these results suggest belowground trait covariation and trade-offs are strongly driven by environmental gradients (de la Riva et al., 2016; Erktan et al., 2018b) and capture different plant strategies to a wide range of environmental conditions (Díaz et al., 2016) that may be in response to a trade-off between growth and survival (Reich, 2014).

## Root plasticity

Phenotypic variation is an important driver of plant performance under different environmental conditions (Caballero et al.). The majority of research on phenotypic variation to date has focused aboveground (e.g., Roscher et al., 2018; Valladares et al., 2002) and we are still far from understanding the full extent to which environmental conditions elicit a plastic response belowground (Freschet et al., 2021). This special issue brings an essential milestone to this research frontier by gathering several empirical studies showing a rapid plastic response of root traits under changing environmental conditions. Fratte et al. demonstrate that mulching favored the establishment of plant communities with lower plasticity through an adaptive convergence between analogous traits at leaf and root levels. Using a multi-generation experiment with an annual herb (*Papaver rhoeas*), March-Salas et al. proved that precipitation predictability promotes intra- and trans-generational plasticity in root traits, observing differential

root trait responses between ancestors and descendants. Moreover, Xu et al. found that, for lianas and vines in tropical ecosystems, phenotypic variation in root diameter in root tips is strongly linked to changes in cortex thickness and cortex cell size rather than on stele diameter variation. These studies widen the characterisation of trait phenotypic variability and decipher the complex and context dependent interactions between root traits and the environment.

## Root and soil interactions

Root-soil interactions occur at multiple spatial and temporal scales and are driven by complex processes occurring between roots, microbes, and the soil environment. Discerning relationships between root traits and root-soil interactions can improve our understanding of plant responses to, and their effects on, the environment (Violle et al., 2007). Song et al. and Borden et al. conduct detailed measurements of rhizosphere enzymatic activity and root-rhizosphere respiration rates, respectively. Both studies consider rhizosphere activity as the result of direct root activity and indirect effects of roots on microbial activity. Song et al. found that the spatial distribution of enzyme activity along the root growth axis was associated with larger root diameter in European beech (*Fagus sylvatica*), and longer root hair length in Norway spruce; and contrasting distributions with distance from rhizoplane suggesting differential contributions of root vs. microbial enzymatic activity. In a field study, Borden et al. found specific root respiration covaried with morphological and chemical root traits, and while microbial abundance in the rhizosphere coordinated with root trait variation, this was not the case in bulk soil.

## Ecosystem functioning and services

Theoretical and empirical evidence predicts that root traits are directly linked with soil structure, nutrient cycling, production and, consequently, ecosystem functioning (Freschet et al., 2021). Thus, roots influence the levels of productive and regulatory ecosystem functions and might directly affect ecosystem services (Freschet et al., 2021), but, as Wang et al. stated in this special issue: “the importance of root traits in ecosystem-level functioning, is increasingly recognized but still not well-understood”. This special issue showcases studies observing the role of root traits as key drivers of ecosystem services, such as phytoremediation, crop productivity and carbon cycle. Fratte et al. demonstrate that mulching application increases below-ground biomass, which may favour the proliferation of microbes devoted to soil organic contaminants’ degradation. In another study with cropped lentil plants in arid nutrient-poor environments, El-hady et al. showed that the application of root activator and phosphorus enhanced plant growth and productivity by invigorating root traits. While Borden et al. identify connections between root trait variation with carbon dioxide emissions from soil. These studies improve our understanding of the direct benefits of plant root systems in delivery of ecosystem services *via* root trait-function relationships.

## Conclusions

This special issue brings together research from multiple fields of root ecology that are unified in their trait based approach. Taken together, this special issue gives a complex but realistic picture of the multidimensional and dynamic roles roots play belowground, defining plant resource uptake strategies and performance, and driving ecosystem functioning and services. However, the ability to scale up fine root trait variation to community and ecosystem functioning level requires more critical investigations that make empirical connections between anatomical, morphological, and physiological characteristics of plant roots with ecosystem scale processes.

## Author contributions

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

## Funding

ER is supported by a María Zambrano contract funded by the Spanish Ministry of Universities and IP was supported by a Ramón y Cajal contract (RYC2021-033081-I) funded by the Ministry of

Science and Innovation, both co-funded by European Union-Next Generation Plan funded by European Union-NextGenerationEU. IP acknowledges funding by the Fundación Séneca (project 20654/JLI/18), co-funded by European Union FEDER funds.

## Acknowledgments

We deeply thank all the authors and reviewers who have participated in this Research Topic.

## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

## Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

## References

- Bergmann, J., Weigelt, A., van der Plas, F., Laughlin, D. C., Kuyper, T. W., Guerrero-Ramirez, N., et al. (2020). The fungal collaboration gradient dominates the root economics space in plants. *Sci. Adv.* 6, eaba3756. doi: 10.1126/sciadv.aba3756
- Caldwell, M. M., Dawson, T. E., and Richards, J. H. (1998). Hydraulic lift: Consequences of water efflux from the roots of plants. *Oecologia* 113, 151–161. doi: 10.1007/s004420050363
- Carmona, C. P., Bueno, C. G., Toussaint, A., Träger, S., Díaz, S., Moora, M., et al. (2021). Fine-root traits in the global spectrum of plant form and function. *Nature* 597, 683–687. doi: 10.1038/s41586-021-03871-y
- de la Riva, E. G., Pérez-Ramos, I. M., Tosto, A., Navarro-Fernández, C. M., Olmo, M., Marañón, T., et al. (2016). Disentangling the relative importance of species occurrence, abundance and intraspecific variability in community assembly: A trait-based approach at the whole-plant level in Mediterranean forests. *Oikos* 125, 354–363. doi: 10.1111/oik.01875
- de la Riva, E. G., Prieto, I., Marañón, T., Pérez-Ramos, I. M., Olmo, M., and Villar, R. (2021). Root economics spectrum and construction costs in Mediterranean woody plants: The role of symbiotic associations and the environment. *J. Ecol.* 109, 1873–1885. doi: 10.1111/1365-2745.13612
- Díaz, S., Kattge, J., Cornelissen, J. H., Wright, I. J., Lavorel, S., Dray, S., et al. (2016). The global spectrum of plant form and function. *Nature* 529, 167–171. doi: 10.1038/nature16489
- Erktan, A., McCormack, M. L., and Roumet, C. (2018a). Frontiers in root ecology: recent advances and future challenges. *Plant Soil* 424, 1–9. doi: 10.1007/s11104-018-3618-5
- Erktan, A., Roumet, C., Bouchet, D., Stokes, A., Paillet, F., and Munoz, F. (2018b). Two dimensions define the variation of fine root traits across plant communities under the joint influence of ecological succession and annual mowing. *J. Ecol.* 106, 2031–2042. doi: 10.1111/1365-2745.12953
- Freschet, G. T., Roumet, C., Comas, L. H., Weemstra, M., Bengough, A. G., Rewald, B., et al. (2021). Root traits as drivers of plant and ecosystem functioning: Current understanding, pitfalls and future research needs. *New Phytol.* 232, 1123–1158. doi: 10.1111/nph.17072
- Freschet, G. T., Violle, C., Roumet, C., and Garnier, E. (2018). "Interactions between soil and vegetation: Structure of plant communities and soil functioning," in *Soils within the critical zone: ecology*. Eds. P. Lemancau and M. Blouin (London, UK: ISTE Editions), 83–104.
- Howard, A. R., Van Iersel, M. W., Richards, J. H., and Donovan, L. A. (2009). Night-time transpiration can decrease hydraulic redistribution. *Plant Cell Environ.* 32, 1060–1070. doi: 10.1111/j.1365-3040.2009.01988.x
- Ma, Z. Q., Guo, D. L., Xu, X. L., Lu, M. Z., Bardgett, R. D., Eissenstat, D. M., et al. (2018). Evolutionary history resolves global organization of root functional traits. *Nature* 555, 94–97. doi: 10.1038/nature25783
- Neumann, R. B., and Cardon, Z. G. (2012). The magnitude of hydraulic redistribution by plant roots: A review and synthesis of empirical and modeling studies. *New Phytol.* 194, 337–352. doi: 10.1111/j.1469-8137.2012.04088.x
- Prieto, I., Armas, C., and Pugnaire, F. I. (2012). Water release through plant roots: New insights into its consequences at the plant and ecosystem level. *New Phytol.* 193, 830–841. doi: 10.1111/j.1469-8137.2011.04039.x
- Prieto, I., Martínez-Tillería, K., Martínez-Manchego, L., Montecinos, S., Squeo, F. A., and Pugnaire, F. I. (2010). Hydraulic lift through transpiration suppression in shrubs from two arid ecosystems: patterns and control mechanisms. *Oecologia* 163, 855–865. doi: 10.1007/s00442-010-1615-3
- Reich, P. B. (2014). The world-wide 'fast-slow' plant economics spectrum: A traits manifesto. *J. Ecol.* 102, 275–301. doi: 10.1111/1365-2745.12211
- Roscher, C., Schumacher, J., Lipowsky, A., Gubsch, M., Weigelt, A., Schmid, B., et al. (2018). Functional groups differ in trait means, but not in trait plasticity to species richness in local grassland communities. *Ecology* 99, 2295–2307. doi: 10.1002/ecy.2447
- Scholz, F. G., Bucci, S. J., Goldstein, G., Moreira, M. Z., Meinzer, F. C., Domec, J. C., et al. (2008). Biophysical and life-history determinants of hydraulic lift in Neotropical savanna trees. *Funct. Ecol.* 22, 773–786. doi: 10.1111/j.1365-2435.2008.01452.X
- Valladares, F., Balaguer, L., Martínez-Ferri, E., Pérez-Corona, E., and Manrique, E. (2002). Plasticity, instability and canalization: Is the phenotypic variation in seedlings of sclerophyll oaks consistent with the environmental unpredictability of Mediterranean ecosystems? *New Phytol.* 156, 457–467. doi: 10.1046/j.1469-8137.2002.00525.x
- Violle, C., Navas, M. L., Vile, D., Kazakou, E., Fortunel, C., Hummel, I., et al. (2007). Let the concept of trait be functional! *Oikos* 116, 882–892. doi: 10.1111/j.2007.0030-1299.15559.x
- Wright, I. J., Reich, P. B., Westoby, M., Ackerly, D. D., Baruch, Z., Bongers, F., et al. (2004). The worldwide leaf economics spectrum. *Nature* 428, 821–827. doi: 10.1038/nature02403



# Root Functional Trait and Soil Microbial Coordination: Implications for Soil Respiration in Riparian Agroecosystems

Kira A. Borden<sup>1,2,3\*</sup>, Tolulope G. Mafa-Attoye<sup>4</sup>, Kari E. Dunfield<sup>4</sup>, Naresh V. Thevathasan<sup>4</sup>, Andrew M. Gordon<sup>4</sup> and Marney E. Isaac<sup>3</sup>

<sup>1</sup> Faculty of Land and Food Systems, The University of British Columbia, Vancouver, BC, Canada, <sup>2</sup> Centre for Sustainable Food Systems, The University of British Columbia, Vancouver, BC, Canada, <sup>3</sup> Department of Physical and Environmental Sciences, University of Toronto Scarborough, Toronto, ON, Canada, <sup>4</sup> School of Environmental Sciences, University of Guelph, Guelph, ON, Canada

## OPEN ACCESS

### Edited by:

Sebastian Leuzinger,  
Auckland University of Technology,  
New Zealand

### Reviewed by:

Ivika Ostonen,  
University of Tartu, Estonia  
Stephen David James Archer,  
Auckland University of Technology,  
New Zealand

### \*Correspondence:

Kira A. Borden  
kira.borden@ubc.ca

### Specialty section:

This article was submitted to  
Functional Plant Ecology,  
a section of the journal  
Frontiers in Plant Science

**Received:** 15 March 2021

**Accepted:** 15 June 2021

**Published:** 08 July 2021

### Citation:

Borden KA, Mafa-Attoye TG,  
Dunfield KE, Thevathasan NV,  
Gordon AM and Isaac ME (2021)  
Root Functional Trait and Soil  
Microbial Coordination: Implications  
for Soil Respiration in Riparian  
Agroecosystems.  
Front. Plant Sci. 12:681113.  
doi: 10.3389/fpls.2021.681113

Predicting respiration from roots and soil microbes is important in agricultural landscapes where net flux of carbon from the soil to the atmosphere is of large concern. Yet, in riparian agroecosystems that buffer aquatic environments from agricultural fields, little is known on the differential contribution of CO<sub>2</sub> sources nor the systematic patterns in root and microbial communities that relate to these emissions. We deployed a field-based root exclusion experiment to measure heterotrophic and autotrophic-rhizospheric respiration across riparian buffer types in an agricultural landscape in southern Ontario, Canada. We paired bi-weekly measurements of in-field CO<sub>2</sub> flux with analysis of soil properties and fine root functional traits. We quantified soil microbial community structure using qPCR to estimate bacterial and fungal abundance and characterized microbial diversity using high-throughput sequencing. Mean daytime total soil respiration rates in the growing season were 186.1 ± 26.7, 188.7 ± 23.0, 278.6 ± 30.0, and 503.4 ± 31.3 mg CO<sub>2</sub>-C m<sup>-2</sup> h<sup>-1</sup> in remnant coniferous and mixed forest, and rehabilitated forest and grass buffers, respectively. Contributions of autotrophic-rhizospheric respiration to total soil CO<sub>2</sub> fluxes ranged widely between 14 and 63% across the buffers. Covariation in root traits aligned roots of higher specific root length and nitrogen content with higher specific root respiration rates, while microbial abundance in rhizosphere soil coordinated with roots that were thicker in diameter and higher in carbon to nitrogen ratio. Variation in autotrophic-rhizospheric respiration on a soil area basis was explained by soil temperature, fine root length density, and covariation in root traits. Heterotrophic respiration was strongly explained by soil moisture, temperature, and soil carbon, while multiple factor analysis revealed a positive correlation with soil microbial diversity. This is a first in-field study to quantify root and soil respiration in relation to trade-offs in root trait expression and to determine interactions between root traits and soil microbial community structure to predict soil respiration.

**Keywords:** absorptive roots, autotrophic respiration, heterotrophic respiration, plant functional traits, root economics spectrum, rhizosphere

## INTRODUCTION

Soil respiration is a massive source of atmospheric carbon dioxide (CO<sub>2</sub>) and agricultural soils have been identified as a major contributor to global warming (Lal, 2003; Schlesinger and Andrews, 2000). Therefore, agricultural land use practices that mitigate and/or reverse net carbon (C) loss from soil need to be increasingly adopted. One such land use practice is the protection and restoration of riparian agroecosystems with trees (i.e., agroforestry) and/or perennial grasses (Tufekcioglu et al., 2003; Thevathasan et al., 2012; Udawatta and Jose, 2012). These “riparian buffers” are well understood in their importance to protect streambanks from erosion, capture nutrient runoff and leaching from adjacent cropping systems, and support biodiversity (Thevathasan et al., 2012; Christen and Dalgaard, 2013). Additionally, these specialized ecosystems can increase C storage in living tree and perennial biomass as well as in soil (Tufekcioglu et al., 2003; Fortier et al., 2013; Oelbermann et al., 2014). However, soil respiration in riparian systems is extremely variable (Tufekcioglu et al., 2001, 1999; Oelbermann et al., 2014; De Carlo et al., 2019), which makes it challenging to fully account for the net ecosystem exchange of C under various buffer types, and the associated plant community transformations, within agricultural landscapes.

Plant roots and soil microbes have complex relationships and interactions that drive their contributions to soil respiration (De Vries et al., 2016; Fry et al., 2019). Autotrophic-rhizospheric respiration ( $R_{a+r}$ ) is a function of roots respiring (root metabolic processes; i.e., autotrophic respiration) and the stimulated microbial activity in the rhizosphere (i.e., rhizospheric respiration) (Bardgett, 2017; Chen et al., 2019). Autotrophic-rhizospheric respiration can range dramatically from 10 to 90% of total soil respiration in vegetated ecosystems (Hanson et al., 2000). On the other hand, heterotrophic respiration ( $R_h$ ) from microbial decomposition of soil organic matter (SOM) can be both independent of plant root function (Ferlian et al., 2017) but also controlled by the quantity and quality of SOM from above and belowground litter inputs (De Long et al., 2019). Thus, roots can have both immediate effects on production of CO<sub>2</sub> via  $R_{a+r}$  and longer-term effects via root turnover and contributions to SOM pools.

Trait-based plant ecology strives to explain plants' response to the environment (“response”) and/or plants' impact on the environment (“effect”) (Lavorel and Garnier, 2002; Violle et al., 2007). This approach has provided major advances in relating key plant traits to biogeochemical processes (De Vries et al., 2016; Borden et al., 2019; Coleman et al., 2020). The strength and importance of root traits in describing CO<sub>2</sub> fluxes from soil is uncertain given the limited information from natural plant communities (De Long et al., 2019). However, general trends do show trade-offs in root trait expression, with specific root length (SRL) and root nitrogen content ( $N_{root}$ ) being positively related with root respiration and decomposition, while root diameter (D) and carbon to nitrogen ratio (C:N<sub>root</sub>) are positively related with root longevity (Sun and Mao, 2011; McCormack et al., 2015; Roumet et al., 2016; Ma et al., 2018). Thus, such covariation among root traits characterize the constraints in

root construction and function across and within plant species (Kong et al., 2014; Prieto et al., 2015; Roumet et al., 2016; Borden and Isaac, 2019). Additionally, these root trait trade-offs may be related to microbial communities, with described relationships of species that form mycorrhizal fungi associations tending to have higher D and lower SRL (Ma et al., 2018; Bergmann et al., 2020). Concurrently, the expanding analytics of the soil microbiome has provided detail and range in capturing the composition of soil microorganisms (Toju et al., 2018). Taken together, these research advancements offer opportunities to systematically integrate plant traits, microbial communities, and agroecosystem processes (Pommier et al., 2018; Isaac and Borden, 2019; Fulthorpe et al., 2020). Yet, these belowground interactions have not been empirically related to respiration rates.

Our study was designed to determine (1) root trait covariation with root respiration rates, (2) root trait covariation with microbial communities, and (3) the subsequent effects of root traits and soil microbial richness and evenness on soil CO<sub>2</sub> emissions in riparian agroecosystems. To do this, we performed a root exclusion experiment in four riparian buffer types (rehabilitated forest and grass buffers and remnant coniferous and mixed forest buffers) characterized by distinct plant communities in order to quantify the components of soil respiration: heterotrophic and autotrophic-rhizospheric respiration. We hypothesize that fundamental trade-offs in root construction and function coordinate with microbial community variation. We also expect that trait covariation, along with some key abiotic soil conditions, is an important explanatory variable for soil respiration.

## MATERIALS AND METHODS

### Study Sites

We carried out a 10-week (May–August 2018) field experiment along Washington Creek, Ontario, Canada (43°18'N 80°33'W). The creek system is at an elevation of ~300 m and has a mean annual temperature of 7.3°C and mean annual precipitation of 784 mm (1981–2010 Station Data; Environment Canada 2019). The creek is spring fed and situated in a region primarily under intensive agriculture of corn-soybean rotations. Soils are loam and classified as Gray Brown Luvisol with parent material composed of glacial till over limestone bedrock (Oelbermann et al., 2014). We maximized the potential range of belowground processes within the same creek system by sampling sites of distinct vegetative communities and variable soil properties. Four riparian buffer types were selected representing different perennial compositions. These buffers were either on sites with a history of managed rehabilitation (>30 years old), or on sites of remnant, old-growth forest: (i) grass and (ii) hardwood forest (referred to hereafter as “rehabilitated forest”) on rehabilitated land, and (iii) coniferous forest, and (iv) mixed forest on remnant land. The creek is alkaline (7.5–8.5) (Oelbermann et al., 2014) and soil pH at these sites ranged from 7.1 to 7.6, with higher values at the younger, rehabilitated grass and forest sites (7.5 and 7.6, respectively) compared to the old-growth forest sites (7.1). Soil inorganic C was also higher at the rehabilitated buffers (8



and 11%) (unpublished data; **Supplementary Table 1**). For more details on study sites refer to De Carlo et al. (2019), Oelbermann et al. (2014), and Mafa-Attoye et al. (2020).

## Soil Respiration

We used the root exclusion method (i.e., difference method) to estimate autotrophic + rhizospheric respiration ( $R_{a+r}$ ) and differentiate  $\text{CO}_2$  from heterotrophic respiration ( $R_h$ ) in soil (Hanson et al., 2000; Lavigne et al., 2003; Kuzyakov and Larionova, 2005). We did so in a nested sampling design: four sampling plots of  $1.0 \times 0.5$  m were established within each buffer type. In each sampling plot, root exclusion sub-plots were created in half the area ( $0.5 \times 0.5$  m) and trenched to 40 cm. Soil within trenched sub-plots was carefully removed and living roots were removed from that soil. Landscaping fabric was inserted to line the exclusion plots to prevent root growth into the exclusion area and soil was gently returned to the exclusion area, with effort made to replace soil at the same depth and with similar compaction (Lavigne et al., 2003). Soil collars were inserted in the center of each section, 2.5 cm into the soil. In the root-exclusion sub-plots, newly established plants were removed by hand throughout the experiment. At the end of the experiment, after digging out the landscaping fabric, we observed roots had not penetrated through the fabric. At each sampling time, any physical disturbance like trenching or adjustment of soil collars was carried out after  $\text{CO}_2$  measurements were taken.

Root exclusion experiments have limitations due to possible effects on soil conditions from soil disturbance and lack of vegetative cover (Hanson et al., 2000; Kuzyakov and Larionova, 2005). Presumably, an increase in respiration from soils occurred immediately after soil disturbance particularly during exclusion plot set-up, but effort was made to protect soil during removal and replacement, to minimize disturbance of soil aggregates, and then during the two-week stabilization period prior to the first measurements of soil respiration. We tested our assumption of similar soil conditions by comparing bulk soil bacteria and fungi abundance, soil moisture, and soil temperature between paired inclusion and exclusion sub-plots on a subset of sampling dates, which is further explained in the statistical analysis section.

Every 2 weeks following soil collar installation, soil  $\text{CO}_2$  flux was measured using a portable infrared gas analyzer (Licor L6400XT) with a soil  $\text{CO}_2$  flux closed chamber. Measurements across all sites were completed on the same day between 09:00 and 14:00 with the order of buffer type and sampling plot randomized. Ambient  $\text{CO}_2$  near the soil surface was measured prior to measurements and used to set target  $\text{CO}_2$  and range. The average of three cycles per soil collar was used to calculate  $\text{CO}_2$  flux. Total soil respiration ( $R_s$ ;  $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ) was measured in the root-inclusion sub-plots, while fluxes of  $\text{CO}_2$  from root-exclusion sub-plots captured heterotrophic respiration ( $R_h$ ;  $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ). Autotrophic + rhizospheric respiration ( $R_{a+r}$ ;  $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ) was calculated as the difference between  $R_s$  and  $R_h$ .

## Soil Physico-Chemical Properties

Soil temperature was measured using a temperature probe (LI-COR #6000-09TC) inserted to a depth of  $\sim 15$  cm near the

soil collar at each sampling time. From each plot, soil and root samples (explained in the following section) were collected using a soil corer of known volume ( $100 \text{ cm}^3$ ) in the top 10 cm of soil within root inclusion areas. Soil moisture was determined on  $\sim 5$  g of field moist soil, dried at  $105^\circ\text{C}$  for 48 h, to calculate gravimetric content. Available nitrate ( $\text{NO}_3^-$ ) and available ammonium ( $\text{NH}_4^+$ ) in 1:10 field fresh soil to KCl solution extractions were measured colorimetrically on a flow injection analyzer (QuikChem8500; Lachat Instruments, Milwaukee, WI, United States). Another subsample of soil was dried and ground and analyzed for total C and N with an elemental analyzer (CN 628, LECO Instruments, Mississauga, ON, Canada). All soil chemical analyses were completed at University of Toronto Scarborough.

## Root Sampling and Analysis

At the start of the experiment, fine roots were removed from root-exclusion sub-plots to sample root traits and corresponding microbial abundance in rhizosphere soil, which is explained in the microbial sampling sections. Over the course of the experiment, and at the time of each soil respiration sampling date, roots were extracted from  $100 \text{ cm}^3$  soil cores collected from the 0–10 cm soil depth. After soil was sub-sampled for soil properties, roots were extracted by washing samples over sieves, further cleaned to remove adhering soil particles, and then processed for further analysis. We focused our analysis on absorptive fine roots, which are most responsible for nutrient uptake and have the highest respiration rates (McCormack et al., 2015), and excluded rhizomes collected from grasses and “transport” roots from woody vegetation (see McCormack et al., 2015; Borden et al., 2020). Image analysis of root morphology (total length and average diameter) was measured using WinRhizo 2019a (Reagent Instruments Inc., Canada), and then standardized using the dry weight biomass of root samples after 48 h at  $60^\circ\text{C}$ . Dried root samples were then ground and analyzed for total C and N using an elemental analyzer (CN 628, LECO Instruments, Mississauga, ON, Canada).

With root length data from each sampling volume, we calculated fine root length density (FRLD;  $\text{cm cm}^{-3}$ ), which excluded rhizomes and root orders  $> 3$  of woody plants. At the scale of individual roots, we measured traits that are positively associated with resource acquisition: specific root length (SRL;  $\text{m g}^{-1}$ ), and root nitrogen content ( $N_{\text{root}}$ ;  $\text{mg g}^{-1}$ ); and root traits positively associated with root tissue longevity: average root diameter ( $D$ ; mm), and root C to N ratio ( $\text{C:N}_{\text{root}}$ ). We also calculated the specific root respiration ( $R_{\text{root}}$ ;  $\text{nmol CO}_2 \text{ g}^{-1} \text{ s}^{-1}$ ), by dividing  $R_{a+r}$  respiration by the absorptive fine root biomass of that same plot on the same sampling date, as a standardized indicator of root-rhizosphere activity (Makita et al., 2012; Roumet et al., 2016; Zhou et al., 2018).

## Microbial Sampling and Analysis

Microbial sampling and analysis occurred separately for soil adhering to roots (rhizosphere) and bulk soil. Rhizosphere soil (DNA) was analyzed for targeted genes to quantify bacteria and fungi abundance. Bulk soil (cDNA) was further analyzed for potential activity of targeted transcripts of bacteria and fungi,

and sequenced in order calculate microbial diversity. Methods for both are described below and a summary of sampling, processing, and analysis steps is provided in **Supplementary Table 2**.

## Microbial Sampling

### *Rhizosphere soil*

For microbial analysis of rhizosphere soil, we collected all roots including attached soil (after shaking) that were exhumed from each root exclusion sub-plot. In the lab, absorptive roots were homogenized and subsampled into three batches from each plot. Rhizosphere soil was separated from the roots using the methods described previously by Donn et al. (2014). The adhering soil and the root samples were vortexed thrice for 30 s each time, and 20 mL from the rhizosphere soil mixture obtained was centrifuged at 5000 r.p.m for 15 min at 5°C. The resulting pellets were stored at −20°C prior to DNA extraction. Roots were rinsed and stored at 4°C until processing, as described in section “Root Sampling and Analysis.”

### *Bulk soil*

For microbial analysis of bulk soil, bi-weekly soil samples were collected from both root exclusion and root inclusion sub-plots on the same day that soil respiration, soil, and roots were sampled. Three random soil samples were collected from 0–10 cm depth, gently homogenized, and ~2 g of composited sample was immediately transferred into pre-weighed sterile tubes containing 3 mL of LifeGuard soil preservation solution (MO BIO Laboratories, Inc., Carlsbad, CA, United States) to stabilize the RNA. Tubes were stored and transported on ice, then transferred to −80°C freezer. Based on soil respiration data we selected two dates for intensive sequencing analysis. We chose dates that showed (i) large CO<sub>2</sub> emission rates representative of the peak of vegetative growth (July 4) and (ii) later in the summer when emissions remained high and stabilized (August 15).

## Nucleic Acid Extraction and Quantitative Real-Time PCR

From rhizosphere soil, DNA was extracted using the PowerSoil DNA Isolation Kit (QiagenR Valencia, CA, United States) following the manufacturer's protocol. From bulk soil samples, RNA and DNA were co-extracted using RNeasy PowerSoil™ Total RNA Kit and DNA Elution Kit (Qiagen®, Valencia, CA, United States) following the manufacturer's instructions. The RNA obtained was subjected to DNase treatment and then reverse transcribed to complementary DNA (cDNA) suitable for qPCR.

The total bacterial (16S rRNA) and fungal (18S rRNA) genes and transcripts from rhizosphere and bulk soil, respectively, were quantified by performing qPCR. Primer pairs 338F/518R (16S rRNA; Fierer et al., 2005) and FF390/FR1 (18S rRNA; Vainio and Hantula, 2000) were used for target genes and transcripts (further details provided with **Supplementary Table 3**). Samples were analyzed in duplicates in 96-well PCR plates with a Bio-Rad CFX detection system (Bio-Rad Laboratories, Inc., Hercules, CA, United States). The PCR efficiency, R<sup>2</sup>, and slope of the standard curve for quantification were 16S (101.9%, 0.99, and −3.27), 18S (99.2%, 0.98, and −3.34).

## Sequencing and Bioinformatics of Bulk Soil Microbial Communities

High-throughput Illumina MiSeq sequencing approach was used to quantify the diversity of bacterial and fungal communities from bulk soil in root inclusion sub-plots. The extracted cDNA was sent to McGill University and Génome Québec Innovation Center, Montréal (Québec) Canada, and analyzed via the Illumina MiSeq platform (Illumina Inc., San Diego, CA, United States) using 515F/806R (bacteria) and ITS1F/ITS2 (fungi) primer sets (White et al., 1990; Gardes and Bruns, 1993; Apprill et al., 2015; Parada et al., 2016). Sequenced data from Illumina fastq files for bacterial 16S rRNA and fungal ITS transcripts were processed and analyzed using Quantitative Insights Into Microbial Ecology (QIIME 2, version 2019.1). The SILVA-132 and UNITE databases were used to assign taxonomy to the amplicon sequence variants (ASVs) for bacteria and fungi, respectively. Alpha diversity metrics such as Shannon's diversity index, Observed ASVs, and Faith's Phylogenetic Diversity and Pielou's evenness were computed from QIIME.

## Statistical Analysis

All statistical analyses were completed in R 3.5.0 (R Core Team., 2019). Our analyzed dataset from four riparian buffer types with four sampling plots each over eight sampling times includes  $n = 120$  soil, root data and respiration measurements,  $n = 38$  bulk soil microbial data, as well as an initial collection of  $n = 14$  paired microbial rhizosphere soil and root trait data. One plot in the rehabilitated forest was omitted due to concern of effects from streambank erosion. Parametric assumptions of normality were evaluated visually and using the Shapiro-Wilk test. When necessary, variables were square root ( $R_s$ ,  $R_h$ ,  $R_{a+r}$ , FRLD, soil available N) or log transformed (roots: SRL,  $N_{root}$ , D, C: $N_{root}$ ,  $R_{root}$ ; soil: moisture, temperature, C, C:N; microbial: 16S, 18S).

To characterize the range of soil physico-chemical properties among the buffer types, variable means are presented in **Supplementary Table 1**. Cumulative daytime CO<sub>2</sub> emissions over an 84-day period (Mg CO<sub>2</sub>-C ha<sup>−2</sup>) were calculated by linear interpolation of the bi-weekly measurements from June 6 and August 29 using the *gasfluxes* package (Fuss, 2019) and differences among the buffer types were evaluated using ANOVA. We also evaluated the assumptions that establishment of root-exclusion sub-plots had minimal effect on abiotic and biotic conditions in soil during our experiment by using paired t-tests on soil moisture, soil temperature, abundance and activity of bacteria and fungi communities (16S and 18S genes and transcripts) between paired inclusion and exclusion sub-plots on the same sampling date.

Using our data set of root traits paired with soil respiration measurements, we evaluated how  $R_{root}$  covaries with root traits in a principal component analysis (PCA) using the *vegan* package (Oksanen et al., 2019). This approach quantifies the strength of covariation and trade-offs in root trait expression on the dominant PCA axes. We evaluated the influence of the type of riparian buffer and sampling date, and their interaction, on coordinated root trait expression (PCA axis scores) using two-way ANOVA. We also assessed overall root trait covariation

with microbial abundance in a second PCA using our dataset containing paired root traits and bacteria and fungi abundances in rhizosphere soil.

We assessed drivers of soil respiration rates in riparian buffers using two approaches. In our first approach using the full data set, linear mixed models (LMM) fitted with REML in the *nlme* package (Pinheiro et al., 2018) quantified how soil and root variables explained  $R_s$  and its components:  $R_h$  and  $R_{a+r}$ . Fixed effects were selected *a priori* based on known dominant controlling factors for microbial and/or root respiration: soil moisture, soil temperature, available N in soil ( $\text{NO}_3^-$  and  $\text{NH}_4^+$ ), soil C, soil C:N, rooting density (FRLD), and we also included overall absorptive root syndromes by using PCA axes scores. We treated sampling plot nested in riparian buffer type as a repeated measure assigned as a random effect. We then assessed the proportion of variance explained by the continuous soil and root variables alone (marginal  $r^2$ ) versus when random effects of riparian buffer and sampling plot are included (conditional  $r^2$ ) (Nakagawa and Schielzeth, 2013). The proportions of variation explained by included random effects were estimated using variance decomposition with the *ape* package (Paradis et al., 2004). In our second approach, using the subset of data from two sampling dates with observations of microbial community structure in bulk soil, we used multiple factor analysis (MFA) to assess how groups of variables: microbial abundances, microbial diversity indices, root traits, and soil properties, covary together or independently with each other and with  $R_h$ . Riparian buffer type and sampling date were assigned as supplementary variables. Basically, in this analysis PCA is performed first separately for each group of variables, then the resulting standardized group-based PCA data are used to perform an overall PCA to evaluate how the groups of variables covary. We evaluated group similarity (correlation) by calculating the RV coefficient and performing a Monte Carlo permutation ( $n = 1000$ ) to test if group correlations were significantly different than when randomly generated. For MFA, we used the “FactoMineR” (Le et al., 2008) and “ade4” (Dray and Dufour, 2007) packages.

## RESULTS

### Total, Heterotrophic, and Autotrophic-Rhizospheric Respiration

Soil respiration rates fluctuated over the growing season in the grass buffer and rehabilitated forest and were generally above that of coniferous forest and mixed forest (Figure 1). Average daytime total soil respiration rates were  $186.1 \pm 26.7$ ,  $188.7 \pm 23.0$ ,  $278.6 \pm 30.0$ , and  $503.4 \pm 31.3$  mg  $\text{CO}_2\text{-C m}^{-2} \text{ h}^{-1}$  in the coniferous, mixed, rehabilitated forest, and grass buffer, respectively. Percent contributions of  $R_{a+r}$  to  $R_s$  ranged from 14 to 63% and was proportionally high in the grass buffer and coniferous forest >40% (Figure 1). Estimated cumulative  $R_h$  emissions from daytime measurements ranged between  $2.1 \pm 2.0$  (coniferous forest) to  $5.3 \pm 1.6$  (grass) Mg  $\text{CO}_2\text{-C ha}^{-1}$  and  $R_{a+r}$  emissions ranged between  $1.5 \pm 1.5$  (mixed forest) to  $5.8 \pm 2.2$  (grass) Mg  $\text{CO}_2\text{-C ha}^{-1}$  (Table 1). There were significant differences among riparian buffer types for

$R_s$  emissions ( $p = 0.009$ ) and  $R_{a+r}$  emissions ( $p = 0.01$ ) but not for  $R_h$  emissions ( $p = 0.09$ ) (Table 1). Grass buffer had significantly higher  $R_{a+r}$  emissions and corresponding  $R_s$  emissions compared to coniferous forest and mixed forest but had non-significant ( $p = 0.13$ ) and marginally significant ( $p = 0.09$ ) differences from rehabilitated forest in  $R_s$  and  $R_{a+r}$  emissions, respectively (Table 1). There were no significant differences in soil moisture, soil temperature, and microbial (16S and 18S) activity or abundance between paired root exclusion and inclusion sub-plots, except for  $0.5^\circ\text{C}$  higher soil temperature in exclusion sub-plots on July 4 ( $p < 0.01$ ) (Supplementary Figure 1).

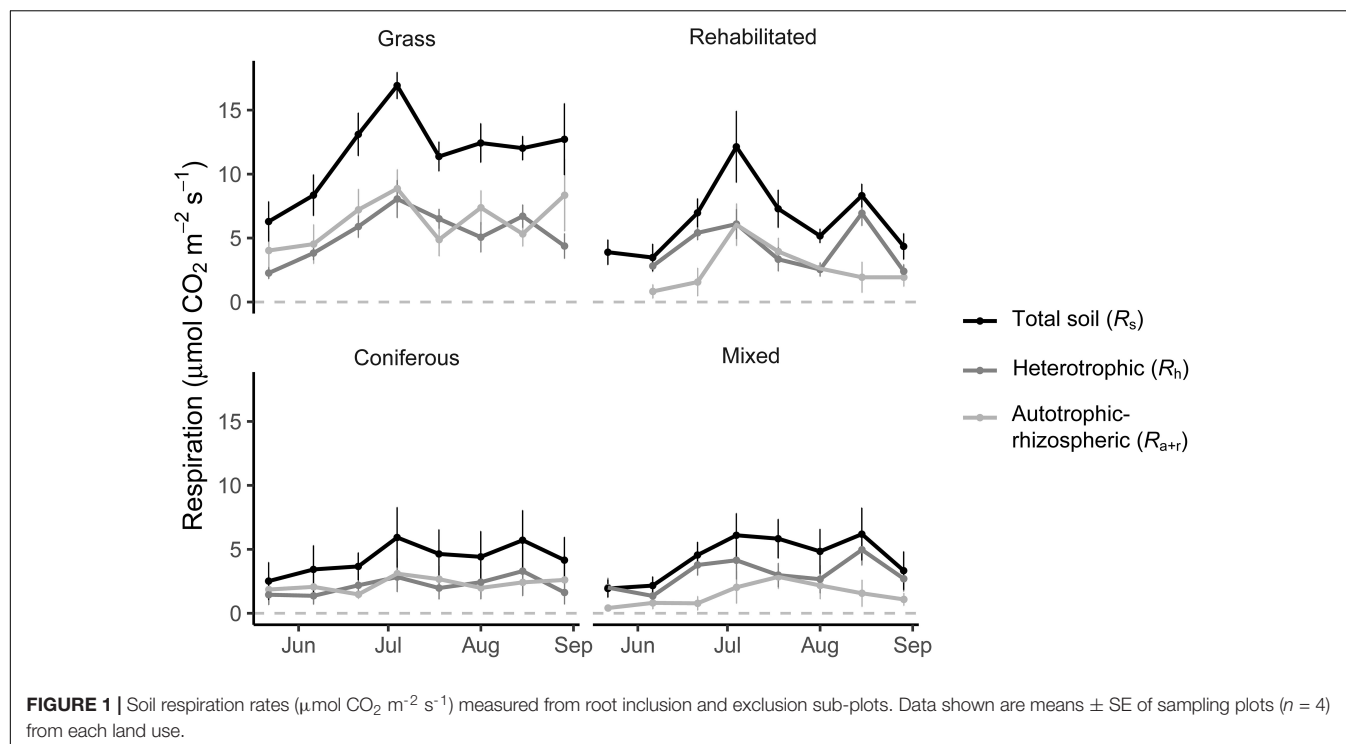
### Covariation of Root Traits and Microbial Communities

Covariation in absorptive fine root traits was well explained by the first principal component axis (~50 to 60% of total covariation) both for our full-season trait data set with respiration rates (Figure 2A) as well as the analysis of roots and microbial communities in rhizosphere soil (Figure 2B). On this axis, roots expressing higher SRL and  $N_{root}$  also had higher  $R_{root}$ , and were in opposition to roots expressing thicker D, higher C:N $_{root}$ . All root traits were significantly correlated with PC1 (Supplementary Table 4). In rhizosphere soil, PCA of root traits and rhizosphere microbial communities indicate that higher abundances of 16S and 18S coordinated with thicker D and higher C:N $_{root}$  on PC1 (Figure 2B). PC2 explained >20% of total covariation (Figure 2) and in the respiration data set, shows a trade off in roots with higher SRL, C:N $_{root}$ , and  $R_{root}$  opposed to roots that have thicker D and higher  $N_{root}$ . For roots with microbial abundance measured in the rhizosphere, C:N $_{root}$  was strongly featured on PC2 in a trade-off with  $N_{ab}$  and 18S (Figure 2B; Supplementary Table 4).

The type of riparian buffer was important in controlling the relative position of individual observations on PC1 ( $F_{3,99} = 82.72$ ;  $p < 0.001$ ) with roots in grass > rehabilitated forest > mixed forest > coniferous forest having relatively higher PC1 scores (i.e., higher SRL, N, and  $R_{root}$ ). Date was also significant ( $F_{1,99} = 3.25$ ;  $p = 0.07$ ) with PC1 scores shifting lower (i.e., thicker D, higher C:N $_{root}$ , and lower  $R_{root}$ ) later in the summer compared to earlier sampling dates. There was no interaction of buffer type  $\times$  date on PC1 scores ( $F_{3,99} = 2.56$ ;  $p = 0.08$ ). For PC2 scores, there were significant effects from buffer type ( $F_{3,99} = 6.96$ ;  $p < 0.001$ ), with PC2 scores relatively higher for grass > mixed > coniferous > rehabilitated forest. Broadly, PC2 scores increased (i.e., higher SRL, C:N $_{root}$ , and  $R_{root}$ ) over the season, with a significant main effect of date ( $F_{1,99} = 20.02$ ;  $p < 0.001$ ) but the extent of this increase over time depended on buffer type, with a significant buffer type  $\times$  date interaction ( $F_{3,99} = 6.85$ ;  $p < 0.001$ ).

### Abiotic and Biotic Soil Environment Variables in Relation to Soil Respiration

Our experiment occurred over a range of soil abiotic soil conditions in the study sites on the same creek system (Supplementary Table 1). In explaining  $R_s$  and its components ( $R_h$  and  $R_{a+r}$ ), soil temperature was consistently a significant



**FIGURE 1** | Soil respiration rates ( $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ) measured from root inclusion and exclusion sub-plots. Data shown are means  $\pm$  SE of sampling plots ( $n = 4$ ) from each land use.

positive predictor (Table 2). Soil moisture was a negative predictor of  $R_s$  and  $R_h$ , while available  $\text{NH}_4^+$  was a negative coefficient for  $R_s$  and soil C was a positive coefficient of  $R_h$ . For  $R_{a+r}$  the only significant explanatory variables other than soil temperature were root variables: positive FRLD and positive PC1 axis scores and PC2 axis scores associated with higher  $R_{root}$  (Table 2). Fixed effect variables explained 49 to 61% of variation in soil respiration and its components, and when also accounting for random effects (i.e., non-measured effects of inherent differences among the riparian buffer types, or among individual sampling plots within each buffer type) improved the explained variance to between 75 and 86% (Table 2). Variance decomposition showed the repeated measure on the sampling plots nested in buffer type contributed the most to the improvement in explained variance, by 20.4, 16.0, and 16.2% for  $R_s$ ,  $R_h$ , and  $R_{a+r}$ , respectively, while buffer type (i.e., other site level differences that were not measured and included as fixed effects) contributed only 2.4, 0.3, and 0.9% for  $R_s$ ,  $R_h$ , and  $R_{a+r}$ , respectively.

Multiple factor analysis revealed grouped variables: root traits and soil properties were significantly correlated ( $RV = 0.32$ ; Table 3), such that roots with thicker D and higher  $\text{C:N}_{root}$  were aligned with soil high in moisture, available  $\text{NH}_4^+$ , and soil C, while roots with higher SRL,  $\text{N}_{root}$ , and  $R_{root}$  were associated with soil with higher available  $\text{NO}_3^-$  (Figure 3). Heterotrophic respiration was related to covariation in soil properties ( $RV = 0.38$ ) and less so to covariation in root traits ( $RV = 0.12$ ) (Table 3). We observed a range of microbial diversity in bulk soil, with significantly higher diversity in the rehabilitated forest buffer than in the coniferous forest buffer (Supplementary Table 5). However, there was no

significant difference in microbial abundances among buffer types (Supplementary Figure 2). In MFA, microbial diversity indices were collectively positively correlated with  $R_h$  ( $RV = 0.35$ ), while microbial abundance was not ( $RV = 0.04$ ) (Table 3). Microbial abundance and diversity in bulk soil were independent to root trait covariation (Figure 3 and Table 3).

## DISCUSSION

### Coordination Belowground? Root Traits and Microbial Communities

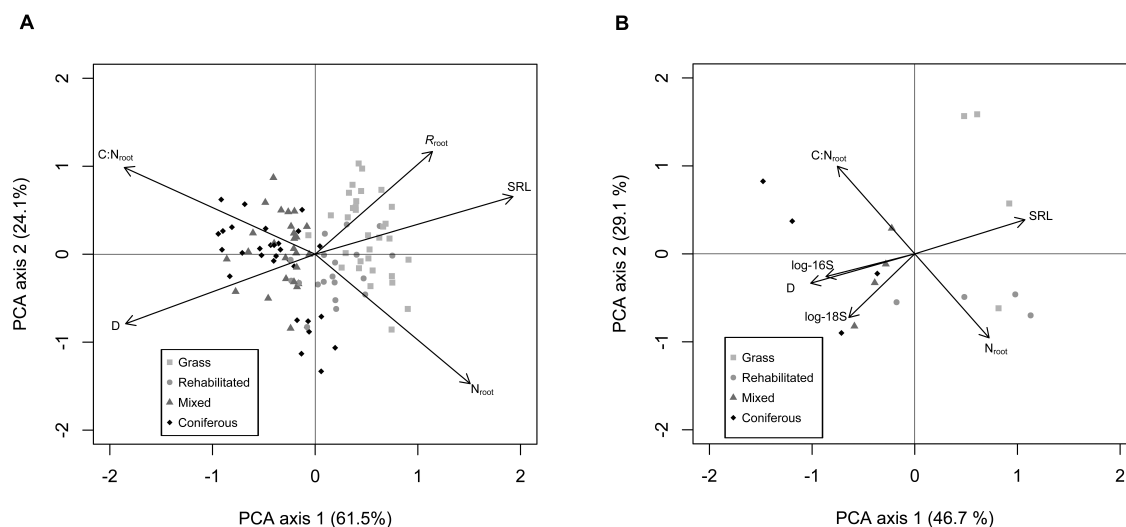
Our results show for the first time, to our knowledge, a dominant root trait axis inclusive of  $R_{root}$ . Specific root respiration was associated with roots expressing higher SRL and  $\text{N}_{root}$

**TABLE 1** | Cumulative soil  $\text{CO}_2$  emissions ( $\text{Mg C ha}^{-1}$ ) between June and August 2018 (84-day sampling period) estimated from measured daytime emissions.

Buffer type	Heterotrophic ( $R_h$ )	Autotrophic-rhizospheric ( $R_{a+r}$ )	Total ( $R_s$ )
Grass	$5.72 \pm 0.87$	$6.46 \pm 1.35$ a	$12.18 \pm 1.25$ a
Rehabilitated	$3.89 \pm 0.53$	$2.51 \pm 0.51$ ab	$6.88 \pm 1.10$ ab
Coniferous	$2.24 \pm 1.09$	$2.29 \pm 0.83$ b	$4.54 \pm 2.00$ b
Mixed	$3.19 \pm 0.96$	$1.57 \pm 0.78$ b	$4.69 \pm 1.39$ b

Same letters indicate no significant differences in respiration component between riparian buffer types.





**FIGURE 2 |** Principal component analysis (PCA) of absorptive fine root traits: D = average root diameter (mm); C:N<sub>root</sub> = root C to N ratio; SRL = specific root length ( $\text{m g}^{-1}$ ); N<sub>root</sub> = root N content ( $\text{mg g}^{-1}$ ); R<sub>root</sub> = specific root-rhizosphere respiration ( $\text{nmol CO}_2 \text{ g}^{-1} \text{ s}^{-1}$ ) from full data set ( $n = 120$ ) **(A)**. PCA of absorptive fine root traits measured with paired rhizosphere soil analysis of bacterial (16S) and fungal (18S) abundance **(B)**.

**TABLE 2 |** Coefficients of linear mixed models to predict total soil respiration ( $R_s$ ), heterotrophic respiration ( $R_h$ ), and autotrophic-rhizospheric respiration ( $R_{a+r}$ ).

	Soil variables							Root variables			Marginal $r^2$	Conditional $r^2$
Soil respiration component	Intercept	log-soil moist.	log-soil temp.	sqrt-soil NO <sub>3</sub> <sup>−</sup>	sqrt-soil NH <sub>4</sub> <sup>+</sup>	log-soil C	log-soil C:N	sqrt-FRLD <sub>ab</sub>	PC1	PC2		
$R_s$	−1.905	<b>−1.463</b>	<b>0.064</b>	0.031	<b>−0.052</b>	0.629	1.521	0.121	<b>0.488</b>	<b>0.438</b>	0.61	0.86
$R_h$	−2.288	<b>−1.906</b>	<b>0.044</b>	0.015	−0.024	<b>1.364</b>	0.294	0.017	0.268	0.117	0.50	0.82
$R_{a+r}$	−2.474	−0.336	<b>0.044</b>	0.032	−0.049	−0.146	2.966	<b>0.178</b>	<b>0.394</b>	<b>0.576</b>	0.49	0.75

Coefficients of fitted fixed effects are reported with significant coefficients in bold. Total variation explained by fixed effects of soil and root variables (marginal  $r^2$ ) and when random effects (sampling plot nested in land use) are included (conditional  $r^2$ ) are shown.

(Figure 2A). This observed root trait covariation support a hypothesized “root economics spectrum” (Kong et al., 2014; Roumet et al., 2016; Borden et al., 2020) related to the C economy in plants (Roumet et al., 2016).

Bacteria and fungi abundance in rhizosphere soil were also aligned with root trait covariation, associating with roots of thicker D and lower SRL, and for fungi abundance, roots with thicker D and higher N<sub>root</sub> (Figure 2B). These results follow previously identified trade-offs in resource acquisition strategies on a root-microbe continuum (Ma et al., 2018; Bergmann et al., 2020) [i.e., when resource acquisition is “outsourced” to associated fungi (Bergmann et al., 2020)]. Conversely, in bulk soil, root trait covariation seemed unrelated to microbial activity (Figure 3). Other studies report mixed results. For instance, De Long et al. (2019) observed that higher root N was positively related with higher fungal and bacterial abundances in bulk soil, while Leff et al. (2018), found no relationships between root traits and bacteria or fungi community composition in bulk soil. In this study, it appears that covariation of soil microbial abundances in rhizosphere soil with root traits may not be due to the underlying microbial abundances in bulk soil, with potential differences in microbial communities between rhizosphere soil and bulk soil (Donn et al., 2014).

We observed generalized shifts in root traits among riparian buffers and sampling dates (Figure 2A). While our study was designed to examine community-level root trait variation, it is likely that not only species composition but also phenological variation and/or intraspecific variation may also have an effect on expression of root traits. For example, nutrient gradients can shift root morphology and anatomy within species (Wang et al., 2017; Borden and Isaac, 2019). Similarly, Donn et al. (2014) found that microbial community structure changed over time in rhizosphere but not in bulk soil, suggesting plant phenology-driven effects on the soil microbiome. As we observed a significant effect of sampling time on root trait expression, it could be expected that the rhizosphere microbial profile and relationship with root traits could vary over the growing season. Additional work is needed to test effects of seasonality.

## Disentangling Belowground Drivers of Soil Respiration

Respiration from heterotrophic and autotrophic processes in soil occur and vary at different spatial and temporal scales (Gomez-Casanovas et al., 2012; Jacinthe and Vidon, 2017). Our study and previous research show high heterogeneity of CO<sub>2</sub> emissions

**TABLE 3 |** RV coefficients (top right of table) between groups of abiotic and biotic environment variables and heterotrophic respiration ( $R_h$ ) from multiple factor analysis (Figure 3).

	$R_h$	Soil properties	Microbial abundance	Microbial diversity	Root traits
$R_h$	–	<b>0.38</b>	0.04	<b>0.35</b>	<b>0.12</b>
Soil properties	0.005	–	0.08	0.18	<b>0.32</b>
Mic. abundance	0.279	0.202	–	0.14	0.04
Mic. diversity	0.001	0.104	0.679	–	0.10
Root traits	0.031	0.002	0.670	0.062	–

Coefficients are in bold if they are significantly ( $p < 0.05$ ) correlated based on simulated  $p$ -values from a Monte Carlo test (bottom left of table).

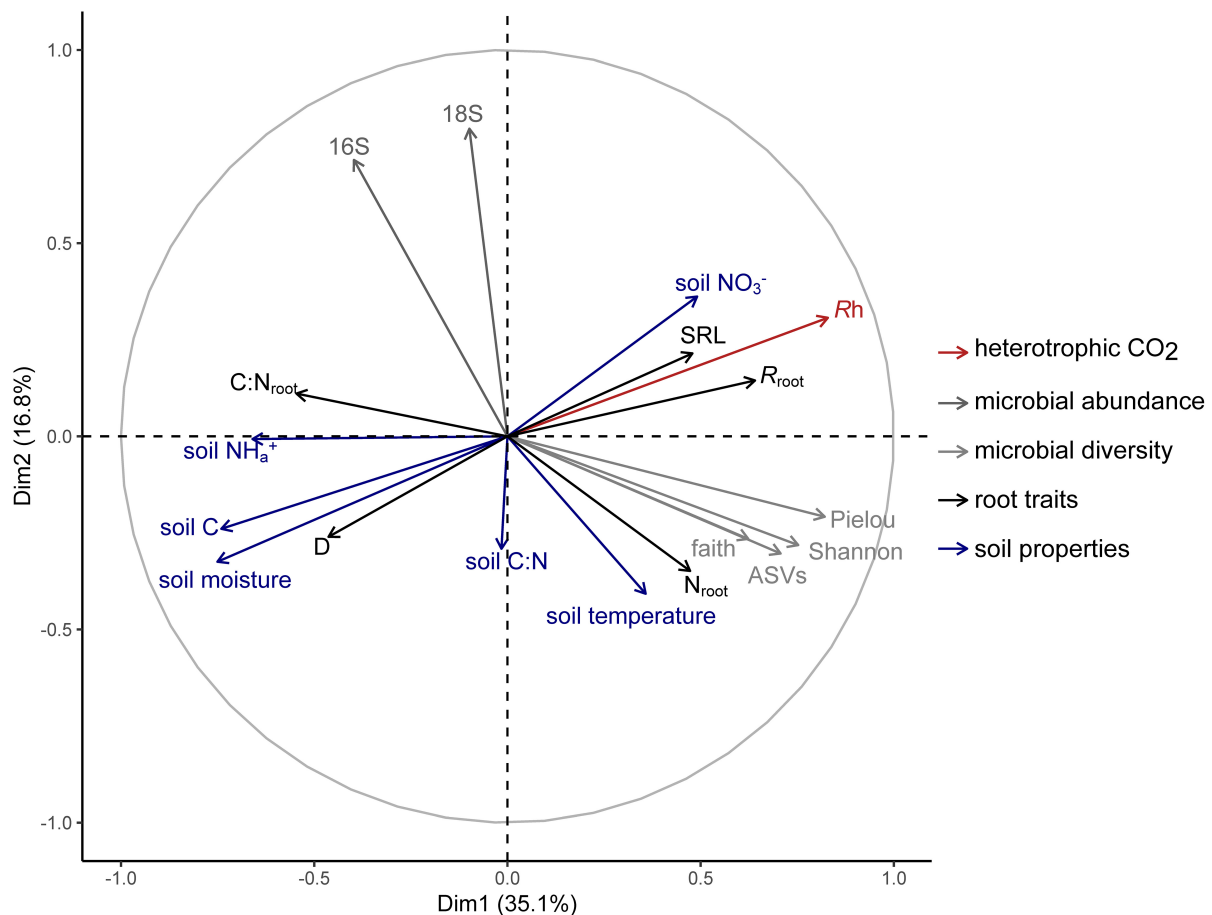
and soil conditions within these riparian buffers (De Carlo et al., 2019). Over the growing season soil respiration varied and peaked in early July, and markedly so in the grass buffer (Figure 1), driven by the high density of roots. Steinauer et al. (2017) found that denser root systems (i.e., amount of roots in soil) and shallower root system (i.e., location of roots in soil) resulted in higher total soil respiration in grasslands. In our study, not only the density of roots in soil but the expressed root traits led to higher  $R_{a+r}$  (Table 2). Thus, organ-level variation in roots may also have a predictable impact on autotroph-rhizosphere respiration measured on a soil area basis, and these patterns may influence CO<sub>2</sub> emissions from soil over a growing season. Broadly, our findings of systematic variation of root traits in predicting respiration rates within and across the studied buffer types contribute to identifying empirical relationships that are meaningful for ecosystem-level analysis across a range of scales and riparian agroecosystems. Notably, root trait covariation remained important predictors of total soil respiration, thus supporting root functional trait integration into biogeochemical models of total soil respiration.

Root respiration is thought to correlate positively with root decomposition rates (Prieto et al., 2016; Roumet et al., 2016). Thus, it would be expected the root traits will relate to how root litter inputs affect heterotrophic respiration. However, in our study, evidence of root effects on soil processes was weak to negligible. Covariation in root traits showed a weak association with  $R_h$  from MFA (Table 3) and, when accounting for individual soil properties, was not a predictor of  $R_h$  in the linear mixed model (Table 2). A lack of association between root traits and microbial respiration in bulk soil could be due to the temporal lag between root turnover and decomposition of roots which is not captured in the time scale of our study. Similarly, Ferlian et al. (2017) found soil stoichiometry, rather than root stoichiometry, to be a main determinant of soil microbial biomass and total soil respiration from soil collected near roots of angiosperm trees. On the other hand, microbial diversity showed a stronger correlation with  $R_h$ . Liu et al. (2020) found systematic trends in microbial communities in predicting total soil respiration rates across distinct rice paddies and corn-wheat cropping environments. A greater diversity of microorganisms in soil may enable decomposition of a wider range of soil organic matter,

thus leading to overall higher rates of respiration from soil (Maron et al., 2018).

Biogeochemical models require identifying key abiotic controls of biological activity that collectively drive C exchange (Fry et al., 2019). The two major abiotic controls of soil respiration: moisture and temperature, which regulate biological activity and gas diffusion in soil, were strong explanatory variables of  $R_h$ . Due to large variability in soil moisture in riparian systems within a buffer zone and over the growing season, soil hydrological conditions may be particularly important in controlling belowground respiration (Gordon et al., 1987; Jacinthe and Vidon, 2017). For root-derived respiration, soil temperature was an important control of  $R_{a+r}$ , reflecting the importance of soil temperature on root growth and activity (Boone et al., 1998). While not empirically tested in this study, relatively high soil pH and soil inorganic C in the rehabilitated buffers compared to the remnant forest buffers, may also control site-level soil respiration rates. On larger soil pH gradients, heterotrophic respiration has been found to increase with higher soil pH, while root respiration decreases (Chen et al., 2016). Additionally, and particularly in the rehabilitated buffers, the proportion of CO<sub>2</sub> emissions from soil originating from carbonates (soil inorganic C) could be significant, and CO<sub>2</sub> from microbial respiration may react with carbonates in soil (Ramnarine et al., 2012). More detailed analysis of sources of CO<sub>2</sub> emissions could reveal complex C exchanges in the soil profile at these sites.

During the timeframe of this field experiment, we assumed that there was negligible influence of root removal on the soil environment between inclusion and exclusion sub-plots, which validated calculated  $R_{a+r}$ . From our comparative test on soil moisture and temperature and total microbial abundance and activity between paired sub-plots, this assumption held. The only exception was on one sampling date when soil temperature in the exclusion sub-plots were warmer than in paired inclusion sub-plots demonstrating the effects of vegetation on regulating soil temperature and considerations when carrying out root exclusion field studies. Shade cloth over exclusion sub-plots may improve the similarity between sub-plots particularly during a peak in summer temperatures and solar irradiance. Gentle removal and replacement of soil in the exclusion plots at matched depths was carried out to avoid disturbing microaggregates and maintain initial soil compaction as best as possible. However, bulk density may have changed, particularly in plots that had a high density of roots, and it is likely there was some disturbance to soil macro- and micro-porosity. Additionally, while total bacteria and fungi abundance and activity were similar between the inclusion and exclusion sub-plots, additional work is needed to understand potential effects on microbial community composition given our finding that  $R_h$  positively correlated with microbial diversity. Despite these possible sources of error, these destructive studies remain a viable method to differentiate heterotrophic from root-derived respiration in the field when isotopic analysis (i.e., <sup>13</sup>C natural abundance or tracer methods) is not applicable or impractical for extensive field-deployed studies.



**FIGURE 3 |** Multiple factor analysis of heterotrophic respiration ( $R_h$ ) and grouped biotic and abiotic soil environment variables: microbial abundance [bacterial (16S) and fungal (18S) abundance]; microbial diversity [Shannon's diversity index, Observed ASVs, Faith's Phylogenetic Diversity, and Pielou's evenness]; root traits [average root diameter (D), root C to N ratio (C:N<sub>root</sub>), specific root length (SRL), root N content ( $N_{root}$ ), and specific root-rhizosphere respiration ( $R_{root}$ )]; and soil properties [soil moisture, soil temperature, total soil C, soil C:N, available  $\text{NO}_3^-$ , and available  $\text{NH}_4^+$ ].

## Riparian Agroecosystem Management Implications

Riparian buffers can lessen the negative impacts of agricultural production on the aquatic environment, but less is known on greenhouse gas emissions to the atmosphere. Therefore, accurate estimates of  $\text{CO}_2$  emissions from riparian agroecosystems are necessary for assessing climate change mitigation versus warming potential. However, the diversity of management and vegetative structure and composition (i.e., in existing forest, new forest, or other perennial buffers) can be challenging to draw generalizable conclusions. In this regard, frameworks that can systematically measure and compare ecosystem processes across diverse riparian systems are useful. De Long et al. (2019) found that plant traits and soil properties improved predictions of C fluxes in the ecosystem (ecosystem respiration and net ecosystem exchange) in plant monocultures but not in mixed communities. In our study, we found evidence of coordinated belowground root-microbe strategies and correlation between root traits and autotrophic-rhizospheric respiration (i.e., root-derived respiration) and total soil respiration, suggesting measurable integration across trophic

levels and an application to improve modeled C based on belowground plant traits.

Distinguishing sources of  $\text{CO}_2$  allows for more refined predictions of climate mitigation and  $\text{CO}_2$  emissions that differ in spatial and temporal scales. Soil respiration in temperate riparian systems is markedly highest during summer months (Oelbermann et al., 2014; De Carlo et al., 2019). Thus, the major differences in  $\text{CO}_2$  emissions among riparian buffer types were likely captured during the sampling period of the present study, although our results do not represent annual patterns. Differences of cumulative emissions based on daytime measurements among buffer types were accentuated by autotrophic-rhizospheric respiration, particularly in a grass buffer with high density of fine roots (Table 1 and Figure 1). Thus, in certain types of riparian buffers, root-derived respiration can substantially elevate total  $\text{CO}_2$  emissions from soil.

However, soil respiration indicates higher biological activity in soil and relates to other important ecological processes. In different studies at the same study sites, nitrous oxide ( $\text{N}_2\text{O}$ ) emissions were relatively low in the rehabilitated grass buffer,

with observed differences in nitrifying bacteria community structure (Mafa-Attoye et al., 2020), while methane (CH<sub>4</sub>) emissions were starkly higher in remnant mixed forest buffer (Buchanan et al., 2020) where soil moisture levels were highest. Given that N<sub>2</sub>O and CH<sub>4</sub> have, respectively, 298 and 25 times the warming potential as CO<sub>2</sub>, full assessment of greenhouse gases fluxes and their trade-offs are required to optimize riparian buffer management for climate change mitigation. In rehabilitating or maintaining existing riparian forests and grassland buffers, a refined understanding of sources of respiration and their interactions with key abiotic controls is essential for accurate estimates of net ecosystem exchange of C and as part of a full assessment of ecosystem services in the agricultural landscape.

## CONCLUSION

This is one of the first field studies to examine interactions between covariation of root traits and soil microbial community abundance and diversity to investigate sources of soil respiration. Our findings reveal strong covariation of root traits with specific root respiration rates and this coordination in root traits was important in predicting soil CO<sub>2</sub> emissions on an area basis. We also show that root traits covaried with microbial abundance in rhizosphere soil but not in bulk soil, while microbial diversity in bulk soil was positively associated with heterotrophic respiration. As respiration from soil represents the major pathway of C transfer from the ecosystem to the atmosphere, strategic management and design of riparian buffers can begin to adopt functional trait approaches to assess the belowground processes that regulate delivery of key ecological services for climate change mitigation.

## DATA AVAILABILITY STATEMENT

The raw sequence data are deposited in NCBI SRA under the project PRJNA718381. The non-sequencing data are

deposited in Scholars Portal Dataverse: <https://doi.org/10.5683/SP2/O1MKRS>.

## AUTHOR CONTRIBUTIONS

KB, MI, KD, TM-A, and NT conceived and planned the experiments. KB and TM-A carried out the experiments and analyzed the data. KB completed the statistical analysis and wrote the manuscript. MI, KD, NT, and AG managed project funding and provided resources to complete the research. All authors provided critical feedback and helped shape the research, analysis, and manuscript.

## ACKNOWLEDGMENTS

We thank the reviewers for their constructive comments which improved the quality of the manuscript. We graciously thank Brian and Elizabeth Tew and Josie and Jens Madsen for access to research sites on their property. We are also grateful for field assistance from Serra Buchanan, Ato Aggrey, Adrianna Wiley, and Sara Low. We thank Astrid Galvez Ciani, Hinson Hin Heng Hon, and Kamini Khosla for their assistance with laboratory analysis, as well as Dasiel Obregon Alvarez for assistance with bioinformatics. Thanks to Enoch Ofosu for providing soil inorganic carbon data for our study sites. We graciously acknowledge financial support for this research from Agriculture and Agri-Food Canada's Agricultural Greenhouse Gases Program as well funding from the Natural Science and Engineering Research Council of Canada (postdoctoral fellowship to KB).

## SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fpls.2021.681113/full#supplementary-material>

## REFERENCES

- Apprill, A., McNally, S., Parsons, R., and Weber, L. (2015). Minor revision to V4 region SSU rRNA 806R gene primer greatly increases detection of SAR11 bacterioplankton. *Aquat. Microb. Ecol.* 75, 129–137. doi: 10.3354/ame01753
- Bardgett, R. D. (2017). Plant trait-based approaches for interrogating belowground function. *Biol. Environ.* 117, 1–13. doi: 10.3318/bioe.2017.03
- Bergmann, J., Weigelt, A., van der Plas, F., Laughlin, D. C., Kuyper, T. W., Guerrero-Ramirez, N., et al. (2020). The fungal collaboration gradient dominates the root economics space in plants. *Sci. Adv.* 6:eaba3756. doi: 10.1126/sciadv.aba3756
- Boone, R. D., Nadelhoffer, K. J., Canary, J. D., and Kaye, J. P. (1998). Roots exert a strong influence on the temperature sensitivity of soil respiration. *Nature* 396, 570–572. doi: 10.1038/25119
- Borden, K. A., Anglaere, L. C. N., Owusu, S., Martin, A. R., Buchanan, S. W., Addo-Danso, S. D., et al. (2020). Soil texture moderates root functional traits in agroforestry systems across a climatic gradient. *Agric. Ecosyst. Environ.* 295:106915. doi: 10.1016/j.agee.2020.106915
- Borden, K. A., and Isaac, M. E. (2019). Management strategies differentially affect root functional trait expression in cocoa agroforestry systems. *Agron. Sustain. Dev.* 39:21. doi: 10.1007/s13593-019-0567-1
- Borden, K. A., Thomas, S. C., and Isaac, M. E. (2019). Variation in fine root traits reveals nutrient-specific acquisition strategies in agroforestry systems. *Plant Soil* 453, 139–151. doi: 10.1007/s11104-019-04003-2
- Buchanan, S. W., Baskerville, M., Oelbermann, M., Gordon, A. M., Thevathasan, N. V., and Isaac, M. E. (2020). Plant diversity and agroecosystem function in riparian agroforests: providing ecosystem services and land-use transition. *Sustainability* 12:568. doi: 10.3390/su12020568
- Chen, D., Li, J., Lan, Z., Hu, S., and Bai, Y. (2016). Soil acidification exerts a greater control on soil respiration than soil nitrogen availability in grasslands subjected to long-term nitrogen enrichment. *Funct. Ecol.* 30, 658–669. doi: 10.1111/1365-2435.12525
- Chen, Y., Li, S., Zhang, Y., Li, T., Ge, H., Xia, S., et al. (2019). Rice root morphological and physiological traits interaction with rhizosphere soil and its effect on methane emissions in paddy fields. *Soil Biol. Biochem.* 129, 191–200. doi: 10.1016/j.soilbio.2018.11.015



- Christen, B., and Dalgaard, T. (2013). Buffers for biomass production in temperate European agriculture: a review and synthesis on function, ecosystem services and implementation. *Biomass Bioenerg.* 55, 53–67. doi: 10.1016/j.biombioe.2012.09.053
- Coleman, B. R., Martin, A. R., Thevathasan, N. V., Gordon, A. M., Isaac, M. E. (2020). Leaf trait variation and decomposition in short-rotation woody biomass crops under agroforestry management. *Agric. Ecosyst. Environ.* 298:106971. doi: 10.1016/j.agee.2020.106971
- De Carlo, N. D., Oelbermann, M., and Gordon, A. M. (2019). Carbon dioxide emissions: spatiotemporal variation in a young and mature riparian forest. *Ecol. Eng.* 138, 353–361. doi: 10.1016/j.ecoleng.2019.07.036
- De Long, J. R., Jackson, B. G., Wilkinson, A., Pritchard, W. J., Oakley, S., Mason, K. E., et al. (2019). Relationships between plant traits, soil properties and carbon fluxes differ between monocultures and mixed communities in temperate grassland. *J. Ecol.* 107, 1704–1719. doi: 10.1111/1365-2745.13160
- De Vries, F. T., Brown, C., and Stevens, C. J. (2016). Grassland species root response to drought: consequences for soil carbon and nitrogen availability. *Plant Soil* 409, 297–331. doi: 10.1007/s11104-016-2964-4
- Donn, S., Kirkegaard, J. A., Perera, G., Richardson, A. E., and Watt, M. (2014). Evolution of bacterial communities in the wheat crop rhizosphere. *Environ. Microbiol.* 17, 610–621. doi: 10.1111/1462-2920.12452
- Dray, S., and Dufour, A. (2007). The ade4 package: implementing the duality diagram for ecologists. *J. Stat. Softw.* 22, 1–20. doi: 10.18637/jss.v022.i04
- Ferlian, O., Wirth, C., and Eisenhauer, N. (2017). Leaf and root C-to-N ratios are poor predictors of soil microbial biomass C and respiration across 32 tree species. *Pedobiologia* 65, 16–23. doi: 10.1016/j.pedobi.2017.06.005
- Fierer, N., Jackson, J. A., Vilgalys, R., and Jackson, R. B. (2005). Assessment of soil microbial community structure by use of taxon-specific quantitative PCR assays. *Appl. Environ. Microbiol.* 71, 4117–4120. doi: 10.1128/AEM.71.7.4117-4120.2005
- Fortier, J., Truax, B., Gagnon, D., and Lambert, F. (2013). Root biomass and soil carbon distribution in hybrid poplar riparian buffers, herbaceous riparian buffers and natural riparian woodlots on farmland. *Springerplus* 2:539. doi: 10.1186/2193-1801-2-539
- Fulthorpe, R., Martin, A. R., and Isaac, M. E. (2020). Root endophytes of coffee (*Coffea arabica*): variation across climatic gradients and relationships with functional traits. *Phytobiomes J.* 4, 27–39. doi: 10.1094/phyto-04-19-0021-r
- Fuss, R. (2019). Gasfluxes: Greenhouse Gas Flux Calculation From Chamber Measurements. R package version 0.4-3. <https://CRAN.R-project.org/package=gasfluxes>.
- Fry, E. L., De Long, J. R., Álvarez Garrido, L., Alvarez, N., Carrillo, Y., Castañeda-Gómez, L., et al. (2019). Using plant, microbe, and soil fauna traits to improve the predictive power of biogeochemical models. *Methods Ecol. Evol.* 10, 146–157. doi: 10.1111/2041-210X.13092
- Gardes, M., and Bruns, T. D. (1993). ITS primers with enhanced specificity for basidiomycetes-application to the identification of mycorrhizae and rusts. *Molec. Ecol.* 2, 113–118. doi: 10.1111/j.1365-294x.1993.tb00005.x
- Gordon, A. M., Schlentner, R. E., and van Cleve, K. (1987). Seasonal patterns of soil respiration and CO<sub>2</sub> evolution following harvesting in the white spruce forest of interior Alaska. *Can. J. Forest Res.* 17, 304–310. doi: 10.1139/x87-051
- Gomez-Casanovas, N., Matamala, R., Cook, D. R., and Gonzalez-Meler, M. A. (2012). Net ecosystem exchange modifies the relationship between the autotrophic and heterotrophic components of soil respiration with abiotic factors in prairie grasslands. *Glob. Chang. Biol.* 18, 2532–2545. doi: 10.1111/j.1365-2486.2012.02721.x
- Hanson, P. J., Edwards, N. T., Garten, C. T., and Andrews, J. A. (2000). Separating root and soil microbial contributions to soil respiration: a review of methods and observations. *Biogeochemistry* 48, 115–146. doi: 10.1023/A:1006244819642
- Isaac, M. E., and Borden, K. A. (2019). Nutrient acquisition strategies in agroforestry systems. *Plant Soil* 444, 1–19. doi: 10.1007/s11104-020-04670-6
- Jacinthe, P. A., and Vidon, P. (2017). Hydro-geomorphic controls of greenhouse gas fluxes in riparian buffers of the White River watershed. *Geoderma* 301, 30–41. doi: 10.1016/j.geoderma.2017.04.007
- Kong, D., Ma, C., Zhang, Q., Li, L., Chen, X., Zeng, H., et al. (2014). Leading dimensions in absorptive root trait variation across 96 subtropical forest species. *New Phytol.* 203, 863–872. doi: 10.1111/nph.12842
- Kuzyakov, Y., and Larionova, A. A. (2005). Root and rhizomicrobial respiration: a review of approaches to estimate respiration by autotrophic and heterotrophic organisms in soil. *J. Plant Nutr. Soil Sci.* 168, 503–520. doi: 10.1002/jpln.200421703
- Lal, R. (2003). Global potential of soil carbon sequestration to mitigate the greenhouse effect. *Crit. Rev. Plant Sci.* 22, 151–184. doi: 10.1080/713610854
- Lavigne, M. B., Boutin, R., Foster, R. J., Goodine, G., Bernier, P. Y., and Robitaille, G. (2003). Soil respiration responses to temperature are controlled more by roots than by decomposition in balsam fir ecosystems. *Can. J. Forest Res.* 33, 1744–1753. doi: 10.1139/X03-090
- Lavorel, S., and Garnier, E. (2002). Predicting changes in community composition and ecosystem functioning from plant traits: revisiting the Holy Grail. *Funct. Ecol.* 16, 545–556. doi: 10.1046/j.1365-2435.2002.00664.x
- Le, S., Josse, J., and Husson, F. (2008). FactoMineR: an R package for multivariate analysis. *J. Stats. Softw.* 25, 1–18. doi: 10.18637/jss.v025.i01
- Leff, J. W., Bardgett, R. D., Wilkinson, A., Jackson, B. G., Pritchard, W. J., De Long, J. R., et al. (2018). Predicting the structure of soil communities from plant community taxonomy, phylogeny, and traits. *ISME J.* 12, 1794–1805. doi: 10.1038/s41396-018-0089-x
- Liu, Y. R., Delgado-Baquerizo, M., Yang, Z., Feng, J., Zhu, J., and Huang, Q. (2020). Microbial taxonomic and functional attributes consistently predict soil CO<sub>2</sub> emissions across contrasting croplands. *Sci. Total Environ.* 702:134885. doi: 10.1016/j.scitotenv.2019.134885
- Ma, Z., Guo, D., Xu, X., Lu, M., Bardgett, R. D., Eissenstat, D. M., et al. (2018). Evolutionary history resolves global organization of root functional traits. *Nature* 555, 94–97. doi: 10.1038/nature25783
- Mafa-Attoye, T. G., Baskerville, M. A., Ofosu, E., Oelbermann, M., Thevathasan, N. V., and Dunfield, K. E. (2020). Riparian land-use systems impact soil microbial communities and nitrous oxide emissions in an agro-ecosystem. *Sci. Total Environ.* 724:138148. doi: 10.1016/j.scitotenv.2020.138148
- Makita, N., Kosugi, Y., Dannoura, M., Takanashi, S., Niiyama, K., Kassim, A. R., et al. (2012). Patterns of root respiration rates and morphological traits in 13 tree species in a tropical forest. *Tree Phys.* 32, 303–312. doi: 10.1093/treephys/tp008
- Maron, P. A., Sarr, A., Kaisermann, A., Lévêque, J., Mathieu, O., Guigue, J., et al. (2018). High microbial diversity promotes soil ecosystem functioning. *Appl. Environ. Microbiol.* 84, e2738–e2717. doi: 10.1128/AEM.02738-17
- McCormack, M. L., Dickie, I. A., Eissenstat, D. M., Fahey, T. J., Fernandez, C. W., Guo, D., et al. (2015). Redefining fine roots improves understanding of below-ground contributions to terrestrial biosphere processes. *New Phytol.* 207, 505–518. doi: 10.1111/nph.13363
- Nakagawa, S., and Schielzeth, H. (2013). A general and simple method for obtaining R<sup>2</sup> from generalized linear mixed-effects models. *Methods Ecol. Evol.* 4, 133–142. doi: 10.1111/j.2041-210x.2012.00261.x
- Oelbermann, M., Raimbault, B. A., and Gordon, A. M. (2014). Riparian land-use and rehabilitation: impact on organic matter input and soil respiration. *Environ. Manage.* 55, 496–507. doi: 10.1007/s00267-014-0410-z
- Oksanen, J., Blanchet, F. G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., et al. (2019). *vegan: Community Ecology Package. R package version 2.5-5.* <https://CRAN.R-project.org/package=vegan>.
- Parada, A. E., Needham, D. M., and Fuhrman, J. A. (2016). Every base matters: assessing small subunit rRNA primers for marine microbiomes with mock communities, time series and global field samples. *Environ. Microbiol.* 18, 1403–1414. doi: 10.1111/1462-2920.13023
- Paradis, E., Claude, J., and Strimmer, K. (2004). APE: analyses of phylogenetics and evolution in R language. *Bioinformatics* 20, 289–290. doi: 10.1093/bioinformatics/btg412
- Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D., and R Core Team. (2018). *nlme: Linear and Nonlinear Mixed Effects Models. R package version 3.1-137.* <https://CRAN.R-project.org/package=nlme>.
- Pommier, T., Cantarel, A., Grigulis, K., Lavorel, S., Legay, N., Baxendale, C., et al. (2018). The added value of including key microbial traits to determine nitrogen-related ecosystem services in managed grasslands. *J. Appl. Ecol.* 55, 49–58.
- Prieto, I., Roumet, C., Cardinael, R., Dupraz, C., Jourdan, C., Kim, J. H., et al. (2015). Root functional parameters along a land-use gradient: evidence of a

- community-level economics spectrum. *J. Ecol.* 103, 361–373. doi: 10.1111/1365-2745.12351
- Prieto, I., Stokes, A., and Roumet, C. (2016). Root functional parameters predict fine root decomposability at the community level. *J. Ecol.* 104, 725–733. doi: 10.1111/1365-2745.12537
- R Core Team. (2019). *R: A Language And Environment For Statistical Computing*. Vienna, Austria: R Foundation for Statistical Computing.
- Ramnarine, R., Wagner-Riddle, C., Dunfield, K. E., and Voroney, R. P. (2012). Contributions of carbonates to soil CO<sub>2</sub> emissions. *Can. J. Soil Sci.* 92, 599–607. doi: 10.4141/CJSS2011-025
- Roumet, C., Birouste, M., Picon-Cochard, C., Ghestem, M., Osman, N., Vrignon-Brenas, S., et al. (2016). Root structure-function relationships in 74 species: evidence of a root economics spectrum related to carbon economy. *New Phytol.* 210, 815–826. doi: 10.1111/nph.13828
- Schlesinger, W. H., and Andrews, J. A. (2000). Soil respiration and the global carbon cycle. *Biogeochemistry* 48, 7–20. doi: 10.1023/A:1006247623877
- Steinauer, K., Fischer, F. M., Roscher, C., Scheu, S., and Eisenhauer, N. (2017). Spatial plant resource acquisition traits explain plant community effects on soil microbial properties. *Pedobiologia* 65, 50–57. doi: 10.1016/j.pedobi.2017.07.005
- Sun, T., and Mao, Z. (2011). Functional relationships between morphology and respiration of fine roots in two Chinese temperate tree species. *Plant Soil* 346, 375–384. doi: 10.1007/s11104-011-0825-8
- Thevathasan, N. V., Gordon, A. M., Bradley, R., Cogliastro, A., Folkard, P., Grant, R., et al. (2012). “Agroforestry research and development in Canada: the way forward,” in *Agroforestry - The Future Of Global Land Use, Advances In Agroforestry*, eds P. K. R. Nair and D. Garrity (Springer Netherlands), 247–283
- Toju, H., Peay, K. G., Yamamichi, M., Narisawa, K., Hiruma, K., Naito, K., et al. (2018). Core microbiomes for sustainable agroecosystems. *Nat. Plants* 4, 247–257. doi: 10.1038/s41477-018-0139-4
- Tufekcioglu, A., Raich, J. W., Isenhardt, T. M., and Schultz, R. C. (2003). Biomass, carbon and nitrogen dynamics of multi-species riparian buffers within an agricultural watershed in Iowa. *Agrofor. Syst.* 57, 187–198. doi: 10.1023/A:1024898615284
- Tufekcioglu, A., Raich, J. W., Isenhardt, T. M., and Schultz, R. C. (2001). Soil respiration within riparian buffers and adjacent crop fields. *Plant Soil* 229, 117–124. doi: 10.1023/A:1004818422908
- Tufekcioglu, A., Raich, J. W. W., Isenhardt, T. M. M., and Schultz, R. C. C. (1999). Fine root dynamics, coarse root biomass, root distribution, and soil respiration in a multispecies riparian buffer in Central Iowa. *Agrofor. Syst.* 44, 163–174. doi: 10.1023/A:1006221921806
- Udawatta, R. P., and Jose, S. (2012). Agroforestry strategies to sequester carbon in temperate North America. *Agrofor. Syst.* 86, 225–242. doi: 10.1007/s10457-012-9561-1
- Vainio, E. J., and Hantula, J. (2000). Direct analysis of wood-inhabiting fungi using denaturing gradient gel electrophoresis of amplified ribosomal DNA. *Mycol. Res.* 104, 927–936. doi: 10.1017/S0953756200002471
- Violle, C., Navas, M. L., Vile, D., Kazakou, E., Fortunel, C., Hummel, I., et al. (2007). Let the concept of trait be functional!. *Oikos* 116, 882–892. doi: 10.1111/j.0030-1299.2007.15559.x
- Wang, G., Liu, F., and Xue, S. (2017). Nitrogen addition enhanced water uptake by affecting fine root morphology and coarse root anatomy of Chinese pine seedlings. *Plant Soil* 418, 177–189. doi: 10.1007/s11104-017-3283-0
- White, T. J., Bruns, T., Lee, S. J. W. T., and Taylor, J. (1990). *Pcr Protocols: A Guide To Methods And Applications*. San Diego: Academic Press. 315–322.
- Zhou, M., Bai, W., Zhang, Y., and Zhang, W. H. (2018). Multi-dimensional patterns of variation in root traits among coexisting herbaceous species in temperate steppes. *J. Ecol.* 106, 2320–2331. doi: 10.1111/1365-2745.12977

**Conflict of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2021 Borden, Mafa-Attoye, Dunfield, Thevathasan, Gordon and Isaac. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.



# Root Community Traits: Scaling-Up and Incorporating Roots Into Ecosystem Functional Analyses

Ruili Wang<sup>1</sup>, Guirui Yu<sup>2,3\*</sup> and Nianpeng He<sup>2,3,4\*</sup>

<sup>1</sup> College of Forestry, Northwest A&F University, Yangling, China, <sup>2</sup> Key Laboratory of Ecosystem Network Observation and Modeling, Institute of Geographic Sciences and Natural Resources Research, Chinese Academy of Sciences, Beijing, China, <sup>3</sup> College of Resources and Environment, University of Chinese Academy of Sciences, Beijing, China, <sup>4</sup> Institute of Grassland Science, Northeast Normal University and Key Laboratory of Vegetation Ecology, Ministry of Education, Changchun, China

**Keywords:** root trait, community-level trait, species-level trait, ecosystem functioning, scale-matching

## INTRODUCTION

Plant roots are important organs responsible for the physical support and for the acquisition of nutrients and water from the soil, which is necessary for plant growth and reproduction. Thus, the morphological, structural, physiological, and biochemical traits of roots theoretically play crucial roles in driving a series of physiological and ecological functions on different levels, from individuals to ecosystems (Bardgett et al., 2014; Laliberté, 2017; McCormack et al., 2017; Freschet et al., 2021). The latest study of Freschet et al. (2021) stated that root traits were central to the maintenance of multiple ecosystem processes and functioning, especially the transformation and circulation of elements and mineral/organic compounds across the spheres. These previous studies provide us the theoretical basis for the linkages between root traits and the different aspects of ecosystem functioning, such as gross primary productivity (GPP) and nutrient-use efficiency (NUE).

## SUBSTANTIAL PROGRESS IN THE STUDY OF ROOT TRAIT VARIATION

Root traits are initially studied in agricultural systems for developing crops with superior growth and high productivity. In recent decades, root traits of natural communities have attracted increasing interest, particularly in the intra- and inter-species variation, trait covariation, strategies of nutrient acquisition, and mechanisms of species coexistence (Eissenstat, 1992; Lambers et al., 2008; Ma et al., 2018; Kong et al., 2019; Freschet et al., 2021). The scientific community has gradually recognized that compared with coarse roots, fine (<2 mm diameter) or absorptive roots (first- to third-order fine roots) are more active in the acquisition of resources at the species level (Pregitzer et al., 2002; McCormack et al., 2015). Classifying roots into functional modules is a key step that promotes the progress in ecological research largely based on root traits, leading to the identification of general principles of root variation among various plant species, environments, and root orders (McCormack et al., 2015; Iversen et al., 2017).

Comparative root ecology has recently shown that plant species are able to regulate the plasticity of their root traits to adapt to the external environment by modulating the morphology, architecture, and microbial associations of fine roots via trade-off between nutrient acquisition efficiency and investment (Kramer-Walter et al., 2016; Ma et al., 2018). For example, in woody plants, the diameter of first-order roots decreases from plants in tropical (stable habitats) to desert (infertile and highly seasonal habitats) biomes, which is accompanied by a reduced reliance on mycorrhizae and thinner roots allowing for an increase in carbon-use efficiency that can aid the

## OPEN ACCESS

### Edited by:

Iván Prieto,  
Spanish National Research  
Council, Spain

### Reviewed by:

Serra-Willow Buchanan,  
University of Toronto  
Scarborough, Canada  
Emily R. Larson,  
University of Bristol, United Kingdom

### \*Correspondence:

Guirui Yu  
yugr@igsnr.ac.cn  
Nianpeng He  
henp@igsnr.ac.cn

### Specialty section:

This article was submitted to  
Functional Plant Ecology,  
a section of the journal  
Frontiers in Plant Science

**Received:** 02 April 2021

**Accepted:** 28 June 2021

**Published:** 22 July 2021

### Citation:

Wang R, Yu G and He N (2021) Root  
Community Traits: Scaling-Up and  
Incorporating Roots Into Ecosystem  
Functional Analyses.  
Front. Plant Sci. 12:690235.  
doi: 10.3389/fpls.2021.690235

colonization of new habitats (Ma et al., 2018). The development of global databases of root traits across different species, biomes, and environmental gradients (such as the Fine-Root Ecology Database, <http://roots.ornl.gov>) using standardized protocols may, thus, be vital for improving our predictive capacity across different ecological scales in the future.

## DISCUSSION

### How to Link Root Traits With Ecosystem Functioning?

Despite the aforementioned substantial advances toward a greater understanding of intra- and inter-species root trait variation, the importance of root traits in ecosystem-level functioning, particularly carbon and nutrient cycling, is increasingly recognized but still not well-understood (Freschet et al., 2021). Understanding and predicting the effects of plant functional traits on certain key ecological processes has been coined as the “holy grail” in ecological research (Lavorel and Garnier, 2002). However, root traits remain underrepresented or non-parameterized in studies on ecosystem models and terrestrial biosphere functioning (Warren et al., 2015), partly because the mismatch between traits and functions is apparent between species and natural communities. Little evidence of the links between root functional traits and community or ecosystem-level functioning has been reported or verified in complex natural communities (van der Plas et al., 2020; Freschet et al., 2021); therefore, scaling up the links between them from organ or species to ecosystem levels may lead to large uncertainties.

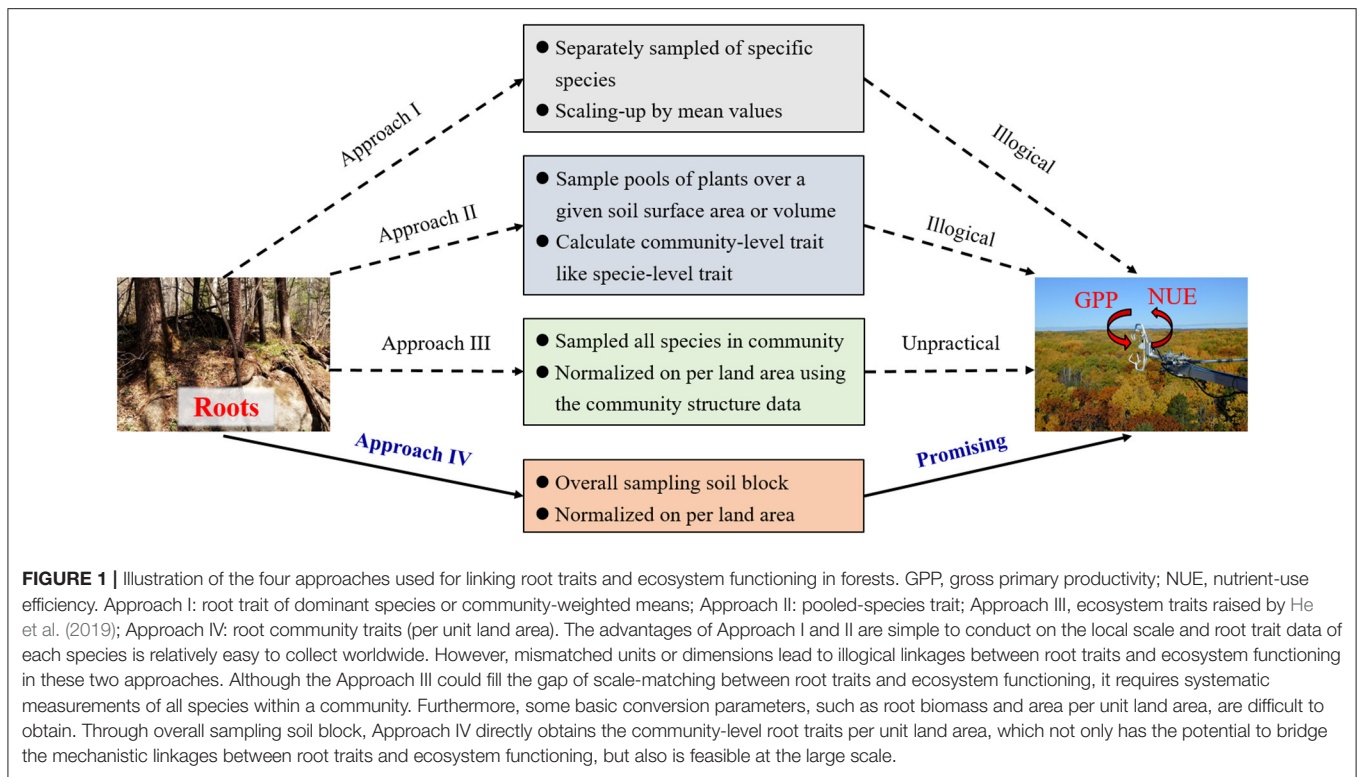
Identifying the links between root traits measured at the organ level and the ecosystem-level functioning, and even integrating these root traits into ecological models and remote sensing techniques are great challenges in ecological studies (Laliberté, 2017; McCormack et al., 2017; Freschet et al., 2021). One common method used in previous studies is to calculate the trait values of the dominant species by averaging or weighting all species' abundances in a community (e.g., community-weighted mean, CWM) to represent the values for an entire plant community (Approach I, **Figure 1**). Following this, the variation in specific root traits and its consequences on higher-level processes and functions could be identified (Lavorel and Garnier, 2002; Violle et al., 2007). This method is feasible for assessing community-level root traits worldwide with the help of the databases of each species' root traits and community structure. However, in practice, three vital difficulties must be overcome at large scales when trying to link community-level root traits with ecosystem functioning. First, it is challenging and laborious to obtain the values of root abundance (or biomass) for each species in natural communities, which is a basic conversion factor in CWM methods. Although plant dominance is typically assessed according to aboveground features (e.g., Mokany et al., 2008; De Long et al., 2019), belowground organs of individual species may not scale proportionally in relation to their aboveground dimension. This case is especially important in ecosystems where most biomass is allocated belowground,

e.g., grasslands and shrubby biomes (Ottaviani et al., 2020). Secondly, these traditional scaling-up approaches are based on the assumption of linear or approximately equal species contributions (Reichstein et al., 2014), and few studies have directly tested and quantified the linkages between organ-level root traits and ecosystem functioning, especially in the complex natural ecosystems (van der Plas et al., 2020; Freschet et al., 2021). As a result, major research challenges still face ecologists when studying the interface between root traits and ecosystem functioning (Freschet et al., 2021). Thirdly and most importantly, mismatched units between ecosystem functioning and CWMs decouple their relationships. Functions at the ecosystem level are generally estimated based on land area by using eddy-flux observations, remote sensing, or ecological modeling. By contrast, the units of root traits based on CWMs remain the same as those of measured plant organ traits, such as root element content ( $\text{g kg}^{-1}$ ) and specific root length (SRL,  $\text{mm}^2 \text{mg}^{-1}$ ). Another method proposed to estimate the community traits is pooled-species approach (Approach II, **Figure 1**), which pools of plants are sampled over a given soil surface area or soil volume and the community-level functional parameters (e.g., SRL; root tissue density, RTD) are directly measured (Klump and Soussana, 2009; Prieto et al., 2016). Compared with the CWM approach, the pooled-species approach is far less time consuming without need to estimate root abundance of each species. However, the pooled-species approach also fails to solve the problem of mismatched units of root traits with ecosystem properties. Such shortcomings of mismatched units or scales limit the development of these traditional methods and their application in ecological models (Warren et al., 2015; McCormack et al., 2017).

Developing a new methodology for quantifying community-level root traits per unit of land area is both theoretically and practically essential to better incorporate root traits into ecological models (He et al., 2019; Liu et al., 2021). This new concept of ecosystem traits (He et al., 2019) should be helpful, because the traits of ecosystems or plant communities are defined as the traits or quantitative characteristics of organisms at the community level and expressed as the intensity (or density) normalized per unit land area (Approach III, **Figure 1**). Foliar area or biomass per unit land area of specific species has been used to calculate foliar physiological parameters of ecosystem traits, such as total stomatal number per unit ground surface area ( $\text{number m}^{-2}$ ), as well as to identify the tight links with the productivity and water-use efficiency of an ecosystem (Wang et al., 2015). The concept of ecosystem traits inspires us to rethink the sampling methods to resolve these questions for natural ecosystems.

However, Approach III will result in larger challenges in calculating community-level root traits because obtaining the basic conversion parameters for scaling up, such as root biomass or area of each plant species, is more difficult for roots than for leaves in natural communities. All evidences support the use of holistic sampling methods, i.e., using root cores or soil blocks under a given soil surface area or volume to identify the morphological, chemical, and physiological traits of roots, and then normalizing the data per unit land area (i.e., root





community traits, Approach IV, **Figure 1**). The advantage of holistic sampling is that we can directly obtain the traits of community-level root entities, which enables us to match the units with ecosystem functioning and to integrate data from field traits with the approaches and technologies used in macroecology. Many studies have used root cores to determine root traits, but most of them only investigated root biomass per unit land area or calculated functional parameters like species-level traits (e.g., Klumpp and Soussana, 2009; Prieto et al., 2016). The concept of root community traits urges us to measure and identify the total spatial and temporal variation in root traits, which may enable us to better link the traits with ecosystem functioning (e.g., GPP or NUE) in the natural ecosystems at a large scale.

## What Is Promising for Root Community Traits in Ecosystem Studies?

Here, we define root community traits as the overall morphological, chemical, and physiological traits of roots in a natural community, and they are normalized per unit land area. The new concept of root community traits is important for plant growth and production for each specific trait or all of them jointly in natural communities. For example, at the species level, SRL, i.e., the length of roots per unit biomass, can indicate the economics of root investment, and high SRL often indicates a high efficiency of nutrient uptake by roots and a high respiration rate (Reich, 2014). As a community-level root trait, the total root length per unit land area (i.e.,

root length density, RLD) could be quantified to indicate the nutrient uptake capacity of a community by belowground roots, and it could account for the variation in ecosystem processes, particularly belowground cycling of carbon and nutrients. Similarly to RLD, we also could estimate the density of total root biomass, area, and chemical contents per unit land area. Therefore, the parameters of root community traits should be widely used in studies at the ecosystem level. We suggest the following four practices in future studies: (1) determining the relationships between community-level root traits and the environment at a large scale and investigating empirical relationships between root traits and ecosystem-level functioning are required to promote ecological models of vegetation dynamics; (2) studies of traditional root traits should be integrated with macro-ecological studies using new technologies (e.g., ground penetrating radar and remote sensing); (3) new perspectives should be offered for understanding the relationships between above- and belowground traits. Whether above- and belowground traits co-vary or exhibit coordinated responses to a changing environment has been intensively debated and remains controversial (Weemstra et al., 2016; Wang et al., 2017). Within a community, tree canopies tend to extend fully and capture more light for photosynthesis to meet the demand for the growth of stems and roots, and in turn, belowground roots provide anchorage and resource acquisition. The concept of root community traits enables us to re-examine the relationship between roots and shoots from the perspective of above- and belowground plant communities; (4) with the help

of molecular and DNA sequencing-based techniques, we can determine the belowground dominance of individual species on the basis of land area. Thus, the mechanisms of community structure assembly and productivity optimization in nature could be explored from a new perspective.

In conclusion, this new idea of root community traits may help us re-examine the multiple roles of plant roots in community assemblages and ecosystem processes, and it can help us resolve the difficulties of traditional scaling-up approaches. In the combination with some new observation technologies (e.g., ground penetrating radar, multispectral and X-ray images), we may track the dynamics of entire root systems over different spatial and temporal scales. More importantly, this new concept of root community traits can help us incorporate root traits into the investigations of ecosystem functioning at large scales, as well as to improve ecological

models, particularly for productivity, nutrient acquisition, and soil carbon cycling.

## AUTHOR CONTRIBUTIONS

NH and GY designed the research. RW, GY, and NH wrote the manuscript. All authors contributed to the article and approved the submitted version.

## FUNDING

This work was supported by the National Natural Science Foundation of China (31988102, 31872690), the National Key R&D Program of China (2017YFA0604803), and the Chinese Academy of Sciences Strategic Priority Research Program (XDA19020302).

## REFERENCES

- Bardgett, R. D., Mommer, L., and De Vries, F. T. (2014). Going underground: root traits as drivers of ecosystem processes. *Trends Ecol. Evol.* 29, 692–699. doi: 10.1016/j.tree.2014.10.006
- De Long, J. R., Jackson, B. G., Wilkinson, A., Pritchard, W. J., Oakley, S., Mason, K. E., et al. (2019). Relationships between plant traits, soil properties, and carbon fluxes differ between monocultures and mixed communities in temperate grassland. *J. Ecol.* 107, 1704–1719. doi: 10.1111/1365-2745.13160
- Eissenstat, D. M. (1992). Costs and benefits of constructing roots of small diameter. *J. Plant Nutr.* 15, 763–782. doi: 10.1080/01904169209364361
- Freschet, G. T., Roumet, C., Comas, L. H., Weemstra, M., Bengough, A. G., Rewald, B., et al. (2021). Root traits as drivers of plant and ecosystem functioning: current understanding, pitfalls, and future research needs. *New Phytol.* 1–36. doi: 10.1111/nph.17072
- He, N., Liu, C., Piao, S., Sack, L., Xu, L., Luo, Y., et al. (2019). Ecosystem traits linking functional traits to macroecology. *Trends Ecol. Evol.* 34, 200–210. doi: 10.1016/j.tree.2018.11.004
- Iversen, C. M., McCormack, M. L., Powell, A. S., Blackwood, C. B., Freschet, G. T., Kattge, J., et al. (2017). A global fine-root ecology database to address below-ground challenges in plant ecology. *New Phytol.* 215, 15–26. doi: 10.1111/nph.14486
- Klump, K., and Soussana, J. F. (2009). Using functional traits to predict grassland ecosystem change: a mathematical test of the response-and-effect trait approach. *Global Change Biol.* 15, 2921–2934. doi: 10.1111/j.1365-2486.2009.01905.x
- Kong, D., Wang, J., Wu, H., Valverde-Barrantes, O. J., Wang, R., Zeng, H., et al. (2019). Nonlinearity of root trait relationships and the root economics spectrum. *Nat. Commun.* 10:2203. doi: 10.1038/s41467-019-10245-6
- Kramer-Walter, K. R., Bellingham, P. J., Millar, T. R., Smissen, R. D., Richardson, S. J., and Laughlin, D. C. (2016). Root traits are multidimensional: specific root length is independent from root tissue density and the plant economic spectrum. *J. Ecol.* 104, 1299–1310. doi: 10.1111/1365-2745.12562
- Laliberté, E. (2017). Below-ground frontiers in trait-based plant ecology. *New Phytol.* 213, 1597–1603. doi: 10.1111/nph.14247
- Lambers, H., Raven, J. A., Shaver, G. R., and Smith, S. E. (2008). Plant nutrient-acquisition strategies change with soil age. *Trends Ecol. Evol.* 23, 95–103. doi: 10.1016/j.tree.2007.10.008
- Lavorel, S., and Garnier, E. (2002). Predicting changes in community composition and ecosystem functioning from plant traits: revisiting the Holy Grail. *Funct. Ecol.* 16, 545–556. doi: 10.1046/j.1365-2435.2002.00664.x
- Liu, C., Li, Y., Yan, P., and He, N. (2021). How to improve the predictions of plant functional traits on ecosystem functioning? *Front. Plant Sci.* 12:622260. doi: 10.3389/fpls.2021.622260
- Ma, Z. Q., Guo, D. L., Xu, X. L., Lu, M. Z., Bardgett, R. D., Eissenstat, D. M., et al. (2018). Evolutionary history resolves global organization of root functional traits. *Nature* 555, 94–97. doi: 10.1038/nature25783
- McCormack, M. L., Dickie, I. A., Eissenstat, D. M., Fahey, T. J., Fernandez, C. W., Guo, D., et al. (2015). Redefining fine roots improves understanding of below-ground contributions to terrestrial biosphere processes. *New Phytol.* 207, 505–518. doi: 10.1111/nph.13363
- McCormack, M. L., Guo, D. L., Iversen, C. M., Chen, W. L., Eissenstat, D. M., Fernandez, C. W., et al. (2017). Building a better foundation: improving root-trait measurements to understand and model plant and ecosystem processes. *New Phytol.* 215, 27–37. doi: 10.1111/nph.14459
- Mokany, K., Ash, J., and Roxburgh, S. (2008). Functional identity is more important than diversity in influencing ecosystem processes in a temperate native grassland. *J. Ecol.* 96, 884–893. doi: 10.1111/j.1365-2745.2008.01395.x
- Ottaviani, G., Molina-Venegas, R., Charles-Dominique, T., Chelli, S., Campetella, G., Canullo, R., et al. (2020). The neglected belowground dimension of plant dominance. *Trends Ecol. Evol.* 35, 763–766. doi: 10.1016/j.tree.2020.06.006
- Pregitzer, K. S., DeForest, J. L., Burton, A. J., Allen, M. F., Ruess, R. W., and Hendrick, R. L. (2002). Fine root architecture of nine North American trees. *Ecol. Monogr.* 72, 293–309. doi: 10.1890/0012-9615(2002)072[0293:FRAONN]2.0.CO;2
- Prieto, I., Stokes, A., and Roumet, C. (2016). Root functional parameters predict fine root decomposability at the community level. *J. Ecol.* 104, 725–733. doi: 10.1111/1365-2745.12537
- Reich, P. B. (2014). The world-wide ‘fast-slow’ plant economics spectrum: a traits manifesto. *J. Ecol.* 102, 275–301. doi: 10.1111/1365-2745.12211
- Reichstein, M., Bahn, M., Mahecha, M. D., Kattge, J., and Baldocchi, D. D. (2014). Linking plant and ecosystem functional biogeography. *Proc. Natl. Acad. Sci. U.S.A.* 111, 13697–13702. doi: 10.1073/pnas.1216065111
- van der Plas, F., Schröder-Georgi, T., Weigelt, A., Barry, K., Meyer, S., Alzate, A., et al. (2020). Plant traits alone are poor predictors of ecosystem properties and long-term ecosystem functioning. *Nat. Ecol. Evol.* 4, 1602–1611. doi: 10.1038/s41559-020-01316-9
- Vielle, C., Navas, M. L., Vile, D., Kazakou, E., Fortunel, C., Hummel, I., et al. (2020). Let the concept of trait be functional! *Oikos* 116, 882–892. doi: 10.1111/j.0030-1299.2007.15559.x
- Wang, R., Wang, Q., Zhao, N., Yu, G., and He, N. (2017). Complex trait relationships between leaves and absorptive roots: coordination in tissue N concentration but divergence in morphology. *Ecol. Evol.* 7, 2697–2705. doi: 10.1002/ece3.2895
- Wang, R., Yu, G., He, N., Wang, Q., Zhao, N., Xu, Z., et al. (2015). Latitudinal variation of leaf stomatal traits from species to community level

- in forests: linkage with ecosystem productivity. *Sci. Rep.* 5:14454; doi: 10.1038/srep14454
- Warren, J. M., Hanson, P. J., Iversen, C. M., Kumar, J., Walker, A. P., and Wulfschleger, S. D. (2015). Root structural and functional dynamics in terrestrial biosphere models—evaluation and recommendations. *New Phytol.* 205, 59–78. doi: 10.1111/nph.13034
- Weemstra, M., Mommer, L., Visser, E. J. W., van Ruijven, J., Kuyper, T. W., Mohren, G. M. J., et al. (2016). Towards a multidimensional root trait framework: a tree root review. *New Phytol.* 211, 1159–1169. doi: 10.1111/nph.14003

**Conflict of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2021 Wang, Yu and He. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.





# Plant Nutrient Contents Rather Than Physical Traits Are Coordinated Between Leaves and Roots in a Desert Shrubland

Xiaoyan Jiang<sup>1</sup>, Xin Jia<sup>1,2\*</sup>, Shengjie Gao<sup>1</sup>, Yan Jiang<sup>1</sup>, Ningning Wei<sup>1</sup>, Cong Han<sup>1</sup>, Tianshan Zha<sup>1,2</sup>, Peng Liu<sup>1,2</sup>, Yun Tian<sup>2</sup> and Shugao Qin<sup>1,2</sup>

<sup>1</sup>Yanchi Research Station, School of Soil and Water Conservation, Beijing Forestry University, Beijing, China, <sup>2</sup>Key Laboratory for Soil and Water Conservation, State Forestry and Grassland Administration, Beijing Forestry University, Beijing, China

## OPEN ACCESS

### Edited by:

Hiroko Kurokawa,  
Forestry and Forest Products  
Research Institute, Japan

### Reviewed by:

Patompong Johns Saengwilai,  
Mahidol University,  
Thailand  
Hui Guo,  
Nanjing Agricultural University,  
China

### \*Correspondence:

Xin Jia  
xinjia@bjfu.edu.cn

### Specialty section:

This article was submitted to  
Functional Plant Ecology,  
a section of the journal  
Frontiers in Plant Science

**Received:** 01 July 2021

**Accepted:** 28 September 2021

**Published:** 26 October 2021

### Citation:

Jiang X, Jia X, Gao S, Jiang Y, Wei N,  
Han C, Zha T, Liu P, Tian Y and  
Qin S (2021) Plant Nutrient Contents  
Rather Than Physical Traits Are  
Coordinated Between Leaves and  
Roots in a Desert Shrubland.  
*Front. Plant Sci.* 12:734775.  
doi: 10.3389/fpls.2021.734775

Although leaf economics spectrum (LES) has been extensively tested with regional and global datasets, the correlation among functional traits of desert plants remains largely unclear. Moreover, examinations on whether and how leaf and root traits are coordinated have yielded mixed results. We investigated variations in leaf and fine-root traits across 48 species in a desert community of northern China to test the hypotheses that (1) the leaf-trait syndrome of plant species in desert shrublands follows the predictions of the global LES, and is paralleled by a similar root-trait syndrome, (2) functional traits related to nutrient contents and resource uptake are tightly coordinated between leaves and fine roots in desert ecosystems where plant growth is limited primarily by dry and nutrient-poor conditions, and (3) traits as well as their relationships vary among functional groups. Our results partially supported the LES theory. Specific leaf area (SLA) was correlated with leaf tissue density, phosphorus content, and carbon-to-nitrogen ratio, but not with leaf nitrogen content. Specific root length (SRL) was not correlated with other fine-root traits, and fine-root traits were largely independent of each other. Therefore, fine-root traits did not mirror the leaf-trait syndrome. Fine-root nitrogen and phosphorus contents, nitrogen-to-phosphorous ratio, and carbon-to-nitrogen ratio all increased with analogous leaf traits, whereas SRL was not correlated with SLA. After phylogenetic effects were considered, nutrient contents and their ratios still displayed stronger coordination between leaves and fine roots than did SRL and SLA. The overall pattern of trait variations and relationships suggested differentiation among functional groups. Our results suggest that despite the absence of a root-trait syndrome, fine-root functions in the studied desert community were probably coordinated with leaf functions with respect to nutrient allocation and use.

**Keywords:** functional trait, plant functional type, specific leaf area, specific root length, stoichiometry

## INTRODUCTION

Leaf functional traits play an important role in plant carbon assimilation, water relations and energy balance (Ackerly et al., 2002), while root traits determine nutrient and water uptake that are crucial for plant survival and growth (McCormack et al., 2015; Weemstra et al., 2016; Wang et al., 2018). According to leaf and root economics spectrum (LES and RES, respectively),

specific leaf area (SLA) and specific root length (SRL) are two key traits that indicate plant resource strategies (Wright et al., 2004; Cheng et al., 2016). A global foliar dataset indicated that 82% of total variance in photosynthetic capacity can be explained by SLA and leaf nitrogen content (LN; Wright et al., 2004; Ali et al., 2016). Specifically, species with high SLA exhibiting high LN, leaf phosphorus content (LP), high photosynthetic rate and short leaf lifespan, and low leaf tissue density (LTD), i.e., a resource-acquisitive strategy. The opposite is for species with low SLA exhibiting conserved traits (Wright et al., 2004; Pérez-Ramos et al., 2012). However, some studies found weak or a lack of correlation between SLA and other leaf traits across species (Zhou et al., 2010; Chen et al., 2013). In addition, existing studies on leaf traits mainly focused on forests and grasslands (He et al., 2008; Fajardo and Siefert, 2016; Hosseini et al., 2019), it remains largely unclear how leaf traits are correlated across species in desert communities.

Unlike leaves, the ongoing progress on fine-root trait correlations revealed a more complex and multidimensional economics space, reflecting a variety of evolutionary pressures and tradeoffs belowground (Kong et al., 2014; Xia et al., 2021). Some studies reported that fine roots of species with high SRL, small diameter, low tissue density (RTD), and high N content (RN) were associated with low construction costs, high respiration rates, and high turnover rates, a pattern analogous to leaf-trait correlations (Reich, 2014; Caplan et al., 2019). Exceptions to this pattern are nonetheless common (Holdaway et al., 2011; Weemstra et al., 2016). For example, Kramer-Walter et al. (2016) reported that SRL was independent of RTD and the plant economic spectrum across the most abundant tree species in New Zealand. Moreover, studies on temperate tree species showed no correlation between SRL and RN (Comas and Eissenstat, 2004) or between root lifespan and SRL or root diameter (Withington et al., 2006). Far less is known about whether there is a root-trait syndrome that parallels the leaf-trait syndrome in desert communities.

For a plant economics spectrum to occur, traits of different plant organs (e.g., leaf and root) must be coordinated in a way that follows evolutionary and biophysical constraints (Reich, 2014; Carvajal et al., 2019). The level of coordination between root and leaf traits can be multidimensional, meaning that certain root traits are coordinated with analogous leaf traits, while other root traits vary independently of leaf traits (Kramer-Walter et al., 2016). Empirical evidence indicates that analogous leaf and root traits can be correlated across species in grasslands on the Inner Mongolian Plateau and the Tibetan Plateau (Geng et al., 2014). However, other studies showed that analogous leaf and root traits were weakly correlated at the global scale (Craine et al., 2005) and that the same plant can have aboveground traits that are correlated with root traits of the opposite growth strategy in temperate grasslands (Personeni and Loiseau, 2004). The correlation between key physical leaf and root traits remains controversial (Cheng et al., 2016). For example, the reported SLA–SRL relationship was positive (Withington et al., 2006), negative (Kembel and Cahill, 2011), or nonsignificant (Chen et al., 2013). The same holds for the correlation between leaf and fine-root nutrient contents (e.g., N and P), which was found to be positive in some studies (Tjoelker et al., 2005; Kerkhoff

et al., 2006; Freschet et al., 2010; Holdaway et al., 2011), but not in others (Withington et al., 2006; Chen et al., 2013). These mixed findings suggest that the coordination between leaf and root traits may be contingent upon environmental conditions (e.g., abiotic stresses and soil properties), species composition, plant functional types (PFTs) and the spatial scale of interest (Geng et al., 2014; Cheng et al., 2016; Weemstra et al., 2016). In desert environments, leaf and fine-root traits are expected to be tightly coordinated because water and nutrient limitation to plant growth requires fine-root functions (i.e., water and nutrient uptake) to match those of leaves (i.e., photosynthesis and transpiration; Carvajal et al., 2019). We currently know little about the relative strength of stoichiometric vs. physical coordination between leaves and roots across desert plants.

Many leaf and root traits have been shown to differ among PFTs that are predefined by growth form (e.g., grass, forb, and woody species), taxonomy (e.g., monocot and eudicot) or functional categories (e.g., legumes, non-legumes; Freschet et al., 2017). Therefore, PFTs may be useful in categorizing species trait syndromes (Tjoelker et al., 2005; Caplan et al., 2019). For example, global analyses showed that graminoids had generally lower fine-root N content and tissue density than forbs, shrubs, and trees (Freschet et al., 2017). A local-scale study in a subarctic flora suggested that differences among growth forms can also be seen for structural traits such as SRL and RTD (Freschet et al., 2010). Although individual leaf and fine-root traits were observed to differ among PFTs, whether trait correlations differ among PFTs in predictable ways remains poorly understood (Tjoelker et al., 2005).

Current knowledge on plant functional traits is mainly obtained from studies on forests and grasslands (Craine et al., 2005; Kong et al., 2014; Cheng et al., 2016; Zhang et al., 2018). Species from the desert region should display strategies favoring higher belowground (water and nutrient) than aboveground (light) resource acquisition compared with species from forests or grasslands (Liu et al., 2010). However, it remains largely unclear whether existing theories are equally applicable to desert plants. Desert shrub communities constitute an ideal system to test the traits correlations because water is considered the main resource limiting plant abundance and distribution (Carvajal et al., 2019). We examined how leaf and fine-root traits are correlated across species and differ among PFTs in a desert shrubland. Specifically, we tested the hypotheses that (1) the leaf-trait syndrome of plant species in desert shrublands follows the predictions of the global LES, and is paralleled by a similar fine root-trait syndrome, (2) functional traits related to nutrient contents and resource uptake are tightly coordinated between leaves and fine roots in desert ecosystems where plant growth is limited primarily by dry and nutrient-poor conditions, and (3) traits as well as their relationships vary among PFTs.

## MATERIALS AND METHODS

### Study Site

This study was conducted at the Yanchi Research Station (37°42′31″N, 107°13′37″E, 1530 m.a.s.l.), Ningxia, northern China.

The site is located at the southern edge of the Mu Us Desert and is characterized by a temperate semiarid continental climate. The mean annual temperature (1954–2020) is 8.4°C, and the mean annual precipitation is 293 mm. Most precipitation (>70%) occurs during June to September (data source: Yanchi Meteorological Station, Yanchi Research Station). The soil is a Arenosols (The FAO-UNESCO soil classification) with a total nitrogen content of 0.1–0.2 g kg<sup>-1</sup> and a soil organic carbon (C) content of about 2.0 g kg<sup>-1</sup>. The landscape of this region is typical of inland dune ecosystems, which are colonized mainly by deciduous perennial shrubs, perennial grasses and annuals (She et al., 2017). The study area experienced severe desertification during the 1960–1990's due to human disturbances (e.g., overgrazing and reclamation). Large-scale conservation practices (e.g., fencing and grazing ban) over the recent two decades have promoted the recovery of natural vegetation (Bai et al., 2018). The studied shrubland community is located in a conserved area in which human activities are negligible and all plants grow naturally. The shrubland community is dominated by a mixture of xerophytic shrub species, including *Artemisia ordosica*, *Hedysarum mongolicum*, and *Salix psammophila*. Most abundant herbaceous species include *Leymus secalinus*, *Stipa glareosa*, *Pennisetum centrasiaticum*, and *Setaria viridis*. Germination and leaf emergence usually start in mid-April, and the growing season ends in around mid-October. Leaf area index (LAI) at mid-growing season varies from year to year, and can exceed 1.0 m<sup>-2</sup> m<sup>2</sup> in most productive years. LAI declines virtually to zero during winter as all plant species are cold-deciduous.

## Field Sampling and Trait Measurements

Four plots (40 m × 40 m, 20 m apart from each other) were set in the studied shrubland community in the spring of 2019. The four plots were considered replicates based on their similarity in topography and soil properties (Supplementary Table 1) as well as in species composition. The similarity in species composition was quantified with the Jaccard index (Qin et al., 2019), which ranged from 0.61 to 0.71. We then ranked all species in each plot by relative abundance and sampled all dominant species (i.e., relative abundance >5%). Following the standard trait collection protocols detailed in Cornelissen et al. (2003) and other studies (Liu et al., 2010; Geng et al., 2014; Mitchell et al., 2017), we sampled five mature individuals of each dominant species (to minimize labor and disturbance) from each plot. A total of 20 individuals (five ind. × four plots) were sampled for most species, while 10–15 individuals were sampled for those which occurred in only two or three plots. For each individual we collected five fully-expanded, fresh and healthy leaves and 10 fine roots (diameter < 2 mm; Cornelissen et al., 2003; Kong et al., 2014; Mitchell et al., 2017). This sampling strategy ensures that all field sampling and measurements can be done during the mid-growing season (from late June to late August), and that sampled species can represent the community assembly. We acknowledge that our small sample size for each species may not be adequate for examining intraspecific trait variations. However, the sampling

method described here has been commonly used to investigate trait variations and relationships across species (Geng et al., 2014).

For shrub species, we carefully excavated the soil (0–30 cm) at the base of each individual whose leaves had been collected, exposing the coarse roots. To ensure fine roots of the target individual were sampled, we followed each coarse root to find the attachment points of fine roots of the target plant. The points at which intact fine roots were attached to the coarse root were then determined using vernier calipers and cut with scissors. For herbaceous species, we carefully collected whole plants back to lab for the separation of leaves and fine roots. Active fine roots (generally have a lighter color and a fully turgid appearance) of each individual were identified according to root color, texture and connection to its shoot (Cheng et al., 2016).

A total of 10 shrub and 38 herbaceous species were investigated, covering 39 genera and 16 families. All sampled species are deciduous, including 33 perennials, three biennials, two annual grasses and 10 annual forbs (Supplementary Table 2). Leaf traits were measured for all 48 species (Supplementary Table 2; Supplementary Figure 1), while fine-root traits were measured for a subset of 43 species as fine roots were difficult to collect for five herbaceous species. We measured functional traits for both leaves and fine roots, including SLA, SRL, LTD, LN, RN, LP, root phosphorous content (RP), leaf and root organic C contents (LC and RC, respectively), and further calculated LN:LP, RN:RP, LC:LN, and RC:RN ratios.

Plant functional traits of sampled species were measured following standardized protocols detailed in Cornelissen et al. (2003). All samples were sealed in plastic bags, placed on ice, and returned to the lab where leaf samples were digitally imaged within 1 h of collection. Leaf thickness was determined with electronic vernier calipers, and leaf area was measured using the Image J software.<sup>1</sup> Leaf volume was calculated as the product of leaf thickness and leaf area. We carefully removed the remaining soil and organic matter from the fine-root samples using deionized water and tweezers. In the absence of a digital image analysis system, we measured the length of fine roots manually. Put the fine-root samples on the glass plate with grid paper and measure its length by straightening both ends with tweezers (Cheng et al., 2005). Leaf and fine-root samples were oven dried at 75°C for 48 h to constant weight and weighed to calculate SLA (leaf area per unit dry mass, cm<sup>2</sup> g<sup>-1</sup>), SRL (fine-root length per unit dry mass m g<sup>-1</sup>) and LTD (leaf dry mass per total volume, g cm<sup>-3</sup>). The C, N contents (g kg<sup>-1</sup>) of leaf and fine-root samples were measured through an elemental analyzer (Vario Max CN Element Analyser, Elementar, Germany) and total P content (g kg<sup>-1</sup>) was analyzed colorimetrically after H<sub>2</sub>SO<sub>4</sub>-H<sub>2</sub>O<sub>2</sub>-HF digestion (John, 1970).

## Statistical Analysis

All data were logarithmically transformed prior to analysis to satisfy the assumption of normality. The N:P and C:N ratios represent mass ratios in this study. Investigated plants

<sup>1</sup><https://imagej.net/Welcome>

were sorted into PFTs for analyzing differences in trait values and bivariate trait relationships among groups. Specifically, they were classified into grasses, forbs, and woody species based on life form, into legumes and non-legumes based on their ability to fix nitrogen, and into monocots and eudicots based on their evolutionary relationships. We did not compare deciduous vs. evergreen species because the latter are virtually absent from our study site due to cold winter. Nor did we compare perennial vs. annual species, as this classification largely confounds that based on life form (i.e., all woody species and most grasses are perennial). Species mean trait values were used for testing trait correlations, due to our focus on trait relationships across species. Bivariate trait relationships were tested with a model II (standardized major axis, SMA) regression, which is commonly used when independent variable is not clearly defined and/or measurement errors exist for both variables (Craine et al., 2005). SMA slopes and y-intercepts were calculated using the “smatr” package of the R software. Due to multiple trait correlations, a principal component analysis (PCA) was performed for all leaf and fine-root traits (“whole-plant PCA” hereafter) to test overall patterns of trait variations (Craine et al., 2005). All variables used in PCA were standardized to a mean of zero and a SD of one. A separate PCA was also performed for all leaf traits (“leaf PCA” hereafter) or all fine-root traits (“root PCA” hereafter), to examine overall trait variations in leaves and fine roots, respectively. Differences in any leaf or fine-root traits among PFTs and species were tested using a nested ANOVA (nested ANOVA), in which functional type was treated as a fixed factor, and species was treated as a random factor nested within functional type. The Tukey HSD method was used for *post hoc* multiple comparisons. Multivariate analysis of variance (MANOVA) was performed to test whether species scores on the first two PCA axes show overall differences among grasses, forbs, and woody species, and Hotelling’s  $T^2$ -test was performed instead when comparing between legumes and non-legumes, or between monocots and eudicots. In addition, ANOVA and *t*-test were performed on species scores to compare PFTs along individual PCA axes.

To remove the effects of phylogenetic relatedness among species (due to shared evolutionary history) on trait variations, we calculated phylogenetically independent contrasts (PIC, Felsenstein, 1985) using the “ape” R package to further evaluate pairwise correlations between leaf and fine-root traits (Kerkhoff et al., 2006; Geng et al., 2014). Investigated species were sorted into genera and families based on the APG III classification using the “plantlist” R package (The Angiosperm Phylogeny Group, 2009), and a supertree for all taxa was built using the freely available software Phylomatic.<sup>2</sup> Because PICs were calculated based on nonnegative  $x$ -axis contrasts, we forced the SMA regressions on PICs through the origin following Kerkhoff et al. (2006). All statistical analyses were conducted in R version 4.0.3 (The R development Core Team). The significance level was set as  $p=0.05$ .

<sup>2</sup><http://www.phylodiversity.net/phyloomatic>

## RESULTS

### Trait Correlations in Leaves and Fine Roots Across All Species

Pairwise trait relationships revealed that SLA was positively correlated with LP, negative correlated with LTD and LC:LN, but not correlated with LN and LN:LP across all species (Figure 1). Surprisingly, LN and LP were not correlated ( $p=0.21$ ). The first two main axes (PC1 and PC2) for the leaf PCA explained 43 and 32% of total variance, respectively, in selected leaf traits. Leaf PCA was generally consistent with pairwise relationships, with PC1 showing that species with low SLA had high LTD and LC:LN, but low LN, LP, and LN:LP (Figure 2A and Table 1). SRL was independent of other fine-root traits (Supplementary Figure 2). PC1 and PC2 for the root PCA explained 52 and 27% of total variance, respectively, in examined fine-root traits. PC1 for the root PCA showed that species with low SRL generally had high RN, and RN:RP and low RP and RC:RN (Figure 2B and Table 1).

### Correlations Between Leaf and Fine-Root Traits Across All Species

Nutrient-related traits (N, P, N:P, and C:N) were all positively correlated between leaves and fine roots (Figures 3A–D). SLA and SRL were unrelated (Figure 3E). After controlling for phylogenetic relatedness among species, N and P contents, and N:P ratio were tightly coordinated between leaves and fine roots, whereas the correlations between SRL and SLA and between LC:LN and RC:RN were marginally significant (Table 2).

The whole-plant PCA revealed two independent sets of correlations (Figure 2C). PC1 accounted for 41% of total variance in leaf and fine-root traits, compared to 24% explained by PC2 (Figure 2C and Table 1). PC1 represented a continuous distribution of species from those that have low N, N:P ratio and high C:N ratio leaves and fine roots to those that have high N, N:P ratio and low C:N ratio leaves and fine roots (Figure 2C and Table 1). PC2 represented a continuum of species from those characterized by high SLA and tissue P but low LTD to those with low SLA and tissue P but high LTD. Moreover, plant scores on the first two axes of the root PCA were, respectively, correlated with their scores on the first two axes of the leaf PCA (for PC1:  $R^2=0.44$ ,  $p<0.01$ ; for PC2:  $R^2=0.26$ ,  $p<0.01$ ).

### Variations in Leaf and Fine-Root Traits Among PFTs

SRL was higher in monocots than in eudicots, and highest in grasses and lowest in woody species (Supplementary Figure 3A). LTD was higher in monocots than in eudicots, and highest in grasses and lowest in forbs (Supplementary Figure 3B). Legumes had generally higher N and lower P (therefore higher N:P and lower C:N) than non-legumes (Supplementary Figure 4). RN and RP were higher in eudicots than in monocots, and highest in forbs and lowest in grasses (Supplementary Figures 4A,B). RC:RN



ratio was higher in monocots than in eudicots, and highest in grasses and lowest in forbs (Supplementary Figure 4D).

Significant correlations between SLA and LTD were found in all PFTs except for monocots and grasses (Table 3). SLA was positively correlated with LN and LP in forbs and eudicots, and was negatively correlated with LC:LN in forbs, eudicots, and non-legumes. LP also increased with SLA in non-legumes. N and P contents were significantly correlated in leaves ( $R^2=0.22$ ,  $p<0.01$ ) and fine roots ( $R^2=0.24$ ,  $p<0.01$ ) of non-legumes, but not in other PFTs. SRL was largely uncorrelated with other fine-root traits in individual PFTs (Supplementary Table 3), exceptions were RN-SRL (positive) and RC:RN-SRL (negative) relationships in legumes, and RP-SRL (positive) and RN:RP-SRL (negative) relationships in eudicots.

None of the PFTs showed significant correlation between SLA and SRL (Figure 3E, other results not shown). Fine-root nutrient contents increased with leaf nutrient contents in forbs, woody species, eudicots, and non-legumes (Figure 3 and Table 4). LN and LC:LN were significantly correlated with RN and RC:RN, respectively, in eudicots but not monocots.

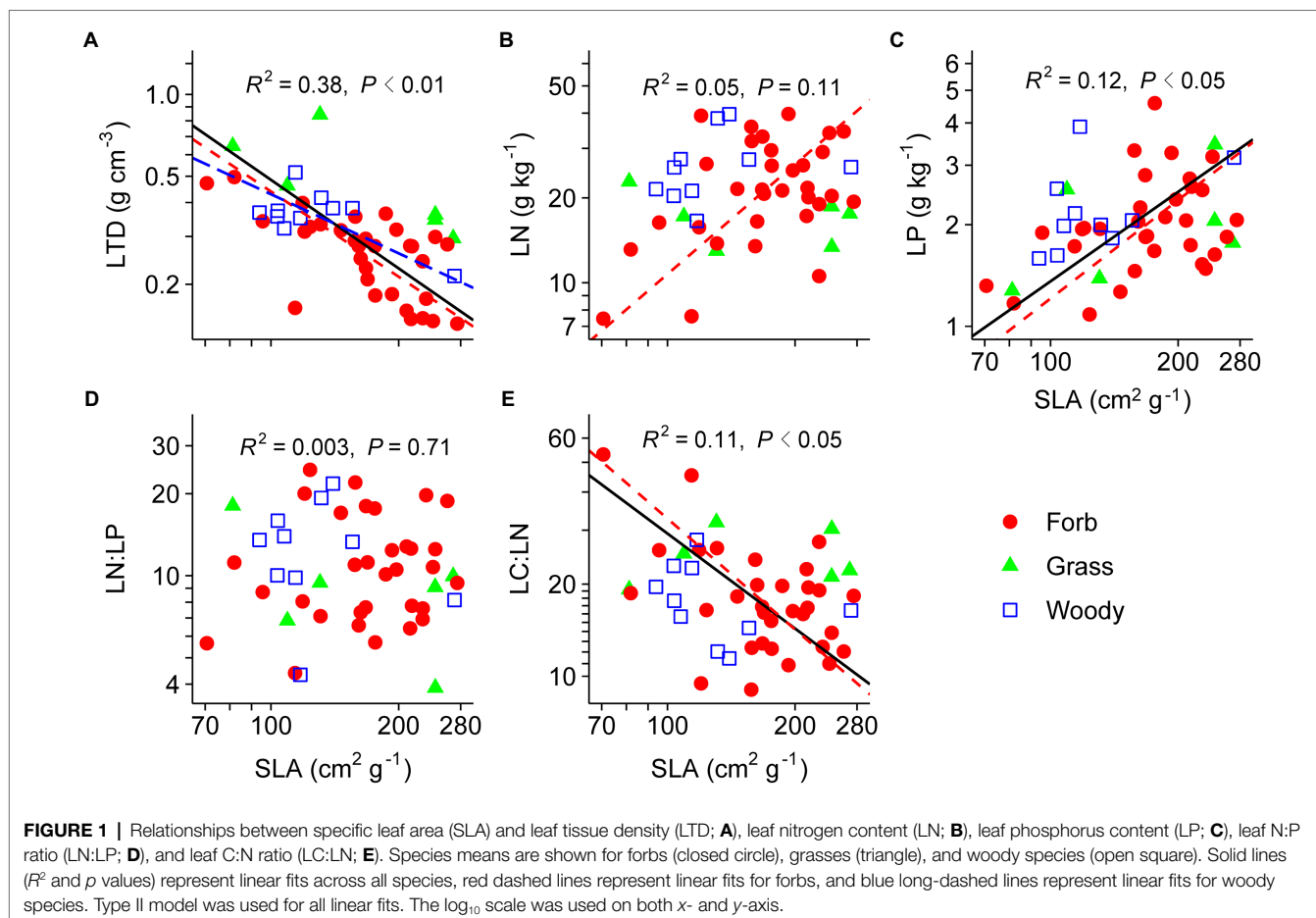
MANOVA and Hotelling's  $T^2$ -test revealed that species scores on the first two main axes (PC1 and PC2) of leaf, root, and whole-plant PCA generally differ among PFTs, except for those for leaf PCA among grasses, forbs, and woody species and

between monocots and eudicots (Supplementary Table 4). ANOVA and  $t$ -test for PC1 showed significant functional type effects on species scores except for leaf traits among grasses, forbs, and woody species; and ANOVA and  $t$ -test for PC2 also generally supported trait differentiation among PFTs, albeit with a few exceptions (Supplementary Table 5).

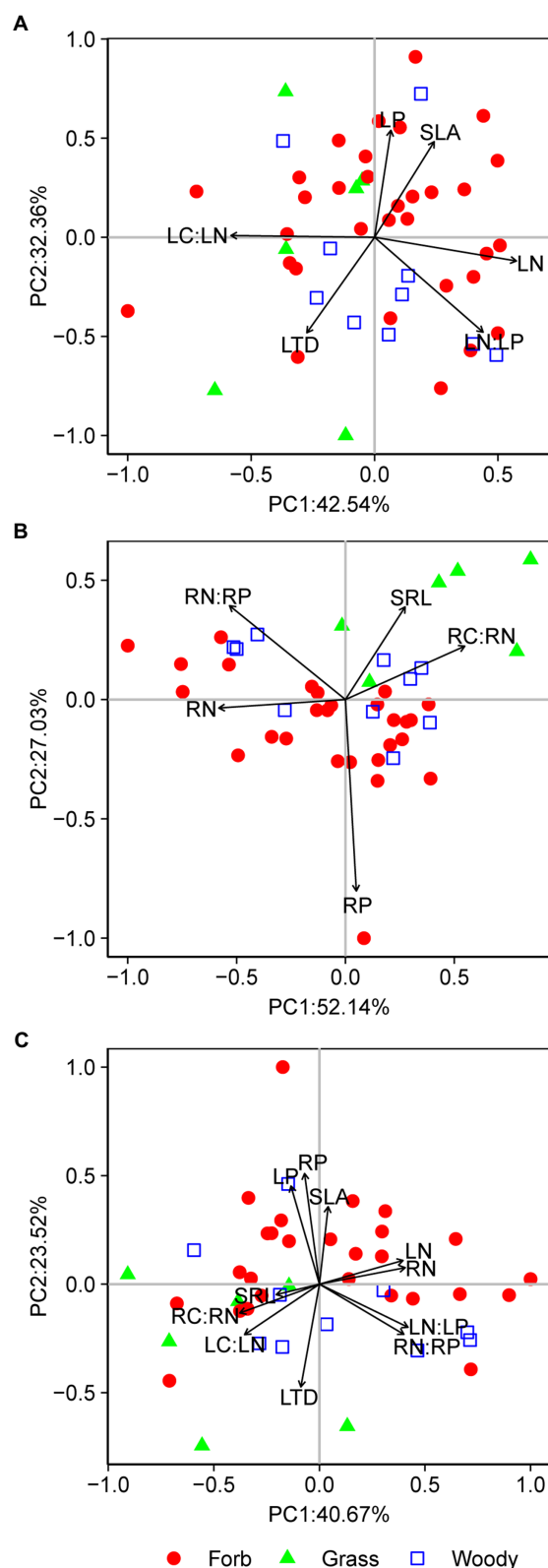
## DISCUSSION

### Do Leaf Traits Conform to the LES Theory?

The LES theory predicts that species with high SLA are characterized by low LTD, high mass-based nutrient contents, high photosynthetic and respiration rates, and short life span, while species with low SLA usually show the opposite pattern of leaf traits (Wright et al., 2004). Our results are partially consistent with our first hypothesis that leaf traits of desert shrubland species follow the LES theory. The correlation between SLA and LN, as predicted by LES, was not supported in this study. Positive correlations between SLA and LN have been widely reported in previous leaf trait studies (Reich, 2014), with the exception of Zhou et al. (2010), who found that SLA and LN were decoupled across dominant species of the Inner Mongolia grassland. The decoupling between LN and LP we found is against the ecological stoichiometry







**FIGURE 2 |** Principal component analyses (PCAs) for leaf traits (A), fine-root traits (B), and whole-plant traits (C). Each data point represents the position of (Continued)

**FIGURE 2 |** a forb (closed circle), grass (triangle), or woody species (open square) in the two-dimensional trait space. SLA, specific leaf area; LTD, leaf tissue density; LN, leaf nitrogen content; LP, leaf phosphorus content; LN:LP, leaf N:P ratio; LC:LN, leaf C:N ratio; SRL, specific root length; RN, fine-root nitrogen content; RP, fine-root phosphorus content; RN:RP, fine-root N:P ratio; and RC:RN, fine-root C:N ratio. The percentages on x- and y-axis indicate the amount of variance explained by the two main axes.

**TABLE 1 |** Coefficients for eigenvectors for main axes of principal component analyses (PCAs) on leaf and/or fine-root traits.

Traits	Leaf or root PC1	Leaf or root PC2	Whole-plant PC1	Whole-plant PC2
SLA	0.24	0.48	0.04	0.36
LTD	-0.27	-0.48	-0.09	-0.47
LN	0.57	-0.12	0.40	0.11
LP	0.07	0.54	-0.13	0.45
LN:LP	0.44	-0.48	0.42	-0.20
LC:LN	-0.58	0.01	-0.35	-0.23
SRL	0.27	0.39	-0.20	-0.05
RN	-0.58	-0.04	0.41	0.08
RP	0.05	-0.80	-0.07	0.51
RN:RP	-0.53	0.39	0.40	-0.23
RC:RN	0.55	0.22	-0.38	-0.13

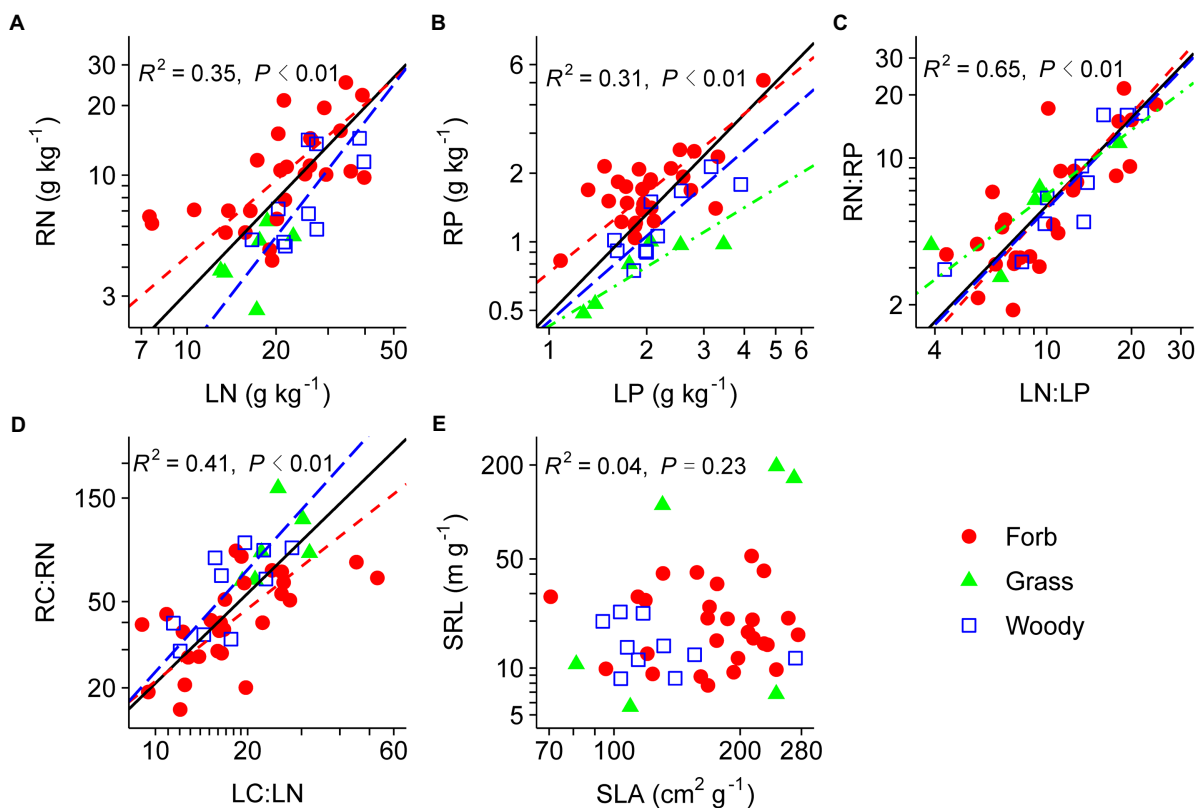
SLA, specific leaf area; LTD, leaf tissue density; LN, leaf nitrogen content; LP, leaf phosphorus content; LN:LP, leaf N:P ratio; LC:LN, leaf C:N ratio; SRL, specific root length; RN, fine-root nitrogen content; RP, fine-root phosphorus content; RN:RP, fine-root N:P ratio; and RC:RN, fine-root C:N ratio.

theory (Zhang et al., 2018), but similar to the finding from a semi-desert community (Grubb et al., 2015).

Such a pattern among leaf traits implies that LN is not necessarily related to the acquisitive strategy in harsh environments (e.g., drylands; He et al., 2008). First, desert species may store part of absorbed N in leaves when plant growth is strongly water-limited (Zhou et al., 2010). Stored N does not contribute directly to the “fast” syndrome. Second, LN is not only involved in photosynthesis, but also comprises compounds that play important non-photosynthetic roles (e.g., defense against herbivory and energy production for metabolism; Osnas et al., 2013). Therefore, the multiple functions of LN (e.g., photosynthesis, storage, and defense) should be explicitly considered in investigating trait variations, trade-offs, and plant strategies in desert communities.

## Is There a Root-Trait Syndrome in Parallel With the Leaf-Trait Syndrome?

The RES assumes that leaf traits are matched by parallel root traits along the acquisitive-conservative resource spectrum (Reich, 2014; Weemstra et al., 2016), and the theory predicts that plant roots with high SRL are also characterized by low LTD but high nutrient contents, turnover rates, and respiration rates. However, the existence of an RES analogous to the LES is currently debated, and evidence has been mixed among and within studies (Withington et al., 2006; Kong et al., 2014; Weemstra et al., 2016). Our results showed generally weak or no correlation between fine-root traits, and thus do not support our hypothesis that the leaf-trait syndrome is paralleled by a



**FIGURE 3 |** Relationships between analogous leaf and fine-root traits across species, including the RN–LN relationship (A), the RP–LP relationship (B), the RN:RP–LN:LP relationship (C), the RC:RN–LC:LN relationship (D), and the SRL–SLA relationship (E). For abbreviations see Figures 1, 2. Species means are shown for forbs (closed circle), grasses (triangle), and woody species (open square). Solid lines ( $R^2$  and  $p$  values) represent linear fits across all species, red dashed lines represent linear fits for forbs, blue long-dashed lines represent linear fits for woody species, and green dot-dashed lines represent linear fits for grasses. Type II model was used for all linear fits. The log<sub>10</sub> scale was used on both x- and y-axis.

**TABLE 2 |** Phylogenetically-independent contrasts between leaf and fine-root traits among desert species.

Root vs. leaf trait	$R^2$	$p$	$n$
logSRL vs. logSLA	0.47	0.05	8
logRN vs. logLN	0.45	<0.01	16
logRP vs. logLP	0.86	<0.01	11
log(RN:RP) vs. log(LN:LP)	0.59	<0.01	24
log(RC:RN) vs. log(LC:LN)	0.30	0.06	11

For abbreviations see Table 1.

similar root-trait syndrome. Similar to our results, some studies in forests and grasslands also found a lack of correlation between SRL and RN (Tjoelker et al., 2005; Chen et al., 2013; Weemstra et al., 2016) and between SRL and RTD (Craine et al., 2001; Chen et al., 2013; Kramer-Walter et al., 2016).

Several reasons may account for the lack of a root-trait syndrome that parallels the leaf-trait syndrome. With regard to methodology, the sampled fine roots (<2 mm in diameter) were not necessarily all absorptive roots. The possible inclusion of fine transport roots (second- or higher-order roots), which

do not represent resource uptake strategies, may confound trait relationships (Withington et al., 2006; McCormack et al., 2015). Root order rather than diameter may be a better proxy for root functioning (McCormack et al., 2015). Therefore, our conclusion of a lack of fine-root trait syndrome is tentative and should be verified in future studies with functional root classifications or order-based analyses. With regard to ecological mechanisms, roots are subjected to multiple constraints especially in desert ecosystems (e.g., uptake of water and multiple nutrients), while leaves are mainly adapted for maximizing carbon gain during their lifetimes (Weemstra et al., 2016). Soil physical and chemical properties in desert regions (such as density, pH, and cation exchange capacity) may present additional limits to root traits that are not present aboveground. In addition, leaf and root traits are not necessarily analogous, because they function differently and might not be related to resource uptake in a similar manner (Weemstra et al., 2016). Finally, resource acquisition by roots is strongly influenced by their interactions with mycorrhizal fungi and other rhizospheric organisms, resulting in selection forces on root traits that are distinct from those on leaf traits (Withington et al., 2006; Reich, 2014). Therefore, a lack of root-trait syndrome that parallels the leaf-trait syndrome across desert shrubland species implies that a

**TABLE 3 |** Relationships between SLA ( $x$ ) and other leaf traits ( $y$ ) for different functional types (PFTs).

$y$	PFT	$a$	$b$	$R^2$
logLTD	Forb (32)	1.73	−1.04***	0.41
	Woody (10)	1.08	−0.72*	0.43
	Eudicot (42)	1.69	−1.02***	0.47
	Legume (13)	1.31	−0.82*	0.31
	Non-legume (35)	1.92	−1.12***	0.41
logLN	Forb (32)	−1.56	1.29**	0.20
	Eudicot (42)	−1.25	1.18*	0.10
logLP	Forb (32)	−1.86	0.97*	0.15
	Eudicot (42)	−1.69	0.91*	0.11
	Non-legume (35)	−1.50	0.84**	0.19
log(LC:LN)	Forb (32)	3.92	−1.20**	0.22
	Eudicot (42)	3.63	−1.09**	0.17
	Non-legume (35)	3.37	−0.94*	0.15

For abbreviations see **Table 1**. Symbols  $a$  and  $b$  represent the intercept and slope of linear regression, respectively,  $R^2$  represents the coefficient of determination. Only significant relationships are shown. Species numbers are included in parentheses. \*Indicates significant linear regression at 0.05 confidence levels.

\*\*Indicates significant linear regression at 0.01 confidence levels.

\*\*\*Indicates significant linear regression at 0.001 confidence levels.

multidimensional root trait framework (Weemstra et al., 2016) that incorporates multiple root functions, multiple constraints on root traits and plant-mycorrhizal interactions may be developed for understanding root-trait variations and correlations in deserts and other stressful environments.

## Are Fine-Root Traits Coordinated With Analogous Leaf Traits?

Our results revealed that plant nutrient contents rather than physical traits (i.e., SLA and SRL) were coordinated between leaves and fine roots in the studied shrubland, a pattern partially in line with our second hypothesis. The whole-plant economics spectrum assumes SRL to be analogous to SLA (Reich, 2014), as these two traits represent resource acquisition capability by fine roots and leaves, respectively. However, available evidence for the linkage between SRL and SLA is mixed at best (Withington et al., 2006). Some studies revealed positive SLA–SRL relationships in woody (Withington et al., 2006; Holdaway et al., 2011) and herbaceous species (Cheng et al., 2016), while other studies found either negative or a lack of correlation between SLA and SRL in different regions and ecosystem types (Kembel and Cahill, 2011; Chen et al., 2013; Geng et al., 2014). Our finding of the nonsignificant SRL–SLA relationship also suggest that SRL may not be the functional analogue of SLA in desert shrublands. In contrast to leaves, the link between root physical traits and resource uptake are not well-established, and SRL might not be a adequate predictor of belowground resource acquisition capacity in desert ecosystems (Eissenstat et al., 2000; Weemstra et al., 2016). Firstly, root functioning (e.g., absorptive vs. transport) is strongly affected by its branching order, even for herbaceous species which do not have as many root branches and complex structures as do woody plants. This may lead

**TABLE 4 |** Correlations between leaf and fine-root traits for different functional types (PFTs).

$y$	$x$	PFT	$a$	$b$	$R^2$
logRN	logLN	Forb (27)	−0.45	1.10***	0.39
		Woody (10)	−1.42	1.65*	0.50
		Eudicot (37)	−0.59	1.17***	0.32
		Non-legume (32)	−0.47	1.01*	0.17
logRP	logLP	Grass (6)	−0.37	0.86*	0.75
		Forb (27)	−0.14	1.16***	0.44
		Woody (10)	−0.35	1.25**	0.67
		Monocot (6)	−0.37	0.86*	0.75
log(RN:RP)	log(LN:LP)	Eudicot (37)	−0.23	1.28***	0.35
		Non-legume (32)	−0.34	1.48***	0.35
		Grass (6)	−0.19	1.02*	0.68
		Forb (27)	−0.73	1.50***	0.62
		Woody (10)	−0.63	1.39***	0.78
		Monocot (6)	−0.19	1.02*	0.68
log(RC:RN)	log(LC:LN)	Eudicot (37)	−0.70	1.46***	0.67
		Non-legume (32)	−0.44	1.18***	0.46
		Forb (27)	0.22	1.11***	0.40
		Woody (10)	−0.19	1.57*	0.52
		Eudicot (37)	0.10	1.24***	0.36
		Non-legume (32)	0.39	1.06**	0.27

For abbreviations see **Table 1**. Symbols  $a$  and  $b$  represent the intercept and slope of linear regression, respectively,  $R^2$  represents the coefficient of determination. Only significant relationships are shown. Species numbers are included in parentheses. \*Indicates significant linear regression at 0.05 confidence levels.

\*\*Indicates significant linear regression at 0.01 confidence levels.

\*\*\*Indicates significant linear regression at 0.001 confidence levels.

to differences in root and leaf physical traits, obscuring the SRL–SLA relationship (Geng et al., 2014; Cheng et al., 2016). In addition, the fine roots of herbaceous species in this study may have a relatively small range of variation in trait values, and as a consequence, the SLA–SRL relationship is likely to be nonsignificant (Geng et al., 2014). Secondly, many desert plants rely on mycorrhizal hyphae to efficiently exploit the soil, and small SRL may support more mycorrhizal fungal colonization per unit root length (Comas et al., 2002; McCormack et al., 2015). Thirdly, root physical traits such as diameter and SRL may be more phylogenetically conservative than leaf physical traits, leading to weak physical coordination between organs (Comas and Eissenstat, 2009; Chen et al., 2013). Therefore, explicit consideration of fine-root functions and assessment of plant-mycorrhizal interactions may aid in the understanding of leaf-root coordination in desert plants.

Plant nutrient contents were significantly positively correlated between leaves and fine roots (**Figures 2C, 3**). These results are in line with previous studies on temperate grasslands (Craine et al., 2005) and forests (Holdaway et al., 2011). Furthermore, the PICs confirmed that the nutrient coordination between leaves and fine roots were not the result of phylogenetic relatedness among studied species (**Table 2**). Such a nutrient-based leaf-root coordination thus reflects the consistency in nutrient uptake and allocation above- and below-ground in desert shrublands. The tight coordination between leaf and root nutrient contents provides the potential to predict belowground stoichiometry from aboveground measurements.

## Do Functional Types Summarize Differences in Traits and Trait Relationships?

Large uncertainties exist on the extent to which trait syndromes are able to differentiate among predefined PFTs (Cheng et al., 2016; Verheijen et al., 2016). Our results support our third hypothesis, showing that PFTs summarized a significant amount of variability in plant traits. Our finding at the local scale is consistent with a recent global synthesis (Verheijen et al., 2016), which demonstrated that PFTs of desert community were differently positioned in the multidimensional trait space. Similarly, Craine et al. (2001) found that grasses and forbs in central Minnesota prairies had distinct trait syndromes. In contrast, Cheng et al. (2016) showed that two key traits, SLA and SRL, were capable of classifying 55 species in the Inner Mongolia grassland into phylogenetically different groups (i.e., early diverged species vs. late diverged species), rather than into distinct PFTs.

Despite the potential of plant traits in discriminating among PFTs, both our results and previous studies reported large variations within PFTs and overlaps between PFTs in plant strategies and traits (Van Bodegom et al., 2012; Verheijen et al., 2013). This indicates that a wide range of strategies may be used by plants within a single PFT to adapt to similar environment conditions, and that some plants may show traits similar to that of other PFTs (Craine et al., 2001). For example, in the studied desert community some grasses such as *Pennisetum centasiaticum* and *Leymus secalinus* were more like forbs in the whole-plant trait space, while some forbs such as *Corispermum hyssopifolium* and *Bassia dasyphylla* had leaves and roots traits that resemble grasses (Figure 2C). We propose that one of the future research challenges in trait-based ecology is to understand what determines the potential of plant traits to functionally differentiate among PFTs, as this potential would allow global or regional vegetation mapping based on trait maps (Van Bodegom et al., 2014; Verheijen et al., 2016).

The ability of plant traits to differentiate among PFTs depends partly on how PFTs are classified. Leaf and roots traits in our study best discriminated between legumes and non-legumes. Another source of uncertainty in differentiating among PFTs is the selection of trait combinations (Verheijen et al., 2016). Differentiations between growth forms (grasses, forbs, and woody species) or evolutionary relationships (monocots and eudicots) were mostly attributed to root rather than leaf traits. Despite the importance of root traits in differentiating among PFTs, variations in some root traits among PFTs are not in line with the presumed RES. For example, low RN and RP in grasses exhibiting high SRL (Supplementary Figures 3, 4), indicating that their roots have relatively low metabolic rates and depend mainly on cost-efficient root structure to acquire soil resources (Reich et al., 2008; Freschet et al., 2017). Future studies should examine which traits and classifications are most relevant to functional differences among PFTs. Incorporation of trait variations among the most relevant classification of PFTs should improve the modelling of plant and ecosystem functioning.

In line with our third hypothesis, PFTs also differed in bivariate relationships between leaf and fine-root traits. Most trait correlations did not hold in all PFTs, suggesting different nutrient absorption and utilization characteristics among PFTs.

Therefore, the influence of PFTs on trait associations and trade-offs should be considered when estimating one trait from another. In addition, differences in bivariate trait relationships among PFTs could provide important insights into the mechanisms governing species effects on ecosystem processes (Tjoelker et al., 2005).

## CONCLUSIONS

Our analyses using 48 species in a desert shrubland community of northern China revealed that variations in leaf traits were partially in line with the predictions of the global LES. Variations in fine-root traits, however, provided little evidence for a RES. The coordination between leaves and fine roots was stronger for nutrient contents and their ratios than for physical traits (i.e., SLA and SRL). In addition, our results illustrate the potential of plant traits to functionally differentiate among PFTs, despite large overlaps among PFTs in plant strategies. We conclude that fine-root functions in the studied desert community are probably coordinated with leaf functions with respect to nutrient allocation and use. Future studies at the regional scale should examine the extent to which our conclusions are applicable across all types of desert communities.

## DATA AVAILABILITY STATEMENT

Data used in this study are available as part of the **Supplementary Material**.

## AUTHOR CONTRIBUTIONS

XinJ designed and led this research. XiaJ and XinJ wrote the draft manuscript and analyzed the data. XiaJ, SG, YJ, NW, and CH performed field sampling and measurements. TZ, PL, YT, and SQ provided editorial advices. All authors contributed to the article and approved the submitted version.

## FUNDING

This study was supported by the National Natural Science Foundation of China (NSFC, nos. 32071843, 31670708, 31901366, and 32071842) and the Fundamental Research Funds for the Central Universities (nos. 2015ZCQ-SB-02, PTYX202122, and PTYX202123).

## ACKNOWLEDGMENTS

We thank Wei Zhang and Shaoyu Guo for their assistance with field sampling and measurements.

## SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fpls.2021.734775/full#supplementary-material>



## REFERENCES

- Ackerly, D. D., Knight, C. A., Weiss, S. B., Barton, K., and Starmer, K. P. (2002). Leaf size, specific leaf area and microhabitat distribution of chaparral woody plants: contrasting patterns in species level and community level analyses. *Oecologia* 130, 449–457. doi: 10.1007/s004420100805
- Ali, A. M., Darvishzadeh, R., Skidmore, A. K., van Duren, I., Heiden, U., and Heurich, M. (2016). Estimating leaf functional traits by inversion of PROSPECT: assessing leaf dry matter content and specific leaf area in mixed mountainous forest. *Int. J. Appl. Earth Obs. Geoinf.* 45, 66–76. doi: 10.1016/j.jag.2015.11.004
- Bai, Y., She, W., Michalet, R., Zheng, J., Qin, S., and Zhang, Y. (2018). Benefactor facilitation and beneficiary feedback effects drive shrub-dominated community succession in a semi-arid dune ecosystem. *Appl. Veg. Sci.* 21, 595–606. doi: 10.1111/avsc.12388
- Caplan, J. S., Meiners, S. J., Flores-Moreno, H., and McCormack, M. L. (2019). Fine-root traits are linked to species dynamics in a successional plant community. *Ecology* 100:e02588. doi: 10.1002/ecy.2588
- Carvajal, D. E., Loayza, A. P., Rios, R. S., Delpiano, C. A., and Squeo, F. A. (2019). A hyper-arid environment shapes an inverse pattern of the fast-slow plant economics spectrum for above-, but not below-ground resource acquisition strategies. *J. Ecol.* 107, 1079–1092. doi: 10.1111/1365-2745.13092
- Chen, W., Zeng, H., Eissenstat, D. M., and Guo, D. (2013). Variation of first-order root traits across climatic gradients and evolutionary trends in geological time. *Glob. Ecol. Biogeogr.* 22, 846–856. doi: 10.1111/geb.12048
- Cheng, J., Chu, P., Chen, D., and Bai, Y. (2016). Functional correlations between specific leaf area and specific root length along a regional environmental gradient in Inner Mongolia grasslands. *Funct. Ecol.* 30, 985–997. doi: 10.1111/1365-2435.12569
- Cheng, Y. H., Han, Y. Z., Wang, Q. C., and Wang, Z. Q. (2005). Seasonal dynamics of fine root biomass, root length density, specific root length and soil resource availability in a *larix gemlini* plantation. *Chin. J. Plant Ecol.* 29, 403–410. doi: 10.17521/cjpe.2005.0053
- Comas, L. H., Bouma, T. J., and Eissenstat, D. M. (2002). Linking root traits to potential growth rate in six temperate tree species. *Oecologia* 132, 34–43. doi: 10.1007/s00442-002-0922-8
- Comas, L. H., and Eissenstat, D. M. (2004). Linking fine root traits to maximum potential growth rate among 11 mature temperate tree species. *Funct. Ecol.* 18, 388–397. doi: 10.1111/j.0269-8463.2004.00835.x
- Comas, L. H., and Eissenstat, D. M. (2009). Patterns in root trait variation among 25 co-existing north American forest species. *New Phytol.* 182, 919–928. doi: 10.1111/j.1469-8137.2009.02799.x
- Cornelissen, J. H. C., Lavorel, S., Garnier, E., Díaz, S., Buchmann, N., Gurvich, D. E., et al. (2003). A handbook of protocols for standardized and easy measurement of plant functional traits worldwide. *Aust. J. Bot.* 51, 335–380. doi: 10.1071/BT02124
- Craine, J. M., Froehle, J., Tilman, D. G., Wedin, D. A., and Chapin, F. S. III (2001). The relationships among root and leaf traits of 76 grassland species and relative abundance along fertility and disturbance gradients. *Oikos* 93, 274–285. doi: 10.1034/j.1600-0706.2001.930210.x
- Craine, J. M., Lee, W. G., Bond, W. J., Williams, R. J., and Johnson, L. C. (2005). Environmental constraints on a global relationship among leaf and root traits of grasses. *Ecology* 86, 12–19. doi: 10.1890/04-1075
- Eissenstat, D. M., Wells, C. E., Yanai, R. D., and Whitbeck, J. L. (2000). Building roots in a changing environment: implications for root longevity. *New Phytol.* 147, 33–42. doi: 10.1046/j.1469-8137.2000.00686.x
- Fajardo, A., and Siefert, A. (2016). Phenological variation of leaf functional traits within species. *Oecologia* 180, 951–959. doi: 10.1007/s00442-016-3545-1
- Felsenstein, J. (1985). Phylogenies and the comparative method. *Am. Nat.* 125, 1–15. doi: 10.1086/284325
- Freschet, G. T., Cornelissen, J. H. C., van Logtestijn, R. S. P., and Aerts, R. (2010). Evidence of the 'plant economics spectrum' in a subarctic flora. *J. Ecol.* 98, 362–373. doi: 10.1111/j.1365-2745.2009.01615.x
- Freschet, G. T., Valverde-Barrantes, O. J., Tucker, C. M., Craine, J. M., McCormack, M. L., Violle, C., et al. (2017). Climate, soil and plant functional types as drivers of global fine-root trait variation. *J. Ecol.* 105, 1182–1196. doi: 10.1111/1365-2745.12769
- Geng, Y., Wang, L., Jin, D., Liu, H., and He, J. S. (2014). Alpine climate alters the relationship between leaf and root morphological traits but not chemical traits. *Oecologia* 175, 445–455. doi: 10.1007/s00442-014-2919-5
- Grubb, P. J., Marañón, T., Pugnaire, F. I., and Sack, L. (2015). Relationships between specific leaf area and leaf composition in succulent and non-succulent species of contrasting semi-desert communities in South-Eastern Spain. *J. Arid Environ.* 118, 69–83. doi: 10.1016/j.jaridenv.2015.03.001
- He, J. S., Wang, L., Flynn, D. F. B., Wang, X., Ma, W., and Fang, J. (2008). Leaf nitrogen: phosphorus stoichiometry across Chinese grassland biomes. *Oecologia* 155, 301–310. doi: 10.1007/s00442-007-0912-y
- Holdaway, R. J., Richardson, S. J., Dickie, I. A., Peltzer, D. A., and Coomes, D. A. (2011). Species- and community-level patterns in fine root traits along a 120000-year soil chronosequence in temperate rain forest. *J. Ecol.* 99, 954–963. doi: 10.1111/j.1365-2745.2011.01821.x
- Hosseini, A., Hosseini, S. M., and Linares, J. C. (2019). Linking morphological and ecophysiological leaf traits to canopy dieback in Persian oak trees from central Zagros. *J. For. Res.* 30, 1755–1764. doi: 10.1007/s11676-018-0805-4
- John, M. K. (1970). Colorimetric determination of phosphorus in soil and plant materials with ascorbic acid. *Soil Sci.* 109, 214–220. doi: 10.1097/00010694-197004000-00002
- Kemmel, S. W., and Cahill, J. F. Jr. (2011). Independent evolution of leaf and root traits within and among temperate grassland plant communities. *PLoS One* 6:e19992. doi: 10.1371/journal.pone.0019992
- Kerckhoff, A. J., Fagan, W. F., Elser, J. J., and Enquist, B. J. (2006). Phylogenetic and growth form variation in the scaling of nitrogen and phosphorus in the seed plants. *Am. Nat.* 168, E103–E122. doi: 10.1086/507879
- Kong, D., Ma, C., Zhang, Q., Li, L., Chen, X., Zeng, H., et al. (2014). Leading dimensions in absorptive root trait variation across 96 subtropical forest species. *New Phytol.* 203, 863–872. doi: 10.1111/nph.12842
- Kramer-Walter, K. R., Bellingham, P. J., Millar, T. R., Smissen, R. D., Richardson, S. J., and Laughlin, D. C. (2016). Root traits are multidimensional: specific root length is independent from root tissue density and the plant economic spectrum. *J. Ecol.* 104, 1299–1310. doi: 10.1111/1365-2745.12562
- Liu, G., Freschet, G. T., Pan, X., Cornelissen, J. H. C., Li, Y., and Dong, M. (2010). Coordinated variation in leaf and root traits across multiple spatial scales in Chinese semi-arid and arid ecosystems. *New Phytol.* 188, 543–553. doi: 10.1111/j.1469-8137.2010.03388.x
- McCormack, M. L., Dickie, I. A., Eissenstat, D. M., Fahey, T. J., Fernandez, C. W., Guo, D., et al. (2015). Redefining fine roots improves understanding of below-ground contributions to terrestrial biosphere processes. *New Phytol.* 207, 505–518. doi: 10.1111/nph.13363
- Mitchell, R. M., Wright, J. P., and Ames, G. M. (2017). Intraspecific variability improves environmental matching, but does not increase ecological breadth along a wet-to-dry ecotone. *Oikos* 126, 988–995. doi: 10.1111/oik.04001
- Osnas, J. L. D., Lichstein, J. W., Reich, P. B., and Pacala, S. W. (2013). Global leaf trait relationships: mass, area, and the leaf economics spectrum. *Science* 340, 741–744. doi: 10.1126/science.1231574
- Pérez-Ramos, I. M., Roumet, C., Cruz, P., Blanchard, A., Autran, P., and Garnier, E. (2012). Evidence for a 'plant community economics spectrum' driven by nutrient and water limitations in a Mediterranean rangeland of southern France. *J. Ecol.* 100, 1315–1327. doi: 10.1111/1365-2745.12000
- Personeni, E., and Loiseau, P. (2004). How does the nature of living and dead roots affect the residence time of carbon in the root litter continuum? *Plant Soil* 267, 129–141. doi: 10.1007/s11104-005-4656-3
- Qin, H., Zhang, Y. B., Dong, G., and Zhang, F. (2019). Altitudinal patterns of taxonomic, phylogenetic and functional diversity of forest communities in mount Guandi, Shanxi, China. *Chin. J. Plant Ecol.* 43, 762–773. doi: 10.17521/cjpe.2018.0088
- Reich, P. B. (2014). The world-wide 'fast-slow' plant economics spectrum: a traits manifesto. *J. Ecol.* 102, 275–301. doi: 10.1111/1365-2745.12211
- Reich, P. B., Tjoelker, M. G., Pregitzer, K. S., Wright, I. J., Oleksyn, J., and Machado, J. L. (2008). Scaling of respiration to nitrogen in leaves, stems and roots of higher land plants. *Ecol. Lett.* 11, 793–801. doi: 10.1111/j.1461-0248.2008.01185.x
- She, W., Bai, Y., Zhang, Y., Qin, S., Liu, Z., and Wu, B. (2017). Plasticity in meristem allocation as an adaptive strategy of a desert shrub under contrasting environments. *Front. Plant Sci.* 8:1933. doi: 10.3389/fpls.2017.01933



- The Angiosperm Phylogeny Group (2009). An update of the angiosperm phylogeny group classification for the orders and families of flowering plants: APG III. *Bot. J. Linn. Soc.* 161, 105–121. doi: 10.1111/j.1095-8339.2009.00996.x
- Tjoelker, M. G., Craine, J. M., Wedin, D., Reich, P. B., and Tilman, D. (2005). Linking leaf and root trait syndromes among 39 grassland and savannah species. *New Phytol.* 167, 493–508. doi: 10.1111/j.1469-8137.2005.01428.x
- Van Bodegom, P. M., Douma, J. C., and Verheijen, L. M. (2014). A fully traits-based approach to modeling global vegetation distribution. *Proc. Natl. Acad. Sci. U. S. A.* 111, 13733–13738. doi: 10.1073/pnas.1304551110
- Van Bodegom, P. M., Douma, J. C., Witte, J. P. M., Ordoñez, J. C., Bartholomeus, R. P., and Aerts, R. (2012). Going beyond limitations of plant functional types when predicting global ecosystem-atmosphere fluxes: exploring the merits of traits-based approaches. *Glob. Ecol. Biogeogr.* 21, 625–636. doi: 10.1111/j.1466-8238.2011.00717.x
- Verheijen, L. M., Aerts, R., Bönsch, G., Kattge, J., and Van Bodegom, P. M. (2016). Variation in trait trade-offs allows differentiation among predefined plant functional types: implications for predictive ecology. *New Phytol.* 209, 563–575. doi: 10.1111/nph.13623
- Verheijen, L. M., Brovkin, V., Aerts, R., Bönsch, G., Cornelissen, J. H. C., Kattge, J., et al. (2013). Impacts of trait variation through observed trait-climate relationships on performance of an earth system model: a conceptual analysis. *Biogeosciences* 10, 5497–5515. doi: 10.5194/bg-10-5497-2013
- Wang, R., Wang, Q., Zhao, N., Xu, Z., Zhu, X., Jiao, C., et al. (2018). Different phylogenetic and environmental controls of first-order root morphological and nutrient traits: evidence of multidimensional root traits. *Funct. Ecol.* 32, 29–39. doi: 10.1111/1365-2435.12983
- Weemstra, M., Mommer, L., Visser, E. J. W., van Ruijven, J., Kuyper, T. W., Mohren, G. M. J., et al. (2016). Towards a multidimensional root trait framework: a tree root review. *New Phytol.* 211, 1159–1169. doi: 10.1111/nph.14003
- Withington, J. M., Reich, P. B., Oleksyn, J., and Eissenstat, D. M. (2006). Comparisons of structure and life span in roots and leaves among temperate trees. *Ecol. Monogr.* 76, 381–397. doi: 10.1890/0012-9615(2006)076[0381:COALS]2.0.CO;2
- Wright, I. J., Reich, P. B., Westoby, M., Ackerly, D. D., Baruch, Z., Bongers, F., et al. (2004). The worldwide leaf economics spectrum. *Nature* 428, 821–827. doi: 10.1038/nature02403
- Xia, M., Valverde-Barrantes, O. J., Suseela, V., Blackwood, C. B., and Tharayil, N. (2021). Coordination between compound-specific chemistry and morphology in plant roots aligns with ancestral mycorrhizal association in woody angiosperms. *New Phytol.* doi: 10.1111/nph.17561 In press
- Zhang, J., He, N., Liu, C., Xu, L., Yu, Q., and Yu, G. (2018). Allocation strategies for nitrogen and phosphorus in forest plants. *Oikos* 127, 1506–1514. doi: 10.1111/oik.05517
- Zhou, P., Geng, Y., Ma, W. H., and He, J. S. (2010). Linkages of functional traits among plant organs in the dominant species of the Inner Mongolia grassland, China. *Chin. J. Plant Ecol.* 34, 7–16. doi: 10.3773/j.issn.1005-264x.2010.01.003

**Conflict of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

**Publisher's Note:** All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Copyright © 2021 Jiang, Jia, Gao, Jiang, Wei, Han, Zha, Liu, Tian and Qin. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.



# The Economics Spectrum Drives Root Trait Strategies in Mediterranean Vegetation

Enrique G. de la Riva<sup>1\*</sup>, José Ignacio Querejeta<sup>2</sup>, Rafael Villar<sup>3</sup>, Ignacio M. Pérez-Ramos<sup>4</sup>, Teodoro Maraño<sup>4</sup>, Javier Galán Díaz<sup>5</sup>, Sergio de Tomás Marín<sup>1</sup> and Iván Prieto<sup>2,6</sup>

<sup>1</sup>Department of Ecology, Brandenburg University of Technology, Cottbus, Germany, <sup>2</sup>Departamento de Conservación de Suelos y Agua, Centro de Edafología y Biología Aplicada del Segura – Consejo Superior de Investigaciones Científicas (CEBAS-CSIC), Murcia, Spain, <sup>3</sup>Área de Ecología, Departamento de Botánica, Ecología y Fisiología Vegetal, Facultad de Ciencias, Córdoba, Spain, <sup>4</sup>Institute of Natural Resources and Agrobiology of Seville (IRNAS), CSIC, Seville, Spain, <sup>5</sup>Doñana Biological Station (EBD), CSIC, Seville, Spain, <sup>6</sup>Departamento de Ecología, Facultad de Biología y Ciencias Ambientales, Universidad de León, León, Spain

## OPEN ACCESS

### Edited by:

Massimiliano Tattini,  
National Research Council (CNR),  
Italy

### Reviewed by:

Lina Fusaro,  
Institute of BioEconomy, Italy  
Martina Pollastrini,  
University of Florence, Italy

### \*Correspondence:

Enrique G. de la Riva  
enga70@gmail.com;  
garciade@b-tu.de

### Specialty section:

This article was submitted to  
Functional Plant Ecology,  
a section of the journal  
Frontiers in Plant Science

**Received:** 09 September 2021

**Accepted:** 27 October 2021

**Published:** 23 November 2021

### Citation:

de la Riva EG, Querejeta JI, Villar R, Pérez-Ramos IM, Maraño T, Galán Díaz J, de Tomás Marín S and Prieto I (2021) The Economics Spectrum Drives Root Trait Strategies in Mediterranean Vegetation. *Front. Plant Sci.* 12:773118. doi: 10.3389/fpls.2021.773118

Extensive research efforts are devoted to understand fine root trait variation and to confirm the existence of a belowground root economics spectrum (RES) from acquisitive to conservative root strategies that is analogous to the leaf economics spectrum (LES). The economics spectrum implies a trade-off between maximizing resource acquisition and productivity or maximizing resource conservation and longevity; however, this theoretical framework still remains controversial for roots. We compiled a database of 320 Mediterranean woody and herbaceous species to critically assess if the classic economics spectrum theory can be broadly extended to roots. Fine roots displayed a wide diversity of forms and properties in Mediterranean vegetation, resulting in a multidimensional trait space. The main trend of variation in this multidimensional root space is analogous to the main axis of LES, while the second trend of variation is partially determined by an anatomical trade-off between tissue density and diameter. Specific root area (SRA) is the main trait explaining species distribution along the RES, regardless of the selected traits. We advocate for the need to unify and standardize the criteria and approaches used within the economics framework between leaves and roots, for the sake of theoretical consistency.

**Keywords:** ecophysiology, fine root strategies, non-mass normalized traits, root anatomy, root economic spectrum, specific root area, specific root length

## INTRODUCTION

Fine roots perform multiple essential functions, including acquisition and storage of soil resources (McCormack and Iversen, 2019). The variation in functional traits of fine roots has become an uprising research topic in the last years aimed at enhancing our understanding of the large diversity of plant belowground uptake strategies (Freschet et al., 2021). Functional trait variation often represents different plant strategies or adaptations to a wide range of environmental conditions (Wright et al., 2004; Díaz et al., 2016), which usually respond to a trade-off between growth and survival (Reich, 2014). This implies that plants invest in their attributes accordingly to an overarching trade-off between maximizing resource acquisition and productivity or

maximizing resource conservation and longevity, the so-called “fast-slow” economics spectrum (Wright et al., 2004; Reich, 2014). This theoretical framework has been successfully linked to plant performance at the leaf level (Wright et al., 2004) but still remains controversial for roots (Freschet et al., 2021).

In the last years, large databases have been built to compile and analyze root trait variation globally (Freschet et al., 2017; Iversen et al., 2017). A search within the largest root database to date (GRoot; Guerrero-Ramírez et al., 2020) showed that the most commonly measured morphological and chemical root traits are specific root length (SRL; measured in 31.7% of all the species in the database), root nitrogen concentration (RN; 27.6%), root diameter (Rdi; 26.0%), and root tissue density (RD; 23.6%), for which their roles in plant functioning and resource economics have been relatively well described (Freschet et al., 2021). By analogy with leaves, plant species growing in more productive environments would have thinner roots with lower tissue density and higher SRL, which enhance a faster return of investments by favoring a higher metabolic activity and respiration at the expense of lower longevity. By contrast, in resource-poor environments the opposite trend of root traits is frequently observed (Roumet et al., 2016; de la Riva et al., 2018). Despite this general trend of root trait covariation, contrasting results have been reported in the literature. Several studies support the existence of a main trend of root trait covariation in line with the root economics spectrum (RES) theory (Prieto et al., 2015; Roumet et al., 2016; de la Riva et al., 2018), whereas other approaches advocate for a multidimensionality of root uptake strategies (Valverde-Barrantes et al., 2015; Weemstra et al., 2016). Recently, evidence supporting a bivariate plane of root specialization called “root economics space” has emerged (McCormack and Iversen, 2019; Bergmann et al., 2020). Within this bidimensional space, the second axis of variation represents the classical trade-off between fast and slow resource uptake strategies (RES, *sensu* Bergmann et al., 2020), while the first axis is defined as a “collaboration” gradient that ranges from species with high SRL and short lifespan that do not depend on mycorrhizal fungi for water and nutrient uptake (“do-it yourself” strategy) to roots with a higher investment in mass per unit of root length (low SRL) that outsource resource uptake *via* symbiotic associations with mycorrhizal fungi as a means to enhance nutrient acquisition. This “outsourcing” strategy has morphological and functional consequences for the roots, as plants with larger Rdi depend more heavily on mycorrhizal fungi for an efficient resource acquisition. However, there is not unanimous agreement about the mechanisms underlying the costs of these root modifications. Thus, a clear explanation for how and why some key fine-root traits sometimes co-vary in a coordinated manner but at other times appear to vary independently is still missing (McCormack and Iversen, 2019).

The lack of consistency in root trait covariation brings up another key aspect that warrants further research, the often reported mismatch between economics spectrum of leaf and root traits. In leaves, the leaf economics spectrum (LES) was defined as the main trend of variation of mass-normalized morphological and physiological traits (e.g., leaf mass per area, photosynthetic capacity), leading to a trade-off between resource

acquisition and conservation that is widely supported at a global scale (Wright et al., 2004; Díaz et al., 2016). This resource-use paradigm has helped to explain leaf trait covariation across species and has been successfully linked to plant strategies both globally and at regional and local scales (Wright et al., 2004; Reich, 2014; Prieto et al., 2018). In parallel, extensive efforts are being carried out to better understand the role of root trait variation and to confirm the existence of a belowground RES, analogous to the LES (Prieto et al., 2015; Roumet et al., 2016; de la Riva et al., 2021). However, the LES and the RES are not always coordinated with each other, and one of the main reasons for these discrepancies could be a potential mismatch between the criteria and traits used to define each of them. Plants show strong multidimensional variation in their “non-mass normalized” attributes whether for leaves or roots [i.e., leaf thickness (Lthick) or root diameter]. However, the widely accepted perspective in the LES approach is that, despite the important roles of leaf tissue density (LD), thickness, area and hydraulic (i.e., stomatal density) or physiological traits (leaf  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$ ) to understand the structure and functioning of leaves (Poorter et al., 2009; Wright et al., 2017; Prieto et al., 2018; Bertolino et al., 2019), neither of these traits are normally more integrative than the specific leaf area (SLA; leaf area per unit of dry mass) or its inverse leaf mass area (LMA; Hodgson et al., 2011; Onoda et al., 2011), which have been considered central attributes of plant strategies. Interspecific variation in SLA is determined by modifications in its underlying components, Lthick and density (Villar et al., 2013; Gratani et al., 2018). However, despite the trade-off between these two components modulating SLA variation, the LES is considered the main trend of functional variation in leaves (Poorter et al., 2009; de la Riva et al., 2016). In contrast to leaves, both mass- and non-mass-based root traits have been frequently and inconsistently analyzed as components of the functional resource uptake strategies, being SRL and its underlying components (RD and diameter) interpreted as a dimension of root variation that is different from the classic economics spectrum, based on mass-normalized traits (i.e., Kramer-Walter et al., 2016; Bergmann et al., 2020). In addition, the historical lack of robust root trait data (Iversen et al., 2017) and the absence of studies considering morphological and physiological traits simultaneously across a large number of species (as can be observed in the GRoot database; Guerrero-Ramírez et al., 2020), has made it very difficult to assert the generality of the RES at the same level as the LES. Therefore, a more precise and rigorous definition of the RES would help to develop a general theory that explains root trait coordination and trade-offs in a wider array of different contexts, using the fewest but most critical attributes of the belowground plant fraction (Reich, 2014).

Based on this background, the present study aimed to explore root trait variation in a large pool of Mediterranean species and assess the main trends of belowground trait diversity under Mediterranean conditions. In a recent study, we have shown evidence that the currency of root economics is the amount of photosynthates required to construct fine roots (root construction costs) that explore the soil for resource acquisition. We observed a main trend of root trait variation, mainly

determined by the covariation between specific root area (SRA), root dry matter content (RDMC), and root density (RD) that showed a clear relationship with root construction costs (de la Riva et al., 2021). Here, we advocate for a more rigorous definition of the main attributes of the economics spectrum, at the root level, that more closely matches the approach used for leaves. This approach will help to define the main planes of root trait variation to better understand which are the most integrative plant strategies encompassing above- and belowground traits. This approach will also help to build a starting point to integrate the complexity of the belowground component in a main trend of root trait variation that defines different resource acquisition-conservation strategies. To do this, we focus on three central hypotheses relevant to the economics spectrum theory in order to evaluate whether it can be broadly extended to roots.

1. The RES is the main trend of root trait covariation in Mediterranean species.
2. Specific root area is a more informative trait than SRL from a plant economics perspective (PES).
3. An anatomical trade-off between tissue density and thickness/diameter exists for both leaves and roots.

To explore these questions, we have compiled a dataset of functional traits with 320 species from Mediterranean – climate areas of the Iberian Peninsula (119 woody and 201 herbaceous) measured using the same protocol. The review and analysis of this dataset will hopefully help to clarify the abovementioned questions and can be a useful complement to the many earlier works it builds upon.

## MATERIALS AND METHODS

### Database

The analyses presented in this manuscript are based on 532 observations from 320 species (119 woody and 201 herbaceous species) with a large phylogenetic diversity (32 different taxonomic orders). The Mediterranean biome is one of the most diverse in the world, with a wide range of environments and plant adaptations; thus, our dataset includes species from arid to sub-humid Mediterranean areas (**Appendix S1**). More importantly, these 320 species encompass ca. 95% of the range of mean variation in LMA encountered globally (Poorter et al., 2009).

To build this database, we selected studies or unpublished data carried out during the last decade by the authors (see **Appendix S1**; de la Riva et al., 2016, 2021 for more details) that contained measurements of eight root (five morphological and three chemical) and six leaf (four morphological and two chemical) functional traits linked to the root- and leaf economics spectrum, respectively. One strength of the database is that leaves and roots were collected on individual trees following the same sampling protocol. For fine roots (<2 mm), we selected SRL ( $\text{m g}^{-1}$ ), SRA ( $\text{m}^2 \text{kg}^{-1}$ ), RDMC ( $\text{g g}^{-1}$ ), RD ( $\text{g cm}^{-3}$ ), Rdi (mm), and the concentration of carbon (RC), nitrogen (RN), minerals, and organic N. For leaves, we selected SLA

( $\text{m}^2 \text{kg}^{-1}$ ), leaf dry matter content (LDMC;  $\text{mg g}^{-1}$ ), Lthick (mm), leaf tissue density (LD, calculated as the ratio of LMA and thickness;  $\text{g ml}^{-1}$ ), and the concentration of leaf nitrogen (LN). In total, we analyzed information of 320 species for root morphological traits (SRL, SRA, RDMC, RD, and Rdi) and for 314 species for leaf morphological traits (SLA, LDMC, Lthick, and LD). Information for leaf and root chemical traits (N and C) was obtained from 64 woody species (from Marañón et al., 2020; de la Riva et al., 2021; 80 observations in total) and minerals and organic N concentration was obtained from 60 species (from de la Riva et al., 2021; 73 observations).

### Data Analyses

All data processing and analyses were done using R 3.6.1. To obtain an overview of the dimensional variation of root and leaf traits (hypothesis 1), we conducted two separate principal component analyses (PCA), one for roots and one for leaves. We used a subset of analogous morphological traits linked to economics spectrum at root (SRL, SRA, RDMC, Rdi, and RD; de la Riva et al., 2021) and leaf level (SLA, LDMC, LD, and Lthick), respectively for the leaf and root PCAs. For these PCAs, and in order to use all the measurements in the database (532), when trait measurements for a given individual were not complete (e.g., there were missing values for one trait, which happened in 15 individuals in total and only for leaf traits), we calculated the mean value for that given trait using the function: `apply {dataset, 2, function (x) ifelse [is.na(x), mean(x, na.rm.=TRUE), x]}`. Additionally, we carried out two separate PCAs for woody and herbaceous species, respectively, which allowed us to assess the influence of the growth forms in the observed patterns.

To control for potential phylogenetic effects on trait covariation, we conducted the same PCAs (leaves and roots and herbaceous and woody species) using phylogenetically independent contrasts and a mean trait value per species for 318 Mediterranean plant species (two ferns species *Equisetum ramosissimum* and *Pteridium aquilinum* were excluded from the analysis because their phylogenetic information was not available in the ALLMB tree used to build the phylogenetic tree for our set of species). We used the “`phyl.pca`” function implemented in the library RPANDA (Morlon et al., 2016) to conduct the phylogenetically informed PCAs. Information to construct the phylogenetic tree of the studied species was obtained from the ALLMB tree (Smith and Brown, 2018; available in [https://github.com/FePhyFoFum/big\\_seed\\_plant\\_trees](https://github.com/FePhyFoFum/big_seed_plant_trees)). The phylogenetic distance of the species (52 in total) that were not found in the ALLMB database were supplanted by the distance of the closest species of the same genus found in the mega-phylogeny tree (see de la Riva et al., 2019 for more details).

To test if the SRA is better proxy than SRL (hypothesis 2), the degree of coordination among SRA and SRL with chemical root traits was determined with Pearson correlations coefficients.

To observe the covariation between SRA with RD and RDi and SLA with LD and Lthick (hypothesis 3), we first run two separate PCAs for each plant organ (leaves and roots) with



these specific traits and the total pool of species (532 observations). Second, we selected species for which root nitrogen was available (64 woody species, 80 observations). We then run three different PCAs in order to compare the patterns obtained within our dataset with those related with the root economic space as defined by Bergmann et al. (2020): (I) a PCA with the same traits proposed by Bergmann et al. (2020); (II) the same PCA, where we replaced SRL by SRA; and (III) the PCA proposed by Bergmann et al. (2020) using analogous leaf and root traits (SRA, RD, and Rdi and SLA, LD, and Lthick).

## RESULTS

### The Root Economics Spectrum Is the Main Trend of Variation in Mediterranean Plants

The results of the general PCA for the 532 observations from 320 species are shown in **Figure 1**. The first PCA axis (58.4% of total trait variation) represents a gradient from SRA and SRL to RDMC and RD, confirming certain coordination among these four root morphological traits (**Figure 1A**). The second PCA axis (25.7% of total trait variation) was mostly determined by the variation in Rdi (**Figure 1A**). Similarly, the PCA of leaf showed the same dimensions of variation as root (**Figure 1B**). That is, the first principal component (PC; 63.4%) was explained by one extreme with high values of SLA, and at the opposite extreme with high values of LDMC and density (LD), while the second PC axis (29.5%) was mainly represented by Lthick variation. When including phylogenetic independent contrast (PIC) in the PCA, the patterns for root and leaf traits were consistent (**Appendix S2**), further confirming that species evolutionary history did not influence the observed patterns. Despite the strong segregation between growth forms along the PCA at root level (herbaceous vs. woody species; one-way ANOVA with the PCA scores of the first component, value of  $p < 0.001$ ), the root PCAs for each growth form showed similar dimensions of variation as in the total dataset (**Figures 1C,D**), with high scores for their respective first PCs (42.7 and 50% of overall variation explained for woody and herbaceous plants, respectively).

### SRA Is a More Informative Trait Than SRL From a Plant Economics Perspective

The Pearson's correlation analyses among morphological and chemical root traits from the subset of woody plants showed that both SRL and SRA were significantly correlated with chemical root traits (value of  $p < 0.05$ ; **Table 1**), such as carbon (C), nitrogen (N), organic Nitrogen (OrgN), and mineral concentration (MinCon). However, the SRA showed higher values of Pearson correlation coefficients than SRL in all cases (**Table 1**).

### There Is an Anatomical Trade-Off Between Tissue Density and Diameter/Thickness

Regarding the links between SRA and SLA with tissue density and root diameter or leaf thickness, the PCAs with the total dataset showed exactly the same trait distribution in both cases

(**Figure 2**). Thus, the first axes of both PCAs were mainly determined by the SRA and SLA variation, respectively, while the second axes were defined by the trade-off between tissue density with diameter (in root) and thickness (in leaf).

By testing the trait distribution proposed by Bergmann's model with the subset of woody species, in which we have nitrogen concentration at leaf and root level (80 observations of 64 species), we confirmed the bivariate relationships underlying a trait space with two main dimensions (**Figure 3A**). The first PCA axis represents the negative correlation between SRL and Rdi, defined by the authors as "collaboration gradient," while the second axis was defined by the variation between RD and root N, defined by the authors as "conservation gradient." However, when SRL was replaced by SRA, the main PCA axis was representative of the resource uptake strategy, with higher values of SRA and RN at one end and RD at the other; whereas the second axis reflected the trade-off between density and diameter. Similarly, when the analogous root and leaf traits are included in Bergman's model, the main PCA axis was mainly defined by the resource uptake strategy (species with higher SLA, SRL, RN, and LN in one end and species with higher LD, RD, Lthick, and Rdi at the other end), highlighting the strong coordination among analogous traits, while the second PCA axis did not show a clear pattern (**Figure 3C**).

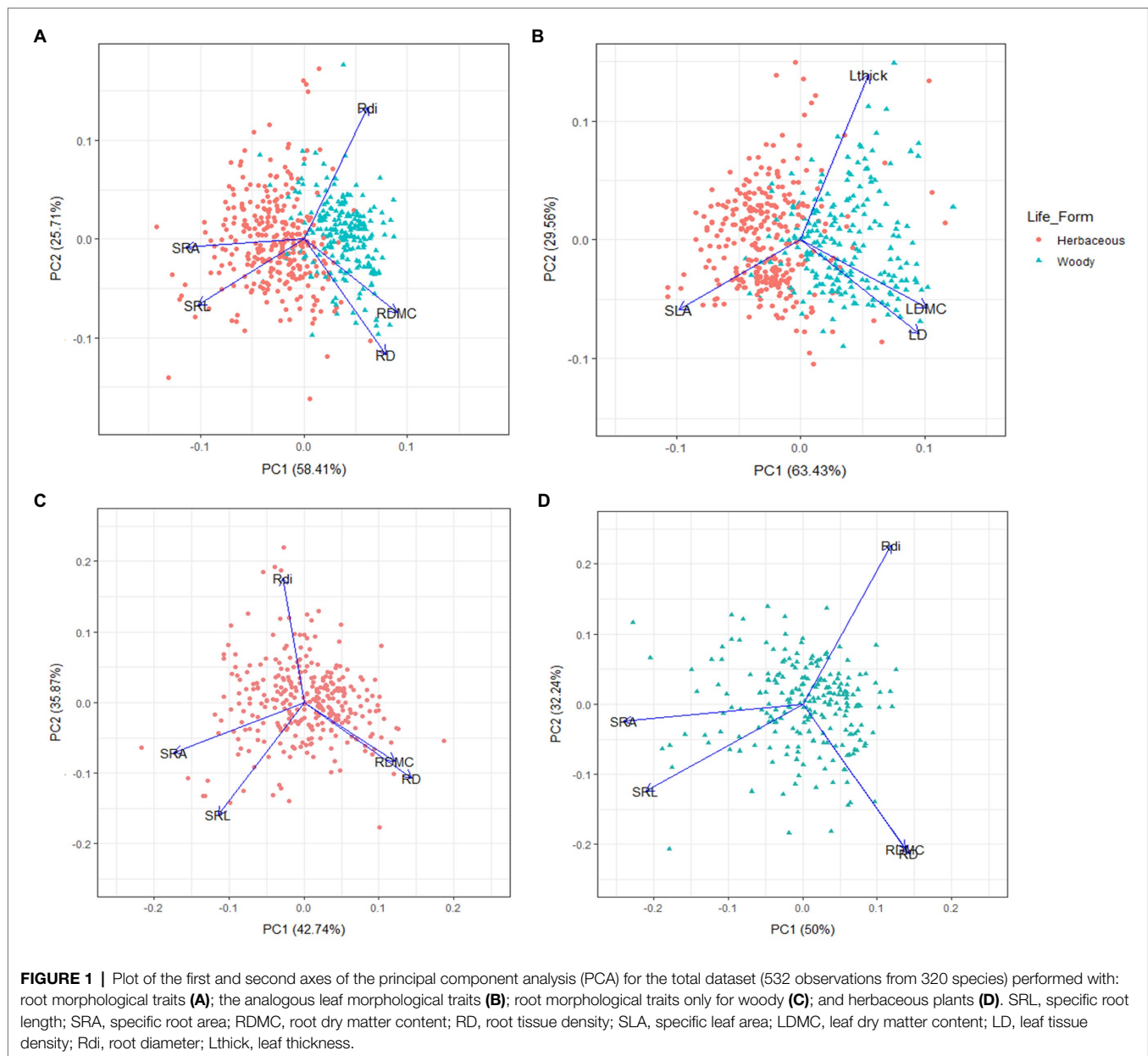
## DISCUSSION

### The Root Economics Spectrum Is the Main Trend of Variation in Mediterranean Plants

Wright et al. (2004) defined as a criterion for the variation along the first PCA axis of the LES that traits should follow (*...the same directionality of trait loadings and similarly high percentage of variance explained by the principal axis*). Following this criterion, the first PCA axis in our dataset represents a main trend of trait covariation with most of the total variance explained by this axis, showing a clear trade-off in the morphological structure of roots in Mediterranean species strongly dominated by mass-normalized traits that we can define as the RES. At the positive end of this axis, we find mainly woody species with high RDMC and low SRA and length (SRL), a syndrome commonly associated with a resource-conservation strategy. At the other end of the specialization gradient, we find mostly herbaceous species with opposite traits, a syndrome frequently associated to resource-acquisitive strategies (Roumet et al., 2016; de la Riva et al., 2018). We obtained similar results when taking into account phylogenetic effects in the PCA. Despite large differences in root trait values between herbaceous and woody species, we found remarkably similar patterns within these life forms separately. This evidence supports that the RES is a general syndrome that holds across and within life forms, at least under Mediterranean conditions.

Defining the RES as a dominant axis of root trait covariation has been challenging (i.e., Kramer-Walter et al., 2016; Weemstra et al., 2016; Bergmann et al., 2020) and the underlying reasons for the discrepancies found across studies, species or environmental gradients are still not clear. For example,





Laughlin et al. (2017) evidenced that ontogenetic and allometric constraints related to divergent responses of traits within species growing across a range of environments may affect the strength of trait coordination across species. Other authors (Shen et al., 2019 and references therein) have suggested that some traits could be constrained by environmental factors, not necessarily related to resource uptake and the economics spectrum (Kramer-Walter et al., 2016; Weemstra et al., 2016) resulting in weak or inconsistent relationships among traits across environments. Another key factor underlying these discrepancies could be that most of these studies on root trait variation have frequently mixed up “non-mass-” and “mass-normalized” traits to develop the root economics spectrum paradigm (i.e., Weemstra et al., 2016), an aspect that we discuss further later.

## SRA Is a More Informative Trait Than SRL From a Plant Economics Perspective

Specific root length is probably one of the most commonly measured traits to characterize the economics spectrum at the belowground level (Ostonen et al., 2007). SRL is the ratio between root length and root dry mass (Ryser, 2006); root length is assumed to be proportional to resource acquisition (foraging or exploitation capacity), while root dry mass should be proportional to construction and maintenance costs (Eissenstat and Yanai, 1997). Hence, thinner and longer roots (higher SRL) are considered as the equivalent of thinner leaves (high SLA), which are less expensive to construct (Villar and Merino, 2001; Villar et al., 2006). In fine roots, SRL is often positively related to root respiration rates, the amount and rate of water and nutrient uptake, and plant growth rates (Eissenstat and Yanai, 1997;

Reich et al., 1998; Tjoelker et al., 2005; Roumet et al., 2016). Therefore, and analogously to SLA in leaves, SRL has been commonly accepted as a key trait to screen and characterize plants according to their ecological strategy and productivity (Freschet et al., 2021).

However, the relationship between SRL and root functioning is not always clear; a fact that often hinders its interpretation and could raise questions for its use as a key explanatory trait of the RES. For example, Eissenstat et al. (2000) did not find any relationship between SRL and root physiology or root lifespan in grasses; Bergmann et al. (2020) found no relationship between SRL and RN or root life span globally, which were instead strongly related to root tissue density. In the late 90s, Ryser (1996, 1998) already argued that from a cost–benefit perspective this mismatch between SRL and root chemical traits is difficult to explain. Within our dataset, we observed

contrasting relationships among anatomical variables involving root length (SRL) or root area (SRA), which could shed some light and explain this apparent contradiction (see details below). One possible explanation is that variations in SRL are tightly coupled with variations in root diameter (McCormack and Iversen, 2019; Bergmann et al., 2020) while variations in root surface area (SRA) are mainly determined by variations in root tissue density (de la Riva et al., 2018). It is frequently assumed that higher SRL enhances soil volume exploration per unit dry mass with a small associated metabolic cost (Ryser, 2006) and that the volume of soil within the influence of a given root is a more important factor than the root surface area; therefore, SRL has been considered a better proxy for resource uptake than SRA (Freschet et al., 2021). However, an increase of the total absorptive surface area per unit mass for a given length in roots should increase the potential to encounter relatively immobile soil resources (McCormack and Iversen, 2019), which may increase the belowground resource uptake capacity. This would be analogous to leaves with high SLA that increase the interception of photosynthetically active radiation to enhance C assimilation. We acknowledge that our studies are observational and, therefore, we cannot provide a mechanistic explanation for the advantage of SRA over SRL as a better proxy of the RES. However, based on 532 observations from 320 species with complete root trait information (SRA and SRL) and the same sampling protocol, we are able to show here that SRA is the main trait explaining the species distribution along the RES, regardless of the selected traits, plant functional groups or phylogenetic effects. In addition, for Mediterranean vegetation SRA is more tightly correlated than SRL with the dry matter content and chemical composition of both roots and leaves (de la Riva et al., 2018, 2021;

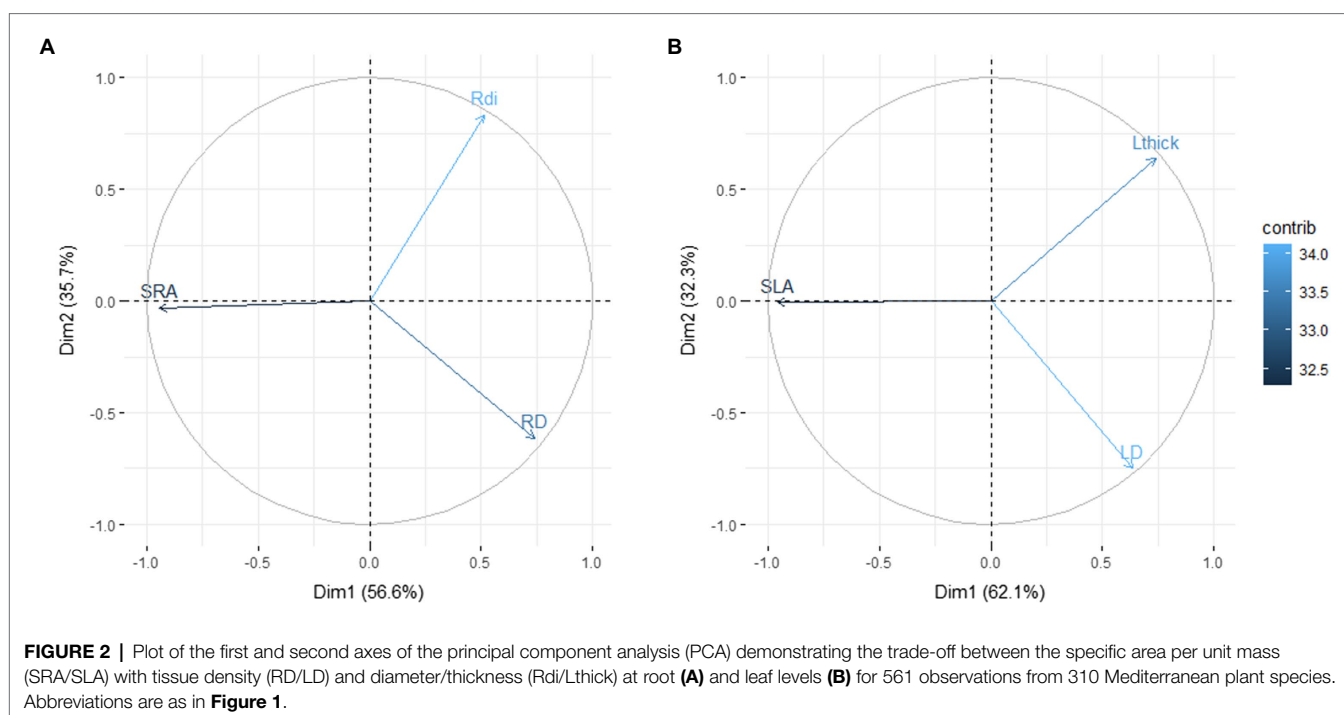
**TABLE 1** | Pearson correlation coefficients for relationships of specific root length (SRL) and specific root area (SRA) with chemical root traits (RN, root nitrogen concentration; RC, root carbon concentration; OrgN, organic nitrogen concentration; and MinCon, mineral concentrations).

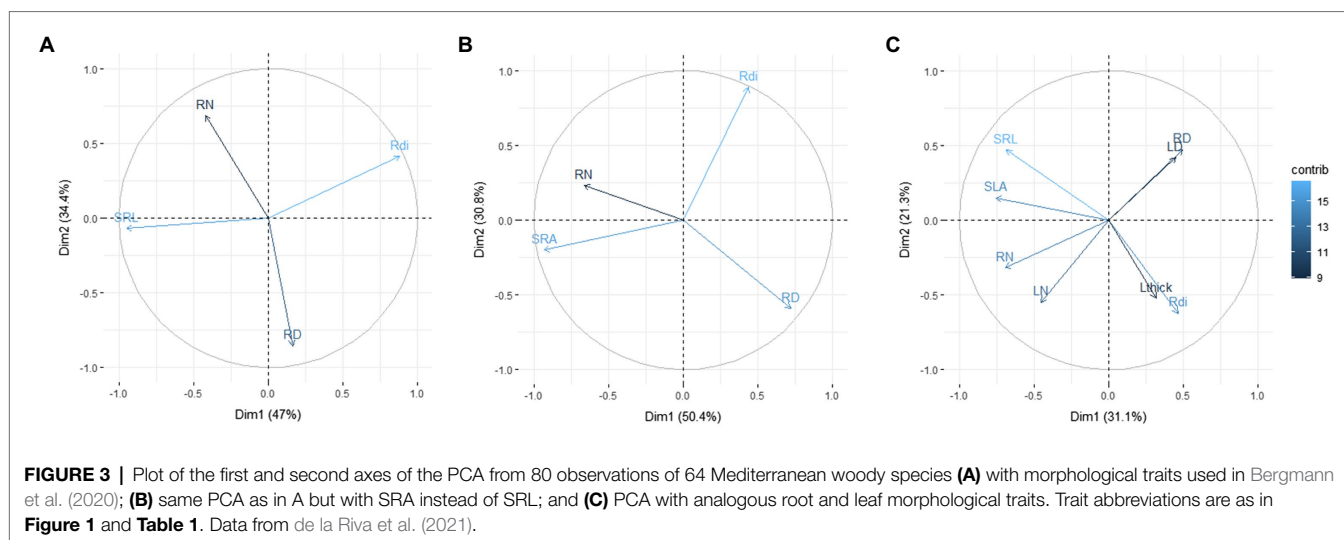
	SRL		SRA	
	<i>r</i>	<i>p</i>	<i>r</i>	<i>p</i>
RN <sup>1</sup>	0.22	<b>0.03</b>	0.39	<b>&lt;0.001</b>
RC <sup>1</sup>	0.28	<b>0.009</b>	0.54	<b>&lt;0.001</b>
OrgN <sup>2</sup>	0.28	<b>0.01</b>	0.48	<b>&lt;0.001</b>
MinCon <sup>2</sup>	0.41	<b>&lt;0.001</b>	0.64	<b>&lt;0.001</b>

Data from Marañón et al. (2020) and de la Riva et al. (2021).

<sup>1</sup>80 observations from 64 woody species.

<sup>2</sup>73 observations from 60 woody species.





Marañón et al., 2020); two features strongly related to metabolic rates and life span (Prieto et al., 2015; Roumet et al., 2016). In a recent study, we were able to show that root construction costs (the currency for root economics) were related to SRA but not to SRL, indicating that area-based rather than length-based traits may provide a better proxy of the investment per unit mass belowground (de la Riva et al., 2021). Unfortunately, SRA is a trait rarely reported in the literature: for instance, SRA was reported only in 7% of the species in the GRoot database (Guerrero-Ramírez et al., 2020), so we are unable to extend this hypothesis to other ecosystems. Therefore, further studies should be conducted in different ecosystem types to examine the role of SRA as a key trait in belowground resource economics (RES). We advocate that SRA is more informative than SRL as a general measure of root resource use efficiency in Mediterranean species, which is consistent with the observed role of its aboveground equivalent SLA as a key trait in leaf economics (Wright et al., 2004; Reich, 2014). However, our proposal by no means neglects the important roles of other root attributes, as SRL, to better understand belowground structure and functioning.

## There Is an Anatomical Trade-Off Between Tissue Density and Diameter/Thickness

Plants can achieve similar SLA or SRA/SRL values with a different investment in density or in leaf thickness/root diameter, which depends on species idiosyncrasies, their mycorrhizal association type and/or their environment (Poorter et al., 2009; de la Riva et al., 2016; John et al., 2017; Bergmann et al., 2020). As we have shown in this paper, analogous traits in roots and leaves generally display similar distributions in the two-dimensional PCA space. That is, the variation between root tissue density and diameter is similar to that between leaf density and thickness, irrespective of other traits considered, whether for roots or for leaves. This bivariate space has an underlying anatomical explanation; both SLA and SRA (or SRL) can be broken down into the product of leaf/root tissue density and leaf lamina thickness/root diameter (Ostonen et al., 2007; Poorter et al., 2009; see **Appendix S3**).

At the leaf level, SLA (or LMA) has been treated as a central attribute of plant strategies due to its strong correlation with photosynthetic and respiration rates, leaf nitrogen concentration and plant growth (Lambers and Poorter, 1992; Wright et al., 2004; Prieto et al., 2018). As a general trend, higher SLA across species is largely determined by a decrease in LD and thickness (Villar et al., 2013; de la Riva et al., 2016; John et al., 2017). But, at the same time, SLA is the inverse of the product of leaf density and thickness (Poorter et al., 2009) and thus, plants may achieve similar SLA values with different proportional investments in both traits, through different combinations in the size and number of cells and organelles (Pyankov et al., 1999; Villar et al., 2013). Overall, plants may increase their leaf thickness by having larger mesophyll cells, or by increasing the proportion of water contained within cells, both of which decrease leaf tissue density (Pyankov et al., 1999; Li et al., 2017). Within woody plants, larger mesophyll cell volumes increase leaf thickness, which may potentially reduce leaf density by diluting the influence of the denser tissues such as veins and the leaf cuticle (John et al., 2017). These authors also observed that the number of mesophyll cell layers influences only leaf thickness, while the cell dry mass density and air space fraction directly influenced leaf density. Therefore, the investment to build a given leaf area per unit dry mass (SLA or LMA) shows a strong dependency on both density and thickness (Gratani et al., 2018). In parallel, SRL (and also SRA) is the inverse of the product between root tissue density and root diameter (Ostonen et al., 2007). Thicker roots (i.e., with larger diameters) in *Pinus sylvestris* (L.) achieved lower root tissue densities by increasing the thickness of the cortex (Zadworny et al., 2017), while an increase of the aerenchyma in *Lotus glaber* (Mill.; Mendoza et al., 2005) and of sclerenchyma tissues in *Paspalum dilatatum* (Poir.; Vasellati et al., 2001) decreased their SRL. Wahl and Ryser (2000) observed that the proportion of cell wall in the stele was the main factor determining the interspecific variation in root density among herbaceous species. Furthermore, previous studies have shown contrasting plastic responses of root tissue density and root diameter to nutrient and water availability (Ryser and Lambers, 1995;

Meier and Leuschner, 2008; Olmo et al., 2014), resulting in overall small variations in SRL despite large structural changes. Hence, different anatomical components may drive the trade-off between density and diameter/thickness, depending on the plant species and the environment.

Recent global analyses have proposed a positive correlation between root diameter and mycorrhizal colonization percentage (Bergmann et al., 2020), because thicker roots may enhance mycorrhizal associations by increasing the cortex thickness (Kong et al., 2019). These authors suggest that higher mycorrhizal dependency may help plants achieve a more efficient resource acquisition, suggesting deviations from a root economics spectrum of acquisitive to conservative belowground strategies. Thus, plants could optimize resource uptake by investing carbon either in abundant thin roots that efficiently explore the soil themselves or in fewer thicker roots that instead favor symbiosis with a mycorrhizal fungal partner. This interpretation assumes that larger root diameter enhances the plant's acquisitive capacity *via* carbon investment in a mycorrhizal fungal partner in return for limiting resources like P or N (McCormack and Iversen, 2019). Mycorrhizal relationships can greatly increase the plant's nutrient uptake; however, in addition to the need to build costly roots (e.g., large root diameters), they also add an extra cost associated with the transfer of C to the fungi to support the symbiosis (between 10 and 20% of net plant C assimilation on average; Allen et al., 2003). Therefore, a higher mycorrhizal root colonization percentage may enhance resource acquisition under poor soil fertility conditions without necessarily implying an acquisitive strategy (Navarro-Fernández et al., 2016). On the contrary, high soil fertility favors plant species with acquisitive root traits (de la Riva et al., 2018) but generally reduces mycorrhizal root colonization, likely because plants do not benefit from this association when soil nutrients are abundant and can be directly acquired by the root without the help of a mycorrhizal partner (Treseder, 2004). Hence, it is not clear how the mycorrhizal collaboration gradient proposed by Bergmann et al. (2020) relates to the differences in mass-normalized traits and root uptake efficiency between fast- and slow-growing species along the RES. A further analysis of our dataset, but using the same traits proposed in Bergmann et al. (2020) yielded a very similar pattern to that found by those authors; that is, a principal axis reflecting the trade-off between SRL and Rdi, and a second axis reflecting the trade-off between RN and root tissue density. However, when we replaced SRL by SRA in the trait space, we observed a reorientation of the bi-dimensional space with the main axis shifting towards the RES (axis 1; **Figure 3B**), and the abovementioned anatomical trade-off included in the second axis. In addition, when we analyzed leaf traits analogous to the root traits selected by Bergmann et al. (2020), a tight covariation between below- and aboveground traits became evident. Our findings suggest that some precaution is advisable with the interpretation of non-mass-based root trait variation (i.e., root diameter). Unfortunately, and similar to that stated for SRA earlier, there is not enough data available at the moment to carry out a comprehensive analysis to understand the covariation between analogous traits at root and leaf levels (e.g., only 59 species in the TRY database have information for all the traits included in **Figure 3C**). Up to date, attending to

our knowledge about Mediterranean vegetation, our overarching perception is that the correlations of SLA and SRA/SRL with anatomy are only partially consistent with the intrinsic anatomical composition of each component separately, illustrating the axiom that “correlation does not imply direct causation” (e.g., John et al., 2017). We would like to stress the importance of an anatomically explicit approach, in which both density and thickness/diameter are taken into account, because plant growth is the increment in dry mass, volume, and length or area that results from the division, expansion, and differentiation of cells (Lambers and Oliveira, 2019). Unfortunately, the links between root anatomy and performance still lack a thorough understanding of the functional relationships between morphological features (Gonzalez-Paleo and Ravetta, 2018).

## CONCLUSION

In summary, fine roots display a wide diversity of forms and properties in Mediterranean vegetation, resulting in a multidimensional root trait space. Our findings highlight how the main trend of variation in this multidimensional root space is largely analogous to the main axis of the leaf economics spectrum. If we accept that plant performance is mainly modulated by mass-normalized leaf traits according to the LES theory, then we advocate that the same approach should be adopted for roots, for the sake of consistency. As we have demonstrated, mass-normalized root traits (especially SRA, RDMC, and RN) are good predictors of the RES in Mediterranean environments. However, wider analysis of morphological and physiological mass-normalized traits encompassing more vegetation types globally should be carried out in order to provide a general conceptual framework that confirms the proposed universal main trend of variation in roots related to the economics spectrum. In addition, we propose that a first step to analyze the trade-off between root density and diameter could be to follow an anatomical approach to understand how and why plants build their fine roots. Thus, further anatomical studies similar to those carried out for leaves (i.e., Villar et al., 2013; de la Riva et al., 2016; John et al., 2017; Li et al., 2017; Gratani et al., 2018), would be necessary to obtain solid conclusions on the role of root anatomical tissues in defining the SRA or SRL of fine roots, which could help clarify the discrepancies between tissue density and root diameter.

## DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

## AUTHOR CONTRIBUTIONS

ER, JQ, and IP conceived the ideas, designed the study, and wrote the first draft. ER, IP, JGD, SM, TM, and IP-R



conducted fieldwork. ER performed statistical analyses. All authors contributed to the article and approved the submitted version.

## FUNDING

This work was financially supported by the German Research Foundation (ECOFUMER, GZ: GA 2899/3-1), Spanish Ministry of Science and Innovation (grant no. CGL2017-82254-R-INTARSU), ECO-MEDIT (CGL2014-53236-R), the projects “Ecología Funcional de los Bosques Andaluces y Predicciones Sobre sus Cambios Futuros (For-Change; UCO-FEDER 18 REF 27943 MOD B; Spain) and Funcionalidad y Servicios Ecosistémicos de los Bosques Andaluces y Normarroquies: Relaciones con la Diversidad Vegetal y Edáfica Ante el Cambio Climático” (P18-RT-3455) by Junta

de Andalucía (Spain), and the Seneca Foundation (project 20654/JLI/18), all cofinanced by European FEDER funds.

## ACKNOWLEDGMENTS

We thank M. Olmo, C. Navarro, and C. Padilla for helping during field work and the IRNAS Analytical Service for chemical analysis of plants.

## SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fpls.2021.773118/full#supplementary-material>

## REFERENCES

- Allen, M. F., Swenson, W., Querejeta, J. I., Egerton-Warburton, L. M., and Treseder, K. K. (2003). Ecology of mycorrhizae: a conceptual framework for complex interactions among plants and fungi. *Annu. Rev. Phytopathol.* 41, 271–303. doi: 10.1146/annurev.phyto.41.052002.095518
- Bergmann, J., Weigelt, A., van der Plas, F., Laughlin, D. C., Kuyper, T. W., Guerrero-Ramirez, N., et al. (2020). The fungal col-laboration gradient dominates the root economics space in plants. *Sci. Adv.* 6:eaba3756. doi: 10.1126/sciadv.aba3756
- Bertolino, L. T., Caine, R. S., and Gray, J. E. (2019). Impact of stomatal density and morphology on water-use efficiency in a changing world. *Front. Plant Sci.* 10:225. doi: 10.3389/fpls.2019.00225
- de la Riva, E. G., Godoy, O., Castro-Díez, P., Gutiérrez-Cánovas, C., and Vilà, M. (2019). Functional and phylogenetic consequences of plant invasion for coastal native communities. *J. Veg. Sci.* 30, 510–520. doi: 10.1111/jvs.12748
- de la Riva, E. G., Marañón, T., Pérez-Ramos, I. M., Navarro-Fernández, C. M., Olmo, M., and Villar, R. (2018). Root traits across environmental gradients in Mediterranean woody communities: are they aligned along the root economics spectrum? *Plant Soil* 424, 35–48. doi: 10.1007/s11104-017-3433-4
- de la Riva, E. G., Olmo, M., Poorter, H., Uberta, J. L., and Villar, R. (2016). Leaf mass per area (LMA) and its relationship with leaf structure and anatomy in 34 Mediterranean woody species along a water availability gradient. *PLoS One* 11:e0148788. doi: 10.1371/journal.pone.0148788
- de la Riva, E. G., Prieto, I., Marañón, T., Perez-Ramos, I. M., Olmo, M., and Villar, R. (2021). Root economics spectrum and construction costs in Mediterranean woody plants: the role of symbiotic associations and the environment. *J. Ecol.* 109, 1873–1885. doi: 10.1111/1365-2745.13612
- Díaz, S., Kattge, J., Cornelissen, J. H., Wright, I. J., Lavorel, S., Dray, S., et al. (2016). The global spectrum of plant form and function. *Nature* 529, 167–171. doi: 10.1038/nature16489
- Eissenstat, D. M., Wells, C. E., Yanai, R. D., and Whitbeck, J. L. (2000). Building roots in a changing environment: implications for root longevity. *New Phytol.* 147, 33–42. doi: 10.1046/j.1469-8137.2000.00686.x
- Eissenstat, D. M., and Yanai, R. D. (1997). The ecology of root lifespan. *Adv. Ecol. Res.* 27, 1–60. doi: 10.1016/S0065-2504(08)60005-7
- Freschet, G. T., Roumet, C., Comas, L. H., Weemstra, M., Bengough, A. G., Rewald, B., et al. (2021). Root traits as drivers of plant and ecosystem functioning: current understanding, pitfalls and future research needs. *New Phytol.* 232, 1123–1158. doi: 10.1111/nph.17072
- Freschet, G. T., Valverde-Barrantes, O. J., Tucker, C. M., Craine, J. M., McCormack, M. L., Violle, C., et al. (2017). Climate, soil and plant functional types as drivers of global fine-root trait variation. *J. Ecol.* 105, 1182–1196. doi: 10.1111/1365-2745.12769
- Gonzalez-Paleo, L., and Ravetta, D. A. (2018). Relationship between photosynthetic rate, water use and leaf structure in desert annual and perennial forbs differing in their growth. *Photosynthetica* 56, 1177–1187. doi: 10.1007/s11099-018-0810-z
- Gratani, L., Varone, L., Crescente, M. F., Catoni, R., Ricotta, C., and Puglielli, G. (2018). Leaf thickness and density drive the responsiveness of photosynthesis to air temperature in Mediterranean species according to their leaf habitus. *J. Arid Environ.* 150, 9–14. doi: 10.1016/j.jaridenv.2017.12.007
- Guerrero-Ramirez, N. R., Mommer, L., Freschet, G. T., Iversen, C. M., McCormack, M. L., Kattge, J., et al. (2020). Global root traits (GRooT) database. *Glob. Ecol. Biogeogr.* 30, 25–37. doi: 10.1111/geb.13179
- Hodgson, J. G., Montserrat-Martí, G., Charles, M., Jones, G., Wilson, P., Shipley, B., et al. (2011). Is leaf dry matter content a better predictor of soil fertility than specific leaf area? *Ann. Bot.* 108, 1337–1345. doi: 10.1093/aob/mcr225
- Iversen, C. M., McCormack, M. L., Powell, A. S., Blackwood, C. B., Freschet, G. T., Kattge, J., et al. (2017). A global fine-root ecology database to address below-ground challenges in plant ecology. *New Phytol.* 215, 15–26. doi: 10.1111/nph.14486
- John, G. P., Scoffoni, C., Buckley, T. N., Villar, R., Poorter, H., and Sack, L. (2017). The anatomical and compositional basis of leaf mass per area. *Ecol. Lett.* 20, 412–425. doi: 10.1111/ele.12739
- Kong, D., Wang, J., Wu, H., Valverde-Barrantes, O. J., Wang, R., Zeng, H., et al. (2019). Nonlinearity of root trait relationships and the root economics spectrum. *Nat. Commun.* 10, 1–9. doi: 10.1038/s41467-019-10245-6
- Kramer-Walter, K. R., Bellingham, P. J., Millar, T. R., Smitsen, R. D., Richardson, S. J., and Laughlin, D. C. (2016). Root traits are multidimensional: specific root length is independent from root tissue density and the plant economic spectrum. *J. Ecol.* 104, 1299–1310. doi: 10.1111/1365-2745.12562
- Lambers, H., and Oliveira, R. S. (2019). “Plant water relations,” in *Plant Physiological Ecology* (Cham: Springer), 187–263.
- Lambers, H., and Poorter, H. (1992). Inherent variation in growth rate between higher plants: a search for physiological causes and ecological consequences. *Adv. Ecol. Res.* 22, 187–261. doi: 10.1016/S0065-2504(08)60148-8
- Laughlin, D. C., Lusk, C. H., Bellingham, P. J., Burslem, D. F., Simpson, A. H., and Kramer-Walter, K. R. (2017). Intraspecific trait variation can weaken interspecific trait correlations when assessing the whole-plant economic spectrum. *Ecol. Evol.* 7, 8936–8949. doi: 10.1002/ece3.3447
- Li, L., Ma, Z., Niinemets, Ü., and Guo, D. (2017). Three key sub-leaf modules and the diversity of leaf designs. *Front. Plant Sci.* 8:1542. doi: 10.3389/fpls.2017.01542
- Marañón, T., Navarro-Fernández, C. M., Gil-Martínez, M., Domínguez, M. T., Madejón, P., and Villar, R. (2020). Variation in morphological and chemical traits of Mediterranean tree roots: linkage with leaf traits and soil conditions. *Plant Soil* 449, 389–403. doi: 10.1007/s11104-020-04485-5
- McCormack, M. L., and Iversen, C. M. (2019). Physical and functional constraints on viable belowground acquisition strategies. *Front. Plant Sci.* 10:1215. doi: 10.3389/fpls.2019.01215
- Meier, I. C., and Leuschner, C. (2008). Genotypic variation and phenotypic plasticity in the drought response of fine roots of European beech. *Tree Physiol.* 28, 297–309. doi: 10.1093/treephys/28.2.297



- Mendoza, R., Escudero, V., and Garcia, I. (2005). Plant growth, nutrient acquisition and mycorrhizal symbioses of a waterlogging tolerant legume (*Lotus glaber* mill.) in a saline-sodic soil. *Plant Soil* 275, 305–315. doi: 10.1007/s11104-005-2501-3
- Morlon, H., Lewitus, E., Condamine, F., Manceau, M., Clavel, J., and Drury, J. (2016). RPANDA: an R package for macroevolutionary analyses on phylogenetic trees. *Methods Ecol. Evol.* 7, 589–597. doi: 10.1111/2041-210X.12526
- Navarro-Fernández, C. M., Pérez-Ramos, I. M., de la Riva, E. G., Vera, J. R., Roumet, C., Villar, R., et al. (2016). Functional responses of Mediterranean plant communities to soil resource heterogeneity: a mycorrhizal trait-based approach. *J. Veg. Sci.* 27, 1243–1253. doi: 10.1111/jvs.12446
- Olmo, M., Lopez-Iglesias, B., and Villar, R. (2014). Drought changes the structure and elemental composition of very fine roots in seedlings of ten woody tree species. Implications for a drier climate. *Plant Soil* 384, 113–129. doi: 10.1007/s11104-014-2178-6
- Onoda, Y., Westoby, M., Adler, P. B., Choong, A. M., Clissold, F. J., Cornelissen, J. H., et al. (2011). Global patterns of leaf mechanical properties. *Ecol. Lett.* 14, 301–312. doi: 10.1111/j.1461-0248.2010.01582.x
- Ostonen, I., Püttsepp, Ü., Biel, C., Alberton, O., Bakker, M. R., Lohmus, K., et al. (2007). Specific root length as an indicator of environmental change. *Plant Biosyst.* 141, 426–442. doi: 10.1080/11263500701626069
- Poorter, H., Niinemets, Ü., Poorter, L., Wright, I. J., and Villar, R. (2009). Causes and consequences of variation in leaf mass per area (LMA): a meta-analysis. *New Phytol.* 182, 565–588. doi: 10.1111/j.1469-8137.2009.02830.x
- Prieto, I., Querejeta, J. I., Segrestin, J., Volaire, F., and Roumet, C. (2018). Leaf carbon and oxygen isotopes are coordinated with the leaf economics spectrum in Mediterranean rangeland species. *Funct. Ecol.* 32, 612–625. doi: 10.1111/1365-2435.13025
- Prieto, I., Roumet, C., Cardinael, R., Dupraz, C., Jourdan, C., Kim, J. H., et al. (2015). Root functional parameters along a land-use gradient: evidence of a community-level eco-nomics spectrum. *J. Ecol.* 103, 361–373. doi: 10.1111/1365-2745.12351
- Pyankov, V. I., Kondratchuk, A. V., and Shipley, B. (1999). Leaf structure and specific leaf mass: the alpine desert plants of the eastern Pamirs, Tadjikistan. *New Phytol.* 143, 131–142. doi: 10.1046/j.1469-8137.1999.00435.x
- Reich, P. B. (2014). The world-wide ‘fast-slow’ plant economics spectrum: a traits manifesto. *J. Ecol.* 102, 275–301. doi: 10.1111/1365-2745.12211
- Reich, P. B., Tjoelker, M. G., Walters, M. B., Vanderklein, D. W., and Buschena, C. (1998). Close association of RGR, leaf and root morphology, seed mass and shade tolerance in seedlings of nine boreal tree species grown in high and low light. *Funct. Ecol.* 12, 327–338. doi: 10.1046/j.1365-2435.1998.00208.x
- Roumet, C., Birouste, M., Picon-Cochard, C., Ghestem, M., Osman, N., Vignion-Brenas, S., et al. (2016). Root structure–function relationships in 74 species: evidence of a root economics spectrum related to carbon economy. *New Phytol.* 210, 815–826. doi: 10.1111/nph.13828
- Ryser, P. (1996). The importance of tissue density for growth and life span of leaves and roots: a comparison of five ecologically contrasting grasses. *Funct. Ecol.* 10, 717–723.
- Ryser, P. (1998). “Intra- and interspecific variation in root length, root turnover and the underlying parameters.” in *Inherent Variation in Plant Growth: Physiological Mechanisms and Ecological Consequences*, eds. H. Lambers, H. Poorter and M. M. I. Van Vuuren (Leiden, Netherlands: Backhuys Publishers), 441–465.
- Ryser, P. (2006). The mysterious root length. *Plant Soil* 286, 1–6. doi: 10.1007/s11104-006-9096-1
- Ryser, P., and Lambers, H. (1995). Root and leaf attributes accounting for the performance of fast-and slow-growing grasses at different nutrient supply. *Plant Soil* 170, 251–265.
- Shen, Y., Gilbert, G. S., Li, W., Fang, M., Lu, H., and Yu, S. (2019). Linking aboveground traits to root traits and local environment: implications of the plant economics spectrum. *Front. Plant Sci.* 10:1412. doi: 10.3389/fpls.2019.01412
- Smith, S. A., and Brown, J. W. (2018). Constructing a broadly inclusive seed plant phylogeny. *Am. J. Bot.* 105, 1–13.
- Tjoelker, M. G., Craine, J. M., Wedin, D., Reich, P. B., and Tilman, D. (2005). Linking leaf and root trait syndromes among 39 grassland and savannah species. *New Phytol.* 167, 493–508. doi: 10.1111/j.1469-8137.2005.01428.x
- Treseder, K. K. (2004). A meta-analysis of mycorrhizal responses to nitrogen, phosphorus, and atmospheric CO<sub>2</sub> in field studies. *New Phytol.* 164, 347–355. doi: 10.1111/j.1469-8137.2004.01159.x
- Valverde-Barrantes, O. J., Smemo, K. A., and Blackwood, C. B. (2015). Fine root morphology is phylogenetically structured, but nitrogen is related to the plant economics spectrum in temperate trees. *Funct. Ecol.* 29, 796–807. doi: 10.1111/1365-2435.12384
- Vasellati, V., Oosterheld, M., Medan, D., and Loreti, J. (2001). Effects of flooding and drought on the anatomy of *Paspalum dilatatum*. *Ann. Bot.* 88, 355–360. doi: 10.1006/anbo.2001.1469
- Villar, R., and Merino, J. (2001). Comparison of leaf construction costs in woody species with differing leaf life-spans in contrasting ecosystems. *New Phytol.* 151, 213–226. doi: 10.1046/j.1469-8137.2001.00147.x
- Villar, R., Ruiz-Robledo, J., De Jong, Y., and Poorter, H. (2006). Differences in construction costs and chemical composition between deciduous and evergreen woody species are small as compared to differences among families. *Plant Cell Environ.* 29, 1629–1643. doi: 10.1111/j.1365-3040.2006.01540.x
- Villar, R., Ruiz-Robledo, J., Uberta, J. L., and Poorter, H. (2013). Exploring variation in leaf mass per area (LMA) from leaf to cell: an anatomical analysis of 26 woody species. *Am. J. Bot.* 100, 1969–1980. doi: 10.3732/ajb.1200562
- Wahl, S., and Ryser, P. (2000). Root tissue structure is linked to ecological strategies of grasses. *New Phytol.* 148, 459–471. doi: 10.1046/j.1469-8137.2000.00775.x
- Weemstra, M., Mommer, L., Visser, E. J., van Ruijven, J., Kuyper, T. W., Mohren, G. M., et al. (2016). Towards a multidimensional root trait framework: a tree root review. *New Phytol.* 211, 1159–1169. doi: 10.1111/nph.14003
- Wright, I. J., Dong, N., Maire, V., Prentice, I. C., Westoby, M., Díaz, G., et al. (2017). Global climatic drivers of leaf size. *Science* 357, 917–921. doi: 10.1126/science.aal4760
- Wright, I. J., Reich, P. B., Westoby, M., Ackerly, D. D., Baruch, Z., Bongers, F., et al. (2004). The worldwide leaf economics spectrum. *Nature* 428, 821–827. doi: 10.1038/nature02403
- Zadworny, M., McCormack, M. L., Żytkowiak, R., Karolewski, P., Mucha, J., and 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. *Glob. Chang. Biol.* 23, 1218–1231. doi: 10.1111/gcb.13514

**Conflict of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

**Publisher’s Note:** All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Copyright © 2021 de la Riva, Querejeta, Villar, Pérez-Ramos, Marañón, Galán Díaz, de Tomás Marín and Prieto. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.



# The Right-Skewed Distribution of Fine-Root Size in Three Temperate Forests in Northeastern China

Cunguo Wang<sup>1</sup>, Ivano Brunner<sup>2</sup>, Junni Wang<sup>1</sup>, Wei Guo<sup>3</sup>, Zhenzhen Geng<sup>3</sup>,  
Xiuyun Yang<sup>4\*</sup>, Zhijie Chen<sup>5\*</sup>, Shijie Han<sup>5</sup> and Mai-He Li<sup>1,2</sup>

<sup>1</sup> Key Laboratory of Geographical Processes and Ecological Security in Changbai Mountains, Ministry of Education, School of Geographical Sciences, Northeast Normal University, Changchun, China, <sup>2</sup> Swiss Federal Institute for Forest, Snow and Landscape Research WSL, Birmensdorf, Switzerland, <sup>3</sup> College of Agronomy, Shenyang Agricultural University, Shenyang, China, <sup>4</sup> College of Forestry, Shanxi Agricultural University, Taigu, China, <sup>5</sup> International Joint Research Laboratory for Global Change Ecology, School of Life Sciences, Henan University, Kaifeng, China

## OPEN ACCESS

### Edited by:

Boris Rewald,  
University of Natural Resources  
and Life Sciences Vienna, Austria

### Reviewed by:

Luciano Pereira,  
University of Ulm, Germany  
Kyotaro Noguchi,  
Tohoku Research Center, Forestry  
and Forest Products Research  
Institute, Japan

### \*Correspondence:

Xiuyun Yang  
xyyang2002@yeah.net  
Zhijie Chen  
chan.chijay@gmail.com

### Specialty section:

This article was submitted to  
Functional Plant Ecology,  
a section of the journal  
Frontiers in Plant Science

**Received:** 08 September 2021

**Accepted:** 14 December 2021

**Published:** 07 January 2022

### Citation:

Wang C, Brunner I, Wang J,  
Guo W, Geng Z, Yang X, Chen Z,  
Han S and Li M-H (2022) The  
Right-Skewed Distribution of  
Fine-Root Size in Three Temperate  
Forests in Northeastern China.  
*Front. Plant Sci.* 12:772463.  
doi: 10.3389/fpls.2021.772463

Trees can build fine-root systems with high variation in root size (e.g., fine-root diameter) and root number (e.g., branching pattern) to optimize belowground resource acquisition in forest ecosystems. Compared with leaves, which are visible above ground, information about the distribution and inequality of fine-root size and about key associations between fine-root size and number is still limited. We collected 27,573 first-order fine-roots growing out of 3,848 second-order fine-roots, covering 51 tree species in three temperate forests (Changbai Mountain, CBS; Xianrendong, XRD; and Maoershan, MES) in Northeastern China. We investigated the distribution and inequality of fine-root length, diameter and area (fine-root size), and their trade-off with fine-root branching intensity and ratio (fine-root number). Our results showed a strong right-skewed distribution in first-order fine-root size across various tree species. Unimodal frequency distributions were observed in all three of the sampled forests for first-order fine-root length and area and in CBS and XRD for first-order fine-root diameter, whereas a marked bimodal frequency distribution of first-order fine-root diameter appeared in MES. Moreover, XRD had the highest and MES had the lowest inequality values (Gini coefficients) in first-order fine-root diameter. First-order fine-root size showed a consistently linear decline with increasing root number. Our findings suggest a common right-skewed distribution with unimodality or bimodality of fine-root size and a generalized trade-off between fine-root size and number across the temperate tree species. Our results will greatly improve our thorough understanding of the belowground resource acquisition strategies of temperate trees and forests.

**Keywords:** branching pattern, fine-root size, first-order fine-root, inequality, root number, trade-off

## INTRODUCTION

Fine roots play a critical role in resource absorption because they are metabolic hotspots associated with symbiotic mycorrhizal fungi (Zadworny and Eissenstat, 2011; McCormack et al., 2020; Kong et al., 2021). Their size (e.g., the diameter of first-order roots) profoundly influences a variety of eco-physiological processes such as lifespan, mortality and decomposition (Eissenstat, 1992;

Pregitzer et al., 2002). Many previous studies have focused on the natural variations in fine-root size and its potential ecological implications on various scales (Comas and Eissenstat, 2009; Kong et al., 2014; Freschet et al., 2017). At the species level, fine-root diameter and length vary from one to two orders of magnitude across tropical and temperate tree species, with larger roots having a thicker cortex and larger stele diameter (Gu et al., 2014; Kong et al., 2014). For a given species, the average diameter and length of an individual fine root increase steadily with root order, but the capacity of resource acquisition significantly decreases (Pregitzer et al., 2002; Guo et al., 2008). Within a root order, roots vary in diameter and length, leading to variation in absorptive capacities for water and nutrients (Polverigiani et al., 2011; Zadworny and Eissenstat, 2011). Born as first-order roots, fibrous roots with a smaller diameter and shorter length are the principal roots for water and nutrient absorption, while pioneer roots with a larger diameter and longer length are built, as the main exploratory roots, to live longer at the expense of absorptive capacity (Zadworny and Eissenstat, 2011). Plants do not put all their “eggs in one basket”, in that large fine roots co-exist with small fine roots (Forde, 2009). Accordingly, variation in the length and diameter of fine roots within a root system can be considered a strategy to reduce ecological niche overlap (e.g., root lifespan overlap) and competition between individual roots, resulting in the exploration of a greater volume of soil (Pagès et al., 1993; Pregitzer et al., 2002).

There is a tremendous amount of evidence that frequency distributions of individual plant height, seed size and leaf size are characteristically non-normal and skewed to the right (Bendel et al., 1989; Moles et al., 2007; Wang et al., 2019). The right-skewed distributions of plant size at different taxonomical levels indicate that small sizes were favored (Dombroskie and Aarssen, 2010). However, to date, the distribution characteristics of fine-root size have not been investigated in detail for forest ecosystems (Wang et al., 2017). Most temperate habitats for trees have environmental conditions where adaptation is conferred through physiological optimization associated directly with many smaller roots and few large ones (Ma et al., 2018). Thin roots have lower construction costs and are more plastic in terms of growth proliferation for resource acquisition than thick roots (Eissenstat, 1991, 1992). Thus, it is necessary to explicitly examine the frequency distribution of fine-root size to improve our ability to predict how trees will respond to a changing climate, and therefore altered resource availability, at the individual root level (Eissenstat and Achor, 1999; Wang et al., 2017). For example, in our previous study we reported that not only the mean but also the variation and the size distribution of the fine roots of *Fraxinus mandschurica* respond to changes in soil nitrogen and water availability (Wang et al., 2017). Furthermore, some plant species show a tendency of bimodal distribution in fine-root diameter (Boot and Mensink, 1990; Eissenstat, 1991; Bouma et al., 2001; Anderson et al., 2007), whereas others follow a unimodal distribution (Scanlan and Hinz, 2010; Hu et al., 2015). In conclusion, coupling the metrics of root functional characteristics with the frequency distribution of fine-root size is considered a powerful approach in assessing water and nutrient uptake behavior of the entire root system and its

interaction with the soil environment (Scanlan and Hinz, 2010; Hu et al., 2015).

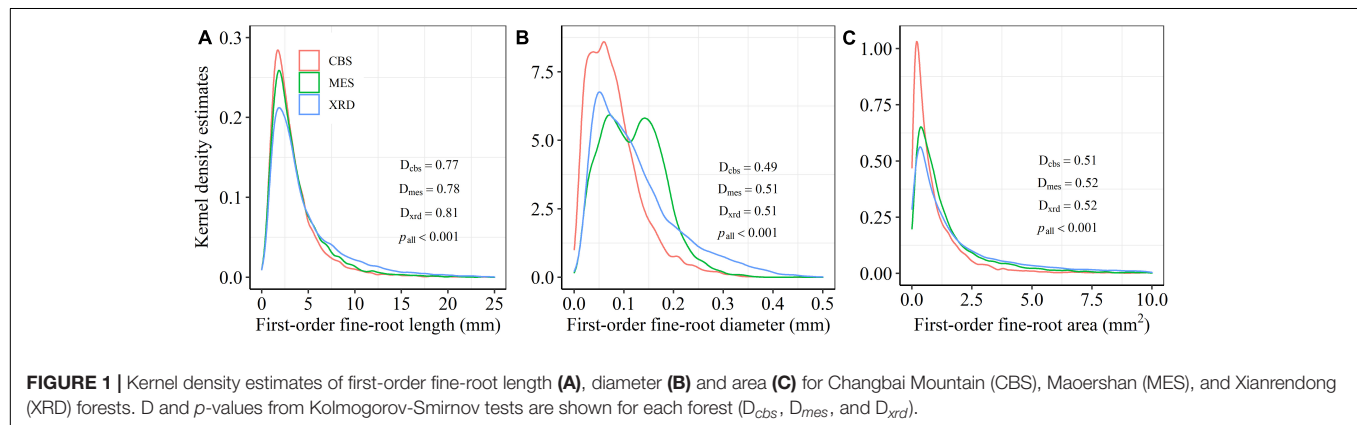
Plants are certainly modular organisms, with recognized capabilities to regulate the size and number of organs at the module scale (Kroon et al., 2005). If a module or structure is relatively small, plants will generally have the capacity to produce more of them (Westoby and Wright, 2003; Deng et al., 2008; Wang et al., 2019). For instance, tree species that produce smaller leaves generally build proportionately more leaves per unit size of the supporting shoot vegetative tissue (i.e., they have a higher leafing intensity), which confirms the generality of the size/number trade-off relationship, regardless of leaf form, leaf habit and habitat (Sun et al., 2006; Kleiman and Aarssen, 2007). As a central component of life-history theory, the fundamental size/number trade-off can be applied to the interpretation of fine-root size variation (Moles et al., 2007). At a specific belowground carbon allocation, trees are expected to produce a highly branched root system capable of rapid and extensive proliferation into resource-rich patches with numerous small roots (Berntson, 1994; Hodge, 2004; Chen et al., 2013). Conversely, a root system with a low level of branching has a higher exploitation potential and the overlap among depletion zones is lower for the few neighboring large roots, which typically have longer life spans and longer nutrient uptake times (Comas and Eissenstat, 2009; Liese et al., 2017). Therefore, fine-root size variation may be mainly constrained to small sizes, not because of the direct adaptability of small roots, but as a necessary trade-off consequence of selection in favor of high root branching (Ding et al., 2020; McCormack et al., 2020).

The temperate mixed forests in Northeastern China account for 35.3 and 34.5% of the national total forested land and standing tree volume, respectively (Wang, 2006). These forests function as a significant carbon sink in the national and global carbon budget (Fang et al., 2001). As the main resource absorption organs, fine roots have a considerable influence on a series of ecological processes, such as net primary production and soil formation in forest ecosystems, and their contributions to forest carbon and nutrient cycling are generally largely mediated by their functional traits, such as fine-root diameter (Freschet et al., 2017, 2021; Ma et al., 2018). However, relatively little is known about the fine-root systems in these large/important forests. In the present study, therefore, we investigated the size characteristics of fine roots in this forested area, along with their frequency distribution, inequality and associations with the number of fine roots. We focused on the first- and second-order roots, due to their important roles in generating variation among root individuals to cope with the instability of forest soil content of nitrogen and water (Pregitzer et al., 2002; Chen et al., 2013; Kong et al., 2014; Wang et al., 2017). Our first objective was to determine whether there is a consistent frequency distribution and inequality or variability in fine-root size at the root individual level within/across temperate tree species. We hypothesized that the diameter, length and area of fine-root individuals would show right-skewed distributions across different tree species and forest ecosystems (asymmetry distribution hypothesis). Next, we aimed to determine whether there is a conspicuous fine-root size/number trade-off below

**TABLE 1** | Summary of the sampling sites in the Changbai Mountain (CBS), Xianrendong (XRD) and Maoershan (MES) forests investigated in this study.

	CBS (Jilin province)	MES (Heilongjiang province)	XRD (Liaoning province)
Location	42°24'N, 127°47'E	45°21'N, 127°30'E	39°54'N, 122°53'E
Climate	Temperate, continental	Temperate, continental monsoon	Humid, warm temperate monsoon
Elevation	738 m a.s.l.	360 m a.s.l.	140 m a.s.l.
MAT	3.5°C	2.8°C	8.7°C
MAP	740 mm	723 mm	1000 mm
Soil	Eutric Cambisol with high organic matter content	Hap-Boric Luvisol with high organic matter content	Haplic Alisol with medium organic matter content

MAT, mean annual temperature; MAP, mean annual precipitation.



ground, similar to the widely established leaf size/number trade-off above ground. We hypothesized that trees in temperate forests of Northeastern China either build relatively thick fine roots but less branched fine-root systems (small root number) or produce thinner fine roots but more highly branched fine-root systems (large root number; size-number hypothesis).

## MATERIALS AND METHODS

### Study Site and Root Collection

For the present study, three temperate natural mixed conifer-broadleaf forests were selected in Northeastern China: Changbai Mountain (CBS), Xianrendong (XRD) and Maoershan (MES) (Table 1). In August 2018, we sampled a total of 51 typical species (19 in XRD, 17 in CBS and 15 in MES), which spanned a wide range of fine-root size (Supplementary Table 1). At least three mature trees were chosen for each species in each forest ecosystem. Roots at 0–20 cm soil depth were carefully excavated near the base of the selected trees (Guo et al., 2008; Kong et al., 2014). Root branches with intact terminal branch orders were cut, transported in plastic bags in a cooler to the laboratory, and frozen at  $-20^{\circ}\text{C}$  until subsequent morphological and chemical analyses.

### Measurements of Fine-Root Traits and Estimations of Inequality in Fine-Root Size

In the laboratory, the sampled roots were classified into first- and second-order fine roots. Most distal roots were named

first-order fine roots, while roots that contained only first-order fine roots were named second-order fine roots (Pregitzer et al., 2002). Here, the term “fine-root size” was defined as the measured values of root length, diameter and area for individual roots. We used branching intensity and ratio as a measure of root number. Branching intensity was defined as the number of first-order roots per centimeter of second-order roots, and branching ratio was defined as the number of first-order roots per second order root (Comas and Eissenstat, 2009; Kong et al., 2014). Branching intensity and ratio were measured for at least 15 second-order roots per individual tree. The collected roots were scanned with an Epson Expression 10000XL scanner (Seiko Epson Corporation, Japan). Measurements were made on the computer screen by mouse clicking on the displayed images using the measuring tools (length of a straight line and a segmented line) provided by ImageJ 1.47 software (National Institutes of Health, United States). Subsamples of fine roots from each species were cleaned and oven-dried at  $60^{\circ}\text{C}$  for 24 h and ground to a fine powder using a ball mill. Carbon and nitrogen concentrations of fine roots were determined with an elemental analyzer (Vario EL Cube; Elementar, Hanau, Germany).

### Statistical Analysis

All analyses were run in R version 4.0.3 (R Core Development Team, 2020). We used the Kruskal-Wallis test (*kruskal.test* function in the “stats” R package) and Kolmogorov-Smirnov test (*ks.test* function in the “stats” R package) to examine differences in fine-root length, diameter and area and their frequency distributions among the three sampling sites. *Post*



**TABLE 2 |** Differences in the size and number, and their inequality/variation, of the first-order fine roots collected from Changbai Mountain (CBS), Xianrendong (XRD) and Maoershan (MES) forests.

	No	Mean $\pm$ SE	Median	Maximum	Minimum	Skewness	Kurtosis	CV	Gini
<b>Changbai mountain forest (CBS)</b>									
FL	9218	3.43 $\pm$ 0.03 <sup>c</sup>	2.63	52.6	0.01	3.27	22.4	0.83	0.30 $\pm$ 0.01 <sup>a</sup>
FD	9218	0.08 $\pm$ 0.01 <sup>c</sup>	0.07	0.60	0.01	1.51	3.86	0.75	0.22 $\pm$ 0.01 <sup>b</sup>
FA	9218	1.10 $\pm$ 0.02 <sup>c</sup>	0.56	42.1	0.01	6.01	58.2	1.71	0.42 $\pm$ 0.02 <sup>a</sup>
BR	1274	7.24 $\pm$ 0.12 <sup>b</sup>	6.00	30.0	1.00	1.40	2.41	0.57	
BI	1274	4.73 $\pm$ 0.09 <sup>b</sup>	4.04	27.0	0.55	2.15	8.15	0.65	
<b>Maoershan forest (MES)</b>									
FL	9203	3.73 $\pm$ 0.03 <sup>b</sup>	2.99	26.9	0.09	2.29	7.56	0.80	0.30 $\pm$ 0.01 <sup>a</sup>
FD	9203	0.12 $\pm$ 0.01 <sup>b</sup>	0.12	0.71	0.01	0.63	1.26	0.50	0.15 $\pm$ 0.01 <sup>c</sup>
FA	9203	1.61 $\pm$ 0.02 <sup>b</sup>	0.91	25.1	0.01	3.70	19.7	1.31	0.35 $\pm$ 0.01 <sup>a</sup>
BR	1156	7.97 $\pm$ 0.13 <sup>a</sup>	7.00	33.0	1.00	1.40	2.56	0.55	
BI	1149	4.98 $\pm$ 0.09 <sup>a</sup>	4.43	34.8	0.46	2.57	18.0	0.61	
<b>Xianrendong forest (XRD)</b>									
FL	9152	4.65 $\pm$ 0.04 <sup>a</sup>	4.17	49.6	0.03	2.42	9.96	0.90	0.29 $\pm$ 0.01 <sup>a</sup>
FD	9152	0.13 $\pm$ 0.01 <sup>a</sup>	0.10	1.03	0.01	1.49	4.15	0.69	0.26 $\pm$ 0.01 <sup>a</sup>
FA	9152	2.34 $\pm$ 0.04 <sup>a</sup>	1.09	65.1	0.01	4.23	32.5	1.52	0.46 $\pm$ 0.02 <sup>a</sup>
BR	1425	6.42 $\pm$ 0.10 <sup>c</sup>	5.00	31.0	1.00	1.54	3.60	0.58	
BI	1425	4.01 $\pm$ 0.08 <sup>c</sup>	3.19	30.8	0.29	2.15	8.13	0.79	

Different letters within a column indicate statistical significance at  $p < 0.05$ . FL, first-order fine-root length (mm); FD, first-order fine-root diameter (mm); FA, first-order fine-root area (mm<sup>2</sup>); BR, branching ratio; BI, branching intensity. No, number of individual fine roots; CV, coefficient of variation.

*hoc* tests were used for mean values of fine-root traits using Fisher's least significant difference criterion to explore differences among the three sampling sites when the Kruskal-Wallis chi-squared value was statistically significant ( $p < 0.05$ ). Kernel density estimates were calculated by weighing the distance of all the data points in each specific location along the distribution (*geom\_density* function in the "ggplot2" R package). The coefficient of variation (CV) and the Gini coefficient (G) (a statistic based on sums of absolute deviations of all observations) have both previously been used to quantify relative size inequality measures of plant traits and populations (He et al., 2005; Zhang and Chen, 2015; Rasmussen and Weiner, 2017). However, the coefficient of variation is more sensitive (less robust) to observations in the right-hand tail of the distribution (Bendel et al., 1989). The Gini coefficient (ranging from zero to one) is a single value that describes a specific degree of evenness, measuring the normalized area between a given Lorenz curve and the perfect evenness line (Weiner and Solbrig, 1984). As an indicator based on Lorenz curves, the Gini coefficient can deepen our insight into the overall degree of size inequality associated with fine-root traits and the relationships between fine-root inequality and distribution pattern (He et al., 2005; Wang et al., 2017). We calculated Gini coefficients with the *Lc* function in the "ineq" R package and plotted Lorenz curves with the *ggplot* function in the "gglorenz" R package with the blessing of "ggplot2" R package (Buckley and Damgaard, 2012; Chen and Cortina, 2020). We used phylogenetically controlled mixed-effects kinship models (*lmeKin* function in the "coxme" R package) with random effects of tree species to explore the relationships between fine-root size (root length, diameter and area) and fine-root number (branching intensity and

branching ratio). Fine-root trait values were log-transformed before analyses. Moreover, a simple correlation analysis (*ggcorr* function in the "GGally" R package) with the Spearman method and a principal component analysis using the spectral decomposition approach (*princomp* function in the "stats" R package) with standardization data were employed to assess the correlations between the fine-root size traits and their inequality. Results from the principal component analysis were visualized graphically using *fviz\_pca\_biplot* function in the "factoextra" R package.

## RESULTS

### Distribution and Inequality in Fine-Root Size

In total, 27,573 first-order fine roots from 3848 second-order fine roots across 51 tree species from the three temperate forest ecosystems were collected to assess the distribution and inequality of fine-root size (**Supplementary Tables 1, 2**). The frequency distributions from the Kolmogorov-Smirnov tests showed marked right-asymmetry for first-order fine-root length, diameter and area for the three forest ecosystems in Northeastern China (**Figure 1**). The positive skewness indicated that the distributions of first- and second-order fine-root size were right-skewed for almost all species, with a systematic right-hand tail of the distributions (**Supplementary Table 2** and **Supplementary Figures 1–3**). The three forest ecosystems showed a similar frequency distribution pattern in first-order fine-root length, with a unimodal value of about 1.80 mm (**Figure 1A**). A markedly bimodal frequency distribution of first-order fine-root diameter was observed in MES only, with one peak at about 0.07 mm



and a second peak at about 0.14 mm (**Figure 1B**). The similar unimodal frequency distribution patterns for first-order fine-root area in the three sites were leptokurtic, with the greatest proportion of small first-order fine-roots appearing in CBS (**Figure 1C**).

There were significant differences in the average length, diameter and area of first-order fine roots among the three forest ecosystems, with the largest roots appearing in XRD and the smallest roots appearing in CBS (**Table 2**). Fine roots in MES exhibited a higher branching intensity and ratio, while fine roots in XRD showed the lowest branching intensity and ratio (**Table 2**). Fine roots of the three forest ecosystems had a similar average coefficient of variation (CV) and Gini coefficient (G) of first-order fine-root length and area. However, significant differences in the Gini coefficients of first-order root diameter were detected, with the highest and lowest values being observed in XRD and MES, respectively (**Table 2** and **Figure 2**). Furthermore, large variations in first- and second-order fine-root size were found both within and among species in the three forest ecosystems (**Supplementary Tables 1, 2**). At the species level, *Phellodendron amurense* produced the largest first- and second-order roots but had smaller variation and inequality in fine-root size (lower CV and G) and lower branching intensity and ratio within each forest ecosystem (**Supplementary Tables 1, 2** and **Supplementary Figures 4–6**). *Sorbaria sorbifolia*, *Corylus mandshurica* and *Quercus mongolica* had the smaller first- and second-order fine roots but had higher variation and inequality in fine-root size (higher CV and G) (**Supplementary Tables 1, 2** and **Supplementary Figures 4–6**). *Ulmus davidiana*, *C. mandshurica* and *Ulmus laciniata* had the highest branching intensity and ratio across the three forest ecosystems (**Supplementary Table 3**).

## Correlations Among Different Fine-Root Traits

First-order fine-root size showed a consistently linear decline with increasing branching intensity and ratio (**Figure 3** and **Supplementary Figure 7**), although the negative relationship between fine-root diameter and branching ratio was not significant in MES (**Supplementary Table 4**). First-order fine-root size was strongly positively correlated with the size of second-order roots (**Supplementary Figure 8**). There were evidently positive correlations among root length, root diameter and root area for both root orders (**Supplementary Figure 8**). The first two trait axes of the principal component analysis accounted for 42.5 and 15.4% of the total variation, respectively (**Figure 4**). Branching intensity and ratio and fine-root size-related parameters had high scores on the first axis, while inequality and variation parameters for first-order fine-root diameter and area had high scores on the second axis (**Figure 4** and **Supplementary Table 5**). Additionally, length inequality and variation, nitrogen and carbon content of first-order fine-roots, and specific root length of first- and second-order fine roots had relatively high scores on the third axis (**Figure 4** and **Supplementary Table 5**). Tree species in XRD tended to have higher fine-root size

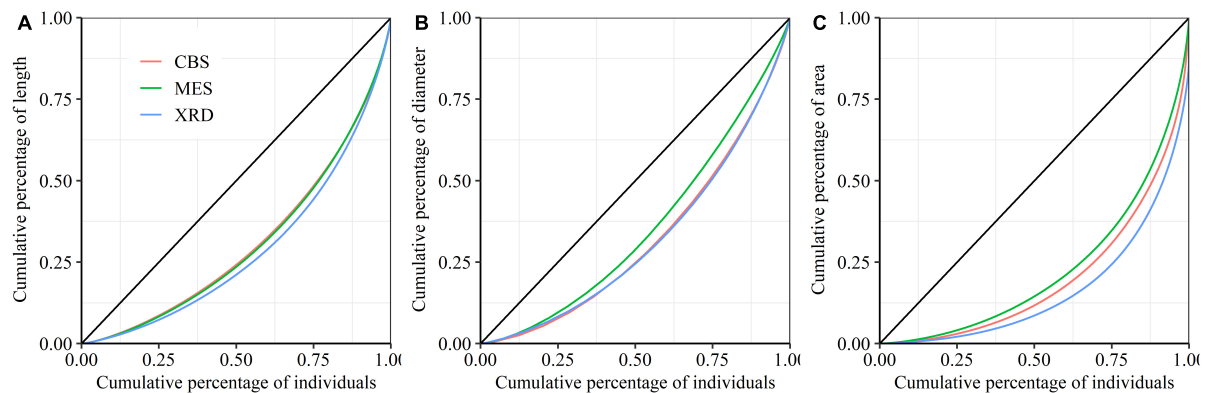
inequality and variation, while those in MES tended to have higher root branching. Fine roots of tree species from CBS scattered on both axes of the principal component analysis (**Figure 4**).

## DISCUSSION

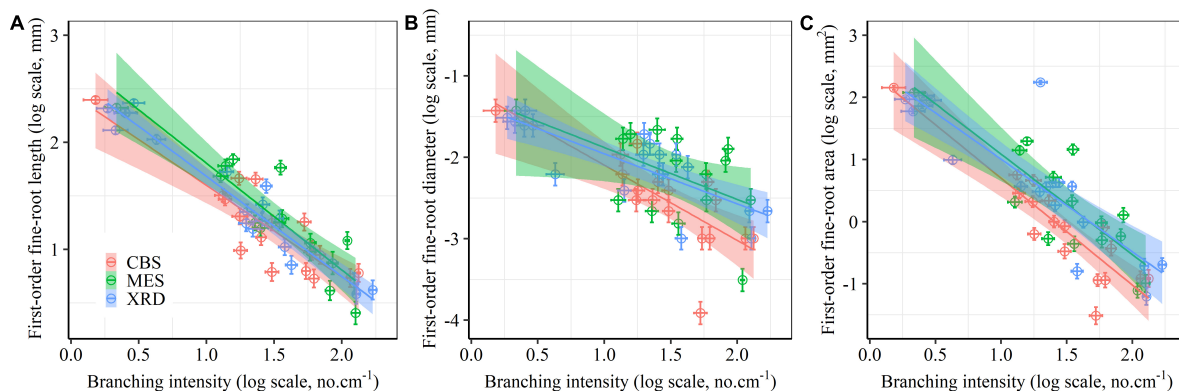
### The Right-Skewed Distribution of Fine-Root Size

In support of our asymmetry distribution hypothesis, we observed noticeably right-skewed distributions of fine-root size (length, diameter and area) at the individual level, with a long tail of larger roots, for the three temperate forest ecosystems and for most of the sampled tree species within each ecosystem in Northeastern China. The preponderance of the right-skewed distribution of plant organ size (small fine-roots in this study) is considered a consequence of the left-wall effect, because the sizes of organs must be greater than zero (Jensen and Zwieniecki, 2013). Similar to leaf size, the strongly right-skewed distributions for fine-root size at the community and species level suggest that most species have experienced natural selection favoring effects of relatively small fine-roots. Stated another way, trees that invest in a small number of coarse roots and many fine roots of small diameter may be better adapted for intensive fine-root growth and high fine-root length densities (Eissenstat, 1992). However, we also noted the diverse skewness and kurtosis of fine-root size among tree species in the three forest ecosystems. For instance, fine-root diameter of *P. amurense* with the negative skewness demonstrated a left-skewed distribution pattern (i.e., many large fine-roots) in both CBS and MES. These findings, similarly to previous studies, suggest that different tree species appear to present contrasting root types (e.g., species with thinner roots *versus* species with thicker roots) to enhance nutrient acquisitions in temperate and subtropical forest ecosystems (Liu et al., 2015; Chen et al., 2016; Cheng et al., 2016). Generally, the change in fine-root size is strongly related with tree's mycorrhizal association, such as arbuscular mycorrhizal or ectomycorrhizal (Bergmann et al., 2020). Like some tree species with thick roots in a subtropical forest (Liu et al., 2015), *P. amurense* in temperate forests producing the largest roots perhaps also relies more on arbuscular mycorrhizal fungi to forage nutrients. Therefore, the integrated consideration of the tree species composition with contrasting specific fine-root and mycorrhizal traits will improve our insights into the skewed distribution of fine-root size across various tree species.

Apart from asymmetry, we found that the unimodal distribution for fine-root size (except for root diameter in MES) was leptokurtic with a narrow peak, reflecting the occurrence of strong environmental filtering in the three temperate forests in Northeastern China. Environmental filtering plays a major role in shaping trait diversity of fine roots in forests, favoring convergence to an optimal trait value (Wang R. et al., 2018). However, a bimodal platykurtic distribution of fine-root diameter was observed in MES, as previously found for other tree and grass species (Boat and Mensink, 1990;



**FIGURE 2 |** Lorenz curves for the length (A), diameter (B) and area (C) of first-order fine roots from Changbai Mountain (CBS), Maoershan (MES) and Xianrendong (XRD) forests.



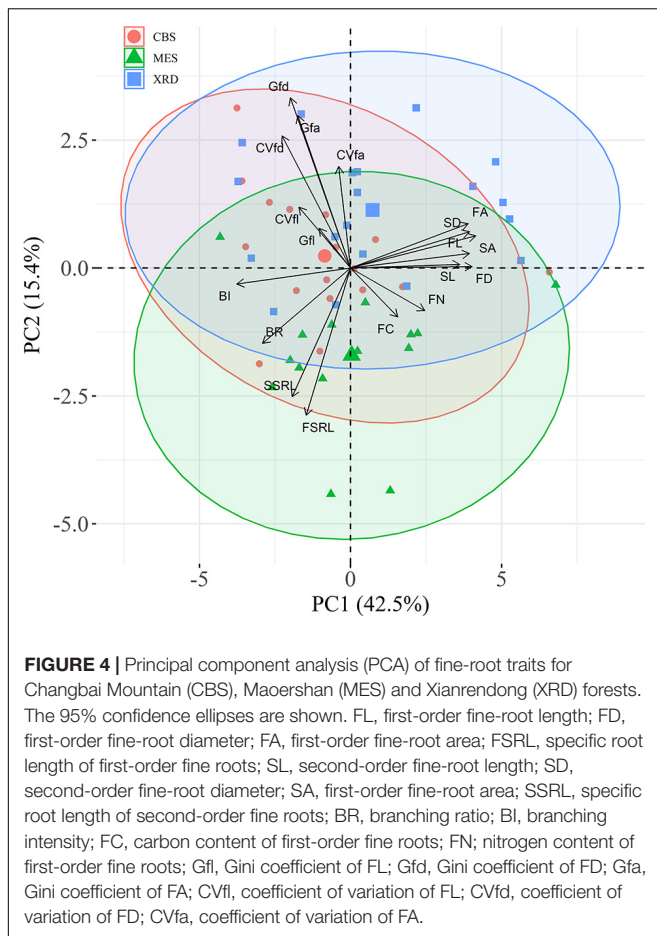
**FIGURE 3 |** Relationships between the size [length (A), diameter (B) and area (C)] of first-order fine roots and branching intensity for Changbai Mountain (CBS), Maoershan (MES) and Xianrendong (XRD) forests. Linear mixed-effects kinship model (Supplementary Table 4) fits are displayed, along with 95% confidence intervals.

Eissenstat, 1991; Bouma et al., 2001; Anderson et al., 2007). Bimodal trait distributions arise when there are multiple optimal trait values, suggesting the co-existence of contrasting functional strategies or the occurrence of stabilizing niche differences among interacting species (Gross et al., 2013; Le Bagousse-Pinguet et al., 2016). The co-occurrence of species with thick roots *versus* species with thin roots was observed at the species level in MES temperate forest. This fits with our above discussions on the skewed-distribution of fine-root size, implying that the maintenance of large variances in fine-root size is also strongly related to the species diversity of the forest community or ecosystem. Although there is a growing body of literature on ecological implications of fine-root diameter (Fort and Freschet, 2020; Valverde-Barrantes et al., 2020), more research on root diameter distributions is required to judge the prevalence of the bimodality observed in MES and to understand fully the functional significance of the bimodal distribution in other plant species. In conclusion, the important characteristics of the right-skewed distributions at the individual level in fine-root size traits reported here provide a basis for inferences about mechanisms of exploitation and acquisition in resources, and

they avoid the loss of information associated with average metrics of root systems.

## Inequality of Fine-Root Size

Our principal component analysis indicated that the second axis associated with fine-root size inequality is likely a critical dimension of the belowground resource uptake strategy in the multidimensional space of fine-root trait variation. Plant responses to environmental changes depend not only on the mean but also on the plasticity and variation of plant traits (Wang et al., 2017; Zhang et al., 2020). Further analyses extending beyond trait means will permit us to explore original relationships among the traits of interest (Russell et al., 2014). Size inequality of individuals exists in most plant populations, and the associated inequality of plant functional traits may have substantial ecological implications (Metsaranta and Lieffers, 2008; Forde, 2009). In this study, we detected significant differences in the Gini coefficients of fine-root diameter among the three forest ecosystems, with the highest and lowest values occurring in XRD and MES, respectively. Changes in fine-root size inequality have been attributed to alterations



**FIGURE 4 |** Principal component analysis (PCA) of fine-root traits for Changbai Mountain (CBS), Maoershan (MES) and Xianrendong (XRD) forests. The 95% confidence ellipses are shown. FL, first-order fine-root length; FD, first-order fine-root diameter; FA, first-order fine-root area; FSRL, specific root length of first-order fine roots; SL, second-order fine-root length; SD, second-order fine-root diameter; SA, first-order fine-root area; SSRL, specific root length of second-order fine roots; BR, branching ratio; BI, branching intensity; FC, carbon content of first-order fine roots; FN, nitrogen content of first-order fine roots; GFI, Gini coefficient of FL; GFD, Gini coefficient of FD; GFA, Gini coefficient of FA; CVFI, coefficient of variation of FL; CVFA, coefficient of variation of FA; CVFD, coefficient of variation of FD.

in the mode of competition among fine-root individuals, leading to changes in the shape of the root size frequency distribution of fine-root systems (Wang et al., 2017). Some previous studies have underlined the importance of competition effects on size inequality and *vice versa* (Chu et al., 2009; Rasmussen and Weiner, 2017). Efficient water availability and higher temperature in XRD are expected to favor larger fine roots growing at relatively higher rates (Kong et al., 2014; Ma et al., 2018), to increase the asymmetric competition in resource utilization by larger fine roots. The harsher environments in MES likely lead to generate many smaller fine roots growing at relatively similar rates, promoting relatively size-symmetric competition among fine-root individuals, and therefore, resource utilization is equal or proportionate to fine-root size (Pagès et al., 1993). Consequently, the biotic factors such as competition, abiotic factors such as nutrient availability and their interactions presented potential effects on size inequality of plant community (Boot and Mensink, 1990; Chu et al., 2009). In addition, we only observed significant differences in size inequality for fine-root diameter, which was always less than that for fine-root length or area in the three forest ecosystems. Therefore, the size inequality of fine-root diameter at the individual root level seems to be a better measure of size inequality, because fine-root diameter is more

stable and more directly linked to the functioning of fine-root systems than fine-root length and area (Chen et al., 2013; Kong et al., 2014).

## Trade-Off Between Fine-Root Size and Fine-Root Number

In support of our size-number hypothesis, we found that fine-root size was negatively correlated with fine-root number (branching intensity and ratio), indicating a trade-off across the investigated tree species between building fewer fine roots with a relatively large diameter and forming thinner fine roots in a more highly branched fine-root system. The results reported here may represent generalized trade-off strategies for fine-root deployment at the individual root level within temperate tree species. The fine-root size/number trade-off is in line with the various well-known compromises between size and number of other organs in plant bodies, such as leaf size/number and seed size/number trade-offs (Westoby and Wright, 2003; Milla, 2009), and with compromises between size and number of individuals at the population level (Deng et al., 2008). The leafing premium hypothesis states that high branching intensity should be rewarded in natural selection, based on the pattern that most species are small-leaved and the negative relationship between leaf size and number (Kleiman and Aarssen, 2007; Leigh et al., 2017). Just as the arrangement and location of leaves on a branch influence the capture of CO<sub>2</sub> and sunlight, the position and organization of most distal roots within the root system (branching pattern) regulate their various ecological and functional aspects, such as the resource-foraging strategy and intra-branch competition (Bentley et al., 2013; Kong et al., 2014; McCormack et al., 2020). A high root branching intensity may indeed bring about selective advantages, given the preponderance of small fine-roots in most tree species (e.g., the strongly positive right-skewed fine-root size frequency distribution observed in this study). Trees can enhance resource absorption not only by producing thinner roots but also by enhancing root branching intensity to rapidly exploit resource-rich soil patches at low fertility sites (Holdaway et al., 2011; Beidler et al., 2015). The fine-root size/number trade-off is one of the fundamental adaptation strategies of plants to environmental changes, and it may therefore have particularly important implications for understanding root size evolution (López-Bucio et al., 2003; Rich and Michelle, 2013; Kong et al., 2014).

We observed that larger fine-root diameters were associated with longer length of fine roots, which appears to be a simply physical relationship (Tyree and Ewers, 1991; McCormack et al., 2020), while root diameter could also be related to other functions such as storage, aerenchyma, etc., Gu et al. (2014). Longer roots, with a greater potential to absorb resources, should be connected to roots with a larger diameter, which effectively represent larger pipes with a lower resistance to conduct those resources (Kong et al., 2014; McCormack et al., 2020). Similarly, the strong, positive correlations in fine-root diameter between first- and second-order fine roots observed across tree species and within the same tree species demonstrate that relatively large first-order fine roots tend to arise from relatively large second-order roots.

## Implications of Fine-Root Size/Number Trade-Off

From an anatomical perspective, smaller fine roots have a less suberized and less lignified hypodermis than larger fine roots, characteristics that are associated with higher construction and maintenance costs (Fitter et al., 1991; Pregitzer et al., 1993; Fort and Freschet, 2020). The lower within-root structural investment for smaller fine roots of temperate tree species allows shorter life spans/quicker turnover and greater plasticity in root growth proliferation (Eissenstat, 1991, 1992), conferring them advantages in seasonally more variable environments (such as MES in this study) in terms of temperature, moisture and nutrient supply (Chen et al., 2013). For instance, small roots with a thinner cortical thickness are likely to have less hydraulic resistance to the lateral transport of resources from the root surface to the vascular bundle (Valverde-Barrantes et al., 2020). From an ecological perspective, roots with a high branching intensity (namely small roots) are capable of rapid and extensive proliferation into resource-rich patches in heterogeneous forest soils (Hodge, 2004; Kong et al., 2014). Moreover, our result of intensive root branching also increases the specific root length of the pool of first- and second-order roots, which are presumed to have high respiration rates and high resource uptake activities (Rewald et al., 2011, 2014; McCormack et al., 2015). It has previously been demonstrated that small roots enhance small-scale foraging and nutrient absorption in the arid temperate zone (Chen et al., 2013). Fine-root clusters with more root tips and branching can accommodate greater colonization by mycorrhizal fungi, which in turn improve the nutrient and water absorption capacity of fine-root systems (Comas and Eissenstat, 2009; Ekblad et al., 2016). In addition, branching pattern differences have various effects on the ability of a fine-root system to capture relatively mobile *versus* immobile soil nutrients (Beidler et al., 2015). A large number of small fine roots are needed to acquire less mobile soil resources rapidly, improving competitive ability (Robinson et al., 1999) but leading to decreases in exploitation efficiency (Berntson, 1994). Radial resistance in fine roots is an important limitation to water and nutrient uptake; therefore, fine roots with a small diameter have a shorter path length for water and nutrient movement to the xylem than large-diameter fine roots (Purushothaman et al., 2013; Wang W. et al., 2018). As a result of selection favoring minimization of transport distances and maximization of exchange surfaces, the formation of very fine roots can be a prime strategy to increase the soil-root exchange surface at a minimal cost (Kong et al., 2017).

In our study, as fine-root length increased the proliferation of root tips decreased, which may be related to the exploitation potential, i.e., the volume of soil exploited per unit volume of fine roots (Chen et al., 2016; McCormack et al., 2020). Compared with small fine roots, large fine roots are more efficient at assimilating diffuse resources through more extensive soil exploration, because low root branching intensity reduces the overlap of depletion zones of neighboring branches and inter-root individual competition (Berntson, 1994; Beidler et al., 2015). Thus, decreased frequency of root tip proliferation events along

a length of fine root may be indicative of a more exploratory growth strategy (Beidler et al., 2015). In other words, there is a clear trade-off between the exploitation efficiency and exploitation potential in fine-root systems. Small fine roots in MES will show a high exploitation efficiency (intensive) but low exploitation potential and are associated with a greater specific root length, more root branching and shorter root life span (fast absorption strategy) (Comas and Eissenstat, 2009; Liese et al., 2017). Inversely, large fine roots in XRD will exhibit a high exploitation potential (extensive) but low exploitation efficiency and are associated with lower specific root length, less root branching and longer root life span (slow absorption strategy) (Zadworny and Eissenstat, 2011; Fort and Freschet, 2020). Together with the similar functional associations identified in leaves, the intensive-extensive continuum in fine-root systems is a key feature of trees and should be considered in order to gain a thorough understanding of the belowground ecological strategies of trees, the assembly processes of forest communities, and the functioning of forest ecosystems (Reich, 2014).

## CONCLUSION

Based on a large dataset of 27,573 individual first-order fine roots from three temperate forest ecosystems in Northeastern China, we explored the size distribution pattern of fine roots and their relationships with fine-root number. Our results reveal the overall generality of a cross-species right-skewed distribution in fine-root size traits, and confirm the existence of a trade-off between the number of fine roots and the size of fine roots of temperate tree species. The observed frequency distributions of fine-root size traits indicate that thinner roots within fine-root systems are favored over thicker roots because of their relative benefits in terms of resource acquisition. We propose that the fine-root size/number trade-off is likely to provide fundamental insight into the variation in patterns of fine-root traits in forest ecosystems. Furthermore, the relationship has important implications regarding the contrasting resource acquisition strategies of thinner and thicker roots within fine-root systems. Coupling information on fine-root size frequency distributions with knowledge on the trade-offs between fine-root functional traits will be a powerful approach in studying the water and nutrient uptake behavior of fine-root system in forest ecosystems.

## DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

## AUTHOR CONTRIBUTIONS

CW and XY conceived the ideas and designed methodology. JW, WG, and ZG collected the data. CW and SH analysed the



data. IB, ZC, and M-HL led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

## FUNDING

This work was financially supported by the National Key R&D Program of China (Grant No. 2019YFA0607301) and the Natural Science Foundation of China (Grant Nos. 41971052 and 42171051).

## REFERENCES

- Anderson, T. M., Starmer, W. T., and Thorne, M. (2007). Bimodal root diameter distributions in Serengeti grasses exhibit plasticity in response to defoliation and soil texture: implications for nitrogen uptake. *Funct. Ecol.* 21, 50–60. doi: 10.1111/j.1365-2435.2006.01192.x
- Beidler, K. V., Taylor, B. N., Strand, A. E., Cooper, E. R., Schönholz, M., and Pritchard, S. G. (2015). Changes in root architecture under elevated concentrations of CO<sub>2</sub> and nitrogen reflect alternate soil exploration strategies. *New Phytol.* 205, 1153–1163. doi: 10.1111/nph.13123
- Bendel, R. B., Higgins, S. S., Teberg, J. E., and Pyke, D. A. (1989). Comparison of skewness coefficient, coefficient of variation, and Gini coefficient as inequality measures within populations. *Oecologia* 78, 394–400. doi: 10.1007/bf00379115
- Bentley, L. P., Stegen, J. C., Savage, V. M., Smith, D. D., Allmen, E. I., Sperry, J. S., et al. (2013). An empirical assessment of tree branching networks and implications for plant allometric scaling models. *Ecol. Lett.* 16, 1069–1078. doi: 10.1111/ele.12127
- Bergmann, J., Weigelt, A., Van der Plas, F., Laughlin, D., Kuyper, T., Guerrero-Ramírez, N., et al. (2020). The fungal collaboration gradient dominates the root economics space in plants. *Sci. Adv.* 6:eaba3756. doi: 10.1126/sciadv.aba3756
- Berntson, G. (1994). Modelling root architecture: are there tradeoffs between efficiency and potential of resource acquisition? *New Phytol.* 127, 483–493. doi: 10.1111/j.1469-8137.1994.tb03966.x
- Boot, R. G. A., and Mensink, M. (1990). Size and morphology of root systems of perennial grasses from contrasting habitats as affected by nitrogen supply. *Plant Soil* 129, 291–299. doi: 10.1007/BF00032425
- Bouma, T. J., Nielsen, K. L., Van Hal, J., and Koutstaal, B. (2001). Root system topology and diameter distribution of species from habitats differing in inundation frequency. *Funct. Ecol.* 15, 360–369. doi: 10.1046/j.1365-2435.2001.00523.x
- Buckley, H. L., and Damgaard, C. (2012). *Lorenz.R: R Code For Drawing Sample Lorenz Curves And To Calculate Gini Coefficients And Lorenz Asymmetry Coefficients [Computer Program]* [Online]. Available Online at: [http://pure.au.dk/portal/en/publications/lorenzr\(21dbe72d-0f9c-4a3b-9a2c-f364828089d3\).html](http://pure.au.dk/portal/en/publications/lorenzr(21dbe72d-0f9c-4a3b-9a2c-f364828089d3).html) [accessed April 5, 2017].
- Chen, J. J., and Cortina, H. (2020). *gglorenz: Plotting Lorenz Curve With The Blessing Of 'ggplot2'*. Available Online at: <https://github.com/jjchern/gglorenz> (accessed May 20, 2020).
- Chen, W., Koide, R. T., Adams, T. S., DeForest, J. L., Cheng, L., and Eissenstat, D. M. (2016). Root morphology and mycorrhizal symbioses together shape nutrient foraging strategies of temperate trees. *Proc. Natl. Acad. Sci. U. S. A.* 113, 8741–8746. doi: 10.1073/pnas.1601006113
- Chen, W., Zeng, H., Eissenstat, D. M., and Guo, D. (2013). Variation of first-order root traits across climatic gradients and evolutionary trends in geological time. *Glob. Ecol. Biogeogr.* 22, 846–856. doi: 10.1111/geb.12048
- Cheng, L., Chen, W., Adams, T. S., Wei, X., Li, L., McCormack, M. L., et al. (2016). Mycorrhizal fungi and roots are complementary in foraging within nutrient patches. *Ecology* 97, 2815–2823. doi: 10.1002/ecy.1514
- Chu, C.-J., Weiner, J., Maestre, F. T., Xiao, S., Wang, Y.-S., Li, Q., et al. (2009). Positive interactions can increase size inequality in plant populations. *J. Ecol.* 97, 1401–1407. doi: 10.1111/j.1365-2745.2009.01562.x

## ACKNOWLEDGMENTS

We thank Zhao Chen, who collected and analyzed fine roots in the field and laboratory.

## SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fpls.2021.772463/full#supplementary-material>

- Comas, L., and Eissenstat, D. (2009). Patterns in root trait variation among 25 co-existing North American forest species. *New Phytol.* 182, 919–928. doi: 10.1111/j.1469-8137.2009.02799.x
- Deng, J., Li, T., Wang, G., Liu, J., Yu, Z., Zhao, C., et al. (2008). Trade-offs between the metabolic rate and population density of plants. *PLoS One* 3:e1799. doi: 10.1371/journal.pone.0001799
- Ding, J., Kong, D., Zhang, Z., Cai, Q., Xiao, J., Liu, Q., et al. (2020). Climate and soil nutrients differentially drive multidimensional fine root traits in ectomycorrhizal-dominated alpine coniferous forests. *J. Ecol.* 108, 2544–2556. doi: 10.1111/1365-2745.13407
- Dombroskie, S. L., and Aarssen, L. W. (2010). Within-genus size distributions in angiosperms: small is better. *Perspect. Plant Ecol. Evol. Syst.* 12, 283–293. doi: 10.1016/j.ppees.2010.06.002
- Eissenstat, D. M. (1991). On the relationship between specific root length and the rate of root proliferation: a field study using citrus rootstocks. *New Phytol.* 118, 63–68. doi: 10.1111/j.1469-8137.1991.tb00565.x
- Eissenstat, D. M. (1992). Costs and benefits of constructing roots of small diameter. *J. Plant Nutr.* 15, 763–782. doi: 10.1080/01904169209364361
- Eissenstat, D. M., and Achor, D. S. (1999). Anatomical characteristics of roots of citrus rootstocks that vary in specific root length. *New Phytol.* 141, 309–321. doi: 10.1046/j.1469-8137.1999.00342.x
- Ekblad, A., Mikusinska, A., Ågren, G. I., Menichetti, L., Wallander, H., Vilgalys, R., et al. (2016). Production and turnover of ectomycorrhizal extramatrical mycelial biomass and necromass under elevated CO<sub>2</sub> and nitrogen fertilization. *New Phytol.* 211, 874–885. doi: 10.1111/nph.13961
- Fang, J. Y., Chen, A. P., Peng, C. H., Zhao, S. Q., and Ci, L. J. (2001). Changes in forest biomass carbon storage in China between 1949 and 1998. *Science* 292, 2320–2322. doi: 10.1126/science.1058629
- Fitter, A., Stickland, T., Harvey, M., and Wilson, G. (1991). Architectural analysis of plant root systems 1. Architectural correlates of exploitation efficiency. *New Phytol.* 118, 375–382. doi: 10.1111/j.1469-8137.1991.tb00018.x
- Forde, B. G. (2009). Is it good noise? The role of developmental instability in the shaping of a root system. *J. Exp. Bot.* 60, 3989–4002. doi: 10.1093/jxb/erp265
- Fort, F., and Freschet, G. T. (2020). Plant ecological indicator values as predictors of fine-root trait variations. *J. Ecol.* 108, 1565–1577. doi: 10.1111/1365-2745.13368
- Freschet, G. T., Roumet, C., Comas, L. H., Weemstra, M., Bengough, A. G., Rewald, B., et al. (2021). Root traits as drivers of plant and ecosystem functioning: current understanding, pitfalls and future research needs. *New Phytol.* 232, 1123–1158. doi: 10.1111/nph.17072
- Freschet, G. T., Valverde-Barrantes, O. J., Tucker, C. M., Craine, J. M., McCormack, M. L., Violle, C., et al. (2017). Climate, soil and plant functional types as drivers of global fine-root trait variation. *J. Ecol.* 105, 1182–1196. doi: 10.1111/1365-2745.12769
- Gross, N., Börger, L., Soriano-Morales, S. I., Le Bagousse-Pinguet, Y., Quero, J. L., García-Gómez, M., et al. (2013). Uncovering multiscale effects of aridity and biotic interactions on the functional structure of Mediterranean shrublands. *J. Ecol.* 101, 637–649. doi: 10.1111/1365-2745.12063
- Gu, J., Xu, Y., Dong, X., Wang, H., and Wang, Z. (2014). Root diameter variations explained by anatomy and phylogeny of 50 tropical and temperate tree species. *Tree Physiol.* 34, 415–425. doi: 10.1093/treephys/tpu019
- Guo, D. L., Xia, M. X., Wei, X., Chang, W. J., Liu, Y., and Wang, Z. Q. (2008). Anatomical traits associated with absorption and mycorrhizal colonization are

- linked to root branch order in twenty-three Chinese temperate tree species. *New Phytol.* 180, 673–683. doi: 10.1111/j.1469-8137.2008.02573.x
- He, Z., Ma, Z., Brown, K. M., and Lynch, J. P. (2005). Assessment of inequality of root hair density in *Arabidopsis thaliana* using the Gini coefficient: a close look at the effect of phosphorus and its interaction with ethylene. *Ann. Bot.* 95, 287–293. doi: 10.1093/aob/mci024
- Hodge, A. (2004). The plastic plant: root responses to heterogeneous supplies of nutrients. *New Phytol.* 162, 9–24. doi: 10.1111/j.1469-8137.2004.01015.x
- Holdaway, R. J., Richardson, S. J., Dickie, I. A., Peltzer, D. A., and Coomes, D. A. (2011). Species and community level patterns in fine root traits along a 120 000 year soil chronosequence in temperate rain forest. *J. Ecol.* 99, 954–963.
- Hu, S., Xiong, D., Huang, J., Wang, W., Huang, C., Deng, F., et al. (2015). Diameter frequency distribution for 0–5 mm roots in six mid-subtropical forests subject to different regeneration approaches. *Acta Ecol. Sin.* 35, 7812–7820. doi: 10.5846/stxb201405050888
- Jensen, K. H., and Zwieniecki, M. A. (2013). Physical limits to leaf size in tall trees. *Phys. Rev. Lett.* 110:018104. doi: 10.1103/PhysRevLett.110.018104
- Kleiman, D., and Aarssen, L. W. (2007). The leaf size/number trade-off in trees. *J. Ecol.* 95, 376–382. doi: 10.1111/j.1365-2745.2006.01205.x
- Kong, D., Ma, C., Zhang, Q., Li, L., Chen, X., Zeng, H., et al. (2014). Leading dimensions in absorptive root trait variation across 96 subtropical forest species. *New Phytol.* 203, 863–872. doi: 10.1111/nph.12842
- Kong, D., Wang, J., Valverde-Barrantes, O. J., and Kardol, P. (2021). A framework to assess the carbon supply-consumption balance in plant roots. *New Phytol.* 229, 659–664. doi: 10.1111/nph.16807
- Kong, D., Wang, J., Zeng, H., Liu, M., Miao, Y., Wu, H., et al. (2017). The nutrient absorption-transportation hypothesis: optimizing structural traits in absorptive roots. *New Phytol.* 213, 1569–1572. doi: 10.1111/nph.14344
- Kroon, H., Huber, H., Stuefer, J., and van Groenendaal, J. (2005). A modular concept of phenotypic plasticity in plants. *New Phytol.* 166, 73–82. doi: 10.1111/j.1469-8137.2004.01310.x
- Le Bagousse-Pinguet, Y., Liancourt, P., Gross, N., de Bello, F., Fonseca, C. R. C., Kattge, J., et al. (2016). Climate, topography and soil factors interact to drive community trait distributions in global drylands. *PeerJ* 4:e1913v1. doi: 10.7287/peerj.preprints.1913v1
- Leigh, A., Sevanto, S., Close, J. D., and Nicotra, A. B. (2017). The influence of leaf size and shape on leaf thermal dynamics: does theory hold up under natural conditions? *Plant Cell Environ.* 40, 237–248. doi: 10.1111/pce.12857
- Liese, R., Alings, K., and Meier, I. C. (2017). Root branching is a leading root trait of the plant economics spectrum in temperate trees. *Front. Plant Sci.* 8:315.
- Liu, B., Li, H., Zhu, B., Koide, R. T., Eissenstat, D. M., and Guo, D. (2015). Complementarity in nutrient foraging strategies of absorptive fine roots and arbuscular mycorrhizal fungi across 14 coexisting subtropical tree species. *New Phytol.* 208, 125–136. doi: 10.1111/nph.13434
- López-Bucio, J., Cruz-Ramírez, A., and Herrera-Estrella, L. (2003). The role of nutrient availability in regulating root architecture. *Curr. Opin. Plant Biol.* 6, 280–287. doi: 10.1016/S1369-5266(03)00035-9
- Ma, Z., Guo, D., Xu, X., Lu, M., Bardgett, R. D., Eissenstat, D. M., et al. (2018). Evolutionary history resolves global organization of root functional traits. *Nature* 555, 94–97. doi: 10.1038/nature25783
- McCormack, M. L., Dickie, I. A., Eissenstat, D. M., Fahey, T. J., Fernandez, C. W., Guo, D., et al. (2015). Redefining fine roots improves understanding of below-ground contributions to terrestrial biosphere processes. *New Phytol.* 207, 505–518. doi: 10.1111/nph.13363
- McCormack, M. L., Kaproth, M. A., Cavender-Bares, J., Carlson, E., Hipp, A. L., Han, Y., et al. (2020). Climate and phylogenetic history structure morphological and architectural trait variation among fine-root orders. *New Phytol.* 228, 1824–1834. doi: 10.1111/nph.16804
- Metsaranta, J. M., and Lieffers, V. J. (2008). Inequality of size and size increment in *Pinus banksiana* in relation to stand dynamics and annual growth rate. *Ann. Bot.* 101, 561–571. doi: 10.1093/aob/mcm320
- Milla, R. (2009). The leafing intensity premium hypothesis tested across clades, growth forms and altitudes. *J. Ecol.* 97, 972–983.
- Moles, A. T., Ackerly, D. D., Tweddle, J. C., Dickie, J. B., Smith, R., Leishman, M. R., et al. (2007). Global patterns in seed size. *Glob. Ecol. Biogeogr.* 16, 109–116. doi: 10.1111/j.1466-8238.2006.00259.x
- Pages, L., Kervella, J., and Chadoeuf, J. (1993). Development of the root system of young peach trees (*Prunus persia* L. Batsch): a morphometrical analysis. *Ann. Bot.* 71, 369–375. doi: 10.1006/anbo.1993.1046
- Polverigiani, S., McCormack, M. L., Mueller, C. W., and Eissenstat, D. M. (2011). Growth and physiology of olive pioneer and fibrous roots exposed to soil moisture deficits. *Tree Physiol.* 31, 1228–1237. doi: 10.1093/treephys/tpr110
- Pregitzer, K. S., DeForest, J. L., Burton, A. J., Allen, M. F., Ruess, R. W., and Hendrick, R. L. (2002). Fine root architecture of nine North American trees. *Ecol. Monogr.* 72, 293–309. doi: 10.1890/0012-9615(2002)072[0293:fraonn]2.0.co;2
- Pregitzer, K. S., Hendrick, R. L., and Fogel, R. (1993). The demography of fine roots in response to patches of water and nitrogen. *New Phytol.* 125, 575–580. doi: 10.1111/j.1469-8137.1993.tb03905.x
- Purushothaman, R., Zaman-Allah, M., Mallikarjuna, N., Pannirselvam, R., Krishnamurthy, L., and Gowda, C. L. L. (2013). Root anatomical traits and their possible contribution to drought tolerance in grain legumes. *Plant Prod. Sci.* 16, 1–8. doi: 10.1626/pp.s.16.1
- R Core Development Team (2020). *R: A Language and Environment for Statistical Computing*. Vienna: R Foundation for Statistical Computing.
- Rasmussen, C. R., and Weiner, J. (2017). Modelling the effect of size-asymmetric competition on size inequality: simple models with two plants. *Ecol. Modell.* 343, 101–108. doi: 10.1016/j.ecolmodel.2016.10.011
- Reich, P. B. (2014). The world-wide ‘fast-slow’ plant economics spectrum: a traits manifesto. *J. Ecol.* 102, 275–301. doi: 10.1111/1365-2745.12211
- Rewald, B., Ephrath, J. E., and Rachmilevitch, S. (2011). A root is a root is a root? Water uptake rates of Citrus root orders. *Plant Cell Environ.* 34, 33–42. doi: 10.1111/j.1365-3040.2010.02223.x
- Rewald, B., Rechenmacher, A., and Godbold, D. L. (2014). It’s complicated: intraroot system variability of respiration and morphological traits in four deciduous tree species. *Plant Physiol.* 166, 736–745. doi: 10.1104/pp.114.240267
- Rich, S. M., and Michelle, W. (2013). Soil conditions and cereal root system architecture: review and considerations for linking Darwin and Weaver. *J. Exp. Bot.* 64, 1193–1208. doi: 10.1093/jxb/ert043
- Robinson, D., Hodge, A., Griffiths, B. S., and Fitter, A. H. (1999). Plant root proliferation in nitrogen-rich patches confers competitive advantage. *Proc. R. Soc. B Biol. Sci.* 266, 431–431. doi: 10.1098/rspb.1999.0656
- Russell, M. B., Woodall, C. W., D’Amato, A. W., Domke, G. M., and Saatchi, S. S. (2014). Beyond mean functional traits: Influence of functional trait profiles on forest structure, production, and mortality across the eastern US. *For. Ecol. Manag.* 328, 1–9. doi: 10.1016/j.foreco.2014.05.014
- Scanlan, C. A., and Hinz, C. (2010). Using radius frequency distribution functions as a metric for quantifying root systems. *Plant Soil* 332, 475–493. doi: 10.1007/s11104-010-0314-5
- Sun, S., Jin, D., and Shi, P. (2006). The leaf size–twig size spectrum of temperate woody species along an altitudinal gradient: an invariant allometric scaling relationship. *Ann. Bot.* 97, 97–107. doi: 10.1093/aob/mcj004
- Tyree, M. T., and Ewers, F. W. (1991). The hydraulic architecture of trees and other woody plants. *New Phytol.* 119, 345–360. doi: 10.1111/j.1469-8137.1991.tb00035.x
- Valverde-Barrantes, O. J., Maherali, H., Baraloto, C., and Blackwood, C. B. (2020). Independent evolutionary changes in fine-root traits among main clades during the diversification of seed plants. *New Phytol.* 228, 541–553. doi: 10.1111/nph.16729
- Wang, C. (2006). Biomass allometric equations for 10 co-occurring tree species in Chinese temperate forests. *For. Ecol. Manag.* 222, 9–16. doi: 10.1016/j.foreco.2005.10.074
- Wang, C., Geng, Z., Chen, Z., Li, J., Guo, W., Zhao, T.-H., et al. (2017). Six-year nitrogen–water interaction shifts the frequency distribution and size inequality of the first-order roots of *Fraxinus mandschurica* in a mixed mature *Pinus koraiensis* forest. *Front. Plant Sci.* 8:1691. doi: 10.3389/fpls.2017.01691
- Wang, C., He, J., Zhao, T.-H., Cao, Y., Wang, G., Sun, B., et al. (2019). The smaller the leaf is, the faster the leaf water loses in a temperate forest. *Front. Plant Sci.* 10:58. doi: 10.3389/fpls.2019.00058
- Wang, R., Wang, Q., Liu, C., Kou, L., Zhao, N., Xu, Z., et al. (2018). Changes in trait and phylogenetic diversity of leaves and absorptive roots from tropical to boreal forests. *Plant Soil* 432, 389–401.

- Wang, W., Wang, Y., Hoch, G., Wang, Z., and Gu, J. (2018). Linkage of root morphology to anatomy with increasing nitrogen availability in six temperate tree species. *Plant Soil* 425, 189–200. doi: 10.1007/s11104-018-3563-3
- Weiner, J., and Solbrig, O. T. (1984). The meaning and measurement of size hierarchies in plant populations. *Oecologia* 61, 334–336. doi: 10.1007/bf00379630
- Westoby, M., and Wright, I. J. (2003). The leaf size-twign size spectrum and its relationship to other important spectra of variation among species. *Oecologia* 135, 621–628. doi: 10.1007/s00442-003-1231-6
- Zadworny, M., and Eissenstat, D. M. (2011). Contrasting the morphology, anatomy and fungal colonization of new pioneer and fibrous roots. *New Phytol.* 190, 213–221. doi: 10.1111/j.1469-8137.2010.03598.x
- Zhang, B., Hautier, Y., Tan, X., You, C., Cadotte, M. W., Chu, C., et al. (2020). Species responses to changing precipitation depend on trait plasticity rather than trait means and intraspecific variation. *Funct. Ecol.* 34, 2622–2633. doi: 10.1111/1365-2435.13675
- Zhang, Y., and Chen, H. Y. H. (2015). Individual size inequality links forest diversity and above-ground biomass. *J. Ecol.* 103, 1245–1252.

**Conflict of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

**Publisher's Note:** All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Copyright © 2022 Wang, Brunner, Wang, Guo, Geng, Yang, Chen, Han and Li. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.



# Evidence of Differences in Covariation Among Root Traits Across Plant Growth Forms, Mycorrhizal Types, and Biomes

Nannan An<sup>1,2</sup>, Nan Lu<sup>1,2\*</sup>, Bojie Fu<sup>1,2,3</sup>, Weiliang Chen<sup>1</sup>, Maierdang Keyimu<sup>1</sup> and Mengyu Wang<sup>1,2</sup>

<sup>1</sup> State Key Laboratory of Urban and Regional Ecology, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences (CAS), Beijing, China, <sup>2</sup> University of Chinese Academy of Sciences, Beijing, China, <sup>3</sup> Faculty of Geographical Science, Beijing Normal University, Beijing, China

## OPEN ACCESS

### Edited by:

Iván Prieto,  
Universidad de León, Spain

### Reviewed by:

Kira A. Borden,  
University of Guelph, Canada  
Enrique García De La Riva,  
Doñana Biological Station, Spanish  
National Research Council (CSIC),  
Spain

### \*Correspondence:

Nan Lu  
nanlv@rcees.ac.cn

### Specialty section:

This article was submitted to  
Functional Plant Ecology,  
a section of the journal  
Frontiers in Plant Science

**Received:** 29 September 2021

**Accepted:** 22 December 2021

**Published:** 28 January 2022

### Citation:

An N, Lu N, Fu B, Chen W,  
Keyimu M and Wang M (2022)  
Evidence of Differences in Covariation  
Among Root Traits Across Plant  
Growth Forms, Mycorrhizal Types,  
and Biomes.  
*Front. Plant Sci.* 12:785589.  
doi: 10.3389/fpls.2021.785589

Fine roots play an important role in plant ecological strategies, adaptation to environmental constraints, and ecosystem functions. Covariation among root traits influence the physiological and ecological processes of plants and ecosystems. Root trait covariation in multiple dimensions at the global scale has been broadly discussed. How fine-root traits covary at the regional scale and whether the covariation is generalizable across plant growth forms, mycorrhizal types, and biomes are largely unknown. Here, we collected six key traits – namely root diameter (RD), specific root length (SRL), root tissue density (RTD), root C content (RCC), root N content (RNC), and root C:N ratio (RCN) – of first- and second-order roots of 306 species from 94 sampling sites across China. We examined the covariation in root traits among different plant growth forms, mycorrhizal types, and biomes using the phylogenetic principal component analysis (pPCA). Three independent dimensions of the covariation in root traits were identified, accounting for 39.0, 26.1, and 20.2% of the total variation, respectively. The first dimension was represented by SRL, RNC, RTD, and RCN, which was in line with the root economics spectrum (RES). The second dimension described a negative relationship between RD and SRL, and the third dimension was represented by RCC. These three main principal components were mainly influenced by biome and mycorrhizal type. Herbaceous and ectomycorrhizal species showed a more consistent pattern with the RES, in which RD, RTD, and RCN were negatively correlated with SRL and RNC within the first axis compared with woody and arbuscular mycorrhizal species, respectively. Our results highlight the roles of plant growth form, mycorrhizal type, and biome in shaping root trait covariation, suggesting that root trait relationships in specific regions may not be generalized from global-scale analyses.

**Keywords:** belowground strategy, fine-root trait, mycorrhizal symbiosis, phylogeny, plant growth form, root economics spectrum



## INTRODUCTION

Fine roots play a multifaceted role in plant resource acquisition, adaptation to environmental changes (Diaz et al., 2004; McCormack et al., 2014), and ecosystem processes (e.g., carbon and nutrient cycling, net primary production, and soil formation) (Cornwell et al., 2008; De Deyn et al., 2008; McCormack et al., 2015). The impact of roots on plant growth and ecosystem processes largely depends on the covariation among the morphological, chemical, and physiological traits of fine roots (Eviner and Chapin III, 2003; Bardgett et al., 2014). Assessing how root traits are interrelated and whether the interrelationships are broadly generalizable can help us better understand belowground resource acquisition strategies and ecosystem functions under environmental change.

Numerous studies have corroborated the generality of the leaf economics spectrum (LES) across plant growth forms, biomes, and spatial scales (Reich et al., 1992, 1999; Wright et al., 2004; Shipley et al., 2006; Hu et al., 2015). However, our understanding of the root economics spectrum (RES) – that demonstrates the unidimensional acquisitive–conservative gradient – lags far behind that of the LES, because conceptual and methodological challenges associated with root traits have impeded data standardization and integration within traits (Delory et al., 2017; Guerrero-Ramírez et al., 2021). The RES hypothesis proposes that roots with an acquisitive strategy are characterized by a thinner diameter, higher specific root length (SRL), and higher root N content (RNC). In contrast, roots with a conservative strategy are represented by a thicker diameter, lower SRL and RNC, and higher root C:N ratio (RCN) (Freschet et al., 2010; Reich, 2014; Roumet et al., 2016). The notion of one-dimensional RES has been supported by some regional studies (Roumet et al., 2016; de la Riva et al., 2018). For example, de la Riva et al. (2021a) found that SRL and specific root area (SRA) were negatively related to root tissue density (RTD) and root dry matter content in Mediterranean vegetation.

However, recent global studies have provided growing evidence for the multiple dimensions of root trait covariation (Bergmann et al., 2020; Weigelt et al., 2021). Two or more independent gradients were found to represent different facets of root trait syndromes (Weemstra et al., 2016). Bergmann et al. (2020) demonstrated two dimensions of the root economics space at the global scale. One was defined as the collaboration gradient represented by a trade-off between root diameter (RD) and SRL, and the other was defined as the conservation gradient represented by the negative relationship between RTD and RNC. This multidimensional trait pattern allows roots to enhance resource uptake from soils through either the construction of thinner fine roots or the development of high mycorrhizal dependency (Bergmann et al., 2020; Weigelt et al., 2021), reflecting the root trait diversity and adaptation to different environments.

The discrepancy of root trait covariation between global and regional studies may be related to the bias in the geographical coverage of the global root trait database (Guerrero-Ramírez et al., 2021) and the scale-dependence of root trait relationships (de la Riva et al., 2016b; Messier et al., 2017). These factors are important because selective pressures vary across spatial

scales (Albert et al., 2010; Liu et al., 2010), and different traits have different sensitivities to the same pressures (Messier et al., 2010; Shiklomanov et al., 2020). For example, recent studies demonstrated that root trait–environment relationships and trait coordination became less clear with decreasing spatial scales (de la Riva et al., 2016b; Liu H. et al., 2019). Moreover, regional studies about the covariation among root traits have also reported contradictory results. For the same set of root traits – namely RD, SRL, SRA, RTD, RNC, root C content (RCC), RCN, stele diameter, and cortex thickness – Liu C. et al. (2019) reported two primary dimensions of trait covariation in the subtropical forests of China, whereas Zhou et al. (2018) demonstrated three main dimensions in the temperate steppes of China. These inconsistencies in results among the regional studies may be related to multiple factors, including the differences in the selection of root traits defining the trait coordination and trade-offs (de la Riva et al., 2021a), species composition (e.g., plant growth form and phylogenetic group) (Wang et al., 2018a; Weigelt et al., 2021), mycorrhizal association type (Comas et al., 2014; Akatsuki and Makita, 2020), and biome type (Wang et al., 2018b; Valverde-Barrantes et al., 2021).

Plant growth form is an important factor shaping root trait syndromes and may influence the root form and function (Freschet et al., 2017; Valverde-Barrantes et al., 2017). For example, Roumet et al. (2016) suggested that the root traits of herbaceous species exhibited a more consistent pattern with the RES hypothesis than those of woody species from temperate, Mediterranean, and tropical biomes in France and China. The potential role of mycorrhizal symbiosis in plant resource uptake strategies is also gaining concerns (Weemstra et al., 2016; Laliberte, 2017; Gao et al., 2021). Ding et al. (2020) found the two dimensions of root trait covariation (that is, RD and SRL vs root nutrients and RTD) in ectomycorrhizal (ECM) species in the eastern Tibetan Plateau and such pattern has been found for arbuscular mycorrhizal (AM) species in a temperate rain forest in New Zealand (Kramer-Walter et al., 2016). To date, there has been no consensus regarding the covariation among root traits across plant growth forms (Adams et al., 2013; Sun et al., 2016) or types of mycorrhizal association (Cheng et al., 2016; Kong et al., 2019).

Another knowledge gap in root ecology involves a comparison of root trait covariation across biomes, as plants adapted to different environmental conditions may exhibit diverse root trait syndromes (Ma et al., 2018; Valverde-Barrantes et al., 2021). According to the RES hypothesis, SRL is expected to be negatively associated with RTD, reflecting the trade-off between root lifespan and construction cost (Roumet et al., 2016; Weemstra et al., 2016). However, this relationship is not observed in all biomes; some studies have described a significant relationship in Mediterranean forests and shrublands and temperate grasslands (de la Riva et al., 2018; Zhou et al., 2018), whereas others studies in temperate rain forests, subtropical forests, and temperate forests have not (Chen et al., 2013; Kong et al., 2014; Kramer-Walter et al., 2016). Therefore, there is a need for comparative studies on the covariation of root traits among multiple biomes (Laughlin et al., 2021).

China is one of the richest countries in terms of plant diversity with ancient origin and complex composition of the flora with



varied types of plant-mycorrhizal symbiosis (Liu et al., 2003; Huang, 2011). It contains multiple biome types, ranging from boreal forests to tropical forests (north to south), and from savannas to alpine tundra (northeast to west and southwest) (Ni, 2001). This variety provides an ideal opportunity to test the root trait covariation and their drivers by including all of the abovementioned factors and to provide an integrated understanding of root trait covariation of the region. We compiled a dataset of six key fine-root traits of 306 species from 94 sampling sites across China. The six root traits were RD, SRL, RTD, RCC, RNC, and RCN, which are widely studied traits related to the RES (Weemstra et al., 2016; Liu C. et al., 2019). These traits are closely associated with plant growth rate, construction cost, and lifespan (Comas and Eissenstat, 2004; Roumet et al., 2016), and reflect the trade-offs between resource acquisition and resource conservation (Reich, 2014). Our objectives were to (1) examine the covariation among the six root traits; and (2) test the generality of the covariation among root traits across plant growth forms, mycorrhizal types, and biomes.

## MATERIALS AND METHODS

### Data Collection

We collected the root trait data of plants in China from the global Fine-Root Ecology Database (FRED<sup>1</sup>) (Iversen et al., 2017) and the published literature (listed in Note 1 in **Supplementary Material**). We focused on first- and second-order roots that are defined by branching order, where the most distal roots are numbered as first order and where second-order roots begin at the junction of two first-order roots (Pregitzer et al., 2002). The first- and second-order roots generally belong to absorptive roots (Guo et al., 2008; Freschet and Roumet, 2017), which have high physiological activity and resource acquisition efficiency (McCormack et al., 2015). Thus, the studies that used diameter-based fine roots (e.g.,  $\leq 1$  mm,  $\leq 2$  mm, and  $\leq 0.5$  mm) and absorptive roots without a clear root branching order were excluded. To ensure data quality and homogeneity, root trait data were obtained according to the following criteria: (1) studies conducted in fields were included and those conducted in croplands, aquatic ecosystems, greenhouses, and laboratories were excluded, in order to minimize the effects of management disturbance; (2) root samples were collected from mature and healthy plant individuals to minimize the effects of ontogeny (Alvarez-Flores et al., 2014); (3) root samples were collected from live roots and data from dead roots were excluded to reduce the confounding effects of root vitality; and (4) root samples were collected from native species and non-native species were excluded. When a species occurred at multiple sampling sites, all site-species trait values were recorded.

Species name and taxonomic nomenclature were standardized and corrected according to the Plant List<sup>2</sup> using the “plantlist” package. A total of 407 site  $\times$  species observations of 306

species from 72 families and 174 genera were collected. The species were classified into seven phylogenetic clades according to APG IV classification (APG, 2016): gymnosperms, chloranthales, monocots, magnoliids, basal eudicots, asterids, and rosids. Basal eudicots include species that are not included in the clades of rosids and asterids. The information on plant growth form (herbaceous and woody) was obtained from the original literature and the TRY-Categorical Traits Dataset<sup>3</sup> (Kattge et al., 2011). Our dataset included 53 herbaceous and 253 woody species, respectively.

The information regarding the type of mycorrhizal association was collected and corrected according to the original literature and previously published databases (Wang and Qiu, 2006; Hempel et al., 2013; Phillips et al., 2013; Valverde-Barrantes et al., 2017; Guerrero-Ramírez et al., 2021). In this study, mycorrhizal types were classified into five groups based on the Global root traits (GRooT) database (Guerrero-Ramírez et al., 2021): AM (221), ECM (68), ericoid mycorrhizal (ERM, 2), AM-ECM (8), and AM-non-mycorrhizal (AM-NM, 7).

The species included in this study accounted for almost all major biomes in China, including forests, grasslands, and alpine tundra. The forests were divided into four biome types: tropical forest (latitude  $< 23.5^\circ\text{N}$ ), subtropical forest (latitude  $23.5^\circ\text{--}34^\circ\text{N}$ ), temperate forest (latitude  $34^\circ\text{--}50^\circ\text{N}$ ), and boreal forest (latitude  $> 50^\circ\text{N}$ ). Species with multiple biome entries were categorized into the biome in which they had the most observations. In this dataset, the tropical forest biome contained 31 species, subtropical forest 144 species, temperate forest 77 species, boreal forest 10 species, grassland 15 species, and the alpine tundra 29 species.

### Construction of the Phylogenetic Tree

The phylogenetic tree was constructed based on the comprehensive angiosperm species-level phylogeny of Zanne et al. (2014) updated by Qian and Jin (2016). The phylogenetic tree was constructed using the “S.PhyloMaker” function in the “phytools” package (Revell, 2012). All 306 species were included in this phylogenetic tree.

### Data Analyses

Before data analyses, all root trait data were  $\log_{10}$ -transformed to meet the assumption of normality. In this study, all data analyses were conducted at the species level, species mean trait values were thus used. We only focus on interspecific trait variation in our analysis.

Blomberg's  $K$  (Blomberg et al., 2003) and Pagel's  $\lambda$  (Pagel, 1999) values were calculated to assess the strength of phylogenetic signals of root traits using the “phytools” package (Revell, 2012). Blomberg's  $K$  values  $> 1$  suggest higher phylogenetic conservatism than expected owing to the Brownian motion, and values  $< 1$  suggest weaker phylogenetic conservatism. Pagel's  $\lambda$  values close to 0 indicate phylogenetic independence, and values closer to 1 indicate that the trait distribution perfectly complies with Brownian motion (Münkemüller et al., 2012).

<sup>1</sup><http://roots.ornl.gov>

<sup>2</sup><http://www.theplantlist.org/>

<sup>3</sup><https://www.try-db.org/TryWeb/Data.php#3>

Correlations among root traits were examined using Pearson's correlation analysis using the "Hmisc" package and phylogenetic independent contrasts (PICs) that account for the phylogenetic relatedness among species using the "picante" package (Felsenstein, 1985). Linear and non-linear regressions were also performed to evaluate the pairwise relationships among root traits. These analyses were conducted for all species and within different plant growth forms, mycorrhizal types, and biomes.

Considering the phylogenetic relatedness among species, phylogenetic principal component analysis (pPCA) was performed to identify the dominant dimensions of root trait covariation using the "phytools" package (Revell, 2012). pPCA is an extended method of PCA that is a powerful multivariate analysis technique and can summarize a set of data on correlated variables with a few composite, uncorrelated principal components (James and McCulloch, 1990). pPCA has been widely used in the determination of independent axes of functional specialization (e.g., Wang et al., 2018a; Liu C. et al., 2019; Bergmann et al., 2020; Weigelt et al., 2021). The Kaiser's eigenvalue greater than 1 rule was used to determine the intrinsic dimensionality of root trait covariation, this is, eigenvalues of the principal components greater than 1 were considered significant (Kaiser, 1958; Tabachnik and Fidell, 1996). This rule was used in previous studies that examined the main axes of plant trait covariation (e.g., Comas and Eissenstat, 2009; Chen et al., 2013; Jager et al., 2015; Kramer-Walter et al., 2016). To test whether the pattern of root trait covariation for all species was similar to those for different plant growth forms, mycorrhizal types, and biomes, the pPCA analysis was further repeated for subsets of plant growth forms (herbaceous and woody), mycorrhizal types (AM and ECM), and biomes (tropical, subtropical, temperate forest types, and alpine tundra). ERM, AM-ECM, AM-NM, boreal forest, and grassland did not perform the pPCA analysis as their sample sizes were small ( $n \leq 15$ ).

To assess the segregation of plant species by their phylogenetic clades, plant growth forms, mycorrhizal types, and biomes along the dominant axes of root trait covariation, one-way ANOVA was used on the scores of species on the pPCA axes and the significance of pairwise differences was tested using Tukey's HSD *post hoc* test.

The variance partitioning analysis was used to determine the relative contributions of plant growth form, mycorrhizal type, and biome according to the pPCA scores of the dominant principal components of root trait covariation for all species. The significances of each factor and their interactions were tested using 999 permutations. The analysis was followed by Legendre and Legendre (2012) using the "vegan" package.

All statistical analyses were conducted using R version 4.0.3 (R Core Team, 2020).

## RESULTS

### Root Trait Covariation

For all species, RD was negatively associated with SRL (Figure 1A and Table 1). RD was not significantly associated with RTD, RNC, RCC, and RCN (Figures 1B–E), but it was negatively associated

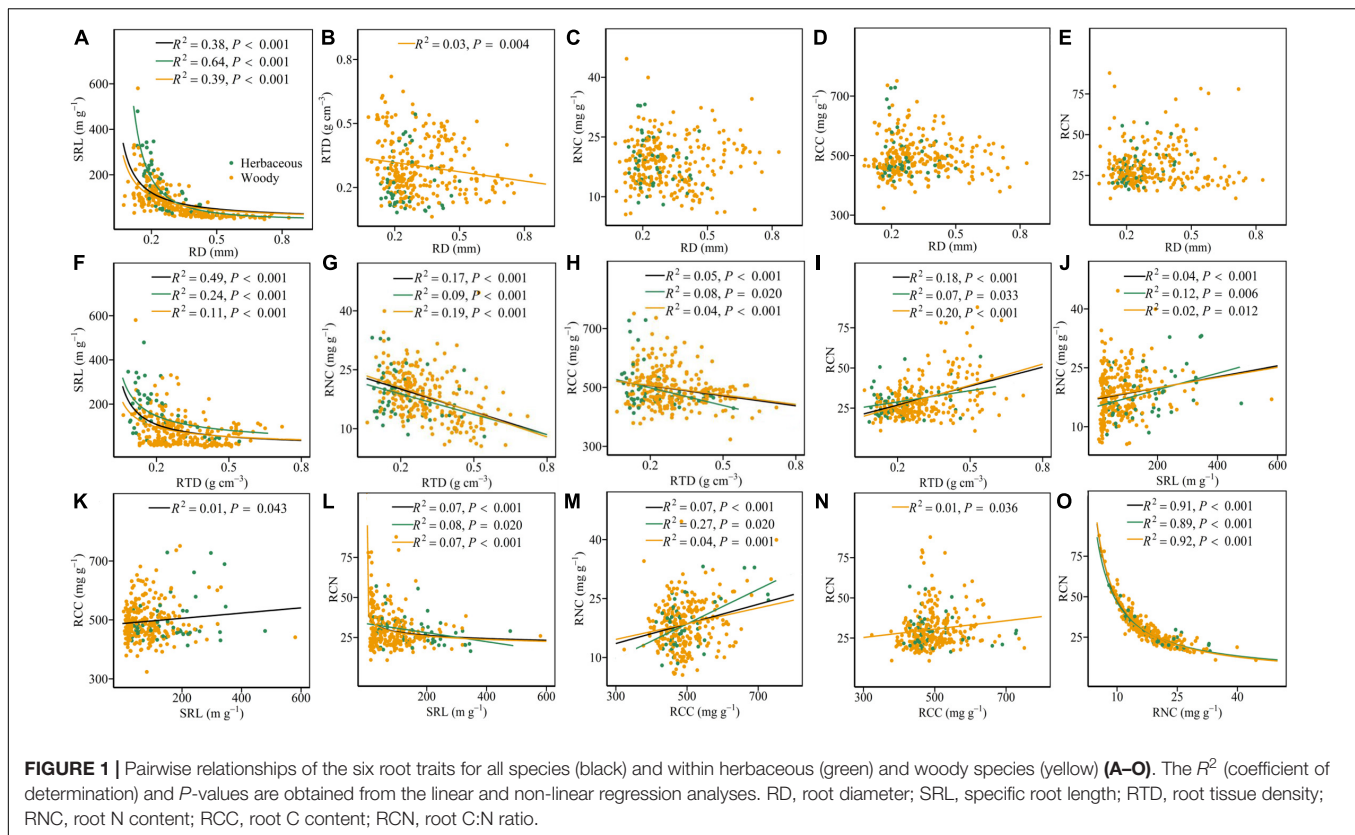
with RTD after considering the phylogenetic information (Table 1). Regardless of the phylogenetic relatedness among species being accounted for, SRL was always negatively and positively associated with RTD and RNC, respectively (Figures 1F,J). RTD, SRL, and RNC were significantly related to RCN (Figures 1I,L,O). However, RCC did not show a significant relationship with RCN (Figure 1N) but it showed significant relationships with RTD, SRL, and RNC (Figures 1H,K,M).

All root traits (except RCC) exhibited significant phylogenetic signals (Table 2). The pPCA results showed that eigenvalues of the first three axes were greater than 1 (Table 2), indicating that the covariation in root traits was represented by three independent dimensions. The first three primary axes accounted for 85.3% of the total variation of root traits. The first PCA axis (PC1) accounted for 39.0% of the total variation and was mainly related to SRL, RTD, RNC, and RCN (Figure 2A). With increasing scores of the PC1 axis, SRL and RNC increased while RTD and RCN decreased. The second PCA axis (PC2) explained 26.1% of the total variation and showed a negative relationship between RD and SRL. The third PCA axis (PC3) accounted for an additional 20.2% of the total variation and was primarily associated with RCC. The species distribution within the trait space showed that gymnosperms had the lowest PC1 scores with higher RTD and RCN values. Magnoliids species had the highest PC2 values with higher RD and lower SRL values whereas monocots and eudicots species had lower PC2 values with lower RD and higher SRL values (Figure 2A and Supplementary Figure 1).

### Root Trait Covariation Across Plant Growth Forms, Mycorrhizal Types, and Biomes

In terms of plant growth form, RD was negatively related to RTD in woody species, but this relationship was not observed in herbaceous species (Figure 1B and Supplementary Figures 2, 3). Compared to the herbaceous group, the woody group exhibited weaker relationships between SRL and RTD and RNC (Figures 1F,G). The ANOVA results showed that herbaceous and woody species showed significant differences in the scores of the first three axes of root trait covariation (i.e., PC1–PC3) ( $P < 0.05$ , Figures 3A,D,G). Herbaceous species had higher scores of the PC1 (e.g., higher SRL and RNC) and the lower scores of the PC2 (e.g., lower RD and higher SRL) compared with woody species. Woody species showed the consistent root trait covariation with the pattern of root trait covariation for all species, representing by three independent dimensions (Supplementary Table 1 and Supplementary Figure 4B). For herbaceous species, the first three axes explained 87.6% of the total variation in root traits (Supplementary Table 1 and Supplementary Figure 4A). Within the first axes, the main difference between herbaceous and woody species was that RD was strongly loaded on PC1 for herbaceous species. In addition, the second dimension was represented by RD, SRL, and RNC; the third dimension was dominated by RCC and RCN for herbaceous species.

The relationships among root traits differed across mycorrhizal types (Supplementary Figures 2, 3, 5). RD



was negatively associated with RTD in AM species but was positively associated with RTD in ECM species (**Supplementary Figure 5B**). RD was negatively related to RNC in ECM species but showed no correlation in AM species (**Supplementary Figure 5C**). Notably, most correlations among root traits were stronger in ECM species than those in AM species. The ANOVA results showed that AM and ECM species showed significant differences in the scores of the PC1 but not for the PC2 and PC3 (**Figures 3B,E,H**). AM species had higher PC1 scores (i.e., higher SRL and RNC values) compared with ECM species. The AM group also showed three independent dimensions of root trait covariation, which was consistent with the pattern for all species (**Supplementary Table 1** and **Supplementary Figure 4C**). ECM species also showed three independent axes of root trait variation. The PC1 axis was dominated by RD, RTD, RCN, SRL, and RNC, the PC2 axis was dominated by RCC, and the PC3 axis was dominated by RD, SRL, and RCN (**Supplementary Table 1** and **Supplementary Figure 4D**).

Moreover, the correlations among root traits differed across biomes (**Supplementary Figures 2, 3, 6**). RD was negatively correlated with RTD in the tropical and subtropical forests, but not in the other biomes. SRL and RNC values decreased with increasing RTD only in the temperate forest. In addition, there were significant differences in the scores of PC1, PC2, and PC3 among biomes ( $P < 0.05$ , **Figures 3C,F,I**) and had different trait syndromes among different biomes (**Figure 2D**). Species in the temperate forest had the highest PC1 scores with higher SRL and RNC values, whereas species in the alpine tundra had the lowest PC1 scores with higher RCN and RTD values. Species in the

tropical and subtropical forests showed higher variations on the PC2 axis compared with other biomes. The dominant dimensions of the covariation in root traits differed greatly across biomes. In the tropical and subtropical forests, the first three axes explained 90.0 and 86.5% of the total variation in root traits, respectively (**Supplementary Table 2** and **Supplementary Figures 4E,F**). In these two forests, the PC1 axis was heavily loaded by RD, RTD, RNC, and RCN, and the PC2 axis was strongly represented by the negative relationships between RD and SRL. In addition, two independent root trait dimensions – representing root morphological traits (i.e., RD, RTD, and SRL) and nutrient traits (i.e., RNC and RCN) – were detected in the alpine tundra (**Supplementary Table 2** and **Supplementary Figure 4H**).

**TABLE 1** | Correlation coefficient matrix of the six root traits using Pearson's correlation analysis (lower-left diagonal) and phylogenetic independent contrasts (PICs, upper-right diagonal) for all species.

	RD	SRL	RTD	RCC	RNC	RCN
RD						
SRL	<b>-0.81***</b>					
RTD	-0.11	<b>-0.39***</b>				
RCC	-0.01		0.11			
RNC	-0.02	<b>0.26***</b>	<b>-0.43***</b>	<b>0.21***</b>		
RCN	0.02	<b>-0.22***</b>	<b>0.36***</b>	<b>0.14*</b>	<b>-0.94***</b>	

Root traits data are  $\log_{10}$ -transformed. The bold indicates that the correlations are significant: \*\*\* $P < 0.001$ ; \* $P < 0.05$ .

RD, root diameter; SRL, specific root length; RTD, root tissue density; RCC, root C content; RNC, root N content; RCN, root C:N ratio.



**TABLE 2 |** Phylogenetic principal component analysis (pPCA) results and phylogenetic signals of the six root traits for all species.

	PC1	PC2	PC3	Blomberg's $K$	$P$ -value	Pagel's $\lambda$	$P$ -value
Eigenvalue	1.53	1.25	1.10				
Variation explained (%)	39.02	26.08	20.22				
RD	-0.25	<b>0.93</b>	0.10	0.12	0.001	0.78	<0.001
SRL	<b>0.65</b>	<b>-0.68</b>	0.16	0.13	0.001	0.72	<0.001
RTD	<b>-0.61</b>	<b>-0.25</b>	-0.43	0.04	0.001	0.67	<0.001
RCC	0.19	0.09	<b>0.86</b>	0.01	0.976	<0.001	1.000
RNC	<b>0.89</b>	0.31	-0.11	0.03	0.029	0.68	<0.001
RCN	<b>-0.82</b>	-0.27	0.49	0.04	0.001	0.65	<0.001
PC1				0.04	0.002	0.67	<0.001
PC2				0.07	0.001	0.79	<0.001
PC3				0.01	0.533	0.27	<0.001

Root traits are  $\log_{10}$ -transformed. Bold indicates the variable loading scores with the greatest load on each component.

RD, root diameter; SRL, specific root length; RTD, root tissue density; RCC, root C content; RNC, root N content; RCN, root C:N ratio; PC1, PC2, and PC3 correspond to the first three main axes of the covariation of root traits based on the pPCA results.

## Relative Effects of Plant Growth Form, Mycorrhizal Type, and Biome on the Root Trait Covariation

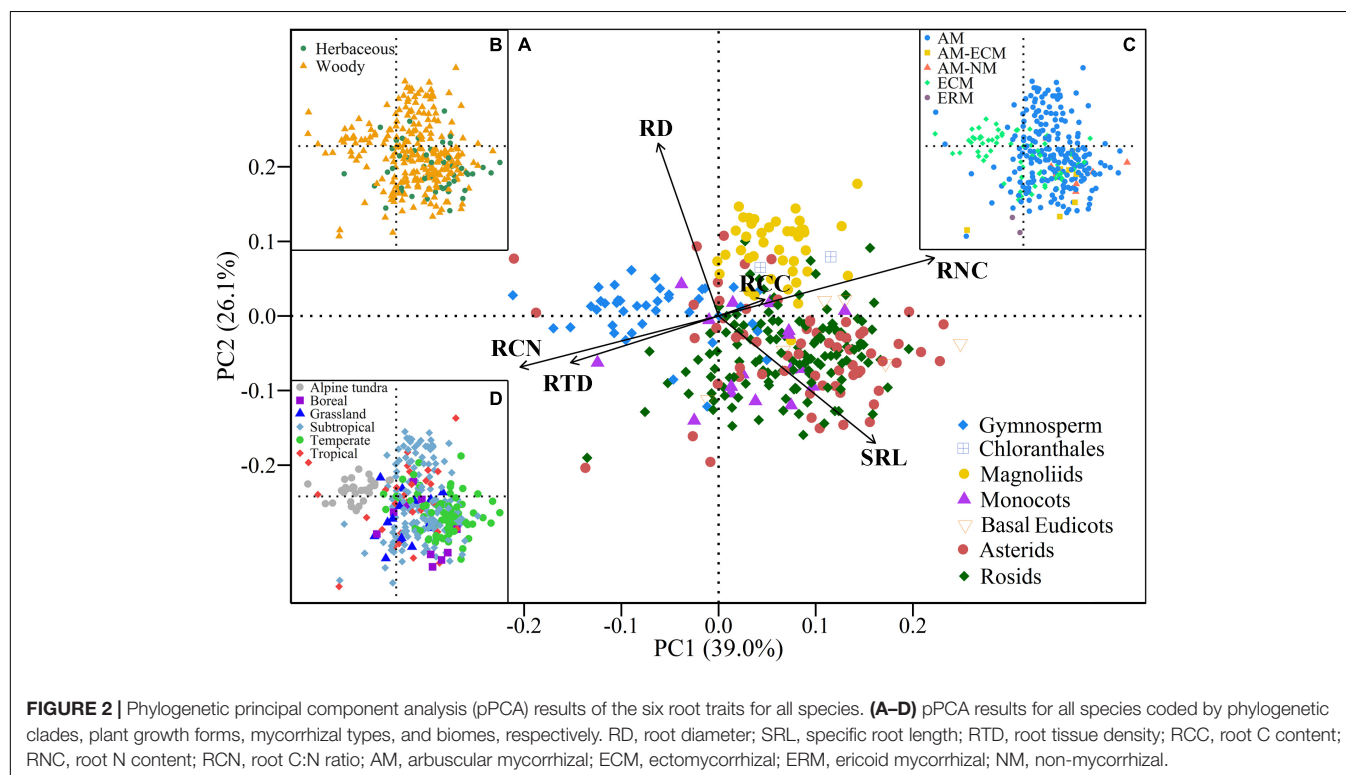
Plant growth form, mycorrhizal type, and biome together accounted for 52.0, 14.1, and 13.8% of the total variation of PC1, PC2, and PC3, respectively (Figure 4). The PC1 axis was mostly

explained by biome alone (33.0%), followed by the interactive effects of biome and mycorrhizal type (14.8%). The PC2 axis was mostly explained by mycorrhizal type alone (7.5%) and biome alone (5.6%), and the PC3 axis was mainly influenced by biome alone (11.8%), followed by plant growth form alone (1.7%).

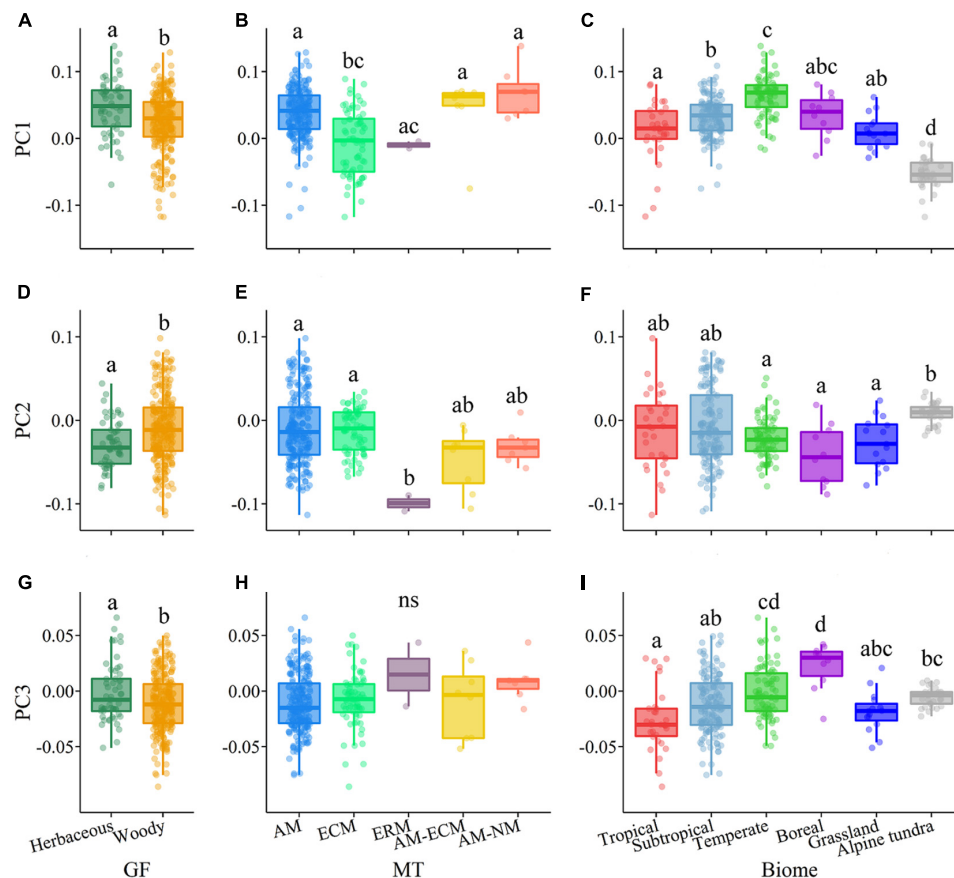
## DISCUSSION

### Multidimensional Pattern of Variation in Root Traits

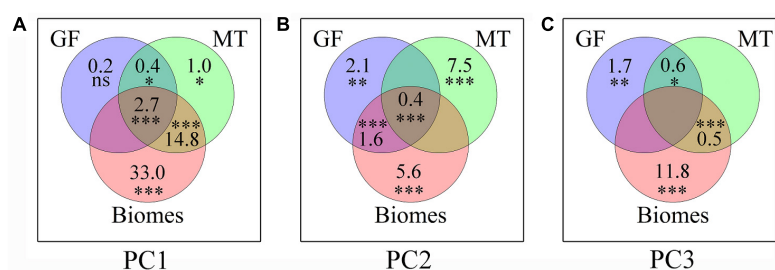
Our study demonstrated three independent dimensions of the covariation among the six root traits in China. The first dimension was dominated by SRL, RTD, RNC, and RCN that were closely interrelated, which is in accord with the RES expectations. This result is not in agreement with the previous studies reporting that RD and SRL were orthogonal to RTD and RNC (Kramer-Walter et al., 2016; Wang et al., 2018a; McCormack and Iversen, 2019; Bergmann et al., 2020). Within the first axis, in one end, species had high RTD, high RCN, low SRL, and low RNC associated with a resource-conservation strategy, whereas in the other end species had high SRL, high RNC, low RTD, and low RCN associated with a resource-acquisitive strategy (Roumet et al., 2016; de la Riva et al., 2018). As the expected from the RES, we found that SRL was negatively associated with RTD and positively related to RNC, suggesting a trade-off between resource acquisition and construction costs of roots (Makita et al., 2012; Li et al., 2019). According to the RES expectations, fine roots with a higher SRL should be associated with a higher uptake activity and metabolic rate (i.e., higher







**FIGURE 3 |** Distribution of plant growth forms, mycorrhizal types, and biomes along the first three main principal components (i.e., PC1–PC3) of the root trait covariation. **(A,D,G)** Plant growth form (GF); **(B,E,H)** mycorrhizal type (MT); **(C,F,I)** biomes. Letters represent statistically significant differences in the average PCs (Tukey's HSD *post hoc* test,  $P < 0.05$ ), such that groups not containing the same letter are different. In the box plots the central line represents the mean; the lower and upper box limits correspond to the 25th and 75th percentiles and the upper (lower) whiskers extend to 1.5 (–1.5) times the interquartile range, respectively. AM, arbuscular mycorrhizal; ECM, ectomycorrhizal; ERM, ericoid mycorrhizal; NM, non-mycorrhizal.



**FIGURE 4 |** Relative contributions (%) of plant growth form, mycorrhizal type, and biome to the first three main principal components (i.e., PC1–PC3) of root trait covariation. The intersections represent variation that is jointly explained by two or more variable categories. **(A)** PC1; **(B)** PC2; **(C)** PC3. The number (lower-right) indicates the variations that are unexplained proportion by these three groups. The significances of each category are tested using 999 permutations. \*\*\* $P < 0.001$ ; \*\* $P < 0.01$ ; \* $P < 0.05$ ; ns  $P > 0.05$ . GF, plant growth form; MT, mycorrhizal type.

RNC) and shorter lifespan (i.e., lower RTD) to maximize resource acquisition per investment (Eissenstat et al., 2000; Weemstra et al., 2016; de la Riva et al., 2021b). RD was not related to RNC, RTD, and RCN that strongly loaded on the first axis, thus RD was decoupled from the first axis. In addition, the

second axis was dominated by a trade-off between RD and SRL, which did not support the previous studies reporting that the variation of root traits was mostly explained by RD and SRL (Wang et al., 2018a; McCormack and Iversen, 2019; Bergmann et al., 2020). Recent studies have reported that RD and SRL were

positively and negatively related to the percentage of mycorrhizal colonization, respectively (Ma et al., 2018; Bergmann et al., 2020), suggesting that roots can enhance resource uptake from soils by constructing either thin-diameter roots with higher SRLs (i.e., “do-it-yourself” strategy), or in contrast thick-diameter roots *via* reliance on mycorrhizal associations (i.e., “outsourcing” strategy) (McCormack and Iversen, 2019; Bergmann et al., 2020). However, roots with a larger mycorrhizal colonization may increase the resource uptake capacity under resource-limited conditions without necessarily implying an acquisitive strategy (Navarro-Fernández et al., 2016). Therefore, it is needed to uncover how this root–mycorrhizal collaboration gradient links to the differences in resource uptake capacity for species with fast- and slow-traits (de la Riva et al., 2021a). Furthermore, we found that RCC formed an additional axis that was decoupled from the first two dimensions. This lack of correlation may be attributable to two reasons. First, root traits that were related to the first two axes displayed significant phylogenetic conservatism, but RCC did not, suggesting that these trait associations are not tightly coupled. Second, in theory, RCC, RNC, and RCN are mathematically interdependent. However, we observed that RNC was strongly associated with RCN, whereas RCC was not. This indicated that RNC, rather than RCC, was more important in the trade-offs between C investment and resource uptake in roots (An et al., 2021). Our results are not consistent with the global and regional studies (Kramer-Walter et al., 2016; Wang et al., 2018a; McCormack and Iversen, 2019; Bergmann et al., 2020), we found that the RES explained the most variation of root traits and the trade-off between RD and SRL loaded on the second axis in China, suggesting that root trait covariation may not be generalized from global-scale and other regional analyses.

Furthermore, our results revealed that the pattern of variation in root traits was closely linked to the phylogenetic structure; that is, species within different phylogenetic clades occupied different locations in the trait space. Gymnosperms generally dominated boreal and subalpine forests that are mainly constrained by low temperatures, as thick roots with high RTD values tend to have high physical robustness to cope with cold environments (Simpson et al., 2016; Wang et al., 2018b; Yahara et al., 2019). Early diverged magnoliids are generally associated with phosphorus-limited tropical and subtropical soils (Ma et al., 2018), resulting in high RD and RNC values (Figure 5). Thick roots maximize the cortex area, supporting more AM colonization as a complementary strategy for nutrient foraging, leading to high RNC (Brundrett, 2002; Comas et al., 2012; Kong et al., 2019). Moreover, recently diverged eudicots species were more concentrated toward lower RD and higher SRL values. This can allow roots to increase their surface area and explore larger soil volumes per unit of C investment – that is, a “do-it-yourself” strategy (Comas et al., 2012; Valverde-Barrantes et al., 2017; Bergmann et al., 2020).

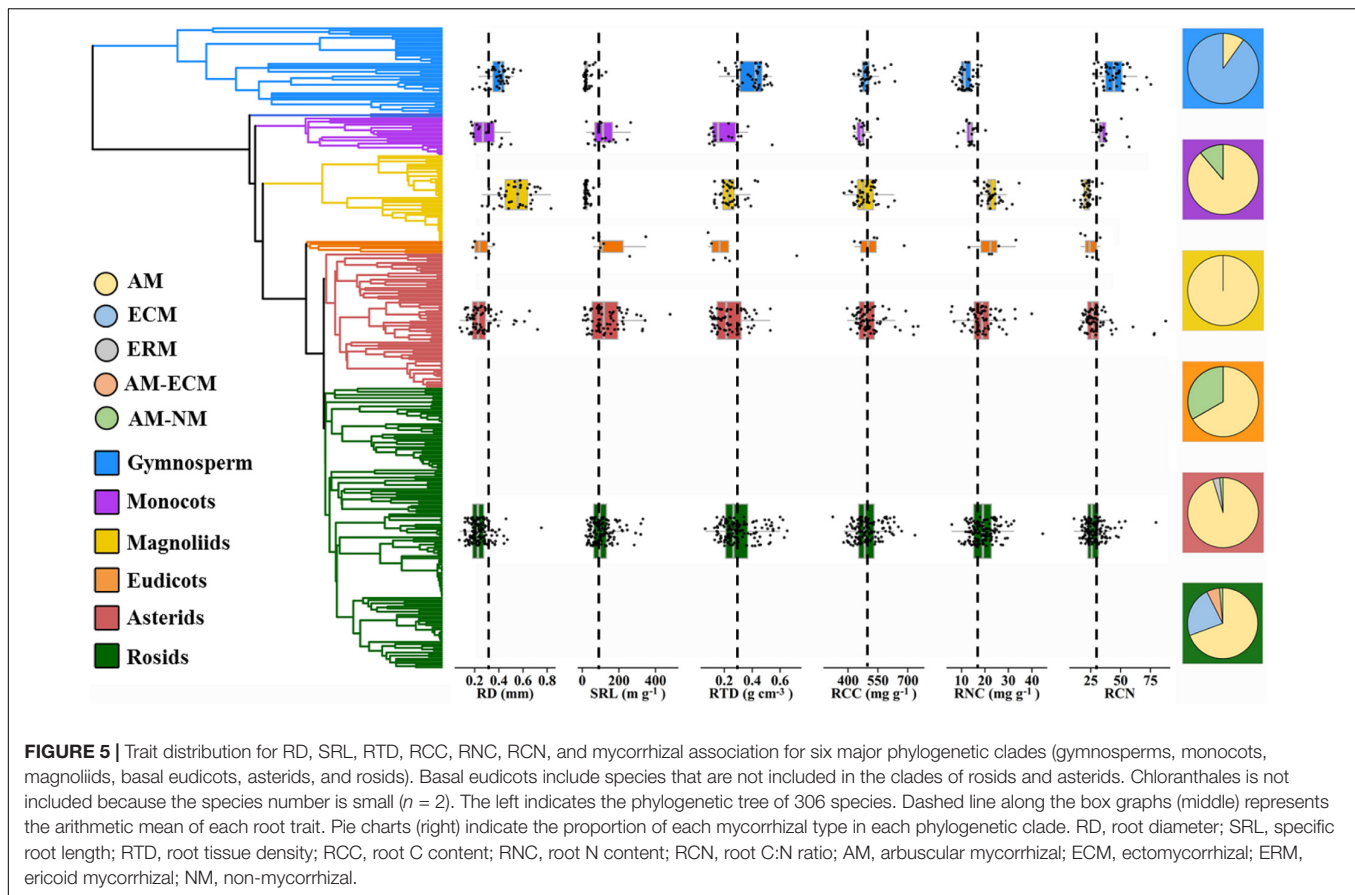
## The Drivers of the Covariation Among Root Traits Biome

Our study showed that biome type was the most important factor influencing the variation of the three independent

dimensions, suggesting that roots have evolved contrasting resource acquisition strategies among different biomes. In addition, we observed that the correlations among root traits varied across biomes. This result was likely due to the differences in environmental constraints (Ostonen et al., 2017) and species composition across biomes (Roumet et al., 2016). Differences in environmental condition and phylogenetic group may cause differences in root trait adjustment (Wang et al., 2018b) and mycorrhizal dependency (Valverde-Barrantes et al., 2016), and changes in both factors could strengthen, weaken, or fully decouple correlations among root traits (Ma et al., 2018; Valverde-Barrantes et al., 2020, 2021). For example, RD was negatively associated with RTD in the tropical and subtropical forests, which is consistent with the study of Valverde-Barrantes et al. (2021). Such a negative relationship can be explained by the anatomical relationships that is commonly observed in leaves (Laughlin, 2014; de la Riva et al., 2016a), plants can have similar specific leaf area (or SRL) values with different proportional investments in leaf tissue density (or RTD) and leaf thickness (or RD) (John et al., 2017; de la Riva et al., 2021a), which would depend on the species identity and their environmental conditions (Olmo et al., 2014). We found that RTD was negatively related to SRL in the temperate forest, suggesting the trade-off between resource acquisition and construction costs of roots (Eissenstat et al., 2000). From the cost-benefit theory, roots with higher SRL and lower RTD would have lower construction costs, higher metabolic rates and faster return of investments (de la Riva et al., 2021b). Furthermore, species in the alpine tundra tended to have more conservative strategies with higher RTD, lower SRL, and RNC, which may be attributable to the ECM-dominated gymnosperms in the alpine tundra in our study. Some root morphological adaptations, such as low cortex area and high branching intensity, are achieved before shifts from AM to alternative mycorrhizal associations (Comas and Eissenstat, 2009; Valverde-Barrantes et al., 2016, 2018). Thus, species associated with ECM fungi were less dependent on the root cortex, and thicker roots with highly lignified stele tissues were closely linked to higher RTD (Guo et al., 2008; Kong et al., 2019). These comparisons among plants in widely disparate biomes from China provide the basis for predicting differences in root trait trade-offs between biomes.

## Mycorrhizal Type

Mycorrhizal type also played an important role in the three main dimensions of root trait variation, especially for the PC2 axis. This result provides new evidence for root–mycorrhizal collaboration gradient representing tradeoffs between “do-it-yourself” and “outsourcing” for resource uptake (McCormack and Iversen, 2019; Bergmann et al., 2020). In agreement with previous studies (Comas et al., 2014; Valverde-Barrantes et al., 2018; de la Riva et al., 2021b), AM species tended to have more acquisitive strategies with higher SRL and lower RTD while ECM species were associated with more conservative strategies with lower SRL and higher RTD within the PC1 axis. This may be due to the differences in anatomical and morphological adaption between AM and ECM species. Species colonized by AM fungi exhibited a rapid resource uptake strategy with a higher investment in root length per unit root mass (i.e., higher SRL)



(Guo et al., 2008; Valverde-Barrantes et al., 2018; Kong et al., 2019). In addition, the first axis was mostly driven by the RES for both AM and ECM species (38.3 and 47.2% of the variance, respectively). However, compared with AM species, ECM species showed a more consistent pattern of the RES because RD was included in the first axis, in which RD and RTD were negatively correlated with SRL, RNC, and RCN. The difference between these two mycorrhizal types may be related to the lower number of species in ECM group compared with AM group. In addition, we found that RD had different associations with RTD and RNC between AM and ECM groups. Among ECM species, RD showed positive and negative relationships with RTD and RNC, respectively, which is in accord with the RES hypothesis (Freschet et al., 2010; Reich, 2014) and also supports the study of Kong et al. (2019) at the global scale. Such relationships in our study could be explained by the typical features of nutrient acquisition in ECM species. ECM species predominately form Hartig nets in the intercellular spaces of root tips and are less dependent on cortex tissue (Brundrett, 2002; Comas et al., 2014), leading to positive correlations between RD and RTD. ECM plants with thin roots have a thick fungal mantle that is relatively rich in N and enhances the N content of thin roots compared to that of thick roots (Kong et al., 2019). Our study demonstrated that AM and ECM species had contrasting root traits syndromes, however, the pattern of root trait variation in these two mycorrhizal types was mostly driven by the RES. These results suggested that the

differences in resource acquisition strategies between them were not only determined by the type of mycorrhizal association. Therefore, further studies should include direct measurements of mycorrhizal colonization (e.g., percentage of mycorrhizal colonization) to confirm the collaboration gradient proposed in the global studies and investigate the trade-offs between root acquisition and conservation in relation to the symbiotic roles presented here.

### Plant Growth Form

We found that plant growth form had a weak effect on the three main axes of root trait variation. Our results are consistent with previous studies reporting that herbaceous species have more acquisitive strategies with thinner RD, higher SRL and RNC compared with woody species (Valverde-Barrantes et al., 2017). In addition, the first axis was dominated by the RES and the second axis was dominated by RD and SRL for both herbaceous and woody species. However, the main difference between these two plant growth forms was that RD was included in the first axis for herbaceous species, leading to herbaceous species with a more consistent pattern with the RES compared with woody species. Such discrepancy between them may be related to two possible reasons. First, RD was negatively related to RTD for woody species, which is in line with previous studies reporting a trade-off between RD and RTD in Mediterranean woody plants (de la Riva et al., 2016b, 2021a). As mentioned above, such a

negative relationship can be explained by the anatomical trade-offs between RD and RTD. Second, Ma et al. (2018) has reported that woody species have approximately 30% more mycorrhizal colonization than herbaceous species for a given RD, suggesting that roots of herbaceous species have become less dependent on mycorrhizae fungi. In addition, herbaceous roots have evolved more efficient trait syndromes (e.g., thinner diameter, higher SRL, and lower RTD), which may change the relationships among root traits. Our results suggested the differences in root ecological strategies among plant growth forms, which can help elucidate the trade-offs between root construction and function and their influences on ecosystem functions.

## Uncertainties and Future Research Needs

The uncertainties of this study lie in four aspects due to the restrictions of data coverage. First, our study focused on commonly studied mycorrhizal statuses (i.e., AM and ECM), some other mycorrhizal types such as ERM, NM, and dual associations were not considered in our analyses owing to the limited number of root samples. The type of mycorrhizal partnership is an important driver of the variation in root traits and their functions (Valverde-Barrantes et al., 2017; Gao et al., 2021). More detailed studies of mycorrhizal status are needed to elucidate how the evolution of root traits and their mycorrhizal associations affect belowground processes in seed plants. Second, the uneven distribution of data across biomes is likely to impede our understanding of the effect of biomes on root resource uptake. In particular, we had a limited number of species in the boreal forest and grassland, which may explain the weak or insignificant relationships among root traits in these biomes. Further work with a wide representation of species from multiple biomes is crucial to improve our understanding of the role of biomes in resource acquisition strategies in fine-root systems. Third, our analysis focused on the interspecific variation in root traits, thus resulting in some uncertainties regarding the importance of intraspecific variation and plasticity in trait-based studies (Jung et al., 2010; Weemstra et al., 2020). Future studies that incorporate the intraspecific trait plasticity will help to elucidate the trade-offs among root traits related to belowground resource acquisition strategies (Isaac et al., 2017). Finally, we considered six root morphological and chemical traits in our study, however, the root trait covariation may be dependent on the trait variables studied. de la Riva et al. (2021a) demonstrated that SRA (i.e., mass-normalized) was more tightly correlated than SRL with the dry matter content and chemical composition of both roots and leaves along the economics spectrum for Mediterranean vegetation. Therefore, it is important to measure and integrate the root trait data based on the standardized approaches, which can be analogous to traits related to the LES, including root morphology (e.g., SRA and root dry matter content), root chemistry (e.g., RNC and lignin), root anatomy (e.g., cortical area and stele area), percentage of mycorrhizal colonization, and root function (e.g., respiration, decomposition, and resources uptake) (Laliberte, 2017). This would allow us to develop a more general integrated framework related to

the trade-offs between root structure and function and their underlying mechanisms.

## CONCLUSION

Our study provides a comprehensive assessment of the covariation among root traits and their generality across plant growth forms, mycorrhizal types, and biomes using the largest root trait database in China. Three independent root trait dimensions were identified, where the first dimension was related to trade-offs between resource acquisition and conservation (i.e., SRL, RTD, RNC, and RCN) that was defined as the RES, the second dimension was related to RD and SRL, and the third dimension was dominated by RCC. Biome and mycorrhizal type were the most important factors in driving the variation of the three main dimensions. Furthermore, the root trait covariation was dependent on plant growth form, mycorrhizal type, and biome. More importantly, we found a more consistent pattern of the root trait variation related to the RES in herbaceous and ECM species compared with woody and AM species, indicating high coordination among root morphological and chemical traits in herbaceous and ECM species. These results demonstrate that the covariation among root traits was more complex across plant growth forms, mycorrhizal types, and biomes at the regional scale than those at the global scale, indicating a critical role of spatial scale in influencing the generality of associations among root traits. Further work on the covariation among root traits at different spatial scales will contribute to our understanding of plant form and function and help predict belowground responses to changing environmental conditions.

## DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

## AUTHOR CONTRIBUTIONS

NA conceived the ideas and collected the data. NA and NL led the writing of the manuscript. All authors contributed critically to the drafts and gave the final approval for publication.

## FUNDING

This work was supported by the National Key R&D Program of China (2017YFA0604700), National Natural Science Foundation of China (41991234), and the International Partnership Program of Chinese Academy of Sciences (121311KYSB20170004).

## SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fpls.2021.785589/full#supplementary-material>



## REFERENCES

- Adams, T. S., McCormack, M. L., and Eissenstat, D. M. (2013). Foraging strategies in trees of different root morphology: the role of root lifespan. *Tree Physiol.* 33, 940–948. doi: 10.1093/treephys/tpt067
- Akatsuki, M., and Makita, N. (2020). Influence of fine root traits on in situ exudation rates in our conifers from different mycorrhizal associations. *Tree Physiol.* 40, 1071–1079. doi: 10.1093/treephys/tpaa051
- Albert, C. H., Thuiller, W., Yoccoz, N. G., Soudant, A., Boucher, F., Saccone, P., et al. (2010). Intraspecific functional variability: extent, structure and sources of variation. *J. Ecol.* 98, 604–613. doi: 10.1111/j.1365-2745.2010.01651.x
- Alvarez-Flores, R., Winkel, T., Anh, N. T. T., and Joffre, R. (2014). Root foraging capacity depends on root system architecture and ontogeny in seedlings of three Andean *Chenopodium* species. *Plant Soil* 380, 415–428. doi: 10.1007/s11104-014-2105-x
- An, N. N., Lu, N., Fu, B. J., Chen, W. L., Keyimu, M., and Wang, M. Y. (2021). Root trait variation of seed plants from China and the primary drivers. *J. Biogeogr.* 48, 2402–2417. doi: 10.1111/jbi.14157
- APG (2016). An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG IV. *Bon. J. Linn. Soc.* 181, 1–20. doi: 10.1111/boj.12385
- Bardgett, R. D., Mommer, L., and De Vries, F. T. (2014). Going underground: root traits as drivers of ecosystem processes. *Trends Ecol. Evol.* 29, 692–699. doi: 10.1016/j.tree.2014.10.006
- Bergmann, J., Weigelt, A., van Der Plas, F., Laughlin, D. C., Kuyper, T. W., Guerrero-Ramirez, N. R., et al. (2020). The fungal collaboration gradient dominates the root economics space in plants. *Sci. Adv.* 6:eaba3756. doi: 10.1126/sciadv.aba3756
- Blomberg, S. P., Garland, T., and Ives, A. R. (2003). Testing for phylogenetic signal in comparative data: behavioral traits are more labile. *Evolution* 57, 717–745. doi: 10.1111/j.0014-3820.2003.tb00285.x
- Brundrett, M. C. (2002). Coevolution of roots and mycorrhizas of land plants. *New Phytol.* 154, 275–304. doi: 10.1046/j.1469-8137.2002.00397.x
- Chen, W. L., Zeng, H., Eissenstat, D. M., and Guo, D. L. (2013). Variation of first-order root traits across climatic gradients and evolutionary trends in geological time. *Glob. Ecol. Biogeogr.* 22, 846–856. doi: 10.1111/geb.12048
- Cheng, L., Chen, W. L., Adams, T. S., Wei, X., Le, L., McCormack, M. L., et al. (2016). Mycorrhizal fungi and roots are complementary in foraging within nutrient patches. *Ecology* 97, 2815–2823. doi: 10.1002/ecy.1514
- Comas, L. H., and Eissenstat, D. M. (2004). Linking fine root traits to maximum potential growth rate among 11 mature temperate tree species. *Funct. Ecol.* 18, 388–397. doi: 10.1111/j.0269-8463.2004.00835.x
- Comas, L. H., and Eissenstat, D. M. (2009). Patterns in root trait variation among 25 co-existing North American forest species. *New Phytol.* 182, 919–928. doi: 10.1111/j.1469-8137.2009.02799.x
- Comas, L. H., Callahan, H. S., and Midford, P. E. (2014). Patterns in root traits of woody species hosting arbuscular and ectomycorrhizas: implications for the evolution of belowground strategies. *Ecol. Evol.* 4, 2979–2990. doi: 10.1002/ece3.1147
- Comas, L. H., Mueller, K. E., Taylor, L. L., Midford, P. E., Callahan, H. S., and Beerling, D. J. (2012). Evolutionary patterns and biogeochemical significance of angiosperm root traits. *Int. J. Plant Sci.* 173, 584–595. doi: 10.1086/665823
- Cornwell, W. K., Cornelissen, J. H., Amatangelo, K., Dorrepaal, E., Eviner, V. T., Godoy, O., et al. (2008). Plant species traits are the predominant control on litter decomposition rates within biomes worldwide. *Ecol. Lett.* 11, 1065–1071. doi: 10.1111/j.1461-0248.2008.01219.x
- De Deyn, G. B., Cornelissen, J. H. C., and Bardgett, R. D. (2008). Plant functional traits and soil carbon sequestration in contrasting biomes. *Ecol. Lett.* 11, 516–531. doi: 10.1111/j.1461-0248.2008.01164.x
- de la Riva, E. G., Marañón, T., Pérez-Ramos, I. M., Navarro-Fernández, C. M., Olmo, M., and Villar, R. (2018). Root traits across environmental gradients in Mediterranean woody communities: are they aligned along the root economics spectrum? *Plant Soil* 424, 35–48. doi: 10.1007/s11104-017-3433-4
- de la Riva, E. G., Tosto, A., Perez-Ramos, I. M., Navarro-Fernandez, C. M., Olmo, M., Anten, N. P. R., et al. (2016b). A plant economics spectrum in Mediterranean forests along environmental gradients: is there coordination among leaf, stem and root traits? *J. Veg. Sci.* 27, 187–199. doi: 10.1111/jvs.12341
- de la Riva, E. G., Olmo, M., Poorter, H., Uberta, J. L., and Villar, R. (2016a). Leaf mass per area (LMA) and its relationship with leaf structure and anatomy in 34 Mediterranean woody species along a water availability gradient. *PLoS One* 11:e0148788. doi: 10.1371/journal.pone.0148788
- de la Riva, E. G., Querejeta, J. I., Villar, R., Pérez-Ramos, I. M., Marañón, T., Galán Díaz, J., et al. (2021a). The economics spectrum drives root trait strategies in Mediterranean vegetation. *Front. Plant Sci.* 12:773118. doi: 10.3389/fpls.2021.773118
- de la Riva, E. G., Prieto, I., Maranon, T., Perez-Ramos, I. M., Olmo, M., and Villar, R. (2021b). Root economics spectrum and construction costs in Mediterranean woody plants: the role of symbiotic associations and the environment. *J. Ecol.* 109, 1873–1885. doi: 10.1111/1365-2745.13612
- Delory, B. M., Weidlich, E. W. A., Meder, L., Lüttge, A., Duijnen, R., Weidlich, R., et al. (2017). Accuracy and bias of methods used for root length measurements in functional root research. *Methods Ecol. Evol.* 8, 1594–1606. doi: 10.1111/2041-210x.12771
- Diaz, S., Hodgson, J. G., Thompson, K., Cabido, M., Cornelissen, J. H. C., Jalili, A., et al. (2004). The plant traits that drive ecosystems: evidence from three continents. *J. Veg. Sci.* 15, 295–304. doi: 10.1111/j.1654-1103.2004.tb02266.x
- Ding, J. X., Kong, D. L., Zhang, Z. L., Cai, Q., Xiao, J., Liu, Q., et al. (2020). Climate and soil nutrients differentially drive multidimensional fine root traits in ectomycorrhizal-dominated alpine coniferous forests. *J. Ecol.* 108, 2544–2556. doi: 10.1111/1365-2745.13407
- Eissenstat, D. M., Wells, C. E., Yanai, R. D., and Whitbeck, J. L. (2000). Building roots in a changing environment: implications for root longevity. *New Phytol.* 147, 33–42. doi: 10.1046/j.1469-8137.2000.00686.x
- Eviner, V. T., and Chapin Iii, F. S. (2003). Functional matrix: a conceptual framework for predicting multiple plant effects on ecosystem processes. *Annu. Rev. Ecol. Syst.* 34, 455–485. doi: 10.1146/annurev.ecolsys.34.011802.132342
- Felsenstein, J. (1985). Phylogenies and the comparative method. *Am. Nat.* 125, 1–15. doi: 10.1086/284325
- Freschet, G. T., and Roumet, C. (2017). Sampling roots to capture plant and soil functions. *Funct. Ecol.* 31, 1506–1518. doi: 10.1111/1365-2435.12883
- Freschet, G. T., Cornelissen, J. H. C., van Logtestijn, R. S. P., and Aerts, R. (2010). Evidence of the 'plant economics spectrum' in a subarctic flora. *J. Ecol.* 98, 362–373. doi: 10.1111/j.1365-2745.2009.01615.x
- Freschet, G. T., Valverde-Barrantes, O. J., Tucker, C. M., Craine, J. M., McCormack, M. L., Violle, C., et al. (2017). Climate, soil and plant functional types as drivers of global fine-root trait variation. *J. Ecol.* 105, 1182–1196. doi: 10.1111/1365-2745.12769
- Gao, J., Zhou, M. Y., Shao, J. J., Zhou, G. Y., Liu, R. Q., Zhou, L. Y., et al. (2021). Fine root trait-function relationships affected by mycorrhizal type and climate. *Geoderma* 394:115011. doi: 10.1016/j.geoderma.2021.115011
- Guerrero-Ramirez, N. R., Mommer, L., Freschet, G. T., Iversen, C. M., McCormack, M. L., Kattge, J., et al. (2021). Global root traits (GRooT) database. *Glob. Ecol. Biogeogr.* 30, 25–37. doi: 10.1111/geb.13179
- Guo, D. L., Xia, M. X., Wei, X., Chang, W. J., Liu, Y., and Wang, Z. Q. (2008). Anatomical traits associated with absorption and mycorrhizal colonization are linked to root branch order in twenty-three Chinese temperate tree species. *New Phytol.* 180, 673–683. doi: 10.1111/j.1469-8137.2008.02573.x
- Hempel, S., Gotzenberger, L., Kuhn, I., Michalski, S. G., Rillig, M. C., Zobel, M., et al. (2013). Mycorrhizas in the Central European flora: relationships with plant life history traits and ecology. *Ecology* 94, 1389–1399. doi: 10.1890/12-1700.1
- Hu, Y. K., Pan, X., Liu, G. F., Li, W. B., Dai, W. H., Tang, S. L., et al. (2015). Novel evidence for within-species leaf economics spectrum at multiple spatial scales. *Front. Plant Sci.* 6:901–909. doi: 10.3389/fpls.2015.00901
- Huang, H. W. (2011). Plant diversity and conservation in China: planning a strategic bioresource for a sustainable future. *Bon. J. Linn. Soc.* 166, 282–300. doi: 10.1111/j.1095-8339.2011.01157.x
- Isaac, M. E., Martin, A. R., de Melo Virginio, Filho, E., Rapidel, B., Rounsard, O., et al. (2017). Intraspecific trait variation and coordination: root and leaf economics spectra in coffee across environmental gradients. *Front. Plant Sci.* 8:1196. doi: 10.3389/fpls.2017.01196
- Iversen, C. M., McCormack, M. L., Powell, A. S., Blackwood, C. B., Freschet, G. T., Kattge, J., et al. (2017). A global Fine-Root Ecology Database to address below-ground challenges in plant ecology. *New Phytol.* 215, 15–26. doi: 10.1111/nph.14486

- Jager, M. M., Richardson, S. J., Bellingham, P. J., Clearwater, M. J., Laughlin, D. C., and De Deyn, G. (2015). Soil fertility induces coordinated responses of multiple independent functional traits. *J. Ecol.* 103, 374–385. doi: 10.1111/1365-2745.12366
- James, F. C., and McCulloch, C. E. (1990). Multivariate analysis in ecology and systematics: panacea or Pandora's Box? *Annu. Rev. Ecol. Sys.* 21, 129–166. doi: 10.1146/annurev.es.21.110190.001021
- John, G. P., Scoffoni, C., Buckley, T. N., Villar, R., Poorter, H., and Sack, L. (2017). The anatomical and compositional basis of leaf mass per area. *Ecol. Lett.* 20, 412–425. doi: 10.1111/ele.12739
- Jung, V., Violle, C., Mondy, C., Hoffmann, L., and Muller, S. (2010). Intraspecific variability and trait-based community assembly. *J. Ecol.* 98, 1134–1140. doi: 10.1111/j.1365-2745.2010.01687.x
- Kaiser, H. F. (1958). The varimax criterion for analytic rotation in factor-analysis. *Psychometrika* 23:187e200. doi: 10.1007/bf02289233
- Kattge, J., Diaz, S., Lavorel, S., Prentice, C., Leadley, P., Bonisch, G., et al. (2011). TRY - a global database of plant traits. *Glob. Change Biol.* 17, 2905–2935. doi: 10.1111/j.1365-2486.2011.02451.x
- Kong, D. L., Ma, C. G., Zhang, Q., Li, L., Chen, X. Y., Zeng, H., et al. (2014). Leading dimensions in absorptive root trait variation across 96 subtropical forest species. *New Phytol.* 203, 863–872. doi: 10.1111/nph.12842
- Kong, D. L., Wang, J. J., Wu, H. F., Valverde-Barrantes, O. J., Wang, R. L., Zeng, H., et al. (2019). Nonlinearity of root trait relationships and the root economics spectrum. *Nat. Commun.* 10:2203. doi: 10.1038/s41467-019-10245-6
- Kramer-Walter, K. R., Bellingham, P. J., Millar, T. R., Smissen, R. D., Richardson, S. J., Laughlin, D. C., et al. (2016). Root traits are multidimensional: specific root length is independent from root tissue density and the plant economic spectrum. *J. Ecol.* 104, 1299–1310. doi: 10.1111/1365-2745.12562
- Laliberte, E. (2017). Below-ground frontiers in trait-based plant ecology. *New Phytol.* 213, 1597–1603. doi: 10.1111/nph.14247
- Laughlin, D. C. (2014). The intrinsic dimensionality of plant traits and its relevance to community assembly. *J. Ecol.* 102, 186–193. doi: 10.1111/1365-2745.12187
- Laughlin, D. C., Mommer, L., Sabatini, F. M., Bruelheide, H., Kuyper, T. W., McCormack, M. L., et al. (2021). Root traits explain plant species distributions along climatic gradients yet challenge the nature of ecological trade-offs. *Nat. Ecol. Evol.* 5, 1123–1134. doi: 10.1038/s41559-021-01471-7
- Legendre, P., and Legendre, L. (2012). *Numerical Ecology*, 3rd English Edn. Amsterdam: Elsevier.
- Li, F. L., Hu, H., McCormack, M. L., Feng, D. F., Liu, X., and Bao, W. K. (2019). Community-level economics spectrum of fine-roots driven by nutrient limitations in subalpine forests. *J. Ecol.* 107, 1238–1249. doi: 10.1111/1365-2745.13125
- Liu, C., Xiang, W. H., Zou, L. M., Lei, P. F., Zeng, Y. L., Ouyang, S., et al. (2019). Variation in the functional traits of fine roots is linked to phylogenetics in the common tree species of Chinese subtropical forests. *Plant Soil* 436, 347–364. doi: 10.1007/s11104-019-03934-0
- Liu, G. F., Freschet, G. T., Pan, X., Cornelissen, J. H. C., Li, Y., and Dong, M. (2010). Coordinated variation in leaf and root traits across multiple spatial scales in Chinese semi-arid and arid ecosystems. *New Phytol.* 188, 543–553. doi: 10.1111/j.1469-8137.2010.03388.x
- Liu, H., Osborne, C. P., Yin, D., Freckleton, R. P., Jiang, G., and Liu, M. (2019). Phylogeny and ecological processes influence grass coexistence at different spatial scales within the steppe biome. *Oecologia* 191, 25–38. doi: 10.1007/s00442-019-04475-0
- Liu, J. G., Ouyang, Z. Y., Pimm, S. L., Raven, P. H., Wang, X. K., Miao, H., et al. (2003). Protecting China's biodiversity. *Science* 300, 1240–1241. doi: 10.1126/science.1078868
- Ma, Z. Q., Guo, D. L., Xu, X. L., Lu, M. Z., Bardgett, R. D., Eissenstat, D. M., et al. (2018). Evolutionary history resolves global organization of root functional traits. *Nature* 555, 94–97. doi: 10.1038/nature25783
- Makita, N., Kosugi, Y., Dannoura, M., Takanashi, S., Niiyama, K., Kassim, A. R., et al. (2012). Patterns of root respiration rates and morphological traits in 13 tree species in a tropical forest. *Tree Physiol.* 32, 303–312. doi: 10.1093/treephys/tps008
- McCormack, M. L., and Iversen, C. M. (2019). Physical and functional constraints on viable belowground acquisition strategies. *Front. Plant Sci.* 10:1215. doi: 10.3389/fpls.2019.01215
- McCormack, M. L., Dickie, I. A., Eissenstat, D. M., Fahey, T. J., Fernandez, C. W., Guo, D. L., et al. (2015). Redefining fine roots improves understanding of below-ground contributions to terrestrial biosphere processes. *New Phytol.* 207, 505–518. doi: 10.1111/nph.13363
- McCormack, M. L., Lavelle, E., and Ma, Z. Q. (2014). Fine-root and mycorrhizal traits help explain ecosystem processes and responses to global change. *New Phytol.* 204, 455–458. doi: 10.1111/nph.13023
- Messier, J., McGill, B. J., and Lechowicz, M. J. (2010). How do traits vary across ecological scales? A case for trait-based ecology. *Ecol. Lett.* 13, 838–848. doi: 10.1111/j.1461-0248.2010.01476.x
- Messier, J., McGill, B. J., Enquist, B. J., and Lechowicz, M. J. (2017). Trait variation and integration across scales: is the leaf economic spectrum present at local scales? *Ecography* 40, 685–697. doi: 10.1111/ecog.02006
- Münkemüller, T., Laverne, S., Bzeznik, B., Dray, S., Jombart, T., Schiffrers, K., et al. (2012). How to measure and test phylogenetic signal. *Methods Ecol. Evol.* 3, 743–756. doi: 10.1111/j.2041-210X.2012.00196.x
- Navarro-Fernández, C. M., Pérez-Ramos, I. M., de la Riva, E. G., Vera, J. R., Roumet, C., Villar, R., et al. (2016). Functional responses of Mediterranean plant communities to soil resource heterogeneity: a mycorrhizal trait-based approach. *J. Veg. Sci.* 27, 1243–1253. doi: 10.1111/jvs.12446
- Ni, J. (2001). A biome classification of China based on plant functional types and the BIOME3 model. *Folia Geobot.* 36, 113–129. doi: 10.1007/BF02803157
- Olmo, M., Lopez-Iglesias, B., and Villar, R. (2014). Drought changes the structure and elemental composition of very fine roots in seedlings of ten woody tree species. Implications for a drier climate. *Plant Soil* 384, 113–129. doi: 10.1007/s11104-014-2178-6
- Ostonen, I., Truu, M., Helmsaari, H. S., Lukac, M., Borken, W., Vanguelova, E., et al. (2017). Adaptive root foraging strategies along a boreal-temperate forest gradient. *New Phytol.* 215, 977–991. doi: 10.1111/nph.14643
- Pagel, M. (1999). Inferring the historical patterns of biological evolution. *Nature* 401, 877–884. doi: 10.1038/44766
- Phillips, R. P., Brzostek, E., and Midgley, M. G. (2013). The mycorrhizal-associated nutrient economy: a new framework for predicting carbon-nutrient couplings in temperate forests. *New Phytol.* 199, 41–51. doi: 10.1111/nph.12221
- Pregitzer, K. S., Deforest, J. L., Burton, A. J., Allen, M. F., and Hendrick, R. R. L. (2002). Fine root architecture of nine north american trees. *Ecol. Monogr.* 72, 293–309. doi: 10.2307/3100029
- Qian, H., and Jin, Y. (2016). An updated megaphylogeny of plants, a tool for generating plant phylogenies and an analysis of phylogenetic community structure. *J. Plant Ecol.* 9, 233–239. doi: 10.1093/jpe/rtv047
- R Core Team (2020). *R: A language and environment for statistical computing. R foundation for statistical computing. Vienna, Austria.* Vienna: R Core Team.
- Reich, P. B. (2014). The world-wide 'fast-slow' plant economics spectrum: a traits manifesto. *J. Ecol.* 102, 275–301. doi: 10.1111/1365-2745.12211
- Reich, P. B., Ellsworth, D. S., Walters, M. B., Vose, J. M., Gresham, C., Volin, J. C., et al. (1999). Generality of leaf trait relationships: a test across six biomes. *Ecology* 80, 1955–1969. doi: 10.1890/0012-9658(1999)080[1955:GOLTRA]2.0.CO;2
- Reich, P. B., Walters, M. B., and Ellsworth, D. S. (1992). Leaf life-span in relation to leaf, plant, and stand characteristics among diverse ecosystems. *Ecol. Monogr.* 62, 365–392. doi: 10.2307/2937116
- Revell, L. J. (2012). Phytools: an R package for phylogenetic comparative biology (and other things). *Methods Ecol. Evol.* 3, 217–223. doi: 10.1111/j.2041-210X.2011.00169.x
- Roumet, C., Birouste, M., Picon-Cochard, C., Ghestem, M., Osman, N., Vriennon-Brenas, S., et al. (2016). Root structure-function relationships in 74 species: evidence of a root economics spectrum related to carbon economy. *New Phytol.* 210, 815–826. doi: 10.1111/nph.13828
- Shiklomanov, A. N., Cowdery, E. M., Bahn, M., Byun, C., Jansen, S., Kramer, K., et al. (2020). Does the leaf economic spectrum hold within plant functional types? A Bayesian multivariate trait meta-analysis. *Ecol. Appl.* 30:2064. doi: 10.1002/eap.2064
- Shipley, B., Lechowicz, M. J., Wright, I., and Reich, P. B. (2006). Fundamental trade-offs generating the worldwide leaf economics spectrum. *Ecology* 87, 535–541. doi: 10.1890/05-1051

- Simpson, A. H., Richardson, S. J., and Laughlin, D. C. (2016). Soil-climate interactions explain variation in foliar, stem, root and reproductive traits across temperate forests. *Glob. Ecol. Biogeogr.* 25, 964–978. doi: 10.1111/geb.12457
- Sun, K., McCormack, M. L., Li, L., Ma, Z. Q., and Guo, D. L. (2016). Fast-cycling unit of root turnover in perennial herbaceous plants in a cold temperate ecosystem. *Sci. Rep.* 6:19698. doi: 10.1038/srep19698
- Tabachnik, B. G., and Fidell, L. S. (1996). *Using multivariate statistics*. New York, NY: Harper Collins.
- Valverde-Barrantes, O. J., Authier, L., Schimann, H., and Baraloto, C. (2021). Root anatomy helps to reconcile observed root trait syndromes in tropical tree species. *Am. J. Bot.* 108, 744–755. doi: 10.1002/ajb2.1659
- Valverde-Barrantes, O. J., Freschet, G. T., Roumet, C., and Blackwood, C. B. (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 Phytol.* 215, 1562–1573. doi: 10.1111/nph.14571
- Valverde-Barrantes, O. J., Horning, A. L., Smemo, K. A., and Blackwood, C. B. (2016). Phylogenetically structured traits in root systems influence arbuscular mycorrhizal colonization in woody angiosperms. *Plant Soil* 404, 1–12. doi: 10.1007/s11104-016-2820-6
- Valverde-Barrantes, O. J., Maherali, H., Baraloto, C., and Blackwood, C. B. (2020). Independent evolutionary changes in fine-root traits among main clades during the diversification of seed plants. *New Phytol.* 228, 541–553. doi: 10.1111/nph.16729
- Valverde-Barrantes, O. J., Smemo, K. A., Feinstein, L. M., Kershner, M. W., and Blackwood, C. B. (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. doi: 10.1007/s00442-017-4044-8
- Wang, B., and Qiu, Y. L. (2006). Phylogenetic distribution and evolution of mycorrhizas in land plants. *Mycorrhiza* 16, 299–363. doi: 10.1007/s00572-005-0033-6
- Wang, R. L., Wang, Q. F., Zhao, N., Xu, Z. W., Zhu, X. J., Jiao, C. C., et al. (2018a). Different phylogenetic and environmental controls of first-order root morphological and nutrient traits: evidence of multidimensional root traits. *Funct. Ecol.* 32, 29–39. doi: 10.1111/1365-2435.12983
- Wang, R. L., Wang, Q. F., Liu, C. C., Kou, L., Zhao, N., Xu, Z. W., et al. (2018b). Changes in trait and phylogenetic diversity of leaves and absorptive roots from tropical to boreal forests. *Plant Soil* 432, 389–401. doi: 10.1007/s11104-018-3816-1
- Weemstra, M., Freschet, G. T., Stokes, A., Roumet, C., and Sayer, E. (2020). Patterns in intraspecific variation in root traits are species-specific along an elevation gradient. *Funct. Ecol.* 35, 342–356. doi: 10.1111/1365-2435.13723
- Weemstra, M., Mommer, L., Visser, E. J., van Ruijven, J., Kuyper, T. W., Mohren, G. M., et al. (2016). Towards a multidimensional root trait framework: a tree root review. *New Phytol.* 211, 1159–1169. doi: 10.1111/nph.14003
- Weigelt, A., Mommer, L., Andraczek, K., Iversen, C. M., Bergmann, J., Bruehlheide, H., et al. (2021). An integrated framework of plant form and function: the belowground perspective. *New Phytol.* 2021:17590. doi: 10.1111/nph.17590
- Wright, I. J., Reich, P. B., Westoby, M., Ackerly, D. D., Baruch, Z., Bongers, F., et al. (2004). The worldwide leaf economics spectrum. *Nature* 428, 821–827. doi: 10.1038/nature02403
- Yahara, H., Tanikawa, N., Okamoto, M., and Makita, N. (2019). Characterizing fine-root traits by species phylogeny and microbial symbiosis in 11 co-existing woody species. *Oecologia* 191, 983–993. doi: 10.1007/s00442-019-04546-2
- Zanne, A. E., Tank, D. C., Cornwell, W. K., Eastman, J. M., Smith, S. A., FitzJohn, R. G., et al. (2014). Three keys to the radiation of angiosperms into freezing environments. *Nature* 506, 89–92. doi: 10.1038/nature12872
- Zhou, M., Bai, W. M., Zhang, Y. S., and Zhang, W. H. (2018). Multi-dimensional patterns of variation in root traits among coexisting herbaceous species in temperate steppes. *J. Ecol.* 106, 2320–2331. doi: 10.1111/1365-2745.12977

**Conflict of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

**Publisher's Note:** All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Copyright © 2022 An, Lu, Fu, Chen, Keyimu and Wang. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.



## OPEN ACCESS

EDITED BY  
Patompong Johns Saengwilai,  
Mahidol University, Thailand

REVIEWED BY  
Eric A. Nord,  
Greenville College, United States  
Hannah Schneider,  
Wageningen University and  
Research, Netherlands  
Christopher Strock,  
The Pennsylvania State University  
(PSU), United States

\*CORRESPONDENCE  
Lei Huang  
mathecolology@163.com

SPECIALTY SECTION  
This article was submitted to  
Functional Plant Ecology,  
a section of the journal  
Frontiers in Plant Science

RECEIVED 12 April 2022  
ACCEPTED 27 June 2022  
PUBLISHED 22 July 2022

CITATION  
Yang G, Huang L and Shi Y (2022)  
Magnitude and determinants of plant  
root hydraulic redistribution: A global  
synthesis analysis.  
*Front. Plant Sci.* 13:918585.  
doi: 10.3389/fpls.2022.918585

COPYRIGHT  
© 2022 Yang, Huang and Shi. This is an  
open-access article distributed under  
the terms of the [Creative Commons  
Attribution License \(CC BY\)](#). The use,  
distribution or reproduction in other  
forums is permitted, provided the  
original author(s) and the copyright  
owner(s) are credited and that the  
original publication in this journal is  
cited, in accordance with accepted  
academic practice. No use, distribution  
or reproduction is permitted which  
does not comply with these terms.

# Magnitude and determinants of plant root hydraulic redistribution: A global synthesis analysis

Guisen Yang<sup>1,2</sup>, Lei Huang<sup>1\*</sup> and Yafei Shi<sup>1,2</sup>

<sup>1</sup>Shapotou Desert Research and Experiment Station, Northwest Institute of Eco-Environmental Resources, Chinese Academy of Sciences, Lanzhou, China, <sup>2</sup>University of Chinese Academy of Sciences, Beijing, China

Plant root hydraulic redistribution (HR) has been widely recognized as a phenomenon that helps alleviate vegetation drought stress. However, a systematic assessment of the magnitude of HR and its drivers at the global scale are lacking. We collected 37 peer-reviewed papers (comprising 47 research sites) published in 1900–2018 and comprehensively analyzed the magnitude of HR and its underlying factors. We used a weighting method to analyze HR magnitude and its effect on plant transpiration. Machine learning algorithms (boosted regression trees) and structural equation modeling were used to determine the influence of each factor on HR magnitude. We found that the magnitude of HR was  $0.249 \text{ mm H}_2\text{O d}^{-1}$  (95% CI, 0.113–0.384) and its contribution to plant transpiration was 27.4% (3–79%). HR varied significantly among different terrestrial biomes and mainly occurred in forests with drier conditions, such as temperate forest ecosystems ( $\text{HR} = 0.502 \text{ mm H}_2\text{O d}^{-1}$ ), where HR was significantly higher than in other ecosystems ( $p < 0.01$ ). The magnitude of HR in angiosperms was significantly higher than that in gymnosperms ( $p < 0.05$ ). The mean magnitude of HR first increased and then decreased with an increase in humidity index; conversely, the mean magnitude of HR decreased with an increase in water table depth. HR was significantly positively correlated with root length and transpiration. Plant characteristics and environmental factors jointly accounted for 61.0% of the variation in HR, and plant transpiration was the major factor that directly influenced HR (43.1% relative importance;  $p < 0.001$ ), and soil texture was an important indirect driver of HR. Our synthesis offers a comprehensive perspective of how plant characteristics and environmental factors influence HR magnitude.

## KEYWORDS

plant roots, hydraulic redistribution, magnitude, influencing factors, terrestrial ecosystems, soil texture

## Introduction

Hydraulic redistribution (HR) is the passive movement of water from moist to dry soil through plant roots, including the lifting of water from the deeper to shallower soil layers (hydraulic lift, HL), the movement of shallow to deep soil layers (downward hydraulic redistribution, DHR), and lateral transportation



(Burgess et al., 1998; Neumann and Cardon, 2012). HR generally occurs in the root-soil interlaced area, where exchanges of mass and energy are the most frequent and active in the soil ecosystem (Leffler et al., 2005; Prieto et al., 2012). The phenomenon is present worldwide within a range of different ecosystems and plant species (Bogie et al., 2018). HR can effectively increase the water content of dry soil, support the vigor and conductivity of fine roots, improve microbial activity, and promote nutrient absorption (Lee et al., 2018; Wang et al., 2018). Early research mainly focused on arid and semi-arid areas, and then gradually expanded to seasonally arid and humid and semi-humid areas (Jackson et al., 2000; Pomazkina and Lubnina, 2002). The latest research reported that HR has been observed in more than 120 plant species from different bioclimatic zones (Fu et al., 2018). HR may affect the net primary productivity of plant communities, vegetation distribution patterns, biogeochemical cycles, and ultimately affect climate (Sun et al., 2018).

Although the amount of water transported through HR is extremely low compared to precipitation, it is increasingly recognized as an important because of its substantial role in the effective water use of plants (Neumann and Cardon, 2012; Lee et al., 2018), such as supporting plant life activities (Fu et al., 2018), prolonging the lifespan of plant fine roots (Meinzer et al., 2004), enhancing the activities of root hairs, and reducing root embolisms under drought stress. The reported average amount of HR varied with species and environmental conditions. For example, the contribution of HR to the upper 100 cm of soil was  $0.7 \text{ mm H}_2\text{O d}^{-1}$  near the middle of the dry season, while in the wet season HR was exceptionally low (Scholz et al., 2010). In addition, it was found that savanna trees in a campo cerrado released about  $0.004 \text{ mm}$  of water per day to the upper soil layers *via* HR, and was  $0.008 \text{ mm}$  per day in a denser savanna site (Scholz et al., 2008a). Empirical estimates of the average magnitude of upward HR span more than an of magnitude, from  $0.04 \text{ mm H}_2\text{O d}^{-1}$  in a Brazilian savanna (Scholz et al., 2010) to  $1.3 \text{ mm H}_2\text{O d}^{-1}$  in New England sugar maples (Emerman and Dawson, 1996). Neumann and Cardon (2012) synthesized that the magnitude of HR varied from  $0.04$  to  $1.30 \text{ mm H}_2\text{O d}^{-1}$ , and  $0.1$  to  $3.23 \text{ mm H}_2\text{O d}^{-1}$  in the empirical and modeling studies. However, a more comprehensive and quantitative analysis of how HR varies across a range of different ecosystems and plant species is lacking.

Most of the studies that investigated HR and its relationship with environmental and biological factors were confined to field or plot scale, and so too were modeling studies (Yu et al., 2013). These studies focused on the magnitude of HR by individual plant species at specific sites and the possible drivers of HR (Bogie et al., 2018; Meunier et al., 2018). Several studies have reported that HR occurs only under certain conditions (Neumann and Cardon, 2012; Hafner et al., 2017). The occurrence and magnitude of HR differs among plant species, even between individuals of the same species under different environments (Neumann and Cardon,

2012). Factors that affect HR include climate (precipitation and evapotranspiration), soil characteristics (soil moisture, soil texture, and land use type; Hafner et al., 2020, and vegetation characteristics (morphological characteristics and distribution of roots, root length, and root water storage ability; Leffler et al., 2005). Although these studies provided an abundance of information at a local scale, they provided little information about the general patterns of HR production at larger spatial scales (Nadezhkina et al., 2015; Yu and D'Odorico, 2015). Moreover, it is difficult to extrapolate these results between sites, and very few studies have attempted to explain the differences in HR magnitude on a regional scale. Therefore, what is the spatial pattern of the magnitude of HR on a global scale? What factors determine the magnitude of HR and how do they affect HR? Understanding these processes could facilitate the evaluation of the significance and effectiveness of HR in plant water use in terrestrial ecosystems. In addition, the quantification of HR could also provide basic data for global groundwater resource assessment and modeling (Zhang and Zwiazek, 2018), determining vegetation water use efficiency and modeling, and other hydrological and surface models (Wang et al., 2018).

Here, we aimed to explore the global patterns of HR to determine which factors are most influential in HR magnitude and to systematically evaluate the responses of HR to its drivers. To accomplish these aims, we compiled a global dataset of 47 HR observations of terrestrial plants (e.g., trees, shrubs, and herbs) extracted from 37 papers published between 1900 and 2018 (Supplementary Data Sheet 1). We used a weighting method to determine the mean magnitude of HR and its contribution to plant transpiration. We used machine learning (boosted regression trees) and structural equation modeling to analyze the influence of each factor on HR quantity.

## Materials and Methods

### Literature search and data compilation

Through the Web of Science, we searched for published literature under “plant root hydraulic redistribution”. We found 400 pieces of literature from 56 countries published between 1900 and 2018. Through title and abstract screening, we excluded papers that only provided qualitative descriptions of HR but did not clearly measure the specific magnitude of HR (DeMalach et al., 2017). In total, 37 papers were identified for integrated analysis, which met our requirements for data extraction, and included 47 research sites and 21 species of plants across five biomes. We also extracted other relevant information in the study, such as soil type, plant root length, sampling date, regional precipitation, evaporation, water table depth, biome type, latitude, longitude, and climate variables. These observations accounted for the amount of HR for different plant species at a specific site,

accounting for the proportion of plant transpiration, and its effect on ecology and hydrology. Furthermore, we calculated the average values of HR (M), sample size (n), standard deviation (SD), and 95% confidence intervals (CI) for the corresponding average HR values. We found that HR research included two common methods: field measurement and model simulation. Since we were interested in the magnitude of HR, we treated independent research conducted by different institutions or researchers at the same location as different research results, including research plots where some field measurements and model studies overlapped. We collected 47 research sites (field studies and model studies, shown in [Supplementary Figure 1](#)).

We adopted the following four criteria to select suitable studies:

- (1) The magnitude of HR and its influencing factors were determined through field or modeling studies.
- (2) The amount of HR by a specific plant species was measured.
- (3) The average value and standard deviation of HR could be directly obtained from the literature or could be calculated indirectly.
- (4) Papers in which field observations occurred during less than a full growing season were excluded.

We extracted the following explanatory variables for each study:

- (1) Location (latitude and longitude)—in cases where the studies did not report the latitude or longitude (5% of study sites), the approximate latitude and longitude were derived by geocoding the name of the location in Google Earth.
- (2) To conduct biome-level analysis, we aggregated the data into seven biomes based on the definitions of the International Geosphere-Biosphere Programme (IGBP): needleleaf or broadleaf forest, temperate forest, deserts or sparsely vegetated, temperate grassland, savannahs, barren land, and shrublands ([Supplementary Figure 2](#); [Xu et al., 2013](#)).
- (3) Humidity index is the ratio of mean annual precipitation to mean annual evapotranspiration. In cases where the original source studies did not report precipitation or evapotranspiration, it was extracted from WorldClim version 2.1 using the site's geographic location (i.e., latitude and longitude).
- (4) Water table depth (m)—for literature that did not report groundwater level information in the study area, the water table depths were extracted from the global patterns of the groundwater table depth dataset ([Fan et al., 2013](#)).
- (5) Plant root length (cm)—the midpoint length of plant roots were calculated as the root length variable for analysis.

## Measurement methods of HR from original literature

### Field measurement

Field measurements were mainly conducted using either of two methods, and the HR unit obtained by the two methods is unified into water volume ( $\text{mm H}_2\text{O d}^{-1}$ ). The soil moisture method divides the plant roots into upper and lower or left and right zones. The circulation of soil moisture in the two zones occurs by means of soil infiltration. One zone is provided with sufficient water supply, and the other zone is deprived of water. HR is estimated by measuring the changes of soil water content ( $\theta$ ) and soil water potential ( $\phi$ ) in the arid zone. The  $\theta$  declines during the day when plant and root demand were highest. At night, HR moves water *via* roots from wetter soil layers to drier soil layers following a water potential gradient. This night-time increase in  $\theta$  in the absence of precipitation is considered to be HR. The magnitude of daily HR was estimated for each sensor and then integrated across the profile to provide total daily HR within the monitored soil layer ([Brooks et al., 2002](#); [Warren et al., 2007](#); [Cleverly et al., 2016](#)).

$$HR = \varnothing_{\max}(\text{day}(x+1)) - \varnothing_{\min}(\text{day } x) \quad (1)$$

Soil water potentials ( $\phi$ ) were usually quantified using thermocouple psychrometers (PST-55, Wescor, Logan, UT) installed soil layers at different depths. The  $\theta$  was quantified using multi-sensor, frequency domain capacitance probes. A statistical program (Sigma Plot 7.101, SPSS Inc., Chicago, IL) was used to fit a simple three-parameter non-linear regression curve to the data at each depth ([Warren et al., 2005](#)):

$$\phi = \frac{-1}{(a + b\theta)^c} \quad (2)$$

where  $a$ ,  $b$ ,  $c$  are parameters determined by the regression.

$$\phi = \phi_{cr} \left( \frac{\theta_r - \theta}{\theta_r - \theta_s} \right)^{\frac{1}{\lambda}} \quad (3)$$

where  $\theta_s$  is the saturated soil volumetric water content,  $\theta_r$  is the residual soil volumetric water content (for very dry soil),  $\phi_{cr}$  is the soil water potential as  $\theta$  approaches saturation, and  $\lambda$  is a parameter related to soil porosity.

The sap flow method involves installing heating and control probes for measuring stem flow on the lateral roots and main roots of plants, respectively. By measuring the temperature difference between the heating probe and the control probe, the liquid flow velocity is calculated, and the total liquid flow per unit time is calculated. The commonly used determination methods include the thermal ratio method (HRM), thermal field deformation method (HFD), and thermal diffusion technology (TDT). Taking the HRM method as an example, several sets

of sensors are placed in the stem and taproot and single sets of sensors are placed in major lateral roots. In addition, the cross-sectional area of the monitored lateral root is extracted. For each species, mean and standard deviation (SD) sap velocity were calculated for all lateral root measurements, and all tap root measurements and these values were then multiplied by the total cross-sectional area of roots in each class of root. HR was quantified as the total volume of water estimated from negative sap flow (i.e., flow directionally away from the trunk) measured on roots. HR was presented as night-time sap flow (g/day) by summing the product of sap velocity by the cross-sectional area of similar lateral roots and water density, instead of volumetric flow velocities (mm/day) because it is difficult to scale up with the size of each individual lateral root monitored. It is difficult to measure sap flow on all roots, so only large lateral roots are instrumented with sap flow sensors (Burgess et al., 2001; Yu et al., 2018).

In addition, the isotope tracer method has also been widely used to study HR, but we did not include them in our analysis because stable isotope tracer technology is mainly used to discover the occurrence of HR, and it is difficult to accurately measure the amount of HR. Finally, the quantity unit of HR obtained by the two methods is unified into water volume (mm H<sub>2</sub>O d<sup>-1</sup>) using this method.

## Model simulation

In addition to field measurements, models can be used to estimate quantify HR. The overall goal of such a model is to capture the influence of soil water content on HR dynamics and magnitude according to the conductivity of soil, soil roots and roots. The original HR model was posited by Ryel et al. (2002), and is now widely used and known as the “Ryel model.” This model also laid a foundation for the establishment of other models in HR research.

Water movement among soil layers by roots has been assumed to occur based on differences in  $\Psi_i$ , with water moving from wetter to drier layers (Caldwell et al., 1998). Water redistributed by roots was modeled as a function of the distribution of active roots, radial conductivity of water between the root-soil interface (rhizosphere conductance), and transpiration activity (Ryel et al., 2002). The Ryel model defines HR as a function of hydraulic conductivity in root water flow path and water potential gradient in different soil layers, and HR (HR<sub>i</sub>) of a certain soil layer  $i$  can be expressed as follows:

$$HR_i = C_{\max} \sum_j (j-i) \max(c_i - c_j) R_{ij} D_{\text{tran}} \quad (4)$$

where  $C_{\max}$  is reduced using an empirical relationship from van Genuchten (range 0–1) as soil water potential  $\Psi$  decreases

(i.e., soil dries) in the source ( $c_j$ ) or the sink ( $c_i$ ) soil layers. Conductance is distributed among soil layers as a function ( $R_{ij}$ ) of root biomass distribution in the layers. Because this approach does not model flow within the root system itself, and therefore does not simulate root water potential, it cannot easily capture the competition for xylem water between atmospheric water demand (via transpiration) and dry soil layers. Ryel et al. (2002) therefore included an “on/off” term,  $D_{\text{tran}}$ , that restricts redistribution to periods with low transpiration demand. For example, Zheng and Wang (2007), Baker et al. (2008), and Wang (2011) adopted Ryel et al.’s (2002) formulation. Scholz et al. (2010) slightly altered the effective conductance calculation to focus on the drying (water-receiving) soil layer’s control over flow. Other models are also used to study HR, such as the big root model (Amenu and Kumar, 2008), macro-meso scale models (Siqueira et al., 2008), and the dynamic root profile model (Schymanski et al., 2008).

## Transpiration of plants

Leaf transpiration losses from the soil were assumed to be primarily limited by the soil-root conductance for water in each layer. The transpiration rate was further limited by the portion of roots within each layer, the sap flow of stems and roots using the heat ratio method (Burgess et al., 2001). Whole-tree crown-related sap flow (equal to transpiration, mm/hr) was calculated by dividing the product sap velocity (cm/hr) and sapwood area (cm<sup>2</sup>) by the crown area (cm<sup>2</sup>). The crown area was calculated as the circular area via measurement of diameter of crown in four directions. Then, the daily transpiration of plants was converted into a unit (mm H<sub>2</sub>O d<sup>-1</sup>) consistent with the HR (Yu et al., 2018).

## Effect size of HR

Confidence intervals (CI) indicate the range within which the true mean (the magnitude of HR) estimates fall in 95% of all possible integrated analyses. The 95% CI was computed using the following equation (Evaristo and McDonnell, 2017):

$$\text{Lower limit} = M - 1.96^* \frac{SD}{\sqrt{n}} \quad (5)$$

$$\text{Upper limit} = M + 1.96^* \frac{SD}{\sqrt{n}} \quad (6)$$

where  $M$  is the average value of HR,  $SD$  is the standard deviation corresponding to the HR value, and  $n$  is the sample size in each study.

The ultimate goal of any integrated analysis is to provide a cross-site comparison and an overall view or effect size (in this

case, the magnitude of HR; Zhang et al., 2021). If the precision across all 37 published papers in our database was equal, we could readily compute the simple mean of all HR estimates. As this was not the case, we needed to compute a weighted mean by assigning weights to the studies. Here, we weighted each study by the inverse of its original (within-study) variance. The weights ( $W_i$ ) allocated to each of the studies are then inversely proportional to the square of the standard deviation (SD) for the  $i$ -th study. This allocates greater weight to studies with smaller standard deviation (Gao and Yohay, 2020). Therefore, the weight calculation formula we used was as follows:

$$W_i = \frac{1}{SD^2} \quad (7)$$

## Data and statistical analysis

We used boosted regression trees (BRT) analysis to estimate the effects of individual predictor variables on HR. Plant transpiration and plant root length were combined as plant characteristics and water table depth, humidity index, mean annual precipitation, and mean annual evapotranspiration metrics were combined to the environmental factors. BRTs are robust to collinearity between variables, variable outliers, and missing data, which is thought to be advantageous in this study as there are many category predictors and little prior information. In addition, BRT has performed well in determining the important independent variables. We performed BRT analysis using the “gbm.step” function in the R “dismo” package (Zhang et al., 2021).

We used GraphPad Prism 6 (GraphPad Software Inc., San Diego, CA, USA) software to complete the HR effect size graphic plotting. The differences of HR in different classifications were measured by one-way analysis of variance (ANOVA) and an independent-sample Kruskal-Wallis test was used to test for differences in HR between soil textures, which were performed using SPSS 22.0 (IBM Corp., Armonk, NY, USA). We created a map using ArcMap 10.6 in ArcGIS 10 (ESRI, Redlands, CA, USA). The fitting curve of the influencing factors was completed using Origin 9.1 (OriginLab Corp., Northampton, MA, USA). We used machine learning (boosted regression tree) to analyze the influence of the individual variables on HR (Zhang et al., 2021). A mixed-model structural equation model (SEM) was constructed using AMOS 21.0 to determine how the magnitude of HR was driven by plant characteristics and environmental factors. Before modeling, we first considered a full model that included all possible environmental factors, and pathways, and eliminated non-significant ones. To test the overall goodness of fit for the SEMs, we used the  $\chi^2$  test and the root mean square error of approximation (RMSEA; García-Palacios et al., 2015).

## Results

### Magnitude of HR and its contribution to plant transpiration demands

We found that the global estimated magnitude of HR was 0.249 mm H<sub>2</sub>O d<sup>-1</sup> (95% CI, 0.113–0.384). However, there is considerable range in HR between studies, from 0.06 mm H<sub>2</sub>O d<sup>-1</sup> in a Brazilian savannah to 1.646 mm H<sub>2</sub>O d<sup>-1</sup> in Western Australia eucalyptus, which reflected site and species-level differences across studies (Figure 1A). In addition, the mean magnitude of HR among the modeling studies was 0.319 mm H<sub>2</sub>O d<sup>-1</sup> ( $n = 12$ ), and for the field studies it the mean magnitude was 0.248 mm H<sub>2</sub>O d<sup>-1</sup> ( $n = 35$ ). The modeling results were significantly higher than those of field measurements (Figure 1B,  $p < 0.01$ ).

The amount of HR in different plant species that accounted for the proportion of daily transpiration of plants was significantly different, ranging from 3 to 79%, with an average value of 27.4% (Figure 2,  $n = 47$ ). Compared with shrubs and trees, the magnitude of HR had a lower influence on plant transpiration in herbs. For example, the smallest influence of the magnitude of HR was observed in *Heteropogon contortus*, in which HR accounted for only 3% of the variation in transpiration. Conversely, in *Quercus robustus*, the largest species, the average daily HR volume accounted for 79% of the daily transpiration volume, which played a very important role in relieving water stress in the dry season and maintaining healthy growth.

### Magnitude of HR in different terrestrial biomes

The HRs at biome and global scales are summarized in Appendix S1. Most of the field sites were in located in North America, South America, and Europe. There were fewer observations for Africa, Russia, Asia, and Antarctica. On a terrestrial biome basis (Figure 3A), HR had the greatest magnitude in temperate forests 0.502 mm H<sub>2</sub>O d<sup>-1</sup> (95% CI, 0.111–0.993) and deserts or sparsely vegetated land 0.216 mm H<sub>2</sub>O d<sup>-1</sup> (95% CI, 0.014–0.475). HR had the smallest magnitude in needleleaf and broadleaf forest 0.100 mm H<sub>2</sub>O d<sup>-1</sup> (95% CI, 0–0.367), temperate grassland 0.098 mm H<sub>2</sub>O d<sup>-1</sup> (95% CI, 0–0.390), and savannahs 0.162 mm H<sub>2</sub>O d<sup>-1</sup> (95% CI, 0.078–0.247). In addition, angiosperms exhibited a greater magnitude of HR at 0.281 mm H<sub>2</sub>O d<sup>-1</sup> (95% CI, 0.053–0.405) than gymnosperms at 0.102 mm H<sub>2</sub>O d<sup>-1</sup> (95% CI, 0–0.323), and this difference was significant (Figure 3B,  $p < 0.01$ ).



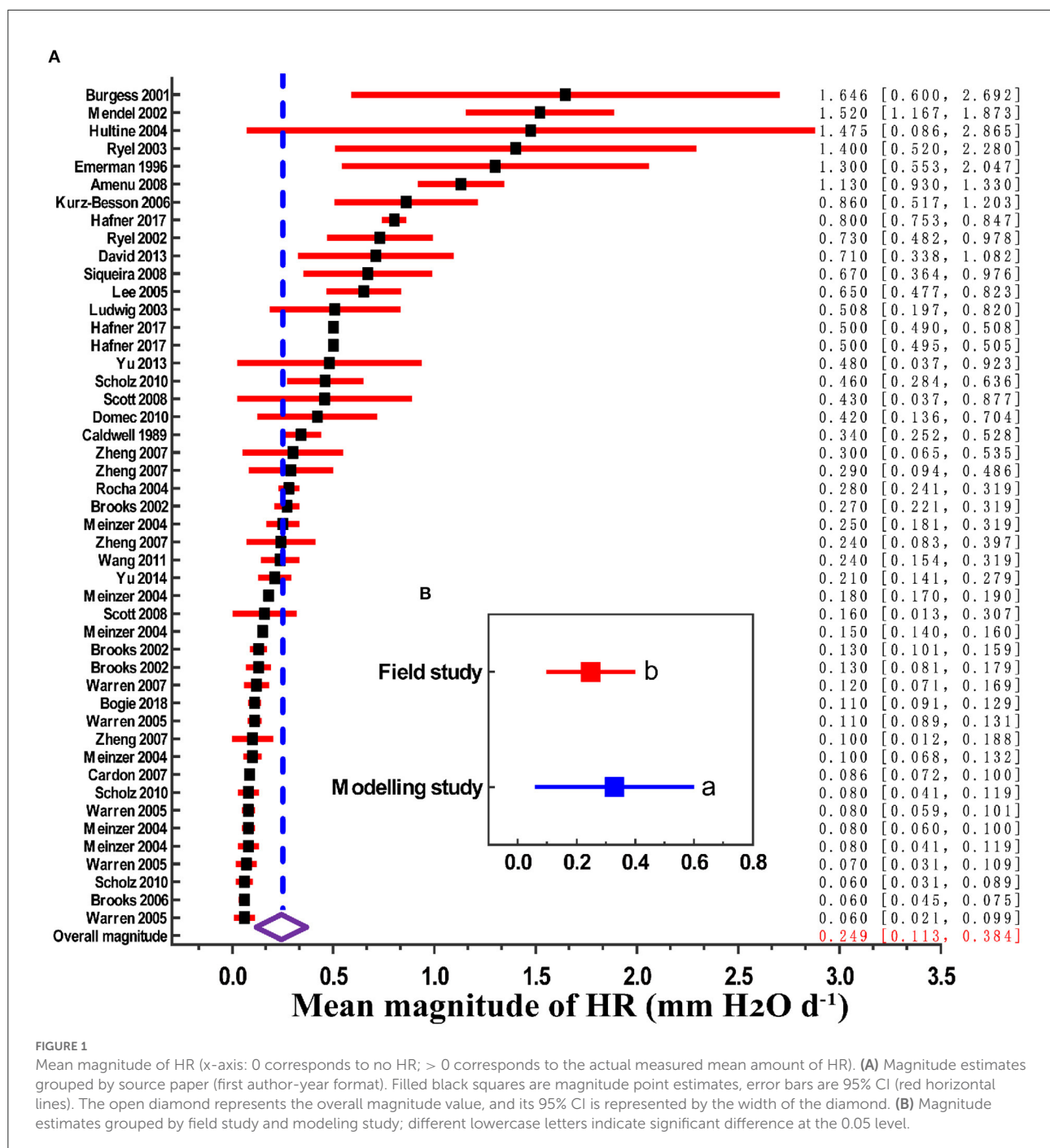


FIGURE 1

Mean magnitude of HR (x-axis: 0 corresponds to no HR; > 0 corresponds to the actual measured mean amount of HR). (A) Magnitude estimates grouped by paper (first author-year format). Filled black squares are magnitude point estimates, error bars are 95% CI (red horizontal lines). The open diamond represents the overall magnitude value, and its 95% CI is represented by the width of the diamond. (B) Magnitude estimates grouped by field study and modeling study; different lowercase letters indicate significant difference at the 0.05 level.

## Factors influencing HR

With an increased humidity index, the amount of HR first increased and then decreased (Figure 4a,  $p < 0.001$ ,  $R^2 = 0.499$ ). The amount of HR decreased as water table depths increased (Figure 4b,  $p < 0.001$ ,  $R^2 = 0.701$ ), but increased with an increase in plant transpiration (Figure 4c,  $p < 0.001$ ,  $R^2 = 0.346$ ) and soil-plant root length (Figure 4d,  $p < 0.001$ ,  $R^2 = 0.514$ ). We

ranked the driving factors using the boosted regression trees. We found that plant transpiration was the major factor influencing HR (relative importance was 43.1 %), followed by plant root length (24.5%), water table depth (23.8%), and humidity index (8.5%; Figure 4f). In addition, we used an ANOVA to compare the relationship between HR and soil texture, and it was found that HR was significantly higher in loam than in sandy soil and clay (Figure 4e,  $p < 0.001$ ).

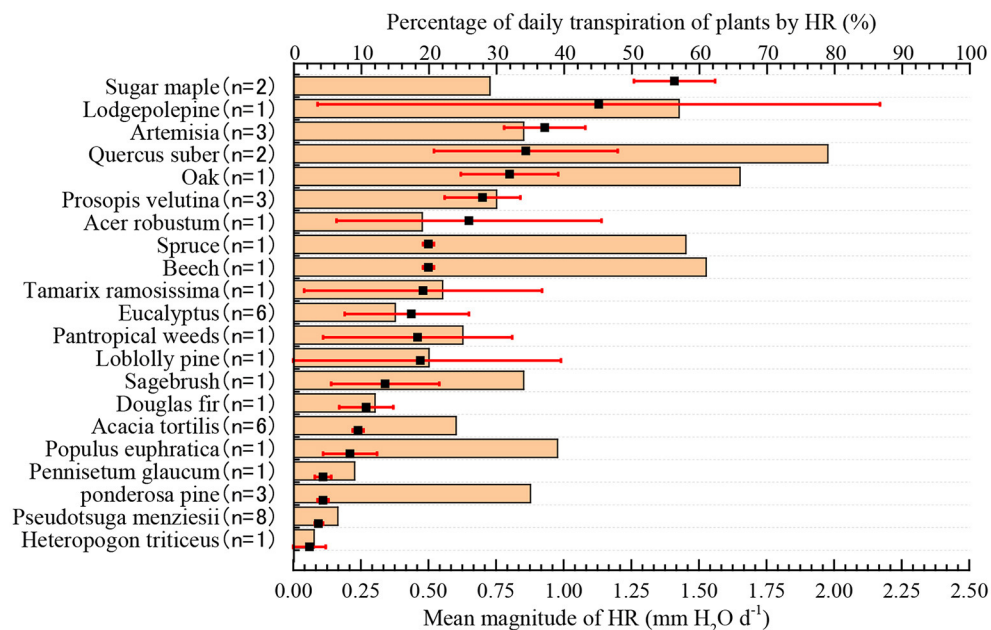


FIGURE 2

The amount of HR and its percentage of average daily transpiration. The filled black square represents the mean plant HR, and the red error bar is a 95% CI. The tan bars represent the percentage of HR in the average daily transpiration of plants.

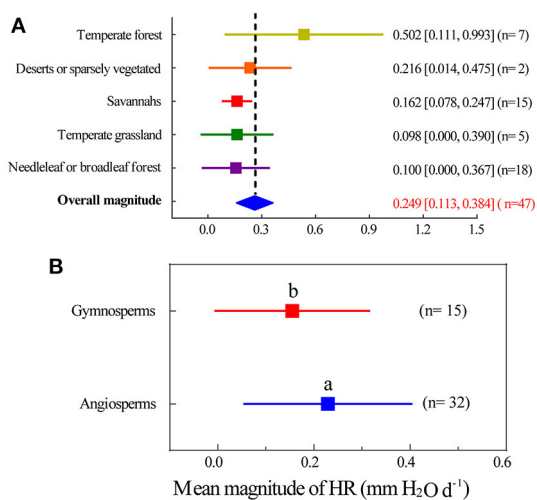


FIGURE 3

Mean magnitude of HR of different vegetation types. (A) Magnitude estimates grouped by different terrestrial biomes. (B) Magnitude estimates grouped by angiosperm and gymnosperm. Filled squares are magnitude point estimates. Error bars are 95% CI. The solid diamond represents the average magnitude of HR and its 95% CI is represented by the width of the diamond. Different lowercase letters indicate significant difference at the 0.05 level.

The SEM linking the mean magnitude of HR with both plant characteristics (e.g., plant seed species and root length) and

environmental factors (e.g., water table depth, humidity index, and average transpiration) as predictors had a good fit to the data and accounted for 61.0% of the variation in HR (Figure 5,  $\chi^2 = 0.277$ , CFI = 1.000,  $P = 0.871$ , RMSEA = 0.000). Soil texture had an important indirect effect on HR. Although the difference in root length between angiosperms and gymnosperms was not significant, their effect on HR was significant. In addition, soil texture was highly correlated with plant transpiration, water table depth, and terrestrial biome. These factors jointly affected the magnitude of HR in plant roots.

## Discussion

### The magnitude of HR and its contribution to plant transpiration water demand

Our study summarized the mean magnitude of HR at the global scale, which is an important step toward a better understanding of regional variation in the magnitude of HR (Zhou et al., 2020; Sian and Menge, 2021). We found that the global estimated magnitude of HR was  $0.249 \text{ mm H}_2\text{O d}^{-1}$ , which is relatively lower than reported by Neumann and Cardon (2012), who found that the mean magnitude of HR was  $0.3 \text{ mm H}_2\text{O d}^{-1}$ . The reason for this difference may be that we used the weighted average method to calculate the magnitude of HR, but this method can more accurately evaluate the errors caused by the difference of sample size in different studies (Veroniki

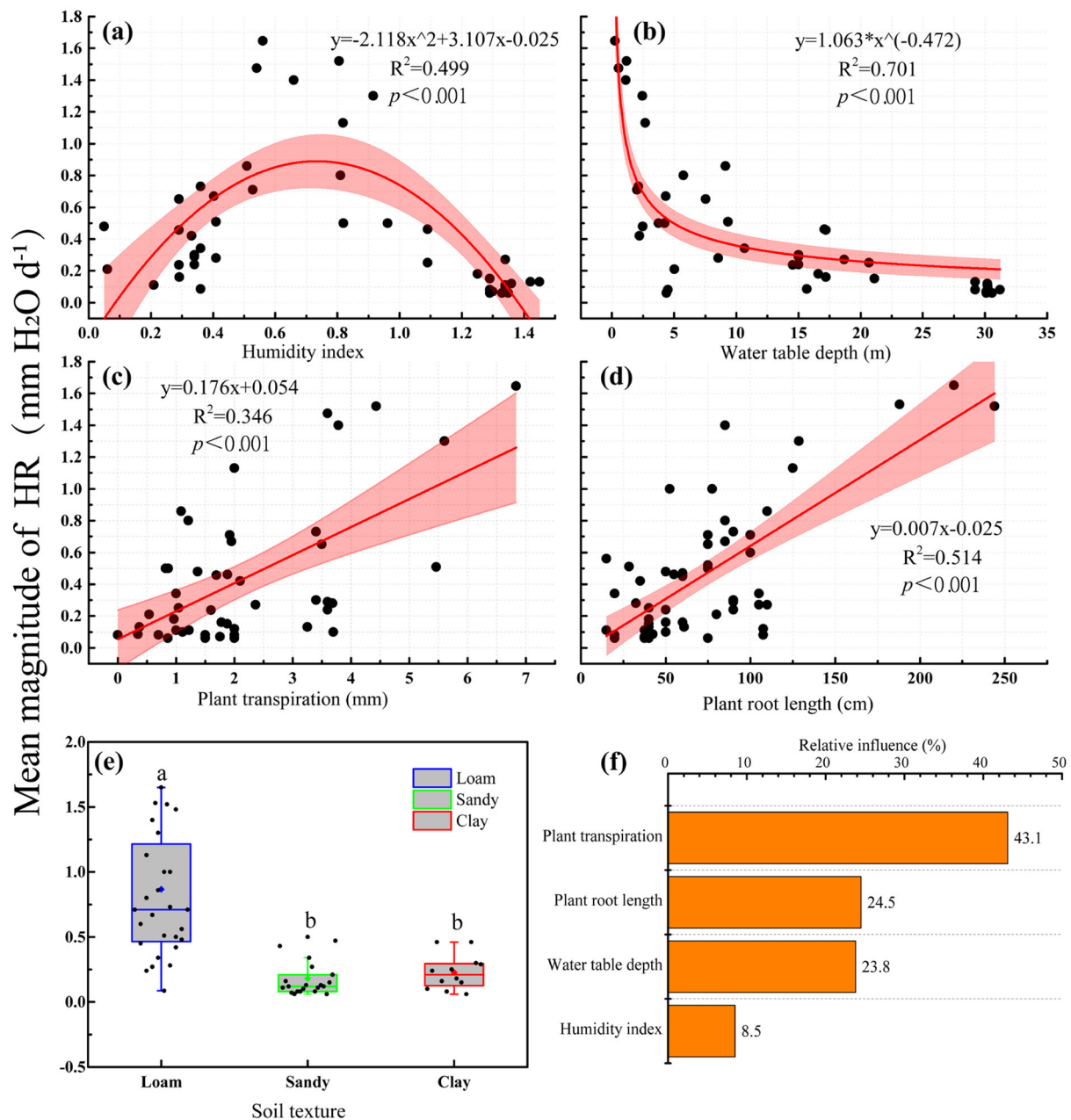


FIGURE 4

The factors influencing HR. Relationship between HR and environmental factors (a–d); (e) influence of soil texture on HR. The boxplots characterize the lower, median, upper quartiles, and the interquartile range (upper quartile–lower quartile), which covers the central 50% of the data. The whiskers represent 95% of the data. The diamond within each boxplot represents the mean and each small circle represents one individual observation. (f) Relative percentages of the influence of vegetation characteristics and environmental factors on HR magnitude. Different lowercase letters indicate significant difference at the 0.05 level.

et al., 2016). In addition, the samples collected in our study were larger. Previous studies of HR were either oriented to specific regions or paid little attention to cross-site influence factors (Prieto et al., 2012; Meunier et al., 2018; Zhang and Zwiazek, 2018). However, our dataset was more comprehensive, and our research results can better represent the actual occurrence of

HR. We also found that the magnitude of HR varied greatly depending upon the ecosystem types (Figure 3A). For example, temperate forests were significantly higher than the other ecosystem types, possibly because this area is mainly distributed in humid and sub-humid areas with seasonal droughts (Scholz et al., 2010), which is more conducive to HR occurrence

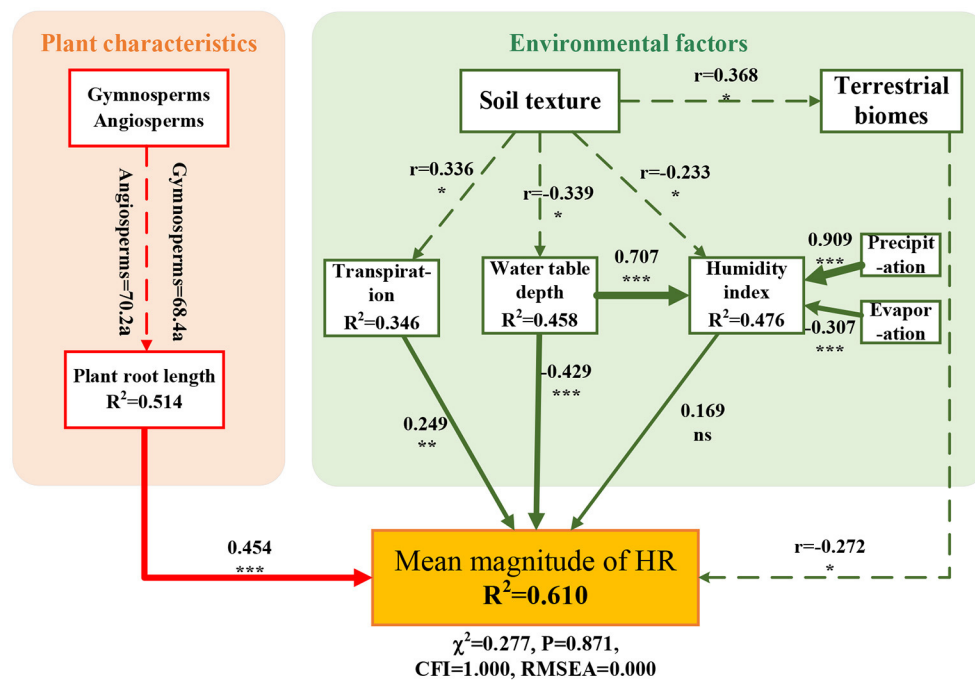


FIGURE 5

Direct and indirect effects of environmental factors on the hydraulic redistribution (HR) magnitude based on structural equation modeling (SEM). SEM fitted with range-standardized coefficients, which link plant type, root length, soil texture, terrestrial biomes, transpiration, water table, and humidity index on the magnitude of HR ( $\chi^2 = 0.277, P = 0.871, CFI = 1.000, RMSEA = 0.000$ ). The number next to the solid arrow represents the normalization coefficient, and the line width corresponds to the strength of the standardized coefficient. Notably, gymnosperms, angiosperms, soil texture, and terrestrial biomes are non-numerical variables. The dashed line shows the correlation between variables, and  $r$  is the correlation coefficient. Letters denote groupings based on *post-hoc* tests. Significance levels are as follows: \*\*\* $p < 0.001$ , \*\* $p < 0.01$ , \* $p < 0.05$ , and ns indicates not significant.

(Neumann and Cardon, 2012). In addition, compared with tropical regions and temperate grasslands, the amount of HR in deserts was also higher. This finding was consistent with the traditional perspective that dry environments are one of the necessary conditions under which to HR occurs (Caldwell et al., 1998; Horton and Hart, 1998). These biome-level estimates of mean magnitude reflect the variability between major habitat types and underline differences across study sites and species (Evaristo and McDonnell, 2017).

Another major finding was that HR in the modeling studies were significantly higher than what was found from the field measurements (Figure 1B). The main reason for this difference could be the sensitivity of modeled HR to root and root-soil conductance (Neumann and Cardon, 2012). The model study quantified (parameterized) HR as a function of water potential between different soil layers (Amenu and Kumar, 2008). Previous studies have reported that the amount of HR is directly proportional to the radial soil-root conductance (Mendel et al., 2002; Wang, 2011). For example, Mendel et al. (2002) found that over the tested range, with each of magnitude increase in radial conductivity of rootlets, HR increased by a factor of 1.4. Thus, this relationship also be a reason for the uncertainty in the HR magnitude research

(Zheng and Wang, 2007). Another reason was that the premise of model simulation research on HR is that the stomata of plants are open during the day and completely closed at night, so the inhibition of transpiration of plants at night on HR was ignored in model research (Dawson et al., 2007). In our study, we used 12 model simulation cases and the weighted average method to further confirm the findings of Siqueira et al. (2008) and Wang (2011). In addition, the amount of HR of angiosperms was significantly higher than that of gymnosperms (Figure 3B). This difference may be because the main conducting elements in angiosperms (xylem vessels) allow for wider variability in element size and wall thicknesses than their conducting element counterparts in gymnosperms (tracheids; Anderegg, 2015). Furthermore, angiosperms have a greater number of parenchyma cells, which are linked to improved hydraulic system efficiency after stressful conditions such as drought (McDowell, 2011). Anatomical differences in the xylem of the two types of plants may explain our finding that angiosperms tend to be more favorable to HR than gymnosperms.

We found that HR accounted for 27.4% of the daily plant transpiration (Figure 2). In fact, this proportion was a very considerable of magnitude. We consider the magnitude of



HR *per se* may be important, however, the proportion of its contribution to plant transpiration would more adequately reflect the eco-hydrologic effects of HR (Sun et al., 2018). If HR contributes a portion of the water required to meet transpiration requirements, the direct hydrologic effect of HR may be significant. Conversely, if HR contributes only a small proportion of transpiration water, it may not have direct and significant hydrologic effects (Neumann and Cardon, 2012). If HR contributes to the transpiration of plants under long-term drought conditions, then HR will have far-reaching significance in promoting ecosystem productivity and protecting plants from drought stress. Previous studies have shown that transpiration increases by 10–40% because of HR in tropical systems (da Rocha et al., 2004), 20–25% in dry and arid environments (Bleby et al., 2010), 19–40% in mesic forests (Jackson et al., 2000), and up to 81% in some Mediterranean ecosystems (Kurz-Besson et al., 2006). However, most of the data provided by such studies are maxima and minima, which do not reliably reflect the average contributions of HR to plant transpiration water requirements. Our study used a weighted average algorithm to address the knowledge gap. In addition, we observed that the proportions of the influence of HR were relatively high in tree and shrub transpiration and low in herb transpiration. This is probably because herbs had shallow roots and were mainly distributed in tropical and subtropical regions where soil water is abundant.

## Analysis on influencing factors of HR

Overall, our synthesis provided a global assessment of how plant characteristics and environmental variables affect HR, which promotes a more comprehensive understanding of the mechanisms of plant root HR (Prieto et al., 2012). We found no significant correlation between annual precipitation and the magnitude of HR. This finding is consistent with the results of previous studies that reported a similar average HR with a mean annual precipitation ranged that from 550 to 2,500 mm (Meinzer et al., 2004). In this case, precipitation intervals and depth may be the key impact factors, which can trigger a cascade of plant physiological responses at different time scales (Huang and Zhang, 2016). However, the amount of HR was significantly correlated with the humidity index (precipitation/evaporation), and as humidity index increased, the amount of HR first increased and then decreased (Figure 4a), which suggested that HR reached an optimal condition when the ratio of precipitation to evaporation reached a certain condition (humidity index = 0.752). In contrast, an extremely dry or humid soil environment was not conducive to the occurrence of HR (Domec et al., 2004). Therefore, after plants have experienced a certain severity or period of drought, the strong transpiration causes the soil moisture to reach a certain level, which stimulates the occurrence of HR and makes it reach a maximum under suitable conditions (Neumann and Cardon, 2012).

The influence of plant root characteristics on the amount of HR has been widely confirmed, and research in this area has focused on the physiological and structural characteristics of plant roots, such as the distribution and pattern of roots or whether the roots have the function of releasing and absorbing water (Scholz et al., 2008b; Wang, 2011). We found that the average root length of plants was significantly positively correlated with the amount of HR (Figure 4d). This finding indicated that when the root length of the plant was longer, the HR was higher than that of plants with shorter roots. Since the heterogeneity of soil moisture content increases with the increase in soil depth, plant root systems must bridge a soil water potential gradient large enough to drive flow. For example, annual herb plants have shorter roots, the difference in shallow soil moisture is smaller, and the magnitude and range of HR are also smaller (Neumann and Cardon, 2012). On the other hand, the long root system of plants is conducive to the absorption and utilization of water sources other than soil moisture, such as river water and deep groundwater, which may increase the amount of HR (Rewald et al., 2015).

Soil texture can influence the potential magnitude of HR (Scholz et al., 2008b; Prieto et al., 2012). Our results demonstrated that the magnitude of HR in loam was significantly higher than in sand or clay (Figure 4e). This may be because the sand content, soil particle size, permeability, and water retention in loam are between sand and clay, which is more conducive to the occurrence of HR (Schymanski et al., 2008). In addition, soil texture affects HR by influencing the soil electrical conductivity and soil moisture. A series of empirical studies showed that with the drying of topsoil, HR first increased to its maximum value, then decreased or remained stable, which suggested that soil texture was important for the maintenance of HR (Warren et al., 2005; Scholz et al., 2008b; Prieto et al., 2010). This phenomenon has also been confirmed in the model simulation (Prieto et al., 2010). The reason may be that in soil with coarse texture, the root-soil contact (electrical conductivity) was more difficult to maintain as a larger aerated pore space would form (Schroder et al., 2008; Schymanski et al., 2008; Prieto et al., 2010).

We found that the amount of HR was negatively correlated with the depth of groundwater, and that the amount of HR decreased as the depth of groundwater increased (Figure 4b). This relationship may be because most of the lateral roots of plants are distributed in the shallow soil layers (Scholz et al., 2008b). A shallower groundwater level is beneficial for increasing the contact area between plant roots and groundwater, thus promoting the amount of HR (Neumann and Cardon, 2012). Studies have shown that New England sugar maple trees maintain a high amount of HR, mainly because the roots of sugar maple trees can reach groundwater (Emerman and Dawson, 1996). Groundwater will increase HR by 0.2 mm H<sub>2</sub>O d<sup>-1</sup> during a simulated drought of up to 100 days in a stand of *Artemisia tridentata* (Ryel et al., 2002). Particularly in an ecosystem where the soil type is sandy, due to the poor

water holding capacity of said soil, the groundwater source provides a sufficient water source for plant HR under drought stress (Neumann and Cardon, 2012). Results showed that increased plant transpiration enhanced the magnitude of HR (Figures 4C,F). Interestingly, in contrast to the above factors that influence HR, plant transpiration is the most significant driver. This is not coincident with Williams et al. (1993), who proposed that low plant transpiration can drive HR during rainstorms. The reason for this explanation is that previous studies focused on comparing the effect of plant transpiration on the amount of HR in certain environments, and thus could not evaluate the impact of plant transpiration on HR in different research sites or plant species (Hafner et al., 2020). Our works provide an insight into the relationship between plant transpiration and the amount of HR in different regions and for different plant species.

## Conclusion

In our synthesis, we determined the magnitude of HR and its contribution to plant transpiration demand and provided a global estimate. The mean magnitude of HR was 0.249 mm H<sub>2</sub>O d<sup>-1</sup>, which accounted for 24.7% of the daily plant transpiration. There were differences in the magnitudes of HR in different biomes. The magnitude of HR in temperate forests was significantly higher than in the other ecosystems. Plant characteristics and environmental factors jointly accounted for 61.0% of the variation in HR. Plant transpiration was the major driver of HR, and we found that soil texture played a key but indirect role in HR. Our study provided new knowledge on the global estimated magnitude of HR and how plant characteristics and environmental factors influence HR magnitude. Further research on HR should focus on the possible synergistic, additive, or antagonistic effects of multiple factors, which will require more empirical studies of multiple factors to clarify the combined effects.

## Data availability statement

The authors declare that the data associated with this study are provided as the Supporting Information Data S1 and R scripts, which are provided via Dryad ([https://datadryad.org/stash/share/fne1XRX1SV-LAMM-JovRfdLRIGDfFwyfGp\\_Gwcssl4](https://datadryad.org/stash/share/fne1XRX1SV-LAMM-JovRfdLRIGDfFwyfGp_Gwcssl4)).

## References

- Amenu, G. G., and Kumar, P. (2008). A model for hydraulic redistribution incorporating coupled soil-root moisture transport. *Hydrol. Earth Syst. Sci.* 12, 55–74. doi: 10.5194/hess-12-55-2008
- Anderegg, W. (2015). Spatial and temporal variation in plant hydraulic traits and their relevance for climate change impacts on vegetation. *New Phytol.* 205, 1008–1014. doi: 10.1111/nph.12907

## Author contributions

GY analyzed the data and wrote the manuscript. LH, GY, and YS conducted data compilation and treatment, data analyzed, and reviewed the manuscript. All authors contributed to the article and approved the submitted version.

## Funding

This study was supported by the National Natural Science Foundation of China (No. 41977420) and Key Research and Development Program of Ningxia Hui Autonomous Region (No. 2021BEG02009).

## Acknowledgments

We would like to thank Russell Doughty at the California Institute of Technology for English editing of the manuscript.

## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

## Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

## Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fpls.2022.918585/full#supplementary-material>

- Baker, I. T., Prihodko, L., Denning, A. S., Goulden, M., Miller, S., and da Rocha, H. R. (2008). Seasonal drought stress in the Amazon: reconciling models and observations. *J. Geophys. Res. Biogeosci.* 113:G00B01. doi: 10.1029/2007JG000644

- Bleby, T. M., McElrone, A. J., and Jackson, R. B. (2010). Water uptake and hydraulic redistribution across large woody root systems to 20 m depth. *Plant Cell Environ.* 33, 2132–2148. doi: 10.1111/j.1365-3040.2010.02212.x

- Bogie, N. A., Bayala, R., Diedhiou, I., Conklin, M. H., Fogel, M. L., Dick, R. P., et al. (2018). Hydraulic redistribution by native sahelian shrubs: bioirrigation to resist in-season drought. *Front. Env. Sci.* 6:98. doi: 10.3389/fenvs.2018.00098
- Brooks, J. R., Meinzer, F. C., Coulombe, R., and Gregg, J. (2002). Hydraulic redistribution of soil water during summer drought in two contrasting Pacific Northwest coniferous forests. *Tree Physiol.* 22, 1107–1117. doi: 10.1093/treephys/22.15-16.1107
- Burgess, S. S. O., Adams, M. A., Turner, N. C., and Ong, C. K. (1998). The redistribution of soil water by tree root systems. *Oecologia* 115, 306–311. doi: 10.1007/s004420050521
- Burgess, S. S. O., Adams, M. A., Turner, N. C., White, D. A., and Ong, C. K. (2001). Tree roots: conduits for deep recharge of soil water. *Oecologia* 126, 158–165. doi: 10.1007/s004420000501
- Caldwell, M. M., Dawson, T. E., and Richards, J. H. (1998). Hydraulic lift: consequences of water efflux from the roots of plants. *Oecologia* 113, 151–161. doi: 10.1007/s004420050363
- Cleverly, J., Eamus, D., Coupe, N. R., Chao, C., Maes, W., and Li, L. (2016). Soil moisture controls on phenology and productivity in a semi-arid critical zone. *Sci. Total Environ.* 568, 1227–1237. doi: 10.1016/j.scitotenv.2016.05.142
- da Rocha, H. R., Goulden, L. M., and Miller, S. D. (2004). Seasonality of water and heat fluxes over a tropical forest in eastern amazonia. *Ecol. Appl.* 14, 22–32. doi: 10.1890/02-6001
- Dawson, T. E., Burgess, S. S. O., Tu, K. P., Oliveira, R. S., Santiago, L. S., Fisher, J. B., et al. (2007). Nighttime transpiration in woody plants from contrasting ecosystems. *Tree Physiol.* 27, 561–575. doi: 10.1093/treephys/27.4.561
- DeMalach, N., Zaady, E., and Kadmon, R. (2017). Contrasting effects of water and nutrient additions on grassland communities: a global meta-analysis. *Glob. Ecol. Biogeogr.* 26, 983–992. doi: 10.1111/geb.12603
- Domec, J. C., Warren, J. M., Meinzer, F. C., Brooks, J. R., and Coulombe, R. (2004). Native root xylem embolism and stomatal closure in stands of Douglas-fir and ponderosa pine: mitigation by hydraulic redistribution. *Oecologia* 141, 7–16. doi: 10.1007/s00442-004-1621-4
- Emerman, S. H., and Dawson, T. E. (1996). Hydraulic lift and its influence on the water content of the rhizosphere: an example from sugar maple, *Acer saccharum*. *Oecologia* 108, 273–278. doi: 10.1007/BF00334651
- Evaristo, J., and McDonnell, J. J. (2017). Prevalence and magnitude of groundwater use by vegetation: a global stable isotope meta-analysis. *Sci. Rep.* 7, 44110. doi: 10.1038/srep44110
- Fan, Y., Li, H., and Miguez-Macho, G. (2013). Global patterns of groundwater table depth. *Science* 339, 940–943. doi: 10.1126/science.1229881
- Fu, C. S., Wang, G. L., Bible, K., Goulden, M. L., Saleska, S. R., Scott, R. L., et al. (2018). Hydraulic redistribution affects modeled carbon cycling via soil microbial activity and suppressed fire. *Global Change Biol.* 24, 3472–3485. doi: 10.1111/gcb.14164
- Gao, J. J., and Yohay, C. (2020). A global meta-analysis of grazing effects on plant richness. *Agr. Ecosyst. Environ.* 302, 107072. doi: 10.1016/j.agee.2020.107072
- García-Palacios, P., Mckie, B. G., Handa, I. T., Frainer, A., and Hättenschwiler, S. (2015). The importance of litter traits and decomposers for litter decomposition: a comparison of aquatic and terrestrial ecosystems within and across biomes. *Funct. Ecol.* 30, 819–829. doi: 10.1111/1365-2435.12589
- Hafner, B. D., Hesse, B. D., Buerle, T. L., and Grams, T. E. E. (2020). Water potential gradient, root conduit size and root xylem hydraulic conductivity determine the extent of hydraulic redistribution in temperate trees. *Funct. Ecol.* 34, 561–574. doi: 10.1111/1365-2435.13508
- Hafner, B. D., Martina, T., Karl-Heinz, H., Marc, G., Rainer, M., and Grams, T. (2017). Hydraulic redistribution under moderate drought among english oak, european beech and norway spruce determined by deuterium isotope labeling in a split-root experiment. *Tree Physiol.* 37, 1–11. doi: 10.1093/treephys/tpx050
- Horton, L. J., and Hart, S. C. (1998). Hydraulic lift: a potentially important ecosystem process. *Trends Ecol. Evol.* 13, 232–235. doi: 10.1016/S0169-5347(98)01328-7
- Huang, L., and Zhang, Z. S. (2016). Effect of rainfall pulses on plant growth and transpiration of two xerophytic shrubs in a revegetated desert area: Tengger Desert, China. *Catena* 137, 269–276. doi: 10.1016/j.catena.2015.09.020
- Jackson, B. R., Schenk, H. J., Jobbágy, E. G., Canadell, J., Colello, G. D., and Dickinson, R. E., et al. (2000). Belowground consequences of vegetation change and their treatment in models. *Ecol. Appl.* 10, 470–483. doi: 10.1890/1051-0761(2000)010[0470:BCOVCA]2.0.CO;2
- Kurz-Besson, C., Otieno, D., do Vale, R. L., Siegwolf, R., Schmidt, M., Herd, A., et al. (2006). Hydraulic lift in cork oak trees in a savannah-type mediterranean ecosystem and its contribution to the local water balance. *Plant Soil*. 282, 361–378. doi: 10.1007/s11104-006-0005-4
- Lee, E., Kumar, P., Barron-Gafford, G. A., Hendryx, S. M., Sanchez-Cañete, E. P., Minor, R. L., et al. (2018). Impact of hydraulic redistribution on multispecies vegetation water use in a semiarid savanna ecosystem: an experimental and modeling synthesis. *Water Resour. Res.* 54, 4009–4027. doi: 10.1029/2017WR021006
- Leffler, A. J., Peek, M. S., Ryel, R. J., Ivans, C. Y., and Caldwell, M. M. (2005). Hydraulic redistribution through the root systems of senesced plants. *Ecology* 86, 633–642. doi: 10.1890/04-0854
- McDowell, N. G. (2011). Mechanisms linking drought, hydraulics, carbon metabolism, and vegetation mortality. *Plant Physiol.* 155, 1051–1059. doi: 10.1104/pp.110.170704
- Meinzer, F. C., Brooks, J. R., Bucci, S., Goldstein, G., Scholz, F. G., and Warren, J. M. (2004). Converging patterns of uptake and hydraulic redistribution of soil water in contrasting woody vegetation types. *Tree Physiol.* 24, 919–928. doi: 10.1093/treephys/24.8.919
- Mendel, M., Hergarten, S., and Neugebauer, H. J. (2002). On a better understanding of hydraulic lift: a numerical study. *Water Resour. Res.* 38, 1–10. doi: 10.1029/2001WR000911
- Meunier, F., Rothfuss, Y., Bariac, T., Biron, P., Richard, P., Durand, J. L., et al. (2018). Measuring and modeling hydraulic lift of lolium multiflorum using stable water isotopes. *Vadose Zone J.* 17, 1–15. doi: 10.2136/vzj2016.12.0134
- Nadezhkina, N., Ferreira, M. I., Conceição, N., Pacheco, C. A., Häusler, M., and David, T. S. (2015). Water uptake and hydraulic redistribution under a seasonal climate: long-term study in a rainfed olive orchard. *Ecophysiology* 8, 387–397. doi: 10.1002/eco.1545
- Neumann, R. B., and Cardon, Z. G. (2012). The magnitude of hydraulic redistribution by plant roots: a review and synthesis of empirical and modeling studies. *New Phytol.* 194, 337–352. doi: 10.1111/j.1469-8137.2012.04088.x
- Pomazkina, L. V., and Lubnina, E. V. (2002). Seasonal and long-term dynamics of the carbon of microbial biomass in plowed soils of the forest-steppe zone in the Baikal region. *Eurasian Soil.* 35, 165–171. doi: 10.1055/s-0030-1263116
- Prieto, I., Armas, C., and Pugnaire, F. I. (2012). Water release through plant roots: new insights into its consequences at the plant and ecosystem level. *New Phytol.* 193, 830–841. doi: 10.1111/j.1469-8137.2011.04039.x
- Prieto, I., Martínez-Tillería, K., Martínez-Manchego, L., Montecinos, S., Pugnaire, F. I., and Squeo, F. A. (2010). Hydraulic lift through transpiration suppression in shrubs from two arid ecosystems: patterns and control mechanisms. *Oecologia* 163, 855–865. doi: 10.1007/s00442-010-1615-3
- Rewald, B., Ephrath, J. E., and Rachmilevitch, S. (2015). A root is a root is a root? Water uptake rates of Citrus root orders. *Plant Cell Environ.* 34, 33–42. doi: 10.1111/j.1365-3040.2010.02223.x
- Ryel, R., Caldwell, M., oder, C., Y., Or, D., and Leffler, A. (2002). Hydraulic redistribution in a stand of *Artemisia tridentata*: evaluation of benefits to transpiration assessed with a simulation model. *Oecologia* 130, 173–184. doi: 10.1007/s004420100794
- Scholz, F. G., Bucci, S. J., Goldstein, G., Moreira, M. Z., Meinzer, F. C., and Domec, J. C., et al. (2008b). Biophysical and life-history determinants of hydraulic lift in neotropical savanna trees. *Funct. Ecol.* 22, 773–786. doi: 10.1111/j.1365-2435.2008.01452.x
- Scholz, F. G., Bucci, S. J., Goldstein, G., Meinzer, F. C., Franco, A. C., and Miralles-Wilhelm, F. (2008a). Temporal dynamics of stem expansion and contraction in savanna trees: withdrawal and recharge of stored water. *Tree Physiol.* 28, 469–480. doi: 10.1093/treephys/28.3.469
- Scholz, F. G., Bucci, S. J., Hoffmann, W. A., Meinzer, F. C., and Goldstein, G. (2010). Hydraulic lift in a Neotropical savanna: experimental manipulation and model simulations. *Agr. Forest. Meteorol.* 150, 629–639. doi: 10.1016/j.agrformet.2010.02.001
- Schroder, T., Javaux, M., Vanderborght, J., Korfgén, B., and Vereecken, H. (2008). Effect of local soil hydraulic conductivity drop using a three-dimensional root water uptake model. *Vadose Zone J.* 7, 1089–1098. doi: 10.2136/vzj2007.0114
- Schymanski, S. J., Sivapalan, M., Roderick, M. L., Beringer, J., and Hutley, L. B. (2008). An optimality-based model of the coupled soil moisture and root dynamics. *Hydrol. Earth Syst. Sci.* 12, 913–932. doi: 10.5194/hess-12-913-2008
- Sian, K. G., and Menge, D. N. L. (2021). Nitrogen-fixing trees increase soil nitrous oxide emissions: a meta-analysis. *Ecology* 102, e03415. doi: 10.1002/ecy.3415
- Siqueira, M., Katul, G., and Porporato, A. (2008). Onset of water stress, hysteresis in plant conductance, and hydraulic lift: scaling soil water dynamics from millimeters to meters. *Water Resour. Res.* 44, W01432. doi: 10.1029/2007WR006094

- Sun, L., Yang, L., Chen, L. D., Zhao, F. K., and Li, S. J. (2018). Hydraulic redistribution and its contribution to water retention during short-term drought in the summer rainy season in a humid area. *J. Hydrol.* 566, 377–385. doi: 10.1016/j.jhydrol.2018.09.032
- Veroniki, A. A., Jackson, D., Viechtbauer, W., Bender, R., Bowden, J., Knapp, G., et al. (2016). Methods to estimate the between-study variance and its uncertainty in meta-analysis. *Res. Synth. Methods* 7, 55–79. doi: 10.1002/jrsm.1164
- Wang, G. L. (2011). Assessing the potential hydrological impacts of hydraulic redistribution in Amazonia using a numerical modeling approach. *Water Resour. Res.* 47, W02528. doi: 10.1029/2010WR009601
- Wang, Y. Y., Jia, B. H., and Xie, Z. H. (2018). Impacts of hydraulic redistribution on eco-hydrological cycles: a case study over the Amazon basin. *Sci China Earth Sci.* 61, 1330–1340. doi: 10.1007/s11430-017-9219-5
- Warren, J. M., Meinzer, F. C., and Brooks, J. R. (2007). Hydraulic redistribution of soil water in two old-growth coniferous forests: quantifying patterns and controls. *New Phytol.* 173, 753–765. doi: 10.1111/j.1469-8137.2006.01963.x
- Warren, J. M., Meinzer, F. C., Brooks, J. R., and Domec, J. C. (2005). Vertical stratification of soil water storage and release dynamics in Pacific Northwest coniferous forests. *Agr. Forest. Meteorol.* 130, 39–58. doi: 10.1016/j.agrformet.2005.01.004
- Williams, K., Caldwell, M. M., and Richards, J. H. (1993). The influence of shade and clouds on the soil-water potential - the buffered behavior of hydraulic lift. *Plant Soil.* 157, 83–95. doi: 10.1007/BF00038751
- Xu, X. F., Thornton, P. E., and Post, W. M. (2013). A global analysis of soil microbial biomass carbon, nitrogen and phosphorus in terrestrial ecosystems. *Glob. Ecol. Biogeogr.* 22, 737–749. doi: 10.1111/geb.12029
- Yu, K., and D'Odorico, P. (2015). Hydraulic lift as a determinant of tree-grass coexistence on savannas. *New Phytol.* 207, 1038–1051. doi: 10.1111/nph.13431
- Yu, T. F., Feng, Q., Si, J. H., Mitchell, P. J., Forster, M. A., Zhang, X. Y., et al. (2018). Depressed hydraulic redistribution of roots more by stem refilling than by nocturnal transpiration for *Populus euphratica* Oliv. *in situ* measurement. *Ecol. Evol.* 8, 2607–2616. doi: 10.1002/ece3.3875
- Yu, T. F., Feng, Q., Si, J. H., Xi, H. Y., and Li, W. (2013). Patterns, magnitude, and controlling factors of hydraulic redistribution of soil water by *Tamarix ramosissima* roots. *J. Arid Land.* 5, 396–407. doi: 10.1007/s40333-013-0173-z
- Zhang, W. Q., and Zwiazek, J. J. (2018). Hydraulic redistribution in slender wheatgrass (*Elymus trachycaulus* Link Malte) and yellow sweet clover (*Melilotus officinalis* L.): potential benefits for land reclamation. *Agron J.* 8, 308. doi: 10.3390/agronomy8120308
- Zhang, Y. F., Wang, X. P., Pan, Y. X., Hu, R., and Chen, N. (2021). Global quantitative synthesis of effects of biotic and abiotic factors on stemflow production in woody ecosystems. *Glob. Ecol. Biogeogr.* 30, 1713–1723. doi: 10.1111/geb.13322
- Zheng, Z., and Wang, G. L. (2007). Modeling the dynamic root water uptake and its hydrological impact at the Reserva Jaru site in Amazonia. *J. Geophys. Res. Biogeosci.* 112, G04012. doi: 10.1029/2007JG000413
- Zhou, Z. H., Wang, C. K., and Luo, Y. Q. (2020). Meta-analysis of the impacts of global change factors on soil microbial diversity and functionality. *Nat. Commun.* 11, 3072. doi: 10.1038/s41467-020-16881-7





## OPEN ACCESS

## EDITED BY

Iván Prieto,  
Universidad de León, Spain

## REVIEWED BY

Andrea Carminati,  
ETH Zürich, Switzerland  
Ray Dybzinski,  
Loyola University Chicago,  
United States  
Frantisek Baluska,  
University of Bonn, Germany

## \*CORRESPONDENCE

Ciro Cabal  
ciro.cabal@gmail.com

## SPECIALTY SECTION

This article was submitted to  
Functional Plant Ecology,  
a section of the journal  
Frontiers in Plant Science

RECEIVED 03 June 2022

ACCEPTED 08 July 2022

PUBLISHED 04 August 2022

## CITATION

Cabal C (2022) Root tragedy of the  
commons: Revisiting the mechanisms  
of a misunderstood theory.  
*Front. Plant Sci.* 13:960942.  
doi: 10.3389/fpls.2022.960942

## COPYRIGHT

© 2022 Cabal. This is an open-access  
article distributed under the terms of  
the [Creative Commons Attribution  
License \(CC BY\)](#). The use, distribution  
or reproduction in other forums is  
permitted, provided the original  
author(s) and the copyright owner(s)  
are credited and that the original  
publication in this journal is cited, in  
accordance with accepted academic  
practice. No use, distribution or  
reproduction is permitted which does  
not comply with these terms.

# Root tragedy of the commons: Revisiting the mechanisms of a misunderstood theory

Ciro Cabal<sup>1,2\*</sup>

<sup>1</sup>High Meadows Environmental Institute, Princeton University, Princeton, NJ, United States,

<sup>2</sup>Department of Biogeography and Global Change, National Museum of Natural Sciences, MNCN,  
CSIC, Madrid, Spain

Fine root density in the soil is a plant functional trait of paramount importance for plant ecology and agriculture. Fine root proliferation by plants involves complex plant strategies that may depend on various abiotic and biotic factors. Concretely, the root tragedy of the commons (RToC) is a behavioral strategy predicted by game theory models in which interacting plants forage for soil resources inefficiently. Generally, researchers assume that the RToC is a proactive competition strategy directly induced by the non-self roots. In this opinion, I recall Hardin's original definition of the tragedy of the commons to challenge this notion. I argue that the RToC is a suboptimal phenotypically plastic response of the plants based on the soil resource information exclusively, and I discuss how this alternative perspective carries important implications for the design of experiments investigating the physiological mechanisms underlying observable plant root responses.

## KEYWORDS

game theory, plant behavioral ecology, plant competition, plant interaction mechanisms, root foraging strategies, root methods

## Introduction

Ecologists use a large array of root functional traits to study plants (Freschet et al., 2021a,b). Root density (i.e., root biomass per volumetric unit of soil) is an important but often neglected plant trait that contains information about the resource investment of plants into foraging belowground (Cabal et al., 2021a). Understanding how plants allocate biomass belowground is important in the context of climate change for a precise assessment of carbon storage in plants (Xia et al., 2017; Qi et al., 2019) and for a more efficient food production (Anten and Vermeulen, 2016; Fréville et al., 2019). Root allocation strategies in plants are complex (McNickle et al., 2016) and may be based on a combination of various abiotic (resources in soil) and biotic (root detection, inter-plant communication, and soil microorganisms) information sources (Novoplansky, 2019; Chen et al., 2020). As a result of such complexity, different plant species may invest more or less into their roots as a response to the presence of non-self roots in the soil (Belter and Cahill, 2015; Postma et al., 2021).

Over the last two decades, the Root Tragedy of the Commons (RToC)—initially a concept with potential to explain fine root density in competitive contexts—has become a controversial idea. Inspired by Donald (1968)’s plant ideotypes, Zhang et al. (1999) published the first game theory model of plant root proliferation as a response to competition resulting in root growth redundancy: plants in their model could increase their yield by reducing root growth. They named this phenomenon an RToC based on Hardin (1968)’s theory. Shortly after, Gersani et al. (2001) observed higher root density at the expense of yield in plants sharing rooting volume with conspecific neighbors as compared with plants growing solo, seemingly validating the RToC experimentally. Root ecologists first replicated their owned/shared experimental design finding similar results (Maina et al., 2002; Falik et al., 2003; O’Brien et al., 2005). Yet, later publications questioned the empirical evidence of an RToC alleging problems with Gersani’s owned/shared design (Laird and Aarssen, 2005; Schenk, 2006; Hess and De Kroon, 2007; Semchenko et al., 2007; Chen et al., 2012, 2015, 2020; McNickle, 2020) (see Box 1).

The ecology of plant interactions is currently moving toward an approach centered on individual plants and their phenotypic plasticity (Bakker et al., 2021; Escudero et al., 2021) but there is very little we know about how plant interactions affect the plastic response of plants to abiotic conditions (Wang and Callaway, 2021). Accounting for the RToC represents a mechanistic approach to the study of phenotypically plastic responses of plants to neighbors, because game theory produces mechanistically informed dynamic predictions. To promote this approach, I briefly revisit the literature from a critical perspective and sticking to the original definition of a “tragedy of the commons” (*sensu* Hardin, 1968). I argue that, while researchers who question the RToC have focused on experimental flaws, there is an underlying dissent between their use of the RToC and Hardin’s definition. They treat the RToC as a proactive competitive strategy, while it is an inevitable consequence of the simplest optimization of resource foraging. This confusion has led to the disparagement of a theory that can be crucial to interpret experimental results correctly and to better understand root-density phenotypic plasticity in the plants.

## Discussion

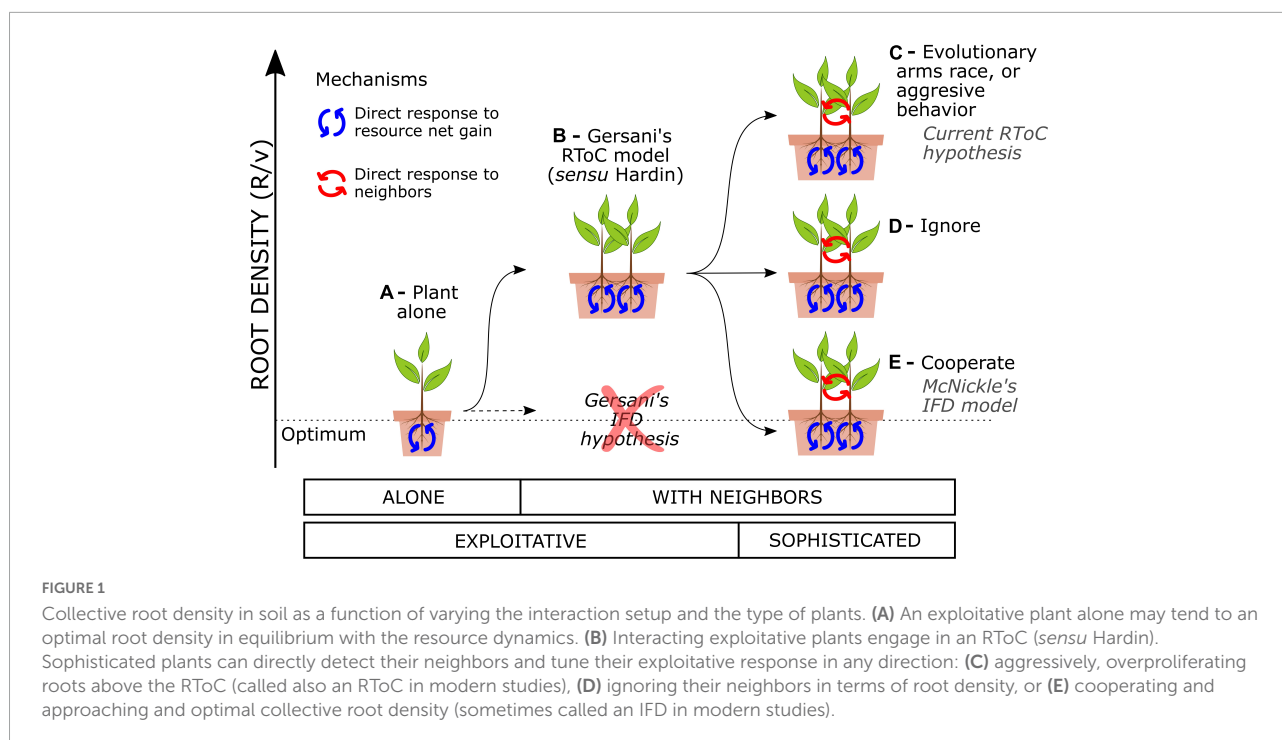
### Defining the root tragedy of the common

Hardin (1968) defined the tragedy of the commons using the famous example of the shared pasture, the herdsman, and the cattle. In this example, each herdsman finds a net reward in adding one more head to his herd above the optimal

cattle density, because the benefits from the animal are for the herdsman alone but the community shares the costs of the decreased pasture quality. The herdsman does not need to do this calculation; he will just realize that adding one more animal is profitable. Since all the herdsman act the same way, they will overexploit the common resource unwittingly, to the detriment of all of them. In a plant root analogy, each individual of plant represents a herdsman, each root unit is like an animal that forages at a cost, and the soil resource plays the role of the pasture.

Plants that are programmed to forage optimally for soil resources disregarding the presence of the neighbors will engage in an RToC. Plants foraging strategies consist in adjusting their root density to the environment through evolutionary fixed traits or phenotypic plasticity. Plants display a large phenotypic plasticity in fine root proliferation (Callaway et al., 2003; Kembel and Cahill, 2005) and will adjust their root density over their lifespan based on both the abiotic information (Hodge, 2004) and the presence of competing neighbors (Craine, 2006). I will herein assume that the most basic plant response is the exploitative response to the abiotic environment, i.e., plants that adjust their root growth to the net resource gain that such roots return (Figure 1A). For instance, an imaginary plant species that had evolved growing with no neighbors for millions of years in heterogeneous soils should become exploitative. Exploitative plants still respond to the presence of neighbors, because the neighbors modify the resource dynamics in soil and the plant must adjust its root density accordingly (Craine, 2005; O’Brien and Brown, 2008; Pierik et al., 2013). Game theory models that assess the response of the purely exploitative plants to the presence of neighbors predict that such plants must engage in an RToC (Zhang et al., 1999; Gersani et al., 2001; Cabal et al., 2020). Gersani et al. (2001) hypothesized that competing exploitative plants could either downregulate root growth to keep collective root density constant as plant population density increases (an “ideal free distribution,” IFD) or follow game theory model predictions and overproliferate roots with respect to the collective optimum (RToC)—not necessarily to the plant alone (Kim et al., 2021). They used the IFD as a null hypothesis to test against the RToC in their experiments with soybeans, finding empirical support for the RToC (Figure 1B).

Plants that have developed a direct response to the presence of neighbors, i.e., that are competing proactively, may use adventitious decision-making algorithms to tune their exploitative response to the present neighbors (hereafter “sophisticated plants”). Evolutionary ecology gives examples of plants evolving fixed suboptimal strategies as a direct response to competitors (Rankin et al., 2007), such as the case of the evolutionary arms race in competition for light which leads to trees investing in trunk wood (Falster and Westoby, 2003; Dybzinski et al., 2011). Also, many publications explicitly state or implicitly assume that plants engage in



an RToC when they overproliferate roots as a response to direct self/non-self root discrimination (see for instance, Hess and De Kroon, 2007; Padilla et al., 2013; Chen et al., 2015, 2020; McNickle, 2020), a strategy that could be called a root aggressive behavior. Both hypotheses, the root arms race and the root aggressive behavior, are reasonable, but neither of them represents an RToC (Figure 1C). Sophisticated plants could also reduce their root density with respect to the exploitative RToC as a response to neighbor presence, either behaviorally or through evolutionary fixed strategies. McNickle and Brown (2014) call such strategy an IFD in their model of plants engaging in collective optimal root foraging strategies (Figure 1E). While understanding both the exploitative and the sophisticated responses of plants has an intrinsic value, the use of the same terminology (RToC and IFD) to name different mechanisms driving plant responses has led to confusion in the field.

According to game theory, only sophisticated plants—not purely exploitative ones—can pursue a collective optimal strategy. Game theory models suggest that the active recognition of other stakeholders and the implementation of complex mechanisms is actually necessary to avoid a tragedy of the commons (He et al., 2015; Murase and Baek, 2018). Likewise, the sophisticated plants need to gather information (other than detecting the mere presence) about each other in order to avoid the RToC and engage in an IFD *sensu* Gersani et al. (2001). This is conspicuous when assessing the cooperative solution of game theory root competition models: the optimization conditions for

every individual depend on the net-gain-generating equations of all the coexisting individuals (Pulliam et al., 1982; McNickle and Brown, 2014; Cabal et al., 2020). Accordingly, plants showing an optimal root density when interacting with each other must be able to measure the total resource net gain of non-self roots in the shared soil. Plants must be unable to avoid engaging in an RToC if they lack the physiological capacity to gather complex information about non-self roots, such as how efficient they are foraging resources. This represents a challenge for maximizing yield in crops by means of controlling root growth (Schneider and Lynch, 2020).

## Identifying the root tragedy of the common

Experimental designs, and in particular, Gersani et al. (2001)'s owned/shared setup, have had a central role in the RToC controversy. The owned/shared experimental design consists in a control treatment in which a plant owns a unit of soil volume (a pot or compartment), and an interaction treatment in which two plants share two units of soil volume. It represents a very convenient experimental design due to its simplicity. Criticisms to this experimental setup are the basis of most studies questioning the classic RToC, but there are good reasons to believe the design is actually correct (see Box 1).

Alternatively, the mesh divider experimental design has become popular (McNickle, 2020) and is often used today

### BOX 1 Gersani's classic-owned/shared experimental design: A defense.

Owned/shared systems were used by Gersani et al. (2001) to produce control and interaction setups with constant total soil volume and nutrients available at the community level. The first limitation of this experimental design was identified by Laird and Aarssen (2005), who noticed that, because the intermingled roots in the shared container are weighted in bulk, owned/shared experimental designs can identify a spurious relationship between the shoot-to-root biomass ratio and the RToC because of the size inequalities leading to an aggregation bias. The confounding effects of changing volume and nutrients available to the plant with neighbor presence were highlighted shortly after (Hess and De Kroon, 2007; Semchenko et al., 2007) triggering most subsequent controversy (see McNickle, 2020 for a comprehensive review). According to these critiques, nutrients can be a confounding factor because, in the interaction treatment, the neighbors will deplete resources leading to lower resource concentration than in the control treatment. To control for this, they suggested keeping the amount of resource available per capita constant across treatments. Soil volume is a confounding factor because increasing rooting volume may promote an increase in root proliferation. Unlike the case of nutrients, rooting volume is not a fungible resource, i.e., all the soil volume is available to all the plants; hence, in the interaction treatment each plant has access to twice the soil volume than in the control. The combination of both factors is found particularly difficult to deal with, and in order to get around these problems researchers have suggested complex designs changing the pot volume and nutrient concentration (Chen et al., 2015), and have developed complex methods to analyze the resulting data (McNickle and Brown, 2014; Chen et al., 2020; McNickle, 2020). In this box, I will support the original approach of Gersani et al. (2001). I defend that the handling of nutrient availability and soil volume in classic owned/shared systems does not represent a problem to experimentally identify an RToC, because the control is not meant to represent the behavior of a plant alone but rather a way to estimate optimal root density.

#### A. Nutrient availability:

Game-theory RToC models are based on basic exploitative plants (Zhang et al., 1999; Gersani et al., 2001; Zea-Cabrera et al., 2006). Take the seminal model by Gersani et al. (2001)'s resource net gain ( $G$ ) for a focal plant ( $i$ ):

$$G_i(u_i, x) = \frac{u_i}{x} H(x) - C(u_i) \quad (1)$$

where  $u_i$  is the root density of each individual plant,  $x$  is the total root density so that  $x = \sum_{i=1}^n u_i$ ,  $H$  is a saturating function that yields the total amount of resources taken up by all  $n$  plants in soil (hence, the first term in the equation yields the resources uptaken by the focal plant), and  $C$  is a cost function. Because each plant optimizes its own net gain selfishly ( $\partial G / \partial u_i = 0$ ), plants in the model adjust their root proliferation to the resource net gain exclusively. Hence, plants engage in an RToC based on the exploitative information only. Indeed, a similar game theory model shows that plants not only increase their root density when a neighbor is present locally (Cabal et al., 2020), but also solo plants will increase their root density identically if the rate of the physical loss of resource rises (Cabal et al., 2021b). The RToC is an exploitative response, which means that it is based in the quasi-equilibrium conditions of the resources entering and exiting the soil, including the resource depletion caused by non-self roots. Adding supplementary resources in the interaction treatment of the owned/shared experiments to compensate for the neighbor-induced resource depletion overrides any possible evidence for an RToC, because it would cancel the mechanisms that trigger the RToC.

#### B. Soil volume:

The concerns regarding rooting volume based on the idea that each plant has access to twice the rooting volume in the interacting treatment are not justified, because in the owned/shared experiments testing for an RToC, the rooting volume is kept constant across treatments at the community level. Gersani et al. (2001) analyzed their root tragedy of the commons (RToC) model in a particular scenario in which both the total number of plants ( $n = 10$ ) and the total rooting volume available to these plants were fixed. They considered three particular cases: ( $N = 1$ ) the soil is partitioned in 10 equal compartments, with each plant having access to one of them (equivalent to their control treatment); ( $N = 2$ ) the soil is partitioned in five equal compartments shared by pairs of plants (equivalent to their interaction treatment); and ( $N = 10$ ) the soil is not partitioned, with all plants having access to all the rooting volume. The value of  $N$  indicates the number of plants sharing each soil partition. A formal definition to the  $H(x)$  and  $C(u)$  functions from Eq. 1 should first be established to be consistent with the equations that Gersani et al. (2001) may have used, with their graphical results as a reference. Let us assume that the nutrient uptake function  $H(x)$  is a saturating function of the following form:

$$H(x) = \frac{\varphi - \varphi e^{-\theta x}}{\theta} \quad (2)$$

and the cost function is a quadratic equation of the following form:

$$C(u) = \alpha u^2 + \beta u \quad (3)$$

As the ESS is the solution that, if adopted by all coexisting plants— $u^* = u_i$  for any  $i$ —maximizes the resource net gain with respect to  $u_i$ , and must satisfy  $\partial G / \partial u_i = 0$ , we can write

$$\frac{N-1}{N} \frac{(\varphi - \varphi e^{-\theta n u^*})}{\theta n u^*} + \frac{1}{N} \varphi e^{-\theta n u^*} = 2\alpha u^* + \beta \quad (4)$$

Using these equations, we can accurately reproduce all the results from Gersani et al. (2001). Unfortunately, there is no analytical solution that can be derived for  $u^*$  in Eq. 4. But, we can approach the solutions numerically for the parameter values of  $\varphi = 0.3$ ,  $\theta = 0.25$ ,  $\alpha = 0.025$ , and  $\beta = 0.025$ , reproducing the authors' original results, obtaining for the control treatment  $u_{N=1}^* \approx 2.6181$  Units of Root ( $R$ ), the interaction treatment  $u_{N=2}^* \approx 3.0174 R$ , and the ten plants sharing a soil volume  $u_{N=10}^* \approx 3.3959 R$ . These values represent the total amount of roots one plant produces in the total soil volume.

Model results do not control for rooting volume in the manner assumed by researchers (i.e., accounting for the rooting volume actually available for each plant in each "pot"). However, this can be calculated by defining a "pot" or unit of rooting volume ( $v$ ) as a tenth of the total soil volume in the model. Thereafter,  $m$  can be defined as the number of rooting volume units per compartment, while the plant root density  $d$  can be defined as the units of root for each plant in each soil compartment ( $R/v$ ). We can calculate each plant's root density in equilibrium using:

$$d^* = \frac{u^*}{m} \quad (5)$$

(Continued)

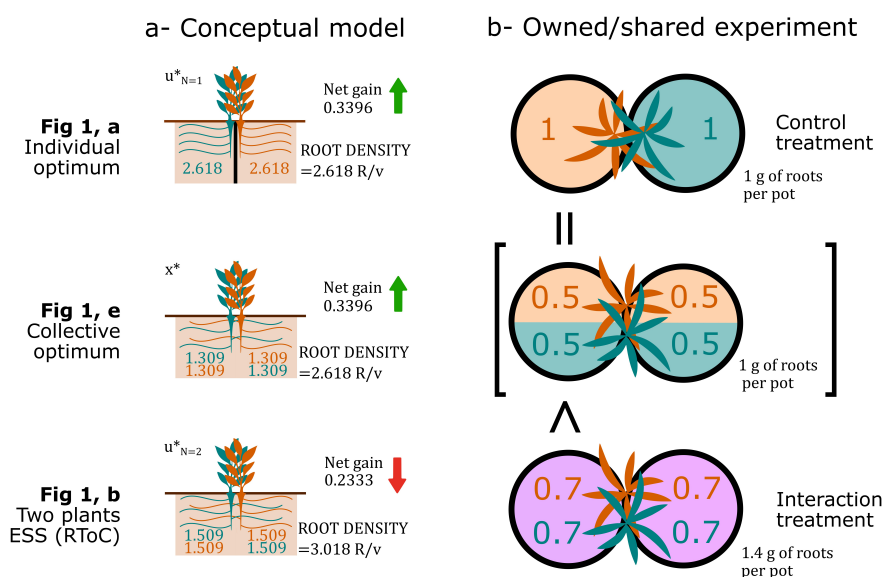


Although root production per plant in the total soil volume increases with competition intensity ( $N$ ), the root density per plant actually decreases; for the control treatment, root density per plant is  $d_{N=1}^* \approx 2.618 R/v$ , and for the interaction treatment, this value is  $d_{N=2}^* \approx 1.509 R/v$ . Nonetheless, such result does not mean that plants are not engaging in an RTToC. The optimal collective rooting strategy  $x^*$  satisfying  $dG_T/dx = 0$  (the optimal root proliferation for any amount of plants sharing a unit of soil volume), where:

$$G_T(x) = H(x) - C(x) \quad (6)$$

is equivalent to the optimal root production of a plant owning a unit of soil volume when plants follow the strategy  $u^*$  satisfying  $dG_i/du_i(N=1) = 0$ . This equality indicates that the maximum collective gain is reached when the root production per plant across all soil space is equivalent to the root production of plants in individual soil compartments. In general, this equality indicates that, given the choice of parameters, a total root density of  $2.6181 R/v$  is optimal, regardless of the number of plants growing roots. Collective gain is optimized at this density, as confirmed by plugging the values in the resource net gain equation: the collective net gains are  $G_{N=1} = 0.3396$ ,  $G_{N=2} = 0.2333$ , and  $G_{N=10} = 0.2032$  resources per plant. In the  $N = 2$  and  $N = 10$  scenarios, plants are engaging in an RTToC by overproliferating their roots with respect to the collective optimal, and are thus inefficiently overexploiting the common resource.

Gersani et al. (2001)'s experiment, and similar owned/shared designs, have been criticized because researchers have interpreted that the root allocation of a plant growing alone in one pot was compared to the root allocation of a plant sharing two pots with a neighbor. The key to understand this type of experimental design relies on correctly interpreting how their model was constructed, and realizing that their control treatment is a proxy for optimal collective root densities. The fundamental assumption of this design is that non-competing plants forage optimally (i.e., they do not have a root overproliferation fixed by an evolutionary arms race), hence, one can calculate the optimal root density (root biomass per unit volume or pot) that maximizes net gain, whether one or several plants share the pot. This root density serves as a base to estimate collective optimal in the two-pot system: If the root density in shared pots is higher, researchers can deduce that competing plants engage in an RTToC (Figure Box 1).



**Figure Box 1:** Results from Gersani et al. (2001), left, conceptual model (values based on the numerical results shown in Box) and, right, experimental results (approximated values from the original paper's results) depicting how the individual optimal is analogous to the collective optimal of the two plants sharing two soil volumes in their approach.

to test for the RTToC (Zhu et al., 2019, 2020; Chen et al., 2021). In a mesh divider setup, each plant owns a partition of a container. Partitions are separated by a permeable mesh in the interaction treatment, whereas in the control treatment, the separation is not permeable. Because resources would only flow across the mesh if diffusion is driven by a nutrient concentration or a water potential gradient (Kirkham, 2014) and plants in each compartment are typically identical, both the compartments must be symmetrical in resource concentration distribution and no force will trigger resource mixing despite the permeability of the mesh. Therefore, the interaction treatment does not differ from the control treatment in terms of soil resource. Contrastingly, other chemical

substances will diffuse freely from one plant's to another's partition (Kong et al., 2018). Hence, mesh divider systems test the effect of non-resource mechanisms controlling for the exploitative response.

When owned/shared experiments detect a root overproliferation, it may be a purely exploitative response, or it could be the combined result of the RTToC, an aggressive strategy, and/or a root arms race. Isolating each phenomenon is crucial to understand mechanistically the plant-foraging strategy.

Isolating the aggressive behavior from the RTToC consists in measuring to what extent the root overproliferation detected in an owned/shared experiment is triggered by direct non-self

roots detection. It is possible to measure that by complementing owned/shared with mesh divider experiments. For instance, Gersani et al. (2001) found root overproliferation in their owned/shared experiment with soybean plants (*Glycine max*) while Chen et al. (2021) found no response in a mesh-divider experiment. The former study demonstrates that soybean plants overproliferated roots with respect to the optimal root density when they interact, yet the latter indicates that this species did not respond directly to the neighbor detection. In conclusion, soybean plants seem to engage in an RToC as classic papers pointed out. Because the fundamental effect of non-self roots on resource dynamics is to increase resource depletion (Schenk, 2006), I suggest growing plants alone and vary the resource decay rate as an alternative experimental design to investigate the purely exploitative response of plants. In this setup, resource decay would emulate non-self root resource depletion without the non-self roots being actually present. While researchers have experimentally assessed the response of plants to soil patches with different resource availability, this is typically done by controlling for resource inputs only (Hodge, 2004). However, the response to resource availability may be quite different when the changes in availability are driven by the inputs or decay rates, only the latter being analogous to depletion by the neighbors (Cabal et al., 2021b).

Isolating the evolutionary root arms race from the RToC consists in determining to what extent any plant, even when growing alone, is overproliferating roots as a fixed trait. On the contrary, this may not be considered a confounding factor in owned/shared experiments because the difference between solo and interacting plants may remain unchanged (the fixed root proliferation happens in both the cases). However, determining whether plants engage in a root arms race seems more challenging than identifying behavioral responses, because we lack manageable control treatments. A possible experiment would consist in performing owned/shared experiments on both the wild plants and their respective cultivars and compare their responses, because we could expect domestic varieties to be bred to avoid an arms race, and maybe also to attenuate the RToC.

## Conclusion

Modern studies consider the RToC as the case in which plants, actively detecting their neighbors, overallocate resources into their roots compared with the plants growing alone. Nevertheless, the classic RToC *sensu* Hardin happens when plants invest more into their roots than the community-level foraging optimal based on the information about soil resource dynamics. While sophisticated strategies based on neighbor detection mechanisms may override and mask the RToC in some species, we must see the RToC as a baseline exploitative

response of all the plants to the interaction with neighbors. Classic owned/shared experiments are a convenient design to identify the trace of an RToC in exploitative plants, but other complementary experiments such as mesh divider systems, varying abiotic resource decay rates, or comparing wild species and their relative cultivars, can provide valuable information to isolate the effects of other mechanisms and phenomena at play.

## Author contributions

The author confirms being the sole contributor of this work and has approved it for publication.

## Funding

This research was funded by the Remedial TE-CM project (Ref. S2018/EMT4338), a project funded by the Regional Government of Madrid, and The Princeton University's Carbon Mitigation Initiative.

## Acknowledgments

Many thanks to Ray Dybzinski for constructive and thought-provoking comments, to Ricardo Martínez-García for his critical review on early versions of the manuscript, to Stephen W. Pacala for several meaningful conversations and extensive support over the past few years, which laid the foundations for this critical paper, and also to Isa, Fer, LuoJun, Yuki, Liana, Matt, and Marilia for conversations on the topic and their invaluable support.

## Conflict of interest

The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

## Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

## References

- Anten, N. P. R., and Vermeulen, P. J. (2016). Tragedies and Crops: understanding Natural Selection To Improve Cropping Systems. *Trends Ecol. Evol.* 31, 429–439. doi: 10.1016/j.tree.2016.02.010
- Bakker, L. M., Barry, K. E., Mommer, L., and van Ruijven, J. (2021). Focusing on individual plants to understand community scale biodiversity effects: the case of root distribution in grasslands. *Oikos* 130, 1954–1966. doi: 10.1111/oik.08113
- Belter, P. R., and Cahill, J. F. (2015). Disentangling root system responses to neighbours: identification of novel root behavioural strategies. *AoB Plants* 7:plv059. doi: 10.1093/aobpla/plv059
- Cabal, C., De Deurwaerder, H., and Matesanz, S. (2021a). Field methods to study the spatial root density distribution of individual plants. *Plant Soil* 462, 25–46. doi: 10.1007/s11104-021-04841-z
- Cabal, C., Martínez-García, R., De Castro Aguilar, A., Valladares, F., and Pacala, S. W. (2021b). Future paths for the “exploitative segregation of plant roots” model. *Plant Signal. Behav.* 16:1891755. doi: 10.1080/15592324.2021.1891755
- Cabal, C., Martínez-García, R., De Castro Aguilar, A., Valladares, F., and Pacala, S. W. (2020). The Exploitative Segregation of Plant Roots. *Science* 370, 1197–1199. doi: 10.1126/science.aba9877
- Callaway, R. M., Pennings, S. C., and Richards, C. L. (2003). Phenotypic plasticity and interactions among plants. *Ecology* 84, 1115–1128.
- Chen, B. J. W., During, H. J., and Anten, N. P. R. (2012). Detect thy neighbor: identity recognition at the root level in plants. *Plant Sci.* 195, 157–167. doi: 10.1016/j.plantsci.2012.07.006
- Chen, B. J. W., During, H. J., Vermeulen, P. J., de Kroon, H., Poorter, H., and Anten, N. P. R. (2015). Corrections for rooting volume and plant size reveal negative effects of neighbour presence on root allocation in pea. *Funct. Ecol.* 29, 1383–1391. doi: 10.1111/1365-2435.12450
- Chen, B. J. W., During, H. J., Vermeulen, P. J., de Kroon, H., Poorter, H., and Anten, N. P. R. (2020). The analysis of plant root responses to nutrient concentration, soil volume and neighbour presence: different statistical approaches reflect different underlying basic questions. *Funct. Ecol.* 34, 2210–2217. doi: 10.1111/1365-2435.13664
- Chen, B. J. W., Huang, L., During, H. J., Wang, X., Wei, J., and Anten, N. P. R. (2021). No neighbour-induced increase in root growth of soybean and sunflower in mesh divider experiments after controlling for nutrient concentration and soil volume. *AoB Plants* 13:lab020. doi: 10.1093/aobpla/plab020
- Craine, J. M. (2005). Reconciling Plant Strategy Theories of Grime and Tilman. *J. Ecol.* 93, 1041–1052. doi: 10.1111/j.1365-2745.2005.01043.x
- Craine, J. M. (2006). Competition for nutrients and optimal root allocation. *Plant Soil* 285, 171–185. doi: 10.1007/s11104-006-9002-x
- Dawkins, R., and Krebs, J. R. (1979). Arms races between and within species. *Proc. R. Soc. Lond. Biol. Sci.* 205, 489–511. doi: 10.1098/rspb.1979.0081
- Donald, C. M. (1968). The breeding of crop ideotypes. *Euphytica* 17, 385–403. doi: 10.1007/BF00056241
- Dybziński, R., Farrior, C., Wolf, A., Reich, P. B., and Pacala, S. W. (2011). Evolutionarily stable strategy carbon allocation to foliage, wood, and fine roots in trees competing for light and nitrogen: an analytically tractable, individual-based model and quantitative comparisons to data. *Am. Nat.* 177, 153–166. doi: 10.1086/657992
- Escudero, A., Matesanz, S., Pescador, D. S., de la Cruz, M., Valladares, F., and Caviries, L. A. (2021). Every little helps: the functional role of individuals in assembling any plant community, from the richest to monospecific ones. *J. Veg. Sci.* 32:e13059. doi: 10.1111/jvs.13059
- Falik, O., Reides, P., Gersani, M., and Novoplansky, A. (2003). Self/non-self discrimination in roots. *J. Ecol.* 91, 525–531. doi: 10.1046/j.1365-2745.2003.00795.x
- Falster, D. S., and Westoby, M. (2003). Plant height and evolutionary games. *Trends Ecol. Evol.* 18, 337–342. doi: 10.1016/S0169-5347(03)00061-2
- Freschet, G. T., Pagès, L., Iversen, C. M., Comas, L. H., Rewald, B., Roumet, C., et al. (2021a). A starting guide to root ecology: strengthening ecological concepts and standardising root classification, sampling, processing and trait measurements. *New Phytol.* 232, 973–1122. doi: 10.1111/nph.17572
- Freschet, G. T., Roumet, C., Comas, L. H., Weemstra, M., Bengough, A. G., Rewald, B., et al. (2021b). Root traits as drivers of plant and ecosystem functioning: current understanding, pitfalls and future research needs. *New Phytol.* 232, 1123–1158. doi: 10.1111/nph.17072
- Fretwell, S. D., and Lucas, H. L. J. (1969). On territorial behavior and other factors influencing habitat distribution in birds. *J. Theor. Dev. Acta Biotheor.* 19, 16–36. doi: 10.1007/BF01601953
- Fréville, H., Roumet, P., Rode, N. O., Rocher, A., Latreille, M., Muller, M. H., et al. (2019). Preferential helping to relatives: a potential mechanism responsible for lower yield of crop variety mixtures? *Evol. Appl.* 12, 1837–1849. doi: 10.1111/eva.12842
- Gersani, M., Brown, J. S., O'Brien, E. E., Maina, G. M., and Abramsky, Z. (2001). Tragedy of the Commons as a Result of Root Competition. *J. Ecol.* 89, 660–669. doi: 10.1046/j.0022-0477.2001.00609.x
- Hardin, G. (1968). The Tragedy of Commons. *Science* 162, 1243–1248. doi: 10.1126/science.162.3859.1243
- He, J. Z., Wang, R. W., Jensen, C. X. J., and Li, Y. T. (2015). Asymmetric interaction paired with a super-rational strategy might resolve the tragedy of the commons without requiring recognition or negotiation. *Sci. Rep.* 5, 26–29. doi: 10.1038/srep07715
- Hess, L., and De Kroon, H. (2007). Effects of rooting volume and nutrient availability as an alternative explanation for root self/non-self discrimination. *J. Ecol.* 95, 241–251. doi: 10.1111/j.1365-2745.2006.01204.x
- Hodge, A. (2004). The plastic plant: root responses to heterogeneous supplies of nutrients. *New Phytol.* 162, 9–24. doi: 10.1111/j.1469-8137.2004.01015.x
- Kemmel, S. W., and Cahill, J. F. (2005). Plant phenotypic plasticity belowground: a phylogenetic perspective on root foraging trade-offs. *Am. Nat.* 166, 216–230. doi: 10.1086/431287
- Kim, B. M., Horita, J., Suzuki, J. I., and Tachiki, Y. (2021). Resource allocation in tragedy of the commons game in plants for belowground competition. *J. Theor. Biol.* 529:110858. doi: 10.1016/j.jtbi.2021.110858
- Kirkham, M. B. (2014). *Principles of soil and plant water relations*. 2nd ed. Amsterdam: Elsevier.
- Kong, C. H., Zhang, S. Z., Li, Y. H., Xia, Z. C., Yang, X. F., Meiners, S. J., et al. (2018). Plant neighbor detection and allelochemical response are driven by root-secreted signaling chemicals. *Nat. Commun.* 9:3867. doi: 10.1038/s41467-018-06429-1
- Laird, R. A., and Aarssen, L. W. (2005). Size inequality and the tragedy of the commons phenomenon in plant competition. *Plant Ecol.* 179, 127–131. doi: 10.1007/s11258-004-6737-4
- Maina, G. G., Brown, J. S., and Gersani, M. (2002). Intra-Plant versus Inter-Plant Root Competition in Beans?: avoidance, resource matching or tragedy of the commons. *Plant Ecol.* 160, 235–247. doi: 10.1023/A:1015822003011
- McNickle, G. G. (2020). Interpreting plant root responses to nutrients, neighbours and pot volume depends on researchers' assumptions. *Funct. Ecol.* 34, 2199–2209. doi: 10.1111/1365-2435.13517
- McNickle, G. G., and Brown, J. S. (2014). An ideal free distribution explains the root production of plants that do not engage in a tragedy of the commons game. *J. Ecol.* 102, 963–971. doi: 10.1111/1365-2745.12259
- McNickle, G. G., Gonzalez-Meler, M. A., Lynch, D. J., Baltzer, J. L., and Brown, J. S. (2016). The world's biomes and primary production as a triple tragedy of the commons foraging game played among plants. *Proc. R. Soc. B Biol. Sci.* 283:20161993. doi: 10.1098/rspb.2016.1993
- Murase, Y., and Baek, S. K. (2018). Seven rules to avoid the tragedy of the commons. *J. Theor. Biol.* 449, 94–102. doi: 10.1016/j.jtbi.2018.04.027
- Novoplansky, A. (2019). What plant roots know? *Semin. Cell Dev. Biol.* 92, 126–133. doi: 10.1016/j.semcdb.2019.03.009
- O'Brien, E. E., and Brown, J. S. (2008). Games roots play: effects of soil volume and nutrients. *J. Ecol.* 96, 438–446. doi: 10.1111/j.1365-2745.2008.01354.x
- O'Brien, E. E., Gersani, M., and Brown, J. S. (2005). Root proliferation and seed yield in response to spatial heterogeneity of below-ground competition. *New Phytol.* 168, 401–412. doi: 10.1111/j.1469-8137.2005.01520.x
- Padilla, F. M., Mommer, L., de Caluwe, H., Smit-Tiekstra, A. E., Wagemaker, C. A. M., Ouborg, N. J., et al. (2013). Early Root Overproduction Not Triggered by Nutrients Decisive for Competitive Success Belowground. *PLoS One* 8:e55805. doi: 10.1371/journal.pone.0055805
- Pierik, R., Mommer, L., and Voeseek, L. A. (2013). Molecular mechanisms of plant competition: neighbour detection and response strategies. *Funct. Ecol.* 27, 841–853. doi: 10.1111/1365-2435.12010
- Postma, J. A., Hecht, V. L., Hikosaka, K., Nord, E. A., Pons, T. L., and Poorter, H. (2021). Dividing the pie: a quantitative review on plant density responses. *Plant Cell Environ.* 44, 1072–1094. doi: 10.1111/pce.13968

- Pulliam, H. R., Pyke, G. H., and Caraco, T. (1982). The scanning behavior of juncos: a game-theoretical approach. *J. Theor. Biol.* 95, 89–103. doi: 10.1016/0022-5193(82)90289-2
- Qi, Y., Wei, W., Chen, C., and Chen, L. (2019). Plant root-shoot biomass allocation over diverse biomes: a global synthesis. *Glob. Ecol. Conserv.* 18:e00606. doi: 10.1016/j.gecco.2019.e00606
- Rankin, D. J., Bargum, K., and Kokko, H. (2007). The tragedy of the commons in evolutionary biology. *Trends Ecol. Evol.* 22, 643–651. doi: 10.1016/j.tree.2007.07.009
- Schenk, H. J. (2006). Root competition: beyond resource depletion. *J. Ecol.* 94, 725–739. doi: 10.1111/j.1365-2745.2006.01124.x
- Schneider, H. M., and Lynch, J. P. (2020). Should Root Plasticity Be a Crop Breeding Target? *Front. Plant Sci.* 11:546. doi: 10.3389/fpls.2020.00546
- Semchenko, M., Hutchings, M. J., and John, E. A. (2007). Challenging the tragedy of the commons in root competition: confounding effects of neighbour presence and substrate volume. *J. Ecol.* 95, 252–260. doi: 10.1111/j.1365-2745.2007.01210.x
- Wang, S., and Callaway, R. M. (2021). Plasticity in response to plant-plant interactions and water availability. *Ecology* 102:e03361. doi: 10.1002/ecy.3361
- Xia, J., Yuan, W., Wang, Y. P., and Zhang, Q. (2017). Adaptive Carbon Allocation by Plants Enhances the Terrestrial Carbon Sink. *Sci. Rep.* 7:3341. doi: 10.1038/s41598-017-03574-3
- Zea-Cabrera, E., Iwasa, Y., Levin, S., and Rodríguez-Iturbe, I. (2006). Tragedy of the commons in plant water use. *Water Resour. Res.* 42:W06D02. doi: 10.1029/2005WR004514
- Zhang, D. Y., Sun, G. J., and Jiang, X. H. (1999). Donald's ideotype and growth redundancy: a game theoretical analysis. *F. Crop. Res.* 61, 179–187. doi: 10.1016/S0378-4290(98)00156-7
- Zhu, L., Xi, N., and Zhang, D. Y. (2020). Lower sensitivity in responses to root competition and soil resource availability in a new wheat cultivar than in an old wheat landrace. *Plant Soil* 450, 557–565. doi: 10.1007/s11104-020-04519-y
- Zhu, Y.-H., Weiner, J., and Li, F.-M. (2019). Root proliferation in response to neighbouring roots in wheat (*Triticum aestivum*). *Basic Appl. Ecol.* 39, 10–14. doi: 10.1016/j.bae.2019.07.001



## Glossary

*Exploitative plant*: A plant or plant species which belowground foraging strategy is based exclusively in maximizing resource net gain, i.e., maximizing the difference between the resource uptake by roots and the cost of building and maintaining such roots, and adjust its root production accordingly.

*Sophisticated plant*: A plant or plant species that can collect more environmental information than an exploitative plant, e.g., self/non-self root discrimination, and use that information to tune its exploitative foraging strategy.

*Self/non-self root discrimination*: The ability of a root to determine whether other surrounding roots are connected to self by active vessel transportation (i.e., belong to the same individual plant) or not.

*Tragedy of the commons sensu Hardin (1968)*: A strategy predicted by game theory models in which several stakeholders sharing a common resource, each adjusting their resource-use strategy only aiming to maximize its net reward (=benefit/uptake - cost/investment), unintentionally and inevitably overexploit the resource to the detriment of all.

*Evolutionary arms race sensu Dawkins and Krebs (1979)*: A strategy predicted by game theory models in the context of evolutionary ecology in which individuals evolve “weapons” (i.e., adaptations designed to harm or outcompete neighbors), engaging in an apparently unlimited escalation of the allocation of resources to such weapons in the course of evolution.

*Root aggressive behavior*: A strategy in which individuals proliferate more roots than exploitative plants when they encounter non-self roots in the soil, as a pre-emptive strategy to deplete soil resources and outcompete the neighbors.

*Ideal free distribution (IFD) sensu Fretwell and Lucas (1969)*: A strategy predicted by bird territoriality models in which ideal free individuals adjust through migration the population density within each habitat so that the suitability in all habitats, which decreases as occupancy increases, remains equal.

—*sensu Gersani et al. (2001)*: They extrapolated this concept to root competition, defining it as a passive habitat selection that leads to no difference in root proliferation or reproductive yield between owners and sharing individuals. This is to be interpreted in the context of their paper, therefore, plants producing as many roots in one soil compartment when owning it as in N soil compartments when sharing them with N plants (hence 1/N times roots per compartment).

—*sensu McNickle and Brown (2014)*: They revisited the concept, but described it as a case in which plants grow roots in proportion to nutrient's availability at each soil location but do not have any direct response to neighbors that is independent of their response to nutrients (the features actually leading to an RTToC!). Nevertheless, their IFD model incorporates cooperative behaviors based on the complex neighbor information (the optimization conditions for every individual depend on the net-gain-generating equations of all the coexisting individuals).



## OPEN ACCESS

## EDITED BY

Patompong Johns Saengwilai,  
Mahidol University, Thailand

## REVIEWED BY

Mohammad Sohulul Islam,  
Hajee Mohammad Danesh Science &  
Technology University, Bangladesh  
Shirley Nichols,  
AgResearch Ltd., New Zealand

## \*CORRESPONDENCE

Khaled A. El-Tarabily  
ktarabily@uaeu.ac.ae  
Synan F. AbuQamar  
sabuqamar@uaeu.ac.ae

## SPECIALTY SECTION

This article was submitted to  
Functional Plant Ecology,  
a section of the journal  
Frontiers in Plant Science

RECEIVED 05 May 2022

ACCEPTED 07 July 2022

PUBLISHED 04 August 2022

## CITATION

Abd El-hady MA, Abd-Elkrem YM,  
Rady MOA, Mansour E, El-Tarabily KA,  
AbuQamar SF and El-temsah ME  
(2022) Impact on plant productivity  
under low-fertility sandy soil in arid  
environment by revitalization of lentil  
roots.  
*Front. Plant Sci.* 13:937073.  
doi: 10.3389/fpls.2022.937073

## COPYRIGHT

© 2022 Abd El-hady, Abd-Elkrem,  
Rady, Mansour, El-Tarabily, AbuQamar  
and El-temsah. This is an open-access  
article distributed under the terms of  
the [Creative Commons Attribution  
License \(CC BY\)](https://creativecommons.org/licenses/by/4.0/). The use, distribution  
or reproduction in other forums is  
permitted, provided the original  
author(s) and the copyright owner(s)  
are credited and that the original  
publication in this journal is cited, in  
accordance with accepted academic  
practice. No use, distribution or  
reproduction is permitted which does  
not comply with these terms.

# Impact on plant productivity under low-fertility sandy soil in arid environment by revitalization of lentil roots

Mohamed A. Abd El-hady<sup>1</sup>, Yasser M. Abd-Elkrem<sup>1</sup>,  
Mohamed O. A. Rady<sup>2</sup>, Elsayed Mansour<sup>3</sup>,  
Khaled A. El-Tarabily<sup>4,5,6\*</sup>, Synan F. AbuQamar<sup>4\*</sup> and  
Mohamed E. El-temsah<sup>1</sup>

<sup>1</sup>Agronomy Department, Faculty of Agriculture, Ain Shams University, Cairo, Egypt, <sup>2</sup>Agronomy Department, Faculty of Agriculture, Fayoum University, Fayoum, Egypt, <sup>3</sup>Agronomy Department, Faculty of Agriculture, Zagazig University, Zagazig, Egypt, <sup>4</sup>Department of Biology, College of Science, United Arab Emirates University, Al Ain, United Arab Emirates, <sup>5</sup>Khalifa Center for Genetic Engineering and Biotechnology, United Arab Emirates University, Al Ain, United Arab Emirates, <sup>6</sup>Harry Butler Institute, Murdoch University, Murdoch, WA, Australia

Lentil is one of the essential legume crops, which provides protein for humans and animals. This legume can improve soil fertility through nitrogen fixation, which is imperative in low-fertility soils. The growth and productivity of lentil could be enhanced through improving nutrition and root revitalization. Therefore, the objective of this study was to assess the impact of root activator (RA) and phosphorus (P) application on morphological, physiological, agronomic, and quality traits of lentil under newly reclaimed low-fertility sandy soil in an arid environment. The RA was applied at four levels of 0 (RA0-untreated control), 1.25 (RA1), 2.5 (RA2), and 3.75 (RA3) l ha<sup>-1</sup>. RA contained 9% potassium humate, 1,600 ppm indole butyric acid, 200 ppm gibberellic acid, and 200 ppm naphthalene acetic acid. The recommended rate of phosphorus (P) fertilization in the newly reclaimed low-fertility sandy soil (75 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup>) was applied, and its amount was increased and decreased by 25 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> vs. non-added control. Thus, P rates were applied at four rates 0 (P0; control), 50 (P1), 75 (P2), and 100 (P3) kg phosphorus pentoxide (P<sub>2</sub>O<sub>5</sub>) ha<sup>-1</sup>. Our results revealed that treated lentil plants with the high levels of both treatments (RA3 and P3) exhibited superiority in root measurements (root length, total number of nodules plant<sup>-1</sup>, number of active nodules plant<sup>-1</sup>, dry weights of active nodules, and total root), nitrogenase activity, chlorophyll *a* and *b*, carotenoids, yield traits, and seed proteins and carbohydrates. However, the recommended P level (75 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup>, P2) under the high level of RA (3.75 l ha<sup>-1</sup>, RA3) displayed non-significant differences in yield traits (plant height, 1,000-seed weight, seed yield ha<sup>-1</sup>) and quality traits (protein and carbohydrate) with

the high P level (100 kg  $P_2O_5$  ha<sup>-1</sup>, P3). Accordingly, its recommended economically and environmentally to use this coapplication of RA3 and P3 in low-fertility soil for better lentil growth, and seed yield and quality.

#### KEYWORDS

carbohydrates, nitrogen uptake, nodulation, phosphorus uptake, proteins, root activator, yield traits

## Introduction

Lentil (*Lens culinaris* Medic.) is an edible legume grown broadly and cultivated for its nutritious seeds (Ganesan and Xu, 2017). Its global cultivated area is almost 5.01 million hectares that produce around 6.53 million tons (FAOSTAT, 2022). It is one of the cheapest sources of vegetable protein and provides a plentiful amount of minerals, fibers, and fundamental amino acids (Khazaei et al., 2019). Additionally, its straw is an alternative unconventional feedstuff for livestock (Jarpa-Parra, 2018). It is a palatable and nourishing feed for animals that contributes significantly to cope with continuously increasing forage demands (Mudgal et al., 2018). Moreover, it fixes atmospheric nitrogen (N<sub>2</sub>) and reduces the utilization of synthetic nitrogenous fertilizers, accordingly diminishes greenhouse gas emissions (Schmidtke et al., 2004; Khazaei et al., 2019). Symbiotic fixation of atmospheric N<sub>2</sub> increases the content of mineral nitrogen (N) in the soil, which remains after lentil harvesting (Hossain et al., 2016). Accordingly, it considerably enriches soil fertility and improves its biological properties, particularly in newly reclaimed low-fertility soils (Gan et al., 2017; Liu et al., 2019; Desoky et al., 2021c).

Newly reclaimed sandy soils suffer from a deficiency of available mineral nutrients, and subsequently low-crop productivity (Mansour et al., 2017; Omar et al., 2022). Hence, numerous endeavors are adopted to elevate legume productivity and quality under newly reclaimed soils (Dhaliwal et al., 2021; El-Mageed et al., 2021). This could be accomplished through improving nutrition approaches, which had a pivotal impact on plant growth and productivity (Mannan et al., 2022). Enhancing root architecture is imperative to ameliorate resource acquisition, water uptake, plant anchoring, and encourage the utilization of soil nutrients (Gahoonia and Nielsen, 2004; Mansour et al., 2021).

Potassium (K) humate is a highly effective plant growth bio-activator for stimulating root development and penetration, photosynthesis efficiency, plant growth, and tolerance against environmental stress (Pradip et al., 2017). Moreover, it contains soluble humic that boosts cell division, synthesis of proteins and nucleic acids, tissue regeneration, and movement of nutrients (Shah et al., 2018; Jindo et al., 2020). Furthermore, the plant growth regulators as butyric acid (BA), gibberellic acid (GA<sub>3</sub>), and naphthalene acetic acid (NAA) promote impacts on roots,

particularly under environmental stresses (Egamberdieva et al., 2017; Sabagh et al., 2021; ElShamey et al., 2022). Growth regulators enhance root initiation, cell division, cell elongation, cell differentiation, vascular tissue, and apical dominance (Singh, 2010; Semida et al., 2021).

Phosphorus (P) is an essential macronutrient for legume development and growth. It plays a decisive role in invigorating biological activities, such as symbiotic N<sub>2</sub> fixation by *Rhizobium*, prolific root hair formation, and nutrient uptake (Míguez-Montero et al., 2020). Furthermore, P application expands the number of root nodules, regulates their growth, enhances nitrogenase activity, and accordingly improves the capacity of N<sub>2</sub> fixation (Gahoonia and Nielsen, 2004). Moreover, it is involved in various fundamental functions, such as cell division, nucleus formation, energy transfer, sugar transformation, nutrient movement, photosynthesis, seed formation, protein synthesis, and crop ripening (Swailem et al., 2021). Accordingly, its application ameliorates root development, biological activities, physiological capabilities, and metabolic functions, which lead to significant enhancement of legume productivity, particularly under newly reclaimed soil conditions (Singh and Singh, 2016).

Different studies have considered the influence of mineral elements on lentil plants. However, further studies are required to explore the responses of lentils to combinations of important elements, such as K humate, indole butyric acid (IBA), GA<sub>3</sub>, and NAA as root activator (RA), as well as P fertilization under newly reclaimed low-fertility sandy soil in arid environments. Therefore, the objective of the present study was to assess the impact of RA and P application at different levels of physiological, morphological, agronomic, and quality traits of lentil under newly reclaimed low-fertility sandy soil in arid environments.

## Materials and Methods

### Experimental site, soil, and climatic conditions

A field experiment was performed at the Higher Institute for Agricultural Cooperation Farm, Regwa region, Alexandria Desert Road, Beheira Governorate, Egypt (30°11'12.0"N, 30°34'32.7"E during the 2018/2019 and 2019/2020 growing seasons. The physical and chemical properties of the

experimental soil were determined before sowing and presented in **Supplementary Table 1**. The experimental soil was sandy throughout the profile (92.1% sand, 1.6% silt, and 6.3% clay), with a pH of 7.5, and the electrical conductivity was  $0.8 \text{ dS m}^{-1}$ .

The available nutrients were 30.72 mg N, 5.12 mg P, and 24.32 mg K  $\text{kg}^{-1}$  of the soil. Accordingly, this soil suffers from a considerable deficiency of available mineral nutrients, particularly NPK compared to fertile soils. The monthly minimum and maximum temperatures and total rainfall for the two growing seasons were obtained from a station close to the experimental site (**Supplementary Table 2**).

## Experimental design and agronomic practices

The RA used in this study contained 9% K humate, 1,600 ppm IBA, 200 ppm GA<sub>3</sub>, and 200 ppm NAA. The RA was applied at four levels, 0 (RA0; untreated control), 1.25 (RA1), 2.5 (RA2), and 3.75 (RA3)  $\text{l ha}^{-1}$  after 20 days after sowing (DAS) as fertigation. The recommended rate in the newly reclaimed low-fertility sandy soil ( $75 \text{ kg P}_2\text{O}_5 \text{ ha}^{-1}$ ) was applied, and this amount was increased and decreased by  $25 \text{ kg P}_2\text{O}_5 \text{ ha}^{-1}$  vs. non-added control. The P was also applied at four levels 0 (P0), 50 (P1), 75 (P2), and 100 (P3)  $\text{kg phosphorus pentoxide (P}_2\text{O}_5) \text{ ha}^{-1}$ .

The P source used was calcium superphosphate that contained 15.5% P<sub>2</sub>O<sub>5</sub>, 19.5% calcium, and 11.5% sulfur at pH 2.0. P fertilizer was added to the soil during its preparation for cultivation. N fertilizer was applied at a rate of  $45 \text{ kg N ha}^{-1}$  as ammonium nitrate (33% N) as fertigation in three equal doses at sowing (0), 15 and 30 DAS. K fertilizer was applied at a rate of  $100 \text{ kg K ha}^{-1}$  as potassium sulfate (K<sub>2</sub>SO<sub>4</sub>, 48% K<sub>2</sub>O) at soil preparation.

The experimental design was conducted using a split-plot arrangement in a randomized complete block design with three replicates. RA was the first factor, assigned to the main plots, and the rate of P was the second factor allocated to the sub-plots. Each plot comprised of five rows, 4 m long and 0.60 m wide, with two seeds were sown per hill, which were spaced 0.15 m apart. Each replicate included 16 plots, i.e., four RA rates  $\times$  four P levels, 16 treatments in total, as shown in **Tables 1–6**. The tested genotype in this experiment was Giza-51, a commercial cultivar in Egypt, and was obtained from the Agricultural Research Center, Giza, Egypt. The sowing was applied during the first week of November in both seasons according to the optimal period for growing lentil in the region.

The seeds were inoculated with the proper strain of *Rhizobium* (*Rhizobium leguminosarum*). All other agricultural practices, comprising drip irrigation to the reference crop evapotranspiration (ET<sub>0</sub>), weed, disease, and pest control,

were performed according to the recommendations of the commercial production of lentil.

## Measurements of root and physiological parameters

After 55 DAS, five plants from each plot were collected randomly to determine root traits. Plants were gently uprooted and washed with tap water to remove the soil from the roots. Then roots were washed with distilled water and blotted with tissue paper. The root length (cm) was measured from the collar region to the tip of the main root, and the number of total nodules was counted on the main and lateral roots. Nodules were detached from the roots and cut into two pieces, and observed for the inside color. Pink/red nodules were recorded as healthy and active in N<sub>2</sub> fixation. The remaining nodules with other colors were classified as inactive to fix N<sub>2</sub>. The active nodules and total root were dried in the oven at 70°C for 48 h and then weighed. Nitrogenase enzyme activity ( $\mu\text{mol C}_2\text{H}_4 \text{ g}^{-1} \text{ nodule DW h}^{-1}$ ) was estimated using an acetylene reduction assay as described by Hardy et al. (1973). At 55 DAS, chlorophyll *a*, chlorophyll *b*, and carotenoids ( $\text{mg g}^{-1} \text{ FW}$ ) were determined according to Hiscox and Israelstam (1979).

## Measurements of seed yield and its attributes

At maturity (130 DAS), ten plants were taken randomly from the inner rows of each plot to measure plant height (cm), number of branches  $\text{plant}^{-1}$ , and number of pods  $\text{plant}^{-1}$ . All plants in each plot were harvested to determine the 1,000-seed weight (g), seed yield ( $\text{kg ha}^{-1}$ ), and biological yield ( $\text{kg ha}^{-1}$ ).

## Measurements of nitrogen and phosphorus

### Measurements of nitrogen

The total N in seed and straw was determined using the Micro-Kjeldahl method as described by Horwitz et al. (2000). N in seed and straw, N uptake, protein percentage, N recovery efficiency (NRE), and N use efficiency (NUE) were calculated according to the following equations.

$$\text{N uptake (kg ha}^{-1}\text{)} = \text{N in seed (kg ha}^{-1}\text{)} + \text{N in straw (kg ha}^{-1}\text{)}$$

$$\text{N in seeds (kg ha}^{-1}\text{)} = \text{Seed N \%} \times \text{seed yield (kg ha}^{-1}\text{)}/100$$

$$\text{N in straw (kg ha}^{-1}\text{)} = \text{Straw N \%} \times \text{straw yield (kg ha}^{-1}\text{)}/100$$



**TABLE 1** Impact of root activator (RA) and phosphorus (P) level on root length (cm), number of nodules plant<sup>-1</sup>, dry weight of active nodules (mg plant<sup>-1</sup>), total root dry weight (mg plant<sup>-1</sup>), and nitrogenase enzyme activity ( $\mu\text{mol C}_2\text{H}_4 \text{ g}^{-1}$  nodule dry weight h<sup>-1</sup>) in lentil plants.

Factor		Root length	Total number of nodules plant <sup>-1</sup>	Number of active nodules plant <sup>-1</sup>	Dry weight of active nodules	Total root dry weight	Nitrogenase enzyme activity
<b>RA</b>							
RA0 (control)		23.12 ± 1.17 <sup>d</sup>	12.85 ± 0.75 <sup>d</sup>	11.48 ± 0.72 <sup>d</sup>	61.71 ± 3.89 <sup>d</sup>	1022 ± 29.42 <sup>d</sup>	7.78 ± 0.18 <sup>d</sup>
RA1 (1.25 l ha <sup>-1</sup> )		28.60 ± 1.61 <sup>c</sup>	15.33 ± 1.13 <sup>c</sup>	13.62 ± 1.00 <sup>c</sup>	73.45 ± 2.73 <sup>c</sup>	1181 ± 27.81 <sup>c</sup>	8.76 ± 0.23 <sup>c</sup>
RA2 (2.50 l ha <sup>-1</sup> )		36.10 ± 1.01 <sup>b</sup>	21.21 ± 1.15 <sup>b</sup>	19.12 ± 1.28 <sup>b</sup>	102.21 ± 2.99 <sup>b</sup>	1314 ± 23.35 <sup>b</sup>	11.13 ± 0.78 <sup>b</sup>
RA3 (3.75 l ha <sup>-1</sup> )		43.57 ± 0.62 <sup>a</sup>	29.38 ± 1.42 <sup>a</sup>	26.75 ± 1.34 <sup>a</sup>	142.97 ± 4.68 <sup>a</sup>	1490 ± 30.19 <sup>a</sup>	15.23 ± 0.72 <sup>a</sup>
<b>P</b>							
P0 (control)		28.01 ± 2.52 <sup>d</sup>	14.22 ± 1.54 <sup>d</sup>	13.24 ± 1.33 <sup>d</sup>	70.05 ± 3.89 <sup>d</sup>	1175 ± 65.53 <sup>c</sup>	8.40 ± 0.49 <sup>d</sup>
P1 (50 kg P <sub>2</sub> O <sub>5</sub> ha <sup>-1</sup> )		31.04 ± 2.78 <sup>c</sup>	18.54 ± 1.99 <sup>c</sup>	15.41 ± 1.85 <sup>c</sup>	81.93 ± 4.87 <sup>c</sup>	1224 ± 59.32 <sup>b,c</sup>	10.38 ± 0.97 <sup>c</sup>
P2 (75 kg P <sub>2</sub> O <sub>5</sub> ha <sup>-1</sup> )		35.14 ± 2.22 <sup>b</sup>	22.36 ± 2.00 <sup>b</sup>	20.33 ± 1.98 <sup>b</sup>	109.47 ± 3.91 <sup>b</sup>	1271 ± 54.61 <sup>b</sup>	11.80 ± 1.03 <sup>b</sup>
P3 (100 kg P <sub>2</sub> O <sub>5</sub> ha <sup>-1</sup> )		37.20 ± 1.84 <sup>a</sup>	23.66 ± 2.15 <sup>a</sup>	21.98 ± 1.96 <sup>a</sup>	118.89 ± 4.65 <sup>a</sup>	1337 ± 50.35 <sup>a</sup>	12.32 ± 1.06 <sup>a</sup>
<b>Interaction</b>							
RA0	P0	18.54 ± 0.08 <sup>o</sup>	9.09 ± 0.05 <sup>n</sup>	8.75 ± 0.18 <sup>c</sup>	47.47 ± 1.62 <sup>c</sup>	953.3 ± 10.17 <sup>i</sup>	6.84 ± 0.11 <sup>k</sup>
	P1	20.35 ± 0.09 <sup>n</sup>	12.12 ± 0.07 <sup>c</sup>	9.63 ± 0.20 <sup>k</sup>	51.23 ± 1.11 <sup>kl</sup>	983.0 ± 13.06 <sup>i</sup>	7.77 ± 0.13 <sup>i,j</sup>
	P2	25.33 ± 0.09 <sup>k</sup>	14.77 ± 0.12 <sup>j</sup>	13.26 ± 0.26 <sup>i</sup>	70.94 ± 2.53 <sup>i</sup>	1039 ± 15.63 <sup>h,i</sup>	8.22 ± 0.12 <sup>g,h,i</sup>
	P3	28.27 ± 0.18 <sup>j</sup>	15.44 ± 0.16 <sup>i</sup>	14.27 ± 0.14 <sup>b</sup>	77.18 ± 1.76 <sup>h</sup>	1114 ± 14.50 <sup>g,h</sup>	8.29 ± 0.11 <sup>g,h</sup>
RA1	P0	22.23 ± 0.10 <sup>m</sup>	10.10 ± 0.06 <sup>m</sup>	10.06 ± 0.21 <sup>j,k</sup>	53.01 ± 1.14 <sup>jk</sup>	1046 ± 13.71 <sup>h,i</sup>	7.60 ± 0.12 <sup>j</sup>
	P1	24.70 ± 0.11 <sup>c</sup>	13.47 ± 0.08 <sup>k</sup>	10.70 ± 0.23 <sup>j</sup>	56.92 ± 1.23 <sup>j</sup>	1180 ± 15.28 <sup>f,g</sup>	8.63 ± 0.14 <sup>g</sup>
	P2	31.87 ± 0.12 <sup>h</sup>	18.47 ± 0.18 <sup>h</sup>	15.90 ± 0.15 <sup>g</sup>	86.34 ± 1.83 <sup>g</sup>	1213 ± 12.02 <sup>e,f,g</sup>	9.20 ± 0.10 <sup>f</sup>
	P3	35.57 ± 0.15 <sup>f</sup>	19.30 ± 0.20 <sup>g</sup>	17.83 ± 0.17 <sup>f</sup>	97.55 ± 1.96 <sup>f</sup>	1283 ± 11.93 <sup>d,e,f</sup>	9.60 ± 0.08 <sup>f</sup>
RA2	P0	31.18 ± 0.21 <sup>i</sup>	15.76 ± 0.06 <sup>i</sup>	14.01 ± 0.10 <sup>h,i</sup>	74.10 ± 1.53 <sup>h,i</sup>	1203 ± 13.83 <sup>e,f,g</sup>	8.03 ± 0.07 <sup>h,i,g</sup>
	P1	35.03 ± 0.24 <sup>g</sup>	19.70 ± 0.08 <sup>g</sup>	16.10 ± 0.11 <sup>g</sup>	85.68 ± 1.61 <sup>g</sup>	1310 ± 10.17 <sup>c,d,e</sup>	9.23 ± 0.08 <sup>f</sup>
	P2	38.10 ± 0.15 <sup>e</sup>	23.73 ± 0.15 <sup>e</sup>	21.80 ± 0.15 <sup>d</sup>	117.98 ± 1.31 <sup>d</sup>	1341 ± 16.23 <sup>c,d</sup>	12.83 ± 0.06 <sup>d</sup>
	P3	40.10 ± 0.11 <sup>d</sup>	25.63 ± 0.16 <sup>d</sup>	24.57 ± 0.17 <sup>c</sup>	131.06 ± 1.94 <sup>c</sup>	1403 ± 11.12 <sup>b,c,d</sup>	14.43 ± 0.14 <sup>c</sup>
RA3	P0	40.10 ± 0.16 <sup>d</sup>	21.94 ± 0.11 <sup>f</sup>	20.16 ± 0.12 <sup>e</sup>	105.64 ± 1.64 <sup>e</sup>	1497 ± 18.63 <sup>a,b</sup>	11.13 ± 0.08 <sup>e</sup>
	P1	44.07 ± 0.17 <sup>c</sup>	28.87 ± 0.14 <sup>c</sup>	25.20 ± 0.15 <sup>c</sup>	133.86 ± 1.81 <sup>c</sup>	1423 ± 14.53 <sup>a,b,c</sup>	15.90 ± 0.11 <sup>b</sup>
	P2	44.87 ± 0.07 <sup>b</sup>	32.46 ± 0.12 <sup>b</sup>	30.37 ± 0.40 <sup>b</sup>	162.61 ± 2.17 <sup>b</sup>	1491 ± 13.02 <sup>a,b</sup>	16.93 ± 0.14 <sup>a</sup>
	P3	45.27 ± 0.08 <sup>a</sup>	34.27 ± 0.21 <sup>a</sup>	31.27 ± 0.18 <sup>a</sup>	169.78 ± 1.01 <sup>a</sup>	1548 ± 17.52 <sup>a</sup>	16.97 ± 0.18 <sup>a</sup>
<b>ANOVA</b>	<b>df</b>	<b>P-value</b>					
RA	3	<0.001	<0.001	<0.001	<0.001	0.001	<0.001
P	3	<0.001	<0.001	<0.001	<0.001	0.001	<0.001
RA × P	9	<0.001	<0.001	<0.001	<0.001	0.032	<0.001

Means followed by different letters under the same factor are significantly different according to Tukey's HSD test ( $p \leq 0.05$ ).

Protein % in seeds was calculated by multiplying N% by a factor of 6.25

$NRE = \text{Total N uptake (kg ha}^{-1}) \times 100 / \text{N applied (kg ha}^{-1})$

$NUE = \text{Seed yield (kg ha}^{-1}) / \text{N applied (kg ha}^{-1})$

## Determination of phosphorus

In an acid mixture of  $\text{HNO}_3$  and  $\text{HClO}_4$ , samples of seeds and straw were digested. Then, P was determined by developing color by color reagent (ammonium molybdate, ammonium vanadate, and nitric acid) with a spectrophotometer ANA-730 at 470 nm wavelength after calibrating with P standards (Horwitz et al., 2000). The accumulated total P in seeds and straw were used to calculate P recovery efficiency (PRE), and P use efficiency (PUE), according to the following equations:

$PRE = \text{Total P uptake (kg ha}^{-1}) \times 100 / \text{applied P (kg ha}^{-1})$

$PUE = \text{Seed yield (kg ha}^{-1}) / \text{applied P (kg ha}^{-1})$

## Statistical analysis

The obtained data were subjected to normality distribution of the residuals and homogeneity of variances prior to analysis of variance (ANOVA) using Shapiro–Wilk and Bartlett's tests (Bartlett, 1937; Shapiro and Wilk, 1965). The combined data of two seasons were subjected to ANOVA using R statistical software version 4.4.1. Differences among the treatments were separated by Tukey's HSD test ( $P \leq 0.05$ ).

Regression analysis was performed between total N and P uptake as dependent variables and root traits as independent variables. A biplot of principal component analysis (PCA) was performed to study the relationship among the studied traits.

## Results

### Root traits

The applied RA, P fertilization, and their interaction displayed significant impacts on the measured root traits; thus, significantly enhanced the root length, number of active nodules  $\text{plant}^{-1}$ , dry weight of active nodules, total root dry weight, and nitrogenase enzyme activity (Table 1). The application of RA at  $3.75 \text{ l ha}^{-1}$  (RA3) and P level of  $100 \text{ kg P}_2\text{O}_5 \text{ ha}^{-1}$  (P3) exhibited the highest values of all root traits. Compared to untreated control (RA0), the RA3 treatment enhanced the root length by 88.5%, the total number of nodules  $\text{plant}^{-1}$  by 128.6%, and number of active nodules  $\text{plant}^{-1}$  by 133.0%. Similarly, the dry weight of active nodules, the dry weight of total root, and nitrogenase activity

increased by 131.6, 45.8, and 95.8%, respectively, when RA3 was applied (Table 1).

The P3 treatment, which is the highest P treatment in this study, also enhanced all the root traits tested. For example, the root length, total number of nodules  $\text{plant}^{-1}$ , number of active nodules  $\text{plant}^{-1}$ , dry weight of active nodules, total root dry weight, and nitrogenase activity increased by 32.8, 66.4, 66.0, 69.6, 13.8, and 46.7%, respectively, compared to P0 treatment (control) (Table 1). The combination of RA3-P2 and RA3-P3 exhibited the highest enhancement in the root length by 142.0 and 144.2%, the total number of nodules  $\text{plant}^{-1}$  by 257.1 and 277.0%, number of active nodules  $\text{plant}^{-1}$  by 247.1 and 257.4%, respectively, compared to the corresponding control treatment (RA0-P0) (Table 1). In addition, the active nodules dry weight increased by 242.5 and 257.5%, total root dry weight increased by 56.5% and 62.4% and the nitrogenase activity increased by 147.5 and 148.1%, respectively (Table 1).

## Physiological parameters

The treatments of RA and P fertilization significantly affected chlorophyll *a*, *b*, and carotenoids contents (Table 2). Increasing the RA rate to  $3.75 \text{ l ha}^{-1}$  (RA3) caused considerable increases in chlorophyll *a*, *b*, and carotenoids by 75.0, 112.0, and 274.2%, respectively, compared to untreated control (RA0). The highest P level exhibited the highest contents of these photosynthetic pigments (Table 2).

For example, the P3 increased the contents of chlorophyll *a*, *b*, and carotenoids in lentil plants by 35.0, 48.4, and 89.6% compared to control plants without any P fertilizer applied (P0). The interactive effect of RA3-P2 and RA3-P3 showed the highest photosynthetic pigments with an increase of 120.9 and 139.5% in chlorophyll *a*, 173.7 and 226.3% in chlorophyll *b*, and 605.6 and 733.3% in carotenoids, respectively, compared to non-treated control (RA0-P0) (Table 2).

## Yield and its attributes

Treatments with either RA or P fertilization significantly affected yield and its attributes in plants (Table 3). The increasing rate of RA to  $3.75 \text{ l ha}^{-1}$  (RA3) enhanced plant height by 71.5%, number of branches  $\text{plant}^{-1}$  by 23.1%, number of pods  $\text{plant}^{-1}$  by 66.2%, 1,000-seed weight by 29.1%, seed yield by 77.4%, and biological yield by 84.5%, compared to those in the RA0 control (Table 3). Likewise, plants treated with the highest P level showed the highest values of yield traits. P3 treatment enhanced plant height by 20.5%, number of branches  $\text{plant}^{-1}$  by 50.3%, number of pods  $\text{plant}^{-1}$  by 36.8%, 1,000-seed

TABLE 2 Impact of root activator (RA) and phosphorus (P) level on chlorophyll *a*, chlorophyll *b*, and carotenoids contents (mg 100 g<sup>-1</sup> fresh weight) in lentil plants.

Factor		Chlorophyll <i>a</i>	Chlorophyll <i>b</i>	Carotenoids
<b>RA</b>				
RA0 (control)		0.52 ± 0.01 <sup>d</sup>	0.25 ± 0.01 <sup>d</sup>	0.31 ± 0.03 <sup>d</sup>
RA1 (1.25 l ha <sup>-1</sup> )		0.61 ± 0.02 <sup>c</sup>	0.28 ± 0.02 <sup>c</sup>	0.38 ± 0.05 <sup>c</sup>
RA2 (2.50 l ha <sup>-1</sup> )		0.82 ± 0.02 <sup>b</sup>	0.44 ± 0.01 <sup>b</sup>	0.83 ± 0.03 <sup>b</sup>
RA3 (3.75 l ha <sup>-1</sup> )		0.91 ± 0.03 <sup>a</sup>	0.53 ± 0.02 <sup>a</sup>	1.16 ± 0.07 <sup>a</sup>
<b>P</b>				
P0 (control)		0.60 ± 0.04 <sup>d</sup>	0.31 ± 0.03 <sup>d</sup>	0.48 ± 0.08 <sup>d</sup>
P1 (50 kg P <sub>2</sub> O <sub>5</sub> ha <sup>-1</sup> )		0.70 ± 0.05 <sup>c</sup>	0.34 ± 0.04 <sup>c</sup>	0.57 ± 0.10 <sup>c</sup>
P2 (75 kg P <sub>2</sub> O <sub>5</sub> ha <sup>-1</sup> )		0.76 ± 0.04 <sup>b</sup>	0.39 ± 0.03 <sup>b</sup>	0.73 ± 0.11 <sup>b</sup>
P3 (100 kg P <sub>2</sub> O <sub>5</sub> ha <sup>-1</sup> )		0.81 ± 0.04 <sup>a</sup>	0.46 ± 0.03 <sup>a</sup>	0.91 ± 0.11 <sup>a</sup>
<b>Interaction</b>				
RA0	P0	0.43 ± 0.009 <sup>k</sup>	0.19 ± 0.007 <sup>k</sup>	0.18 ± 0.01 <sup>i</sup>
	P1	0.50 ± 0.010 <sup>j,k</sup>	0.21 ± 0.008 <sup>k</sup>	0.21 ± 0.01 <sup>i</sup>
	P2	0.57 ± 0.010 <sup>i</sup>	0.26 ± 0.005 <sup>i,j</sup>	0.36 ± 0.01 <sup>h</sup>
	P3	0.58 ± 0.008 <sup>h,i</sup>	0.32 ± 0.005 <sup>g,h</sup>	0.50 ± 0.01 <sup>g</sup>
RA1	P0	0.48 ± 0.010 <sup>k</sup>	0.21 ± 0.008 <sup>k</sup>	0.20 ± 0.01 <sup>i</sup>
	P1	0.56 ± 0.012 <sup>j</sup>	0.24 ± 0.009 <sup>j,k</sup>	0.24 ± 0.01 <sup>i</sup>
	P2	0.66 ± 0.011 <sup>g,h</sup>	0.30 ± 0.006 <sup>h,i</sup>	0.42 ± 0.02 <sup>g,h</sup>
	P3	0.73 ± 0.010 <sup>f</sup>	0.38 ± 0.006 <sup>f</sup>	0.67 ± 0.02 <sup>f</sup>
RA2	P0	0.71 ± 0.013 <sup>f,g</sup>	0.37 ± 0.008 <sup>f,g</sup>	0.69 ± 0.02 <sup>e,f</sup>
	P1	0.81 ± 0.015 <sup>d,e</sup>	0.42 ± 0.009 <sup>e,f</sup>	0.78 ± 0.02 <sup>d,e</sup>
	P2	0.86 ± 0.012 <sup>c,d</sup>	0.46 ± 0.011 <sup>c,d,e</sup>	0.87 ± 0.02 <sup>d</sup>
	P3	0.90 ± 0.007 <sup>b,c</sup>	0.50 ± 0.003 <sup>b,c</sup>	0.97 ± 0.003 <sup>c</sup>
RA3	P0	0.76 ± 0.012 <sup>e,f</sup>	0.45 ± 0.006 <sup>d,e</sup>	0.83 ± 0.06 <sup>d</sup>
	P1	0.92 ± 0.015 <sup>b,c</sup>	0.50 ± 0.007 <sup>b,c</sup>	1.04 ± 0.08 <sup>c</sup>
	P2	0.95 ± 0.008 <sup>b</sup>	0.52 ± 0.005 <sup>b</sup>	1.27 ± 0.03 <sup>b</sup>
	P3	1.03 ± 0.037 <sup>a</sup>	0.62 ± 0.033 <sup>a</sup>	1.50 ± 0.05 <sup>a</sup>
<b>ANOVA</b>	<b>df</b>	<b>P-value</b>		
RA	3	<0.001	<0.001	<0.001
P	3	<0.001	<0.001	<0.001
RA × P	9	0.008	0.053	<0.001

Means followed by different letters under the same factor are significantly different according to Tukey's HSD test ( $p \leq 0.05$ ).

weight by 19.1%, seed yield by 52.9%, and biological yield by 32.0%, compared to plants without P application (P0) (Table 3).

The combinations of RA and P fertilization displayed significant effects on yield traits. The application of RA3-P3 and RA3-P2 on plants exhibited the highest values for all evaluated agronomic traits (Table 3). We noticed that these interactions, RA3-P2 and RA3-P3, resulted in an enhancement in plant height by 106.4 and 107.7%, number of branches plant<sup>-1</sup> by 56.3 and 83.3%, number of pods plant<sup>-1</sup> by 125.4 and 128.4%, 1,000-seed weight by 48.0 and 47.6%, seed yield by 169.5 and 171.2%, and biological yield by 131.4 and 139.7%, respectively, compared to non-treated control RA0-P0 (Table 3).

## Quality traits

Seed protein yield (SPY) and seed carbohydrate yield (SCY) of lentil plants were significantly affected by RA, P fertilization, and their interaction (Table 4). Thus, the highest values of SPY and SCY in plants treated with RA were recorded by RA3 displaying 163.8% and 92.3%, respectively, compared to those in RA0 (Table 4). Among all P treatments, plants treated with P3 showed the highest values of SPY and SCY by 87.2% and 66.7%, respectively, compared to plants treated with no P fertilizer (P0) (Table 4).

The interaction between RA3 with P2 and RA3 with P3 achieved the highest values of SPY, surpassing the untreated control (RA0-P0) by 382.6 and 389.4%, respectively. SCY was

TABLE 3 Impact of root activator (RA) and phosphorus (P) levels on lentil seed yield and its attributes.

Factor		Plant height (cm)	Number of branches plants <sup>-1</sup>	Number of pods plant <sup>-1</sup>	1000 seed weight (g)	Seed yield (kg ha <sup>-1</sup> )	Biological yield (kg ha <sup>-1</sup> )
<b>RA</b>							
RA0 (control)		28.57 ± 0.88 <sup>d</sup>	3.42 ± 0.14 <sup>c</sup>	28.02 ± 1.24 <sup>d</sup>	24.60 ± 0.41 <sup>d</sup>	835 ± 39.5 <sup>d</sup>	2318 ± 71.95 <sup>d</sup>
RA1 (1.25 l ha <sup>-1</sup> )		34.67 ± 1.38 <sup>c</sup>	4.02 ± 0.21 <sup>b</sup>	38.69 ± 2.18 <sup>c</sup>	28.13 ± 0.56 <sup>c</sup>	1193 ± 63.27 <sup>c</sup>	3721 ± 127.96 <sup>c</sup>
RA2 (2.50 l ha <sup>-1</sup> )		42.96 ± 0.77 <sup>b</sup>	4.24 ± 0.19 <sup>a</sup>	45.09 ± 1.07 <sup>b</sup>	30.57 ± 0.80 <sup>b</sup>	1406 ± 65.85 <sup>b</sup>	4146 ± 124.86 <sup>b</sup>
RA3 (3.75 l ha <sup>-1</sup> )		49.01 ± 0.92 <sup>a</sup>	4.21 ± 0.22 <sup>a</sup>	46.58 ± 1.08 <sup>a</sup>	31.75 ± 0.57 <sup>a</sup>	1481 ± 71.03 <sup>a</sup>	4276 ± 99.67 <sup>a</sup>
<b>P</b>							
P0 (control)		34.35 ± 2.68 <sup>d</sup>	3.24 ± 0.7 <sup>d</sup>	32.78 ± 2.38 <sup>d</sup>	25.86 ± 0.71 <sup>d</sup>	952 ± 63.33 <sup>d</sup>	3021 ± 208.51 <sup>d</sup>
P1 (50 kg P <sub>2</sub> O <sub>5</sub> ha <sup>-1</sup> )		38.43 ± 2.57 <sup>c</sup>	3.49 ± 0.09 <sup>c</sup>	38.26 ± 2.33 <sup>c</sup>	28.49 ± 0.73 <sup>c</sup>	1146 ± 60.87 <sup>c</sup>	3589 ± 234.99 <sup>c</sup>
P2 (75 kg P <sub>2</sub> O <sub>5</sub> ha <sup>-1</sup> )		41.05 ± 2.13 <sup>b</sup>	4.29 ± 0.09 <sup>b</sup>	42.50 ± 2.18 <sup>b</sup>	29.89 ± 1.06 <sup>b</sup>	1361 ± 93.78 <sup>b</sup>	3863 ± 241.24 <sup>b</sup>
P3 (100 kg P <sub>2</sub> O <sub>5</sub> ha <sup>-1</sup> )		41.38 ± 2.07 <sup>a</sup>	4.87 ± 0.11 <sup>a</sup>	44.83 ± 2.12 <sup>a</sup>	30.80 ± 0.86 <sup>a</sup>	1456 ± 86.01 <sup>a</sup>	3989 ± 259.56 <sup>a</sup>
<b>Interaction</b>							
RA0	P0	24.04 ± 0.11 <sup>j</sup>	2.88 ± 0.004 <sup>j</sup>	21.97 ± 0.33 <sup>m</sup>	22.69 ± 0.17 <sup>h</sup>	632 ± 2.59 <sup>m</sup>	1925 ± 11.40 <sup>n</sup>
	P1	27.96 ± 0.12 <sup>i</sup>	3.03 ± 0.005 <sup>i</sup>	26.75 ± 0.40 <sup>c</sup>	24.65 ± 0.19 <sup>g</sup>	824 ± 3.38 <sup>c</sup>	2341 ± 13.86 <sup>m</sup>
	P2	30.73 ± 0.19 <sup>h</sup>	3.74 ± 0.004 <sup>e,f</sup>	30.60 ± 0.24 <sup>j</sup>	24.74 ± 0.12 <sup>g</sup>	900 ± 7.98 <sup>k</sup>	2498 ± 7.85 <sup>c</sup>
	P3	31.54 ± 0.17 <sup>g</sup>	4.03 ± 0.03 <sup>d</sup>	32.75 ± 0.31 <sup>i</sup>	26.34 ± 0.24 <sup>f</sup>	985 ± 9.01 <sup>j</sup>	2511 ± 13.17 <sup>c</sup>
RA1	P0	27.96 ± 0.12 <sup>i</sup>	3.20 ± 0.005 <sup>h</sup>	28.53 ± 0.42 <sup>k</sup>	25.22 ± 0.19 <sup>g</sup>	903 ± 3.70 <sup>k</sup>	2961 ± 14.53 <sup>k</sup>
	P1	32.90 ± 0.15 <sup>f</sup>	3.44 ± 0.005 <sup>g</sup>	35.67 ± 0.53 <sup>h</sup>	28.33 ± 0.21 <sup>e</sup>	1128 ± 4.63 <sup>i</sup>	3525 ± 12.88 <sup>i</sup>
	P2	38.90 ± 0.25 <sup>e</sup>	4.45 ± 0.005 <sup>c</sup>	43.10 ± 0.34 <sup>e</sup>	28.53 ± 0.18 <sup>e</sup>	1262 ± 8.41 <sup>g</sup>	4096 ± 12.87 <sup>g</sup>
	P3	38.93 ± 0.18 <sup>e</sup>	4.97 ± 0.04 <sup>b</sup>	47.47 ± 0.46 <sup>c</sup>	30.43 ± 0.14 <sup>c</sup>	1479 ± 9.82 <sup>d</sup>	4304 ± 10.08 <sup>e</sup>
RA2	P0	38.73 ± 0.11 <sup>e</sup>	3.49 ± 0.03 <sup>g</sup>	39.38 ± 0.17 <sup>g</sup>	26.33 ± 0.20 <sup>f</sup>	1102 ± 6.13 <sup>i</sup>	3459 ± 10.98 <sup>j</sup>
	P1	43.03 ± 0.12 <sup>d</sup>	3.84 ± 0.03 <sup>e</sup>	45.27 ± .020 <sup>d</sup>	30.27 ± 0.24 <sup>c</sup>	1299 ± 7.23 <sup>f</sup>	4193 ± 13.31 <sup>f</sup>
	P2	44.93 ± 0.14 <sup>c</sup>	4.46 ± 0.005 <sup>c</sup>	46.77 ± 0.23 <sup>c</sup>	32.73 ± 0.13 <sup>b</sup>	1578 ± 6.76 <sup>c</sup>	4403 ± 14.37 <sup>d</sup>
	P3	45.13 ± 0.12 <sup>c</sup>	5.18 ± 0.04 <sup>a</sup>	48.93 ± 0.14 <sup>b</sup>	32.93 ± 0.15 <sup>a,b</sup>	1644 ± 5.50 <sup>b</sup>	4527 ± 8.84 <sup>b</sup>
RA3	P0	46.64 ± 0.29 <sup>b</sup>	3.41 ± 0.05 <sup>g</sup>	41.24 ± 0.25 <sup>f</sup>	29.20 ± 0.31 <sup>d</sup>	1173 ± 8.26 <sup>h</sup>	3738 ± 7.03 <sup>h</sup>
	P1	49.83 ± 0.31 <sup>a</sup>	3.65 ± 0.06 <sup>f</sup>	45.37 ± 0.28 <sup>d</sup>	30.73 ± 0.27 <sup>c</sup>	1333 ± 9.07 <sup>e</sup>	4297 ± 9.78 <sup>e</sup>
	P2	49.63 ± 0.12 <sup>a</sup>	4.50 ± 0.08 <sup>c</sup>	49.53 ± 0.28 <sup>a,b</sup>	33.57 ± 0.16 <sup>a</sup>	1703 ± 10.76 <sup>a</sup>	4455 ± 11.42 <sup>c</sup>
	P3	49.93 ± 0.18 <sup>a</sup>	5.28 ± 0.02 <sup>a</sup>	50.17 ± 0.18 <sup>a</sup>	33.50 ± 0.15 <sup>a</sup>	1714 ± 7.68 <sup>a</sup>	4614 ± 10.03 <sup>a</sup>
<b>ANOVA</b>	<b>df</b>	<b>P-value</b>					
RA	3	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
P	3	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
RA × P	9	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Means followed by different letters under the same factor are significantly different according to Tukey's HSD test ( $p \leq 0.05$ ).



TABLE 4 Impact of root activator (RA) and phosphorus (P) levels on protein and carbohydrate yields of lentil seeds.

Factor		Protein yield (kg ha <sup>-1</sup> )	Carbohydrate yield (kg ha <sup>-1</sup> )
<b>RA</b>			
RA0 (control)		143.87 ± 10.96 <sup>d</sup>	414.96 ± 23.77 <sup>d</sup>
RA1 (1.25 l ha <sup>-1</sup> )		230.47 ± 19.95 <sup>c</sup>	680.20 ± 42.23 <sup>c</sup>
RA2 (2.50 l ha <sup>-1</sup> )		341.80 ± 22.47 <sup>b</sup>	770.83 ± 41.43 <sup>b</sup>
RA3 (3.75 l ha <sup>-1</sup> )		379.52 ± 22.89 <sup>a</sup>	797.83 ± 43.03 <sup>a</sup>
<b>P</b>			
P0 (control)		186.87 ± 21.40 <sup>d</sup>	478.92 ± 35.35 <sup>d</sup>
P1 (50 kg P <sub>2</sub> O <sub>5</sub> ha <sup>-1</sup> )		247.75 ± 25.50 <sup>c</sup>	635.73 ± 40.91 <sup>c</sup>
P2 (75 kg P <sub>2</sub> O <sub>5</sub> ha <sup>-1</sup> )		311.27 ± 34.95 <sup>b</sup>	750.91 ± 56.13 <sup>b</sup>
P3 (100 kg P <sub>2</sub> O <sub>5</sub> ha <sup>-1</sup> )		349.77 ± 30.94 <sup>a</sup>	799.43 ± 51.72 <sup>a</sup>
<b>Interaction</b>			
RA0	P0	92.75 ± 1.25 <sup>c</sup>	293.55 ± 1.82 <sup>c</sup>
	P1	133.30 ± 1.79 <sup>k</sup>	405.64 ± 2.25 <sup>k</sup>
	P2	157.34 ± 1.78 <sup>j</sup>	454.52 ± 2.77 <sup>j</sup>
	P3	192.11 ± 1.55 <sup>i</sup>	506.13 ± 2.70 <sup>i</sup>
RA1	P0	147.22 ± 1.98 <sup>j</sup>	465.96 ± 2.89 <sup>j</sup>
	P1	202.22 ± 2.12 <sup>i</sup>	669.47 ± 3.16 <sup>f</sup>
	P2	244.87 ± 2.32 <sup>h</sup>	735.09 ± 3.35 <sup>e</sup>
	P3	327.56 ± 1.97 <sup>e</sup>	850.28 ± 4.82 <sup>d</sup>
RA2	P0	235.05 ± 1.70 <sup>h</sup>	560.82 ± 5.03 <sup>h</sup>
	P1	311.44 ± 1.58 <sup>f</sup>	734.82 ± 6.59 <sup>e</sup>
	P2	395.24 ± 1.56 <sup>c</sup>	880.19 ± 6.04 <sup>c</sup>
	P3	425.46 ± 2.08 <sup>b</sup>	907.48 ± 4.09 <sup>b</sup>
RA3	P0	272.47 ± 1.22 <sup>g</sup>	595.35 ± 7.77 <sup>g</sup>
	P1	344.03 ± 1.59 <sup>d</sup>	732.97 ± 9.57 <sup>e</sup>
	P2	447.62 ± 1.99 <sup>a</sup>	929.18 ± 5.07 <sup>a</sup>
	P3	453.95 ± 1.52 <sup>a</sup>	933.83 ± 9.91 <sup>a</sup>
<b>ANOVA</b>	<b>df</b>	<b>P-value</b>	
RA	3	<0.001	<0.001
P	3	<0.001	<0.001
RA × P	9	<0.001	<0.001

Means followed by different letters under the same factor are significantly different according to Tukey's HSD test ( $p \leq 0.05$ ).

also increased by 216.5 and 218.1%, respectively, compared to RA0-P0 (Table 4).

### Nitrogen parameters

All studied N parameters were significantly affected by the RA, P fertilization, and their interaction. The parameters were increased as the rates of the RA or levels of P increased; thus, the highest measurements were recorded at 3.75 l ha<sup>-1</sup> (RA3) or 100 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> (P3), compared to RA0 or P0 control treatments (Table 5). Our results displayed considerable enhancement of N% in seed, N% in straw, total N uptake, NRE, and NUE values in plants treated with the highest rate of RA (RA3) by 50.2, 42.4, 164.8, 164.7,

and 77.3%, respectively, compared to the untreated control (RA0) (Table 5).

Lentil plants supplemented with the highest P level (P3) boosted the N% in seed by 24.5%, N% in straw by 21.6%, total N uptake by 78.8%, NRE by 78.7%, and NUE by 52.8% as compared to non-added P control (P0) in the same order (Table 5). Moreover, the highest amounts of N% in seeds were found in RA3-P2 and RA3-P3 treatments, exhibiting a 79.1% and 80.4% increase, respectively, when compared to the untreated control (RA0-P0) (Table 5). Likewise, treatments of RA3-P2 and RA3-P3 enhanced N% in straw by 56.7 and 70.0%, respectively, compared to the untreated control. Total N uptake, NRE, and NUE values exceeded the values of the untreated control (RA0-P0) by 351.0, 350.7, and 169.6%, respectively, in response to RA3-P2 treatment, and

**TABLE 5** Impact of root activator (RA) and phosphorus (P) levels on N in seed (%), N in straw (%), total N uptake (kg ha<sup>-1</sup>), N recovery efficiency (NRE), and N use efficiency (NUE).

Factor		N in seeds	N in straw	Total N uptake	NRE	NUE
<b>RA</b>						
RA0 (control)		2.71 ± 0.086 <sup>d</sup>	0.33 ± 0.009 <sup>d</sup>	27.95 ± 1.94 <sup>d</sup>	79.85 ± 5.55 <sup>d</sup>	23.86 ± 1.12 <sup>d</sup>
RA1 (1.25 l ha <sup>-1</sup> )	3.03 ± 0.095 <sup>c</sup>	0.38 ± 0.011 <sup>c</sup>	46.56 ± 3.78 <sup>c</sup>	133.04 ± 10.80 <sup>c</sup>	34.09 ± 1.80 <sup>c</sup>	
RA2 (2.50 l ha <sup>-1</sup> )		3.85 ± 0.083 <sup>b</sup>	0.46 ± 0.009 <sup>b</sup>	67.29 ± 4.05 <sup>b</sup>	192.26 ± 11.58 <sup>b</sup>	40.16 ± 1.88 <sup>b</sup>
RA3 (3.75 l ha <sup>-1</sup> )		4.07 ± 0.065 <sup>a</sup>	0.47 ± 0.008 <sup>a</sup>	74.02 ± 3.95 <sup>a</sup>	211.48 ± 11.29 <sup>a</sup>	42.30 ± 2.02 <sup>a</sup>
<b>P</b>						
P0 (control)		3.02 ± 0.070 <sup>d</sup>	0.37 ± 0.017 <sup>d</sup>	37.81 ± 4.26 <sup>d</sup>	108.04 ± 12.19 <sup>d</sup>	27.21 ± 1.80 <sup>d</sup>
P1 (50 kg P <sub>2</sub> O <sub>5</sub> ha <sup>-1</sup> )		3.36 ± 0.095 <sup>c</sup>	0.41 ± 0.020 <sup>c</sup>	49.91 ± 5.20 <sup>c</sup>	142.60 ± 14.86 <sup>c</sup>	32.74 ± 1.73 <sup>c</sup>
P2 (75 kg P <sub>2</sub> O <sub>5</sub> ha <sup>-1</sup> )		3.53 ± 0.079 <sup>b</sup>	0.42 ± 0.018 <sup>b</sup>	60.51 ± 6.46 <sup>b</sup>	172.88 ± 18.47 <sup>b</sup>	38.88 ± 2.67 <sup>b</sup>
P3 (100 kg P <sub>2</sub> O <sub>5</sub> ha <sup>-1</sup> )		3.76 ± 0.068 <sup>a</sup>	0.45 ± 0.017 <sup>a</sup>	67.59 ± 6.02 <sup>a</sup>	193.12 ± 17.20 <sup>a</sup>	41.59 ± 2.45 <sup>a</sup>
<b>Interaction</b>						
RA0	P0	2.35 ± 0.033 <sup>k</sup>	0.30 ± 0.002 <sup>j</sup>	18.75 ± 0.23 <sup>n</sup>	53.58 ± 0.87 <sup>n</sup>	18.05 ± 0.07 <sup>m</sup>
	P1	2.59 ± 0.036 <sup>j</sup>	0.32 ± 0.017 <sup>h,j</sup>	26.14 ± 0.25 <sup>m</sup>	74.70 ± 0.93 <sup>m</sup>	23.53 ± 0.09 <sup>c</sup>
	P2	2.80 ± 0.016 <sup>h,i</sup>	0.34 ± 0.009 <sup>h</sup>	30.54 ± 0.13 <sup>c</sup>	87.25 ± 0.97 <sup>c</sup>	25.72 ± 0.31 <sup>k</sup>
	P3	3.12 ± 0.046 <sup>g</sup>	0.37 ± 0.010 <sup>g</sup>	36.36 ± 0.72 <sup>k</sup>	103.89 ± 1.16 <sup>k</sup>	28.15 ± 0.25 <sup>j</sup>
RA1	P0	2.61 ± 0.037 <sup>i,j</sup>	0.33 ± 0.007 <sup>h,i</sup>	30.32 ± 0.46 <sup>c</sup>	86.62 ± 1.33 <sup>c</sup>	25.79 ± 0.10 <sup>k</sup>
	P1	2.87 ± 0.040 <sup>h</sup>	0.37 ± 0.008 <sup>g</sup>	41.20 ± 0.63 <sup>j</sup>	117.72 ± 1.80 <sup>j</sup>	32.24 ± 0.13 <sup>i</sup>
	P2	3.10 ± 0.029 <sup>g</sup>	0.39 ± 0.003 <sup>f</sup>	50.33 ± 0.27 <sup>h</sup>	143.81 ± 0.79 <sup>h</sup>	36.07 ± 0.24 <sup>g</sup>
	P3	3.54 ± 0.053 <sup>e,f</sup>	0.43 ± 0.004 <sup>e</sup>	64.41 ± 0.69 <sup>f</sup>	184.02 ± 1.12 <sup>f</sup>	42.25 ± 0.65 <sup>d</sup>
RA2	P0	3.41 ± 0.032 <sup>f</sup>	0.42 ± 0.006 <sup>e,f</sup>	47.38 ± 0.34 <sup>i</sup>	135.38 ± 0.99 <sup>i</sup>	31.47 ± 0.17 <sup>i</sup>
	P1	3.84 ± 0.036 <sup>c,d</sup>	0.46 ± 0.006 <sup>c,d</sup>	63.03 ± 0.46 <sup>f</sup>	180.08 ± 1.12 <sup>f</sup>	37.11 ± 0.20 <sup>f</sup>
	P2	4.01 ± 0.030 <sup>b,c</sup>	0.47 ± 0.009 <sup>b</sup>	76.61 ± 0.33 <sup>d</sup>	218.88 ± 0.96 <sup>d</sup>	45.09 ± 0.19 <sup>c</sup>
	P3	4.14 ± 0.031 <sup>a,b</sup>	0.49 ± 0.005 <sup>a,b</sup>	82.15 ± 0.35 <sup>c</sup>	234.72 ± 1.01 <sup>c</sup>	46.97 ± 0.15 <sup>b</sup>
RA3	P0	3.72 ± 0.055 <sup>de</sup>	0.44 ± 0.002 <sup>d,e</sup>	54.80 ± 0.45 <sup>g</sup>	156.58 ± 1.43 <sup>g</sup>	33.51 ± 0.37 <sup>h</sup>
	P1	4.13 ± 0.061 <sup>a,b</sup>	0.48 ± 0.003 <sup>b</sup>	69.27 ± 0.77 <sup>e</sup>	197.92 ± 1.07 <sup>e</sup>	38.08 ± 0.43 <sup>e</sup>
	P2	4.21 ± 0.023 <sup>a,b</sup>	0.47 ± 0.010 <sup>b,c</sup>	84.56 ± 0.55 <sup>b</sup>	241.59 ± 1.58 <sup>b</sup>	48.66 ± 0.36 <sup>a</sup>
	P3	4.24 ± 0.026 <sup>a</sup>	0.51 ± 0.016 <sup>a</sup>	87.45 ± 0.48 <sup>a</sup>	249.85 ± 1.05 <sup>a</sup>	48.98 ± 0.21 <sup>a</sup>
<b>ANOVA</b>	<b>df</b>	<b>P-value</b>				
RA	3	<0.001	<0.001	<0.001	<0.001	<0.001
P	3	<0.001	<0.001	<0.001	<0.001	<0.001
RA × P	9	<0.001	0.013	<0.001	<0.001	<0.001

Means followed by different letters under the same factor are significantly different according to Tukey's HSD test ( $p \leq 0.05$ ).

366.4, 366.3, and 171.4%, respectively, in response to RA3-P3 treatment (Table 5).

## Phosphorus parameters

The P contents in seed and straw were also estimated to evaluate P physiological parameters affected by the application of the RA and/or P fertilization. The results revealed the application of RA3 enhanced P% in seed, P% in straw, total P uptake, PRE, and PUE values in lentil plants by 120.0, 144.4, 315.7, 310.6, and 73.5%, respectively, compared to RA0 control plants (Table 6). When plants were supplied with P3, there were significant increases in

P% in seed, P% in straw, and total P uptake by 51.6, 60.5, and 107.8%, respectively, compared to those of P0 treatment (Table 6).

Thus, the interactive treatment between RA and P fertilization significantly impacted P parameters. The combined RA3-P3 enhanced P% in seeds, P% in straw, and total P uptake in plants by 252.9, 285.7, and 428.7%, respectively, in comparison to those plants that received no treatment (RA0-P0) (Table 6). Similarly, RA3-P2 boosted P% in seeds, P% in straw, and total P uptake in plants by 235.3, 257.1, and 751.3%, respectively, compared to untreated control (Table 6). However, the highest PRE and PUE values were obtained in RA3-P1, followed by RA3-P2 and RA3-P3 combined treatment (Table 6).

**TABLE 6** Impact of root activator (RA) and phosphorus (P) levels on P in seeds (%), P in straw (%), total P uptake (kg ha<sup>-1</sup>), P recovery efficiency (PRE), and P use efficiency (PUE).

Factor		P in seed	P in straw	total P uptake	PRE	PUE
<b>RA</b>						
RA0 (control)		0.25 ± 0.021 <sup>d</sup>	0.09 ± 0.004 <sup>d</sup>	3.45 ± 0.34 <sup>d</sup>	5.36 ± 0.86 <sup>d</sup>	12.78 ± 0.98 <sup>d</sup>
RA1 (1.25 l ha <sup>-1</sup> )		0.29 ± 0.018 <sup>c</sup>	0.10 ± 0.005 <sup>c</sup>	6.20 ± 0.73 <sup>c</sup>	9.57 ± 0.80 <sup>c</sup>	18.06 ± 1.17 <sup>c</sup>
RA2 (2.50 l ha <sup>-1</sup> )		0.48 ± 0.017 <sup>b</sup>	0.12 ± 0.004 <sup>b</sup>	10.22 ± 0.68 <sup>b</sup>	15.97 ± 1.03 <sup>b</sup>	21.15 ± 1.38 <sup>b</sup>
RA3 (3.75 l ha <sup>-1</sup> )		0.55 ± 0.015 <sup>a</sup>	0.22 ± 0.016 <sup>a</sup>	14.34 ± 0.83 <sup>a</sup>	22.01 ± 1.15 <sup>a</sup>	22.17 ± 1.46 <sup>a</sup>
<b>P</b>						
P0 (control)		0.31 ± 0.040 <sup>d</sup>	0.10 ± 0.09 <sup>c</sup>	5.42 ± 0.93 <sup>d</sup>		
P1 (50 kg P <sub>2</sub> O <sub>5</sub> ha <sup>-1</sup> )		0.37 ± 0.046 <sup>c</sup>	0.12 ± 0.010 <sup>b</sup>	7.54 ± 1.23 <sup>c</sup>	15.09 ± 1.22 <sup>a</sup>	22.92 ± 2.17 <sup>a</sup>
P2 (75 kg P <sub>2</sub> O <sub>5</sub> ha <sup>-1</sup> )		0.43 ± 0.036 <sup>b</sup>	0.15 ± 0.013 <sup>a</sup>	10.00 ± 1.31 <sup>b</sup>	13.33 ± 1.25 <sup>b</sup>	18.15 ± 1.88 <sup>b</sup>
P3 (100 kg P <sub>2</sub> O <sub>5</sub> ha <sup>-1</sup> )		0.47 ± 0.031 <sup>a</sup>	0.16 ± 0.012 <sup>a</sup>	11.26 ± 1.35 <sup>a</sup>	11.26 ± 0.97 <sup>c</sup>	14.56 ± 1.46 <sup>c</sup>
<b>Interaction</b>						
RA0	P0	0.17 ± 0.013 <sup>j</sup>	0.07 ± 0.002 <sup>k</sup>	1.95 ± 0.10 <sup>j</sup>		
	P1	0.20 ± 0.013 <sup>i,j</sup>	0.08 ± 0.002 <sup>j,k</sup>	2.83 ± 0.14 <sup>h</sup>	5.67 ± 0.29 <sup>g</sup>	16.47 ± 0.07 <sup>f</sup>
	P2	0.29 ± 0.010 <sup>h</sup>	0.10 ± 0.001 <sup>i</sup>	4.10 ± 0.016 <sup>g</sup>	5.55 ± 0.20 <sup>g</sup>	12.00 ± 0.15 <sup>h</sup>
	P3	0.34 ± 0.015 <sup>g</sup>	0.10 ± 0.001 <sup>i</sup>	4.87 ± 0.20 <sup>g</sup>	4.87 ± 0.18 <sup>g</sup>	9.85 ± 0.09 <sup>i</sup>
RA1	P0	0.19 ± 0.014 <sup>j</sup>	0.08 ± 0.003 <sup>k</sup>	3.26 ± 0.17 <sup>h</sup>		
	P1	0.23 ± 0.017 <sup>i</sup>	0.09 ± 0.003 <sup>j</sup>	4.67 ± 0.25 <sup>g</sup>	9.34 ± 0.32 <sup>f</sup>	22.57 ± 0.10 <sup>c</sup>
	P2	0.34 ± 0.010 <sup>g</sup>	0.11 ± 0.001 <sup>h</sup>	7.44 ± 0.08 <sup>f</sup>	9.92 ± 0.19 <sup>f</sup>	16.83 ± 0.11 <sup>e,f</sup>
	P3	0.42 ± 0.016 <sup>f</sup>	0.12 ± 0.001 <sup>g,h</sup>	9.44 ± 0.33 <sup>e</sup>	9.44 ± 0.28 <sup>f</sup>	14.79 ± 0.23 <sup>g</sup>
RA2	P0	0.39 ± 0.010 <sup>f</sup>	0.10 ± 0.002 <sup>i</sup>	6.71 ± 0.11 <sup>f</sup>		
	P1	0.48 ± 0.012 <sup>d,e</sup>	0.12 ± 0.002 <sup>f,g</sup>	9.79 ± 0.16 <sup>e</sup>	19.58 ± 0.35 <sup>c</sup>	25.98 ± 0.14 <sup>b</sup>
	P2	0.52 ± 0.012 <sup>c,d</sup>	0.13 ± 0.001 <sup>e,f</sup>	11.78 ± 0.15 <sup>d</sup>	15.71 ± 0.26 <sup>d</sup>	21.04 ± 0.10 <sup>d</sup>
	P3	0.53 ± 0.013 <sup>c</sup>	0.13 ± 0.003 <sup>e</sup>	12.61 ± 0.20 <sup>c,d</sup>	12.61 ± 0.22 <sup>e</sup>	16.44 ± 0.09 <sup>f</sup>
RA3	P0	0.48 ± 0.018 <sup>e</sup>	0.16 ± 0.003 <sup>d</sup>	9.74 ± 0.19 <sup>e</sup>		
	P1	0.55 ± 0.019 <sup>b,c</sup>	0.19 ± 0.003 <sup>c</sup>	12.88 ± 1.18 <sup>c</sup>	25.77 ± 0.21 <sup>a</sup>	26.65 ± 0.20 <sup>a</sup>
	P2	0.57 ± 0.013 <sup>a,b</sup>	0.25 ± 0.004 <sup>b</sup>	16.61 ± 0.23 <sup>b</sup>	22.15 ± 0.37 <sup>b</sup>	22.71 ± 0.17 <sup>c</sup>
	P3	0.60 ± 0.012 <sup>a</sup>	0.27 ± 0.003 <sup>a</sup>	18.11 ± 0.26 <sup>a</sup>	18.11 ± 0.28 <sup>c</sup>	17.14 ± 0.11 <sup>e</sup>
<b>ANOVA</b>		<b>Df</b>	<b>P-value</b>			
RA		3	<0.001	<0.001	<0.001	<0.001
P		3	<0.001	<0.001	<0.001	<0.001
RA × P		9	<0.001	<0.001	<0.001	<0.001

Means followed by different letters under the same factor are significantly different according to Tukey's HSD test ( $p \leq 0.05$ ).

## Regression analysis

The regression analysis exhibited a positive linear relationship between the total N uptake with the root length, number of active nodules plant<sup>-1</sup>, and the nitrogenase enzyme activity in lentil (Figures 1A–C). From  $R^2$  values, it was observed that the three root traits were highly associated with total N uptake. Likewise, the three root parameters displayed a positive linear relationship with total P uptake (Figures 1D–F).

## Interrelationship among the assessed treatments and traits

PCA was employed to study the relationship among the assessed treatments and traits, as displayed in Figure 2. The first two PCAs exhibited 90.75% of the variability. The PCA1

accounted for 77.94% of the variation and was associated with the level of assessed treatments of RA and P application from the untreated control (RA0-P0) on the extreme left to the highest level on the extreme right (RA3-P3) (Figure 2).

The highest two levels of both treatments of RA (RA2 and RA3) and P fertilization (P2 and P3) had slight multi-dimensional space as exhibited by the small distances of plots along PCA1, compared to the corresponding controls (RA0 and P0) and the low RA1 and P1 levels which were spread apart and with more dissimilarity (Figure 2). The evaluated root, physiological, agronomic, and quality traits were positively associated with the high levels of RA (RA2 and RA3) and P fertilization (P2 and P3) on the PCA1, which is consistent with the obtained results in Tables 1–6. Thereupon, the PCA biplot is emphasizing the foregoing displayed results (Figure 2).

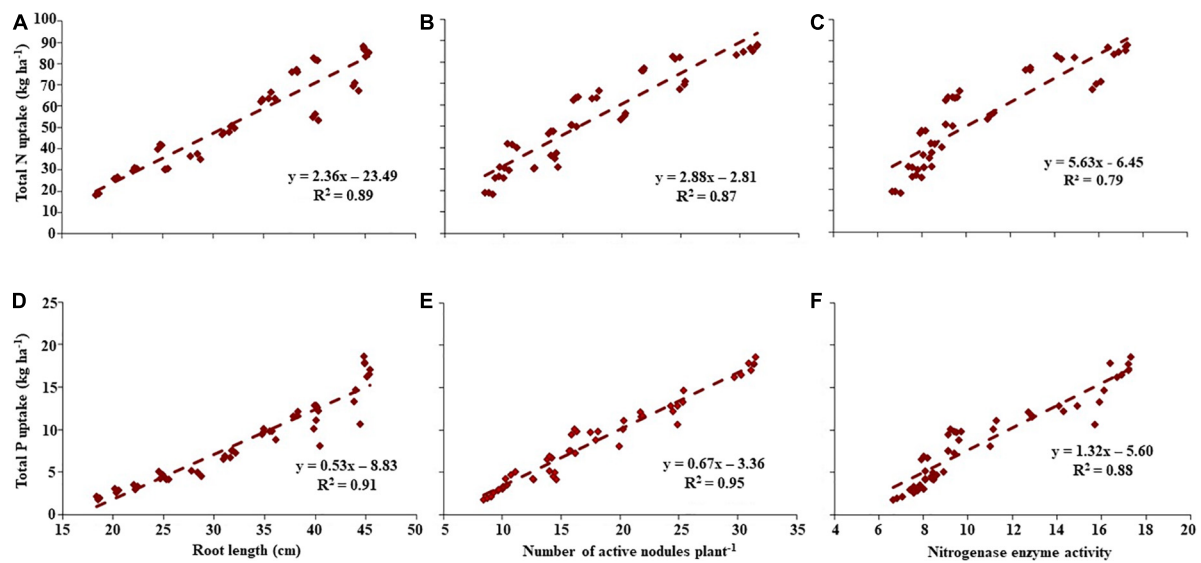


FIGURE 1

Regression relationship between total nitrogen (N) or phosphorus (P) uptake and root traits. Regression relationship of total (A–C) N and (D–F) P uptake on (A,D) root length; (B,E) number of active nodules plant<sup>-1</sup>; and (C,F) nitrogenase enzyme activity.

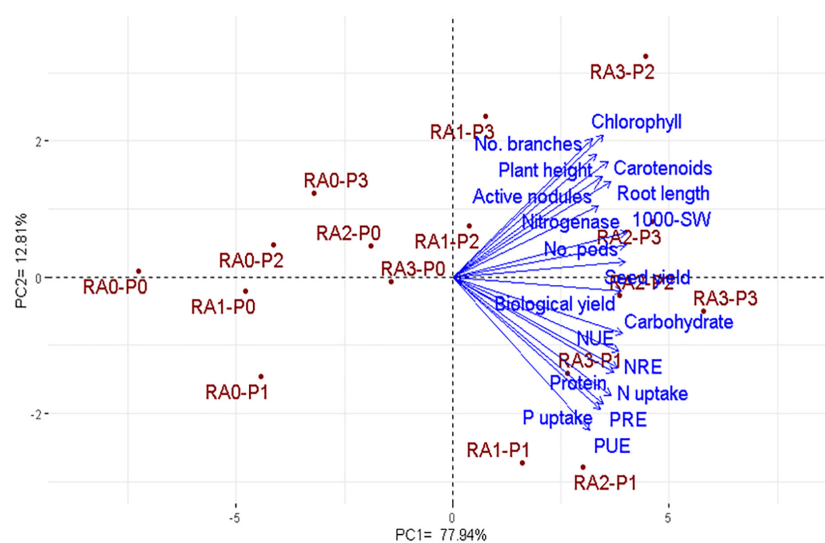


FIGURE 2

Principal component analysis (PCA) biplot for the assessed treatments of RA and P applications and the evaluated traits of lentil over the two growing seasons. RA, root activator; P, phosphorus; NUE, N use efficiency; NRE, N recovery efficiency; PUE, P use efficiency; PRE, P recovery efficiency.

## Discussion

Newly reclaimed sandy soils suffer from nutrient deficiency and various environmental stresses (Mansour et al., 2021). Accordingly, it is imperative to find ecofriendly approaches to enhance plant growth and production under these poor conditions (Desoky et al., 2021b). The present study was performed in newly reclaimed low-fertility sandy soil containing

a very low concentration of nutrients, particularly P and N, compared to the other normal fertile soils. Hence, the impact of RA and P application in different levels were assessed on lentil morphological, physiological, agronomic, and quality characteristics.

The recommended P rate in the newly reclaimed low-fertility sandy soil was 75 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup>; in addition to the lower (50 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup>) and higher (100 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup>) treatment



rates were applied. The obtained results revealed that the three P levels significantly boosted the root traits (root length, number of active nodules, dry weight of active nodules, total root dry weight, and nitrogenase activity) with the maximum values from the highest P level. Roots can control water and nutrient uptake and provide anchoring and mechanical support. P is a critical element for stimulating root development and growth (Singh and Singh, 2016; Du et al., 2022), and plays a crucial role in cell division, metabolic activities, nucleus formation, nodulation, N<sub>2</sub> fixation, as well as starch utilization (Singh et al., 2005; Singh and Singh, 2016). The applied P, particularly the highest level (P3), considerably enhanced all root traits and exhibited strong root growth. Vigorous root system with high root length, active nodules, and dry weight ensured better efficiency in uptaking macro and micronutrients (Wang et al., 2016; Su et al., 2022).

Furthermore, P3 enhanced N<sub>2</sub> fixation efficiency and N uptake by increasing the number of active nodules, nitrogenase activity, and dry weight of active nodules. The relative superiority of root traits associated with P3 treatment was in agreement with previous results. In this respect, Singh et al. (2005) have demonstrated that each increment in P fertilization can enhance the main root length and dry weight. P application enhanced primary root length, total surface area, total root tips, root forks, total dry weight, and root dry weight in lentil plants (Ramtekey et al., 2021). Similarly, the increase in P levels stimulated the N<sub>2</sub> fixation efficiency and N uptake in lentils by improving active nodules and their dry weight plant<sup>-1</sup> (Jindal et al., 2008; Rasheed et al., 2010). Mohamed et al. (2021) have also disclosed that increasing the P level boosted the root dry weight and absorption of macronutrients in common bean.

The RA application substantially stimulated all root traits with relative superiority to RA3. One strategy to enhance crop acquisition efficiency of low-available mineral elements, such as N, P, K, Fe, Mn, Cu, Zn, and Mo, in sandy soil is to improve root traits (Gahoonia and Nielsen, 2004; Gahoonia et al., 2006). In the current study, we applied the RA, which contained IBA, GA<sub>3</sub>, NAA, and K humate. Previously, the application of the plant growth promoters IBA, GA<sub>3</sub>, and NAA enhanced root growth, expansion of root hairs, and cotyledon cells (Chhun et al., 2004; Elmongy et al., 2018). The plant growth regulators have been reported to inhibit primary root elongation but stimulate lateral prolific root hair formation (Pulok et al., 2015). Besides, K humate has a positive impact on the root growth and a number of nodules; thus, this effectively enhances N<sub>2</sub> fixation and nutrient uptake (Rafique et al., 2021). Improving root traits attributed to the application of plant growth promoters has been reported extensively in other studies (Chhun et al., 2004; Pulok et al., 2015; Elmongy et al., 2018; Mao et al., 2018).

The plant pigments, including chlorophyll *a*, *b*, and carotenoids, are indispensable in the photosynthesis process, which provide the essential requirements for plant development (Desoky et al., 2021d; Khan et al., 2021). The RA and P applications significantly enhanced chlorophyll and carotenoids

content compared to untreated control. The positive impact of RA on the contents of chlorophyll *a*, *b*, and carotenoids could be attributed to the applied plant regulators IBA, GA<sub>3</sub>, and NAA (Piotrowska-Niczyporuk et al., 2012). The plant growth regulators improve the source-sink relationship through stimulating transportation and distribution of accumulates, enhancing photosynthesis and sink formation, and invigorating the photo-assimilates translocation (Li et al., 2016; Mustafa et al., 2016; Desoky et al., 2021a; Rafique et al., 2021). Likewise, the P application induced higher contents of chlorophyll *a*, *b* and carotenoids compared to the non-added control. P is an imperative nutritional component, and its lack markedly influences plant pigments (Frydenvang et al., 2015; Carstensen et al., 2018). The high level of both treatments (RA3 and P3) exhibited the maximum values of photosynthesis pigments.

Plant growth and productivity are resulted from the integration of different metabolic and physiological responses. RA and P applications exhibited positive impacts on the root and physiological parameters. Both treatments increased the root length, number of active nodules, and nitrogenase activity, which enhanced N<sub>2</sub> fixation and total N uptake. Likewise, RA and P applications increased the contents of chlorophyll *a*, *b*, and carotenoids. In particular, the highest levels of RA and P application displayed the maximum enhancement in root traits and physiological parameters; thus, this was reflected in boosting nutrient absorption and plant growth. Correspondingly, the high level of RA (3.75 l ha<sup>-1</sup>) and P fertilization (100 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup>) displayed the uppermost plant height, number of branches plant<sup>-1</sup>, number of pods plant<sup>-1</sup>, 1,000-seed weight, seed yield, and biological yield.

Similarly, the recommended P level (75 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup>, P2) and the high level of RA (3.75 l ha<sup>-1</sup>, RA3) displayed non-significant differences in plant height, number of branches plant<sup>-1</sup>, 1,000-seed weight, and seed yield, compared to the high P level (100 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup>, P3). For economic and environmental purposes, it is highly recommended to use the coapplication of P2 and RA3. In this context, several studies have reported substantial improvement in seed yield and its attributes by applying plant growth promoters (Balyan and Singh, 2005; Singh et al., 2005; Togay et al., 2008; Rafique et al., 2021). Similarly, the role of P application in enhancing seed yield and its components has been previously elucidated (Singh et al., 2005; Togay et al., 2008; Nget et al., 2022; Yang et al., 2022).

Increasing N and protein content in lentil seeds was emphatically associated with applying RA and phosphorous fertilization compared to untreated control. The highest levels of RA3 and P3 displayed the uppermost values of quality parameters. Expanding protein concentration in seeds increases the nutritional value of lentil seeds. The protein increment in lentil seeds could be resulted from promoting N<sub>2</sub> fixation, biological activities, and physiological capabilities (Togay et al., 2008; Rasheed et al., 2010; Sital et al., 2011).

## Conclusion

The current research clarified the potential morphological, physiological, agronomic, and quality parameters of lentil plants to different levels of RA and P fertilization. RA and P applications enhanced lentil growth and productivity compared to untreated control by invigorating root traits, nodulation, and physiological parameters. The highest yield traits (plant height, 1,000-seed weight, seed yield  $\text{ha}^{-1}$ ) and quality characters (protein and carbohydrate) were achieved by the coapplication of RA and P fertilization at  $3.75 \text{ l ha}^{-1}$  and  $75 \text{ kg P}_2\text{O}_5 \text{ ha}^{-1}$  with no significant differences with  $100 \text{ kg P}_2\text{O}_5 \text{ ha}^{-1}$ . Subsequently, it is recommended to apply RA and P fertilization to lentil plants at the aforementioned rates to enhance plant growth, yield, and quality and improve agricultural and environmental sustainability under newly reclaimed low-fertility soil.

## Data availability statement

The original contributions presented in this study are included in the article/**Supplementary material**, further inquiries can be directed to the corresponding author/s.

## Author contributions

MAE-h, YA-E, MR, KE-T, SA, and ME-t conceived and designed the experiments. EM, KE-T, and SA analyzed the data and drafted the manuscript. MAE-h, EM, KE-T, SA, and ME-t wrote and edited the final manuscript. All authors read and approved the final version of the manuscript.

## References

- Balyan, J. K., and Singh, M. (2005). Effect of seed inoculation, different levels of irrigation and phosphorus on nodulation and root growth development of lentil. *Res. Crop.* 6, 32–34.
- Bartlett, M. S. (1937). Properties of sufficiency and statistical tests. *Proc. R. Soc. A Math. Phys. Eng. Sci.* 160, 268–282. doi: 10.1098/rspa.1937.0109
- Carstensen, A., Herdean, A., Schmidt, S. B., Sharma, A., Spetea, C., Pribil, M., et al. (2018). The impacts of phosphorus deficiency on the photosynthetic electron transport chain. *Plant Physiol.* 177, 271–284. doi: 10.1104/pp.17.01624
- Chhun, T., Taketa, S., Tsurumi, S., and Ichii, M. (2004). Different behaviour of indole-3-acetic acid and indole-3-butyric acid in stimulating lateral root development in rice (*Oryza sativa* L.). *Plant Growth Regul.* 43, 135–143. doi: 10.1023/B:GROW.0000040120.37448.53
- Desoky, E.-S. M., Mansour, E., El-Sobky, E.-S. E., Abdul-Hamid, M. I., Taha, F. T., Elakkad, H. A., et al. (2021c). Physio-biochemical and agronomic responses of faba beans to exogenously applied nano-silicon under drought stress conditions. *Front. Plant Sci.* 12:637783. doi: 10.3389/fpls.2021.637783
- Desoky, E.-S. M., Mansour, E., Ali, M. M. A., Yasin, M. A. T., Abdul-Hamid, M. I. E., Rady, M. M., et al. (2021b). Exogenously used 24-epibrassinolide promotes drought tolerance in maize hybrids by improving plant and water productivity in an arid environment. *Plants* 10:354. doi: 10.3390/plants10020354
- Desoky, E.-S. M., Merwad, A.-R. M. A., Abo El-Maati, M. F., Mansour, E., Arnaut, S. M. A. I., Awad, M. F., et al. (2021d). Physiological and biochemical mechanisms of exogenously applied selenium for alleviating destructive impacts induced by salinity stress in bread wheat. *Agronomy* 11:926. doi: 10.3390/agronomy11050926
- Desoky, E.-S. M., Elrys, A. S., Mansour, E., Eid, R. S. M., Selem, E., Rady, M. M., et al. (2021a). Application of biostimulants promotes growth and productivity by fortifying the antioxidant machinery and suppressing oxidative stress in faba bean under various abiotic stresses. *Sci. Hortic.* 288:110340. doi: 10.1016/j.scienta.2021.110340
- Dhaliwal, S. S., Sharma, V., Shukla, A. K., Kaur, J., Verma, V., Singh, P., et al. (2021). Enrichment of zinc and iron micronutrients in lentil (*Lens culinaris* Medik.) through biofortification. *Molecules* 26:7671. doi: 10.3390/molecules26247671
- Du, M., Zhang, W., Gao, J., Liu, M., Zhou, Y., He, D., et al. (2022). Improvement of root characteristics due to nitrogen, phosphorus, and potassium interactions increases rice (*Oryza sativa* L.) yield and nitrogen use efficiency. *Agronomy* 12:23. doi: 10.3390/agronomy12010023

## Acknowledgments

KE-T thank the library at Murdoch University, Australia for the valuable online resources and comprehensive databases and thanks Abu Dhabi Research Award (AARE2019) for the Research Excellence-Department of Education and Knowledge (21S105) for support. SA also thank Khalifa Center for Biotechnology and Genetic Engineering-UAEU (Grant number: 31R286) for their support.

## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

## Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

## Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fpls.2022.937073/full#supplementary-material>

- Egamberdieva, D., Wirth, S. J., Alqarawi, A. A., Abd-Allah, E. F., and Hashem, A. (2017). Phyto-hormones and beneficial microbes: essential components for plants to balance stress and fitness. *Front. Microbiol.* 8:2104. doi: 10.3389/fmicb.2017.02104
- El-Mageed, T. A. A., Belal, E. E., Rady, M. O. A., El-Mageed, S. A. A., Mansour, E., Awad, M. F., et al. (2021). Acidified biochar as a soil amendment to drought stressed (*Vicia faba* L.) plants: influences on growth and productivity, nutrient status, and water use efficiency. *Agronomy* 11:1290. doi: 10.3390/agronomy11071290
- Elmogy, M. S., Cao, Y., Zhou, H., and Xia, Y. (2018). Root development enhanced by using in-dole-3-butyric acid and naphthalene acetic acid and associated biochemical changes of *in vitro* *Azalea* microshoots. *J. Plant Growth Regul.* 37, 813–825. doi: 10.1007/s00344-017-9776-5
- ElShamey, E. A. Z., Hamad, H. S., Alshallash, K. S., Alghuthaymi, M. A., Ghazy, M. I., Sakran, R. M., et al. (2022). Growth regulators improve outcrossing rate of diverse rice cytoplasmic male sterile lines through affecting floral traits. *Plants* 11:1291. doi: 10.3390/plants11101291
- FAOSTAT (2022). *Food and Agriculture Organization of the United Nations statistical database*. Available online at: <http://www.fao.org/faostat/en/#data> [Accessed January 22, 2022].
- Frydenvang, J., Van Maarschalkerweerd, M., Carstensen, A., Mundus, S., Schmidt, S. B., Pedas, P. R., et al. (2015). Sensitive detection of phosphorus deficiency in plants using chlorophyll a fluorescence. *Plant Physiol.* 169, 353–361. doi: 10.1104/pp.15.00823
- Gahoonia, T. S., Ali, O., Sarker, A., Nielsen, N. E., and Rahman, M. M. (2006). Genetic variation in root traits and nutrient acquisition of lentil genotypes. *J. Plant Nutr.* 29, 643–655. doi: 10.1080/01904160600564378
- Gahoonia, T. S., and Nielsen, N. E. (2004). Root traits as tools for creating phosphorus efficient crop varieties. *Plant Soil* 260, 47–57. doi: 10.1023/B:PLSO.0000030168.53340.bc
- Gan, Y., Hamel, C., Kutcher, H. R., and Poppy, L. (2017). Lentil enhances agroecosystem productivity with increased residual soil water and nitrogen. *Renew. Agric. Food Syst.* 32, 319–330. doi: 10.1017/S1742170516000223
- Ganesan, K., and Xu, B. (2017). Polyphenol-rich lentils and their health promoting effects. *Int. J. Mol. Sci.* 18:2390. doi: 10.3390/ijms18112390
- Hardy, R. W. F., Burns, R. C., and Holsten, R. D. (1973). Applications of the acetylene-ethylene assay for measurement of nitrogen fixation. *Soil Biol. Biochem.* 5, 47–81. doi: 10.1016/0038-0717(73)90093-X
- Hiscox, J. D., and Israelstam, G. F. (1979). A method for the extraction of chlorophyll from leaf tissue without maceration. *Can. J. Bot.* 57, 1332–1334. doi: 10.1139/b79-163
- Horwitz, W., Chichilo, P., and Reynolds, H. (2000). *Official Methods Of Analysis*, 17th Edn. Gaithersburg: The Association of Official Analytical Chemists.
- Hossain, Z., Wang, X., Hamel, C., Knight, J. D., Morrison, M. J., and Gan, Y. (2016). Biological nitrogen fixation by pulse crops on semiarid Canadian prairies. *Can. J. Plant Sci.* 97, 119–131. doi: 10.1139/cjps-2016-0185
- Jarpa-Parra, M. (2018). Lentil protein: a review of functional properties and food application. An overview of lentil protein functionality. *Int. J. Food Sci.* 53, 892–903. doi: 10.1111/ijfs.13685
- Jindal, C., Khanna, V., and Sharma, P. (2008). Impact of *Rhizobium* and PSB inoculation on P-economy, symbiotic parameters and yield of lentil (*Lens culinaris* Medikus). *J. Res. Punjab Agric. Univ.* 45, 1–3.
- Jindo, K., Canellas, L. P., Albacete, A., Santos, L. F. D., Rocha, R. L. F., Baia, D. C., et al. (2020). Interaction between humic substances and plant hormones for phosphorous acquisition. *Agronomy* 10:640. doi: 10.3390/agronomy10050640
- Khan, M. I. R., Khan, N. A., Jahan, B., Goyal, V., Hamid, J., Khan, S., et al. (2021). Phosphorus supplementation modulates nitric oxide biosynthesis and stabilizes the defence system to improve arsenic stress tolerance in mustard. *Plant Biol.* 23, 152–161. doi: 10.1111/plb.13211
- Khazaei, H., Subedi, M., Nickerson, M., Martínez-Villaluenga, C., Frias, J., and Vandenberg, A. (2019). Seed protein of lentils: current status, progress, and food applications. *Foods* 8:391. doi: 10.3390/foods8090391
- Li, W., Xiong, B., Wang, S., Deng, X., Yin, L., and Li, H. (2016). Regulation effects of water and nitrogen on the source-sink relationship in potato during the tuber bulking stage. *PLoS One* 11:e0146877. doi: 10.1371/journal.pone.0146877
- Liu, K., Blackshaw, R. E., Johnson, E. N., Hossain, Z., Hamel, C., St-Arnaud, M., et al. (2019). Lentil enhances the productivity and stability of oilseed-cereal cropping systems across different environments. *Eur. J. Agron.* 105, 24–31. doi: 10.1016/j.eja.2019.02.005
- Mannan, M., Tithi, M. A., Islam, M. R., Al Mamun, M. A., Mia, S., Rahman, M. Z., et al. (2022). Soil and foliar applications of zinc sulfate and iron sulfate alleviate the destructive impacts of drought stress in wheat. *Cereal Res. Commun.* doi: 10.1007/s42976-022-00262-5
- Mansour, E., Mahgoub, H. A. M., Mahgoub, S. A., El-Sobky, E.-S. E. A., Abdul-Hamid, M. I., Kamara, M. M., et al. (2021). Enhancement of drought tolerance in diverse *Vicia faba* cultivars by inoculation with plant growth-promoting rhizobacteria under newly reclaimed soil conditions. *Sci. Rep.* 11:24142. doi: 10.1038/s41598-021-02847-2
- Mansour, E., Merwad, A. M. A., Yasin, M. A. T., Abdul-Hamid, M. I. E., El-Sobky, E. E. A., and Oraby, H. F. (2017). Nitrogen use efficiency in spring wheat: genotypic variation and grain yield re-sponse under sandy soil conditions. *J. Agric. Sci.* 155, 1407–1423. doi: 10.1017/S0021859617000600
- Mao, J.-P., Zhang, D., Zhang, X., Li, K., Liu, Z., Meng, Y., et al. (2018). Effect of exogenous in-dole-3-butyric acid (IBA) application on the morphology, hormone status, and gene expression of developing lateral roots in *Malus hupehensis*. *Sci. Hortic.* 232, 112–120. doi: 10.1016/j.scienta.2017.12.013
- Míguez-Montero, M. A., Valentine, A., and Pérez-Fernández, M. A. (2020). Regulatory effect of phosphorus and nitrogen on nodulation and plant performance of leguminous shrubs. *AoB Plants* 12, 1–11. doi: 10.1093/aobpla/plz047
- Mohamed, H. I., El-Sayed, A. A., Rady, M. M., Caruso, G., Sekara, A., and Abdelhamid, M. T. (2021). Coupling effects of phosphorus fertilization source and rate on growth and ion accumulation of common bean under salinity stress. *PeerJ* 9:e11463. doi: 10.7717/peerj.11463
- Mudgal, V., Mehta, M. K., and Rane, A. S. (2018). Lentil straw (*Lens culinaris*): an alternative and nutritious feed resource for kids. *Anim. Nutr.* 4, 417–421. doi: 10.1016/j.aninu.2018.04.009
- Mustafa, A., Hussain, A., Naveed, M., Ditta, A., Nazli, Z.-E.-H., and Sattar, A. (2016). Response of okra (*Abelmoschus esculentus* L.) to soil and foliar applied L-tryptophan. *Soil Environ.* 35, 76–84.
- Nget, R., Aguilar, E. A., Cruz, P. C. S., Reaño, C. E., Sanchez, P. B., Reyes, M. R., et al. (2022). Re-sponses of soybean genotypes to different nitrogen and phosphorus sources: impacts on yield components, seed yield, and seed protein. *Plants* 11:298. doi: 10.3390/plants11030298
- Omar, M., Rabie, H. A., Mowafi, S. A., Othman, H. T., Abd El-Moneim, D., Alharbi, K., et al. (2022). Multivariate analysis of agronomic traits in newly developed maize hybrids grown under different agro-environments. *Plants* 11:1187. doi: 10.3390/plants11091187
- Piotrowska-Niczyporuk, A., Bajguz, A., Zambrzycka, E., and Godlewska-Żyłkiewicz, B. (2012). Phytohormones as regulators of heavy metal biosorption and toxicity in green alga *Chlorella vulgaris* (Chlorophyceae). *Plant Physiol. Biochem.* 52, 52–65. doi: 10.1016/j.plaphy.2011.11.009
- Pradip, T., Rajhans, V., Sunil, K., and Balwan. (2017). Effect of potassium humate and bio-inoculants on growth and yield of cowpea (*Vigna unguiculata* (L.) Walp). *Ecol. Environ.* 35, 1494–1498.
- Pulok, M. A. I., Rahman, M. M., Haque, M. N., Chakraborty, R., and Ali, M. (2015). Effect of growth regulators on germination and vigor of lentil seeds. *J. Biosci. Agric. Res.* 3, 8–14.
- Rafique, M., Naveed, M., Mustafa, A., Akhtar, S., Munawar, M., Kaukab, S., et al. (2021). The combined effects of gibberellic acid and rhizobium on growth, yield and nutritional status in chickpea (*Cicer arietinum* L.). *Agronomy* 11:105. doi: 10.3390/agronomy11010105
- Ramtekey, V., Bansal, R., Aski, M. S., Kothari, D., Singh, A., Pandey, R., et al. (2021). Genetic variation for traits related to phosphorus use efficiency in lent species at the seedling stage. *Plants* 10:2711. doi: 10.3390/plants10122711
- Rasheed, M., Jilani, G., Shah, I. A., Najeeb, U., and Iqbal, T. (2010). Improved lentil production by utilizing genetic variability in response to phosphorus fertilization. *Acta Agric. Scand B. Soil Plant Sci.* 60, 485–493. doi: 10.1080/09064710903183562
- Sabagh, A. E., Mbarki, S., Hossain, A., Iqbal, M. A., Islam, M. S., Raza, A., et al. (2021). Potential role of plant growth regulators in administering crucial processes against abiotic stresses. *Front. Agron.* 3:648694. doi: 10.3389/fagro.2021.648694
- Schmidtke, K., Neumann, A., Hof, C., and Rauber, R. (2004). Soil and atmospheric nitrogen uptake by lentil (*Lens culinaris* Medik.) and barley (*Hordeum vulgare* ssp. nudum L.) as monocrops and intercrops. *Field Crops Res.* 87, 245–256. doi: 10.1016/j.fcr.2003.11.006
- Semida, W. M., El-Mageed, T. A. A., Abdalla, R. M., Hemida, K. A., Howladar, S. M., Leilah, A. A. A., et al. (2021). Sequential antioxidants foliar application can alleviate negative consequences of salinity stress in *Vicia faba* L. *Plants* 10:914.
- Shah, Z. H., Rehman, H. M., Akhtar, T., Alsamadany, H., Hamooh, B. T., Mujtaba, T., et al. (2018). Humic substances: determining potential molecular regulatory processes in plants. *Front. Plant Sci.* 9:263. doi: 10.3389/fpls.2018.00263
- Shapiro, S. S., and Wilk, M. B. (1965). An analysis of variance test for normality (complete samples). *Biometrika* 52, 591–611. doi: 10.2307/2333709

- Singh, K. K., Srinivasarao, C., and Ali, M. (2005). Root growth, nodulation, grain yield, and phosphorus use efficiency of lentil as influenced by phosphorus, irrigation, and inoculation. *Commun. Soil Sci. Plant Anal.* 36, 1919–1929. doi: 10.1081/CSS-200062501
- Singh, N., and Singh, G. (2016). Response of lentil (*Lens culinaris* Medikus) to phosphorus—a review. *Agric. Rev.* 37, 27–34. doi: 10.18805/ar.v37i1.9261
- Singh, S. P. (2010). Response of plant growth regulator on growth and yield of fenugreek (*Trigonella foenum-graecum* L.). *Asian J. Hort.* 5, 234–236.
- Sital, J., Kaur, K., Sharma, S., Sandhu, J. S., and Singh, S. (2011). Effect of *Rhizobium* inoculation, phosphorus and nitrogen supplements on protein quality in developing lentil (*Lens culinaris*) seeds. *Indian J. Agric. Biochem.* 24, 17–22.
- Su, R., Zhang, Z., Chang, C., Peng, Q., Cheng, X., Pang, J., et al. (2022). Interactive effects of phosphorus fertilization and salinity on plant growth, phosphorus and sodium status, and tartrate exudation by roots of two alfalfa cultivars. *Ann. Bot.* 129, 53–64. doi: 10.1093/aob/mcab124
- Swailam, M. A., Mowafy, S. A. E., El-Naggar, N. Z. A., and Mansour, E. (2021). Agronomic responses of diverse bread wheat genotypes to phosphorus levels and nitrogen forms in a semiarid environment. *SABRAO J. Breed. Genet.* 53, 592–608. doi: 10.54910/sabrao2021.53.4.4
- Togay, Y., Togay, N., and Dogan, Y. (2008). Research on the effect of phosphorus and molybdenum applications on the yield and yield parameters in lentil (*Lens culinaris* Medic.). *Afr. J. Biotechnol.* 7, 1256–1260.
- Wang, Y., Thorup-Kristensen, K., Jensen, L. S., and Magid, J. (2016). Vigorous root growth is a better indicator of early nutrient uptake than root hair traits in spring wheat grown under low fertility. *Front. Plant Sci.* 7:865. doi: 10.3389/fpls.2016.00865
- Yang, J.-X., Richards, R. A., Jin, Y., and He, J. (2022). Both biomass accumulation and harvest index drive the yield improvements in soybean at high and low phosphorus in south-west China. *Field Crops Res.* 277:108426.



## OPEN ACCESS

## EDITED BY

Kira A. Borden,  
University of Guelph,  
Canada

## REVIEWED BY

Lara Reale,  
University of Perugia,  
Italy  
Yangbo He,  
Huazhong Agricultural University,  
China

## \*CORRESPONDENCE

Wenna Wang  
wwwnefu@163.com

<sup>†</sup>These authors have contributed equally to this work and share first authorship

## SPECIALTY SECTION

This article was submitted to  
Functional Plant Ecology,  
a section of the journal  
Frontiers in Plant Science

RECEIVED 04 June 2022

ACCEPTED 15 August 2022

PUBLISHED 31 August 2022

## CITATION

Xu H, Wang S, Tang L, Wang Y, Li Z and  
Wang W (2022) Differential influence of  
cortex and stele components on root tip  
diameter in different types of tropical  
climbing plants.  
*Front. Plant Sci.* 13:961214.  
doi: 10.3389/fpls.2022.961214

## COPYRIGHT

© 2022 Xu, Wang, Tang, Wang, Li and  
Wang. This is an open-access article  
distributed under the terms of the [Creative  
Commons Attribution License \(CC BY\)](#). The  
use, distribution or reproduction in other  
forums is permitted, provided the original  
author(s) and the copyright owner(s) are  
credited and that the original publication in  
this journal is cited, in accordance with  
accepted academic practice. No use,  
distribution or reproduction is permitted  
which does not comply with these terms.

# Differential influence of cortex and stele components on root tip diameter in different types of tropical climbing plants

Haiwu Xu<sup>1†</sup>, Siyuan Wang<sup>2†</sup>, Liang Tang<sup>1</sup>, Yan Wang<sup>3</sup>,  
Zhongyue Li<sup>3</sup> and Wenna Wang<sup>1\*</sup>

<sup>1</sup>Key Laboratory of Germplasm Resources of Tropical Special Ornamental Plants of Hainan Province, College of Forestry, Hainan University, Haikou, China, <sup>2</sup>School of Forestry, Northeast Forestry University, Harbin, China, <sup>3</sup>Taishan Forest Ecosystem Research Station of State Forestry Administration, State Forestry and Grassland Administration Key Laboratory of Silviculture in downstream areas of the Yellow River, College of Forestry, Shandong Agricultural University, Taian, China

Climbing plants are an abundant and taxonomically diverse plant group that competes intensely with trees and thus substantially affects forest diversity and structure. The growth and physiology of climbing plants largely depend on their root tip structure and function. However, little is known regarding the mechanisms through which anatomical traits regulate root tip diameter in climbing plants. Therefore, our study sought to explore the relationships between root tip diameter and seven anatomical traits (e.g., cortex thickness and stele diameter) in three lianas and three vine species sampled from a tropical forest in Hainan. Root tip diameter was significantly positively correlated with cortex thickness ( $r=0.94-0.99$ ) and stele diameter ( $r=0.72-0.94$ ) within species, especially with cortex thickness. Cortex thickness was significantly positively correlated with mean cortical cell diameter in six species ( $r=0.72-0.93$ ), but was only correlated with the number of cortical cell layers in three species ( $r=0.42-0.66$ ). Stele diameter displayed significant positive correlations with mean conduit diameter ( $r=0.58-0.88$ ) and the number of conduits per stele ( $r=0.50-0.66$ , except for *Cyclea hypoglauca*), and was negatively correlated with conduit density in all species ( $r=-0.65$  to  $-0.77$ ). The correlations between cortical cells and conduit traits and root tip diameter were similar to that with cortex thickness and stele diameter, respectively. Compared with vines, liana root tips showed closer relationships between root diameter and cortex thickness and stele diameter, and between cortex thickness and mean diameter of cortical cells. Moreover, the root tip of lianas possesses significantly higher stele proportion and denser conduits, significantly lower cortex proportion, and smaller conduit size than those of vines. However, the specific conductivity was similar. Overall, these results suggest that the cortex is the main driver for the change in root tip diameter rather than the stele. Nevertheless, both factors were responsible for variations in diameter-related traits when compared with number-related traits, with lianas and vines exhibiting distinct regulatory mechanisms.

## KEYWORDS

root tip diameter, anatomical trait, relationship, lianas, vines



## Introduction

Climbing plants constitute an abundant and taxonomically diverse plant group that competes intensely with trees and thus substantially affect forest diversity and structure (Phillips et al., 2002; Wright et al., 2004; Schnitzer and Bongers, 2011). This is because climbing plants are capable of forming a dense carpet covering the tree canopy, accounting for approximately 20% of the total leaf area of the forest (Putz, 1983) and substantially hindering the light acquisition of individual trees (Putz, 1984; Schnitzer and Bongers, 2002; Ingwell et al., 2010). Furthermore, climbing plants experience less physiological stress than trees during seasonal drought (Smith-Martin et al., 2019), and steadily increase in abundance and biomass in the tropics (Collins et al., 2016). Therefore, they severely reduce tree growth rates, fecundity, and survival in forests (Schnitzer et al., 2005). Thus, studying the physiological and ecological functions of climbing plants would provide key insights into the dynamics of interspecific competition and vegetation composition in forest ecosystems.

The growth and physiological functions of plants largely depend on the structure and function of the root tips, which are the primary structure absorbing water and nutrients from soils (Pregitzer et al., 2002; Guo et al., 2008b). The structure and function of root tips are mainly characterized by functional traits of root tip diameter, cortex thickness and stele diameter (Guo et al., 2008b; Kong et al., 2014). Nevertheless, very few studies have investigated the functional traits of climbing plant root tips. Drawing upon the study of self-supporting plants, root diameter is a direct functional trait that reflects root physiology in herbaceous and woody species (Burton et al., 2012; Comas et al., 2012; Bowsher et al., 2016), with thicker root tips exhibiting weaker absorption but stronger transportation (Guo et al., 2008b; Jia et al., 2010). This is because the cross-sectional size of the root tip can vary depending on its anatomical structure (i.e., cortex and stele), which are important tissues responsible for the absorption and vertical transport of individual plants, respectively (Esau, 1977; Genre et al., 2008; Guo et al., 2008b; Comas et al., 2012). In the study of self-supporting plants, root tip diameter change was more strongly influenced by the variation of cortex thickness or cortical cross-sectional area rather than stele diameter in both herbaceous and woody species (Hummel et al., 2007; Gu et al., 2014a). One of the important reason is that root cortex accounts for a larger proportion in the cross-section area of root tips (Esau, 1977; Guo et al., 2008b; Gu et al., 2014a). Wang et al. (2020) studied on three temperate climbing plant species and found that cortex occupied a larger proportion of the cross-sectional area of root tip diameter than stele. It can be expected that cortex has stronger effects than stele in the regulation of root diameter in climbing plants, but this has not yet been investigated.

Additionally, few studies on climbing plants have investigated the mechanism of changes of the cortex thickness and stele diameter. In self-supporting plants, the variation of root tip cortex thickness and stele diameter are fundamentally caused by the changes in cortical cell and conduit traits (e.g., size and number)

(Dong et al., 2015; Wang et al., 2018). Previous studies on self-supporting plants have reported that root physiology varied widely when different features (i.e., diameter- or number-related traits) of cortical cells and conduits were altered. For example, increasing the conduit and cortical cell diameters rather than increasing the number of conduits and cortical cell layers can more effectively improve vertical transport (Tyree and Ewers, 1991) and reduce metabolic consumption in roots (Chimungu et al., 2014), respectively. Climbing plants are characterized as the extremely wide vessels in their tall and slender stem compared to trees (Ewers et al., 1990; Hacke et al., 2000; Hu et al., 2010; Schnitzer and Bongers, 2011), therefore it appears very likely that the diameter-related traits of anatomical components (i.e., cortical cells and conduits) play more important roles than number-related traits in the regulation of cortex thickness and stele diameter, respectively, and thus contribute greatly to the variations in root tip diameter for this type of plants. However, whether this regulation exists in the root tips of climbing plants has not yet been investigated.

Lianas (woody climbing plants) and vines (herbaceous climbing plants) are two distinct types of climbing plants that show obvious differences in their life cycle, geographical distribution, and sensitivity to environmental changes (Jiménez-Castillo et al., 2006; Hu et al., 2010; Angyalossy et al., 2012). However, few studies have focused on the differences between lianas and vines in the distribution of anatomical structure and the structure–function linkage of the root tips. Drawing upon the findings of self-supporting plants, 50 tropical and temperate tree species (Gu et al., 2014a) showed a higher interspecific variation in root tip diameter than 14 herbaceous Mediterranean species (Hummel et al., 2007), while the proportion of stele diameter in root diameter was more stable and higher among the woody species (mean and range of 25.7 and 23.5% ~ 28.8%, respectively) than herbaceous species (mean and range of 21.9 and 11.8% ~ 40.1%, respectively). These results suggest that, in self-supporting plants, the association of root diameter with anatomical traits appeared stronger in woody plants than in herbaceous plants. Woody and herbaceous plants were widely accepted to be distinctly different in life cycles and evolutionary histories (Ma et al., 2018), it seems likely that the effect caused by the difference between woody and herbaceous plants on root structure and function should be stronger than those caused by the difference between climbing and self-supporting plants. However, it is still unclear whether climbing plants have similar anatomical patterns to that of self-supporting plants, i.e., root tips of woody plants (i.e., lianas in climbing plants) have a greater proportion of stele and tighter association of root diameter with anatomical traits than herbaceous plants (i.e., vines in climbing plants).

The objectives of this study were to investigate the relationships between root tip diameter and several vital anatomical traits, as well as to explore the differences in these intraspecific relationships and anatomical structures between lianas and vines. Our study characterized three liana species (*Podranea ricasoliana*, *Cyclea hypoglauca*, and *Tetrastigma planicaule*) and three vine species

(*Psychotria serpens*, *Passiflora caerulea*, and *Merremia hainanensis*) that inhabit the Limu Mountain Nature Reserve (Hainan, China), a representative tropical forest (Figure 1). For each plant type, the root tip diameters vary in a species-dependent manner, with root diameter of 111.3–207.9  $\mu\text{m}$  and 106.5–183.3  $\mu\text{m}$  between the thickest and the thinnest root species for vines and lianas, respectively (Figure 2). The root tip diameter and the anatomical traits, including cortex thickness, stele diameter, mean diameter of cortical cells, number of cortical cell layers, mean conduit diameter, number of conduits per stele, conduit density, and specific conductivity ( $K_s$ ) were investigated in each species. By analyzing the association between root tip diameter and these anatomical traits within and among species, the regulatory mechanism of the anatomical traits on the root tip diameter of lianas and vines were investigated and our hypotheses were further tested. Therefore, this study could have important theoretical implications for understanding the physiological and ecological functions of climbing plants in tropical forests.

## Materials and methods

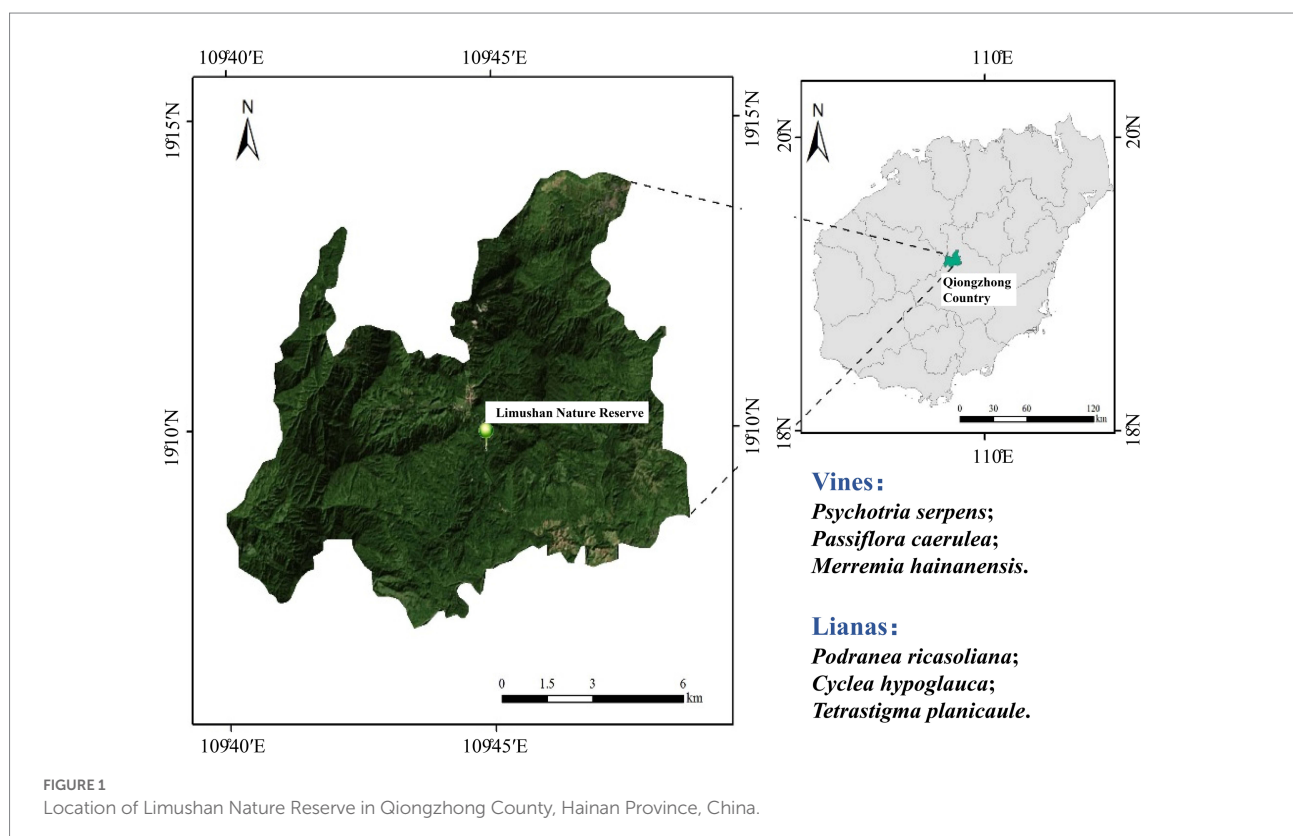
### Study site

The research site was located in the Limushan Nature Reserve (109°39′–109°49′E, 19°07′–19°14′N), in Qiongzong County, Hainan Province, China. The region has a tropical monsoon

climate, with an average annual temperature of 23.1°C. The annual accumulated temperature is 8489.4°C, with the highest and lowest temperatures of 38.2°C and 1.5°C occurring in June and January, respectively (Wang et al., 2004). The rainy season occurs from May to October, with a precipitation of 1,809 mm, accounting for 83% of the annual precipitation. The region has an annual evaporation of 1,391–1,426 mm, a relative humidity of 87–88%, an annual sunshine duration of 1773.5–1918.3 h, and an annual average wind speed of 1.1 m/s (Qin, 2020). The study site was located mid-slope on a hill (100–200 m above sea level). The site soils are laterite and lateritic red, with a pH value of approximately 5, an organic matter ratio of 2.20%, and concentrations of total carbon, total nitrogen, total phosphorus, and total potassium of 1.28, 0.10, 0.08, and 2.24%, respectively (the soil data were provided by the Soil and Fertilizer Station, a section of the Department of Agriculture, Hainan Province, China).

### Root sampling

Our study characterized three woody liana species (*P. ricasoliana*, *C. hypoglauca*, and *T. planicaule*) and three herbaceous vine species (*P. serpens*, *P. caerulea*, and *M. hainanensis*) that grew at the study site. At the end of July 2019, for each species, three individual plants had a similar age or at a similar growth stage were randomly selected. The age (or growth stage) of the individual plant of vines and lianas was identified by



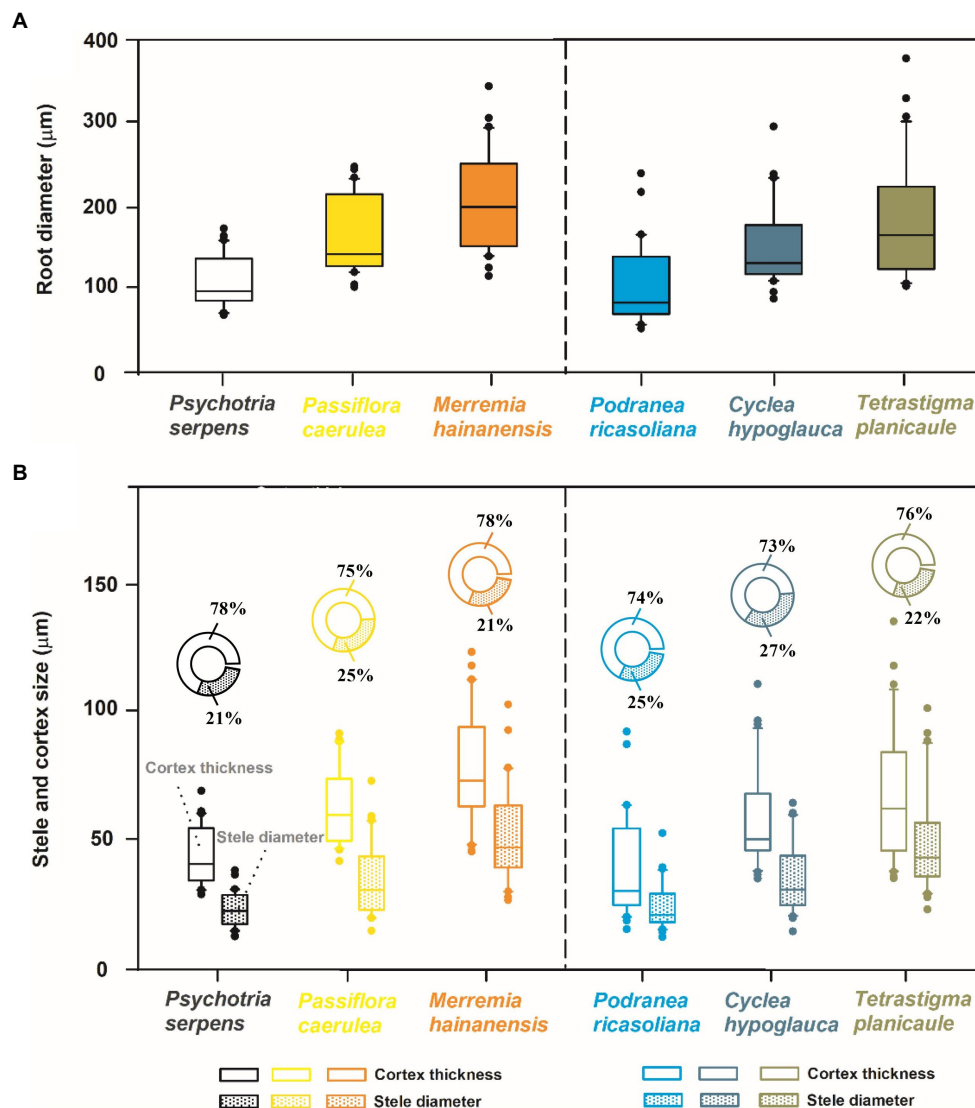


FIGURE 2

Mean values of root tip diameter (A), cortex thickness, stele diameter and the proportion of these two anatomical traits to root tip diameter (B) in three vine (herbaceous climbing plants) and three liana (woody climbing plants) species in tropical forest ( $n=30$ ).

the overall plant growth and stem diameter. In addition, for lianas, the same growth stage of individual plants could also be determined through the similar degree of lignification of stem and branches. The fine root segments of these individual plants were sampled as follows. First, the surface soil around the plants was gently removed by hand, and a spade or steel fork was used to pry the soil loose without damaging the roots of the study plants. Along with excavation of the main root system of each target plant, the surrounding soils were carefully loosened by hand to locate lateral root branches attached to the main roots. Complete fine root segments per plant from the 0–20 cm soil depths were randomly selected and carefully cut at the base using a pair of branch shears. After careful removal of soil particles, the fine root segments were immersed in formalin-aceto-alcohol (FAA) solution (90 ml of 50% ethanol, 5 ml of 100% glacial acetic acid,

and 5 ml of 37% methanol) in reagent bottles, then placed on ice and immediately transported to the laboratory. The reagent bottles were stored at 4°C and the root anatomy was subsequently studied over a 3-month period.

## Measurement of root tip anatomical traits

In the laboratory, three complete fine root segments were randomly selected and removed from the FAA solution, then washed in deionized water to remove impurities. Root tips, identified as distal non-woody roots, were carefully dissected from the root samples using forceps, following the procedures described by Pregitzer et al. (2002) (Supplementary Figure S1). For each

species, total 30–50 root tips from the three individual plants were collected and their surfaces were cleaned using a soft brush. A series of chemical processes, such as dehydration, dealcoholization, xylene removal, and embedding were applied to all root tip samples. Afterward, the root tips were embedded in paraffin, and slides with 8- $\mu$ m-thick root sections were prepared using a microtome and then stained with safranin-fast green (2%; Gu et al., 2014a; Wang et al., 2018). Next, the slides were photographed under a compound microscope (BX-51; Olympus Corporation, Tokyo, Japan). Thirty intact cross sections, i.e., each from an individual root, were randomly selected as replications and for the measurement of functional traits. A range of functional traits, including root tip diameter, cortex thickness, stele diameter, mean diameter of cortical cells, number of layers of cortical cells, mean conduit diameter, and number of conduits per stele were measured using the Motic Images Advanced 3.2 software (Motic Corporation, Zhejiang, China). For each root tip, the mean value of each anatomical trait was obtained from measurements in three directions across the root cross-section. Based on the assumption that the stele is a perfect circle, the stele area was calculated using the circle area formula, taking the average stele diameter as the diameter of the circle. The conduit density was calculated as the ratio of the number of conduits per stele to the stele area (Kong et al., 2016). The specific conductivity ( $K_s$ ) was calculated using Hagen–Poiseuille's law (Tyree and Ewers, 1991):

$$K_s = (\pi \rho / 128 \eta A w) \sum_{i=1}^n (d_i^4)$$

where  $K_s$  is theoretical axial conductivity along a root tip,  $\rho$  is the density of water (where temperature was set at 18°C, consistent with the root respiration measurement),  $\eta$  is the dynamic viscosity,  $d$  is the diameter of the  $i$ th conduit, and  $n$  is the number of the conduits in the xylem.

## Data analysis

Thirty individual root tips, i.e., ten individual roots from each of the three individual plants, for each species were treated as the unit for data analysis in the current study. For each species, the average value and standard error of the root diameter and each anatomical trait were calculated from 30 replicates (i.e., 30 individual roots). One-way factorial analysis of variance ( $p=0.05$ ) was used to analyze the interspecific differences in functional traits of the root tips. Bivariate correlations among traits were evaluated using Pearson's correlation coefficients ( $r$ ) in the SPSS software (2010, V. 19.0, SPSS Inc., Cary, NC, United States), including the relationships (1) between root diameter versus both cortex thickness and stele diameter; (2) between cortex thickness versus both of mean cortical cell diameter and number of cortical cell layers; and (3) between stele diameter versus mean conduit diameter, number of conduits per stele, and conduit density. Additionally, these

relationships were all examined by linear regressions and the adjusted regression coefficients ( $R^2$ ) were obtained. For each group of the intraspecific relationship, the data analyses were performed with 30 replicates for each species. The slopes of the regressions between root diameter and cortex thickness and stele diameter were compared *via* analysis of covariance (ANCOVA) in SPSS. Root tip functional traits were compared between vines and lianas using one-way factorial analysis of variance ( $p=0.05$ ) based on 90 replicates (i.e., 30 individual roots from each of the three species) for each life form. The interrelationships ( $n=6$ , i.e., six species) between root tip diameter and multiple anatomical traits, such as cortex thickness, stele diameter, mean diameter of cortical cells, number of cortical cell layers, mean conduit diameter, and number of conduits per stele, were analyzed by principal component analysis (PCA) in the CANOCO software (CANOCO V. 4.5).

## Results

### Correlations of cortex thickness and stele diameter with root tip diameter

The effects of cortex and stele morphology on root tip diameter can be analyzed by exploring the relationships between root tip diameter and cortex thickness and stele diameter. At the intra-species level, root tip cortex thickness and stele diameter both increased linearly with root diameter in six climbing plants ( $r=0.94$ – $0.99$  and  $r=0.72$ – $0.94$ , respectively;  $p<0.01$ , Figure 3). The slope for cortex thickness was much steeper than that for stele diameter ( $p<0.01$ ). The associations of root tip diameter with cortex thickness and with stele diameter were both strongest in *P. ricasoliana* ( $r=0.99$  and  $r=0.94$ , respectively) among different species, but weakest in *P. caerulea* ( $r=0.94$ , for the association with cortex thickness) and *P. serpens* ( $r=0.72$  for the association with stele diameter).

Among the three species for each vine and liana studied herein, there were increases in cortex thickness and stele diameter with root diameter (Figure 2). Such consistent trends among species resulted in the positive relationships between the root tip diameter and cortex thickness and stele diameter among the six climbing plants, with tighter associations between root tip diameter and cortex thickness (Figure 4). According to PCA, root diameter, cortex thickness, and stele diameter all contributed greatly to the first dimension, which could account for 82.7% of the total variation (Figure 4).

### Correlations of cortical cell traits and conduit traits with root tip diameter

The effects of cortical cell traits on cortex thickness and thus on root tip diameter were examined by exploring the relationships between cortical cell traits (i.e., mean diameter of cortical cells and number of cortical cell layers) and cortex thickness and root tip diameter. Our findings indicated that the mean diameter of



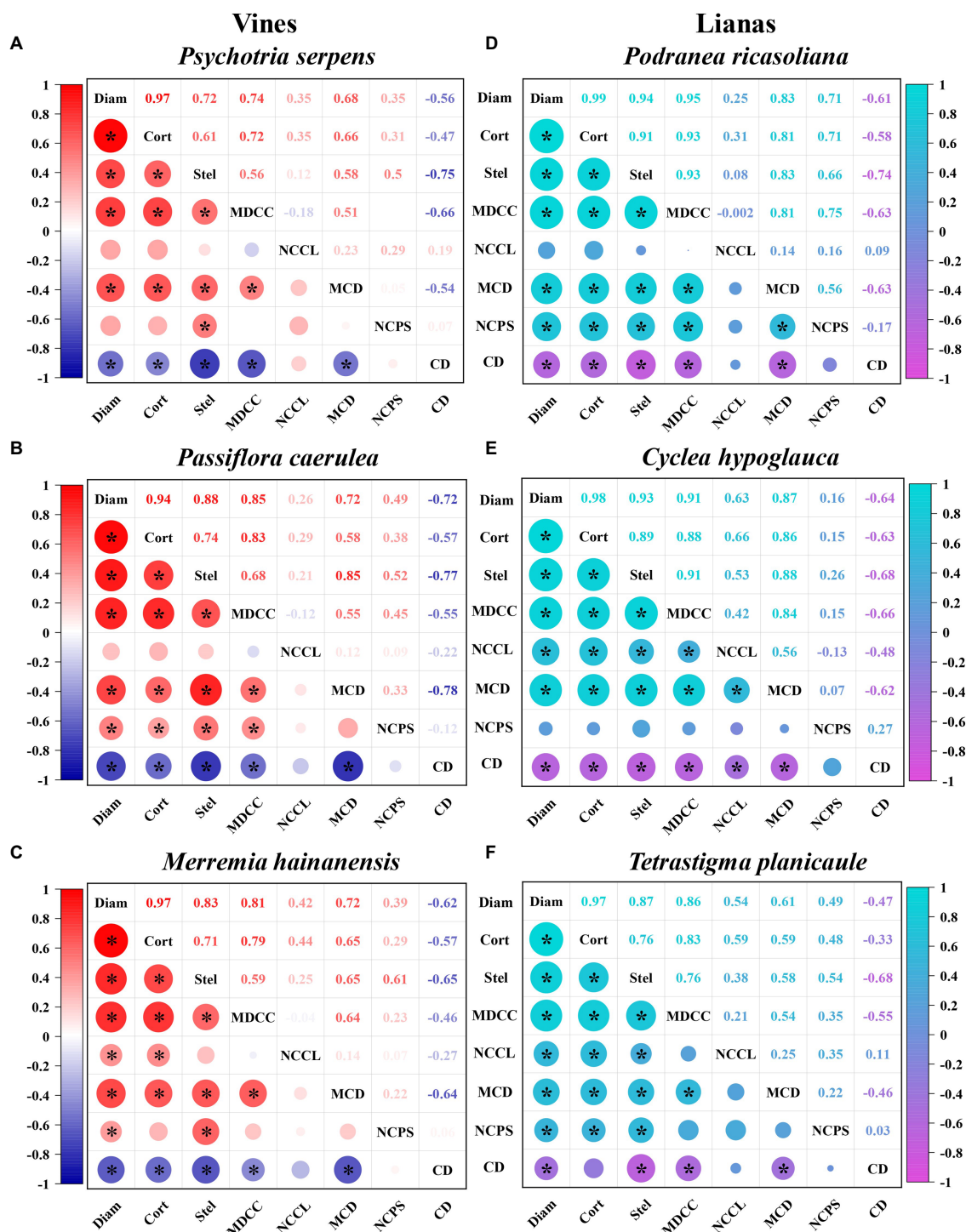


FIGURE 3

Intraspecific correlations of root tip diameter with cortex thickness, stele diameter, and cortical cells and conduit traits in three vine (A–C) (herbaceous climbing plants) and three liana (D–F) (woody climbing plants) species in tropical forest ( $n=30$ ). Color intensity represents the strength of the correlation, figures represent Pearson's correlation coefficients ( $r$ ), and significant correlations ( $p<0.05$ ) are indicated by "\*\*". See Supplementary Tables S2, S3 for adjusted regression coefficients ( $R^2$ ). Diam: root diameter; Cort: cortex thickness; Stel: stele diameter; MDCC: mean diameter of cortical cell; NCCL: number of cortical cell layer; MCD: mean conduit diameter; NCPS: number of conduits per stele; CD: conduit density.

cortical cells of root tips had significant positive correlations with cortex thickness ( $r=0.72\text{--}0.93$ ,  $p<0.01$ ) and with root tip diameter ( $r=0.74\text{--}0.95$ ,  $p<0.01$ ; Figure 3) within each species. In contrast,

the intra-relationships between the number of cortical cell layers and cortex thickness and root tip diameter were relatively weaker, with significant effects observed only in the root tips of



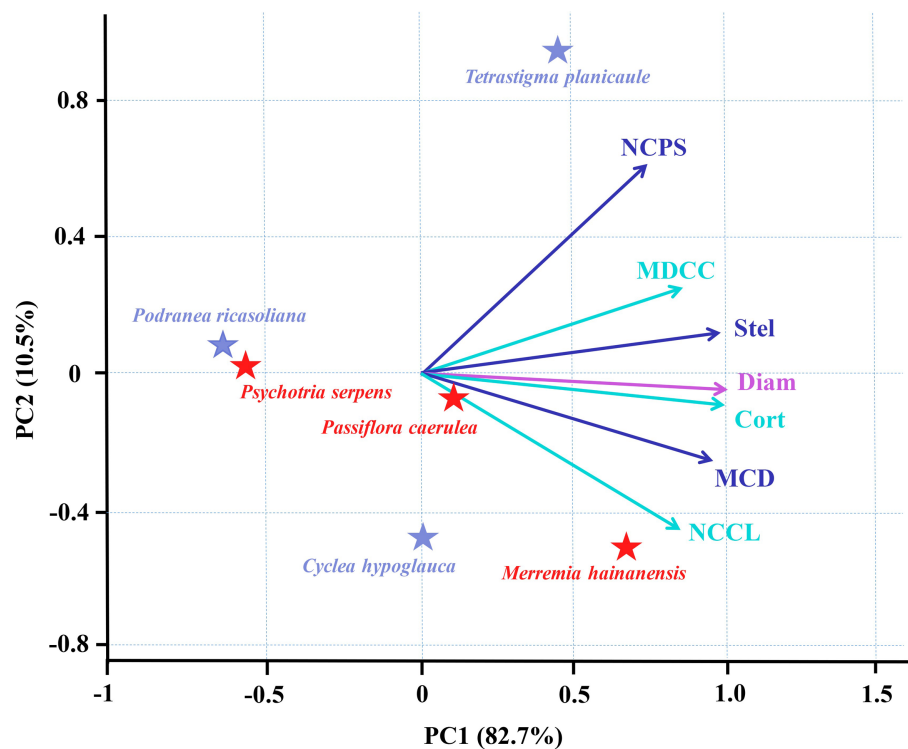


FIGURE 4

Principal component analyses (PCA) for root diameter and six anatomical traits of root tips in three vines (herbaceous climbing plants) and three lianas (woody climbing plants) in tropical forest ( $n=6$ ). All the abbreviations are the same as those described in Figure 3.

*C. hypoglauca* ( $r=0.66$  and  $r=0.63$ , respectively), *T. planicaule* ( $r=0.59$  and  $r=0.54$ , respectively), and *M. hainanensis* ( $r=0.44$  and  $r=0.42$ , respectively).

The influence of conduit traits on stele diameter and thus on root tip diameter was assessed by exploring the relationships between conduit traits (i.e., mean conduit diameter, number of conduits per stele, and conduit density) and stele diameter and root tip diameter. The mean conduit diameter of the root tips showed positive correlations with stele diameter ( $r=0.58$ – $0.88$ ,  $p<0.05$ ) and with root tip diameter ( $r=0.61$ – $0.87$ ,  $p<0.05$ ) at the intraspecific level (Figure 3). However, the number of conduits per stele showed significant intra-associations with both stele diameter and root tip diameter only in *P. ricasoliana* ( $r=0.66$  and  $r=0.71$ , respectively), *P. caerulea* ( $r=0.52$  and  $r=0.49$ , respectively), *M. hainanensis* ( $r=0.61$  and  $r=0.39$ , respectively), and *T. planicaule* ( $r=0.54$  and  $r=0.49$ , respectively). In contrast, in *C. hypoglauca* and *P. serpens*, the number of conduits per stele was significantly correlated with the stele diameter at the intra-species level only in the latter species ( $r=0.50$ ) but was not correlated with root tip diameter in either species. Unlike the mean conduit diameter and the number of conduits per stele, conduit density was negatively correlated with both stele diameter ( $r=-0.65$  to  $-0.77$ ) and root tip diameter ( $r=-0.47$  to  $-0.72$ ) at the intraspecific level (Figure 3).

Based on the current limited number of observations, for each vine and liana, the interspecific variations of cortical cell and conduit traits are dependent on species and life forms. For

example, the mean diameter of cortical cell and number of cortical cell layer was unchanged among three species of vines, but in lianas, it was more dependent on species (Supplementary Table S1). Among all six climbing plants examined herein, the root tip cortex thickness and root tip diameter were both positively correlated with the mean diameter of the cortical cells and the number of cortical cell layers (Figure 4). Consistent with the intraspecific associations, the mean diameter of cortical cells exhibited closer interspecific associations with cortex thickness and root tip diameter compared with the number of cortical cell layers. At the species level, stele diameter and root tip diameter were both positively correlated with the conduit diameter and the number of conduits per stele, with closer correlations with the conduit diameter (Figure 4). The PCA results demonstrated that the mean diameter of cortical cells and mean conduit diameter contributed greatly to the first dimension but their contribution was only slightly weaker than that of root tip diameter, cortex thickness, and stele diameter, all of which accounted for 82.7% of the total variation (Figure 4). In contrast, the number of cortical cell layers and number of conduits per stele contributed largely to the second dimension, which accounted for only 10.5% of the total variation. These results suggest that the first and second dimensions of the PCA were dominated by the diameter-and number-related traits, respectively, with diameter-related traits contributing substantially more to the total variation compared to the number-related traits.

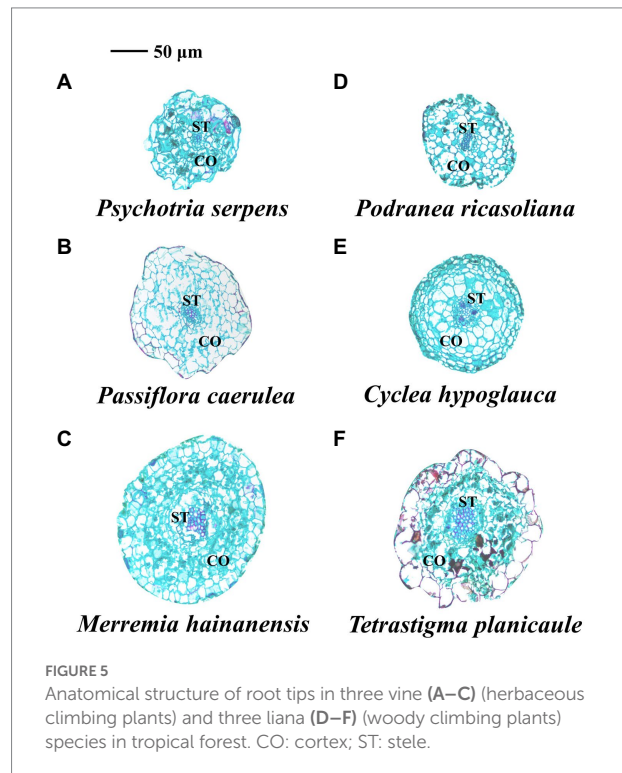
## Difference between vines and lianas

For vines and lianas, the root tip diameter, cortex thickness, and stele diameter exhibited similar trends among species. The root tip diameter values exhibited the following ascending order in vine species: *P. serpens* < *P. caerulea* < *M. hainanensis*. The root tip diameter of lianas had the following order: *P. ricasoliana* < *C. hypoglauca* < *T. planicaule* (Figures 2, 5). Based on the small sample size in the current study, the differences in root tip diameter, cortex thickness, and stele diameter were not significant between these two plant types (Figure 2). But the ratio of bilateral cortex thickness to root tip diameter (3.6% higher) and conduit diameter (12.0% higher) in vines were significantly higher than in lianas (Table 1). However, lianas exhibited 9.6% higher stele diameter to root tip diameter ratios, 22.2% greater conduit numbers, and 23.8% higher conduit density compared with vines, which were all significantly different between life forms ( $p < 0.05$ ). In contrast, no significant difference was observed for the  $K_s$  between lianas and vines (Table 1). Note that the replicates are from only three individual plants per species limited these comparisons between life forms. Furthermore, the correlations between these functional traits also varied considerably between the two plant types. Specifically, the correlation coefficients between root tip diameter and cortex thickness and stele diameter, and between cortex thickness and mean diameter of the cortical cells were generally higher in lianas ( $r = 0.87\text{--}0.99$ ) than in vines ( $r = 0.72\text{--}0.97$ , Figure 3).

## Discussion

### Cortex thickness has a stronger influence on the changes in root tip diameter compared to stele diameter

Our findings indicated that the root tip cortex thickness and stele diameter of the six climbing plants were significantly positively correlated with root tip diameter within and among species, with closer relationships observed between cortex thickness and root tip diameter (Figures 3, 4; Supplementary Table S2). These results confirmed the first hypothesis that cortex have stronger effects than stele in the regulation of root diameter in climbing plants. However, for climbing plants, studies on the linkage of root tip diameter with anatomical traits are extremely lacking. But Wang et al. (2020) studied three temperate climbing species and found that cortex thickness of root tips had more similar interspecific variations to root tip diameter than stele diameter. This may indirectly support our findings. Closer inter-associations between root tip diameter and cortex thickness have also been reported in trees, for example, among 27 tropical tree species and 96 subtropical tree species (Kong et al., 2014; Gu et al., 2014a). However, regarding the intraspecific relationships, the relationship strength in climbing plants was different from those of the temperate tree species examined by Wang et al. (2018). Specifically, the authors found that the correlation strengths of root diameter with cortex thickness and stele diameter



**FIGURE 5**  
Anatomical structure of root tips in three vine (A–C) (herbaceous climbing plants) and three liana (D–F) (woody climbing plants) species in tropical forest. CO: cortex; ST: stele.

Anatomical traits	Vines		Lianas	
	Mean	CV%	Mean	CV%
Cortex proportion and cortical cell traits				
Ratio of bilateral cortex thickness to root tip diameter (%)	76.94a	1.97	74.27b	1.51
Mean diameter of cortical cell (μm)	10.58a	9.83	9.73a	24.29
Number of cortical cell layer	5.88a	15.93	5.83a	8.3
Stele proportion and conduit traits				
Ratio of stele diameter to root tip diameter (%)	22.44b	11.18	24.81a	10.42
Mean conduit diameter (μm)	4.61a	17.7	4.12b	16.64
Number of conduits per stele	15.29b	37.45	19.66a	45.58
Conduit density (no. mm <sup>-2</sup> )	0.02b	39.03	0.03a	33.88
$K_s$ (Kg m <sup>-1</sup> Mpa <sup>-1</sup> s <sup>-1</sup> )	0.17a	27.11	0.15a	40.14

Significant differences ( $p < 0.05$ ) between vines and lianas are indicated by different lower-case letters.

were more dependent on species. It is not clear whether such a disagreement is caused by climate factors or the inherent differences between plant types (i.e., trees and climbing plants). However, our study consistently identified a stronger influence of cortex thickness on root tip diameter in both lianas and vines, suggesting that the root tips of climbing plants preferentially invest in a thicker cortex rather than a thicker stele during root development. Species with a thick cortex generally possess better water and nutrient acquisition capacities because a thick cortex provides ample space for

mycorrhizal colonization (Comas et al., 2012; Zhou et al., 2021). Therefore, we speculate that the strong influence of cortex thickness on root tip diameter in climbing plants enhances the resource acquisition capacities of individual plants.

There are mainly three reasons for the stronger relationship between cortex thickness and root tip diameter at the inter- and intra-specific levels in climbing plants. First, the greater proportion of cortex in the cross-sectional area of root tips, as found in the current and previous studies (Hummel et al., 2007; Gu et al., 2014a; Wang et al., 2020), could directly explain their closer linkage with root tip diameter. Second, the cortex is wrapped around the stele and therefore is more susceptible to environmental stress (Kondo et al., 2000; Enstone et al., 2003; Lux et al., 2004; Gu et al., 2014a). The high sensitivity and susceptibility of the cortex to environmental changes caused by its lateral location may explain its closer correlation with root tip diameter in climbing plants. Previous studies on tropical species also confirmed that the change in root diameter caused by water deficiencies was more consistent with changes in cortex thickness compared to stele diameter in *Astragalus gombiformis* and *Stipa lagascae* (Boughalleb et al., 2014, 2015). Third, the strength of the relationship between root tip diameter and cortex thickness in climbing plants may be related to their unique root physiological regulation strategy. Specifically, changes in root cortex thickness are typically accompanied by multifaceted physiological changes in roots, such as water and nutrient uptake, lateral transport resistance, and energy expenditure (Lascaris and Deacon, 1991; Elliott et al., 1993; Huang and Eissenstat, 2000; Kong et al., 2017; Schneider et al., 2017). Therefore, the stronger linkage between cortex thickness and root tip diameter in climbing plant root tips could be an effective strategy to modulate plant growth and physiology. However, the individual root samples were taken from only three individual plants per species growing in the similar locations, and therefore the variation of root tip functional traits observed in the current study may describe a lot of intra-individual variation. But the variation of the environment could have been greater for some of these species than others. The generality of these results and whether they apply in other species of climbing plants will require more extensive investigations with large sample numbers.

## Diameter-related traits of cortical cells and conduits have a stronger influence on root tip diameter than number-related traits

The results of this study confirmed the second hypothesis that the diameter-related traits of cortical cells and conduits play more important roles in the regulation of cortex thickness and stele diameter, respectively, than the number-related traits, and therefore contribute greatly to the variation of root tip diameter in climbing plants (Figures 3, 4; Supplementary Tables S2, S3). There has been a lack of studies on the associations of anatomical traits of root tips in climbing plants. But previous studies on self-supporting plants have reported that the change in cortical cell diameter represents a lower energy expenditure compared to the number of cortical cell layers, because the former and latter are attributable to variations in vacuole

size and the cytoplasm, respectively (Chimungu et al., 2014). Cortical cell diameter was also found to respond more sensitively than the number of cortical cell layers when the roots of *Zea mays* (Chimungu et al., 2014; Saengwilai et al., 2014), *Malus hupehensis*, and *Malus xiaojinensis* (Wang, 2012) experienced environmental stresses such as water, nitrogen, and zinc deficiencies. Therefore, the closer relationship between cortical cell diameter and root tip diameter observed in our study indicated that the root tip of climbing plants plays a crucial role in regulating growth and metabolism in a cost-efficient manner. When comparing the diameter and number of conduits, the former plays a more important role in determining stele diameter and then influencing root tip diameter. Based on Hagen–Poiseuille's law, the enlargement of conduit diameter can efficiently improve root transport (Tyree and Ewers, 1991). Furthermore, it appears that climbing plant root tips could enlarge their size to efficiently improve their transport ability with increasing root tip diameter. Climbing plants are thought to be more vulnerable to environmental stresses (e.g., drought) than other plant forms (Ewers, 1985; Ewers et al., 1990; Jiménez-Castillo et al., 2006; Hu et al., 2010), due to their extremely wide vessels, which result in higher risks of embolisms than small vessels in self-supporting plants (Ewers et al., 1990; Hacke et al., 2000). The close linkage between conduit diameter and root tip diameter in our study further illustrates that the vulnerabilities of climbing plants to environmental stresses are likely severer in thick-root species and individuals with well-developed root tips. This, however, requires further investigation due to the small number of observations in the current study.

In addition, we noted that the root tip stele diameter was negatively associated with conduit density in climbing plants, which was the opposite to those with conduit diameter and number (Figures 3, 4; Supplementary Table S3). These findings are in agreement with previous studies conducted on subtropical tree species (Kong et al., 2014). Species with large conduit density and small conduits are considered to have weak transport but larger long-term water use efficiency and better adaptability to arid conditions (Poorter et al., 2010; Kong et al., 2014; Pfautsch et al., 2016a,b; Zhou et al., 2021). In our study, the correlations between conduit traits and root tip diameter were similar to those with stele diameter, suggesting that the species of climbing plants with thinner root tips would have more advantages in water and nutrient uptake compared to vertical transport, which is consistent with previous studies on self-supporting plants (Roumet et al., 2016). These findings suggest that conduit traits are likely responsible for the trade-offs of absorption and transport in the root tips of climbing plants.

## Higher stele proportion and closer relationships between functional traits in lianas (woody climbing plants) than vines (herbaceous climbing plants)

In the present study, root tips of lianas have smaller but denser conduits in a thicker stele when compared to vines with a similar root tip diameter, and the associations of root diameter with anatomical traits were stronger in lianas than in vines (Figure 3;

Table 1; Supplementary Table S2), supported our third hypothesis. These features of liana root tips may be an important adaptation of anatomical structure in response to climatic stress. Lianas are more sensitive to water and temperature stresses induced by climate factors than vines due to their longer life cycle and lower belowground storage capacities (Jiménez-Castillo et al., 2006; Hu et al., 2010; Angyalossy et al., 2012). Therefore, these closer relationships between root tip diameter and anatomical traits in the root tips of lianas appear to be an effective strategy to better cope with climate-induced environmental stressors such as drought and cold stress. The wide vessels of climbing plants are typically vulnerable to drought- and cold-induced embolism (Hu et al., 2010). Our findings indicated that lianas had significantly smaller but denser conduits in a thicker stele compared to vines, which may reduce the damage caused by climatic stress (e.g., drought and cold conditions). Surprisingly, no significant difference was observed for the  $K_s$  between lianas and vines at the similar root tip diameters. Therefore, for similar diameter of root tips of lianas and vines, the difference in xylem observed between the two plant types suggests that these climbing plants possess diverse vertical transport strategies but similar transport abilities.

Compared with climatic changes, Bhattarai and Vetaas (2003) reported that herbaceous species, including vines, may be more sensitive to local environmental stresses such as nutrient and water deficiencies in soil. For self-supporting species, woody plants can grow deeper in soil to search for water under local drought conditions, whereas herbaceous plants remain in shallow soils and therefore fully experience the adverse effects of drought conditions (Ansley et al., 2014). In climbing plants, the root tips of vines possess significantly higher ratios of bilateral cortex thickness to root diameter when compared to lianas with similar root tip diameters (Table 1). Therefore, we inferred that vine root tips tend to develop a thicker cortex during growth in order to cope with local environmental stress, thus providing ample space for both the temporary storage of water and minerals and mycorrhizal colonization (Comas et al., 2012). Similarly, for self-supporting species, root N uptake rate has been reported to be higher in herbaceous plants than in woody plants (Ma et al., 2018). Therefore, vines and lianas possess distinct growth strategies, which was reflected in the differences in root tip anatomical cross-sectional structure, as well as in the correlations of these anatomical traits with root tip diameter. Taken together, our findings provide important insights into the influence of different anatomical structures on root tip size in lianas and vines in tropical ecosystems. This study is a detailed investigation of anatomical trait relationships of a limited number of study plants, but more work is needed to understand the extent of these relationships and patterns across environmental gradients and among other climbing plants.

## Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

## Author contributions

WW and SW conceived and designed this research project. HX, WW, and LT performed the data analysis. WW, SW, YW, and ZL completed the field work. HX and SW performed the laboratory experiment and have contributed equally to this work and share first authorship. WW and LT supervised this work. HX, SW, LT, YW, ZL, and WW contributed to the revisions and comments concerning the manuscript. All authors contributed to the article and approved the submitted version.

## Funding

This research was funded by the National Natural Science Foundation of China (31901301), Hainan Provincial Natural Science Foundation of China for High-level Talents (2019RC159), and Scientific Research Foundation for Hainan University [KYQD (ZR)1987].

## Acknowledgments

The authors thank Hongfeng Wang for work conducted in the field and in the laboratory, and Jiacun Gu and Zhengquan Wang for improvements to the manuscript. We also thank reviewers and the editor for their valuable work and comments.

## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

## Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

## Supplementary material

The Supplementary material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fpls.2022.961214/full#supplementary-material>



## References

- Angyalossy, V., Angeles, G., Pace, M. R., Lima, A. C., Dias-Leme, C. L., Lohmann, L. G., et al. (2012). An overview of the anatomy, development and evolution of the vascular system of lianas. *Plant Ecol. Divers.* 5, 167–182. doi: 10.1080/17550874.2011.615574
- Ansley, R. J., Boutton, T. W., and Jacoby, P. W. (2014). Root biomass and distribution patterns in a semi-arid mesquite savanna: responses to long-term rainfall manipulation. *Rangel. Ecol. Manag.* 67, 206–218. doi: 10.2111/REM-D-13-00119.1
- Bhattarai, K. R., and Vetaas, O. R. (2003). Variation in plant species richness of different life forms along a subtropical elevation gradient in the Himalayas, East Nepal. *Glob. Ecol. Biogeogr.* 12, 327–340. doi: 10.1046/j.1466-822X.2003.00044.x
- Boughalleb, F., Abdellaoui, R., Ben-Brahim, N., and Neffati, M. (2014). Anatomical adaptations of *Astragalus gombiformis* Pomel under drought stress. *Open Life Sci.* 9, 1215–1225. doi: 10.2478/s11535-014-0353-7
- Boughalleb, F., Abdellaoui, R., Haddad, Z., and Neffati, M. (2015). Anatomical adaptations of the desert species *Stipa lagascae* against drought stress. *Biologia* 70, 1042–1052. doi: 10.1515/biolog-2015-0125
- Bowsher, A. W., Miller, B. J., and Donovan, L. A. (2016). Evolutionary divergences in root system morphology, allocation, and nitrogen uptake in species from high-versus low-fertility soils. *Funct. Plant Biol.* 43, 129–140. doi: 10.1071/FP15162
- Burton, A. J., Jarvey, J. C., Jarvi, M. P., Zak, D. R., and Pregitzer, K. S. (2012). Chronic N deposition alters root respiration-tissue N relationship in northern hardwood forests. *Glob. Chang. Biol.* 18, 258–266. doi: 10.1111/j.1365-2486.2011.02527.x
- Chimungu, J. G., Brown, K. M., and Lynch, J. P. (2014). Large root cortical cell size improves drought tolerance in maize. *Plant Physiol.* 166, 2166–2178. doi: 10.1104/pp.114.250449
- Collins, C. G., Wright, S. J., and Wurzbarger, N. (2016). Root and leaf traits reflect distinct resource acquisition strategies in tropical lianas and trees. *Oecologia* 180, 1037–1047. doi: 10.1007/s00442-015-3410-7
- Comas, L. H., Mueller, K. E., Taylor, L. L., Midford, P. E., Callahan, H. S., and Beerling, D. J. (2012). Evolutionary patterns and biogeochemical significance of angiosperm root traits. *Int. J. Plant Sci.* 173, 584–595. doi: 10.1086/665823
- Dong, X. Y., Wang, H. F., Gu, J. C., Wang, Y., and Wang, Z. Q. (2015). Root morphology, histology and chemistry of nine fern species (Pteridophyta) in a temperate forest. *Plant Soil* 393, 215–227. doi: 10.1007/s11104-015-2484-7
- Elliott, G. A., Robson, A. D., and Abbott, L. K. (1993). Effects of phosphate and nitrogen application on death of the root cortex in spring wheat. *New Phytol.* 123, 375–382. doi: 10.1111/j.1469-8137.1993.tb03748.x
- Enstone, D. E., Peterson, C. A., and Ma, F. (2003). Root endodermis and exodermis: structure, function, and responses to the environment. *J. Plant Growth Regul.* 21, 335–351. doi: 10.1007/s00344-003-0002-2
- Esau, K. (1977). *Anatomy of Seed Plants. 2nd Edn.* New York, NY: Wiley, 215–255.
- Ewers, F. W. (1985). Xylem structure and water conduction in conifer trees, dicot trees, and lianas. *IAWA Bulletin* 6, 309–317.
- Ewers, F. W., Fisher, J. B., and Chiu, S. T. (1990). A survey of vessel dimensions in stems of tropical lianas and other growth forms. *Oecologia* 84, 544–552. doi: 10.1007/BF00328172
- Genre, A., Chabaud, M., Faccio, A., Barker, D. G., and Bonfante, P. (2008). Prepenetration apparatus assembly precedes and predicts the colonization patterns of arbuscular mycorrhizal fungi within the root cortex of both *Medicago truncatula* and *Daucus carota*. *Plant Cell* 20, 1407–1420. doi: 10.1105/tpc.108.059014
- Gu, J. C., Xu, Y., Dong, X. Y., Wang, H. F., and Wang, Z. Q. (2014a). Root diameter variations explained by anatomy and phylogeny of 50 tropical and temperate tree species. *Tree Physiol.* 34, 415–425. doi: 10.1093/treephys/tpu019
- Guo, D. L., Xia, M. X., Wei, X., Chang, W. J., Liu, Y., and Wang, Z. Q. (2008b). Anatomical traits associated with absorption and mycorrhizal colonization are linked to root branch order in twenty-three Chinese temperate tree species. *New Phytol.* 180, 673–683. doi: 10.1111/j.1469-8137.2008.02573.x
- Hacke, U. G., Sperry, J. S., and Pittermann, J. (2000). Drought experience and cavitation resistance in six shrubs from the Great Basin, Utah. *Basic Appl. Ecol.* 1, 31–41. doi: 10.1078/1439-1791-00006
- Hu, L., Li, M. G., and Li, Z. (2010). Geographical and environmental gradients of lianas and vines in China. *Glob. Ecol. Biogeogr.* 19, 554–561. doi: 10.1111/j.1466-8238.2010.00527.x
- Huang, B., and Eissenstat, D. M. (2000). Linking hydraulic conductivity to anatomy in plants that vary in specific root length. *J. Am. Soc. Hortic. Sci.* 125, 260–264. doi: 10.21273/JASHS.125.2.260
- Hummel, I., Vile, D., Violle, C., Devaux, J., Ricci, B., Blanchard, A., et al. (2007). Relating root structure and anatomy to whole-plant functioning in 14 herbaceous Mediterranean species. *New Phytol.* 173, 313–321. doi: 10.1111/j.1469-8137.2006.01912.x
- Ingwell, L. L., Joseph Wright, S., Becklund, K. K., Hubbell, S. P., and Schnitzer, S. A. (2010). The impact of lianas on 10 years of tree growth and mortality on Barro Colorado Island, Panama. *J. Ecol.* 98, 879–887. doi: 10.1111/j.1365-2745.2010.01676.x
- Jia, S. X., Wang, Z. Q., Li, X. P., Sun, Y., Zhang, X., and Liang, A. (2010). N fertilization affects on soil respiration, microbial biomass and root respiration in *Larix gmelinii* and *Fraxinus mandshurica* plantations in China. *Plant Soil* 333, 325–336. doi: 10.1007/s11104-010-0348-8
- Jiménez-Castillo, M., Wiser, S. K., and Lusk, C. H. (2006). Elevational parallels of latitudinal variation in the proportion of lianas in woody floras: Elevational variation in floristic importance of lianas. *J. Biogeogr.* 34, 163–168. doi: 10.1111/j.1365-2699.2006.01570.x
- Kondo, M., Aguilar, A., Abe, J., and Morita, S. (2000). Anatomy of nodal roots in tropical upland and lowland rice varieties. *Plant Prod. Sci.* 3, 437–445. doi: 10.1626/pp.3.437
- Kong, D. L., Ma, C. G., Zhang, Q., Li, L., Chen, X. Y., Zeng, H., et al. (2014). Leading dimensions in absorptive root trait variation across 96 subtropical forest species. *New Phytol.* 203, 863–872. doi: 10.1111/nph.12842
- Kong, D. L., Wang, J. J., Kardol, P., Wu, H. F., Zeng, H., Deng, X. B., et al. (2016). Economic strategies of plant absorptive roots vary with root diameter. *Biogeosciences* 13, 415–424. doi: 10.5194/bg-13-415-2016
- Kong, D. L., Wang, J. J., Zeng, H., Liu, M. Z., Miao, Y., Wu, H. F., et al. (2017). The nutrient absorption–transportation hypothesis: optimizing structural traits in absorptive roots. *New Phytol.* 213, 1569–1572. doi: 10.1111/nph.14344
- Lascaris, D., and Deacon, J. W. (1991). Relationship between root cortical senescence and growth of wheat as influenced by mineral nutrition, *Idriella bolleyi* (Sprague) von Arx and pruning of leaves. *New Phytol.* 118, 391–396. doi: 10.1111/j.1469-8137.1991.tb00020.x
- Lux, A., Luxova, M., Abe, J., and Morita, S. (2004). Root cortex: structural and functional variability and responses to environmental stress. *Root Res.* 13, 117–131. doi: 10.3117/rootres.13.117
- Ma, Z. Q., Guo, D. L., Xu, X. L., Lu, M. Z., Bardgett, R. D., Eissenstat, D. M., et al. (2018). Evolutionary history resolves global organization of root functional traits. *Nature* 555, 94–97. doi: 10.1038/nature25783
- Pfautsch, S., Harbusch, M., Wesolowski, A., Smith, R., Macfarlane, C., Tjoelker, M. G., et al. (2016a). Climate determines vascular traits in the ecologically diverse genus *eucalyptus*. *Ecol. Lett.* 19, 240–248. doi: 10.1111/ele.12559
- Pfautsch, S., Macfarlane, C., Harbusch, M., Wesolowski, A., Smith, R., Boer, M., et al. (2016b). Vessel diameter and related hydraulic traits of 31 *eucalyptus* species arrayed along a gradient of water availability. *Ecology* 97:1626. doi: 10.1890/16-0147.1
- Phillips, O. L., Martinez, R. V., Arroyo, L., Baker, T. R., Killeen, T., Lewis, S. L., et al. (2002). Increasing dominance of large lianas in Amazonian forests. *Nature* 418, 770–774. doi: 10.1038/nature00926
- Poorter, L., McDonald, I., Alarcón, A., Fichtler, E., Licona, J., Peña-Claros, M., et al. (2010). The importance of wood traits and hydraulic conductance for the performance and life history strategies of 42 rainforest tree species. *New Phytol.* 185, 481–492. doi: 10.1111/j.1469-8137.2009.03092.x
- Pregitzer, K. S., Deforest, J. L., Burton, A. J., Allen, M. F., Ruess, R. W., and Hendrick, R. L. (2002). Fine root architecture of nine north American trees. *Ecol. Monogr.* 72, 293–309. doi: 10.1890/0012-9615(2002)072[0293:FRAONN]2.0.CO;2
- Putz, F. E. (1983). Liana biomass and leaf area of a “tierra firme” forest in the Rio Negro basin, Venezuela. *Biotropica* 15, 185–189. doi: 10.2307/2387827
- Putz, F. E. (1984). The natural history of lianas on Barro Colorado Island, Panama. *Ecology* 65, 1713–1724. doi: 10.2307/1937767
- Qin, X. T. (2020). *Flora Analysis of Bryophytes in Limushan Nature Reserve*. Haikou: Hainan University Press.
- Roumet, C., Birouste, M., Picon-Cochard, C., Ghestem, M., Osman, N., Vigneron Brenas, S., et al. (2016). Root structure–function relationships in 74 species: evidence of a root economics spectrum related to carbon economy. *New Phytol.* 210, 815–826. doi: 10.1111/nph.13828
- Saengwilai, P., Nord, E. A., Chimungu, J. G., Brown, K. M., and Lynch, J. P. (2014). Root cortical aerenchyma enhances nitrogen acquisition from low-nitrogen soils in maize. *Plant Physiol.* 166, 726–735. doi: 10.1104/pp.114.241711
- Schneider, H. M., Wojciechowski, T., Postma, J. A., Brown, K. M., Lücke, A., Zeisler, V., et al. (2017). Root cortical senescence decreases root respiration, nutrient content and radial water and nutrient transport in barley: cortical senescence



reduces respiration, nutrient content and radial transport. *Plant Cell Environ.* 40, 1392–1408. doi: 10.1111/pce.12933

Schnitzer, S. A., and Bongers, F. (2002). The ecology of lianas and their role in forests. *Trends Ecol. Evol.* 17, 223–230. doi: 10.1016/S0169-5347(02)02491-6

Schnitzer, S. A., and Bongers, F. (2011). Increasing liana abundance and biomass in tropical forests: emerging patterns and putative mechanisms: increasing lianas in tropical forests. *Ecol. Lett.* 14, 397–406. doi: 10.1111/j.1461-0248.2011.01590.x

Schnitzer, S. A., Kuzee, M. E., and Bongers, F. (2005). Disentangling above- and below-ground competition between lianas and trees in a tropical forest. *J. Ecol.* 93, 1115–1125. doi: 10.1111/j.1365-2745.2005.01056.x

Smith-Martin, C. M., Bastos, C. L., Lopez, O. R., Powers, J. S., and Schnitzer, S. A. (2019). Effects of dry-season irrigation on leaf physiology and biomass allocation in tropical lianas and trees. *Ecology* 100:e02827. doi: 10.1002/ecy.2827

Tyree, M. T., and Ewers, F. W. (1991). The hydraulic architecture of trees and other woody plants. *New Phytol.* 119, 345–360. doi: 10.1111/j.1469-8137.1991.tb00035.x

Wang, J. H. (2012). *The Morphological and Physiological Responses of Apple Rootstocks to Zinc-Deficiency Stress and Regulation of IAA in the Root Growth*. Taian: Shandong Agriculture University Press.

Wang, W. N., Wang, Y., Hoch, G., Wang, Z. Q., and Gu, J. C. (2018). Linkage of root morphology to anatomy with increasing nitrogen availability in six temperate tree species. *Plant Soil* 425, 189–200. doi: 10.1007/s11104-018-3563-3

Wang, L. J., Hong, M. L., Wang, J. C., Lv, S. Q., and Shi, H. T. (2004). Diversity and Fauna Analysis of Amphibians in Limushan Nature Reserve of Hainan Province. *Chinese J. Zool.* 39, 54–57.

Wang, Y. M., Wang, Y., Wang, S. Y., Gao, G. Q., and Gu, J. C. (2020). Fine root anatomical and morphological traits of three temperate liana species in northeastern China. *J. Beijing For. Univ.* 42, 42–49. doi: 10.12171/j.1000-1522.20190419

Wright, S. J., Calderón, O., Hernández, A., and Paton, S. (2004). Are lianas increasing in importance in tropical forests? A 17-year record from Panama. *Ecology* 85, 484–489. doi: 10.1890/02-0757

Zhou, M., Bai, W., Li, Q., Guo, Y., and Zhang, W. H. (2021). Root anatomical traits determined leaf-level physiology and responses to precipitation change of herbaceous species in a temperate steppe. *New Phytol.* 229, 1481–1491. doi: 10.1111/nph.16797



## OPEN ACCESS

## EDITED BY

Patompong Johns Saengwilai,  
Mahidol University, Thailand

## REVIEWED BY

M. Teresa Boquete,  
University of Santiago de Compostela,  
Spain  
Jiacun Gu,  
Northeast Forestry University, China

## \*CORRESPONDENCE

Martí March-Salas  
martimarchsalas@gmail.com

## SPECIALTY SECTION

This article was submitted to  
Functional Plant Ecology,  
a section of the journal  
Frontiers in Plant Science

RECEIVED 19 July 2022

ACCEPTED 24 October 2022

PUBLISHED 14 November 2022

## CITATION

March-Salas M, Scheepens JF,  
van Kleunen M and Fitze PS (2022)  
Precipitation predictability affects  
intra- and trans-generational plasticity  
and causes differential selection on  
root traits of *Papaver rhoeas*.  
*Front. Plant Sci.* 13:998169.  
doi: 10.3389/fpls.2022.998169

## COPYRIGHT

© 2022 March-Salas, Scheepens, van  
Kleunen and Fitze. This is an open-  
access article distributed under the  
terms of the [Creative Commons  
Attribution License \(CC BY\)](https://creativecommons.org/licenses/by/4.0/). The use,  
distribution or reproduction in other  
forums is permitted, provided the  
original author(s) and the copyright  
owner(s) are credited and that the  
original publication in this journal is  
cited, in accordance with accepted  
academic practice. No use,  
distribution or reproduction is  
permitted which does not comply with  
these terms.

# Precipitation predictability affects intra- and trans-generational plasticity and causes differential selection on root traits of *Papaver rhoeas*

Martí March-Salas <sup>1,2,3\*</sup>, J. F. Scheepens <sup>1</sup>,  
Mark van Kleunen <sup>4,5</sup> and Patrick S. Fitze <sup>2,3</sup>

<sup>1</sup>Plant Evolutionary Ecology, Faculty of Biological Sciences, Goethe University Frankfurt, Frankfurt am Main, Germany, <sup>2</sup>Department of Biodiversity and Evolutionary Biology, Museo Nacional de Ciencias Naturales (MNCN-CSIC), Madrid, Spain, <sup>3</sup>Department of Biodiversity and Ecologic Restoration, Instituto Pirenaico de Ecología (IPE-CSIC), Jaca, Spain, <sup>4</sup>Ecology, Department of Biology, University of Konstanz, Konstanz, Germany, <sup>5</sup>Zhejiang Provincial Key Laboratory of Plant Evolutionary Ecology and Conservation, Taizhou University, Taizhou, China

Climate forecasts show that in many regions the temporal distribution of precipitation events will become less predictable. Root traits may play key roles in dealing with changes in precipitation predictability, but their functional plastic responses, including transgenerational processes, are scarcely known. We investigated root trait plasticity of *Papaver rhoeas* with respect to higher versus lower intra-seasonal and inter-seasonal precipitation predictability (i.e., the degree of temporal autocorrelation among precipitation events) during a four-year outdoor multi-generation experiment. We first tested how the simulated predictability regimes affected intra-generational plasticity of root traits and allocation strategies of the ancestors, and investigated the selective forces acting on them. Second, we exposed three descendant generations to the same predictability regime experienced by their mothers or to a different one. We then investigated whether high inter-generational predictability causes root trait differentiation, whether transgenerational root plasticity existed and whether it was affected by the different predictability treatments. We found that the number of secondary roots, root biomass and root allocation strategies of ancestors were affected by changes in precipitation predictability, in line with intra-generational plasticity. Lower predictability induced a root response, possibly reflecting a fast-acquisitive strategy that increases water absorbance from shallow soil layers. Ancestors' root traits were generally under selection, and the predictability treatments did neither affect the strength nor the direction of selection. Transgenerational effects were detected in root biomass and root weight ratio (RWR). In presence of lower predictability, descendants significantly reduced RWR compared to ancestors, leading to an increase in performance. This points to a change in root allocation in order to maintain or increase the descendants' fitness. Moreover, transgenerational plasticity existed in maximum rooting depth and root biomass, and the less predictable treatment promoted the lowest coefficient

of variation among descendants' treatments in five out of six root traits. This shows that the level of maternal predictability determines the variation in the descendants' responses, and suggests that lower phenotypic plasticity evolves in less predictable environments. Overall, our findings show that roots are functional plastic traits that rapidly respond to differences in precipitation predictability, and that the plasticity and adaptation of root traits may crucially determine how climate change will affect plants.

#### KEYWORDS

acquisition strategy, adaptive transgenerational plasticity, annual plants, inter-seasonal predictability, multi-generation experiment, root allocation strategy, root functional traits, selection gradients

## Introduction

Current climate change entails rising global temperatures, longer and more frequent dry periods, and changes in weather predictability (Xu et al., 2020; IPCC, 2021). Although global mean precipitation is expected to remain rather constant in the near future, precipitation patterns will change, leading to a reduction in precipitation predictability at different temporal scales, *i.e.*, among days, weeks, seasons or years (Tonkin et al., 2017; Xu et al., 2020). The degree of intrinsic precipitation predictability (*i.e.*, the degree of temporal autocorrelation among precipitation events; Pennekamp et al., 2019) will determine the amount and timing of water availability for plants. This may cause plants to change their nutrient and water acquisition strategy, plastically modulate their traits, and eventually may result in adaptation to the new conditions (Yin et al., 2022).

According to evolutionary theory, the evolution of increased plasticity would be favoured under temporally variable but predictable environments (Caro et al., 2016; McNamara et al., 2016). In contrast, reduced plasticity would evolve in temporally variable but less predictable environments, since plastic changes may be misaligned with differential selective pressures over generations (Lande, 2009; Botero et al., 2015; Tufto, 2015; Leung et al., 2020). A recent experiment suggested that higher predictability in natural environments may contribute to the evolution of transgenerational plasticity in reproductive traits (*i.e.*, to the offspring's plastic response to the ancestor's environment) (Yin et al., 2022). Other experiments showed that transgenerational responses in phenological and fitness-related traits to different degrees of predictability are possible (Franch-Gras et al., 2017; March-Salas and Fitze, 2019; March-Salas et al., 2019; Colicchio and Herman, 2020; March-Salas et al., 2021a).

Evolutionary ecology has primarily focused on phenological (e.g. flowering start) and performance (e.g. aboveground

biomass, number of flowers, number of seeds) traits to determine how plants adapt to environmental changes. However, root traits may also play an important role for overall plant development and tolerance of or resistance to changing environmental conditions. Root traits could be under strong natural selective pressure, and vary over generations reflecting adaptation to dynamic water availability (Zhou et al., 2019; Yamauchi et al., 2021). Roots can also rapidly sense fluctuations in water availability and may help species to deal with temporal shifts in the means and predictability of precipitation (Zhou et al., 2019; March-Salas et al., 2021b). However, how the maternal environment determines the transgenerational expression of the root traits of progeny and adaptive responses in future environments remains largely unknown (Donelson et al., 2018).

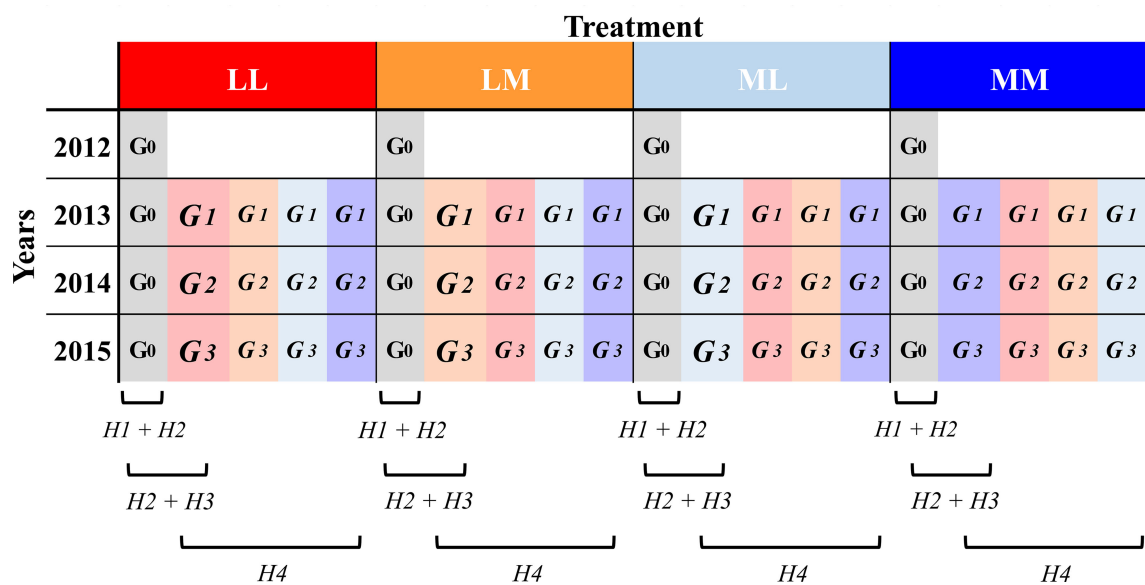
Plastic responses of roots can however be costly (DeWitt et al., 1998), for instance through re-allocation of resources from other plant structures to roots (Manenti et al., 2015). A reduction of resource investment into root biomass may be a response to stressful conditions, potentially helping plants to maintain or even increase their short-term performance by re-allocation into above-ground structures (Lundgren and Des Marais, 2020). Additionally, in response to specific environmental cues, plants may promote within-root allocation-shifts. For instance, a constant water availability may favor deeper and bigger roots and increased branching density but fewer fine roots (Wu et al., 2016; March-Salas et al., 2021b). Promoting lateral root branching while reducing primary root depth may also help plants to withstand stressful conditions such as water limitation or high salinity levels (Ambastha et al., 2020; Gallego-Tévar et al., 2022). This should be especially true in fast-cycling plants with acquisitive strategies, since their roots should acquire temporally available resources in shallow soil layers to guarantee a rapid increase in performance (Hermans et al., 2006; Weemstra et al., 2016; Gallego-Tévar et al., 2022). Despite their relatively small root systems, many annual plants

use acquisitive strategies to favour rapid growth and fast reproduction under stressful conditions (Gallego-Tévar et al., 2022). So, evolutionary experiments that focus on root traits are needed to increase our understanding of how plants deal with changing environments.

In a multi-generation experiment with the annual herb *Papaver rhoeas* L. (Papaveraceae) grown under semi-natural outdoor conditions, we manipulated intra-seasonal (among days) and inter-seasonal (among spring and summer) precipitation predictability. We grew four consecutive generations, each year in the same as well as in contrasting predictability regime to which the maternal plants were exposed. In addition, the ancestral generation (the original generation that had not experienced any of the experimental treatments) was sown in each of the four years under all predictability regimes. With this experimental design, first, we investigated whether and how roots of ancestors respond plastically (*i.e.*, intra-generational plasticity; *e.g.* Sobral et al., 2021) to more *versus* less intra- and inter-seasonal precipitation predictability within four consecutive experimental years. Second, we tested whether root traits are subject to selection. Third, we tested for transgenerational effects to the different predictability regimes (*i.e.*, genetically as well as non-genetically inherited effects when plants grow in the same treatment over generations; March-Salas et al., 2019). In other words, by comparing ancestors and descendants in the same year

and treatment, we investigated whether precipitation predictability promoted trait differentiation in descendants, and whether there was a change in the root trait response between ancestors and descendants that is mediated by the precipitation predictability treatment. This would point to a transgenerational response to a specific environment. Here, we also tested whether the observed differences in root traits between ancestors and descendants led to higher performance of the descendants, what would point to an adaptive change. Fourth, by comparing descendants that grew in the same or contrasting treatment as their mother, we tested whether the descendants' response to the treatments depended on the maternal treatment, which will point to differences in transgenerational plasticity due to the predictability regime (Figure 1).

We hypothesized (H1) that roots of ancestors plastically respond to different degrees of precipitation predictability, with greater root response and investment in root biomass in environments that fluctuate less predictably in order to make instantaneous use of temporally rare events of water availability; (H2) that the considered root traits (maximum rooting depth, number of secondary roots, root biomass, and the relative investment to each of them) affect plant fitness and are under strong selection; (H3) that differences between ancestors and descendants in root traits occur when inter-generational predictability is high and when current environmental conditions



**FIGURE 1**  
Design of the evolution experiment. Ancestors (G<sub>0</sub>) and the three descendant generations (G<sub>1-3</sub>) were subjected to four different precipitation predictability treatment combinations (LL, LM, ML, MM; see methods) in their year of growth (*i.e.*, Treatment). Ancestors (in grey) were grown from 2012 to 2015 and descendants (highlighted in the maternal treatment colour but lighter than the treatment in the growing year) from 2013 to 2015. Below, the brackets refer to the experimental lines (*i.e.*, generation and treatment) involved in the assessment of each of the four main hypothesis (H) we addressed in this work (see Introduction).



are more predictable; and (H4) that transgenerational plasticity is favoured in more predictable conditions.

## Material and methods

### Experimental setup

This study was conducted during four consecutive years (2012–2015) under natural conditions at the experimental field station ‘El Boalar’ (42°33′N, 0°37′W, 705 m a.s.l.; Jaca, Huesca, Spain). The experimental setup consisted of 16 enclosures of 100 m<sup>2</sup> each, which were covered and separated with a mesh and a 1 m tall metal wall. The setup included an automated watering system consisting of one sprinkler in each of the four corners of each enclosure, providing homogenous precipitation in the whole enclosure and allowing us to control the quantity and timing of the watering events (March-Salas et al., 2019). Within each of these enclosures, we established a plot of 7.2 m<sup>2</sup> that was surrounded by a vertical mosquito net (30 cm aboveground and 10 cm belowground) to protect plants from herbivory (e.g. snails, slugs). In each experimental year, the top 30 cm of the soil of each plot was manually ploughed, loosened, homogenised and flattened before sowing.

### Study species

We used the annual herb *Papaver rhoeas* L. (common poppy Papaveraceae), a wide-spread and fast-growing species with acquisitive and exploitative strategies (McNaughton and Harper, 1964; Pérez-Ramos et al., 2019), which naturally occurs in the surroundings of the study site. The species’ height is between 10 and 50 cm, its flowers are bright red and its fruits are a capsule that usually contain hundreds of >1 mm long seeds (Franklin-Tong and Franklin, 1992). It has a slender primary root with multiple lateral secondary roots (Figure S1). The original seed lot of *P. rhoeas* was obtained in 2011 from 50 maternal plants of a nearby population (~60 km away) that experiences higher humidity and otherwise a similar climate as the study site (March-Salas et al., 2019). The seeds from the original seed lot were mixed and are hereafter referred to as ancestors or the ancestral generation. Ancestral seeds were sown in each of the treatments in the four experimental years to be able to disentangle treatment-differences among years from overall treatment effects as well as year control (see below). To preclude potential maternal bias in genetic differentiation among ancestors, we accounted for differences in average seed weight and in the coefficient of variation (CV) in seed weight among the groups of seeds that we sowed in the different treatments and years, i.e., we grouped the seeds so that there were no differences in seed weight mean and CV between the 16 groups – 4 treatment combinations × 4 years.

### Precipitation predictability treatment

The field site’s natural precipitation predictability was manipulated by means of the automated watering system, to simulate higher *versus* lower intrinsic precipitation predictability at two different temporal scales: intra-seasonal (among days) and inter-seasonal (between spring and summer) predictability.

More (M) and less (L) predictable intra-seasonal predictability was simulated by applying 14 supplemental watering events each week at regular or random time-intervals, respectively (March-Salas et al., 2019). Both treatments consisted of the same total amount of precipitation (natural + supplemental) but exhibited a different degree of temporal autocorrelation among precipitation events, i.e., they contrasted in their intrinsic precipitation predictability. Differences in intrinsic predictability of precipitation was calculated by means of permutation entropy, which is inversely related to intrinsic predictability (Pennekamp et al., 2019). The combination of natural and experimental precipitation resulted in a permutation entropy of 0.86 in L and 0.77 in M. Permutation entropy in M was equal to the one of natural precipitation (March-Salas et al., 2019). L thus had 11.69% lower intrinsic precipitation predictability than M (and than natural precipitation). The simulated subtle but significant differences in predictability are in line with theoretical conditions that should lead to adaptive transgenerational plasticity: existence of natural environmental heterogeneity and predictability of an intermediate frequency and without extreme events (Burgess and Marshall, 2014; Uller et al., 2015; Yin et al., 2022).

In spring of each year, from April to late June (hereafter referred to as ‘early treatment’), eight plots were subjected to M and another eight plots to L predictable predictability. In summer, from July to late September (hereafter referred to as ‘late treatment’), half of the plots of each early treatment were exposed to the same (MM, LL) or to the other (ML, LM) treatment, simulating higher (MM, LL) and lower (LM, ML) inter-seasonal predictability. This thus resulted in a two-factorial design with four precipitation predictability treatment combinations: (1) more predictable precipitation during the whole experimental period (MM), (2) less predictable during the whole experimental period (LL), (3) more predictable during spring and less predictable during summer (ML), and (4) less predictable during spring and more predictable during summer (LM). Each treatment combination was applied to four independent plots.

In each experimental year, the total precipitation (natural + supplemental) falling in the experimental set up was within the natural range recorded: (a) at the field site in the previous ten years, (b) in the area of origin of the ancestor seeds, and (c) in the species distribution range (McNaughton and Harper, 1964; March-Salas et al., 2019; March-Salas et al., 2021a). The study site had high potential evapotranspiration and plants were

exposed to water stress from April to September (Figure S2). In the experimental plots, plants were as well exposed to water stress, although less pronounced (for further information on climatic and soil conditions of the study site, see supplementary text and Figure S2).

## Sowing procedure and evolution experiment

In each of the four experimental years, seeds of *P. rhoeas* were individually sown in April in each of the 16 plots. In each plot, three seeds were planted in each of 84 planting positions aligned in a quadratic grid and separated by 20 cm. Each position was differentiated with a metal stick, which was labelled with the seed origin. When more than one seed emerged per position, seedlings were thinned to one.

In 2012, randomly selected ancestor seeds were planted in the 84 positions, and from 2013 to 2015, ancestor and descendant seeds (*i.e.*, the descendant seed generation 1 in 2013, generation 2 in 2014 and generation 3 in 2015) were both sown in the same plot (March-Salas et al., 2019). More specifically, from 2013 to 2015, randomly selected ancestor seeds were sown in 21 positions per plot and descendant seeds in 63 positions per plot. In the case of descendants, from each maternal line (*i.e.*, descendants originating from the same mother) seeds were sown in the same treatment combination as their mother and also in the other three treatment combinations. In the first descendant generation, we included seeds from 12 maternal plants per treatment line, except for seeds from the ML treatment, for which only seeds from 3 maternal plants could be included given that all other maternal plants did not produce enough seeds. For the subsequent generations, we included seeds from 6 maternal plants per treatment line. To avoid bias among used and not used mothers and among used and not used seeds of the same mother, we tested that there were no phenotypic differences between selected and not selected mothers, no differences in seed weight between selected and not selected seed lots, and no significant interaction between the factor 'selected/not selected' and the treatments combinations (all  $p \geq 0.2$ ). Moreover, to make sure that treatment-induced transgenerational effects are not confounded with transgenerational responses to plot-specific conditions, seeds were never planted in the plot in which their mother had been growing previously.

## Plant measurements

At the end of a plant's life cycle (*i.e.*, when all fruits of a given plant were ripe), we individually harvested above-ground biomass and root biomass. Roots were carefully dug out and washed to remove the substrate (Figure S1). For each plant, the

length of the longest root was measured as an estimate of maximum rooting depth (precision: 1 mm) and the number of secondary roots (all lateral root branches >0.5 cm long) was counted. The dry mass of the above-ground and root biomass (in g) was determined after drying at 50 °C for three days. To test whether the treatments affected root allocation strategies, the following root traits were calculated: (1) root weight ratio (RWR; root biomass/total biomass); (2) relative rooting depth (maximum rooting depth/root biomass); and (3) relative root branching (number of secondary roots/root biomass).

## Statistical analysis

Four main types of analysis were conducted using *R* version 4.0.3 (R Development Core Team, 2020) and Linear Mixed-effects Models (LMMs) implemented in the *lme4* package and the *lmer* function (Bates et al., 2015).

First (H1), we tested for treatment effects on root traits of the ancestral generation across the four years (*i.e.*, intra-generation plasticity across four years). The six measured traits (root biomass, maximum rooting depth, number of secondary roots, RWR, relative rooting depth and relative root branching) were included as response variables in six separate models. As fixed factors, we included early treatment (less predictable *versus* more predictable), late treatment (less predictable *versus* more predictable), year (2012, 2013, 2014, 2015) and their two- and three-way interactions, and as random factor we included 'plot\_year' (a factor referring to the plot  $\times$  year combination), as described in Bates et al. (2015). A significant treatment effect would indicate intra-generational plasticity; a significant year effect would indicate differences among years irrespective of the treatment, and a significant treatment(s)  $\times$  year interaction would indicate that the treatment effect depends on the year.

Second (H2), the selective regime (the type and the strength of natural selection) to which the root traits were exposed was analysed using selection gradients (Lande and Arnold, 1983). Analysing the selective regime allows to test if the measured root traits affect plant performance, and therefore, whether they can be considered as functional traits (Violle et al., 2007). As a performance proxy, we used total biomass, because it is generally positively related to seed number (Shipley et al., 2016; Younginger et al., 2017). We could not have precise data on seed number for all plants due to potential loss of seeds from capsules that had opened before collection. However, for the collected data, seed number was strongly correlated with total plant biomass ( $F_{1,455} = 249.48$ ,  $P < 0.001$ ,  $\rho = 0.6$ ). Prior to the analyses, total biomass and all root variables were *ln*-transformed and standardized to a mean of zero and a standard deviation of one to obtain standardized selection gradients (Lande and Arnold, 1983). First, only ancestral plants were analysed. Early treatment, late treatment and year (2012, 2013, 2014, 2015) were modelled as fixed factors, and the

root trait of interest was included as a covariate. To account for non-independence of plants measured in the same year and in the same plot, 'plot\_year' was included as random factor. To test for directional, stabilizing or disruptive selection, linear and quadratic terms of the root variable of interest were included. The full model also contained all two- and three-way interactions between treatments and the covariates. To test whether selective regimes differed among ancestors and descendants, an additional set of analyses was conducted in which ancestors and descendants both sown in 2013, 2014 and 2015 were analysed together. In these analyses, the generation (ancestor *versus* descendant) and its interactions with the other variables were added to the above-mentioned model as well as the ID of the maternal line as random effect.

Third (H3), we tested whether root traits of the descendant generations differed from those of the ancestral generation grown in the same year, and whether differences depended on the predictability treatment. We included early treatment, late treatment, generation (ancestral *versus* descendants), year (2013, 2014, 2015) and their two-, three- and four-way interactions as fixed factors. The ID of the maternal line and 'plot\_year' were modelled as random factors. A significant generation factor would indicate differences between ancestors and descendants independent of treatment, and a significant generation  $\times$  treatment interaction would indicate that predictability treatment effect depends on the studied generation.

Fourth (H4), we tested whether treatment effects in descendants depended on their mothers' treatment by only analysing descendants. The maternal treatment combination, the descendants' treatment combination, the two-way interaction, and year were included as fixed factors, whereas the ID of the maternal line and 'plot\_year' were included as random factors. A significant maternal treatment effect would indicate that the maternal treatment manifested in the descendants independent of the descendant treatments; a significant descendant treatment effect would indicate treatment differences independent from maternal environment; a significant maternal  $\times$  descendant treatment interaction would indicate that the outcome of the descendants' treatment depends on the treatment to which their mothers were exposed, pointing to transgenerational plasticity. Moreover, we calculated the coefficient of variation (the ratio of the standard deviation to the mean, based on means,  $CV_m$ ) among treatments in descendants for each maternal treatment as a quantitative estimator of plasticity (Schlichting, 1986; Valladares et al., 2000), as well as the overall CV of ancestors and of descendants (Table S1).

In all models, we tested the assumptions of normality and homogeneity of variance of the residuals using the Shapiro-Wilk test and the Bartlett test, respectively. If the residuals were not normally distributed, we transformed the response variable (see Tables 1, 2). In the case of heteroscedasticity, we applied a

TABLE 1 Precipitation predictability treatment effects on root traits of ancestors.

Parameter	df	N of secondary roots			Maximum rooting depth <sup>a</sup>			Root biomass <sup>b</sup>		
		Chi <sup>2</sup>	P		Chi <sup>2</sup>	P		Chi <sup>2</sup>	P	
Early [M]	1	0.693	0.405		0.931	0.335		1.311	0.252	
Late [M]	1	1.669	0.196		0.000	0.995		1.736	0.188	
Year	3	140.493	<0.001	***	109.640	<0.001	***	76.550	<0.001	***
Early $\times$ Late	1	4.825	0.028	*	1.821	0.177		2.805	0.094	.
Early $\times$ Year	3	2.697	0.441		2.063	0.559		4.659	0.199	
Late $\times$ Year	3	9.121	0.028	*	6.172	0.104		13.488	0.004	**
Early $\times$ Late $\times$ Year	3	1.115	0.773		2.980	0.395		16.777	<0.001	***
Parameter	df	Root weight ratio <sup>c</sup>			Relative root branching <sup>d</sup>			Relative rooting depth <sup>d</sup>		
		Chi <sup>2</sup>	P		Chi <sup>2</sup>	P		Chi <sup>2</sup>	P	
Early [M]	1	0.484	0.487		8.647	0.003	**	1.140	0.286	
Late [M]	1	0.437	0.509		1.717	0.190		10.517	0.001	**
Year	3	27.642	<0.001	***	13.595	0.004	**	29.809	<0.001	***
Early $\times$ Late	1	1.610	0.205		0.233	0.630		1.563	0.211	
Early $\times$ Year	3	4.091	0.252		3.008	0.390		4.478	0.214	
Late $\times$ Year	3	2.972	0.396		3.081	0.299		7.102	0.069	.
Early $\times$ Late $\times$ Year	3	55.815	<0.001	***	3.319	0.174		6.285	0.094	.

transformations: <sup>a</sup> $\sqrt{0.4}$ ; <sup>b</sup> $\sqrt{0.1}$ ; <sup>c</sup> $\sqrt{0.6}$ ; <sup>d</sup>log.

Linear Mixed-effects Models (LMMs) included Early treatment, Late treatment, Year and their two- and three-way interactions. Transformations applied to the response variable are indicated. Significance is shown as \*  $0.05 > P \geq 0.01$ ; \*\*  $0.01 > P \geq 0.001$ ; \*\*\*  $P < 0.001$ , and . reflects marginal effects ( $0.1 > P \geq 0.05$ ). Sample size was 458.

Response variables are number (N) of secondary roots, maximum rooting depth, root biomass, root weight ratio (RWR), relative root branching, and relative rooting depth.

TABLE 2 Root transgenerational plasticity to precipitation predictability treatments.

Parameter	df	N of secondary roots <sup>a</sup>			Maximum rooting depth <sup>b</sup>			Root biomass <sup>c</sup>		
		Chi <sup>2</sup>	P		Chi <sup>2</sup>	P-value		Chi <sup>2</sup>	P	
Year	2	162.112	<0.001	***	2488.959	<0.001	***	85.861	<0.001	***
Maternal treatment	3	3.925	0.270		5.762	0.124		2.612	0.455	
Descendants treatment	3	6.636	0.084	.	6.460	0.091	.	5.853	0.119	
Maternal × Descendants	9	10.625	0.302		17.715	0.039	*	20.247	0.016	*
Parameter	df	Root weight ratio <sup>b</sup>			Relative root branching <sup>a</sup>			Relative rooting depth <sup>a</sup>		
		Chi <sup>2</sup>	P		Chi <sup>2</sup>	P		Chi <sup>2</sup>	P	
Year	2	5.217	0.074	.	16.736	<0.001	***	1092.349	<0.001	***
Maternal treatment	3	3.933	0.269		3.054	0.383		6.191	0.103	
Descendants treatment	3	1.174	0.759		8.743	0.033	*	3.630	0.304	
Maternal × Descendants	9	14.435	0.097	.	10.604	0.304		14.136	0.118	

transformations: <sup>a</sup>log; <sup>b</sup>√0.5; <sup>c</sup>√0.2.  
 Linear Mixed-effects Models (LMMs) included Year, Maternal treatment, Descendants treatment, Generation and the two-way interaction between Maternal and Descendants treatment. Transformations applied to the response variable are indicated. Significance is shown as \* 0.05 > P ≥ 0.01; \*\* 0.01 > P ≥ 0.001; \*\*\* P < 0.001, and . reflects marginal effects (0.1 > P ≥ 0.05). Sample size was 640.  
 Response variables are number (N) of secondary roots, maximum rooting depth, root biomass, root weight ratio (RWR), relative root branching, and relative rooting depth.

weighted least square regression (Strutz, 2016) by including weights (1/variance) into the model, using the extract model *weights* command. Whenever there were significant main effects containing more than two factor levels or significant interactions, we applied Tukey's *post-hoc* contrasts using the *lsmeans* package to understand which levels differed (Lenth, 2016). Moreover, in all statistical analyses, the most parsimonious model was determined using stepwise backward elimination. Sample size per treatment, year and generation of ancestors, pure descendant lines, and descendants from all treatment combinations are shown in Table S2.

## Results

### Effects of predictability treatments on root traits of ancestors

In the ancestral generation, which was sown in each year (2012–2015), the predictability treatment affected the response of all measured root traits except the maximum rooting depth. The number of secondary roots was significantly lower in ML than in the other treatment combinations (Figure 2A), as indicated by a significant early × late treatment interaction (Table 1). Moreover, although in 2013 plants in the more predictable late treatment (M) tended to have more secondary roots than in the less predictable late treatment (L; P = 0.06), late treatments did not differ in the other years (P ≥ 0.79), as indicated by a significant late treatment × year (Table 1). Root biomass was significantly higher in LL and LM than in ML in 2012 and 2013, and higher in LL than in ML and LM in 2015 (all P < 0.05; Figure 2B), as indicated by a significant three-way interaction

between early treatment, late treatment and year (Table 1). However, no significant differences among treatments existed in 2014, and all other contrasts in 2012, 2013 and 2015 were not significant (P ≥ 0.17). For maximum rooting depth, there were no significant treatment effects (Table 1). Root weight ratio (RWR) was significantly higher in LL than in ML and LM in 2015 (Figure 2C), as indicated by a significant three-way interaction between early treatment, late treatment and year (Table 1). However, other treatments did not significantly differ in 2015, and all *post-hoc* contrasts in the other years were not significant (P ≥ 0.15). Relative root branching was significantly higher in the M than in the L early treatment (Early [M] = 0.449 ± 0.153 [Estimate ± SE]), and it was not affected by the late treatment (Table 1). Relative rooting depth was significantly higher in the L late treatment (Late [M] = -0.535 ± 0.171), and it was not affected by the early treatment (Table 1). So, overall, plants invested less biomass in roots and less in rooting depth but had increased root branching in the M treatment than in the L treatment, particularly if the treatment was imposed in spring.

### Selection acting on root traits

Selection gradients for the ancestral generation revealed significant non-linear relationships of total biomass (hereafter referred as 'performance') with maximum rooting depth (quadratic: Estimate ± SE = 0.158 ± 0.019, P < 0.001; linear: -0.817 ± 0.180, P < 0.001), RWR (quadratic: -0.087 ± 0.022, P < 0.001; linear: 0.670 ± 0.199, P < 0.001) and relative rooting depth (quadratic: 0.099 ± 0.016, P < 0.001, P < 0.001; linear: -1.784 ± 0.179, P < 0.001). Significant linear (but no quadratic) relationships also existed for the number of secondary roots

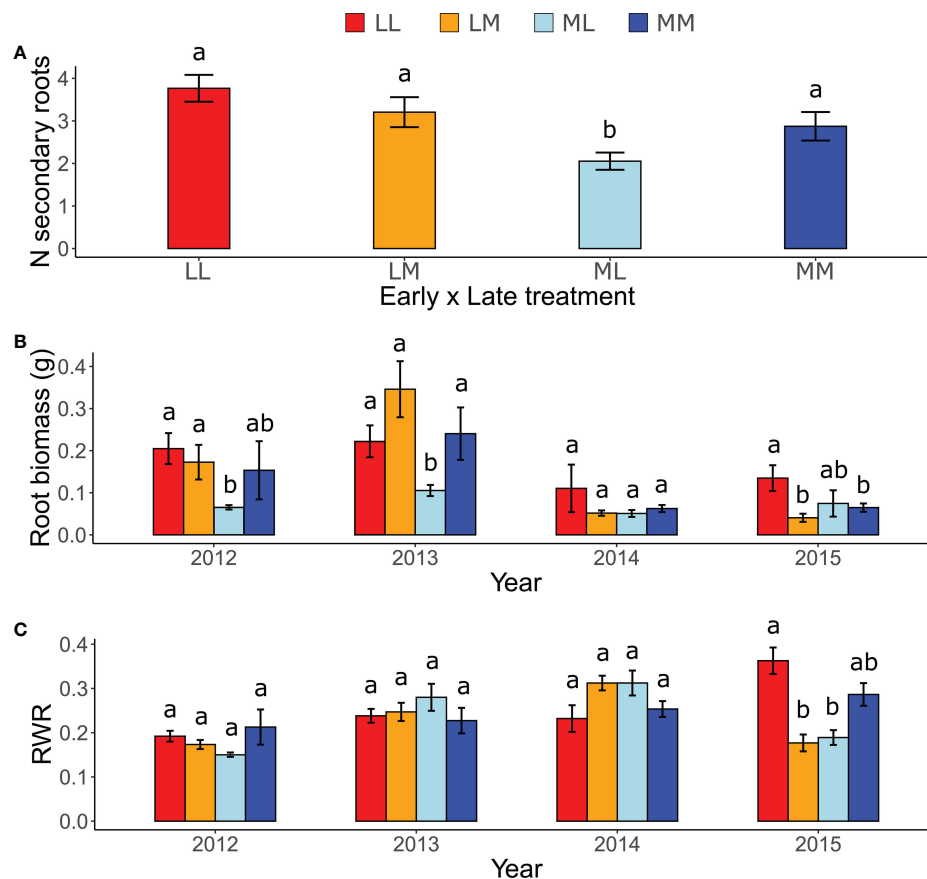


FIGURE 2

Effects of inter-seasonal predictability on the number of secondary roots, root biomass and root weight ratio (RWR) of ancestors. Interaction between: (A) Early treatment  $\times$  Late treatment on the number (N) of secondary roots; (B) Early treatment, Late treatment, and Year on root biomass; and (C) Early treatment, Late treatment, and Year on RWR. Means  $\pm$  SE are shown in the bar plots. Significant *post-hoc* contrasts among treatment combinations (and among treatments within the same year in B and C) are indicated with letters.

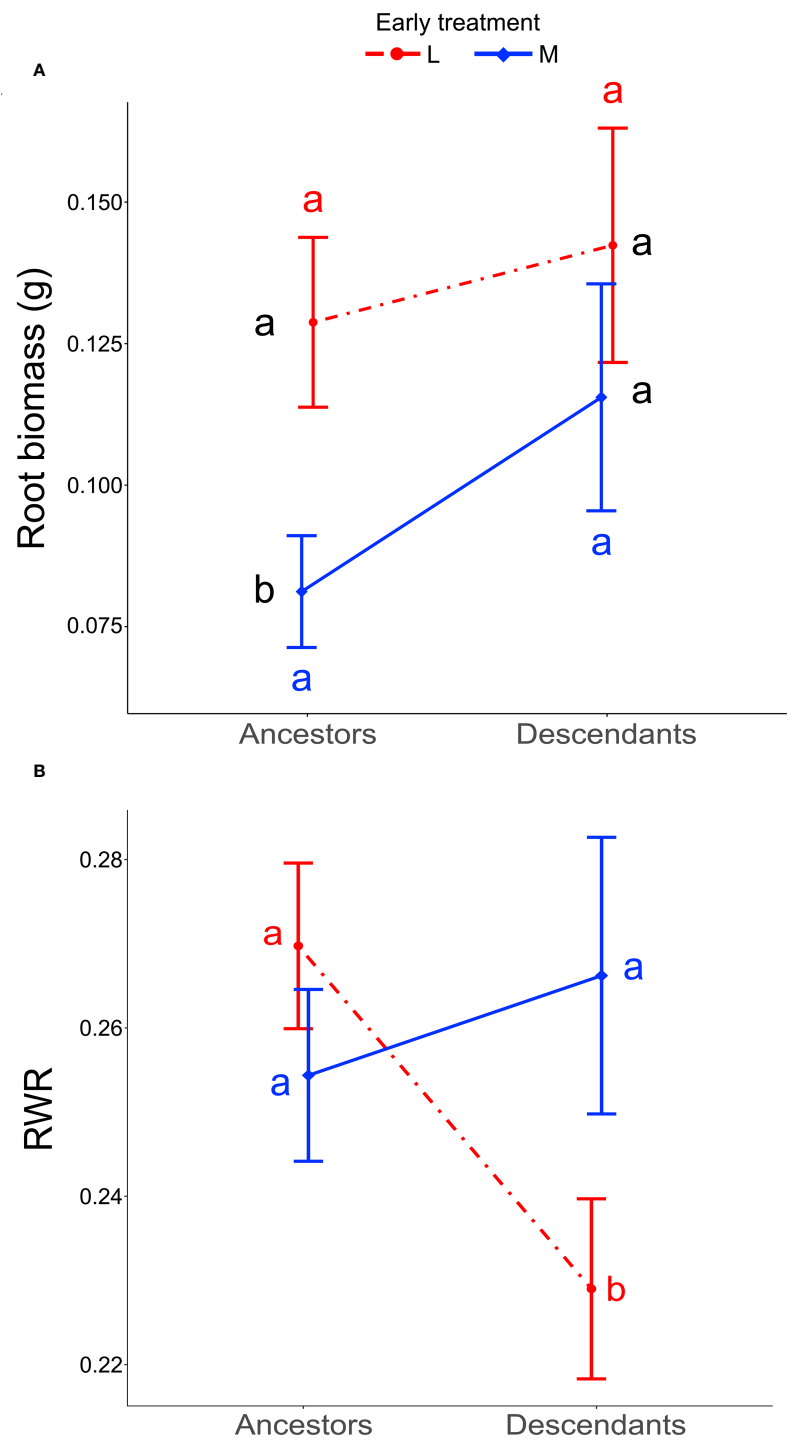
( $0.613 \pm 0.039$ ,  $P < 0.001$ ) and relative root branching ( $-0.016 \pm 0.001$ ,  $P < 0.001$ ). No significant differences in selection gradients existed among treatments (*i.e.* no significant interactions with treatment:  $\chi_1^2 \leq 3.21$ ,  $P \geq 0.08$ ) in any of the root traits. Selection gradients for the number of secondary roots showed that performance, in terms of total biomass, increased with increasing number of secondary roots (Figure S3A). Performance decreased to 2.6 g (in the transformed data) and then increased again with increasing maximum rooting depth. The relationship between performance and RWR was convex and showed that performance was highest at a RWR of 3.9 (in the transformed data; Figure S4A). The relationship between performance and relative root branching was linear, and performance declined with increasing relative root branching (Figure S4B). The relationship between performance and relative rooting depth was concave and the higher the investment into rooting depth the smaller was the decline in performance (Figure S4C). Additionally, selection gradients for the different root

traits did not significantly differ among ancestors and descendants (all:  $\chi_1^2 \leq 3.81$ ,  $P \geq 0.05$ ). So, overall, root traits were generally under selection, and the predictability treatments did neither affect the strength nor the direction of selection.

## Transgenerational effect mediated by precipitation predictability

When testing for trait differences between ancestors and descendants subjected to high inter-seasonal predictability (only possible from 2013 to 2015), we found a significant two-way interaction between early treatment and generation (*i.e.*, ancestor vs. descendant) for root biomass ( $\chi_1^2 = 5.10$ ,  $P = 0.024$ ) and for RWR ( $\chi_1^2 = 4.86$ ,  $P = 0.027$ ). Root biomass of ancestors was significantly larger in the less compared to the more predictable early treatment (*post-hoc* contrast:  $P = 0.012$ ), but no significant differences existed between early treatment





**FIGURE 3**  
Differences between ancestors and descendants in root biomass and RWR. Shown are means  $\pm$  SE of the two significant two-way interactions: Early treatment  $\times$  Generation (ancestors *versus* descendants) on root biomass (A) and RWR (B). Black letters represent *post-hoc* contrasts between both treatments within ancestors or within descendants. Red and blue letters represent *post-hoc* contrasts between ancestors and descendants in the less and in the more predictable treatment, respectively.

levels in descendants in all three years (contrast:  $P = 0.4$ ; Figure 3A). There were no significant differences between ancestors and descendants in number of secondary roots and maximum rooting depth (main effect and all interactions including generation and treatment:  $P \geq 0.05$ ). RWR of descendants was significantly lower than RWR of ancestors in the less predictable treatment ( $P = 0.022$ ), whereas no differences existed between ancestors and descendants in the more predictable treatment ( $P = 0.22$ ; Figure 3B). The significant difference in the less predictable early treatment led to a 13.42% increase in performance of descendants compared to ancestors (Figure 4). There were neither significant differences between ancestors and descendants in relative root branching and relative rooting depth nor for the interaction of generation with the late treatment in root biomass and RWR (main effect and all interactions including generation and treatment:  $P \geq 0.1$ ). So, overall, early predictability treatment induced changes between ancestors and descendants in root biomass and RWR but not in other root traits, which maintained or increased performance in descendants compared to in ancestors.

When testing for transgenerational plasticity to the different precipitation predictability treatments, we observed that the interaction between maternal treatment and descendants treatment was significant for maximum rooting depth and root biomass but not for the other root variables (Table 2 and Figure 5). In both traits, there were no significant contrasts among descendant treatments within the same maternal

treatment, but the results show that the effect of the descendants' treatment depended on the maternal treatment. Descendants' treatments significantly affected relative root branching (*i.e.*, independent of maternal treatment) and marginally affected the number of secondary roots and maximum rooting depth, but descendants' treatments did not affect the other traits (Table 2). *Post-hoc* contrasts revealed that relative root branching was higher in ML than in the other descendant treatment combinations ( $P = 0.044$ ; Figure 5E). Moreover, the lowest coefficients of variation ( $CV_m$ ) among treatments of descendants occurred in the maternal LL treatment in all measured root traits except for relative rooting depth, where the lowest  $CV_m$  occurred in the maternal MM treatment (Table S1). The greatest  $CV_m$  occurred in the maternal MM treatment for number of secondary roots and relative root branching, in the maternal ML treatment for root biomass and RWR, and in the maternal LM treatment for maximum rooting length and relative rooting depth (Table S1). So, overall, transgenerational plasticity existed in maximum rooting depth and root biomass, and the root response of descendants was not greater under the same (compared to different) maternal predictability treatment. Also, the coefficients of variation showed that generally the lowest plasticity is found in the maternal LL treatment. In addition, CVs of the descendants were greater than the CVs of the ancestors in all root traits except for number of secondary roots, suggesting greater plasticity in descendants than in ancestors.

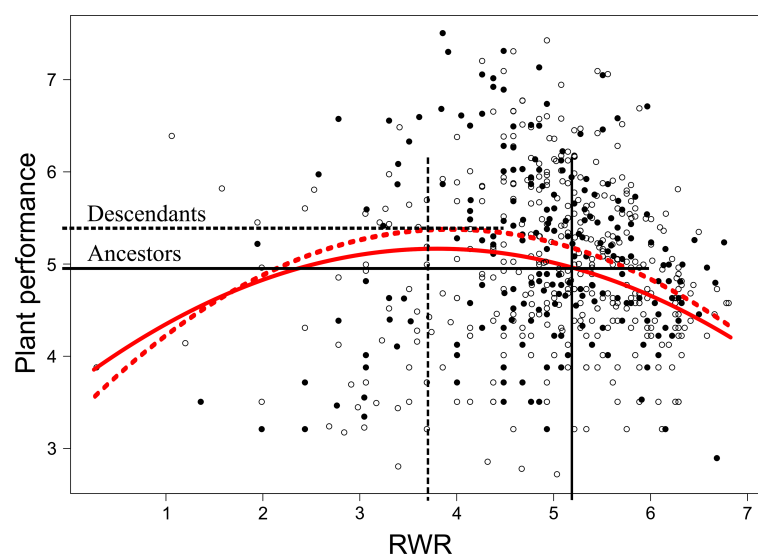


FIGURE 4

Non-linear selection gradients on root weight ratio (RWR) for ancestors and descendants in the less predictable early treatment. Solid and dashed red lines represent ancestors and descendants selection gradients, respectively. White and black dots represent data of ancestors and descendants, respectively. Average RWR of ancestors and descendants exposed to the less predictable early treatment (see Figure 3B) are indicated with vertical black lines and differences in plant performance between ancestors and descendants are indicated with horizontal black lines.

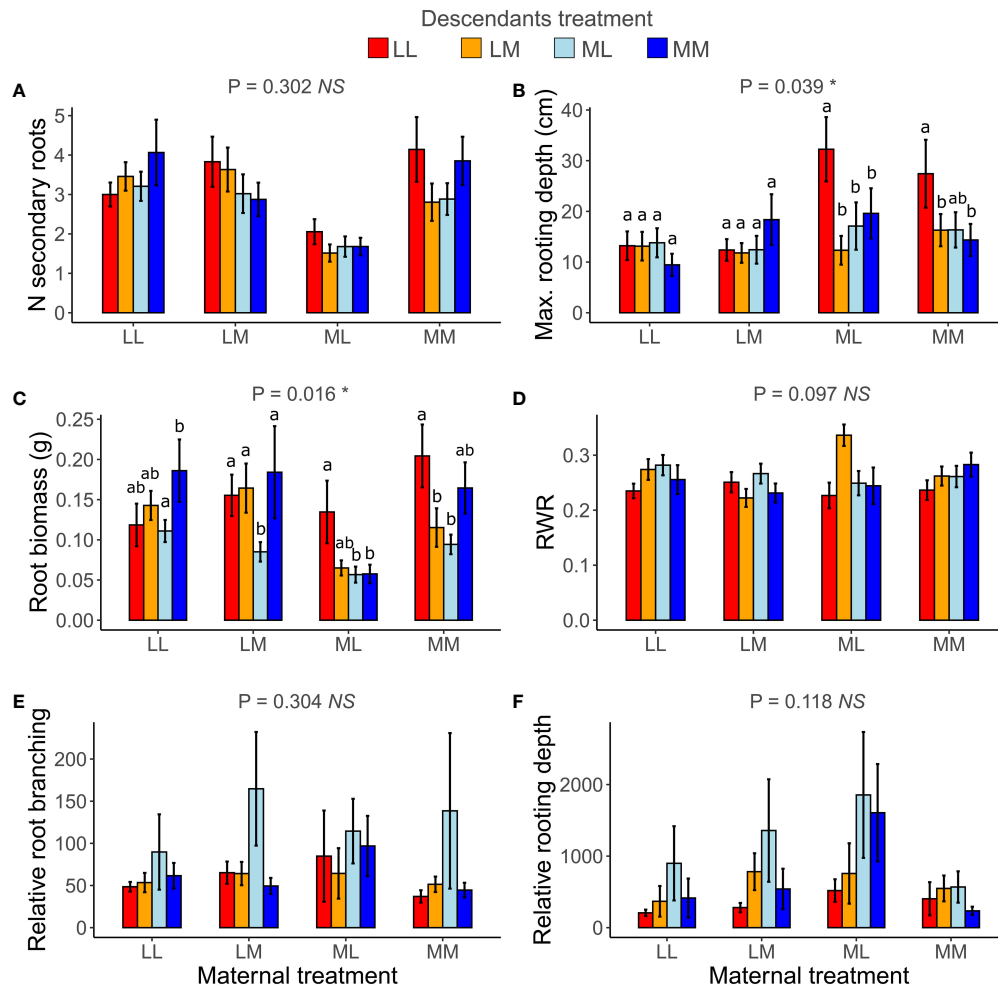


FIGURE 5

Transgenerational plasticity of roots in response to precipitation predictability treatments. Maternal treatment x Descendants treatment interaction in number (N) of secondary roots (A), maximum (max.) rooting depth (B), root biomass (C), root weight ratio (RWR) (D), relative root branching (E), and relative rooting depth (F). Means  $\pm$  SE are shown in the bar plots. Significance of the Maternal treatment x Descendants treatment interaction is shown above each graph. Significant post-hoc contrasts among treatment combinations from the same maternal treatment are indicated with letters.

## Discussion

Our multi-generation experiment demonstrates that precipitation predictability elicits intra- and trans-generational plasticity in root traits, and we observed differential root trait responses between ancestors and descendants of *Papaver rhoeas*. In ancestors, the magnitude of intra-generation plasticity most often depended on the year, and roots responded to lower predictability to increase water absorbance from shallow soil layers (*i.e.*, with greater number of secondary roots). Under high inter-generational predictability, ancestors and descendants exhibited differential root allocation strategies. In the less predictable early treatment, the relative investment into roots (Root weight ratio: RWR) was smaller in descendants compared

to ancestors, reflecting a strategy that descendants used to increase their performance. These results are in line with studies suggesting that *P. rhoeas* can increase its average fitness and competitive ability during drought (e.g. Pérez-Ramos et al., 2019) and with a meta-analysis showing that transgenerational effects should be favoured in fast-growing short-lived organisms such as annual plants (Yin et al., 2019). The results also point to transgenerational plasticity, given that maternal predictability influenced the descendants' plasticity in root traits. Moreover, transgenerational plasticity in root traits was lower when the maternal environment was less predictable, in line with theory and experiments with other systems (McNamara et al., 2016; Yin et al., 2019; Leung et al., 2020; Rescan et al., 2020). Our study thus provides experimental

evidence for plastic adaptation in root traits in response to changes in precipitation predictability.

## Effects of predictability treatments on intra-generation root plasticity

The number of secondary roots, root biomass and root allocation strategies of ancestors were mediated by changes in precipitation predictability, revealing intra-generational plasticity. Treatment differences were absent in the maximum rooting depth and manifested only in a single year in the RWR. The number of secondary roots was lower in ML compared to the other treatment combinations, and LL exhibited, on average, the highest number (Figure 2A). These results may indicate a fast-acquisitive strategy to avoid drought by increasing water absorbance from shallow soil layers (Hickman, 1975; Koevoets et al., 2016; Weemstra et al., 2016). It also suggests that plants may adopt a less acquisitive strategy under more predictable water availability (Prieto et al., 2015; Weemstra et al., 2016). In line with our first hypothesis (H1), despite variation among years, root biomass was generally higher in LL conditions than in other treatment combinations (but not significantly higher in all years and between all treatments; see Figure 2B). Moreover, RWR was significantly higher in LL in 2015 (but no treatment differences existed in other years; Figure 2C), and relative rooting depth was significantly higher in the less predictable late treatment. These responses support the functional equilibrium hypothesis (Brouwer, 1983), which states that plants should allocate more to absorptive organs when resources are temporally scarce. Contrary, relative root branching was greater in the more predictable early treatment, which is in line with studies suggesting that more constant water availability favors increased root branching, *i.e.*, increased root density (e.g. Wu et al., 2016).

The observed plastic changes in roots with respect to predictability treatments are in agreement with the high plasticity found in root traits under environmental change (Hodge, 2004; Bardgett et al., 2014), but other experiments manipulating the amount of precipitation found little plasticity in root traits of herbaceous plants (e.g. Zhou et al., 2019). These differences in root plasticity between studies may be due to species-specific responses or due to differences in the applied treatment. For instance, previous studies have tested the effect of drought events on the root system, whereas we assessed root plasticity with regard to precipitation predictability while keeping the amount of obtained water constant among treatments.

## Selection acting on root traits

In line with the second hypothesis (H2), significant selection was acting on all measured root traits, showing that the

measured root traits are functionally relevant (since they affect fitness; Violle et al., 2007). The number of secondary roots and relative root branching were under directional selection, whereas maximum rooting depth and relative rooting depth were under disruptive and RWR under stabilizing selection. Predictability treatments did not differently affect the strength or direction of selection acting on the measured traits. This implies that *P. rhoeas* may adopt the same strategies to increase performance under different degrees of intra- and inter-seasonal precipitation predictability.

Selection analyses also allow to understand which strategy optimizes performance, and they can reveal whether treatment-induced differences in trait averages led to an increase or decrease (*i.e.*, a constraint) in plant performance (Chevin, 2013). In ancestors, relative rooting depth was significantly higher in the less predictable late treatment while maximum rooting depth did not differ among treatments. The selection gradients showed that plant performance decreased with increasing relative rooting depth (Figure S4C) and that higher relative rooting depth under lower predictability only marginally decreased performance. *Papaver rhoeas* exposed to less predictable late treatment thus invested relatively more into rooting depth, but according to the selection gradients, this change in investment did not induce substantial performance benefits or losses. Moreover, ancestors of *P. rhoeas* reduced their relative root branching in the less predictable early treatment and the number of secondary roots was significantly bigger in LL and LM compared to ML (Figure 2A). According to the selection gradients, lower relative root branching and higher number of secondary roots resulted in higher performance. These findings indicate that, when conditions are less predictable, plants adjust their allocation to roots to maintain or increase their performance, revealing the functional role of roots to cope with different degrees of environmental predictability.

## Transgenerational effects mediated by precipitation predictability

Precipitation predictability elicited transgenerational effects in root biomass and RWR. In ancestors, root biomass was significantly higher in the less predictable early treatment compared to the more predictable early treatment, and in descendants no significant treatment differences existed (Figure 3A), pointing to transgenerational treatment-induced changes that minimized differences detected in ancestors. On average, root biomass was greater in descendants compared to ancestors under both less and more predictable early treatments. However, in line with our third hypothesis (H3), under more predictable precipitation, the average increase in root biomass from ancestors to descendants was greater than under less predictable conditions, suggesting that more predictable conditions allow for a stronger transgenerational response (McNamara et al., 2016; Dong

et al., 2018). This suggests that transgenerational effects with respect to environmental predictability may balance effects on fitness over the course of generations (Donelson et al., 2018; Yin et al., 2019). Moreover, this indicates that high inter-generational predictability promotes gradual changes and may facilitate the evolution of transgenerational responses (Burgess and Marshall, 2014; McNamara et al., 2016; Yin et al., 2022). Under less predictable early treatment, descendants exhibited lower RWR than ancestors, whereas in more predictable early treatment no differences existed between ancestors and descendants (Figure 3B). The reduced RWR in descendants under less predictable conditions led to a 13.42% increase in performance (Figure 4), which points to an adaptive transgenerational effect (Herman and Sultan, 2011). This confirms that, in presence of natural environmental heterogeneity, intermediate environmental predictability, and in absence of extreme events, adaptive transgenerational effects may likely to occur (Burgess and Marshall, 2014; Uller et al., 2015; Yin et al., 2022).

The absence of treatment effects during the late growth phase and the absence of inter-seasonal predictability effects on the transgenerational response suggests that transgenerational effects principally occurred with respect to early growth conditions (when plants are most sensitive to environmental changes; Burton and Metcalfe, 2014). This is in line with results on phenological and fitness traits from previous experiments (March-Salas et al., 2019) and with a meta-analysis showing strongest transgenerational effects when environmental cues affect the juvenile phase (Yin et al., 2019). Moreover, there was no evidence for transgenerational changes in the investment in root branching and in root length, which suggests that plasticity in root traits might be conserved to successfully cope with differences in the predictability of precipitation.

Our experiment shows that precipitation predictability can drive transgenerational plasticity in root traits. All maternal predictability treatments promoted different root phenotypes among the descendants' predictability treatments (Figure 5), and descendants generally showed a greater plasticity than ancestors (Table S1). In all measured root traits except for relative rooting depth (Table S1) the maternal LL treatment promoted a lower coefficient of variation in descendants compared to the other maternal treatments involving more predictable precipitation (i.e., MM, ML, LM). This suggests that lower phenotypic plasticity evolves in less predictable environments, supporting previous findings in other systems (Leung et al., 2020; Rescan et al., 2020). This is also in line with simulations (McNamara et al., 2016), with experiments measuring reproductive traits of *Arabidopsis thaliana* (Yin et al., 2022) and with our own hypothesis (H4), suggesting that higher predictability generally favours transgenerational plasticity. In addition, descendants generally reduced their root response when less predictable conditions persisted over generations. This shows that plants can change their strategy if they grow under LL over consecutive generations. This is in line with the lower RWR in descendants compared to ancestors when conditions are less predictable. Our

results also indicate that changes in plant strategies over generations may be favoured by the ancestors' environment, in order to increase overall plant performance in the following generations (McIntyre and Strauss, 2014), and that adaptive transgenerational responses also occur under less predictable conditions (Donelson et al., 2018; Yin et al., 2019). However, a common-garden experiment will unravel whether root responses may be adaptive or merely plastic. Moreover, the direction and strength of transgenerational effect may vary across taxa, traits and developmental stages (Yin et al., 2019), or in presence of extreme events (Beier et al., 2012; Uller et al., 2015). Therefore, using model species and comparing subtle *versus* extreme changes in precipitation predictability may show more general and robust responses.

## Conclusion

Empirical evidence for evolutionary changes in response to differences in predictability is rare, especially in root traits. Our multi-generation experiment demonstrates that roots respond highly plastically to different degrees of precipitation predictability, that they are under strong selection pressure, and that transgenerational effects can enhance performance of descendants depending on the root trait and the predictability of environmental conditions. Lower predictability can enhance root responses and does not implicitly hinder a plant's performance, since *P. rhoeas* altered its strategies involving root traits to maintain or even increase performance when reduced predictability persisted over generations. This points to adaptive transgenerational plasticity. However, a common-garden experiment growing offspring from all treatments under common conditions should be performed to confirm these findings. Overall, our findings show that even subtle changes in predictability elicit intra- and trans-generational plastic responses in root traits, highlighting the importance of environmental predictability as an evolutionary driver of transgenerational responses in plant populations.

## Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

## Author contributions

PF and MM-S designed the study with inputs from MK. MM-S implemented the study, measured the different traits and collected the samples with inputs from PF. MM-S analysed the data and wrote the manuscript, with PF, MK and JS contributing to revisions. All authors contributed to the article and approved the submitted version.



## Funding

Funding was provided by the Spanish Ministry of Economy and Competitiveness (CGL2012-32459, CGL2016-76918 to PSF) and the Swiss National Science Foundation (PPOOP3\_128375, PP00P3\_152929/1 to PSF). MM-S was supported by a PhD grant (BES-2013-062910) financed by the Spanish Ministry of Economy and Competitiveness. Publication costs were covered by Goethe University Frankfurt.

## Acknowledgments

We thank Guillermo Mercé, Blanca Santamaría, Diana Íñigo and Miguel Moreno for practical assistance, and María Urieta and Helena Clavero for technical assistance. We also thank Federico Fillat, Luis Villar, Paloma Mas and Guillem Masó for their scientific support.

## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

## Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

## Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fpls.2022.998169/full#supplementary-material>

## References

- Ambastha, V., Friedmann, Y., and Leshem, Y. (2020). Laterals take it better—emerging and young lateral roots survive lethal salinity longer than the primary root in *arabidopsis*. *Sci. Rep.* 10 (1), 1–11. doi: 10.1038/s41598-020-60163-7
- Bardgett, R. D., Mommer, L., and De Vries, F. T. (2014). Going underground: Root traits as drivers of ecosystem processes. *Trends Ecol. Evol.* 29, 692–699. doi: 10.1016/j.tree.2014.10.006
- Bates, D., Mächler, M., Bolker, B. M., and Walker, S. C. (2015). Fitting linear mixed-effects models using lme4. *J. Stat. Software* 67, 1–48. doi: 10.18637/jss.v067.i01
- Beier, C., Beierkuhnlein, C., Wohlgemuth, T., Penuelas, J., Emmett, B., Körner, C., et al. (2012). Precipitation manipulation experiments—challenges and recommendations for the future. *Ecol. Lett.* 15 (8), 899–911. doi: 10.1111/j.1461-0248.2012.01793.x
- Botero, C. A., Weissing, F. J., Wright, J., and Rubenstein, D. R. (2015). Evolutionary tipping points in the capacity to adapt to environmental change. *Proc. Natl. Acad. Sci.* 112, 184–189. doi: 10.1073/pnas.1408589111
- Brouwer, R. (1983). Functional equilibrium: Sense or nonsense? *Neth. J. Agric. Sci.* 31 (4), 335–348. doi: 10.18174/njas.v31i4.16938

### SUPPLEMENTARY FIGURE 1

Root diversity in example individuals of *Papaver rhoeas* from the experiment. From left to right: roots with decreasing numbers of secondary roots. The scale bar represents 50 mm.

### SUPPLEMENTARY FIGURE 2

Temperature, potential evapotranspiration and precipitation at the study site. (A) Average daily temperature per month for each of the four experimental years. Colors and dot symbols correspond to the different experimental years and dotted lines to second order polynomial regressions. (B) Average potential evapotranspiration (PET) per month at the field site (Atlas Climático Digital de Aragón). The dotted line corresponds to a second order polynomial regression. (C) Difference between monthly precipitation (P) and potential evapotranspiration (PET) at the field site (red dots and red dotted line) and including the irrigated amount of water (yellow dots and yellow dotted line). Dotted lines correspond to second order polynomial regressions.

### SUPPLEMENTARY FIGURE 3

Selection acting on root traits of ancestors. Model predictions of selection gradients are shown for number of secondary roots (A) and maximum rooting depth (B). Since no significant interactions with treatments existed (see 'Results'), only significant linear (A) and quadratic (B) predictions are shown.

### SUPPLEMENTARY FIGURE 4

Selection acting on root traits indicating root allocation strategies of ancestors. Selection gradients are shown for root weight ratio (RWR) (A), relative root branching (B), and relative rooting depth (C). Since no significant interactions with treatment existed (see 'Results'), model predictions of significant quadratic (A, C) and linear (B) relationships are shown.

### SUPPLEMENTARY TABLE 1

Means and coefficients of variation of measured root traits depending on maternal predictability treatments. The means of all root traits are shown for each of the descendant treatments depending maternal treatment, and also for each descendant treatment independent of the maternal treatment. The coefficient of variation (the ratio of the standard deviation to the mean, based on means,  $CV_m$ ) among treatments in descendants for each maternal treatment is also shown as well as the overall CV of ancestors ( $CV_a$ ) and the overall CV of descendants ( $CV_d$ ).

### SUPPLEMENTARY TABLE 2

Sample size per treatment, year and generation. The sample size per treatment and year is presented for the ancestral plants, and the sample size per treatment and generation is presented for the descendants that were subjected to the same treatment for four generations (referred to as 'descendants – pure lines') and for the descendants from all treatment combinations over generations used for the analysis on transgenerational plasticity. The hypothesis (H) tested for each group of data is shown.

- Burgess, S. C., and Marshall, D. J. (2014). Adaptive parental effects: the importance of estimating environmental predictability and offspring fitness appropriately. *Oikos* 123 (7), 769–776. doi: 10.1111/oik.01235
- Burton, T., and Metcalfe, N. B. (2014). Can environmental conditions experienced in early life influence future generations? *Proc. R. Soc. B.* 281 (1785), 20140311. doi: 10.1098/rspb.2014.0311
- Caro, S. M., Griffin, A. S., Hinde, C. A., and West, S. A. (2016). Unpredictable environments lead to the evolution of parental neglect in birds. *Nat. Commun.* 7 (1), 1–10. doi: 10.1038/ncomms10985
- Chevin, L. M. (2013). Genetic constraints on adaptation to a changing environment. *Evol.* 67, 708–721. doi: 10.1111/j.1558-5646.2012.01809.x
- Colicchio, J. M., and Herman, J. (2020). Empirical patterns of environmental variation favor adaptive transgenerational plasticity. *Ecol. Evol.* 10 (3), 1648–1665. doi: 10.1002/ece3.6022
- DeWitt, T. J., Sih, A., and Wilson, D. S. (1998). Costs and limits of phenotypic plasticity. *Trends Ecol. Evol.* 13 (2), 77–81. doi: 10.1016/S0169-5347(97)01274-3
- Donelson, J. M., Salinas, S., Munday, P. L., and Shama, L. N. (2018). Transgenerational plasticity and climate change experiments: where do we go from here? *Glob. Change Biol.* 24 (1), 13–34. doi: 10.1111/gcb.13903
- Dong, B. C., Van Kleunen, M., and Yu, F. H. (2018). Context-dependent parental effects on clonal offspring performance. *Front. Plant Sci.* 9, 1824. doi: 10.3389/fpls.2018.01824
- Franch-Gras, L., Garcia-Roger, E. M., Serra, M., and Carmona, M. J. (2017). Adaptation in response to environmental unpredictability. *Proc. R. Soc. B.* 284 (1868), 20170427. doi: 10.1098/rspb.2017.0427
- Franklin-Tong, V. E., and Franklin, F. C. H. (1992). Gametophytic self-incompatibility in papaver rhoeas L. *Sex Plant Reprod.* 5 (1), 1–7. doi: 10.1007/BF00714552
- Gallego-Tévar, B., Hidalgo-Galvez, M. D., Cambrollé, J., Martínez-Muñoz, M., Villar-Godoy, A., and Pérez-Ramos, I. M. (2022). Transgenerational responses to climate change in Mediterranean annual species with contrasting functional strategies. *Environ. Exp. Bot.* 196, 104817. doi: 10.1016/j.envexpbot.2022.104817
- Hermans, C., Hammond, J. P., White, P. J., and Verbruggen, N. (2006). How do plants respond to nutrient shortage by biomass allocation? *Trends Plant Sci.* 11, 610–617. doi: 10.1016/j.tplants.2006.10.007
- Herman, J. J., and Sultan, S. E. (2011). Adaptive transgenerational plasticity in plants: case studies, mechanisms, and implications for natural populations. *Front. Plant Sci.* 2, 102. doi: 10.3389/fpls.2011.00102
- Hickman, J. C. (1975). Environmental unpredictability and plastic energy allocation strategies in the annual polygonum cascadenae (Polygonaceae). *J. Ecol.* 63, 689–701. doi: 10.2307/2258745
- Hodge, A. (2004). The plastic plant: root responses to heterogeneous supplies of nutrients. *New Phytol.* 162, 9–24. doi: 10.1111/j.1469-8137.2004.01015.x
- IPCC (2021) *Assessment report 6 climate change 2021: The physical science basis*. Available at: <https://www.ipcc.ch/report/ar6/wg1/>.
- Koevoets, I. T., Venema, J. H., Elzenga, J. T. M., and Testerink, C. (2016). Roots withstanding their environment: exploiting root system architecture responses to abiotic stress to improve crop tolerance. *Front. Plant Sci.* 7, 1–19. doi: 10.3389/fpls.2016.01335
- Lande, R. (2009). Adaptation to an extraordinary environment by evolution of phenotypic plasticity and genetic assimilation. *J. Evol. Biol.* 22 (7), 1435–1446. doi: 10.1111/j.1420-9101.2009.01754.x
- Lande, R., and Arnold, S. J. (1983). The measurement of selection on correlated characters. *Evol.* 37, 1210–1226. doi: 10.1111/j.1558-5646.1983.tb00236.x
- Lenth, R. V. (2016). Least-squares means: The {R} package lsmeans. *J. Stat. Software* 69, 1–33. doi: 10.18637/jss.v069.i01
- Leung, C., Rescan, M., Grulois, D., and Chevin, L. M. (2020). Reduced phenotypic plasticity evolves in less predictable environments. *Ecol. Lett.* 23 (11), 1664–1672. doi: 10.1111/ele.13598
- Lundgren, M. R., and Des Marais, D. L. (2020). Life history variation as a model for understanding trade-offs in plant–environment interactions. *Curr. Biol.* 30 (4), R180–R189. doi: 10.1016/j.cub.2020.01.003
- Manenti, T., Loeschcke, V., Moghadam, N. N., and Sørensen, J. G. (2015). Phenotypic plasticity is not affected by experimental evolution in constant, predictable or unpredictable fluctuating thermal environments. *J. Evol. Biol.* 28 (11), 2078–2087. doi: 10.1111/jeb.12735
- March-Salas, M., Fandos, G., and Fitze, P. S. (2021a). Effects of intrinsic environmental predictability on intra-individual and intra-population variability of plant reproductive traits and eco-evolutionary consequences. *Ann. Bot.* 127 (4), 413–423. doi: 10.1093/aob/mcaa096
- March-Salas, M., and Fitze, P. S. (2019). A multi-year experiment shows that lower precipitation predictability encourages plants' early life stages and enhances population viability. *PeerJ* 7, e6443. doi: 10.7717/peerj.6443
- March-Salas, M., van Kleunen, M., and Fitze, P. S. (2019). Rapid and positive responses of plants to lower precipitation predictability. *Proc. R. Soc. B.* 286 (1913), 20191486. doi: 10.1098/rspb.2019.1486
- March-Salas, M., van Kleunen, M., and Fitze, P. S. (2021b). Effects of intrinsic precipitation-predictability on root traits, allocation strategies and the selective regimes acting on them. *Oikos* 2022(1). doi: 10.1111/oik.07970
- McIntyre, P. J., and Strauss, S. Y. (2014). Phenotypic and transgenerational plasticity promote local adaptation to sun and shade environments. *Evol. Ecol.* 28 (2), 229–246. doi: 10.1007/s10682-013-9670-y
- McNamara, J. M., Dall, S. R., Hammerstein, P., and Leimar, O. (2016). Detection vs. selection: integration of genetic, epigenetic and environmental cues in fluctuating environments. *Ecol. Lett.* 19 (10), 1267–1276. doi: 10.1111/ele.12663
- McNaughton, I. H., and Harper, J. L. (1964). Papaver I. *J. Ecol.* 52 (3), 767–793. doi: 10.2307/2257860
- Pennekamp, F., Iles, A. C., Garland, J., Brennan, G., Brose, U., Gaedke, U., et al. (2019). The intrinsic predictability of ecological time series and its potential to guide forecasting. *Ecol. Monogr.* 89 (2), e01359. doi: 10.1002/ecm.1359
- Pérez-Ramos, I. M., Matías, L., Gómez-Aparicio, L., and Godoy, Ó. (2019). Functional traits and phenotypic plasticity modulate species coexistence across contrasting climatic conditions. *Nat. Commun.* 10 (1), 1–11. doi: 10.1038/s41467-019-10453-0
- Prieto, I., Roumet, C., Cardinale, R., Dupraz, C., Jourdan, C., Kim, J. H., et al. (2015). Root functional parameters along a land-use gradient: evidence of a community-level economics spectrum. *J. Ecol.* 103 (2), 361–373. doi: 10.1111/1365-2745.12351
- R Development Core Team (2020). *R: A language and environment for statistical computing* (Vienna, Austria). Available at: <https://www.R-project.org/>.
- Rescan, M., Grulois, D., Ortega-Aboud, E., and Chevin, L. M. (2020). Phenotypic memory drives population growth and extinction risk in a noisy environment. *Nat. Ecol. Evol.* 4 (2), 193–201. doi: 10.1038/s41559-019-1089-6
- Schlichting, C. D. (1986). The evolution of phenotypic plasticity in plants. *Annu. Rev. Ecol. Syst.* 17, 667–693. doi: 10.1146/annurev.es.17.110186.003315
- Shipley, B., De Bello, F., Cornelissen, J. H. C., Laliberté, E., Laughlin, D. C., and Reich, P. B. (2016). Reinforcing loose foundation stones in trait-based plant ecology. *Oecologia* 180 (4), 923–931. doi: 10.1007/s00442-016-3549-x
- Sobral, M., Sampedro, L., Neylan, I., Siemens, D., and Dirzo, R. (2021). Phenotypic plasticity in plant defense across life stages: inducibility, transgenerational induction, and transgenerational priming in wild radish. *Proc. Natl. Acad. Sci.* 118 (33), e2005865118. doi: 10.1073/pnas.2005865118
- Strutz, T. (2016). *Data fitting and uncertainty: A practical introduction to weighted least squares and beyond. 2nd edition* (Wiesbaden: Springer Vieweg).
- Tonkin, J. D., Bogan, M. T., Bonada, N., Rios-Touma, B., and Lytle, D. A. (2017). Seasonality and predictability shape temporal species diversity. *Ecology* 98 (5), 1201–1216. doi: 10.1002/ecy.1761
- Tufto, J. (2015). Genetic evolution, plasticity, and bet-hedging as adaptive responses to temporally autocorrelated fluctuating selection: A quantitative genetic model. *Evol.* 69 (8), 2034–2049. doi: 10.1111/evo.12716
- Uller, T., English, S., and Pen, I. (2015). When is incomplete epigenetic resetting in germ cells favoured by natural selection? *Proc. R. Soc. B.* 282 (1811), 20150682. doi: 10.1098/rspb.2015.0682
- Valladares, F., Martínez-Ferri, E., Balaguer, L., Pérez-Corona, E., and Manrique, E. (2000). Low leaf-level response to light and nutrients in Mediterranean evergreen oaks: a conservative resource-use strategy? *New Phyt.* 148 (1), 79–91. doi: 10.1046/j.1469-8137.2000.00737.x
- Violle, C., Navas, M. L., Vile, D., Kazakou, E., Fortunel, C., Hummel, I., et al. (2007). Let the concept of trait be functional! *Oikos* 116 (5), 882–892. doi: 10.1111/j.0030-1299.2007.15559.x
- Weemstra, M., Mommer, L., Visser, E. J. W., Van Ruijven, J., Kuyper, T. W., Mohren, G. M. J., et al. (2016). Towards a multidimensional root trait framework: A tree root review. *New Phytol.* 211, 1159–1169. doi: 10.1111/nph.14003
- Wu, Q., Pagès, L., and Wu, J. (2016). Relationships between root diameter, root length and root branching along lateral roots in adult, field-grown maize. *Ann. Bot.* 117 (3), 379–390. doi: 10.1093/aob/mcv185
- Xu, L., Zhang, C., Chen, N., Moradkhani, H., Chu, P.-S., and Zhang, X. (2020). Potential precipitation predictability decreases under future warming. *Geophys. Res. Lett.* 47, e2020GL090798. doi: 10.1029/2020GL090798
- Yamauchi, T., Pedersen, O., Nakazono, M., and Tsutsumi, N. (2021). Key root traits of poaceae for adaptation to soil water gradients. *New Phytol.* 229 (6), 3133–3140. doi: 10.1111/nph.17093

Yin, J., Lin, X., Yao, J., Li, Q. Q., and Zhang, Y. Y. (2022). Genotypic variation of transgenerational plasticity can be explained by environmental predictability at origins. *Oikos* 2022 (5), e09006. doi: 10.1111/oik.09006

Yin, J., Zhou, M., Lin, Z., Li, Q. Q., and Zhang, Y. Y. (2019). Transgenerational effects benefit offspring across diverse environments: A meta-analysis in plants and animals. *Ecol. Lett.* 22 (11), 1976–1986. doi: 10.1111/ele.13373

Younginger, B. S., Sirová, D., Cruzan, M. B., and Ballhorn, D. J. (2017). Is biomass a reliable estimate of plant fitness? *Appl. Plant Sci.* 5 (2), 1600094. doi: 10.3732/apps.1600094

Zhou, M., Wang, J., Bai, W., Zhang, Y., and Zhang, W. H. (2019). The response of root traits to precipitation change of herbaceous species in temperate steppes. *Funct. Ecol.* 33 (10), 2030–2041. doi: 10.1111/1365-2435.13420



## OPEN ACCESS

EDITED BY  
Ivika Ostonen,  
University of Tartu, Estonia

REVIEWED BY  
Tianshan Zha,  
Beijing Forestry University, China  
Mattia Terzaghi,  
University of Bari Aldo Moro, Italy

\*CORRESPONDENCE  
Rodica Pena  
r.pena@reading.ac.uk

SPECIALTY SECTION  
This article was submitted to  
Functional Plant Ecology,  
a section of the journal  
Frontiers in Plant Science

RECEIVED 05 July 2022  
ACCEPTED 26 October 2022  
PUBLISHED 16 November 2022

CITATION  
Song B, Razavi BS and Pena R (2022)  
Contrasting distribution of enzyme  
activities in the rhizosphere of  
European beech and Norway spruce.  
*Front. Plant Sci.* 13:987112.  
doi: 10.3389/fpls.2022.987112

COPYRIGHT  
© 2022 Song, Razavi and Pena. This is  
an open-access article distributed under  
the terms of the [Creative Commons  
Attribution License \(CC BY\)](#). The use,  
distribution or reproduction in other  
forums is permitted, provided the  
original author(s) and the copyright  
owner(s) are credited and that the  
original publication in this journal is  
cited, in accordance with accepted  
academic practice. No use,  
distribution or reproduction is  
permitted which does not comply with  
these terms.

# Contrasting distribution of enzyme activities in the rhizosphere of European beech and Norway spruce

Bin Song<sup>1,2</sup>, Bahar S. Razavi<sup>3,4</sup> and Rodica Pena<sup>2,5\*</sup>

<sup>1</sup>School of Geography and Ocean Science, Nanjing University, Nanjing, China, <sup>2</sup>Forest Botany and Tree Physiology, University of Göttingen, Göttingen, Germany, <sup>3</sup>Department of Soil and Plant Microbiome, Institute of Phytopathology, University of Kiel, Kiel, Germany, <sup>4</sup>Department of Agriculture Soil Science, University of Göttingen, Göttingen, Germany, <sup>5</sup>Department of Sustainable Land Management, School of Agriculture, Policy and Development, University of Reading, Reading, United Kingdom

Recent policies and silvicultural management call for forest regeneration that involve the selection of tree species able to cope with low soil nutrient availability in forest ecosystems. Understanding the impact of different tree species on the rhizosphere processes (e.g., enzyme activities) involved in nutrient mobilisation is critical in selecting suitable species to adapt forests to environmental change. Here, we visualised and investigated the rhizosphere distribution of enzyme activities (cellobiohydrolase, leucine-aminopeptidase, and acid phosphomonoesterase) using zymography. We related the distribution of enzyme activities to the seedling root morphological traits of European beech (*Fagus sylvatica*) and Norway spruce (*Picea abies*), the two most cultivated temperate tree species that employ contrasting strategies in soil nutrient acquisition. We found that spruce showed a higher morphological heterogeneity along the roots than beech, resulting in a more robust relationship between rhizoplane-associated enzyme activities and the longitudinal distance from the root apex. The rhizoplane enzyme activities decreased in spruce and increased in beech with the distance from the root apex over a power-law equation. Spruce revealed broader rhizosphere extents of all three enzymes, but only acid phosphomonoesterase activity was higher compared with beech. This latter result was determined by a larger root system found in beech compared with spruce that enhanced cellobiohydrolase and leucine-aminopeptidase activities. The root hair zone and hair lengths were significant variables determining the distribution of enzyme activities in the rhizosphere. Our findings indicate that spruce has a more substantial influence on rhizosphere enzyme production and diffusion than beech, enabling spruce to better mobilise nutrients from organic sources in heterogeneous forest soils.

## KEYWORDS

root morphology, traits, rhizoplane, zymography, temperate forests, gamma-irradiated soil

## Introduction

In forest ecosystems, anthropogenic pollution and climate change have resulted in nutrient limitation for trees (Jonard et al., 2015; Greaver et al., 2016; Marchi et al., 2018; Xue et al., 2022). This is particularly critical to seedling survival and growth in forest regeneration (Baier et al., 2006; Collet and Le Moguedec, 2007; Wagner et al., 2010). Forestry considers specific management practices (e.g., selective felling) to increase the benefic influence of mother trees on seedling nutrient acquisition through mycorrhizal mycelial networks (Figueiredo et al., 2021). However, empirical evidence suggests that mycorrhizal networking does not necessarily contribute to the seedling establishment and their nutritional improvement (Högberg and Högberg, 2022). In the absence of mature trees (e.g., regeneration in clear felling), seedlings perform better than in the presence of conspecific trees (Högberg and Högberg, 2022). One of the main mechanisms trees employ to alleviate nutrient limitation is the formation of a nutritional symbiosis with mycorrhizal fungi. The trees supply the fungi with photosynthetically fixed carbon in exchange for enhanced nutrient acquisition (Smith and Read, 2010). Young seedlings struggle to associate with mycorrhizal fungi in the first months of growth until the first flush of leaves becomes mature (Harley & Waid, 1955; Warren Wilson and Harley, 1983), and later, during the early stages, seedlings have a meagre mycorrhization rate (Harley, 1939; Warren Wilson and Harley, 1983; Newton and Pigott, 1991; Zeleznik et al., 2007; Pena et al., 2013; Nahberger et al., 2021). Therefore, the young seedlings, which are the most susceptible to nutrient deficiency among all other stages in the life of a tree, in the absence of mycorrhizal partners, must develop different strategies to overcome the nutrient limitation. One of these strategies is to intensify the rhizosphere processes, particularly the enzyme activities, which catalyze the nutrient release from soil organic matter (Marschner et al., 2011; Dijkstra et al., 2013; AminiTabrizi et al., 2022). The rhizosphere was broadly defined as the volume of soil influenced by root activity (Hiltner, 1904; Hinsinger, 1998). The term has been refined to comprise the endo- and ecto-rhizosphere, which consists of the rhizoplane (i.e., the root surface) and rhizosphere soil (Reinhold-Hurek et al., 2015; York et al., 2016). The ecto-rhizosphere is a hotspot of microbial activities and soil organic matter decomposition sustained by plant rhizodeposition (Ge et al., 2017; Macia-Vicente et al., 2020).

The plant influences the rhizosphere in vertical and horizontal directions (Luster et al., 2009; Minz et al., 2013; Wen et al., 2022). The roots are composed of segments that differ in their degree of development and differentiation showing specific physiological and biochemical characteristics: root cap, root tip, elongation zone, root-hair zone, mature zone, and sites of lateral root emergence. This root functional heterogeneity controls the root spatial abilities to take up nutrients (Luster

et al., 2009; Hinsinger et al., 2011; Helliwell et al., 2017; Wang et al., 2022a). However, its effects on the spatial distribution of enzyme activities in the rhizosphere have been rarely investigated.

In the rhizosphere, the enzyme activities are determined by synergistic effects of plant and root-associated or soil microorganisms (Hinsinger et al., 2006; Kuzyakov and Razavi, 2019; Ren et al., 2021). The microorganisms rely on rhizodeposits (exudates, mucilage, or border cell loss, McCully, 1999; Brzostek et al., 2013; Zwetsloot et al., 2018) as easily accessible carbon (C) sources (Kuzyakov et al., 2000; Kuzyakov and Cheng, 2001; Huang et al., 2019; Herre et al., 2022). Thus, the plants contribute to the rhizosphere enzyme activities either directly by releasing enzymes or indirectly by influencing microbial abundance or activity through variation in the quantity and quality of rhizodeposition (Haichar et al., 2008; Pei et al., 2016; Uroz et al., 2016a; Zwetsloot et al., 2018). Furthermore, as the rhizoplane acts as a “regulatory gate” of microbial entry to the endosphere (van der Heijden and Schlaeppi, 2015), the plants also involve immunity and signalling mechanisms in controlling microbial distribution on the rhizoplane (Dupuy and Silk, 2016; Schmidt et al., 2018).

The rhizosphere enzyme activities are commonly interpreted as a response of plants and microorganisms to nutrient demand (Burns et al., 2013; Kang et al., 2022). The spatial distribution of enzyme activities in the rhizosphere may contribute to or reflect the plant ability to cope with nutrient limitations through better exploitation of soil resources (Hinsinger et al., 2011; Hummel et al., 2021). A comprehensive understanding of enzyme activities in the rhizosphere in relation to root morphological traits is a valuable contribution to selecting more adapted tree species to cope with nutrient limitations at young seedling stages.

European beech (*Fagus sylvatica* L.) and Norway spruce (*Picea abies* Karst) are the dominant species in temperate forest ecosystems in Central Europe (FAO, 2010; Leuschner and Ellenberg, 2017). They differ in terms of the chemical properties of the rhizosphere, root morphology and architecture, and the strategy by which they enhance nutrient acquisition (Wang et al., 2001; Calvaruso et al., 2014; Kolstad et al., 2016; Odriozola et al., 2020). Norway spruce has a shallow root system that commonly proliferates in the uppermost, organic-rich, soil layers, which contrasts with the beech tend toward a heart-shaped root system that branches out in both the upper and deeper soil layers (Schmid, 2002). In response to resource limitation, beech has a high level of plasticity in root biomass partitioning, a strategy not pursued by spruce, which has limited root plasticity (Matjaž and Primož, 2010; Schall et al., 2012). Nevertheless, spruce has a higher nutrient requirement and, consequently, higher nutrient depletion in the rhizosphere than beech (Wang et al., 2001).

In this study, we aimed to investigate the spatial distribution of enzyme activities on the rhizoplane and the rhizosphere



enzyme activity extents (i.e., the horizontal distance from the root centre to bulk soil at which the enzyme activity decreases to a constant value) in relation with root morphological traits in European beech and Norway spruce young seedlings.

Rhizosphere enzyme activities change with plant nutrient requirements and physiology (Marschner et al., 2011), root morphology (Razavi et al., 2016; Giles et al., 2018; Ma et al., 2018a; Ma et al., 2018b), or rhizosphere microbial activity (Steinauer et al., 2016; Zhang et al., 2019; Kante et al., 2021). All these factors vary with plant species, life history or phylogeny (García et al., 2004; Minz et al., 2013; Brtnický et al., 2021; Uroz et al., 2016b). Therefore, we hypothesised that (1) spatial distribution of enzyme activities in the rhizoplane is related to root morphological traits, and (2) Norway spruce show higher rhizosphere enzyme activities and broader rhizosphere extents than European beech. In this way, the spruce may complement its strategy of gaining access to organically bound nutrients at the expense of investment in root growth in deeper soil horizons that is the case with beech.

To test these hypotheses, we used a microcosm experiment where European beech and Norway spruce seedlings were planted in the soil, where fungi were absent. We used a  $\gamma$ -irradiated forest soil amended with soil-solution bacteria. In this way, we simulate the natural seedling establishment in the absence of ectomycorrhizal fungi that may influence rhizosphere enzyme activities (Firsching & Claassen, 1996).

To evaluate the enzyme activities and their spatial distribution in the rhizosphere, we employed zymography. For the investigation, we selected three enzymes, which are common in the temperate forest soil (Baldrian and Štursová, 2011), catalyse reactions of C, N, or P cycles and partially enabled us to disentangle the contribution of plant and microorganisms to the activity distribution pattern: cellobiohydrolase (CBH), which is mainly secreted by microorganisms to degrade cellulose into soluble sugars (Payne et al., 2015; Sanaullah et al., 2016); leucine-aminopeptidase (LAP), which targets proteinaceous compounds to release amino acids; and acid phosphomonoesterase (AP), which hydrolyses organic P-compounds to phosphates. Leucine-aminopeptidase and AP are secreted by both plants and microorganisms (Turner and Haygarth, 2005; Nannipieri et al., 2011).

## Material and methods

### Experimental setup

European beech (*Fagus sylvatica* L.) and Norway spruce (*Picea abies* L. Karst) seedlings were grown in rhizoboxes for three months until the first flush of leaves was mature. This is also the stage when the root system is well-developed and considered fully developed for the season (Harley, 1940; Wilcox, 1968; Warren Wilson and Harley, 1983). The plants

were obtained from seeds (Niedersächsische Landesforsten, Forstamt Oerrel, Germany), which were germinated on moist filter paper at 4°C in darkness for one week. When the radicles reached a length of 1–2 cm, the seedlings were sterilised following the procedure described by Pena et al. (2013). In short, after removal of the seed coats, the seedlings were immersed in a water solution of 0.1% fungicide and 0.1% tetracycline for 24 h (Proplant, Arysta LifeScience, Düsseldorf, Germany) (Duchefa Biochemie, Haarlem, Holland). The seedlings were thoroughly rinsed and then transferred immediately to rhizoboxes, where they were planted at a depth of 5 cm.

The rhizoboxes had inner dimensions of 21.5 × 11.4 × 3.6 cm and could be easily opened from the front. They were filled with sieved soil (mesh size 1000  $\mu$ m) to a bulk density of 1.5 g cm<sup>-3</sup>. This soil was loamy Haplic Luvisol, obtained by collecting the Ah horizon to a depth of 20 cm from a mature beech stand in the Hainich forest in Central Germany (51°04'46"N, 10°27'08"E). The soil contained 58 g kg<sup>-1</sup> sand and 301 g kg<sup>-1</sup> clay, with 36 g kg<sup>-1</sup> of organic C and 3 g kg<sup>-1</sup> total N, at a pH of 5.0 (Solly et al., 2014).

The soil was sterilised by  $\gamma$ -irradiation on two occasions at 30 kGy with a one-week interval between treatments (BGS Beta-Gamma-Service GmbH & Co, Wiehl, Germany). To minimise residual enzyme activities, the soil was kept in tightly closed containers at 4°C for one year (Lensi et al., 1991). Nevertheless, the bias of abiotic enzymes stabilised in the soil matrix (Nannipieri et al., 2018) was low as we used the same soil in all treatments.

The soil fungal contamination was monitored by spreading a 1.0 ml fresh soil solution on a Petri dish containing modified Melin-Norkrans (MMN) agar medium and incubating the plates in darkness at 18°C for three weeks. Slight bacterial growth did occur, but no fungal growth was observed.

Before planting the soil, the rhizoboxes were amended with a bacterial culture stemming from a soil solution from the same forest site. The bacterial culture preparation was conducted according to the procedure described by (Nguyen et al., 2017). Each rhizobox was inoculated with 1.0 ml of bacterial solution diluted with 25 ml H<sub>2</sub>O.

After planting, the rhizoboxes (n=4) were transferred to a climate chamber maintained at a constant temperature of 22 ± 1°C, humidity 60%, and a daily light period of 14 h with an active photosynthetic radiation intensity of 250  $\mu$ mol m<sup>-2</sup> s<sup>-1</sup>. The plants were watered 2x/week with approximately 50 ml H<sub>2</sub>O per rhizobox. During the growth period, these boxes were inclined at an angle of 50° to facilitate root growth along their fronts.

### Soil zymography

The spatial distribution of enzyme activities on the rhizoplane and in the rhizosphere soil was determined *in situ*

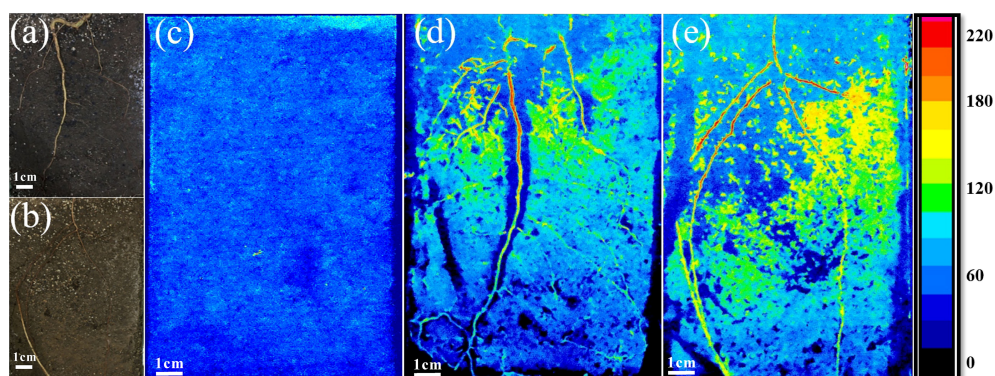
using direct soil zymography before planting and when the plants were three-months-old (Figure 1). Soil zymography was conducted following the protocol from Razavi et al. (2019), with the measurements of cellobiohydrolase (CBH) (EC 3.2.1.91); leucine-aminopeptidase (LAP) (EC 3.4.11.1); and acid phosphomonoesterase (AP) (EC 3.1.3.2) activities. The applied method is based on the visualisation of enzyme activities using hydrophilic polyamide membranes saturated with either 4-methylumbelliferone (MUF)-substrates (pH 6.5) or 7-amino-4-methylcoumarin (AMC)-substrates (pH 7.2). The substrates become fluorescent when hydrolysed by the specific enzyme (Dong et al., 2007). The membranes (Tao Yuan, China) were 100  $\mu\text{m}$  thick with a pore size of 0.45  $\mu\text{m}$ . Cellobiohydrolase activity was detected using 4-methylumbelliferyl- $\beta$ -D-cellobioside (MUF-C), LAP activity by L-leucine-7-amino-4-methylcoumarin hydrochloride (AMC-L), and AP activity by 4-methylumbelliferyl-phosphate (MUF-P) substrate. MUF substrates were dissolved separately to a concentration of 12 mM in an MES buffer ( $\text{C}_6\text{H}_{13}\text{NO}_4\text{SNa}0.5$ , Sigma-Aldrich, Darmstadt, Germany). AMC substrate was dissolved in TRIZMA ( $\text{C}_4\text{H}_{11}\text{NO}_3 \cdot \text{HCl}$ ,  $\text{C}_4\text{H}_{11}\text{NO}_3$ , Sigma-Aldrich, Darmstadt, Germany). The rhizoboxes were carefully opened, and the membrane, previously saturated with the enzyme-specific substrate, was applied directly to the root surface. For each rhizobox, a soil zymography test was performed separately for each enzyme in the following order: CBH, AP, and LAP (Ma, Liu, et al., 2018). Each membrane was incubated on the soil surface for one hour and then gently lifted off with tweezers. After incubation, the membranes were placed on a transparent laser imaging cover (35  $\times$  43 cm, Carestream, NY, USA), transferred to the darkroom, and photographed under ultraviolet (UV) illumination at an excitation wavelength of 355 nm and an emission wavelength of 460 nm. The camera was a Sony DSC-RX100m5 (Sony, Tokyo, Japan) with 35 mm focal

length and 20 megapixels, mounted on a Rocwing Pro Copy Stand (Rocwing Co, London, UK). To ensure uniform illumination of the membranes, three 22W purple fluorescent lamps (Damar Worldwide 4 LLC, Aurora, U.S.A.) were fitted to the camera as a source of UV light. For all measurements, the distance, aperture, and shutter speed of the camera were set to 250 mm, f/5.0 and 1/250 sec, respectively.

## Calibration and validation of soil zymograms

To analyse and quantify the zymogram pictures, a standard calibration curve was plotted relating the enzyme activity to the gray-value of zymogram fluorescence using serial dilutions of MUF (0, 0.01, 0.05, 0.1, 0.2, 0.4, 0.6, 0.8, and 1.0 mM) and AMC (0, 0.01, 0.1, 0.2, 0.5, 1.0, 2.0, 3.0, 4.0, 5.0, and 6.0 mM). The membranes were cut into 4  $\text{cm}^2$  pieces and soaked in these solutions for one hour. The amount of MUF and AMC on an area basis was calculated based on the size of the membrane and the volume of solution taken up (Guber et al., 2018). The membranes were placed in the transparent laser imaging cover and photographed in UV light at the camera setting described above. These calibration membranes were imaged under UV light following the same procedure as with the rhizobox samples.

The zymogram quantification was conducted with a Matlab toolbox (MathWorks, Natick, Massachusetts, USA). The fluorescence spots on the zymogram images show the areas in which the substrate was enzymatically hydrolysed. The intensity of fluorescence is proportional to the enzyme activity. To calculate the enzyme activity, the clearly visible, not overlapping roots were selected and subsequently, skeletonised and plotted using the Thinning and Image acquisition (Euclidean distance map) functions (Zarebanadkouki et al., 2014). Images were



**FIGURE 1**  
Roots of seedlings of European beech (A) and Norway spruce (B) grown in the rhizoboxes. Examples of zymographs showing the spatial distribution of acid phosphomonoesterase (AP) activity before planting (C), and in the rhizosphere of European beech (D) and Norway spruce (E). Side colour maps are proportional to the enzyme activities ( $\text{pmol mm}^{-2} \text{h}^{-1}$ ).

transformed to 16-bit grayscale images as matrices and corrected for light variations and camera noise (Razavi et al., 2016; Wei et al., 2019a). The total activity of every single enzyme in the rhizosphere was calculated by assessing the sum total of the pixel values in the region between the root centre and border of the rhizosphere. The grey-value from the blank region of samples was set as the background, and, subsequently, subtracted from the total pixel value. To calculate the enzyme activities, the *Analysis* function of ImageJ Fiji (National Institutes of Health, USA) was employed. On the rhizoplane, the measurements included 340 points up to a longitudinal distance of 7.0 cm from the root tip. In the rhizosphere soil, the enzyme activities were measured laterally from the root centre at seven points, equally distributed over 1.8 mm. The lateral measurements were conducted on seven vertical levels, and subsequently, the mean value for each horizontal point was derived. The standard calibration curve was used to convert the all grey-values from the various images to enzyme activities using STATISTICA software (Dell, Texas, USA) according to the procedure described by Guber et al. (2018) and Razavi et al. (2019).

The enzyme activities were measured on the rhizoplane and in the rhizosphere soil laterally from the taproot in beech and all (three to five) main roots in spruce. In the latter case, the mean values were calculated and used in the analysis. The reason for using all main roots in spruce was the formation of horizontal side roots of similar size to the taproot that is a characteristic of spruce seedlings (Puhe, 2003). In young beech seedlings, on the contrary, the taproot comprises the main part of the root system, with the lateral roots becoming functionally important only later in the ontogeny (Harley, 1939; Harley, 1940). The visual assessment of zymograms confirmed that the most intense enzyme activities occurred on the tap root in beech and all main (tap and side) roots in spruce (Figure 1).

## Sampling and analysis of root morphology

Following the zymography measurements, the plants were harvested and separated into the shoot and root segments. The roots were carefully washed to remove all soil particles, briefly dried with paper tissues, and weighed to register the fresh biomass. After the morphological analysis, the roots were separated into fine and coarse roots, and aliquots of both categories were oven-dried at 60°C to constant weight; these were then used for assessing the dry biomass.

A root morphological analysis was conducted using WinRHIZO software (Regent Instruments Inc., Ottawa, Canada). The entire roots were evenly spread within a thin layer of tap water in a transparent tray and scanned at a resolution of 400 dpi using a flatbed scanner (ScanMaker i800plus, Microtek, China). The images of these scanned root samples were saved in the TIF format and then imported to

WinRHIZO software. The software was set up as suggested by Lobet et al. (2011) and Pierret et al. (2013): *Image mode* 8-bit Gray; *Resolution* 400 dpi; *Scale* 100%; *Calibration* intrinsic for scanner; *Root/background* and *Colour analysis* based on grey levels (threshold)—Manual—Dark on white background; *Particle* cleaning on. The WinRHIZO data output included total root length, main root length, surface area, mean diameter, root volume, number of tips, and number of forks. The 7 cm long roots, which were analysed for enzyme activities, were excised and placed in a Petri dish with tap water and photographed (Leica DFC 420 C; Leica, Wetzlar, Germany) under a stereomicroscope (Leica M205 FA). The extent of the root elongation zone (the area from the apex until the first root hairs) and root hair zone, root hair length, and root diameter were measured along the root fragments at the points where enzyme activities were measured using the tool MRI Root Hair Tools implemented in ImageJ ([https://github.com/MontpellierRessourcesImagerie/imagej\\_macros\\_and\\_scripts/wiki/MRI\\_Root\\_Hair\\_Tools](https://github.com/MontpellierRessourcesImagerie/imagej_macros_and_scripts/wiki/MRI_Root_Hair_Tools)).

## Calculations and data analysis

The normalised enzyme activity was calculated by dividing the total enzyme activity by the root surface area, as described by Ma et al. (2018b).

The nutrition acquisition ratio in the rhizosphere was calculated as following: C/N acquisition =  $\ln(\text{CBH})/\ln(\text{LAP})$ , N/P acquisition =  $\ln(\text{LAP})/\ln(\text{AP})$ , and C/P acquisition =  $\ln(\text{CBH})/\ln(\text{AP})$  (Wei et al., 2019b; Karhu et al., 2022).

The enzyme activity ( $y$ ), as a function of vertical distance from the root tip ( $x$ ), was fitted by a power function (Niklas, 1994):

$$y = ax^b$$

where  $a$  is the allometric coefficient, and  $b$  is the regression coefficient or scaling exponent.

The enzyme activity ( $y$ ) as a function of horizontal distance from the root centre ( $x$ ) was fitted by applying the sigmoid Hill equation:

$$y = \min + \frac{(\max - \min)}{1 + (x/EC_{50})^{-\text{Hill slope}}}$$

The criteria of equation choice were the best description of the observed pattern of data distribution. The curve fitting was conducted with Origin (Pro) software (OriginLab Corporation, Northampton, MA, USA).

The differences between the plant species concerning the root characteristics, enzyme activities and scaling exponents of fitted curves (Sokal and Rohlf, 2013) were assessed using Student's  $t$ -test or one-factor ANOVA;  $p$ -values < 0.05 were used to indicate significant differences between the means. Levene's and Shapiro-Wilk's tests were used to check for data homogeneity

and normal distribution. Principal Components Analysis (PCA) was employed to evaluate the relationships between root morphological characteristics and enzyme activities. The analyses were conducted with R 3.6.0 statistical software using the following functions: `levene.test()` and `qqPlot()`, `car`-package (Fox and Weisberg, 2018); `shapiro.test()`, `aov()`, `TukeyHSD()`, `t-test()`, `stats`-package; and `princomp()`, `vegan`-package (R Core Team, 2020).

## Results

### Variation in root morphology of European beech and Norway spruce

Total root length and surface area were four and ten times larger in beech than in spruce (Table 1). The number of root tips and biomass were also 10 times higher in beech than in spruce (Table 1).

In beech, the mean root diameter was 20% larger than in spruce (Table 1), and the taproot increased with the distance from the root apex (Table 2). In spruce, no relationship between root diameter and the distance from root apex was apparent (Table 2).

The root hair zone started at  $0.13 \pm 0.005$  mm and  $0.33 \pm 0.021$  mm distance from the root apex in beech and spruce, respectively. In beech, the root hair length (RHL) was in the range of 0.03 to 0.04 mm (Figure 2). A relatively small percentage (2.4%) of root hairs reached longer lengths. Root hair length was unrelated to the longitudinal distance from the root apex (Table 2). In spruce, RHL was more heterogeneous, ranging from 0.2 to 0.5 although about 70% of the root hairs showed an RHL ranging from 0.3 to 0.5 mm (Figure 2). Root hair length significantly increased with the longitudinal distance from the root apex (Table 2). Thus, various root characteristics of beech (e.g., length, surface area, diameter, and biomass) were

superior to those of spruce, even though spruce had overall longer root hairs than beech (Figure 2).

### The longitudinal distribution of enzyme activities on the rhizoplane

The spatial distributions of enzyme activities on the rhizoplane in relationship with the distance from the root apex were described by a power-law in beech and an inverse power-law equation in spruce (Figure 3). The order of scaling was about ten times higher in spruce than in beech (Figure 3), indicating a larger difference in enzyme activities along the roots in spruce than in beech. The largest difference in the scaling order between beech ( $0.03 \pm 0.002$ ) and spruce ( $0.34 \pm 0.01$ ) was in LAP activity. In beech, LAP activity slightly increased from  $180 \pm 3$  (pmol cm<sup>-2</sup>h<sup>-1</sup>) at the root apex to  $210 \pm 1$  (pmol cm<sup>-2</sup>h<sup>-1</sup>) at 7.0 cm from the root tip (Figure 3C), while in spruce it decreased from  $188 \pm 5$  (pmol cm<sup>-2</sup>h<sup>-1</sup>) to  $27 \pm 4$  (pmol cm<sup>-2</sup>h<sup>-1</sup>) at the same distance from the root apex (Figure 3D). The trend was similar for CBH activities (scaling order,  $0.08 \pm 0.003$  in beech;  $0.21 \pm 0.009$  in spruce, Figures 3A, B) and AP ( $0.05 \pm 0.004$  in beech;  $0.31 \pm 0.010$  in spruce, Figures 3E, F).

The patterns of the spatial distribution of enzyme activities along the root growth axis were associated with the increase in root diameter in beech, and a decrease in root hair length in spruce, as the two parameters were correlated with the distance from the root apex (Table 2). To investigate whether this association with root parameters was independent of tree species, we evaluated the association of root hair length in beech (Figure S1) and root diameter size in spruce (Figure S2) with distance from the root apex in distinct root zones, characterised by specific classes of root hair lengths or root diameters, respectively. In beech, in the root areas in which no root hairs occurred, - primarily at the root apex, the power-law equation did not adequately describe the spatial relationship concerning any of the measured enzyme activities (SE = 0.120 for CBH; SE = 0.140

TABLE 1 Root characteristics of young beech and spruce seedling.

	Beech	Spruce
Total length (cm)	1196 ± 47 b	216.4 ± 33.1 a
Taproot length (cm)	19.55 ± 0.98 b	13.87 ± 1.13 a
Surface area (cm <sup>2</sup> )	189.9 ± 7.8 b	28.73 ± 4.51 a
Mean Diameter (mm)	0.51 ± 0.02 b	0.40 ± 0.01 a
Volume (cm <sup>3</sup> )	2.64 ± 0.08 b	0.28 ± 0.06 a
Tips	4452 ± 207 b	732 ± 75.3 a
Forks	6775 ± 487 b	689 ± 51 a
Root hair length (mm)*	0.06 ± 0.01 a	0.32 ± 0.03 b
Biomass (g)	3.32 ± 0.29 b	0.41 ± 0.01 a

\*Values obtained by stereomicroscope image analysis.

Values represent the mean (± SD). Different letters indicate significant statistical differences between European beech and Norway spruce obtained by Student's t-test,  $p < 0.05$ . N = 4.



TABLE 2 Correlation matrix (Spearman *r*) of root hair length and root diameter with the longitudinal distance from the root apex.

	Root hair length	Diameter
Distance from the root tip		
Beech	0.010	<b>0.180</b>
Spruce	<b>0.400</b>	0.020

Bolded numbers indicate a significant relationship between European beech and Norway spruce,  $p < 0.05$ . ( $N = 340$ ).

for LAP;  $SE = 0.226$  for AP, [Figure S1](#)). Similarly, in the root areas where root hair length exceeded 0.04 mm, the power-law function failed to fit the data ( $SE = 0.047$  for CBH;  $SE = 0.030$  for LAP;  $SE = 0.119$  for AP, [Figure S1](#)). The root areas of root hair lengths of 0.3 and 0.4 mm, representing the largest rhizoplane area, revealed a close relationship between enzyme activities and the distance from the root ([Figure S1](#)). This relationship was particularly prominent in CBH activity, which increased with the distance from root apex both in the 0.3 mm-root hair length ( $0.08 \pm 0.005$ ) and 0.4 mm-root hair length root areas ( $0.07 \pm 0.002$ , [Figure S1](#)).

In spruce, in areas of the smallest ( $\leq 0.24$  mm) and largest ( $\geq 0.28$  mm) root diameter size ([Figure S2](#)), the distribution of enzyme activities poorly fitted a power-law equation. In the root areas where the diameter was 0.25 cm, 0.26 cm, and 0.27 cm, the enzyme activities followed the power-law relationship concerning the distances from the root apex ( $0.018 \leq SE \leq 0.038$ ; [Figure S2](#)).

### The relationships between root morphology and enzyme activities in the rhizosphere soil

Enzyme activities in the soil assessed from zymograms before planting were in a similar range of 18 to 19 pmol cm<sup>-1</sup> h<sup>-1</sup> for CBH and LAP, and AP, respectively ([Figures 1, 4](#)). In the rhizosphere of three-month-old beech seedlings, CBH and LAP activities were about twice as high as in the spruce rhizosphere

([Figure 4A](#)). Acid phosphomonoesterase activity, on the contrary, was higher in the spruce than beech rhizosphere ([Figures 4A, 1](#)). Carbon/Nitrogen acquisition ratio (ratio of C-acquiring to N-acquiring enzyme activities) was close to 1.0 and did not differ between beech and spruce ([Table S1](#)). Acquisition ratios of C/P and N/P were higher in beech than in spruce, with values <1.0 in the spruce ([Table S1](#)).

When the enzyme activity values were normalised to the root surface area, no differences occurred between the spruce and beech rhizosphere CBH and LAP activities ([Figure 4B](#)). However, AP activity was about four times higher in the spruce than in the beech rhizosphere ([Figure 4B](#)).

In beech, CBH, LAP, and AP activities were positively associated with the first principal component (PC1, 55.7%) together with root diameter and volume ([Table S2](#), [Figure 5](#)). All other root parameters were also positively associated with PC1 but to a lower magnitude. In spruce, only LAP activity was associated with PC1 (65.7%), together with all root morphological traits ([Table S2](#), [Figure 5](#)). The second PC (21.7%) had largely negative associations with LAP and AP activities, and root diameter ([Table S2](#), [Figure 5](#)).

### The extents of enzyme activities in the rhizosphere soil

The profile of rhizosphere enzyme activities as a function of the outward distance from the root centre was described by the

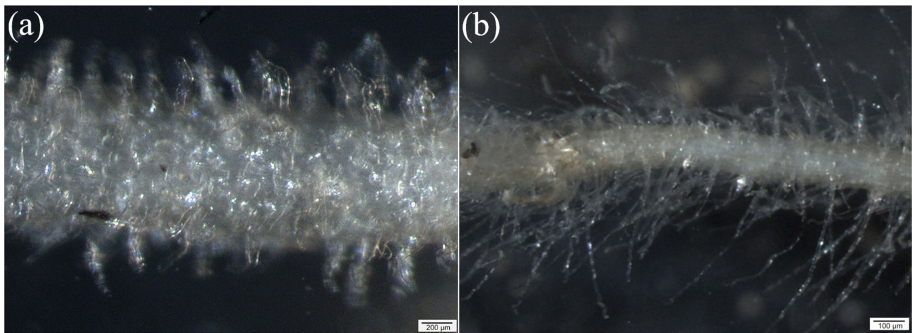


FIGURE 2 Stereomicroscope images of root hair zone of European beech (A) and Norway spruce (B).



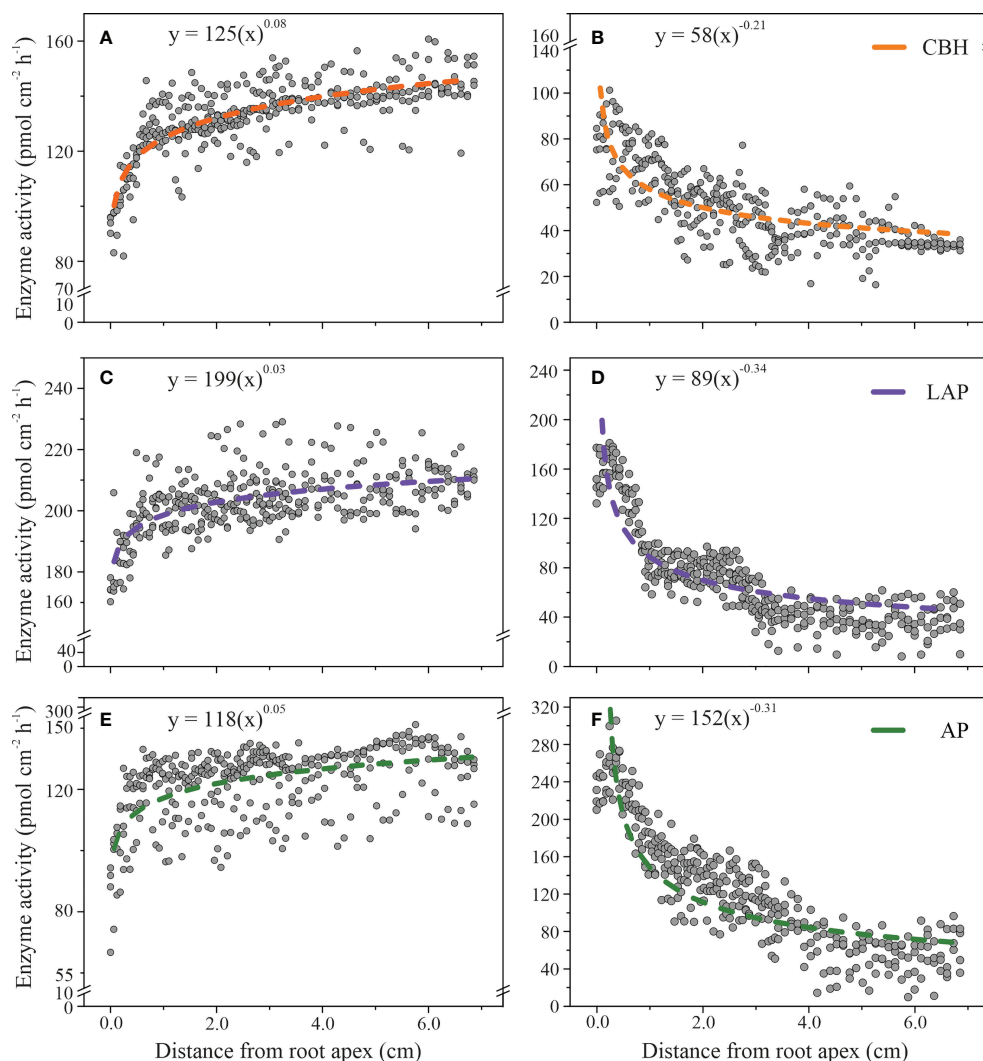


FIGURE 3

Enzyme activities on the rhizoplane as a function of the longitudinal distance from the root apex to the distal root zone in European beech (A, C, E) and Norway spruce (B, D, F): cellobiohydrolase (CBH), leucine-aminopeptidase (LAP), and acid phosphomonoesterase (AP). The models include data from 85 individual measuring points per plant. (N = 4).

Hill equation. This took on different forms in the beech and spruce samples (Figure 6). For all measured enzymes – both in beech and spruce – the maximum value was apparent on the root surface and started to decrease at the root radius edge (Figure 6). An exception was the beech LAP activity, which gradually decreased from the root centre toward the end of the root radius (Figure 6).

The inflexion point of the CBH activity function from the root centre was 0.5 mm for beech and 0.4 mm for spruce (Figure 6). The rhizosphere extent of CBH activity differed ( $P = 0.038$ ) between beech ( $0.72 \pm 0.05$  mm) and spruce ( $0.80 \pm 0.04$  mm). There was a more abrupt decrease in beech CBH activity (Hill coef. = 6.5) in response to the distance from the root centre as compared to spruce CBH activity (Hill coef. = 3.0 for spruce,

Figure 6). The shape of the beech CBH activity curve indicated that the decrease in CBH activity started where the root hair area ended (Figure 6A). The influence of root hairs also became apparent through the linear correlation between the extent of RHL and rhizosphere CBH activity (Table 3).

Leucine-aminopeptidase activity in the beech rhizosphere continued until  $0.78 \pm 0.03$  mm but with a gradual decrease (Hill coef. = 2.5) (Figure 6A). Likewise, in spruce, LAP activity was present until  $0.84 \pm 0.11$  mm (Hill coef. = 3.6) (Figure 6B). In both species, LAP activity was not related to root hair lengths (Table 3).

Hill AP activity curve in the beech and spruce rhizospheres produced different shapes as compared to those of CBH and LAP (Figures 6A, B). The inflexion points of the curve from the

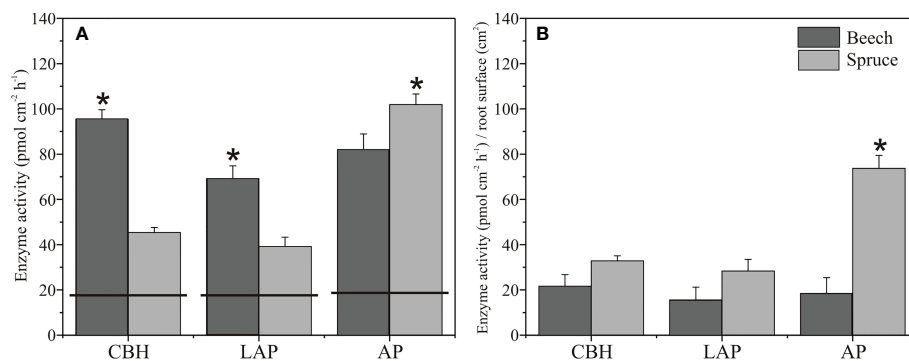


FIGURE 4

Enzyme activities in the rhizosphere of European beech and Norway spruce. Total (A) and root surface area-normalised (B) activity of cellobiohydrolase (CBH), leucine-aminopeptidase (LAP), and acid phosphomonoesterase (AP). The horizontal lines on the bars indicate the enzyme activities in the soil before tree planting. Bars represent mean values, and error bars represent standard error of the mean (N = 4). Asterisks indicate significant differences for means of enzyme activities between beech and spruce,  $p < 0.05$ .

root centre corresponded to 0.6 mm for beech and 0.7 mm for spruce (Figure 6). Acid phosphomonoesterase activity displayed the broadest rhizosphere distribution among the three enzyme activities, both in beech (distance from the root centre =  $1.22 \pm 0.07$  mm) and spruce ( $1.58 \pm 0.03$  mm), with significant differences ( $p < 0.005$ ) between the tree species (Figure 6). The curve shape indicated that there was no effect of the root hair size on AP activity (Figures 6A, B). There were no linear relationships between AP activity and the length of root hairs (Table 3).

In beech, C/P and C/N acquisition ratios decreased  $< 1.0$  with decreasing CBH activity at the edge of root hairs, while N/P ratio remained constant in the rhizosphere soil (Figure 6A, inset). In spruce, all acquisition ratios were maintained constant, close to 1.0 (Figure 6B, inset).

## Discussion

In this study, we investigated the spatial distribution of enzyme activities on the rhizoplane and in the rhizosphere soil of European beech and Norway spruce in relationship with root morphology. In accordance with previous studies (Bolte and Villanueva, 2006; Schall et al., 2012), we found that the size of the root system (i.e., root length, surface, mean diameter, biomass, and number of forks and root tips) was more extensive in beech than spruce. This finding confirms the beech nutrient uptake strategy to massively proliferate in exploiting a large soil volume (Leuschner et al., 2001).

The taproot zones, considered a proxy of longitudinal root heterogeneity, were more evident in spruce than in beech. The root apex, elongation zone, and root hair zones were broader in

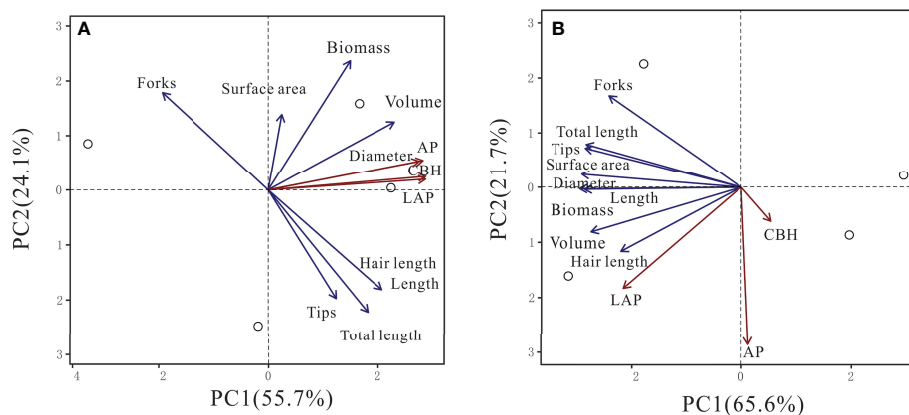
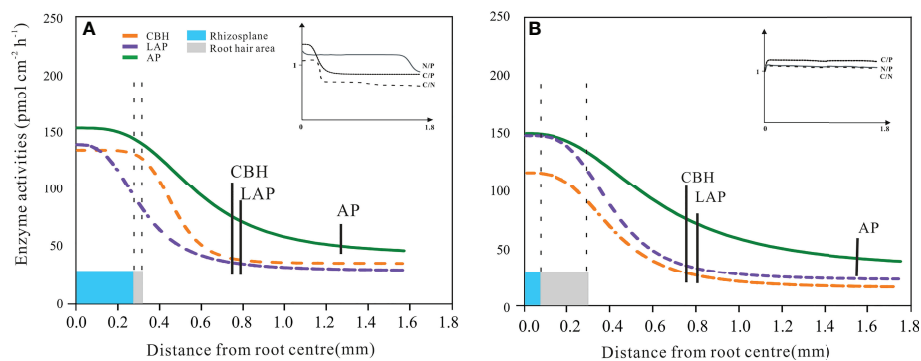


FIGURE 5

Principal component analysis (PCA) of cellobiohydrolase (CBH), leucine-aminopeptidase (LAP), and acid phosphomonoesterase (AP) activities in relationship with root traits in beech (A) and spruce (B) rhizosphere. (N = 4).



**FIGURE 6** Distribution of enzyme activities in the European beech (A) and Norway spruce (B) rhizosphere. The curves represent the Hill function fitting the enzyme activity related to the horizontal distance from the root centre. Cellobiohydrolase (CBH), leucine-aminopeptidase (LAP), and acid phosphomonoesterase (AP). The vertical dotted lines represent the size of the root radius (blue area) and root hair length (grey area). The vertical solid bars represent the end of the extent of enzyme activity for CBH, LAP, and AP. The models include the mean data from 7 vertical measuring points per radial measurement point per plant. (N = 4). Error bars of enzyme activities were omitted to improve visualisation; the standard errors were always less than 10% of the activity values.

spruce than in beech. The root hair lengths presented a higher variation and were on average more than ten times greater in spruce than in beech.

The enzyme activities on the rhizoplane increase with root diameter and decrease with root hair lengths

The results confirmed our first hypothesis that the spatial distribution of enzyme activities in the rhizoplane is related to root morphological traits. We found that the spatial distribution of enzyme activities along the root growth axis was associated with the increase in root diameter in beech, and a decrease in root hair length in spruce.

Distribution of CBH, LAP or AP activities on the rhizoplane in relation to the longitudinal distance from the root apex followed a power law and an inverse power law equation in beech and spruce, respectively. Razavi et al. (2016) have also reported differences in the distribution of enzyme activities along rhizoplane among herbaceous plants, but the contrast was less apparent than in the selected tree species. However, the order of scaling was relatively small in beech compared with spruce, suggesting a relative homogeneity in the spatial distribution of enzyme activities along

the beech roots. This result was explained by the more homogeneous morphological appearance of beech roots compared with spruce. The root morphological and physiological heterogeneity results in the alteration of the chemical composition of exudates (Jaeger et al., 1999; Wen et al., 2022) or generate micro-niches, where the mucilage and other exudates may accumulate, favouring microbial activity (Massalha et al., 2017; Schmidt et al., 2018; Li et al., 2020; Xiong et al., 2021). As the enzymes cleave complex organic compounds from rhizodeposits into absorbable nutrients for plants and microorganisms (Nannipieri et al., 2007; Nannipieri et al., 2011), the quantity and quality of rhizodeposits are likely contributing to the spatial distribution of enzyme activities on the rhizoplane. A particularly important component of root heterogeneity is the root hair zone that prevails in comparison with other root zones in exchange fluxes with the environment, mainly nutrient uptake (Bidel et al., 2000; Laporte et al., 2013; Nguyen, 2003), or the release of exudates (Holz et al., 2018). The root hair zone supports the presence and wave-like distribution of microorganisms (Semenov et al., 1999; Zelenev et al., 2000). This explains our findings in beech roots of a stronger relationship between enzyme activities and distance from the root tip in the root hair zone than in other zones (Figure S1). Although we cannot disentangle the contribution of root or microbial activity to the rhizoplane

**TABLE 3** Pearson's correlations between root hair lengths and rhizosphere extents of cellobiohydrolase (CBH), leucine aminopeptidase (LAP), and acid phosphomonoesterase (AP) in European beech and Norway spruce. N = 28.

	CBH	LAP	AP
Root hair length			
Beech	0.833	0.228	0.020
Spruce	0.690	0.320	0.010

enzyme activities, we posit that the root hair presence likely stimulated microbially- than plant-derived enzymes. We developed this proposition as in the root hair zone the microbe-specific CBH showed a more robust relationship than the dual (plant and microbially derived) AP activity.

In spruce, we found a decrease in enzyme activities from the root apex to the elongation zone and further to the root hair zone. The root hair lengths increased with the distance from the apex. The high enzymatic activity at the root apex has been previously described (Kuzyakov & Razavi, 2019) and possibly explained by a higher microbial enzymatic activity (Kuzyakov et al., 2000) triggered by the high amount of released exudates at the root apex (Watt et al., 2006; Hinsinger et al., 2009). However, we cannot exclude that the higher apical activities are due to a larger contribution of plant-released enzymes. Root apical parts are involved in taking up specific nutrients (Häussling et al., 1988) and exude enzymes more intensively than mature parts (Godlewski and Adamczyk, 2007). Moreover, plant and microbial uptake of nutrients (i.e., phosphorus) spatially differ along with the roots: the root apical part is reserved for the plant, while the root hair zone is for microbial P uptake (Marschner et al., 2011; Spohn and Kuzyakov, 2013). We speculate that the plant-released enzymes significantly contribute to the spatial distribution of enzyme activities on the rhizoplane. In support of this assumption, we found a lesser relationship between microbe-specific CBH activity and distance from the root tip than AP and LAP, which both plants and microorganisms can release.

## The rhizosphere CBH and LAP, but not AP activities were higher in beech than in spruce

The more extensive root system of beech triggered higher CBH and LAP activities in beech than in spruce rhizosphere. We obtained no differences in CBH and LAP activities between beech and spruce by considering the differences in root surface area. However, AP activity was higher in spruce than beech, regardless of the root size. This result was surprising because in beech, similarly to CBH and LAP, and in accordance with other studies (Meller et al., 2020), AP activity was largely positively associated with root morphological parameters, particularly root mean diameter and root volume. We may explain this discrepancy through the complexity of factors that marginal the rhizosphere AP activity (Nannipieri et al., 2011; Margalef et al., 2017; Nannipieri et al., 2018). The physiological status of the plant (Clausing et al., 2021), species or genotype identity (Denton et al., 2006; Razavi et al., 2016; Ma et al., 2018b; Meller et al., 2020), or soil phosphorus level (Hofmann et al., 2016; Wang et al., 2022b) contribute to AP activity in the rhizosphere.

The acid phosphatase was the only enzyme which supported the first part of our second hypothesis that enzyme activities are

higher in spruce than in beech rhizosphere. Rejsek et al. (2012) have reported higher AP activity in spruce than beech forests because of differences in plant-released AP activity. In contrast, microbial-derived AP activity was similar in beech and spruce soil (Rejsek et al., 2012). Other studies also have suggested a high contribution of the plant over microbial AP activity in the rhizosphere (Nannipieri et al., 2011; Rejsek et al., 2012; Spohn & Kuzyakov, 2013; Hou et al., 2015).

Cellobiohydrolase and LAP activities were higher in beech than in spruce rhizosphere primarily because of the root size. Cellobiohydrolase CBH was related to root morphology in beech, but not in spruce, while LAP was the only enzyme related to root morphological parameters both in beech and spruce. In contrast with AP activity that may consist of a significant plant-derived contribution, CBH (Payne et al., 2015; Sanaullah et al., 2016) and LAP (Zhang et al., 2019; Greenfield et al., 2020) activities in the rhizosphere are likely produced by microbial enzymes. Thus, CBH and LAP activities were related to the root size as microbial activity relies on root exudation (Kuzyakov and Razavi, 2019) that positively correlates with the root size (Badri and Vivanco, 2009; Kuzyakov and Razavi, 2019) and root morphology (Meier et al., 2020). Moreover, CBH is involved in plant cell degradation by microorganism's penetration in the endosphere (Schmidt et al., 2018) and the degradation of dead root fragments (Berlemont and Martiny, 2013; López-Mondéjar et al., 2016). A larger root surface and amount of dead material that increases with the root size may contribute to the observed higher CBH activity in beech than in the spruce rhizosphere.

The nutrient acquisition ratio representing the microbial investment in acquiring nutrients to maintain their internal stoichiometry for C/N was similar between beech and spruce and close to 1.0, indicating no microbial C vs N limitations (Sinsabaugh and Shah, 2012). The acquisition ratios of C/P and N/P were < 1.0 and higher in spruce than in beech. However, we cannot interpret them in the way of P limitation, although a higher AP activity is commonly related to P depletion (Nannipieri et al., 2011), as AP activity was related to trees and not related to microorganism requirements.

## Broader AP and CBH activity extents in spruce than beech rhizosphere soil

The extents of enzyme activities in the rhizosphere were in the order AP > LAP > CBH in both species. This finding is consistent with those of Razavi et al. (2016) and Ma et al. (2018b). They reported the same pattern in herbaceous plants, likely reflecting the plant and microbial demand for nutrients or, in the case of microorganisms, the availability of rhizodeposits, which represent their primary energy source (Burns et al., 2013).

The rhizosphere extents of AP activity were more significant in spruce than beech, supporting the higher AP activity found in the spruce rhizosphere. Taken together, these results indicate a

higher potential to mobilise P from organic sources for spruce than beech. We speculate that better access to organic P reflects the spruce strategy to cope with nutrient limitation at the expense of investment in root development (Matjaž and Primož, 2010; Schall et al., 2012). Nevertheless, caution is due here as the measured enzyme activities are potential values, which do not indicate the *in situ* rates of enzymatically catalysed reactions and are not representative enough of a biogeochemical process that involves numerous enzymes (Nannipieri et al., 2018).

Surprisingly, in contrast to rhizosphere CBH activity, CBH extents were greater in spruce than in beech rhizosphere. We found a positive relationship between CBH activity extents and root hair lengths that benefited spruce, which have longer root hairs than beech. A similar association was also apparent in herbaceous plants (Ma, et al., 2018). We explain this pattern by CBH particularity of being released by microorganisms, whose activities are increased by the potential rhizosphere enrichment in exudates produced by root hairs (Czarnota et al., 2003; Datta et al., 2011). Nevertheless, the relationship between the rhizosphere enzyme extent and root hair length was apparent for CBH but not for LAP and AP activities. This finding emphasises the presence of high amounts of polysaccharides, which may occur in the root hair-build rhizosheath (York et al., 2016) or may result from the fast root hair turnover (Tester and Leigh, 2001). In beech, the decrease in CBH activity resulted in a reduction of C/N acquisition ratio, which may indicate a potential increase in N mineralisation, improving soil N availability (but see Mori (2020) for the relevance of enzymatic stoichiometry). This proposition is also based on the observation that although no differences in LAP extents were apparent between beech and spruce, we may emphasise a higher ability of spruce than beech to release LAP. We observed that the Hill curve's shape showed an abrupt decline in LAP activity compared with AP and CBH activities. In beech, the decline was in the proximity of the root centre while in spruce at the edge of the root radius. Greenfield et al. (2020) have shown that plant-release LAP activity occurs on the root surface but not in the rhizosphere, where microbial LAP activity is mainly present. We may link the observed LAP activity decline to the moment when the plant-released LAP activity ceased.

This study shows that spatial distribution of the rhizosphere enzyme activities differs between European beech and Norway spruce young seedlings which may result in different abilities to acquire nutrients and cope with nutrient limitations. The differences were apparent both in the form and strength of the relationship between the rhizoplane enzyme activities and also with regard to the extent of enzyme activities in the rhizosphere. Spruce seedlings showed higher variability in the spatial distribution of enzyme activity in the rhizoplane and rhizosphere soil than beech seedlings. In contrast, beech seedlings showed a larger positive association of enzyme activities with root morphology. We speculate that these abilities enable spruce to mobilise nutrients from heterogeneous forest soils better than beech, which in compensation, has higher plasticity to adjust biomass

partitioning and root morphology and enhance enzyme activities. Beech is more successful in natural regeneration than spruce (Pretzsch et al., 2015). Spruce regeneration demands favourable light and climatic conditions to overcome the seedling establishment (Diaci, 2002; Hanssen et al., 2003; Mottet et al., 2021). However, under climate change, the strategy of spruce to mobilise nutrients by investing less in root biomass and more in enzyme distribution might surpass the beech strategy.

## Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

## Author contributions

BS and RP conceived and designed the study, analysed data, and drafted the manuscript; BR interpreted the results, and revised the manuscript and data presentations. All authors contributed to the article and approved the submitted version.

## Funding

We also acknowledge financial support from the China Scholarship Council (CSC) through a PhD scholarship to BS (No. 201506300046) and Deutsche Forschungsgemeinschaft (DFG) Priority Program 1374 "Infrastructure-Biodiversity-Exploratories" (PE 2256/3-1).

## Acknowledgments

We thank Huanying Feng for lab help and Huili Shi for support with the sampling. We are grateful to Andrea Polle for her continuous support and Mark Tibbett for the critical reading of the manuscript.

## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

## Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or



claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

## Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fpls.2022.987112/full#supplementary-material>

### SUPPLEMENTARY FIGURE 1

Distribution of enzyme activities on the rhizoplane of European beech. The curves present the power-law fitting of enzyme activities as a

function of vertical distance from the root apex in the regions with different root hair length (RHL). Cellobiohydrolase (CBH); leucine-aminopeptidase (LAP); acid phosphomonoesterase (AP). RHL = 0 mm (A–C); RHL = 0.03 mm (D–F); RHL = 0.04 mm (G–I); RHL > 0.04 mm (J–L). The models include data from 85 individual measuring points per plant. (N = 4).

### SUPPLEMENTARY FIGURE 2

Distribution of enzyme activities on the rhizoplane of Norway spruce. The curves present the power-law fitting of enzyme activities as a function of vertical distance from the root apex in the regions of different root diameter (RD). Cellobiohydrolase (CBH); leucine-aminopeptidase (LAP); acid phosphomonoesterase (AP). RD ≤ 0.24 mm (A–C); RD = 0.25 mm (D–F); RD = 0.26 mm (G, H, I); RD = 0.27 mm (J–L); RD ≥ 0.28 mm (M–O).

## References

- AminiTabrizi, R., Dontsova, K., Grachet, N. G., and Tfaily, M. M. (2022). Elevated temperatures drive abiotic and biotic degradation of organic matter in a peat bog under oxic conditions. *Sci. Total Environ.* 804, 150045. doi: 10.1016/j.scitotenv.2021.150045
- Badri, D. V., and Vivanco, J. M. (2009) Regulation and function of root exudates. *Plant Cell Environ.* 32, 666–681. doi: 10.1111/j.1365-3040.2009.01926.x
- Baier, R., Ettl, R., Hahn, C., and Göttlein, A. (2006). Early development and nutrition of Norway spruce (*Picea abies* (L.) karst.) seedlings on different seedbeds in the Bavarian limestone Alps - a bioassay. *Ann. For. Sci.* 63, 339–348. doi: 10.1051/forest:2006014
- Baldrian, P., and Štursová, M. (2011). Enzymes in forest soils, in: G. Shukla and A. Varma (Eds.), *Soil Enzymology, Soil Biology*. Berlin, Heidelberg: Springer, 61–73. doi: 10.1007/978-3-642-14225-3\_4
- Berlemont, R., and Martiny, A. C. (2013). Phylogenetic distribution of potential cellulases in bacteria. *Appl. Environ. Microbiol.* 79, 1545–1554. doi: 10.1128/AEM.03305-12
- Bidel, L. P., Renault, P., Pagès, L., and Rivièrè, L. M. (2000). Mapping meristem respiration of *Prunus persica* (L.) batsch seedlings: potential respiration of the meristems, O<sub>2</sub> diffusional constraints and combined effects on root growth. *J. Exp. Bot.* 51, 755–768. doi: 10.1093/jexbot/51.345.755
- Bolte, A., and Villanueva, I. (2006). Interspecific competition impacts on the morphology and distribution of fine roots in European beech (*Fagus sylvatica* L.) and Norway spruce (*Picea abies* (L.) karst.). *Eur. J. For. Res.* 125, 15–26. doi: 10.1007/s10342-005-0075-5
- Brtnicky, M., Kintl, A., Hammerschmidt, T., Mustafa, A., Elbl, J., Kucerik, J., et al. (2021). Clover species specific influence on microbial abundance and associated enzyme activities in rhizosphere and non-rhizosphere soils. *Agronomy-Basel* 11, 2214. doi: 10.3390/agronomy11112214
- Brzostek, E. R., Greco, A., Drake, J. E., and Finzi, A. C. (2013). Root carbon inputs to the rhizosphere stimulate extracellular enzyme activity and increase nitrogen availability in temperate forest soils. *Biogeochemistry* 115, 65–76. doi: 10.1007/s10533-012-9818-9
- Burns, R. G., DeForest, J. L., Marxsen, J., Sinsabaugh, R. L., Stromberger, M. E., Wallenstein, M. D., et al. (2013). Soil enzymes in a changing environment: current knowledge and future directions. *Soil Biol. Biochem.* 58, 216–234. doi: 10.1016/j.soilbio.2012.11.009
- Calvaruso, C., Collignon, C., Kies, A., and Turpault, M. P. (2014). Seasonal evolution of the rhizosphere effect on major and trace elements in soil solutions of Norway spruce (*Picea abies* karst) and beech (*Fagus sylvatica*) in an acidic forest soil. *Open J. Soil Sci.* 4, 323–336. doi: 10.4236/ojss.2014.49034
- Clausing, S., Pena, R., Song, B., Müller, K., Mayer-Gruner, P., Marhan, S., et al. (2021). Carbohydrate depletion in roots impedes phosphorus nutrition in young forest trees. *New Phytol.* 229, 2611–2624. doi: 10.1111/nph.17058
- Collet, C., and Le Moguedec, G. (2007). Individual seedling mortality as a function of size, growth and competition in naturally regenerated beech seedlings. *Forestry: Int. J. For. Res.* 80, 359–370. doi: 10.1093/forestry/cpm016
- Czarnota, M. A., Paul, R. N., Weston, L. A., and Duke, S. O. (2003). Anatomy of sorgoleone-secreting root hairs of sorghum species. *Int. J. Plant Sci.* 164, 861–866. doi: 10.1086/378661
- Datta, S., Kim, C. M., Pernas, M., Pires, N. D., Proust, H., Tam, T., et al. (2011). Root hairs: development, growth and evolution at the plant-soil interface. *Plant Soil* 346, 1–14. doi: 10.1007/s11104-011-0845-4
- Denton, M. D., Sasse, C., Tibbett, M., and Ryan, M. H. (2006). Root distributions of Australian herbaceous perennial legumes in response to phosphorus placement. *Funct. Plant Biol.* 33, 1091–1102. doi: 10.1071/FP06176
- Diaci, J. (2002). Regeneration dynamics in a Norway spruce plantation on a silver fir-beech forest site in the Slovenian Alps. *For. Ecol. Manage.* 161, 27–38. doi: 10.1016/S0378-1127(01)00492-3
- Dijkstra, F. A., Carrillo, Y., Pendall, E., and Morgan, J. A. (2013). Rhizosphere priming: a nutrient perspective. *Front. Microbiol.* 4. doi: 10.3389/fmicb.2013.00216
- Dong, S., Brooks, D., Jones, M. D., and Grayston, S. J. (2007). A method for linking *in situ* activities of hydrolytic enzymes to associated organisms in forest soils. *Soil Biol. Biochem.* 39, 2414–2419. doi: 10.1016/j.soilbio.2007.03.030
- Dupuy, L. X., and Silk, W. K. (2016). Mechanisms of early microbial establishment on growing root surfaces. *Vadose Zone J.* 15, 1–13. doi: 10.2136/vzj2015.06.0094
- FAO (2010). “Global forest resources assessment 2010 - main report,” in *FAO forestry paper*, vol. 163. Available at: <http://www.fao.org/3/i1757e/i1757e00.htm>.
- Figueiredo, A. F., Boy, J., and Guggenberger, G. (2021). Common mycorrhizae network: A review of the theories and mechanisms behind underground interactions. *Front. Fungal Biol.* 30. doi: 10.3389/ffunb.2021.735299
- Firsching, B. M., and Claassen, N. (1996). Root phosphatase activity and soil organic phosphorus utilization by Norway spruce [*Picea abies* (L.) karst.]. *Soil Biol. Biochem.* 28, 1417–1424. doi: 10.1016/S0038-0717(96)00149-6
- Fox, J., and Weisberg, S. (2018). *An r companion to applied regression* (SAGE Publications Inc). Available at: <https://us.sagepub.com/en-us/nam/an-r-companion-to-applied-regression/book246125>.
- García, J. A. L., Domenech, J., Santamaria, C., Camacho, M., Daza, A., and Mañero, F. J. G. (2004). Growth of forest plants (pine and holm-oak) inoculated with rhizobacteria: relationship with microbial community structure and biological activity of its rhizosphere. *Environ. Exp. Bot.* 52, 239–251. doi: 10.1016/j.envexpbot.2004.02.003
- Ge, T. D., Wei, X. M., Razavi, B. S., Zhu, Z. K., Hu, Y. J., Kuzyakov, Y., et al. (2017). Stability and dynamics of enzyme activity patterns in the rice rhizosphere: effects of plant growth and temperature. *Soil Biol. Biochem.* 113, 108–115. doi: 10.1016/j.soilbio.2017.06.005
- Giles, C. D., Dupuy, L., Boitt, G., Brown, L. K., Condron, L. M., Darch, T., et al. (2018). Root development impacts on the distribution of phosphatase activity: improvements in quantification using soil zymography. *Soil Biol. Biochem.* 116, 158–166. doi: 10.1016/j.soilbio.2017.08.011
- Godlewski, M., and Adamczyk, B. (2007). The ability of plants to secrete proteases by roots. *Plant Physiol. Biochem.* 45, 657–664. doi: 10.1016/j.plaphy.2007.06.001
- Greaver, T. L., Clark, C. M., Compton, J. E., Vallano, D., Talhelm, A. F., Weaver, C. P., et al. (2016). Key ecological responses to nitrogen are altered by climate change. *Nat. Climate Change* 6, 836–843. doi: 10.1038/nclimate3088
- Greenfield, L. M., Hill, P. W., Paterson, E., Baggs, E. M., and Jones, D. L. (2020). Do plants use root-derived proteases to promote the uptake of soil organic nitrogen? *Plant Soil* 456, 355–367. doi: 10.1007/s11104-020-04719-6
- Guber, A., Kravchenko, A., Razavi, B. S., Uteau, D., Peth, S., Blagodatskaya, E., et al. (2018). Quantitative soil zymography: mechanisms, processes of substrate and enzyme diffusion in porous media. *Soil Biol. Biochem.* 127, 156–167. doi: 10.1016/j.soilbio.2018.09.030

- Haichar, F. Z., Marol, C., Berge, O., Rangel-Castro, J. I., Prosser, J. I., Balesdent, J., et al. (2008). Plant host habitat and root exudates shape soil bacterial community structure. *ISME J.* 2, 1221–1230. doi: 10.1038/ismej.2008.80
- Hanssen, K. H., Granhus, A., Brække, F. H., and Haverbaaen, O. (2003). Performance of sown and naturally regenerated *Picea abies* seedlings under different scarification and harvesting regimens. *Scandinavian J. For. Res.* 18, 351–361. doi: 10.1080/02827580310005973
- Harley, J. L. (1939). The early growth of beech seedlings under natural and experimental conditions. *J. Ecol.* 27, 384–400. doi: 10.2307/2256365
- Harley, J. L. (1940). A study of the root system of the beech in woodland soils, with especial reference to mycorrhizal infection. *J. Ecol.* 28, 107–117. doi: 10.2307/2256165
- Harley, J. L., and Waid, J. S. (1955). The effect of light upon the roots of beech and its surface population. *Plant Soil* 7, 96–112. doi: 10.1007/BF01343547
- Häussling, M., Jorns, C. A., Lehmbecker, G., Hecht-Buchholz, Ch., and Marschner, H. (1988). Ion and water uptake in relation to root development in Norway spruce (*Picea abies* (L.) karst.). *J. Plant Physiol.* 133, 486–491. doi: 10.1016/S0176-1617(88)80042-7
- Helliwell, J. R., Sturrock, C. J., Mairhofer, S., Craigon, J., Ashton, R. W., Miller, A. J., et al. (2017). The emergent rhizosphere: imaging the development of the porous architecture at the root-soil interface. *Sci. Rep.* 7, 14875. doi: 10.1038/s41598-017-14904-w
- Herre, M., Heitkoetter, J., Heinze, S., Rethemeyer, J., Preusser, S., Kandler, E., et al. (2022). Differences in organic matter properties and microbial activity between bulk and rhizosphere soil from the top- and subsoils of three forest stands. *Geoderma* 409, 115589. doi: 10.1016/j.geoderma.2021.115589
- Hiltner, L. (1904). Ueber neuere erfahrungen und probleme auf dem gebiete der bodenbakteriologie und unter besonderer Berücksichtigung der grundung und brache. *Arb. Deut. Landw. Gesell.* 98, 59–78.
- Hinsinger, P. (1998). How do plant roots acquire mineral nutrients? chemical processes involved in the rhizosphere. *Adv. Agron.* 225, 225–265. doi: 10.1016/S0065-2113
- Hinsinger, P., Bengough, A. G., Vetterlein, D., and Young, I. M. (2009). Rhizosphere: biophysics, biogeochemistry and ecological relevance. *Plant Soil* 321, 117–152. doi: 10.1007/s11104-008-9885-9
- Hinsinger, P., Brauman, A., Devau, N., Gérard, F., Jourdan, C., Laclau, J.-P., et al. (2011). Acquisition of phosphorus and other poorly mobile nutrients by roots. where do plant nutrition models fail? *Plant Soil* 348, 29. doi: 10.1007/s11104-011-0903-y
- Hinsinger, P., Plassard, C., and Jaillard, B. (2006). Rhizosphere: A new frontier for soil biogeochemistry. *J. Geochem. Explor.* 88, 210–213. doi: 10.1016/j.jexplo.2005.08.041
- Hofmann, K., Heuck, C., and Spohn, M. (2016). Phosphorus resorption by young beech trees and soil phosphatase activity as dependent on phosphorus availability. *Oecologia* 181, 369–379. doi: 10.1007/s00442-016-3581-x
- Högborg, P., and Högborg, M. N. (2022). Does successful forest regeneration require the nursing of seedlings by nurse trees through mycorrhizal interconnections? *For. Ecol. Manag.* 516, 120252. doi: 10.1016/j.foreco.2022.120252
- Holz, M., Zarebanadkouki, M., Kuzyakov, Y., Pausch, J., and Carminati, A. (2018). Root hairs increase rhizosphere extension and carbon input to soil. *Ann. Bot.* 121, 61–69. doi: 10.1093/aob/mcx127
- Hou, E., Chen, C., Wen, D., and Liu, X. (2015). Phosphatase activity in relation to key litter and soil properties in mature subtropical forests in China. *Sci. Total Environ.* 515, 83–91. doi: 10.1016/j.scitotenv.2015.02.044
- Huang, R., Zhang, Z., Xiao, X., Zhang, N., Wang, X., Yang, Z., et al. (2019). Structural changes of soil organic matter and the linkage to rhizosphere bacterial communities with biochar amendment in manure fertilized soils. *Sci. Total Environ.* 692, 333–343. doi: 10.1016/j.scitotenv.2019.07.262
- Hummel, C., Boitt, G., Santner, J., Lehto, N. J., Condon, L., and Wenzel, W. W. (2021). Co-Occurring increased phosphatase activity and labile p depletion in the rhizosphere of lupinus angustifolius assessed with a novel, combined 2D-imaging approach. *Soil Biol. Biochem.* 153, 107963. doi: 10.1016/j.soilbio.2020.107963
- Jaeger, C. H., Lindow, S. E., Miller, W., Clark, E., and Firestone, M. K. (1999). Mapping of sugar and amino acid availability in soil around roots with bacterial sensors of sucrose and tryptophan. *Appl. Environ. Microbiol.* 65, 2685–2690. doi: 10.1128/AEM.65.6.2685-2690.1999
- Jonard, M., Fürst, A., Verstraeten, A., Thimonier, A., Timmermann, V., Potočić, N., et al. (2015). Tree mineral nutrition is deteriorating in Europe. *Global Change Biol.* 21, 418–430. doi: 10.1111/gcb.12657
- Kang, H. B., Xue, Y., Yan, C. L., Lu, S., Yang, H., Zhu, J. Q., et al. (2022). Contrasting patterns of microbial nutrient limitations between rhizosphere and bulk soil during stump sprout restoration in a clear-cut oak forest. *For. Ecol. Manage.* 515, 120241. doi: 10.1016/j.foreco.2022.120241
- Kante, M., Riah-Anglet, W., Cliquet, J. B., and Trinsoutrot-Gattin, I. (2021). Soil enzyme activity and stoichiometry: linking soil microorganism resource requirement and legume carbon rhizodeposition. *Agronomy-Basel* 11, 2131. doi: 10.3390/agronomy11112131
- Karhu, K., Alaei, S., Li, J., Merilä, P., Ostonen, I., and Bengtson, P. (2022). Microbial carbon use efficiency and priming of soil organic matter mineralization by glucose additions in boreal forest soils with different C:N ratios. *Soil Biol. Biochem.* 167, 108615. doi: 10.1016/j.soilbio.2022.108615
- Kolstad, A. L., Asplund, J., Nilsson, M. C., Ohlson, M., and Nybakken, L. (2016). Soil fertility and charcoal as determinants of growth and allocation of secondary plant metabolites in seedlings of European beech and Norway spruce. *Environ. Exp. Bot.* 131, 39–46. doi: 10.1016/j.envexpbot.2016.06.013
- Kuzyakov, Y., and Cheng, W. (2001). Photosynthesis controls of rhizosphere respiration and organic matter decomposition. *Soil Biol. Biochem.* 33, 1915–1925. doi: 10.1016/S0038doi
- Kuzyakov, Y., Friedel, J. K., and Stahr, K. (2000). Review of mechanisms and quantification of priming effects. *Soil Biol. Biochem.* 32, 1485–1498. doi: 10.1016/S0038-0717(00)00084-5
- Kuzyakov, Y., and Razavi, B. S. (2019). Rhizosphere size and shape: temporal dynamics and spatial stationarity. *Soil Biol. Biochem.* 135, 343–360. doi: 10.1016/j.soilbio.2019.05.011
- Laporte, M. A., Denaix, L., Pagès, L., Sterckeman, T., Flénet, F., Dauguet, S., et al. (2013). Longitudinal variation in cadmium influx in intact first order lateral roots of sunflower (*Helianthus annuus* L.). *Plant Soil* 372, 581–595. doi: 10.1007/s11104-013-1756-3
- Lensi, R., Lescure, C., Steinberg, C., Savoie, J.-M., and Faurie, G. (1991). Dynamics of residual enzyme activities, denitrification potential, and physico-chemical properties in a  $\gamma$ -sterilized soil. *Soil Biol. Biochem.* 23, 367–373. doi: 10.1016/0038-0717(91)90193-N
- Leuschner, C., and Ellenberg, H. (2017). *Ecology of central European forests: vegetation ecology of central Europe, volume I* (Springer International Publishing). Available at: <https://www.springer.com/de/book/9783319430409>.
- Leuschner, C., Hertel, D., Coners, H., and Büttner, V. (2001). Root competition between beech and oak: a hypothesis. *Oecologia* 126, 276–284. doi: 10.1007/s004420000507
- Li, M., Schmidt, J. E., LaHue, D. G., Lazicki, P., Kent, A., Machmuller, M. B., et al. (2020). Impact of irrigation strategies on tomato root distribution and rhizosphere processes in an organic system. *Front. Plant Sci.* 11. doi: 10.3389/fpls.2020.00360
- Lobet, G., Pagès, L., and Draye, X. (2011). A novel image-analysis toolbox enabling quantitative analysis of root system architecture. *Plant Physiol.* 157, 29–39. doi: 10.1104/pp.111.179895
- López-Mondéjar, R., Zühlke, D., Becher, D., Riedel, K., and Baldrian, P. (2016). Cellulose and hemicellulose decomposition by forest soil bacteria proceeds by the action of structurally variable enzymatic systems. *Sci. Rep.* 6, 25279. doi: 10.1038/srep25279
- Luster, J., Göttlein, A., Nowack, B., and Sarret, G. (2009). Sampling, defining, characterising and modeling the rhizosphere-the soil science tool box. *Plant Soil* 321, 457–482. doi: 10.1007/s11104-008-9781-3
- Macía-Vicente, J. G., Nam, B., and Thines, M. (2020). Root filtering, rather than host identity or age, determines the composition of root-associated fungi and oomycetes in three naturally co-occurring brassicaceae. *Soil Biol. Biochem.* 146, 107806. doi: 10.1016/j.soilbio.2020.107806
- Ma, X., Liu, Y., Zarebanadkouki, M., Razavi, B. S., Blagodatskaya, E., and Kuzyakov, Y. (2018). Spatiotemporal patterns of enzyme activities in the rhizosphere: Effects of plant growth and root morphology. *Biol. Fertility Soils* 54, 819–828. doi: 10.1007/s00374-018-1305-6
- Marchi, E., Chung, W., Visser, R., Abbas, D., Nordfjell, T., Mederski, P. S., et al. (2018). Sustainable forest operations (SFO): a new paradigm in a changing world and climate. *Sci. Total Environ.* 634, 1385–1397. doi: 10.1016/j.scitotenv.2018.04.084
- Margalef, O., Sardans, J., Fernández-Martínez, M., Molowny-Horas, R., Janssens, I. A., Ciais, P., et al. (2017). Global patterns of phosphatase activity in natural soils. *Sci. Rep.* 7, 1337. doi: 10.1038/s41598-017-01418-8
- Marschner, P., Crowley, D., and Rengel, Z. (2011). Rhizosphere interactions between microorganisms and plants govern iron and phosphorus acquisition along the root axis—model and research methods. *Soil Biol. Biochem.* 43, 883–894. doi: 10.1016/j.soilbio.2011.01.005
- Massalha, H., Korenblum, E., Malitsky, S., Shapiro, O. H., and Aharoni, A. (2017). Live imaging of root-bacteria interactions in a microfluidics setup. *Proc. Natl. Acad. Sci.* 114, 4549–4554. doi: 10.1073/pnas.1618584114
- Matjaž, Č., and Primož, S. (2010). Root distribution of under-planted European beech (*Fagus sylvatica* L.) below the canopy of a mature Norway spruce stand as a function of light. *Eur. J. For. Res.* 129, 531–539. doi: 10.1007/s10342-009-0352-9

- Ma, X., Zarebanadkouki, M., Kuzyakov, Y., Blagodatskaya, E., Pausch, J., and Razavi, B. S. (2018b). Spatial patterns of enzyme activities in the rhizosphere: effects of root hairs and root radius. *Soil Biol. Biochem.* 118, 69–78. doi: 10.1016/j.soilbio.2017.12.009
- McCully, M. E. (1999). Roots in soil: unearthing the complexities of roots and their rhizospheres. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 50, 695–718. doi: 10.1146/annurev.arplant.50.1.695
- Meier, I. C., Tückmantel, T., Heitkötter, J., Müller, K., Preusser, S., Wrobel, T. J., et al. (2020). Root exudation of mature beech forests across a nutrient availability gradient: the role of root morphology and fungal activity. *New Phytol.* 226, 583–594. doi: 10.1111/nph.16389
- Meller, S., Frossard, E., Spohn, M., and Luster, J. (2020). Plant nutritional status explains the modifying effect of provenance on the response of beech sapling root traits to differences in soil nutrient supply. *Front. Forests Global Change* 3. doi: 10.3389/fgc.2020.535117
- Minz, D., Ofek, M., and Hadar, Y. (2013). “Plant rhizosphere microbial communities,” in *The prokaryotes: Prokaryotic communities and ecophysiology*. Eds. E. Rosenberg, E. F. DeLong, S. Lory, E. Stackebrandt and F. Thompson (Berlin Heidelberg: Springer), 215–243. doi: 10.1007/978-3-642-30123-0\_38
- Mori, T. (2020). Does coenzymatic stoichiometry really determine microbial nutrient limitations? *Soil Biol. Biochem.* 146, 107816. doi: 10.1016/j.soilbio.2020.107816
- Mottet, M.-J., Lambert, M.-C., and DeBlois, J. (2021). Natural regeneration of Norway spruce, an introduced species, in and around plantations in Quebec, Canada. *For. Ecol. Manage.* 498, 119553. doi: 10.1016/j.foreco.2021.119553
- Nahberger, T. U., Benucci, G. M. N., Kraigher, H., and Grebenc, T. (2021). Effect of earthworms on mycorrhization, root morphology and biomass of silver fir seedlings inoculated with black summer truffle (*Tuber aestivum* vittad.). *Sci. Rep.* 11, 6167. doi: 10.1038/s41598-021-85497-8
- Nannipieri, P., Ascher-Jenull, J., Ceccherini, M. T., Landi, L., Pietramellara, G., Renella, G., et al. (2007). Microbial diversity and microbial activity in the rhizosphere. *Ciencia Del Suelo* 25, 89–97.
- Nannipieri, P., Gagnoni, L., Landi, L., and Renella, G. (2011). “Role of phosphatase enzymes in soil,” in *Phosphorus in action: Biological processes in soil phosphorus cycling*. Eds. E. Bütemann, A. Oberson and E. Frossard (Berlin Heidelberg: Springer), 215–243. doi: 10.1007/978-3-642-15271-9\_9
- Nannipieri, P., Trasar-Cepeda, C., and Dick, R. P. (2018). Soil enzyme activity: a brief history and biochemistry as a basis for appropriate interpretations and meta-analysis. *Biol. Fertility Soils* 54, 11–19. doi: 10.1007/s00374-017-1245-6
- Newton, A. C., and Pigott, C. D. (1991). Mineral nutrition and mycorrhizal infection of seedling oak and birch. *New Phytol.* 117, 45–52. doi: 10.1111/j.1469-8137.1991.tb00943.x
- Nguyen, C. (2003). Rhizodeposition of organic C by plants: Mechanisms and controls. *Sustain. Agric.* 23, 375–396. doi: 10.1051/agro:2003011
- Nguyen, D., Pena, R., and Polle, A. (2017). Impact of ectomycorrhizal community composition and soil treatment on inorganic nitrogen nutrition and performance of beech (*Fagus sylvatica* L.) provenances. *Trees* 31, 1891–1904. doi: 10.1007/s00468-017-1594-7
- Niklas, K. J. (1994). *Plant Allometry: The Scaling of Form and Process* (Chicago: University of Chicago Press.).
- Odrizola, I., Martinovic, T., Bahnmann, B. D., Rysanek, D., Masinova, T., Sedlak, P., et al. (2020). Stand age affects fungal community composition in a central European temperate forest. *Fungal Ecol.* 48, 100985. doi: 10.1016/j.funeco.2020.100985
- Payne, C. M., Knott, B. C., Mayes, H. B., Hansson, H., Himmel, M. E., Sandgren, M., et al. (2015). Fungal cellulases. *Chem. Rev.* 115, 1308–1448. doi: 10.1021/cr500351c
- Pei, Z., Eichenberg, D., Bruelheide, H., Kröber, W., Kühn, P., Li, Y., et al. (2016). Soil and tree species traits both shape soil microbial communities during early growth of Chinese subtropical forests. *Soil Biol. Biochem.* 96, 180–190. doi: 10.1016/j.soilbio.2016.02.004
- Pena, R., Simon, J., Rennenberg, H., and Polle, A. (2013). Ectomycorrhiza affect architecture and nitrogen partitioning of beech (*Fagus sylvatica* L.) seedlings under shade and drought. *Environ. Exp. Bot.* 87, 207–217. doi: 10.1016/j.envexpbot.2012.11.005
- Pierret, A., Gonkhamdee, S., Jourdan, C., and Maeght, J. L. (2013). IJ\_Rhizo: an open-source software to measure scanned images of root samples. *Plant Soil* 373, 531–539. doi: 10.1007/s11104-013-1795-9
- Pretzsch, H., Biber, P., Uhl, E., and Dauber, E. (2015). Long-term stand dynamics of managed spruce–fir–beech mountain forests in central Europe: structure, productivity and regeneration success. *Forestry: Int. J. For. Res.* 88, 407–428. doi: 10.1093/forestry/cpv013
- Puhe, J. (2003). Growth and development of the root system of Norway spruce (*Picea abies*) in forest stands—a review. *For. Ecol. Manage.* 175, 253–273. doi: 10.1016/S0378-1127(02)00134-2
- Razavi, B. S., Zarebanadkouki, M., Blagodatskaya, E., and Kuzyakov, Y. (2016). Rhizosphere shape of lentil and maize: spatial distribution of enzyme activities. *Soil Biol. Biochem.* 96, 229–237. doi: 10.1016/j.soilbio.2016.02.020
- Razavi, B., Zhang, X., Bilyera, N., Guber, A., and Zarebanadkouki, M. (2019). Soil zymography: simple and reliable? review of current knowledge and optimization of the method. *Rhizosphere* 11, 100161. doi: 10.1016/j.rhisph.2019.100161
- R Core Team (2020) *R: The R project for statistical computing*. Available at: <https://www.r-project.org/>.
- Reinhold-Hurek, B., Büniger, W., Burbano, C. S., Sabale, M., and Hurek, T. (2015). Roots shaping their microbiome: global hotspots for microbial activity. *Annu. Rev. Phytopathol.* 53, 403–424. doi: 10.1146/annurev-phyto-082712-102342
- Rejsek, K., Vranova, V., and Formanek, P. (2012). Determination of the proportion of total soil extracellular acid phosphomonoesterase (E.C. 3.1.3.2) activity represented by roots in the soil of different forest ecosystems. *Sci. World J.* 250805. doi: 10.1100/2012/250805
- Ren, C. J., Zhou, Z. H., Guo, Y. X., Yang, G. H., Zhao, F. Z., Wei, G. H., et al. (2021). Contrasting patterns of microbial community and enzyme activity between rhizosphere and bulk soil along an elevation gradient. *Catena* 196, 104921. doi: 10.1016/j.catena.2020.104921
- Sanaullah, M., Razavi, B. S., Blagodatskaya, E., and Kuzyakov, Y. (2016). Spatial distribution and catalytic mechanisms of  $\beta$ -glucosidase activity at the root-soil interface. *Biol. Fertility Soils* 52, 505–514. doi: 10.1007/s00374-016-1094-8
- Schall, P., Lödige, C., Beck, M., and Ammer, C. (2012). Biomass allocation to roots and shoots is more sensitive to shade and drought in European beech than in Norway spruce seedlings. *For. Ecol. Manage.* 266, 246–253. doi: 10.1016/j.foreco.2011.11.017
- Schmid, I. (2002). The influence of soil type and interspecific competition on the fine root system of Norway spruce and European beech. *Basic Appl. Ecol.* 3, 339–346. doi: 10.1078/1439-1791-00116
- Schmidt, H., Nunan, N., Höck, A., Eickhorst, T., Kaiser, C., Woebken, D., et al. (2018). Recognizing patterns: spatial analysis of observed microbial colonization on root surfaces. *Front. Environ. Sci.* 6. doi: 10.3389/fenvs.2018.00061
- Semenov, A. M., van Bruggen, A. H. C., and Zelenev, V. V. (1999). Moving waves of bacterial populations and total organic carbon along roots of wheat. *Microbial Ecol.* 37, 116–128. doi: 10.1007/s002489900136
- Sinsabaugh, R. L., and Shah, J. J. F. (2012). Coenzymatic stoichiometry and ecological theory. *Annu. Rev. Ecol. Evolution Syst.* 43, 313–343. doi: 10.1146/annurev-ecolsys-071112-124414
- Smith, S. E., and Read, D. J. (2010). *Mycorrhizal Symbiosis* (London: Academic Press).
- Sokal, R., and Rohlf, F. (2013). *Biometry: the principles and practice of statistics in biological research*. New York: W.H. Freeman and Company.
- Solly, E. F., Schöning, I., Boch, S., Kandeler, E., Marhan, S., Michalzik, B., et al. (2014). Factors controlling decomposition rates of fine root litter in temperate forests and grasslands. *Plant Soil* 382, 203–218. doi: 10.1007/s11104-014-2151-4
- Spohn, M., and Kuzyakov, Y. (2013). Distribution of microbial- and root-derived phosphatase activities in the rhizosphere depending on p availability and C allocation—coupling soil zymography with 14C imaging. *Soil Biol. Biochem.* 67, 106–113. doi: 10.1016/j.soilbio.2013.08.015
- Steinauer, K., Chatzinotas, A., and Eisenhauer, N. (2016). Root exudate cocktails: the link between plant diversity and soil microorganisms? *Ecol. Evol.* 6, 7387–7396. doi: 10.1002/eece3.2454
- Tester, M., and Leigh, R. A. (2001). Partitioning of nutrient transport processes in roots. *J. Exp. Bot.* 52, 445–457. doi: 10.1093/jexbot/52.suppl\_1.445
- Turner, B. L., and Haygarth, P. M. (2005). Phosphatase activity in temperate pasture soils: Potential regulation of labile organic phosphorus turnover by phosphodiesterase activity. *Sci. Total Environ.* 344, 27–36. doi: 10.1016/j.scitotenv.2005.02.003
- Uroz, S., Buee, M., Deveau, A., Mieszkun, S., and Marten, F. (2016a). Ecology of the forest microbiome: highlights of temperate and boreal ecosystems. *Soil Biol. Biochem.* 103, 471–488. doi: 10.1016/j.soilbio.2016.09.006
- Uroz, S., Oger, P., Tisserand, E., Cébron, A., Turpault, M.-P., Buee, M., et al. (2016b). Specific impacts of beech and Norway spruce on the structure and diversity of the rhizosphere and soil microbial communities. *Sci. Rep.* 6, 27756. doi: 10.1038/srep27756
- van der Heijden, M. G. A., and Schlaeppli, K. (2015). Root surface as a frontier for plant microbiome research. *Proc. Natl. Acad. Sci. U.S.A.* 112, 2299–2300. doi: 10.1073/pnas.1500709112
- Wagner, S., Collet, C., Madsen, P., Nakashizuka, T., Nyland, R. D., Sagheb-Talebi, K., et al. (2010). Beech regeneration research: From ecological to



silvicultural aspects. *For. Ecol. Manag., The ecology and silviculture of beech: from gene to landscape*. 259, 2172–2182. doi: 10.1016/j.foreco.2010.02.029

Wang, C. G., Brunner, I., Wang, J. N., Guo, W., Geng, Z. Z., Yang, X. Y., et al. (2022a). The right-skewed distribution of fine-root size in three temperate forests in northeastern China. *Front. Plant Sci.* 12. doi: 10.3389/fpls.2021.772463

Wang, Z., Göttlein, A., and Bartonek, G. (2001). Effects of growing roots of Norway spruce (*Picea abies* [L.] karst.) and European beech (*Fagus sylvatica* L.) on rhizosphere soil solution chemistry. *J. Plant Nutr. Soil Sci.* 164, 35–41. doi: 10.1002/1522-2624(200102)164

Wang, M. M., Wu, Y. C., Zhao, J. Y., Liu, Y., Chen, Z., Tang, Z. Y., et al. (2022b). Long-term fertilization lowers the alkaline phosphatase activity by impacting the *phoD*-harboring bacterial community in rice-winter wheat rotation system. *Sci. Total Environ.* 821, 153406. doi: 10.1016/j.scitotenv.2022.153406

Warren Wilson, J., and Harley, J. L. (1983). The development of mycorrhiza on seedlings of *fagus sylvatica* L. *New Phytol.* 95, 673–695. doi: 10.1111/j.1469-8137.1983.tb03532.x

Watt, M., Silk, W. K., and Passioura, J. B. (2006). Rates of root and organism growth, soil conditions, and temporal and spatial development of the rhizosphere. *Ann. Bot.* 97, 839–855. doi: 10.1093/aob/mcl028

Wei, X., Razavi, B. S., Hu, Y., Hu, Y., Xu, X., Zhu, Z., et al. (2019b). C/P stoichiometry of dying rice root defines the spatial distribution and dynamics of enzyme activities in root-detritusphere. *Biol. Fertility Soils* 55, 251–263. doi: 10.1007/s00374-019-01345-y

Wei, X., Zhu, Z., Wei, L., Wu, J., and Ge, T. (2019a). Biogeochemical cycles of key elements in the paddy-rice rhizosphere: microbial mechanisms and coupling processes. *Rhizosphere* 10, 100145. doi: 10.1016/j.rhisph.2019.100145

Wen, Z., White, P. J., Shen, J., and Lambers, H. (2022). Linking root exudation to belowground economic traits for resource acquisition. *New Phytol.* 233, 1620–1635. doi: 10.1111/nph.17854

Wilcox, H. E. (1968). Morphological studies of the root of red pine, *pinus resinosa* A. growth characteristics and patterns of branching. *Am. J. Bot.* 55, 247–254. doi: 10.1002/j.1537-2197.1968.tb06968.x

Xiong, Q., Hu, J., Wei, H., Zhang, H., and Zhu, J. (2021). Relationship between plant roots, rhizosphere microorganisms, and nitrogen and its special focus on rice. *Agriculture-Basel* 11, 234. doi: 10.3390/agriculture11030234

Xue, Y., Kang, H. B., Cui, Y. X., Lu, S., Yang, H., Zhu, J. Q., et al. (2022). Consistent plant and microbe nutrient limitation patterns during natural vegetation restoration. *Front. Plant Sci.* 13. doi: 10.3389/fpls.2022.885984

York, L. M., Carminati, A., Mooney, S. J., Ritz, K., and Bennett, M. J. (2016). The holistic rhizosphere: integrating zones, processes, and semantics in the soil influenced by roots. *J. Exp. Bot.* 67, 3629–3643. doi: 10.1093/jxb/erw108

Zarebanadkouki, M., Kroener, E., Kaestner, A., and Carminati, A. (2014). Visualization of root water uptake: quantification of deuterated water transport in roots using neutron radiography and numerical modeling. *Plant Physiol.* 166, 487–499. doi: 10.1104/pp.114.243212

Zelenev, V. V., van Bruggen, A. H. C., and Semenov, A. M. (2000). BACWAVE: a spatial-temporal model for traveling waves of bacterial populations in response to a moving carbon source in soil. *Microbial Ecol.* 40, 260–272. doi: 10.1007/s002480000029

Zelezniak, P., Hrenko, M., Then, C., Koch, N., Grebenc, T., Levanic, T., et al. (2007). CASIROZ: root parameters and types of ectomycorrhiza of young beech plants exposed to different ozone and light regimes. *Plant Biol. (Stuttgart Germany)* 9, 298–308. doi: 10.1055/s-2006-955916

Zhang, X., Dippold, M. A., Kuzyakov, Y., and Razavi, B. S. (2019). Spatial pattern of enzyme activities depends on root exudate composition. *Soil Biol. Biochem.* 133, 83–93. doi: 10.1016/j.soilbio.2019.02.010

Zwetsloot, M. J., Kessler, A., and Bauerle, T. L. (2018). Phenolic root exudate and tissue compounds vary widely among temperate forest tree species and have contrasting effects on soil microbial respiration. *New Phytol.* 218, 530–541. doi: 10.1111/nph.15041



## OPEN ACCESS

## EDITED BY

Enrique García De La Riva,  
Doñana Biological Station (CSIC),  
Spain

## REVIEWED BY

Tianshan Zha,  
Beijing Forestry University, China  
Gabriela Woźniak,  
University of Silesia in Katowice,  
Poland

## \*CORRESPONDENCE

Michele Dalle Fratte  
michele.dallefratte@gmail.com

## SPECIALTY SECTION

This article was submitted to  
Functional Plant Ecology,  
a section of the journal  
Frontiers in Plant Science

RECEIVED 06 October 2022

ACCEPTED 07 November 2022

PUBLISHED 24 November 2022

## CITATION

Dalle Fratte M, Montagnoli A, Anelli S,  
Armiraaglio S, Beatrice P, Ceriani A,  
Liprerì E, Miali A, Nastasio P and  
Cerabolini BEL (2022) Mulching in  
lowland hay meadows drives an  
adaptive convergence of above- and  
below-ground traits reducing plasticity  
and improving biomass: A possible  
tool for enhancing phytoremediation.  
*Front. Plant Sci.* 13:1062911.  
doi: 10.3389/fpls.2022.1062911

## COPYRIGHT

© 2022 Dalle Fratte, Montagnoli, Anelli,  
Armiraaglio, Beatrice, Ceriani, Liprerì,  
Miali, Nastasio and Cerabolini. This is an  
open-access article distributed under  
the terms of the [Creative Commons  
Attribution License \(CC BY\)](#). The use,  
distribution or reproduction in other  
forums is permitted, provided the  
original author(s) and the copyright  
owner(s) are credited and that the  
original publication in this journal is  
cited, in accordance with accepted  
academic practice. No use,  
distribution or reproduction is  
permitted which does not comply with  
these terms.

# Mulching in lowland hay meadows drives an adaptive convergence of above- and below-ground traits reducing plasticity and improving biomass: A possible tool for enhancing phytoremediation

Michele Dalle Fratte<sup>1\*</sup>, Antonio Montagnoli<sup>1</sup>, Simone Anelli<sup>2</sup>,  
Stefano Armiraaglio<sup>3</sup>, Peter Beatrice<sup>1</sup>, Alex Ceriani<sup>1</sup>,  
Elia Liprerì<sup>3</sup>, Alessio Miali<sup>1</sup>, Paolo Nastasio<sup>2</sup>  
and Bruno Enrico Leone Cerabolini<sup>1</sup>

<sup>1</sup>Department of Biotechnologies and Life Sciences (DBSV), University of Insubria, Varese, Italy,

<sup>2</sup>Ente Regionale per i Servizi all'Agricoltura e alle Foreste della Lombardia (ERSAF), Milan, Italy,

<sup>3</sup>Municipality of Brescia - Museum of Natural Sciences, Brescia, Italy

We aimed to understand the effect of mulching (i.e., cutting and leaving the crushed biomass to decompose in situ) on above- and below-ground plant functional traits and whether this practice may be a potential tool for enhancing the phytoremediation of lowland hay meadows. To this aim, we evaluated at the community level seven years of mulching application in a PCBs and HMs soil-polluted Site of National Interest (SIN Brescia-Caffaro) through the analysis of the floristic composition and the above- and below-ground plant traits. We found that the abandonment of agricultural activities led to a marked increase in the soil organic carbon and pH, and the over-imposed mulching additionally induced a slight increase in soil nutrients. Mulching favored the establishment of a productive plant community characterized by a more conservative-resource strategy, a higher biomass development, and lower plasticity through an adaptive convergence between above- and below-ground organs. In particular, the analysis of the root depth distribution highlighted the key role of roots living in the upper soil layer (10 cm). Mulching did not show a significant effect on plant species known to be effective in terms of PCB phytoremediation. However, the mulching application appears to be a promising tool for enhancing the root web that functions as the backbone for the proliferation of microbes devoted to organic contaminants' degradation and selects a two-fold number of plant species known to be metal-tolerant.



However, besides these potential positive effects of the mulching application, favoring species with a higher biomass development, in the long term, may lead to a biodiversity reduction and thus to potential consequences also on the diversity of native species important for the phytoremediation.

#### KEYWORDS

**fine-root traits, functional traits, global plant spectrum, heavy metals, leaf economics spectrum, phytoremediation, PCB, root economics spectrum**

## 1 Introduction

Phytoremediation is the use of plants to remediate pollutants in contaminated soil, water, and air. It encompasses several methods for contaminant degradation, removal, or immobilization (Mackova et al., 2010). In the last 30 years, many examples described plant species accumulating and/or metabolizing organic xenobiotics, (see Mackova et al., 2010 and references therein). Despite having lots of information about the use of plants for phytoremediation purposes, much work is still necessary to forecast all aspects of its beneficial application (McCutcheon and Schnoor, 2003; Macek et al., 2004; Mackova et al., 2010), especially for recalcitrant soil contaminants such as persistent organic pollutants (POPs) and heavy metals (HMs). Regarding phytoremediation, the rhizosphere is of particular relevance since it hosts microbial communities with different metabolism that depend on the chemicals released from plant roots (McNear, 2013; Reinhold-Hurek et al., 2015). However, field testing is still needed to extend the theoretical knowledge and the practical experience learned from model plants to native plant communities.

Many hydrophobic organic compounds, including PCBs, although banned since the 1970s, are priority soil contaminants because of their toxicity and tendency to persist in soils/sediments and to escape biological degradation (Passatore et al., 2014). Therefore, PCBs phytoremediation has attracted increasing attention (Mackova et al., 2009; Mackova et al., 2010; Vergani et al., 2017; Jiang et al., 2022). At the same time, the problem of HMs' pollution is becoming more and more severe with increasing industrialization even because, unlike organic substances, HMs are completely non-biodegradable (Mackova et al., 2010; Ali et al., 2013). Several investigations have shown that PCBs and HMs can be translocated from soil to various parts of the plants and can accumulate in higher concentrations in particular tissues than in others. Plants can uptake HMs from the soil through plant roots and translocate them to shoots (Tangahu et al., 2011; Ali et al., 2013). Conversely, plants can accumulate PCBs from the soil into the roots (Mackova et al., 2009; Terzaghi et al., 2019; Terzaghi et al., 2020) and from air

into leaves even if the roots-to-leaves transfer is generally limited by the high hydrophobicity of these chemicals (Collins et al., 2006). However, the sorption of metalloids, metals, and organic compounds, is also controlled by the soil organic matter (Cornelissen et al., 2005; Branzini and Zubillaga, 2012). The application of carbon-rich charcoal-like materials such as biochar and activated carbon has been proposed, for example, as a tool for the *in-situ* stabilization of organic contaminants in soils (Beesley et al., 2011; Denyes et al., 2013). However, the relative increase in soil carbon fractions due to charcoal-like materials amendment can vary depending on the environmental conditions (Chagas et al., 2022). It is, therefore, necessary to test other methods for increasing soil organic matter based on material collected *in situ* and less affected by the multiple local factors.

Mulching has been used since the 1990s as a low-cost alternative to grazing or conventional mowing for abandoned grasslands maintenance (Mašková et al., 2009). The method consists in cutting the above-ground biomass and crushing the clippings into pieces several centimeters long that are left on the site to decompose and release a large proportion of their mineral nutrient content (Gaisler et al., 2004; Doležal et al., 2011; Metsoja et al., 2012). There have been several studies dealing with the effects of mulching on grasslands/meadows (Gaisler et al., 2019; and references therein), but only a few of these were conducted over a long period (Moog et al., 2002; Mašková et al., 2009; Römermann et al., 2009; Gaisler et al., 2013; Oelmann et al., 2017; Gaisler et al., 2019). Although mulching can represent an alternative way of increasing soil carbon content in grassland soils (Jordán et al., 2010), it has never been considered a potential tool for enhancing phytoremediation in contaminated areas. Indeed, compared to traditional management practices (i.e., grazing and/or mowing), mulching leaves above-ground biomass to decompose *in situ* and thus can be beneficial in two ways: for the phytoremediation of soil contaminants and to avoid the problem of the disposal of the contaminated biomass. However, it is not still clear whether alternative management treatments in grasslands, such as mulching, can lead to changes in floristic and functional composition.

Although mulching can have similar effects to traditional management on the floristic composition due to the removal of taller plants and prevention of succession (Pavlů et al., 2016; Gaisler et al., 2019), there is contrasting evidence about its impact on species richness (Moog et al., 2002; Mašková et al., 2009; Doležal et al., 2011; Gaisler et al., 2013; Gaisler et al., 2019). Also, its effect on the above- and below-ground plant community structure and functions represented by plant functional traits can be much different (Kahmen et al., 2002; Kahmen and Poschlod, 2008; Römermann et al., 2009; Doležal et al., 2011; Gaisler et al., 2019). Plant functional traits are key features of individual organisms related to their fitness and responses to environmental conditions (Dalle Fratte et al., 2019b) and are indeed strongly related to management measures (De Bello et al., 2010; Lavorel et al., 2011). In herbaceous plant communities, management types characterized by intermediate levels of disturbance can facilitate average levels of competitiveness, stress tolerance and ruderality, while an excessive productivity load can lead to more competitive communities (Grime, 2006a; Cerabolini et al., 2016). A slight increase in competitive and acquisitive plants with mulching treatment has been observed (Moog et al., 2002; Römermann et al., 2009; Doležal et al., 2011). Since mulching has been associated with higher soil nutrient levels (Oelmann et al., 2017) the development of more productive plant communities is reasonable, but this may negatively affect species richness (Grime, 2006a; Pierce, 2014).

Differences in land use types and management are essential for plant rooting (Ravenek et al., 2016; Tasser et al., 2021), but a complete evaluation of the effects of mulching on above- and below-ground plant traits (e.g., fine-root traits) coordination remain poorly characterized. Crucially, there is still no information concerning the effects of mulching on below-ground plant functional traits. Mowing and fertilization, which can be considered the two main factors of mulching, have been applied separately, but only the fertilization resulted in a considerable root traits shift (Leuschner et al., 2013). The increment of soil organic matter due to mulching treatment may reduce the survival of individual roots, increasing the fine-root turnover rate (Pritchard et al., 2010), resulting in a general suppression of fine-root length independently of the diameter class considered (Simiele et al., 2022). Trait-based approaches emerged in the last decades to address different ecological questions from the individual to plant-community level (Díaz et al., 2016; Bruelheide et al., 2018). However, a complete understanding of the trade-offs between above- and below-ground functional traits is difficult to reach (Carmona et al., 2021; De la Riva et al., 2021; Weigelt et al., 2021), also because root traits remain poorly investigated (Kattge et al., 2020). Specifically, Carmona et al. (2021) pointed out that the trade-offs between above-ground and fine-root traits seem decoupled. Indeed, their coordination may strongly influence plant competition, community structure, and plant-environment

interactions (Tumber-Dávila et al., 2022). In particular, the more productive niche imposed by mulching may drive a functional convergence of plant traits (Grime, 2006b), and this should involve both above- and below-ground traits. Thus, it becomes crucial to investigate these two components of plant communities to get a full view of the adaptive ways of plant communities to changes in environmental features due to global changes or different management regimes.

Following the imposed abandonment in the 1990s of agricultural areas in the Site of National Interest (SIN) Brescia-Caffaro (Northern Italy) due to soil contamination by organic and inorganic pollutants, mulching has recently been introduced as a potential tool for phytoremediation of soil contaminants. In this study we focused on the effects of mulching on above- and below-ground traits of these communities, and we hypothesized that: 1) mulching favors the establishment of plant communities typical of stable hay meadows, 2) mulching drives the selection of a more productive plant community by reducing the species richness, 3) mulching led to an adaptive convergence between above- and below-ground traits at the plant community level, 4) mulching can select plant species that are metal-tolerant and/or active in PCB degradation. To test our multiple hypothesis, we analyzed the floristic-vegetation composition, above- and below-ground biomass, and community-level leaves and fine-root traits of the plant communities of the abandoned agricultural areas, and we compared their values between areas subject to mowing and mulching and areas subject to traditional mowing.

## 2 Methods

### 2.1 Study site and plot selection

We carried out this study in the agricultural areas of the Site of National Interest (SIN) Brescia-Caffaro, Northern Italy (45° 32.365' N, 10° 11.123' E), a site heavily contaminated by the polluted exhaust water of the factory, which was used for more than 50 years for irrigation (Di Guardo et al., 2020). The Caffaro factory was one of the larger PCB producers in Europe until 1984. In that year, the surrounding areas were found to be heavily contaminated by PCBs of more than 80 congeners, including PCB 209, the decachlorinated PCB at concentrations in the order of tens of mg kg<sup>-1</sup> of total PCBs (Terzaghi et al., 2019), but also PCDDs, PCDFs, DDT and its isomers, metalloids and HMs (e.g., As up to 79 mg Kg<sup>-1</sup>, Hg up to 4 mg Kg<sup>-1</sup>, and Pb up to 447 mg Kg<sup>-1</sup>) exceeding the threshold concentration of contamination (Di Guardo et al., 2017). Therefore, the agricultural activity on the site has been banned for the last four decades. Since 2014, mowing and mulching have been introduced every 3 to 4 times a year. The cut biomass is then crushed into pieces 5 – 10 cm long and left in place to favor the coverage of herbaceous species capable of developing

rhizosphere degradation processes while preventing the settlement of woody plant species.

Within the same pedologic unit, we selected two sampling areas corresponding to the control (NM; no mulching) and mulching-treated (MU) areas (Figure 1). These soils are deep silty loam with a poor skeleton in the topsoil, typical of coarse limestone floods (Fluventic Hapludolls; Soil Survey Staff, 2014). In 2014 they were characterized by greater cation exchange capacity (CEC) in the upper part (0–40 cm), pH values falling within the range 7.6–7.8, and almost stable up to 100 cm depth, with higher soil organic carbon (SOC), total soil nitrogen and sulfur content (respectively,  $N_{tot}$  and  $S_{tot}$ ) in the upper 10 cm compared to the depth 60–100 cm. Soil calcium and magnesium content (Ca and Mg) showed higher values in the first 30 cm depth and lower values at greater depth, while soil iron and potassium content (Fe and K) were almost stable along the depth (Table 1 and Supplementary Figure 1). The maximum concentration of organic contaminants in the soils of the study

site is usually within the first 40 cm of soil (Di Guardo et al., 2020).

Both NM and MU areas had an extension of about 1.1 ha (Figure 1). The NM area was characterized by the mowing practices carried out regularly over the years, which implies the removal of cut material. Before mulching treatment (2014), the study site was homogeneously characterized by synanthropic and ruderal vegetation. This vegetation type was related to the cultivation abandonment that started in the 1980s. The SIN agricultural area has a plain surface with a negligible elevation difference, less than 70 cm for the study site (Figure 1). Indeed, we focused on a single field of the study site to avoid any potential bias due to the legacy of different past agricultural activities. Moreover, the spatial distribution of NM and MU areas has been based on the ongoing management activities established by the responsible organization (ERSAF – Ente Regionale per i servizi all'Agricoltura e alle Foreste, Lombardia).

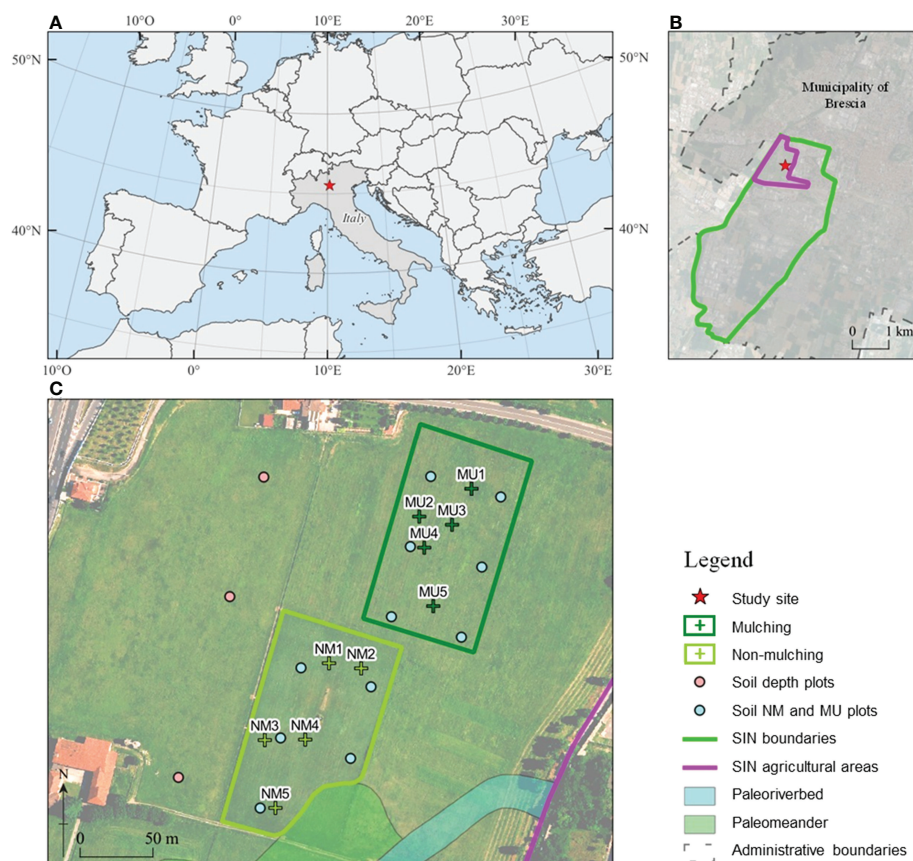


FIGURE 1

Location of the National Relevance Site (SIN) Brescia-Caffaro in Northern Italy (A) with a detail of the contaminated agricultural areas (B), and of the control (NM; no mulching) and mulching-treated (MU) areas sampled within the study site (C). Crosses indicates the location of the vegetation sampling points, and circles indicate the location of the soil sampling points: pink, soil sampled each 10 cm layer up to 100 cm depth in 2014, light blue, mixed homogeneous soil sampled up to 40 cm in 2014 and 2020.

TABLE 1 Average values ( $\pm$  standard deviation) of the main physico-chemical soil properties in relation to soil depth layers of the three points sampled in 2014 up to 100 cm depth (see Figure 1).

soil depth	sand (%)	silt (%)	clay (%)	CEC (meq 100g <sup>-1</sup> )	pH	SOC (g Kg <sup>-1</sup> )	N <sub>tot</sub> (g Kg <sup>-1</sup> )	S (g Kg <sup>-1</sup> )	Ca (g Kg <sup>-1</sup> )	Fe (g Kg <sup>-1</sup> )	Mg (g Kg <sup>-1</sup> )	K (g Kg <sup>-1</sup> )
0-10	57.00 $\pm$ 10.34	35.60 $\pm$ 9.70	7.38 $\pm$ 0.99	24.63 $\pm$ 1.94	7.76 $\pm$ 0.19	20.27 $\pm$ 5.98	2.18 $\pm$ 0.32	0.38 $\pm$ 0.02	32.07 $\pm$ 7.20	28.2 $\pm$ 1.5	22.93 $\pm$ 3.04	11.40 $\pm$ 0.53
10-20	55.34 $\pm$ 9.91	35.00 $\pm$ 8.65	9.63 $\pm$ 1.44	23.63 $\pm$ 1.19	7.59 $\pm$ 0.08	13.73 $\pm$ 8.95	1.53 $\pm$ 0.17	0.28 $\pm$ 0.04	28.73 $\pm$ 5.25	25.87 $\pm$ 4.16	21.43 $\pm$ 4.97	10.16 $\pm$ 1.67
20-30	52.17 $\pm$ 12.76	38.34 $\pm$ 12.95	9.52 $\pm$ 0.86	22.67 $\pm$ 1.17	7.80 $\pm$ 0.26	14.67 $\pm$ 7.68	1.42 $\pm$ 0.30	0.29 $\pm$ 0.01	31.63 $\pm$ 5.76	28.87 $\pm$ 2.87	22.77 $\pm$ 3.18	9.78 $\pm$ 1.67
30-40	52.60 $\pm$ 7.49	36.43 $\pm$ 6.26	10.96 $\pm$ 1.28	22.60 $\pm$ 1.39	7.76 $\pm$ 0.09	15.00 $\pm$ 6.01	1.08 $\pm$ 0.33	0.26 $\pm$ 0.04	34.53 $\pm$ 6.32	28.4 $\pm$ 2.49	26.17 $\pm$ 4.76	10.97 $\pm$ 3.02
40-60	46.60 $\pm$ 3.24	41.87 $\pm$ 4.27	11.50 $\pm$ 1.01	21.43 $\pm$ 1.36	7.79 $\pm$ 0.07	10.63 $\pm$ 5.19	0.68 $\pm$ 0.17	0.20 $\pm$ 0.02	35.50 $\pm$ 8.25	27.97 $\pm$ 1.42	26.97 $\pm$ 5.56	10.94 $\pm$ 1.94
60-80	48.52 $\pm$ 7.02	40.03 $\pm$ 5.61	11.43 $\pm$ 1.45	18.70 $\pm$ 1.31	7.86 $\pm$ 0.03	10.73 $\pm$ 7.71	0.42 $\pm$ 0.30	0.17 $\pm$ 0.03	42.77 $\pm$ 2.20	27 $\pm$ 2.17	28.33 $\pm$ 3.71	10.55 $\pm$ 1.36
80-100	43.56 $\pm$ 6.78	44.93 $\pm$ 6.66	11.47 $\pm$ 0.76	16.33 $\pm$ 1.10	7.96 $\pm$ 0.15	7.08 $\pm$ 6.13	0.25 $\pm$ 0.01	0.14 $\pm$ 0.03	49.83 $\pm$ 1.46	25.13 $\pm$ 2.68	29.80 $\pm$ 4.39	9.54 $\pm$ 0.55
ALL	50.83 $\pm$ 8.60	38.89 $\pm$ 7.67	10.27 $\pm$ 1.74	21.43 $\pm$ 3.01	7.79 $\pm$ 0.16	13.16 $\pm$ 7.02	1.08 $\pm$ 0.68	0.24 $\pm$ 0.08	36.44 $\pm$ 8.46	27.35 $\pm$ 2.56	25.49 $\pm$ 4.69	10.48 $\pm$ 1.58

CEC, cation exchange capacity; SOC, organic carbon; Ntot, total nitrogen; Stot, total sulfur; Ca, calcium; Fe, iron; Mg, magnesium; K, potassium.

## 2.2 Sampling and data collection

### 2.2.1 Experimental design and floristic-vegetation analysis

During the 2021 within each area (NM and MU), five points were randomly selected for vegetation analysis, using a minimum distance of 20 m from each other and an internal buffer of 10 m to avoid any disturbances due to edge effects. Each point corresponds to the center of a 4 x 4 m squared plot for a total of ten floristic-vegetation relevés (five in the NM and five in the MU areas). The coverage of all vascular plant species was determined by visual estimation and recorded directly on a percentage scale. The plant species evaluated for their phytoremediation capability of PCB-contaminated soils (Vergani et al., 2017) and known as metal-tolerant (Landolt et al., 2010) were listed for each relevé.

The nomenclature used for plant species is based on the most recent checklist of the native and alien vascular plants of Italy (Bartolucci et al., 2018; Galasso et al., 2018). Within each plot, a 2 x 2 m core area was selected and divided into 16 subplots of 50 x 50 cm to measure the biomass. Two of these subplots were randomly selected for a total of 20 biomass samples (ten in the NM and ten in the MU areas). The floristic-vegetation relevés and the biomass sampling were carried out on the same days (24 and 25 June 2021), coinciding with the estimated peak biomass. In the center of each subplot, we then sampled 10 cm soil cores (diameter and height of 4 and 10 cm, respectively), reaching a depth of 40 cm using a motor-driven core drill. We selected a depth of 10 cm interval because this is the standard unit for sampling root biomass considering depth increments (e.g., Fort et al., 2013; Freschet et al., 2021a; Baronti et al., 2022).

### 2.2.2 Above-ground traits analysis

For each species, leaf functional traits representative of the plant size and economics (i.e., leaf area, LA; leaf dry matter content, LDMC; specific leaf area, SLA; leaf nitrogen content, LNC) were obtained from the authors' datasets (FIFTH and LIFTH, see Cerabolini et al., 2010; Dalle Fratte et al., 2021) accessible through the TRY database (Kattge et al., 2020, <https://www.trydb.org>; see datasets n. 227, 228, 229, 371, 372 and the forthcoming 467). Above-ground standing crop and litter (Al-Mufti et al., 1977) were sampled in the whole 50 x 50 cm subplot using an electric lawn mower and oven dried (70°C for 24 h) to obtain the above-ground dry weight (AGDW).

### 2.2.3 Below-ground traits analysis

The soil cores were immediately stowed in a portable refrigerator and then stored in the laboratory at 4°C until their analysis. Each sample of soil cores was placed in a nylon bag (400  $\mu$ m mesh) closed at one end with a zip tie; each nylon bag was inserted into the washing machine drum and automatically



washed with cold water to let the soil sieve out so that only roots and stones remained within the nylon bag (adapted from Benjamin and Nielsen, 2004). Within each washed nylon bag, all the fine-root material was separated from the rest (organic and mineral fraction of the soil and stone materials) using tweezers and a stereomicroscope.

The root samples were immersed in water in order to avoid drying and consequent shrinkage and scanned at a resolution of 800 dpi with a calibrated scanner coupled with a transparency unit (Epson Expression 10,000 XL). We estimated the below-ground dry weight (BGDW) of each 10 cm soil layer by drying it in an oven at 70°C for 24 h. Finally, the scanned images were analyzed with WinRhizo Pro V. 2007d software (Regent Instruments Inc., Quebec, Canada) to obtain morphological data such as root length (RL) and mean root diameter (MRD), as well as RL in each diameter class of 0.1 mm interval.

## 2.2.4 Soil chemical analysis

In 2014 and 2020 we also analyzed soil characteristics such as pH, SOC and  $N_{tot}$  in the upper 40 cm of soil in 11 points distributed within each sampling area (five in the NM and six in the MU areas) (Figure 1). We sampled soil cores up to a depth of 40 cm; the entire length was mixed and used for the chemical analysis.

## 2.3 Data analysis

To determine the mulching effect on the floristic composition of plant communities we calculated the indicator species for each of the two groups of relevés (NM vs. MU areas) using the “multipatt” function of the “indicspecies” R package (De Cáceres and Legendre, 2009); we applied the correlation index based on abundance data (r.g) which is more sensitive to the local ecological context.

For each soil depth layer (0 - 10, 10 - 20, 20 - 30, and 30 - 40 cm) we calculated the following root traits: below-ground dry weight (BGDW), mean root diameter (MRD), root length (RL), specific root length (SRL), and fine-root percentage (FRP). The FRP was calculated as the percentage of RL with a diameter < 0.1 mm (Fort et al., 2013); SRL was calculated by dividing the RL by BGDW (Ostonen et al., 2007; Pérez-Harguindeguy et al., 2013; Montagnoli et al., 2019). For each root trait and class of soil depth, we first calculated the average value between the two subplots, and we then summarized the same traits at the plot-level (0 - 40 cm) as following: RL and BGDW were summed among all soil depth layers, while SRL, MRD and FRP were calculated as the average value among all soil depth layers. The same procedure was also used to calculate the total RL in each class of diameter.

For each relevé we calculated the community weighted mean (CWM) of leaf traits, using as weight the estimated percentage coverage of each species. To estimate the AGDW of each plot we

also calculated the average value between the two subplots. The total biomass for each plot was calculated as the sum of the AGDW and BGDW of each plot. In each of the two groups of relevés (NM vs. MU areas) we then analyzed the relations between biodiversity (i.e., species richness) and productivity (i.e., total biomass) fitting a generalized linear model (GLM) by means of the function “glm” of the “stats” base R package. We compared the community-level traits (both above- and below-ground) between NM and MU areas by means of the Wilcoxon test.

A redundancy analysis (RDA) was performed to determine the relations between community-level explanatory variables (i.e., CWM of leaf traits and community-level root traits, above- and below-ground biomass) that resulted to have a significant effect on the previous analysis and floristic composition of plant communities. For this aim, we used the function “rda” of the “vegan” R package (Oksanen et al., 2022). Before RDA, data were scaled to unit variance. We tested the significance of each constrained axis independently through a permutation test based on 999 randomizations of the rows of the environmental matrix to preserve the correlation between environmental variables (De Bello et al., 2021).

We then compared the differences of soil physico-chemical properties along depth (3 points in 2014) and between NM and MU (12 points in 2014 vs. 2020) by means of the Dunn’s test multiple comparisons using the function “dunn.test” of the package “dunn.test” (Dinno, 2017). The same analysis was also used to compare root traits in relation to soil depth.

All the analyses were done using the R software (R Core Team, 2021).

## 3 Results

### 3.1 Floristic composition and vegetation structure

Considering all the relevés we found a total of 38 vascular plant species, 27 in the NM area and 33 in the MU area (Table 2). The indicator plant species of the MU area were *Arrhenatherum elatius*, *Sorghum halepense*, *Galium mollugo*, and *Convolvulus sepium*, while indicator species in the control area were *Daucus carota*, *Trifolium pratense*, and *Achillea roseoalba* (Table 2). The analyzed relevés did not highlight native plant species known for their significance in conservation or exotic plant species belonging to the regional blacklist of invasive alien species (LR 10/2008 and subsequent updates).

We found 25 metal-tolerant species and seven species of particular interest for the phyto-rhizoremediation of PCB-contaminated soils (Table 2). Among the metal-tolerant species, three were exclusive (i.e., present in a single area only) of the NM area, and seven of the MU area. Indicator plant species recognized as metal-tolerant for the NM area were



**TABLE 2** Synoptic table of the frequency and average coverage of the species in the 10 floristic-vegetation relevés carried out in the control (no-mulching; NM) and mulching-treated (MU) areas.

Species name	Indicator species		Frequency (%)		Coverage (%)		Phyto-rhizoremediation	Metal tolerant
			NM	MU	NM	MU		
<i>Daucus carota</i> L.	Dau_car	** (NM)	100	100	52	6	p	m
<i>Trifolium pratense</i> L. subsp. pratense	Tri_pra	* (NM)	100	80	20	4	p	m
<i>Achillea roseoalba</i> Ehrend.	Ach_ros	* (NM)	100	60	20	3		
<i>Avena barbata</i> Pott ex Link	Ave_bar		80	.	1	.		
<i>Myosotis arvensis</i> (L.) Hill subsp. arvensis	Myo_arv		40	.	1	.		m
<i>Picris hieracioides</i> L. subsp. hieracioides	Pic_hie		20	.	1	.		
<i>Trifolium repens</i> L.	Tri_rep		20	.	1	.		m
<i>Trifolium campestre</i> Schreb.	Tri_cam		20	.	1	.		m
<i>Arrhenatherum elatius</i> (L.) P.Beauv. ex J.Presl & C.Presl subsp. elatius	Arr_ela	* (MU)	100	100	44	73		m
<i>Sorghum halepense</i> (L.) Pers.	Sor_hal	** (MU)	100	100	10	40		
<i>Galium mollugo</i> L.	Gal_mol	** (MU)	100	100	2	10		m
<i>Convolvulus sepium</i> L.	Con_sep	** (MU)	.	100	.	10		m
<i>Medicago lupulina</i> L.	Med_lup		.	60	.	1	p	m
<i>Vicia sativa</i> L.	Vic_sat		.	40	.	1		
<i>Verbena officinalis</i> L.	Ver_off		.	40	.	2		
<i>Rumex crispus</i> L.	Rum_cri		.	40	.	1	p	m
<i>Rumex acetosa</i> L. subsp. acetosa	Rum_ace		.	40	.	1		m
<i>Veronica persica</i> Poir.	Ver_per		.	40	.	1		m
<i>Clematis vitalba</i> L.	Cle_vit		.	40	.	1		m
<i>Hypericum perforatum</i> L.	Hyp_per		.	20	.	2		
<i>Taraxacum</i> F.H.Wigg. sect. <i>Taraxacum</i>	Tar_off		.	20	.	1		m
<i>Lathyrus</i> sp.	Lat_sp.		.	20	.	1		
<i>Erigeron annuus</i> (L.) Desf.	Eri_ann		100	80	6	2		m
<i>Cerastium brachypetalum</i> Desp. ex Pers. subsp. brachypetalum	Cer_bra		100	80	1	1		m
<i>Lotus corniculatus</i> L. subsp. corniculatus	Lot_cor		80	100	13	8		m
<i>Plantago lanceolata</i> L.	Pla_lan		100	60	10	2		m
<i>Lolium perenne</i> L.	Lol_per		100	40	13	4	p	m
<i>Dactylis glomerata</i> L. subsp. glomerata	Dac_glo		80	20	2	1		m
<i>Convolvulus arvensis</i> L.	Con_arv		80	20	9	5		m
<i>Cirsium arvense</i> (L.) Scop.	Cir_arv		40	100	4	6	p	m
<i>Medicago sativa</i> L.	Med_sat		40	40	8	2	p	
<i>Salvia pratensis</i> L. subsp. pratensis	Sal_pra		80	60	19	4		
<i>Clinopodium vulgare</i> L. subsp. vulgare	Cli_vul		40	20	1	3		
<i>Holcus lanatus</i> L. subsp. lanatus	Hol_lan		60	40	6	1		m
<i>Crepis vesicaria</i> L.	Cre-ves		40	20	1	1		
<i>Carex divulsa</i> Stokes	Car_div		20	40	1	2		
<i>Lysimachia arvensis</i> (L.) U.Manns & Anderb. subsp. arvensis	Lys_arv		20	40	1	1		m
<i>Bellis perennis</i> L.	Bel_per		20	20	1	1		m

The last two columns indicate the species of interest for the phytoremediation of PCB contaminated soils (Vergani et al., 2017) and metal-tolerant species (M indicator, Landolt et al., 2010). \*p-value < 0.05, \*\*p-value < 0.01.

*Daucus carota* and *Trifolium pratense*, and for the MU area were *Arrhenatherum elatius*, *Convolvulus sepium*, and *Galium mollugo* (Table 2). For both NM and MU areas, the remaining plant species had low frequency (< 2%) or scarce coverage (<

5%) and were not exclusive to one specific area (e.g., *Lolium perenne*, *Holcus lanatus*; Table 2). The species of interest for phyto-rhizoremediation were two in the NM area (*Daucus carota* and *Trifolium pratense*) and two in the MU area

(*Cirsium arvense* and *Medicago lupulina*). The three other species of interest for phyto-rhizoremediation (*Lolium perenne*, *Medicago sativa*, and *Rumex crispus*) had high frequency (> 40%) but no significant coverage (< 15%) in both NM and MU areas (Table 2).

### 3.2 Above- and below-ground traits

The total biomass (above- plus below-ground) was significantly ( $p < 0.05$ ) higher in the MU area (mean  $619.3 \pm \text{SD } 82.5 \text{ g m}^{-2}$ ) than in the NM area (mean  $1335.3 \pm \text{SD } 210.6 \text{ g m}^{-2}$ ) (Figure 2A). On the contrary, there was no significant difference in species richness between the NM (mean  $17.8 \pm \text{SD } 2.0$  No.) and MU area (mean  $17.8 \pm \text{SD } 3.7$  No.) (Figure 2B). For the MU area, plant species richness exhibited a significant ( $p < 0.01$ ) linear decrease with the increase of total biomass (Figure 2C). On the contrary, in the NM area, we did not find significant relationship between species richness and biomass (Figure 2C). The species richness was higher at intermediate values of the total biomass (plot MU5), and it was lower in correspondence of both the lowest and the highest total biomass values (Figure 2C).

The analysis of above-ground traits showed significantly ( $p < 0.01$ ) higher values of AGDW and LDMC in the MU area (Figures 3A, D). On the contrary, SLA and LNC were found significantly ( $p < 0.01$ ) lower in the MU than in the NM areas (Figures 3C, E). Finally, LA did not show any significant difference (Figure 3B).

Concerning the below-ground traits, BGDW ( $p < 0.1$ ) and SRL ( $p < 0.01$ ) showed a significant increase and decrease,

respectively, in relation to mulching treatment (Figures 3F, H). All other traits did not show significant differences (Figures 3G, I, J).

According to the rooting depth distribution, BGDW was not different between NM and MU areas for all the soil depth layers analyzed (Figure 4A). In the NM area, BGDW did not differ between the first (0 – 10 cm) and the second (10 – 20 cm) soil layer while significantly ( $p < 0.05$ ) decreasing at greater soil depth (20 – 30 and 30 – 40 cm). For the MU area, BGDW in the upper soil layer (0 – 10 cm) was significantly ( $p < 0.05$ ) higher than in the lower soil depth layer (10 – 20 cm). At further depth (20 – 30 cm and 30 – 40 cm) BGDW in the MU area did not significantly differ (Figure 4A).

RL was not different between NM and MU areas for all the soil depth layers analyzed (Figure 4B). In the NM area, RL in the first soil layer was significantly ( $p < 0.05$ ) higher only compared to the third and fourth depth layers (20 – 30 cm and 30 – 40 cm, respectively), but RL did not differ among different soil layers, except the first one. The same pattern was also observable in the MU area, even if the third and fourth layers showed significantly ( $p < 0.05$ ) lower values of RL compared to the second layer (10 – 20 cm, Figure 4B).

In the NM area, SRL was significantly ( $p < 0.05$ ) higher than in the MU area only for the upper soil layer (0 – 10 cm) while at further depth there was no difference (Figure 4C). In the NM area, SRL did not differ between the first, second and third layers, while it was significantly ( $p < 0.05$ ) higher at the lowest soil depth (30 – 40 cm). In the MU area, SRL in the upper soil layer was significantly ( $p < 0.05$ ) lower only compared to the third layer, but there was no difference comparing the other soil depth layers (Figure 4C).

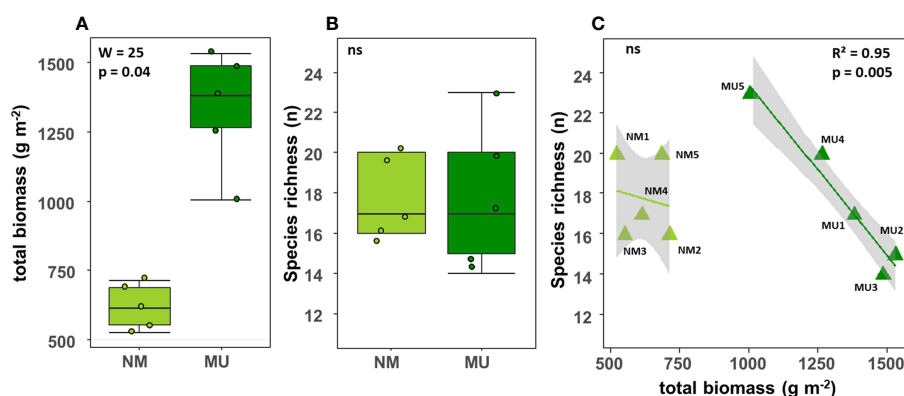


FIGURE 2

Comparison of the total biomass (A) and species richness (B) between control (NM; no mulching) and mulching-treated (MU) areas, and relationship between species richness and total biomass (C) in the 10 sampling plots. The lines in figure C indicate the best fitting and its 95% confidence interval obtained through a generalized linear model (GLM). The boxplot indicates the median (line in the middle of the boxes), the interquartile range (boxes) and 1.5 times the interquartile range (whiskers). Results of the Wilcoxon test (A, B) or GLM model (C) are reported in each subfigure; the  $R^2$  value is the pseudo- $R^2$  of the GLM model; ns, not significant ( $p > 0.05$ ).

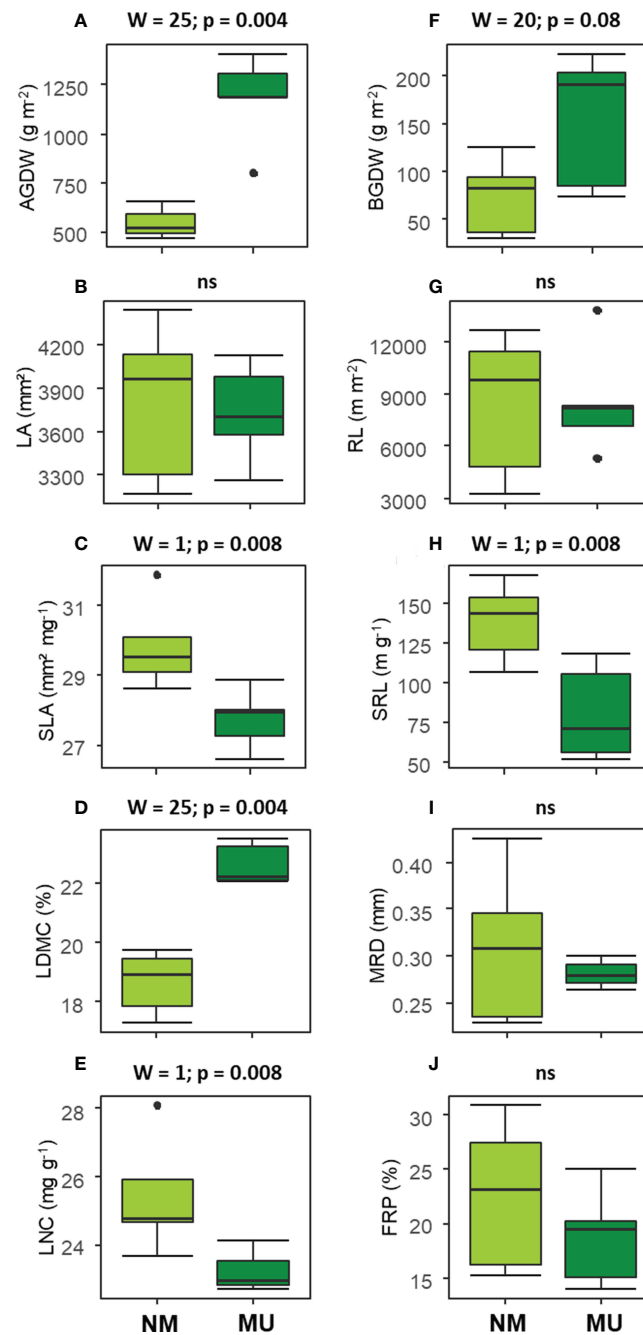


FIGURE 3

Comparison of the community-level above- (A–E) and below-ground traits (F–J) between control (NM; no mulching) and mulching (MU) in the 10 sampling plots. The boxplot indicates the median (line in the middle of the boxes), the interquartile range (boxes), 1.5 times the interquartile range (whiskers) and outliers (circle). Results of the Wilcoxon test are reported in each subfigure; ns, not significant ( $p > 0.1$ ). AGDW and BGDW, above- and below- ground dry weight, FRP, fine-root percentage, LA, community weighted mean (CWM) of leaf area, LDMC, CWM of leaf dry matter content, LNC, CWM of leaf nitrogen content, MRD, mean root diameter, SLA, CWM of specific leaf area, SRL, specific root length, RL, root length.

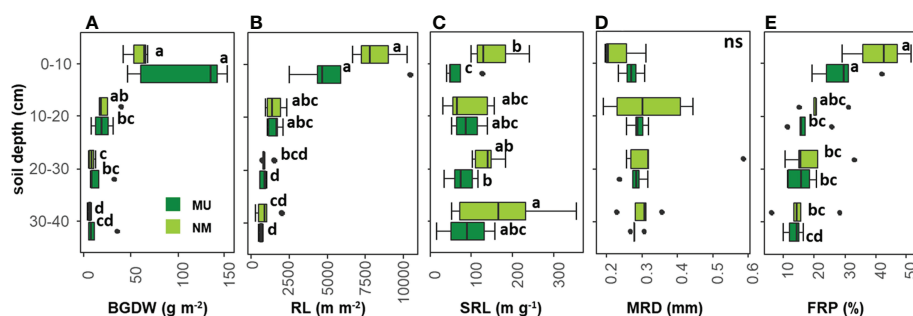


FIGURE 4

Comparison of community-level below-ground traits between control (NM; no mulching) and mulching-treated (MU) areas for each soil depth layer. The boxplot indicates the median (line in the middle of the boxes), the interquartile range (boxes), 1.5 times the interquartile range (whiskers) and outliers (circle). Small letters indicate the results of the Dunn test *post-hoc* comparisons; ns, not significant ( $p > 0.05$ ). BGDW, below-ground dry weight (A); RL, root length (B); SRL, specific root length (C); MRD, mean root diameter (D); FRP, fine-root percentage (E).

MRD did not show significant differences between NM and MU areas for all the soil depth layers, and among the different soil depth layers in both NM and MU areas (Figure 4D).

FRP was not different between NM and MU areas for all the soil depth layers (Figure 4E). In the NM area, FRP in the first soil layer was significantly ( $p < 0.05$ ) higher only compared to the third and fourth depth layers (20 – 30 cm and 30 – 40 cm, respectively), but it did not differ among

different soil layers, except the first one. In the MU area, FRP in the first soil layer was significantly ( $p < 0.05$ ) higher compared to all the other soil layers, but it did not differ among the other soil layers (Figure 4E).

According to different root diameter classes, both NM and MU areas showed higher values of RL within the very-fine fraction ( $d < 0.4$  mm). The NM area had significantly ( $p < 0.05$ , except  $p < 0.1$  for the class 1.3 – 1.4 mm) higher values of

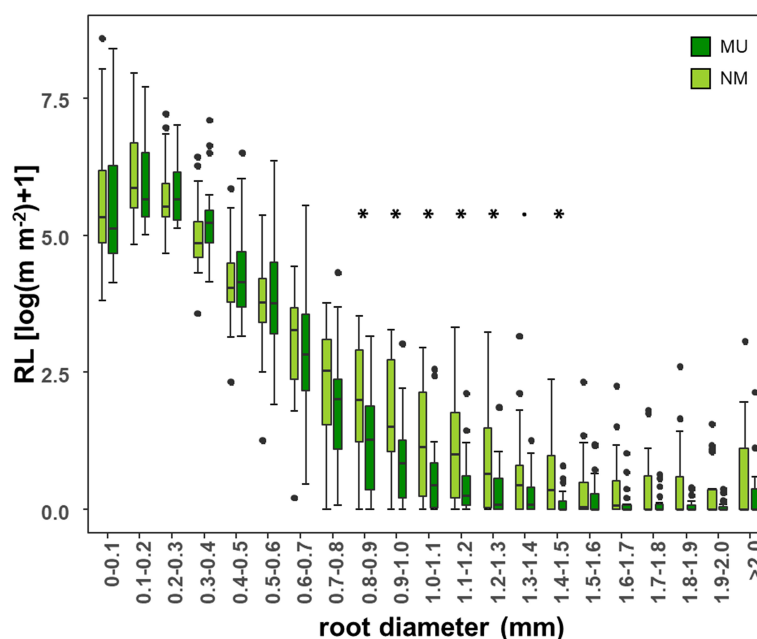


FIGURE 5

Comparison of root length (RL) within each class of root diameter at the community-level between control (NM; no mulching) and mulching-treated (MU) areas in the 10 sampled plots. RL is plotted as logarithmic only for facilitating the visualization of smaller values. The boxplot indicates the median (line in the middle of the boxes), the interquartile range (boxes), 1.5 times the interquartile range (whiskers) and outliers (circle). Results of the Wilcoxon test are reported only if significant at  $p < 0.05$  (asterisk) or  $p < 0.1$  (point).

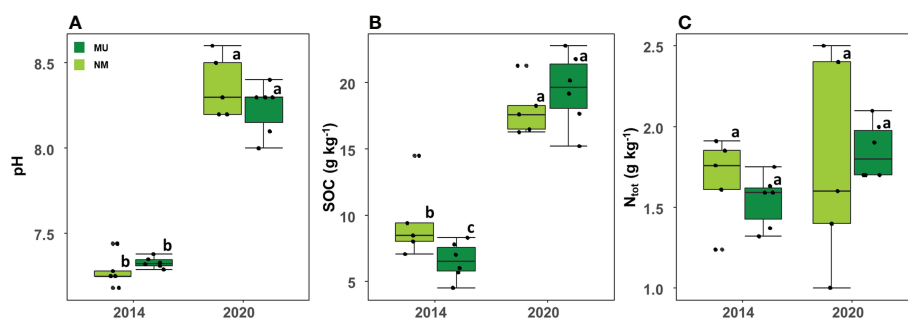


FIGURE 6

Comparison of soil pH (A), organic carbon (SOC; B) and total nitrogen content ( $N_{tot}$ ; C) between control (NM; no mulching) and mulching-treated (MU) areas in the 11 soil plots sampled in the years 2014 and 2020. Small letters indicate the results of the Dunn test *post-hoc* comparisons.

RL compared to the MU one for the roots falling within the diameter range of 0.8 – 1.5 mm (Figure 5).

correlating with SLA, LNC, and SRL (Figure 7). Finally, the functional space of the NM relevés was wider than MU ones.

### 3.3 Soil chemical properties

Soil pH was significantly ( $p < 0.01$ ) higher in 2020 (mean  $8.3 \pm SD 0.2$ ) than in 2014 (mean  $7.3 \pm SD 0.1$ ) independently of the treatment, but it did not differ between the NM and MU areas in the two sampled time-points (Figure 6A).

SOC also was significantly ( $p < 0.01$ ) higher in 2020 (mean  $18.8 \pm SD 2.5 \text{ g Kg}^{-1}$ ) compared to 2014 (mean  $7.9 \pm SD 2.6 \text{ g Kg}^{-1}$ ) independently of the treatment (Figure 6B). In 2014 SOC was significantly higher in the NM area than in the MU one, while in 2020 SOC was slightly higher in the MU area, even if without statistical significance (Figure 6B).

No differences were detected between 2014 and 2020 for  $N_{tot}$  independently of the treatment (Figure 6C). Although without significant difference, the MU area showed a slight increase of  $N_{tot}$  from 2014 to 2020, and a simultaneous slight decrease in the NM area (Figure 6C).

### 3.4 Redundancy analysis (RDA)

Plant traits significantly affected by the mulching treatment were used for the RDA (see Figure 4). These traits explained 72% of the total variance of the vegetation dataset (i.e., constrained variance), and the first two axes represented 58% of this variation (Figure 7 and Supplementary Table 1). Moreover, only the first axis showed a significant effect ( $F = 3.3$ ,  $p < 0.05$ ).

All the MU relevés were displayed on the left side of the ordination diagram and were positively correlated with both above- and below-ground biomass (AGDW and BGDW) and LDMC (Figure 7). On the opposite, the NM relevés were displayed on the right side of the ordination diagram

## 4 Discussion

Our results highlighted that the plant communities of the abandoned agricultural fields of the Site of National Interest (SIN) Brescia-Caffaro are today typical of hay meadows of the class *Molinio-Arrhenatheretea* in both NM and MU areas. This was expectable due to the natural succession of spontaneous vegetation after the abandonment of agricultural activities (Prévosto et al., 2011; Sojneková and Chytrý, 2015). In particular, we found evidence that seven years of mulching favors the establishment of vegetation *facies* greatly dominated by *Arrhenatherum elatius*, thus indicating a stable vegetation *facies* of hay meadows. Moreover, our results confirmed that mulching improves the total biomass of the analyzed plant communities, both above- and below-ground (Figures 2, 3), representing more productive and competitive vegetation. In a relatively fertile *A. elatius* community, we found a significant deviation in the floristic composition of the treated area (i.e., mulching - MU) compared to the control one (i.e., no mulching - NM). Comparable observations linked the floristic changes to the presence of eutrophic soils (Moog et al., 2002), which support the abundance of competitive strategist species (Grime, 2006a). On the contrary, in the control area, the vegetation evolved toward a hay meadow dominated by *Daucus carota*, representing a more ruderal vegetation *facies* (Table 2). In the MU area, although species richness was not significantly affected, we observed a significant linear decrease with the increase of the biomass (Figure 2C), thus resembling the more productive extreme of the humped-back curve (Grime, 1973; Pierce, 2014; Cerabolini et al., 2016). Such evidence suggests that long-term mulching application could lead to a reduction of biodiversity. Other long-term mulching



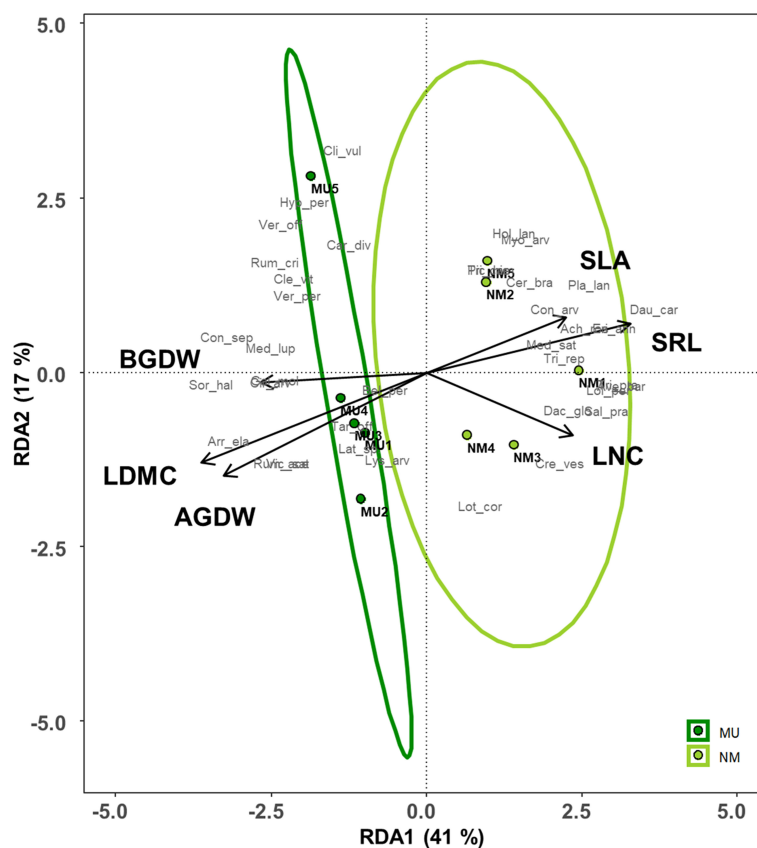


FIGURE 7

Redundancy analysis (RDA) ordination of the plant community composition comparing control (NM; no mulching) and mulching-treated (MU) areas in relation to the community-level plant functional traits that were significantly affected by the mulching treatment (see Figures 3). The circles represent the 95% confidence interval. Legend: AGDW and BGDW, above- and below- ground dry weight, LDMC, community weighted mean (CWM) of leaf dry matter content, LNC, CWM of leaf nitrogen content, SLA, CWM of specific leaf area, SRL, specific root length. Supporting data are reported in Supplementary Table 1.

experiments reported similar findings for semi-natural meadows dominated by *Festuca rubra* or *A. elatius* (Gaisler et al., 2013; Gaisler et al., 2019; respectively). Therefore, we may assert that mulching affects the floristic composition of plant communities favoring potentially dominant species at the expense of subordinate species. Altogether, these findings supported our first two hypothesis.

Since 2014, we observed a simultaneous increase in soil pH and SOC in both NM and MU areas (Figure 6). This finding might be due to the increment of soil nutrients and the release of disturbance due to the abandonment of agricultural activity (Novara et al., 2014; Nadal-Romero et al., 2021). Despite more fertile soils (i.e., higher carbon content) are usually associated with lower pH values in temperate grassland ecosystems (Kidd et al., 2017), the release of soil disturbance may have enhanced the soil biota community and related bioturbation (Kurganova et al., 2019). An increase in soil pH has been, for example, attributed to top-soil bioturbation (Dostál et al., 2005; Jílková, 2008; Desie et al., 2020), which in turn facilitates the

phytoavailability of metals (Leveque et al., 2014) as well as other organic contaminants, such as PCBs that are mainly transported with solid material (Cousins et al., 1999).

When both SOC and  $N_{tot}$  parameters were compared between NM and MU areas, we found that in 2014 the MU area had slightly lower values than the NM one, while an opposite trend was observed seven years later (2020), with higher values in the MU area (Figure 6). Although this trend was observable, differences among data were not statistically significant, likely due to the mid-term study period (2014–2020), which is still not enough to denote appreciable differences. Indeed, it has been demonstrated that long-term mulching can significantly increase the soil organic matter and available nutrients due to the degradation of mulching materials (Jordán et al., 2010; Doležal et al., 2011). Moreover, in upland meadows, a higher nutrient content has also been associated with additional nitrogen input through  $N_2$  fixation of legumes favored by mulching, such as *T. repens* (Gaisler et al., 2004; Pavlů et al., 2016). In our study, legume species were equally spread in

both NM and MU areas (Table 2), suggesting that mulching effects on legume coverage might be related to elevation where meadows are located. However, all the *Trifolium* species were associated with the NM area (Table 2), which in turn was characterized by low graminoids abundance, supporting the nitrogen-based competitive trade-off between grass and clover (Schwinning and Parsons, 1996).

Moreover, the accumulation of litter characterized the mulching areas with a continuous soil cover over the season. In turn, the soil cover may reduce the water runoff, soil loss, and POP mobilization and be partly responsible for different species composition compared to the control area (Moog et al., 2002; Jordán et al., 2010). However, for the same vegetation type and climatic conditions, the decomposition time of the organic matter is four weeks circa (Moog et al., 2002 and references therein), indicating a lack of effects related to the litter accumulation. Finally, mulching reduces water evaporation and increases infiltration (Gupta and Gupta, 1986), generally enhancing soil water conservation (Jun et al., 2014; Li et al., 2020). These new ecological conditions characterizing the MU area, in our study may be related to optimal condition for the spread of competitive and invasive species such as *Sorghum halepense* (Table 2; Dalle Fratte et al., 2019a).

Data of above- and below- ground traits showed a coordinated arrangement in the bi-dimensional space of the RDA along the first axis, which represents the economics spectrum (Wright et al., 2004; Reich, 2014), and the existence of trait syndromes at the plant community level (Zanzottera et al., 2020), which was independent of the treatment (Figure 7 and Supplementary Table 1). These findings showed an adaptative convergence between leaf and root economics spectrum and, thus, a coordination of above- and below-ground organs. In detail, mulching treatment seemed to select species with a higher biomass development both above- and below-ground, supporting our third hypothesis. On the contrary, plant community of control area had a higher SLA and SRL. Mulching thus selected a conservative and more productive plant community that might be related to a higher biomass development while, the control area was characterized by acquisitive and less productive plant community, which adopted a coordinated above- and below- ground plastic strategy. Finally, we might assert that in our lowland hay meadows, SRL is the below-ground equivalent to SLA since their role is plastically enhance resource acquisition (Eissenstat and Yanai, 1997). Indeed, other authors found similar results at the species-level across different biomes (Freschet et al., 2010; Fort et al., 2013; De la Riva et al., 2019; Shen et al., 2019; Baronti et al., 2022).

Concerning the leaf economics spectrum, results reported in the literature are controversial. In oligotrophic mountain meadows, Doležal et al. (2011) found that mulching promotes species characterized by acquisitive strategies. On the contrary, in line with our findings, Oomes et al. (1996) detected an

increase in dry matter production with mulching treatment. For the root economics spectrum (Ostonen et al., 2007; Weemstra et al., 2016), SRL is often regarded as a core trait reflecting the extent of soil exploration, in search of water and nutrients, per unit cost of biomass allocation (Freschet et al., 2021b). Indeed, the RL is assumed to be proportional to resource acquisition, while the biomass should be proportional to construction and maintenance (Ostonen et al., 2007; De la Riva et al., 2021). In Mediterranean plants the root economics spectrum has been identified as the main axis of variation (Montagnoli et al., 2019; De la Riva et al., 2021), but we are not aware of studies that investigated its relationship with mulching treatment.

Although SRL was higher in control area than in mulching one, we did not detect significant changes in RL and MRD (Figures 3G, I) at the whole 2 mm diameter class. Interestingly, when the 0.1 mm root diameter classes were considered (Figure 5), plants in the MU area had lower values of RL for the roots falling within the diameter range of 0.8 – 1.5 mm. This variation occurred in pioneer fine-roots representing the framework functioning in nutrient and water transport (Montagnoli et al., 2021). On the contrary, in the NM area, plants need to lengthen the fine-root system and enhance nutrient acquisition with fibrous roots, which are thin in diameter, lower in carbon costs, and higher in absorptive function overall lowering the SRL value. Moreover, we found differences in root traits at different soil depths analyzed (Figure 4). Mulching affected the fine-root traits with higher magnitude in the upper soil layer (0 - 10 cm). Indeed, in the upper soil layer, plants in the NM area had a lower root mass and a longer fine-root system (i.e., higher SRL) than in the MU one, although this difference was not statistically significant (Figure 4B). Thus, in the upper soil layer, mulching selects species with a shorter root population (i.e., lower RL) that is mostly dedicated to water transport (pioneer roots with larger diameter) and low SRL, highlighting the fundamental role played by the surface fine-roots (Baronti et al., 2022) growing with higher water and nutrient contents as in the case of the MU area (Jordán et al., 2010; Doležal et al., 2011). On the contrary, plant species of the NM area, whose soil is characterized by less water and nutrient content, have longer fine-roots and lower fine-root biomass (Figure 4A) since they are mostly dedicated to water and nutrient acquisition (fibrous roots). Fine-root biomass and length decreased at deeper soil layers (10 - 20, 20 - 30, and 30 - 40 cm), and the mulching effect was negligible.

According to plant species composition, mulching did not favor the development of species known as effective for PCBs phytoremediation. However, since the below-ground biomass was higher in the MU area, we could speculate that mulching facilitates the PCB phytoremediation through the increase of root system biomass development, representing the supporting backbone for the microbial communities, which in turn carry out the phytodegradation activity (Passatore et al., 2014;

Terzaghi et al., 2022). Indeed, roots of some species can grow with immobile soil contaminants (PCBs, PAHs) and release cometabolites (i.e., flavonoids) during the entire lifespan (e.g., Leigh et al., 2002), fostering the growth and activity of degradative microbes. In turn, the presence of contaminants may induce a shift in the resident soil microbial community selecting the degradative populations (Mackova et al., 2010; Di Guardo et al., 2017).

Vergani et al. (2017), in a recent review study, identified plant species effective for PCB phytoremediation, and some of those species were part of the indicator species composing vegetation only of the NM area (Table 2). On the contrary, in the case of the MU area, these species were present but not among the indicator ones, thus suggesting that the biodiversity decline associated with the mulching application could negatively impact the diversity and abundance of native species important for phytoremediation. Since the mentioned review study (Vergani et al., 2017) focuses mainly on commercial species, we cannot exclude that there are equivalent wild native species with unknown phytoremediation potential (e.g., Slater et al., 2011). For example, *Festuca arundinacea*, a well-known species for phytoremediation, was not recorded in our study plots. However, this species has a high degree of physiognomic analogy with other dominant species found in the MU area, such as *Arrhenatherum elatius* or *Sorghum halepense* (Table 2). These analogies suggest the possible use of native species for testing their phytoremediation potential. In addition, native species with high phytoremediation potential (e.g., *Medicago sativa*; Mackova et al., 2010; Jing et al., 2018) could be enhanced in their coverage and associated with tools for organic pollutants stabilization (Denyes et al., 2013; Baronti et al., 2022). Finally, and more importantly, the mulching treatment seems to favor the development of a higher number of metal-tolerant plant species, which in turn could enhance the phytoremediation of polluted soils. Our findings thus indicate that the mulching practice can be a suitable method for the remediation of heavy metal-polluted soils, confirming comparable observations in forest soils (Kiikkilä et al., 2001) and the high potential of native plants for phytoremediation (Nouri et al., 2011; Wang et al., 2022). All together these findings led us to partially support our fourth hypothesis.

## 5 Conclusions

Our study pointed out that the abandonment of agricultural activities within the soil-polluted Site of National Interest (SIN Brescia-Caffaro) led to a marked increase in the soil organic carbon and pH. The over-imposed mulching (i.e., cutting the biomass and leaving the crushed clippings to decompose in situ) additionally induced a slight increase in soil nutrients. These new ecological conditions favored the establishment of a more

productive plant community with a more conservative-resource strategy typical of stable hay meadows and dominated by *Arrhenatherum elatius* and *Sorghum halepense*.

Above- and below-ground plant traits showed a coordinated variation at the community level, highlighting an adaptive convergence between the leaf and root economics spectrum. Mulching selected species with a higher biomass development both above- and below-ground, while the plant community of the control area had a higher SLA and SRL. Plants subjected to the mulching treatment showed a reduction of the root population mainly functioning in nutrient and water transport (i.e., lower pioneer roots' length).

Although mulching did not select native plant species beneficial for soil PCBs remediation, the observed increase of the root web (i.e., below-ground biomass) might be functional to the proliferation of microbes devoted to contaminants' degradation. At the same time, mulching treatment selected a two-fold number of plant species known to be metal-tolerant, suggesting that it can be a suitable method for selecting relevant species for the remediation of HMs-polluted soils. However, our data suggest that the long-term mulching application could lead to a biodiversity decline with potential impacts also on the diversity of native species important for phytoremediation.

## Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

## Author contributions

MDF, AMo, and BC conceived the research project and developed the study plan. MDF, AMo, SAN, SAr, and BC were responsible for the field sampling. PN provided primary funding. MDF, AMo, and BC dealt with the methodological approach and the experimental design. MDF, AMo, and BC were responsible for the data collection and interpretation. AMo, PB, and AMi dealt with root sampling, processing, and data arrangement. MDF, AC, SAr, EL, and BC dealt with vegetation analysis. MDF and BC dealt with the data of leaf traits. MDF performed the data analysis and chart visualization, and wrote the manuscript draft. AMo dealt with the draft revision process. MDF and AMo finalized the manuscript. All authors contributed to the article and approved the submitted version.

## Funding

This study was funded by ERSAP Lombardia (Ente Regionale per i Servizi all'Agricoltura e alle Foreste).

## Acknowledgments

Authors thank Mario Ferrari for his valuable help in the floristic determination. MDF, AMo, and BC acknowledge the Department of Biotechnology and Life Science, University of Insubria, for providing the necessary support to the joint research project.

## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

## References

- Ali, H., Khan, E., and Sajad, M. A. (2013). Phytoremediation of heavy metals—concepts and applications. *Chemosphere* 91, 869–881. doi: 10.1016/j.chemosphere.2013.01.075
- Al-Mufti, M. M., Sydes, C. L., Furness, S. B., Grime, J. P., and Band, S. R. (1977). A quantitative analysis of shoot phenology and dominance in herbaceous vegetation. *J. Ecol.* 65, 759–791. doi: 10.2307/2259378
- Baronti, S., Magno, R., Maienza, A., Montagnoli, A., Ungaro, F., and Vaccari, F. P. (2022). Long term effect of biochar on soil plant water relation and fine roots: Results after 10 years of vineyard experiment. *Sci. Tot. Environ.* 851, 158225. doi: 10.1016/j.scitotenv.2022.158225
- Bartolucci, F., Peruzzi, L., Galasso, G., Albano, A., Alessandrini, A., Ardenghi, N. M. G., et al. (2018). An updated checklist of the vascular flora native to Italy. *Plant Biosyst.* 152, 179–303. doi: 10.1080/11263504.2017.1419996
- Beesley, L., Moreno-Jiménez, E., Gomez-Eyles, J. L., Harris, E., Robinson, B., and Sizmur, T. (2011). A review of biochars' potential role in the remediation, revegetation and restoration of contaminated soils. *Environ. pollut.* 159, 3269–3282. doi: 10.1016/j.envpol.2011.07.023
- Benjamin, J. G., and Nielsen, D. C. (2004). A method to separate plant roots from soil and analyze root surface area. *Plant Soil* 267, 225–234. doi: 10.1007/S11104-005-4887-3
- Branzini, A., and Zubillaga, M. S. (2012). Comparative use of soil organic and inorganic amendments in heavy metals stabilization. *Appl. Environ. Soil Sci.* 2012. doi: 10.1155/2012/721032
- Bruehlheide, H., Dengler, J., Purschke, O., Lenoir, J., Jiménez-Alfaro, B., Hennekens, S. M., et al. (2018). Global trait–environment relationships of plant communities. *Nat. Ecol. Evol.* 2, 1906–1917. doi: 10.1038/s41559-018-0699-8
- Carmona, C. P., Bueno, C. G., Toussaint, A., Träger, S., Diaz, S., Moora, M., et al. (2021). Fine-root traits in the global spectrum of plant form and function. *Nature* 597, 683–687. doi: 10.1038/s41586-021-03871-y
- Cerabolini, B. E. L., Brusa, G., Ceriani, R. M., de Andreis, R., Luzzaro, A., and Pierce, S. (2010). Can CSR classification be generally applied outside Britain? *Plant Ecol.* 210, 253–261. doi: 10.1007/S11258-010-9753-6
- Cerabolini, B. E. L., Pierce, S., Verginella, A., Brusa, G., Ceriani, R. M., and Armiraglio, S. (2016). Why are many anthropogenic agroecosystems particularly species-rich? *Plant Biosyst.* 150, 550–557. doi: 10.1080/11263504.2014.987848
- Chagas, J. K. M., de Figueiredo, C. C., and Ramos, M. L. G. (2022). Biochar increases soil carbon pools: Evidence from a global meta-analysis. *J. Environ. Manage.* 305, 114403. doi: 10.1016/j.jenvman.2021.114403
- Collins, C., Fryer, M., and Grosso, A. (2006). Plant uptake of non-ionic organic chemicals. *Environ. Sci. Technol.* 40, 45–52. doi: 10.1021/ES0508166
- Cornelissen, G., Gustafsson, Ö., Bucheli, T. D., Jonker, M. T. O., Koelmans, A. A., and van Noort, P. C. M. (2005). Extensive sorption of organic compounds to black carbon, coal, and kerogen in sediments and soils: Mechanisms and consequences for distribution, bioaccumulation, and biodegradation. *Environ. Sci. Technol.* 39, 6881–6895. doi: 10.1021/ES050191B
- Cousins, I. T., Geva, B., and Jones, K. C. (1999). Measuring and modelling the vertical distribution of semi-volatile organic compounds in soils. I: PCB and PAH soil core data. *Chemosphere* 39, 2507–2518. doi: 10.1016/S0045-6535(99)00164-2
- Dalle Fratte, M., Bolpagni, R., Brusa, G., Caccianiga, M., Pierce, S., Zanzottera, M., et al. (2019a). Alien plant species invade by occupying similar functional spaces to native species. *Flora* 257, 151419. doi: 10.1016/j.flora.2019.151419
- Dalle Fratte, M., Brusa, G., Pierce, S., Zanzottera, M., and Cerabolini, B. E. L. (2019b). Plant trait variation along environmental indicators to infer global change impacts. *Flora* 254, 113–121. doi: 10.1016/j.flora.2018.12.004
- Dalle Fratte, M., Pierce, S., Zanzottera, M., and Cerabolini, B. E. L. (2021). The association of leaf sulfur content with the leaf economics spectrum and plant adaptive strategies. *Funct. Plant Biol.* 48, 924–935. doi: 10.1071/FP20396
- De Bello, F., Carmona, C. P., Dias, A. T., Götzenberger, L., Moretti, M., Berg, M. P., et al. (2010). *Handbook of trait-based ecology: from theory to R tools*. (Cambridge, England: Cambridge University Press). doi: 10.1017/9781108628426
- De Bello, F., Lavorel, S., Diaz, S., Harrington, R., Cornelissen, J. H. C., Bardgett, R. D., et al. (2010). Towards an assessment of multiple ecosystem processes and services via functional traits. *Biodivers. Conserv.* 19, 2873–2893. doi: 10.1007/s10531-010-9850-9
- De Cáceres, M., and Legendre, P. (2009). Associations between species and groups of sites: Indices and statistical inference. *Ecol* 90, 3566–3574. doi: 10.1890/08-1823.1
- De la Riva, E. G., Prieto, I., and Villar, R. (2019). The leaf economic spectrum drives leaf litter decomposition in Mediterranean forests. *Plant Soil* 435, 353–366. doi: 10.1007/s11104-018-3883-3
- De la Riva, E. G., Querejeta, J. I., Villar, R., Pérez-Ramos, I. M., Marañón, T., Galán Díaz, J., et al. (2021). The economics spectrum drives root trait strategies in Mediterranean vegetation. *Front. Plant Sci.* 12. doi: 10.3389/fpls.2021.773118/FULL
- Denyes, M. J., Rutter, A., and Zeeb, B. A. (2013). *In situ* application of activated carbon and biochar to PCB-contaminated soil and the effects of mixing regime. *Environ. pollut.* 182, 201–208. doi: 10.1016/j.envpol.2013.07.016
- Desie, E., van Meerbeek, K., de Wandeler, H., Bruehlheide, H., Domisch, T., Jaroszewicz, B., et al. (2020). Positive feedback loop between earthworms, humus form and soil pH reinforces earthworm abundance in European forests. *Funct. Ecol.* 34, 2598–2610. doi: 10.1111/1365-2435.13668
- Diaz, S., Kattge, J., Cornelissen, J. H. C., Wright, I. J., Lavorel, S., Dray, S., et al. (2016). The global spectrum of plant form and function. *Nature* 529, 167–171. doi: 10.1038/nature16489
- Di Guardo, A., Raspa, G., Terzaghi, E., Vergani, L., Mapelli, F., Borin, S., et al. (2020). PCB Vertical and horizontal movement in agricultural soils of a highly contaminated site: Role of soil properties, cultivation history and PCB physico-chemical parameters. *Sci. Tot. Environ.* 747, 141477. doi: 10.1016/j.scitotenv.2020.141477
- Di Guardo, A., Terzaghi, E., Raspa, G., Borin, S., Mapelli, F., Chouaia, B., et al. (2017). Differentiating current and past PCB and PCDD/F sources: The role of a

## Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

## Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fpls.2022.1062911/full#supplementary-material>



large contaminated soil site in an industrialized city area. *Environ. pollut.* 223, 367–375. doi: 10.1016/j.envpol.2017.01.033

Dinno, A. (2017). *Dunn.test: Dunn's test of multiple comparisons using rank sums. r package version 1.3.5*. Available at: <https://cran.r-project.org/web/packages/dunn.test/>

Doležal, J., Mašková, Z., Lepš, J., Steinbachová, D., de Bello, F., Klimešová, J., et al. (2011). Positive long-term effect of mulching on species and functional trait diversity in a nutrient-poor mountain meadow in central Europe. *Agric. Ecosyst. Environ.* 145, 10–28. doi: 10.1016/j.agee.2011.01.010

Dostál, P., Březnová, M., Kozlíčková, V., Herben, T., and Kovář, P. (2005). Ant-induced soil modification and its effect on plant below-ground biomass. *Pedobiologia* 49, 127–137. doi: 10.1016/j.pedobi.2004.09.004

Eissenstat, D. M., and Yanai, R. D. (1997). The ecology of root lifespan. *Adv. Ecol. Res.* 27, 1–60. doi: 10.1016/S0065-2504(08)60005-7

Fort, F., Jouany, C., and Cruz, P. (2013). Root and leaf functional trait relations in poaceae species: Implications of differing resource-acquisition strategies. *J. Plant Ecol.* 6, 211–219. doi: 10.1093/jpe/pts034

Freschet, G. T., Cornelissen, J. H. C., van Logtestijn, R. S. P., and Aerts, R. (2010). Evidence of the “plant economics spectrum” in a subarctic flora. *J. Ecol.* 98, 362–373. doi: 10.1111/j.1365-2745.2009.01615.x

Freschet, G. T., Pagès, L., Iversen, C. M., Comas, L. H., Rewald, B., Roumet, C., et al. (2021a). A starting guide to root ecology: strengthening ecological concepts and standardising root classification, sampling, processing and trait measurements. *New Phytol.* 232 (3), 973–1122. doi: 10.1111/nph.175721

Freschet, G. T., Roumet, C., Comas, L. H., Weemstra, M., Bengough, A. G., Rewald, B., et al. (2021b). Root traits as drivers of plant and ecosystem functioning: current understanding, pitfalls and future research needs. *New Phytol.* 232, 1123–1158. doi: 10.1111/NPH.17072

Gaisler, J., Hejčman, M., and Pavlů, V. (2004). Effect of different mulching and cutting regimes on the vegetation of upland meadow. *Plant Soil Environ.* 50, 324–331. doi: 10.17221/4039-PSE

Gaisler, J., Pavlů, V., Nwaogu, C., Pavlů, K., Hejčman, M., and Pavlů, V. V. (2019). Long-term effects of mulching, traditional cutting and no management on plant species composition of improved upland grassland in the Czech republic. *Grass Forage Sci.* 74, 463–475. doi: 10.1111/gfs.12408

Gaisler, J., Pavlů, V., Pavlů, L., and Hejčman, M. (2013). Long-term effects of different mulching and cutting regimes on plant species composition of festuca rubra grassland. *Agric. Ecosyst. Environ.* 178, 10–17. doi: 10.1016/j.agee.2013.06.010

Galasso, G., Conti, F., Peruzzi, L., Ardenghi, N. M. G., Banfi, E., Celesti-Grapow, L., et al. (2018). An updated checklist of the vascular flora alien to Italy. *Plant Biosyst.* (Cambridge, England: Cambridge University Press) 152, 556–592. doi: 10.1080/11263504.2018.1441197

Grime, J. P. (1973). Competitive exclusion in herbaceous vegetation. *Nature* 242, 344–347. doi: 10.1038/242344a0

Grime, J. P. (2006a). *Plant strategies, vegetation processes, and ecosystem properties*. 2nd ed (Chichester, West Sussex: John Wiley & Sons).

Grime, J. P. (2006b). Trait convergence and trait divergence in herbaceous plant communities: Mechanisms and consequences. *J. Veg. Sci.* 17, 255–260. doi: 10.1111/j.1654-1103.2006.tb02444.x

Gupta, J. P., and Gupta, G. K. (1986). Effect of tillage and mulching on soil environment and cowpea seedling growth under arid conditions. *Arid. Land Res. Manag.* 7, 233–240. doi: 10.1080/15324988709381141

Jiang, L., Zhang, D., Song, M., Guan, G., Sun, Y., Li, J., et al. (2022). The positive role of root decomposition on the bioremediation of organic pollutants contaminated soil: A case study using PCB-9 as a model compound. *Soil Biol. Biochem.* 171, 108726. doi: 10.1016/j.soilbio.2022.108726

Jílková, V. (2008). The effect of ants on soil properties and processes (Hymenoptera: Formicidae related papers. *Myrmecol. News* 11, 191–199.

Jing, R., Fusi, S., and Kjellerup, B. v. (2018). Remediation of polychlorinated biphenyls (PCBs) in contaminated soils and sediment: State of knowledge and perspectives. *Front. Environ. Sci.* 6. doi: 10.3389/FENVS.2018.00079/BIBTEX

Jordán, A., Zavala, L. M., and Gil, J. (2010). Effects of mulching on soil physical properties and runoff under semi-arid conditions in southern Spain. *Catena* 81, 77–85. doi: 10.1016/J.CATENA.2010.01.007

Jun, F., Yu, G., Quanjui, W., Malhi, S. S., and Yangyang, L. (2014). Mulching effects on water storage in soil and its depletion by alfalfa in the loess plateau of northwestern China. *Agric. Water Manag.* 138, 10–16. doi: 10.1016/J.AGWAT.2014.02.018

Kahmen, S., and Poschold, P. (2008). Effects of grassland management on plant functional trait composition. *Agric. Ecosyst. Environ.* 128, 137–145. doi: 10.1016/j.agee.2008.05.016

Kahmen, S., Poschold, P., and Schreiber, K. F. (2002). Conservation management of calcareous grasslands. changes in plant species composition and response of functional traits during 25 years. *Biol. Conserv.* 104, 319–328. doi: 10.1016/S0006-3207(01)00197-5

Kattge, J., Bönisch, G., Diaz, S., Lavorel, S., Prentice, I. C., Leadley, P., et al. (2020). TRY plant trait database – enhanced coverage and open access. *Glob. Chang. Biol.* 26, 119–188. doi: 10.1111/gcb.14904

Kidd, J., Manning, P., Simkin, J., Peacock, S., and Stockdale, E. (2017). Impacts of 120 years of fertilizer addition on a temperate grassland ecosystem. *PLOS One* 12, e017463. doi: 10.1371/JOURNAL.PONE.0174632

Kiikkilä, O., Perkiömäki, J., Barnette, M., Derome, J., Pennanen, T., Tulisalo, E., et al. (2001). *In situ* bioremediation through mulching of soil polluted by a copper-nickel smelter. *J. Environ. Qual.* 30, 1134–1143. doi: 10.2134/JEQ2001.3041134X

Kurganova, I., Merino, A., Lopes de Gerenyu, V., Barros, N., Kalinina, O., Giani, L., et al. (2019). Mechanisms of carbon sequestration and stabilization by restoration of arable soils after abandonment: A chronosequence study on phaeozems and chernozems. *Geoderma* 354, 113882. doi: 10.1016/J.GEODERMA.2019.113882

Landolt, E., Bäumler, B., Erhardt, A., Hegg, O., Klötzli, F., Lämmler, W., et al. (2010). Flora indicativa. Ecological indicator values and biological attributes of the flora of Switzerland and the Alps. ökologische zeigerwerte und biologische kennzeichen zur flora der schweiz und der alpen. *Haupt Verlag, Bern*.

Lavorel, S., Grigulis, K., Lamarque, P., Colace, M. P., Garden, D., Girel, J., et al. (2011). Using plant functional traits to understand the landscape distribution of multiple ecosystem services. *J. Ecol.* 99, 135–147. doi: 10.1111/j.1365-2745.2010.01753.x

Leigh, M. B., Fletcher, J. S., Fu, X., and Schmitz, F. J. (2002). Root turnover: An important source of microbial substrates in rhizosphere remediation of recalcitrant contaminants. *Environ. Sci. Technol.* 36, 1579–1583. doi: 10.1021/ES015702I/ASSET/IMAGES/LARGE/ES015702IF00004.JPEG

Leuschner, C., Gebel, S., and Rose, L. (2013). Root trait responses of six temperate grassland species to intensive mowing and NPK fertilisation: A field study in a temperate grassland. *Plant Soil* 373, 687–698. doi: 10.1007/S11104-013-1836-4

Leveque, T., Capowiez, Y., Schreck, E., Xiong, T., Foucault, Y., and Dumat, C. (2014). Earthworm bioturbation influences the phytoavailability of metals released by particles in cultivated soils. *Environ. pollut.* 191, 199–206. doi: 10.1016/J.ENVPOL.2014.04.005

Li, S., Wang, F., Chen, M., Liu, Z., Zhou, L., Deng, J., et al. (2020). Mowing alters nitrogen effects on the community-level plant stoichiometry through shifting plant functional groups in a semi-arid grassland. *Environ. Res. Lett.* 15, 074031. doi: 10.1088/1748-9326/AB8A87

Macek, T., Francová, K., Kochánková, L., Lovecká, P., Ryslavá, E., Rezek, J., et al. (2004). Phytoremediation: Biological cleaning of a polluted environment. *Rev. Environ. Health* 19, 63–82. doi: 10.1515/REVEH.2004.19.1.63/MACHINEREADABLECITATION/RIS

Mackova, M., Dowling, D., and Macek, T. (2010). *Phytoremediation rhizoremediation* (Dordrecht, The Netherlands: Springer).

Mackova, M., Prouzova, P., Stursa, P., Ryslava, E., Uhlik, O., Beranova, K., et al. (2009). Phyto/rhizoremediation studies using long-term PCB-contaminated soil. *Environ. Sci. pollut. Res.* 16, 817–829. doi: 10.1007/S11356-009-0240-3/TABLES/7

Mašková, Z., Doležal, J., Květ, J., and Zemek, F. (2009). Long-term functioning of a species-rich mountain meadow under different management regimes. *Agric. Ecosyst. Environ.* 132, 192–202. doi: 10.1016/j.agee.2009.04.002

McCutcheon, S., and Schnoor, J. L. (2003). *Phytoremediation: Transformation and control of contaminants* (Hoboken, New Jersey: John Wiley & Sons, Inc).

McNear, D. H. (2013). The rhizosphere - roots, soil and everything in between. *Nat. Educ. Knowledge* 4, 1.

Metsoja, J. A., Neuenkamp, L., Pihu, S., Vellak, K., Kalwij, J. M., and Zobel, M. (2012). Restoration of flooded meadows in Estonia - vegetation changes and management indicators. *Appl. Veg. Sci.* 15, 231–244. doi: 10.1111/J.1654-109X.2011.01171.X

Montagnoli, A., Baronti, S., Alberto, D., Chiatante, D., Scippa, G. S., and Terzaghi, M. (2021). Pioneer and fibrous root seasonal dynamics of vitis vinifera l. are affected by biochar application to a low fertility soil: A rhizobox approach. *Sci. Tot. Environ.* 751, 141455. doi: 10.1016/J.SCITOTENV.2020.141455

Montagnoli, A., Dumroese, R. K., Terzaghi, M., Onelli, E., Scippa, G. S., and Chiatante, D. (2019). Seasonality of fine root dynamics and activity of root and shoot vascular cambium in a quercus ilex l. forest (Italy). *For. Ecol. Manage.* 431, 26–34. doi: 10.1016/J.FORECO.2018.06.044

Moog, D., Poschold, P., Kahmen, S., and Schreiber, K. F. (2002). Comparison of species composition between different grassland management treatments after 25 years. *Appl. Veg. Sci.* 5, 99–106. doi: 10.1111/J.1654-109X.2002.TB00539.X



- Nadal-Romero, E., Rubio, P., Kremyda, V., Absalah, S., Cammeraat, E., Jansen, B., et al. (2021). Effects of agricultural land abandonment on soil organic carbon stocks and composition of soil organic matter in the central Spanish pyrenees. *Catena* 205, 105441. doi: 10.1016/j.catena.2021.105441
- Nouri, J., Lorestan, B., Yousefi, N., Khorasani, N., Hasani, A. H., Seif, F., et al. (2011). Phytoremediation potential of native plants grown in the vicinity of ahangaran lead-zinc mine (Hamedan, Iran). *Environ. Earth Sci.* 62, 639–644. doi: 10.1007/s12665-010-0553-Z
- Novara, A., la Mantia, T., Rühl, J., Badalucco, L., Kuzyakov, Y., Gristina, L., et al. (2014). Dynamics of soil organic carbon pools after agricultural abandonment. *Geoderma* 235–236, 191–198. doi: 10.1016/j.geoderma.2014.07.015
- Oelmann, Y., Brauckmann, H. J., Schreiber, K. F., and Broll, G. (2017). 40 years of succession or mulching of abandoned grassland affect phosphorus fractions in soil. *Agric. Ecosyst. Environ.* 237, 66–74. doi: 10.1016/j.agee.2016.12.014
- Oksanen, J., Blanchet, F. G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., et al. (2022). Vegan: Community ecology package. *R package version 2.6-4*. Available at: <https://github.com/vegandevs/vegan>.
- Oomes, M. J. M., Olf, H., and Altena, H. J. (1996). Effects of vegetation management and raising the water table on nutrient dynamics and vegetation change in a wet grassland. *J. Appl. Ecol.* 33, 576–588. doi: 10.2307/2404986
- Ostonen, I., Püttsepp, Ü., Biel, C., Alberton, O., Bakker, M. R., Lohmus, K., et al. (2007). Specific root length as an indicator of environmental change. *Plant Biosyst.* 141, 426–442. doi: 10.1080/11263500701626069
- Passatore, L., Rossetti, S., Juwarkar, A. A., and Massacci, A. (2014). Phytoremediation and bioremediation of polychlorinated biphenyls (PCBs): State of knowledge and research perspectives. *J. Hazard Mater.* 278, 189–202. doi: 10.1016/j.jhazmat.2014.05.051
- Pavlu, L., Gaisler, J., Hejman, M., and Pavlu, V. v. (2016). What is the effect of long-term mulching and traditional cutting regimes on soil and biomass chemical properties, species richness and herbage production in dactylis glomerata grassland? *Agric. Ecosyst. Environ.* 217, 13–21. doi: 10.1016/j.agee.2015.10.026
- Pérez-Harguindeguy, N., Díaz, S., Garnier, E., Lavorel, S., Poorter, H., Jaureguiberry, P., et al. (2013). New handbook for standardised measurement of plant functional traits worldwide. *Aust. J. Bot.* 61, 167–234. doi: 10.1071/BI12225
- Pierce, S. (2014). Implications for biodiversity conservation of the lack of consensus regarding the humped-back model of species richness and biomass production. *Funct. Ecol.* 28, 253–257. doi: 10.1111/1365-2435.12147
- Prévost, B., Kuiters, L., Bernhardt-Römermann, M., Dölle, M., Schmidt, W., Hoffmann, M., et al. (2011). Impacts of land abandonment on vegetation: Successional pathways in European habitats. *Folia Geobot.* 46, 303–325. doi: 10.1007/s12224-010-9096-z
- Pritchard, S. G., Maier, C. A., Johnsen, K. H., Grabman, A. J., Chalmers, A. P., and Burke, M. K. (2010). Soil incorporation of logging residue affects fine-root and mycorrhizal root-tip dynamics of young loblolly pine clones. *Tree Physiol.* 30, 1299–1310. doi: 10.1093/TREEPHYS/TPQ067
- Ravenek, J. M., Mommer, L., Visser, E. J. W., van Ruijven, J., van der Paauw, J. W., Smit-Tiekstra, A., et al. (2016). Linking root traits and competitive success in grassland species. *Plant Soil* 407, 49–53. doi: 10.1007/s11104-016-2843-Z
- R Core Team (2021). R: A language and environment for statistical computing. *R foundation for Statistical Computing*, Vienna, Austria. Available at: <https://www.R-project.org/>.
- Reich, P. B. (2014). The world-wide “fast-slow” plant economics spectrum: A traits manifesto. *J. Ecol.* 102, 275–301. doi: 10.1111/1365-2745.12211
- Reinhold-Hurek, B., Bünge, W., Burbano, C. S., Sabale, M., and Hurek, T. (2015). Roots shaping their microbiome: Global hotspots for microbial activity. *Annu. Rev. Phytopathol.* 53, 403–424. doi: 10.1146/ANNUREV-PHYTO-082712-102342
- Römermann, C., Bernhardt-Römermann, M., Kleyer, M., and Poschlod, P. (2009). Substitutes for grazing in semi-natural grasslands-do mowing or mulching represent valuable alternatives to maintain vegetation structure? *J. Vegetation Sci.* 20, 1086–1098. doi: 10.1111/j.1654-1103.2009.01106.x
- Schwinning, S., and Parsons, A. J. (1996). Analysis of the coexistence mechanisms for grasses and legumes in grazing systems. *J. Ecol.* 84, 799–813. doi: 10.2307/2960553
- Shen, Y., Gilbert, G. S., Li, W., Fang, M., Lu, H., and Yu, S. (2019). Linking aboveground traits to root traits and local environment: Implications of the plant economics spectrum. *Front. Plant Sci.* 10. doi: 10.3389/fpls.2019.01412
- Simiele, M., de Zio, E., Montagnoli, A., Terzaghi, M., Chiatante, D., Scippa, G. S., et al. (2022). Biochar and/or compost to enhance nursery-produced seedling performance: A potential tool for forest restoration programs. *Forests* 13, 550. doi: 10.3390/F13040550
- Slater, H., Gouin, T., and Leigh, M. B. (2011). Assessing the potential for rhizoremediation of PCB contaminated soils in northern regions using native tree species. *Chemosphere* 84 (2), 199–206. doi: 10.1016/j.chemosphere.2011.04.058
- Soil Survey Staff (2014). *Keys to soil taxonomy. 12th ed* (Washington, DC: USDA-Natural Resources Conservation Service).
- Sojneková, M., and Chytrý, M. (2015). From arable land to species-rich semi-natural grasslands: Succession in abandoned fields in a dry region of central Europe. *Ecol. Eng.* 77, 373–381. doi: 10.1016/j.ecoleng.2015.01.042
- Tangahu, B. V., Sheikh Abdullah, S. R., Basri, H., Idris, M., Anuar, N., and Mukhlisin, M. (2011). A review on heavy metals (As, Pb, and Hg) uptake by plants through phytoremediation. *Int. J. Chem. Eng.* 2011. doi: 10.1155/2011/939161
- Tasser, E., Gamper, S., Walde, J., Obojes, N., and Tappeiner, U. (2021). Evidence for the importance of land use, site characteristics and vegetation composition for rooting in European Alps. *Sci. Rep.* 11, 1–15. doi: 10.1038/s41598-021-90652-2
- Terzaghi, E., Raspa, G., Zanardini, E., Morosini, C., Anelli, S., Armiraglio, S., et al. (2022). Life cycle exposure of plants considerably affects root uptake of PCBs: Role of growth strategies and dissolved/particulate organic carbon variability. *J. Hazard Mater.* 421, 126826. doi: 10.1016/j.jhazmat.2021.126826
- Terzaghi, E., Vergani, L., Mapelli, F., Borin, S., Raspa, G., Zanardini, E., et al. (2019). Rhizoremediation of weathered PCBs in a heavily contaminated agricultural soil: Results of a biostimulation trial in semi field conditions. *Sci. Tot. Environ.* 686, 484–496. doi: 10.1016/j.scitotenv.2019.05.458
- Terzaghi, E., Vitale, C. M., Salina, G., and di Guardo, A. (2020). Plants radically change the mobility of PCBs in soil: Role of different species and soil conditions. *J. Hazard Mater.* 388, 121786. doi: 10.1016/j.jhazmat.2019.121786
- Tumber-Dávila, S. J., Schenk, H. J., Du, E., and Jackson, R. B. (2022). Plant sizes and shapes above and belowground and their interactions with climate. *New Phytol* 235, 1032–1056. doi: 10.1111/nph.18031
- Vergani, L., Mapelli, F., Zanardini, E., Terzaghi, E., di Guardo, A., Morosini, C., et al. (2017). Phyto-rhizoremediation of polychlorinated biphenyl contaminated soils: An outlook on plant-microbe beneficial interactions. *Sci. Tot. Environ.* 575, 1395–1406. doi: 10.1016/j.scitotenv.2016.09.218
- Wang, Q., Sun, Q., Wang, W., Liu, X., Song, L., and Hou, L. (2022). Effects of different native plants on soil remediation and microbial diversity in jiu-long iron tailings area, Jiangxi. *Forests* 13, 1106. doi: 10.3390/f13071106
- Weemstra, M., Mommer, L., Visser, E. J. W., van Ruijven, J., Kuyper, T. W., Mohren, G. M. J., et al. (2016). Towards a multidimensional root trait framework: a tree root review. *New Phytol.* 211, 1159–1169. doi: 10.1111/NPH.14003
- Weigelt, A., Mommer, L., Andrzejczak, K., Iversen, C. M., Bergmann, J., Bruehlheide, H., et al. (2021). An integrated framework of plant form and function: the belowground perspective. *New Phytol.* 232, 42–59. doi: 10.1111/nph.17590
- Wright, I. J., Reich, P. B., Westoby, M., Ackerly, D. D., Baruch, Z., Bongers, F., et al. (2004). The worldwide leaf economics spectrum. *Nature* 428, 821–827. doi: 10.1038/nature02403
- Zanzottera, M., Dalle Fratte, M., Caccianiga, M., Pierce, S., and Cerabolini, B. E. L. (2020). Community-level variation in plant functional traits and ecological strategies shapes habitat structure along succession gradients in alpine environment. *Comm. Ecol.* 21, 55–65. doi: 10.1007/s42974-020-00012-9

# Frontiers in Plant Science

Cultivates the science of plant biology and its applications

The most cited plant science journal, which advances our understanding of plant biology for sustainable food security, functional ecosystems and human health.

## Discover the latest Research Topics

[See more →](#)

### Frontiers

Avenue du Tribunal-Fédéral 34  
1005 Lausanne, Switzerland  
[frontiersin.org](https://frontiersin.org)

### Contact us

+41 (0)21 510 17 00  
[frontiersin.org/about/contact](https://frontiersin.org/about/contact)

