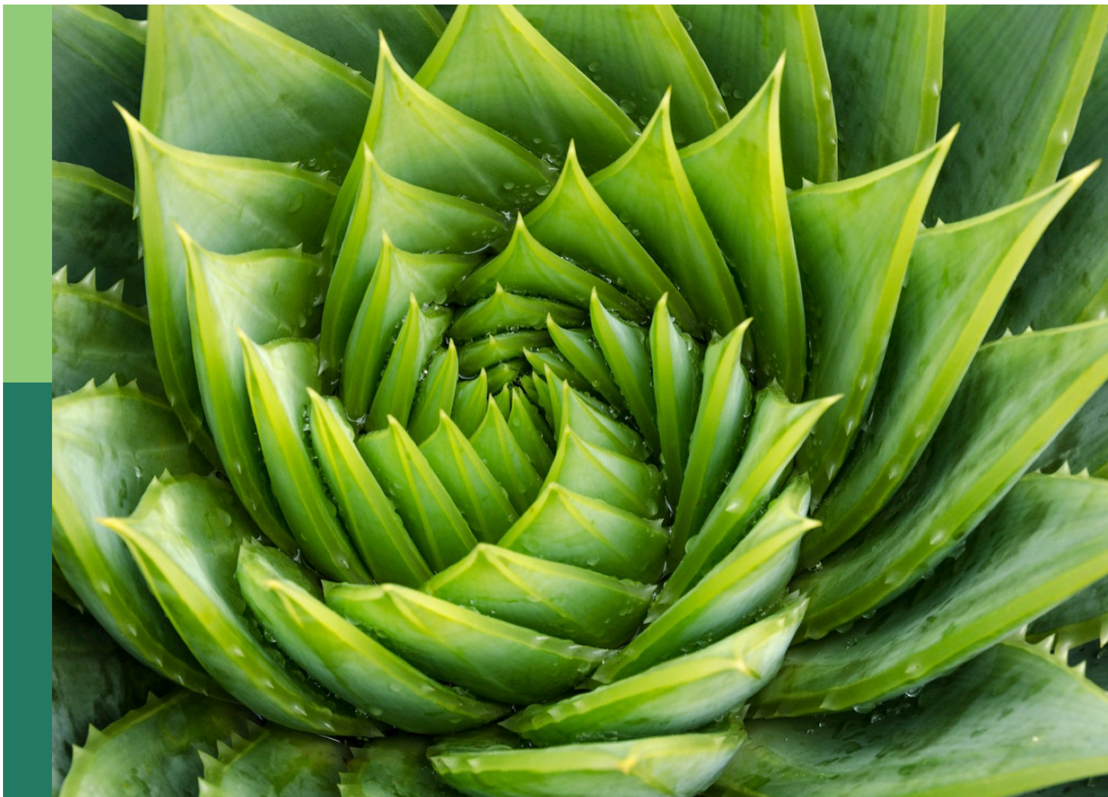
Research Topics

Plant diversity: the key to ecosystem resilience in a changing world

Edited by
Claudia Bita-Nicolae and Praveen Dhyani

Published in
Frontiers in Plant Science



ISSN 1664-8714
ISBN 978-2-8325-5936-9
DOI 10.3389/978-2-8325-5936-9

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

Plant diversity: the key to ecosystem resilience in a changing world

Topic editors
Claudia Bita-Nicolae — Institute of Biology Bucharest of the Romanian Academy, Romania
Praveen Dhyani — University of Koblenz, Germany

Citation
Bita-Nicolae, C., Dhyani, P., eds. (2025). *Plant diversity: the key to ecosystem resilience in a changing world*. Lausanne: Frontiers Media SA.
doi: 10.3389/978-2-8325-5936-9

Table of contents

04 Editorial: Plant diversity: the key to ecosystem resilience in a changing world
Claudia Bita-Nicolae and Praveen Dhyani

07 Divergent Abiotic Stressors Drive Grassland Community Assembly of Tibet and Mongolia Plateau
Jianming Wang, Mingxu Li, Li Xu, Congcong Liu, Pu Yan and Nianpeng He

19 Linking functional composition moments of the sub-Mediterranean ecotone with environmental drivers
Sergio de Tomás Marín, Javier Galán Díaz, Jesús Rodríguez-Calcerrada, Iván Prieto and Enrique G. de la Riva

34 Response of grassland ecosystem function to plant functional traits under different vegetation restoration models in areas of karst desertification
Shuzhen Song, Kangning Xiong and Yongkuan Chi

51 Impact of meteorological variability on diurnal and seasonal net ecosystem productivity in a desert riparian forest ecosystem
Dexiong Teng, Xuewei Gong, Xuemin He, Jingzhe Wang, Guanghui Lv, Jinlong Wang and Xiaodong Yang

68 Root exudation under maize/soybean intercropping system mediates the arbuscular mycorrhizal fungi diversity and improves the plant growth
Shu Zhang, Shumin Li, Lingbo Meng, Xiaodan Liu, Yuhang Zhang, Shuchang Zhao and Haobing Zhao

86 Agroecology and beyond: enhancing ecosystem services provided by natural vegetation and inventing "service weeds"
Ioannis Gazoulis, Panagiotis Kanatas, Stavros Zannopoulos, Metaxia Kokkini, Vasiliki Kontogeorgou, Nikolaos Antonopoulos and Ilias Travlos

91 Shallow tillage mitigates plant competition by increasing diversity and altering plant community assembly process
Zihao Li, Jirong Qiao, Zhuofan Li, Xiaowei Gao, Guangyu Hong, Haifeng Yang, Ercha Hu, Chenming Liu, Xiaojiang Wang, Guanzhi Liu and Runhong Gao

102 Strict biennial lifecycle and anthropogenic interventions affect temporal genetic differentiation in the endangered endemic plant, *Pedicularis hallaisanensis*
Seongjun Kim, Byoung-Doo Lee, Chang Woo Lee, Hwan-Joon Park, Jung Eun Hwang, Hyeong Bin Park, Young-Joong Kim, Daeyoung Jeon and Young-Jun Yoon

111 Effects of short- and long-term plant functional group removal on alpine meadow community niche
Jingjing Wei, Zhonghua Zhang, Li Ma, Xue Hu, Haze Ade, Hongye Su, Zhengchen Shi, Honglin Li and Huakun Zhou

frontiers | Frontiers in Plant Science

TYPE Editorial
PUBLISHED 13 January 2025
DOI 10.3389/fpls.2024.1534119

Editorial: Plant diversity: the key to ecosystem resilience in a changing world

Claudia Bita-Nicolae[1]* and Praveen Dhyani[2]

[1]Taxonomy, Ecology & Nature Conservation Department, Institute of Biology Bucharest of the Romanian Academy, Bucharest, Romania, [2]Institute for Integrated Natural Sciences, University of Koblenz, Koblenz, Germany

KEYWORDS

plant communities, ecosystem stability, abiotic stressors, climatic conditions, biodiversity

Editorial on the Research Topic
Plant diversity: the key to ecosystem resilience in a changing world

OPEN ACCESS

EDITED AND REVIEWED BY
Sebastian Leuzinger,
Auckland University of Technology,
New Zealand

*CORRESPONDENCE
Claudia Bita-Nicolae
claudia.bita@ibiol.ro

RECEIVED 25 November 2024
ACCEPTED 03 December 2024
PUBLISHED 13 January 2025

CITATION
Bita-Nicolae C and Dhyani P (2025) Editorial: Plant diversity: the key to ecosystem resilience in a changing world.
Front. Plant Sci. 15:1534119.
doi: 10.3389/fpls.2024.1534119

In a world shaped by climate fluctuations (Ditlevsen et al., 2002), natural disasters and changing human impacts (Benevolenza and DeRigne, 2019), the richness and diversity of plant species within ecosystems are key factors influencing the resilience of the ecosystem. Plants are fundamental species that, at the base of the trophic pyramid, provide food, shelter and resources to many other species (Christenhusz and Byng, 2016). Our Research Topic, "Plant Diversity: The Key to Ecosystem Resilience in a Changing World", explored in depth the complex interplay between plant diversity and ecosystem resilience (Wang et al.; Song et al.; de Tomás Marín et al., Zhang et al.; Wei et al.). We revealed the remarkable ways diverse plant communities support productivity (Teng et al., Li et al., Kim et al.), facilitate nutrient cycling and enhance soil stability (Zhou et al., 2024), thereby strengthening an ecosystem's ability to withstand and recover from a range of disturbances (Gazoulis et al.). These insights contribute significantly to conservation strategies and land management paradigms, guiding conserving and revitalizing ecosystem stability in our planet's changing global transformations (Li et al.; Kim et al.).

Ecological research showed that diverse plant communities are essential for the stability of ecosystems (Wang et al.; de Tomás Marín et al.). They provide essential functions such as nutrient cycling, pest and disease resistance, habitat provision and support for pollination and reproduction. These multiple benefits enhance an ecosystem's ability to respond to challenges and sustain its vitality in the face of adversity (Song et al.). In contrast, simplified landscapes with reduced plant diversity are inherently more vulnerable and less able to respond to the changing conditions of our world (Zhou et al., 2024).

Wang et al. investigated how abiotic stressors influence community assemblages in grasslands on the Tibetan Plateau and Mongolian Plateau by examining the distribution of plant traits (height, specific leaf area and leaf dry matter content). The study emphasized that future climate change, including warming and changing rainfall patterns, will affect all communities differently in regions with distinct climatic conditions. At the same time, Song et al. explored the role of plant functional traits in restoring ecosystem functions in karst desertification areas. In this context, de Tomás Marín et al. examined how environmental conditions influence plant community assemblages in sub-Mediterranean ecotones in central Spain. It analyzes functional traits in six plant communities, finding that community type is the main driver of differences in functional structure. Intraspecific trait variability, rather than species turnover, plays a key role in functional changes. The study suggests that ecotones are sensitive to minor environmental changes, leading to changes in plant and functional composition. Teng et al. investigated the net ecosystem productivity of a desert riparian forest over seven years, focusing on how meteorological factors like solar radiation, temperature, humidity, and vapor pressure deficit influence net ecosystem productivity. The findings showed significant daily and seasonal variations in net ecosystem productivity, with a circadian rhythm linked to meteorological conditions. Diurnal temperature and vapor pressure deficit changes were particularly impactful, affecting net ecosystem productivity's daily amplitude and phase. The study highlights how climate variability influences ecosystem carbon dynamics, offering insights into the potential effects of climate change on arid ecosystems.

There were studies highlighting the importance of biodiversity and ecological processes in different contexts. Li et al. examined how shallow tillage affects species diversity and community dynamics in the Mu Us Desert. It found that shallow tillage increases species and phylogenetic diversity and alters community structure by reducing plant competition and enhancing stochastic processes like dispersal limitation. The study suggests selecting species with high adaptability and low niche overlap for effective ecological restoration. Gazoulis et al. discussed how incorporating non-crop plants, especially weeds, into agroecosystems can improve sustainability and resilience. Properly managing weeds through practices like mowing or grazing can provide valuable ecosystem services. Overcoming challenges related to training and quantifying these services requires research, education, and policy support. On the other hand, Kim et al. focused on the endangered plant *Pedicularis hallaisanensis* in Korea, which has a biennial lifecycle and distinct flowering cohorts. Human-induced habitat changes have reduced its genetic diversity. The study recommends minimizing anthropogenic impacts and including individuals from both flowering cohorts in conservation efforts to preserve genetic diversity. Zhang et al. found that intercropping soybean and maize significantly enhanced root exudates, lowered soil pH, improved nutrient availability (e.g., nitrogen and phosphorus), and increased arbuscular mycorrhizal fungi (AMF) colonization, improving AMF community composition. Metabolomics and sequencing analysis showed that root exudates in this system are closely linked to AMF and soil nutrient dynamics. These findings suggest that increased root exudates in intercropping systems enhance AMF composition, boost soil fertility, and sustain intercropping benefits.

Finally, Wei et al. investigated the effects of plant functional group removal on community biodiversity and niche dynamics in an alpine meadow over 3 and 10 years. Results showed that removing plant functional groups led to changes in species niches. Over time, removing Gramineae and Cyperaceae reduced their numbers, narrowed their niche widths, and decreased niche overlap. The loss of species diversity caused significant changes in the niches of remaining species, with increased negative species associations. Niche differences, driven by resource allocation, were vital in shaping species composition dynamics in the community.

The authors contributing to this research explored how plant diversity influences ecosystem resilience, including its impact on nutrient cycling, productivity and interactions with other species. The authors analyzed how different disturbances, from natural events to human-induced activities, interact with plant diversity to form resilience. Recent advances in ecological modeling, remote sensing technologies, and genetic analysis have allowed further exploration of these dynamics.

Author contributions

CB-N: Writing – original draft, Conceptualization. PD: Writing – review & editing, Validation.

Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article. This editorial was funded for CBN by project numbers RO1567-IBB01/2024 from the Institute of Biology Bucharest of the Romanian Academy.

Acknowledgments

We are very grateful to the article authors who contributed to this Research Topic, the reviewers for evaluating the work, and the Frontiers editorial staff for their guidance and production assistance. We also thank Carly Cowell for her involvement.

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

References

Benevolenza, M. A., and DeRigne, L. (2019). The impact of climate change and natural disasters on vulnerable populations: A systematic review of literature. *J. Hum. Behav. Soc. Environ.* 29, 266–281. doi: 10.1080/10911359.2018.1527739

Christenhusz, M. J., and Byng, J. W. (2016). The number of known plants species in the world and its annual increase. *Phytotaxa* 261, 201–217. doi: 10.11646/phytotaxa.261.3.1

Ditlevsen, P. D., Ditlevsen, S., and Andersen, K. K. (2002). The fast climate fluctuations during the stadial and interstadial climate states. *Ann. Glaciol.* 35, 457–462. doi: 10.3189/172756402781816870

Zhou, H., Shi, H., Yang, Y., Feng, X., Chen, X., Xiao, F., et al. (2024). Insights into plant salt stress signaling and tolerance. *J. Genet. Genom.* 51 (1), 16–34. doi: 10.1016/j.jgg.2023.08.007

frontiers in Plant Science

ORIGINAL RESEARCH
published: 03 January 2022
doi: 10.3389/fpls.2021.715730

Divergent Abiotic Stressors Drive Grassland Community Assembly of Tibet and Mongolia Plateau

***Jianming Wang*[1]*, Mingxu Li*[1]**, Li Xu*[1]*, Congcong Liu*[1]*, Pu Yan*[1] *and Nianpeng He*[1,2,3]**

[1] Key Laboratory of Ecosystem Network Observation and Modeling, Institute of Geographic Sciences and Natural Resources Research, Chinese Academy of Sciences, Beijing, China, [2] College of Resources and Environment, University of Chinese Academy of Sciences, Beijing, China, [3] Key Laboratory of Vegetation Ecology, Ministry of Education, Northeast Normal University, Changchun, China

Multiple ecological processes simultaneously govern community assembly, but it remains unclear how abiotic stressors regulate the relative importance of these processes among different biogeographic regions. Therefore, we conducted a comprehensive study on the responses of community assembly to varying environmental gradients, using the mean, variance, skewness, and kurtosis of plant height (height), specific leaf area (SLA) and leaf dry matter content (LDMC) distributions on the Tibetan Plateau (TP) and the Mongolian Plateau (MP). Our results showed that the prevalence of trait convergence across all grasslands in both TP and MP seem to be the result of abiotic filtering or weaker competitive exclusion etc. These trait-convergence assembly processes decrease the functional dispersion but increase the evenness of the trait frequency distribution. The mean, variance, skewness, and kurtosis responses of grassland communities to abiotic stress varied between the TP and MP. On average, plant trait distribution was mainly driven by temperature on the TP, and low-temperature stress altered the community assembly rules. In contrast, water availability shaped plant trait frequency distributions on the MP, and drought stress mediated the balance between different assembly processes. Our results provide empirical evidence that divergent abiotic stressors regulate the grassland community assembly on the TP and MP. Together, our study speculates that different aspects of future climate change, such as climate warming and changing precipitation patterns, on community assembly are dependent on regional climatic regimes.

Keywords: trait, community assembly, abiotic filtering, weaker competitive exclusion, stress, climate change

Edited by:
Jian-Guo Huang,
South China Botanical Garden,
Chinese Academy of Sciences (CAS),
China

Reviewed by:
Réka Kiss,
Hungarian Academy of Sciences,
Hungary
Hanxue Liang,
Shanxi University, China

****Correspondence:***
Mingxu Li
mingxuli@igsnrr.ac.acn

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

Received: *27 May 2021*
Accepted: *06 December 2021*
Published: *03 January 2022*

Citation:
Wang J, Li M, Xu L, Liu C, Yan P and He N (2022) Divergent Abiotic Stressors Drive Grassland Community Assembly of Tibet and Mongolia Plateau. Front. Plant Sci. 12:715730. doi: 10.3389/fpls.2021.715730

INTRODUCTION

Elucidating the mechanisms and drivers of plant community assembly is a key challenge in ecology (Keddy, 1992; Vellend, 2010; Yao et al., 2021). However, many studies suggest that the traditional taxon-based approach is unable to adequately describe the influence of climate on plant community assembly processes (McGill et al., 2006; Götzenberger et al., 2012; Purschke et al., 2013; Cadotte and Tucker, 2017). Functional traits characterize the ecological strategies that species use to respond to environmental variations (Dìaz and Cabido, 2001; Violle et al., 2007). In nature, plant communities along environmental gradients exhibit distinct functional trait frequency distributions (Wieczynski et al., 2019; Liu et al., 2020), and the shifts in trait distributions are linked to community assembly processes and their responses to climate change (Enquist et al., 2015). Neutral theory posits that all individuals in a community are ecologically equivalent, and stochastic processes produce a random local trait frequency distribution (Cadotte and Tucker, 2017; Perronne et al., 2017). In contrast, a non-random trait frequency distribution pattern is observed if deterministic processes dominate (Kraft and Ackerly, 2010). Recent studies have suggested that multiple processes regulate community assembly, and changes in the relative strength of these processes may induce substantial shifts in trait distributions (Enquist et al., 2015). Therefore, exploring the influence of the underlying processes on trait distribution may provide new insights into community assembly mechanisms.

Abiotic filtering and biotic interactions have long been recognized as the predominant forces of community assembly (Emerson and Gillespie, 2008; Swenson et al., 2012). Abiotic filtering tend to preferentially select species with specific traits enter into community, resulting in coexistence of functionally similar species (i.e., trait convergence). Limiting similarity holds that biotic interactions, such as competition and parasitism, may prevent species from being too similar, leading to the niche differentiation of coexisting species possessing dissimilar traits (Macarthur and Levins, 1967; Cornwell and Ackerly, 2009; Bernard-Verdier et al., 2012). However, weaker competitor hypothesis believes that competitive interaction also leads to trait convergence patterns, because species bearing traits associated with low competitive ability are likely to be excluded by highly competitive species sharing traits conferring higher fitness, resulting the coexistence of functionally similar species (Grime, 2006; Mayfield and Levine, 2010). Additionally, facilitative interactions have been found to result in trait convergence among existing species (Moeller, 2004). Both the importance of abiotic filtering and biotic interactions has been demonstrated across different scales and ecosystems (Ding et al., 2019; Fang et al., 2019; Luo et al., 2021). However, their relative roles might be shaped by abiotic stressors (Lhotsky et al., 2016; Luo et al., 2019). Bernard-Verdier et al. (2012) found that resource availability mediated assembly rules in a Mediterranean rangeland. Furthermore, different abiotic stressors may influence species strategies differently. For example, resource availability is the main driver of different strategies along the leaf economic spectrum under high precipitation, whereas hydraulic constraints prevail under arid conditions (Blanchard et al., 2019). Therefore, testing the effect of multiple abiotic stressors on trait distribution provides a great opportunity to understand and predict the response of community assembly to climate change (Le Bagousse-Pinguet et al., 2017). Nevertheless, how multiple abiotic stressors jointly drive variations in the distribution of traits within communities across different spatial scales remains unclear.

Large-scale tests of trait–environment relationships have focused on trait distributions that span multiple biomes and biogeographic regions (Bruelheide et al., 2018). However, each region often has unique historical and biogeographic features (such as species pool, soil, and climate) (Zhang et al., 2016). These complex differences in historical and biogeographic conditions among different regions may be confounded in testing local community assembly processes (Ricklefs and He, 2016). As the direction and rates of trait evolution, dispersal, and speciation differ among biogeographic regions (Weir and Schluter, 2007; Cooper and Purvis, 2010), the manner in which species strategies respond to abiotic stressors may depend on the environmental regime (Muscarella et al., 2016; Crous et al., 2018). For example, water availability generally determines biodiversity in regions with high energy inputs, whereas temperature is more important for cold areas or higher altitudes (Hawkins et al., 2003; Kreft and Jetz, 2007). However, few studies have focused on elucidating how trait frequency distributions in different regions with different limiting factors are affected by abiotic stressors.

To explore how multiple abiotic stressors affect plant community assembly among regions, regional-scale transect surveys were conducted across major grassland types within two representative Eurasian grassland biogeographic regions (**Figure 1**). The Tibetan Plateau (TP), referred to as the "the world's roof," covers the highest alpine grasslands, which experience low temperatures as a result of their altitude (Liu et al., 2018). Recently, the rate of climate warming on the TP has been more than twice the global average (Ma et al., 2017; Yao, 2019). The Mongolian Plateau (MP) covers the largest regions of Eurasian temperate grasslands, which are characterized by extremely limited water and nutrient availability (Wang J. et al., 2021). MP is also expanding owing to climate warming and frequent extreme weather events (Easterling et al., 2000; Dai, 2013). Both TP and MP have continuous natural vegetation gradients, which range from desert grassland to typical grassland to meadow, and similar precipitation gradients from west to east, making them ideal systems for conducting biogeographic comparisons of the responses of community assembly to climate change.

Here, the frequency distribution of three key functional traits of 80 grassland communities was quantified using the four important moments (i.e., mean, variance, skewness, and kurtosis) in each region (**Figure 1**). We specifically address the following questions: (1) What is the relative importance of stochastic and deterministic processes in these two regions? (2) Does the response of trait frequency distribution to abiotic stressors differ between the two regions? (3) What are the foremost abiotic stressors regulating community assembly processes in two regions?

MATERIALS AND METHODS

Study Regions

In 2018, field investigations were conducted across the alpine and temperate regions of the eastern part of the Eurasian grasslands, including the TP and MP (**Figure 1**). In each region, an east–west transect was established, ranging from arid to mesic grasslands with varied soil, climatic, and vegetation conditions. The transect on the TP includes three dominant vegetation types (desert grassland, typical grassland, and meadow) with decreasing mean annual temperature (MAT, ca. 0.8 to −2.96°C)

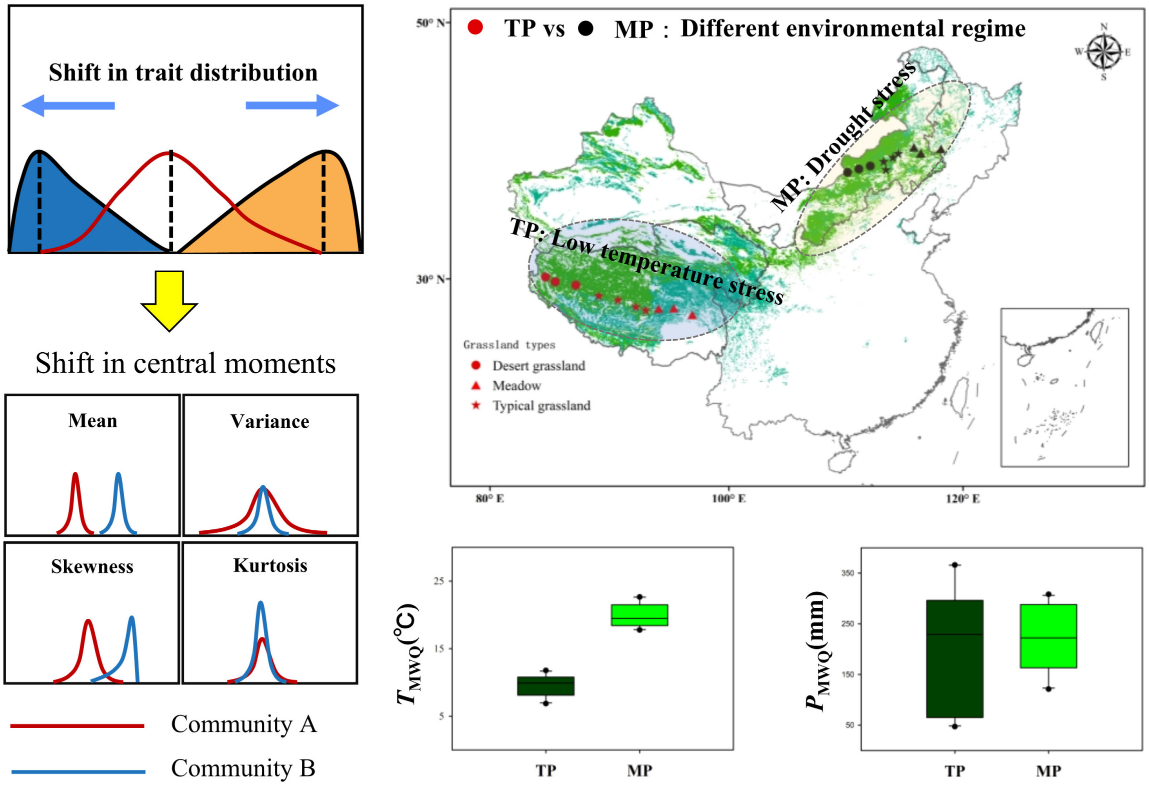


FIGURE 1 | Two comparative grassland transects on the Tibetan Plateau (TP) and the Mongolian Plateau (MP). They represent different environmental regimes. Each transect covers meadows, typical grasslands, and desert grasslands. The shifts in central moments of trait distribution represent the difference in intrinsic structure between two communities.

and increasing mean annual precipitation (MAP, ca. 75–606 mm) from desert grassland to meadows. On the TP, desert grasslands were dominated by *Stipa tianschanica var. gobica* and *Ajania fruticulose,* typical grasslands were dominated by *Stipa purpurea* and *Stipa capillata*, and meadows were dominated by *Kobresia pygmaea* and *Potentilla saundersiana*, among others. The transect on the inner MP includes three dominant vegetation types (desert grassland, typical grassland, and meadow) with increasing MAP (ca. 183–425 mm) from desert grassland to meadows, whereas average MAT was the highest in meadows (ca. 3.91–6.64°C), followed by desert grasslands (ca. 1.81–3.81°C), and the lowest in typical grasslands (ca. 0.98–1.59°C). On the MP, desert grasslands were dominated by *Stipa breviflora* and *Stipa klemenzii;* typical grasslands were dominated by *Stipa grandis* and *Artemisia frigida*, and meadows were dominated by *Stipa baicalensis* and *Leymus chinensis*. The Wilcoxon test showed that the soil and climatic conditions were significantly different between the two grassland transects (**Figure 1** and **Supplementary Figure 1**). Together, these transects were ideal systems for examining the macroscale drivers of community assembly.

Field Sampling

At each east–west transect, 10 survey sites were randomly selected along the vegetation gradient, including three for desert grassland, four for typical grassland, and three for meadow. At each site, eight 1 m × 1 m quadrats were randomly established within a 1 km × 1 km sampling area, and the geographic coordinates and elevations of each quadrat were recorded using a Global Positioning System (GPS) device. In total, 24 quadrats of desert grassland, 32 quadrats of typical grassland, and 24 quadrats of meadow were selected in each biogeographic region. For each quadrat, all plant species and their individuals were identified, and the plant coverage of each species was visually estimated. The height of each species was determined by measuring the height of 50 randomly selected individuals from each site. Approximately 50 mature but non-senescent leaves with little damage were collected from different locations at each site (outside of each quadrat) to determine leaf dry matter content (LDMC) and SLA. Plant samples were detached to measure plant height, SLA, and LDMC using a previously described procedure (Cornelissen et al., 2003; Pérez-Harguindeguy et al., 2013). The "species mean" trait values were calculated by averaging the trait values of all repetitions of a given species sampled across 10 sites. Aboveground biomass was clipped by species in each 1 m × 1 m quadrats, and dead parts were removed and combined with plant litter. The leaf and aboveground biomass samples were carefully cleaned and oven-dried at 60°C. The aboveground biomass of each species within each quadrat was measured. According to soil heterogeneity, 20–30 soil cores (10 cm in depth) were randomly collected in each quadrat and subsequently mixed into a composite sample.

Soil and Climate Data

All soil samples were air-dried after being sieved (2 mm mesh), and visible roots and organic debris were removed. We summarized soil parameters at each quadrat using soil total nitrogen (S_{TN}) and phosphorus (S_{TP}) content, and soil pH (S_{pH}). S_{pH} was determined using a 1:2.5 (v/v) soil water aqueous extract. The S_{TN} and S_{TP} contents have been calculated in a previous study (Zhang et al., 2020).

As climate variables, the mean temperature of the warmest quarter (T_{MWQ}) and temperature seasonality (T_S) were selected to represent the temperature stress; the precipitation of the wettest quarter (P_{MWQ}), and precipitation seasonality (P_S) were selected to represent the water stress. T_S indicates the difference between the annual maximum and minimum temperatures, whereas P_S reflects the differences in the seasonal distribution of precipitation between locations in the form of alternating dry and wet seasons. These climatic data were obtained from the WorldClim global climate database using geographic coordinates for each site (with a resolution of 1 km × 1 km).[1]

Trait Frequency Distribution

Three key plant traits, namely, height, SLA, and LDMC, were used in this study. Plant height is a central trait for plant ecological strategies and is strongly correlated with seed mass, life span, and time to maturity (Zhang et al., 2016). Height is key to a species' carbon gain strategy because it is a major determinant of the species' competitive ability to light (Díaz et al., 2016). SLA describes the amount of leaf area for light capture per unit of biomass invested, which reflects the trade-offs between leaf structural attributes, carbon gain, and nutrient content (Wright et al., 2004). High SLA values are generally recorded in resource-rich environments, whereas low values are recorded in resource-poor environments (Freschet et al., 2010; Pérez-Harguindeguy et al., 2013). LDMC quantifies leaf tissue density and nutrient retention capacity. High LDMC values indicate a preference for conserving nutrients. Species with high levels of LDMC have tough leaves that are highly resistant to hazards (Freschet et al., 2010; Lienin and Kleyer, 2012). Height and SLA are two key independent axes of plant ecological strategies (Westoby, 1998; Le Bagousse-Pinguet et al., 2017). SLA is a function of LDMC and leaf thickness, while LDMC may give more meaningful information (Pérez-Harguindeguy et al., 2013). For example, SLA misses the majority of its ecological explanation in species whose photosynthetic organs do not have the typical planar form, LDMC remains well defined (Hodgson et al., 2011).

The trait frequency distribution of plant communities was quantified using four moments: mean, variance, skewness, and kurtosis (Enquist et al., 2015). The mean is the average location of plant community traits as a result of environmental selection and species adaptation, whereas the variance reflects the range or dispersion of trait values within a local community (Gross et al., 2017). Skewness and kurtosis are the shape moments of the trait frequency distribution, which represent the rarity and evenness of trait values within local communities (Butterfield and Munson, 2016; Wieczynski et al., 2019). We calculated the community-weighted mean, variance, skewness, and kurtosis (all weighted by the relative aboveground biomass of species) of height, SLA, and LDMC for each community.

$$\mathrm{Mean_j}{=}\textstyle\sum_i^n P_i \mathrm{Ti} \tag{1}$$

$$Variance_j = \sum_i^n P_i(\mathrm{Ti} - \mathrm{Mean}_j)^2 \tag{2}$$

$$Skewness_j = \sum_i^n \frac{P_i(\mathrm{Ti} - \mathrm{Mean}_j)^{\,3}}{Variance_j^{3/2}} \tag{3}$$

$$Kurtosis_j = \sum_i^n \frac{P_i(\mathrm{Ti} - \mathrm{Mean}_j)^4}{Variance_j^2} \tag{4}$$

where P_i and T_i are the relative aboveground biomass and the trait value of species i, respectively, in community j and n is the total number of species within community j. For each community, the sum of the relative aboveground biomass is equal to 100%.

Null Models

In this study, null model was used to identify the relative dominance of deterministic and stochastic processes (Swenson, 2014). All species occurring in 80 plots of each region were regarded as species pools for that region. Then, we randomly shuffled the functional trait values using these species pools and generated 999 randomized communities. Subsequently, the standardized effect size (SES) of each trait moment calculated as the difference between the observed value and the mean value of the null communities divided by the standard deviation of value of the null communities:

$$\mathrm{SES} = \frac{\left(parameter_{observed} - mean\left(parameter_{null}\right)\right)}{standard\ deviation\ \left(paramatern_{ull}\right)}. \tag{5}$$

Due to the non-normality of the variables, the Wilcoxon test was conducted to test the significant deviations of the observed parameters (i.e., mean, variance, skewness, and kurtosis) from null expectations (SES = 0). Community assembly was considered non-random if SES was significantly different from 0 (Kraft and Ackerly, 2010).

Community-weighted variance (CWV) is equal to the functional dispersion defined by Rao's quadratic entropy estimated using Euclidian dissimilarities (Rao, 1982; De Bello et al., 2009). Therefore, the SES values of the variances in height, SLA, and LDMC frequency distributions were used to further test the relative strength of different deterministic processes in regulating grassland community assembly. The SES of a CWV less than 0 indicates trait convergence among coexisting species (Blanchard et al., 2019), whereas an SES value greater than 0 indicates trait divergence patterns (Bernard-Verdier et al., 2012). Trait divergence and convergence patterns may result from

---

[1] http://www.worldclim.org

multiple community assembly processes, such as abiotic filtering and competitive and facilitative interactions (Moeller, 2004; Grime, 2006; Cornwell and Ackerly, 2009; Mayfield and Levine, 2010). To avoid uncertainties in the accepted usage of the term "assembly process," the trait-convergence and trait-divergence assembly processes were adopted in this study following the method of (Pillar et al., 2009).

Statistical Analysis

Four moments (mean, variance, skewness, and kurtosis) of trait distribution and seven environmental variables (T_{MWQ}, T_S, P_{MWQ}, P_S, S_{TN}, S_{TP}, and S_{pH}) were used in the statistical analysis. Prior to the analysis, all the explanatory variables were standardized. The data for variance and kurtosis of the trait frequency distribution were log-transformed to improve non-normality. The explanatory variables were divided into temperature stress, water availability, and soil attributes. The Wilcoxon test was employed to explore the differences in environmental variables and the moments of trait distribution between the TP and the MP. General linear and quadratic regression models were used to evaluate the relationship between the moments of trait frequency distribution and abiotic variables. Akaike information criterion (AIC) values were used to determine the better-fitting model (with a 10-unit reduction in the AIC value).

Stepwise multiple regression was used to further examine the most important drivers of the moments of the trait frequency distribution. The quadratic terms of the explanatory variables were also included in the initial models to account for possible quadratic relationships. To prevent data overfitting, all variables were subjected to forward selection until $P < 0.05$ for all explanatory variables. Variables were removed according to the criterion of a variance inflation factor > 3 to further avoid strong collinearity among variables. Hierarchical partitioning was applied to explore the independent effect of each variable using an R package.

RESULTS

Shift in Functional Trait Frequency Distribution Along Environmental Gradients

Both the mean and variance of the height, SLA, and LDMC frequency distributions were lower on the TP than on the MP (**Supplementary Figure 2**). However, there were no differences in skewness and kurtosis values. Furthermore, the responses of the four moments of height, SLA, and LDMC distributions to temperature stress and water availability differed between the TP and MP (**Figure 2**, **Supplementary Figures 3–6**, and **Supplementary Tables 1–3**). Both all moments of height and SLA distribution, and the skewness of LDMC distribution were more strongly related to T_{MWQ} or T_S in TP. In contrast, both all moments of height and LDMC distribution, skewness and kurtosis of SLA distribution showed stronger correlations with P_{MWQ} or P_S in MP. Notably, the variance and kurtosis of LDMC distribution were only significantly related to P_S in TP, while the mean and variance of SLA were more strongly related to T_{MWQ} in MP.

The mean height was positively related to T_{MWQ} on the TP but showed a concave relationship on the MP (**Supplementary Table 1**). The mean height increased with P_{MWQ} on the MP but decreased on the TP. The variances in height had opposing responses to T_{MWQ} between the TP and MP. Both the skewness and kurtosis of the SLA were significantly correlated with P_{MWQ} on the MP ($P < 0.01$) but only with T_{MWQ} on the TP ($P < 0.01$) (**Supplementary Table 2**). The skewness of the LDMC distribution was positively related to P_S on the TP but negatively related to P_S on the MP (**Supplementary Table 3**). The shifts in the height and SLA distributions along the soil pH and nutrient gradients also varied between the TP and MP. For example, the mean and variance of both height and SLA increased with soil pH on the TP but not on the MP.

Drivers of Trait Frequency Distribution

Stepwise multiple regression results showed that the four moments of the height, SLA, and LDMC frequency distributions are dependent on climatic factors on both the TP and MP (**Supplementary Tables 4**, **5**). However, the relative influence of water- and temperature-related factors varied between the TP and MP. On the TP, T_S and T_{MWQ} individually explained 0–47.30, 7.67–40.77, and 0–21.32% of the variation in the four moments of the height, SLA, and LDMC distributions, respectively (**Figure 3** and **Supplementary Figure 7**), whereas water-related factors individually explained 0–13.69%, 0–9.46%, and 4.9–24.41% of the variation in those three trait distributions. On the MP, P_S and P_{MWQ} individually explained 30.28–61.21%, 6.02–25.40%, and 7.35–50.62% of the variations in the four moments of the height, SLA, and LDMC distributions, respectively (**Figure 3** and **Supplementary Figure 7**), whereas T_S and T_{MWQ} explained 0–22.08%, 0–62.04%, and 0–22.99% of the variations in those three trait distributions. These results indicate that both water- and temperature-related factors significantly influence the trait distributions on the TP and MP. However, on average, temperature-related factors determine the trait distributions on the TP, whereas water-related factors have greater influence on the trait distributions on the MP.

Influence of Different Ecological Processes on Community Assembly

The results of the null model and Wilcoxon test demonstrated that almost all moments of height, SLA, and LDMC (except for mean and skewness of SLA) significantly deviated from their null expectations (SES = 0) on the TP ($P < 0.05$, **Figure 4** and **Supplementary Figure 8**). Similarly, the variance and kurtosis of height significantly deviated from the null expectations on the MP. Furthermore, the mean SESs of the kurtosis of height, SLA, and LDMC distributions across all grassland communities on both the TP and MP were significantly less than 0 (**Figure 4**). The majority of grassland communities exhibited convergent frequency distributions for height (81.25%), SLA (88.75%), and LDMC (68.75%) on the TP (**Figure 5**). Convergent SLA, LDMC,

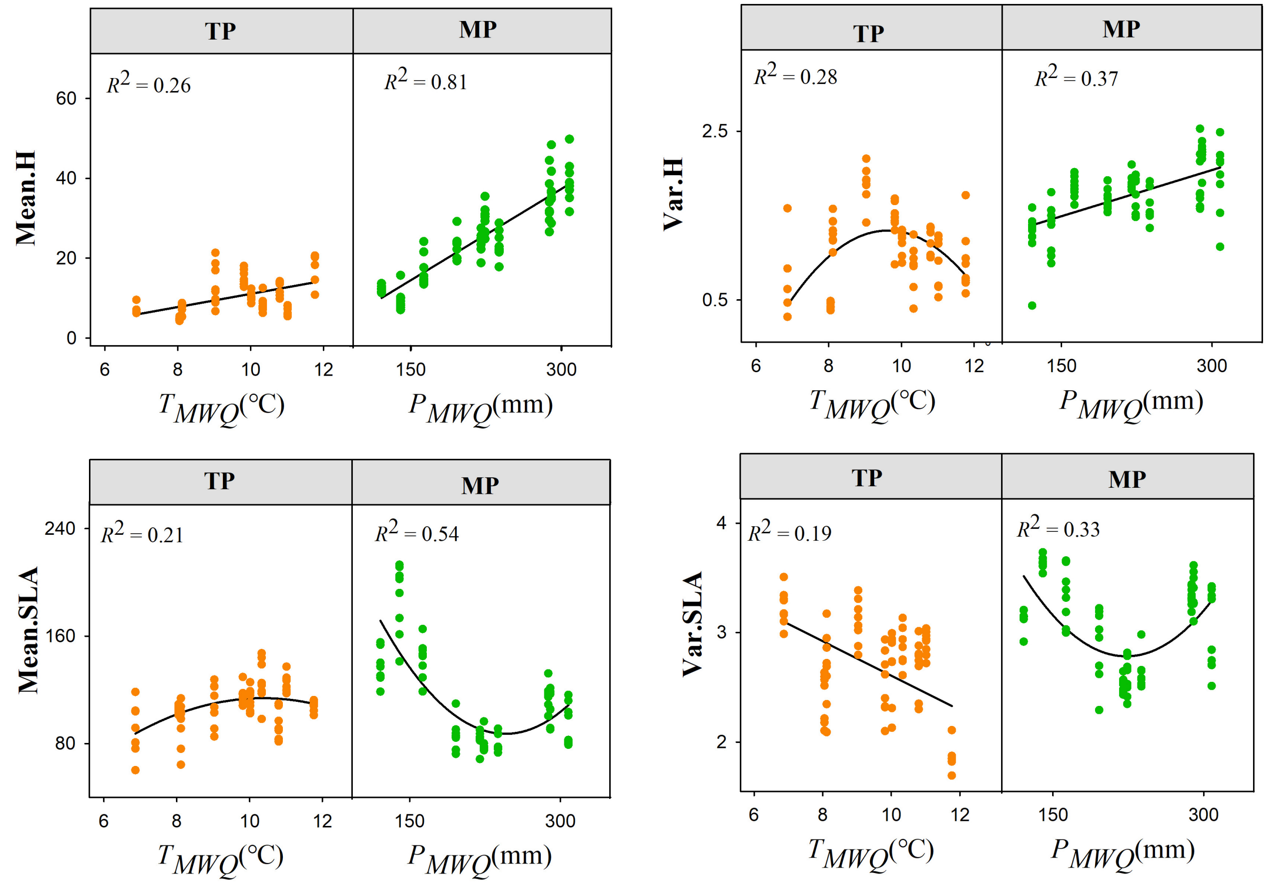


FIGURE 2 | Shifts in the trait frequency distributions for height (H) and specific leaf area (SLA) within grassland communities along environmental gradients. Mean.H and Mean.SLA represent community-weighted mean of height and SLA, respectively; Var.H and Var.SLA represent community-weighted variance of height and SLA, respectively.

and height distributions were also observed in 86.25, 65, and 75% of the grassland communities on the MP, respectively. The Wilcoxon test results further demonstrated lower observed variances of height, SLA, and LDMC than expected on both the TP and MP, when trait variance was evaluated as the mean value across all local grassland communities.

DISCUSSION

Dominant Roles of Trait-Convergence Process in Driving Community Assembly

To elucidate the relative contributions of deterministic and stochastic processes in grassland community assembly across the TP and MP, we determined the differences between the observed and expected values for dominance, dispersion, rarity, and evenness of trait distributions. Our results demonstrated that almost all moments of the height, SLA, and LDMC frequency distributions significantly deviated from the null expectations, suggesting the dominance of deterministic processes (Kraft and Ackerly, 2010; Liu et al., 2020). More importantly, our results further demonstrated that trait convergence was more prevalent than trait divergence across grassland communities of the TP and MP, implying the widespread coexistence of functionally similar species within the grassland community. This indicates that trait-convergence processes, such as abiotic filtering or weaker competitive exclusion, govern grassland community assembly (Grime, 2006; Mayfield and Levine, 2010; Backhaus et al., 2021). Previous studies on forests have reported the prevalence of trait divergence in tree assemblages (Luo et al., 2019, 2021), while study on desert steppe observed a convergent trait frequency distribution (Wang X. et al., 2021). The interpretation for those discrepancy between forest and grassland might be that, harsh grassland environment leads to a stronger abiotic filtering or weaker competitive exclusion, which reduce the range of trait values (convergence; De Bello et al., 2009; Kraft and Ackerly, 2010), while limiting similarity dominates in weaker stressful forest, thereby results in a high trait diversity (divergence; Mayfield and Levine, 2010). This supports the viewpoints of environment stress governs the relative roles of different assembly processes, which resulting in different trait distribution patterns (Enquist et al., 2015; Lhotsky et al., 2016).

The mean SESs of the kurtosis of height, SLA, and LDMC distributions across all communities of the TP and MP were

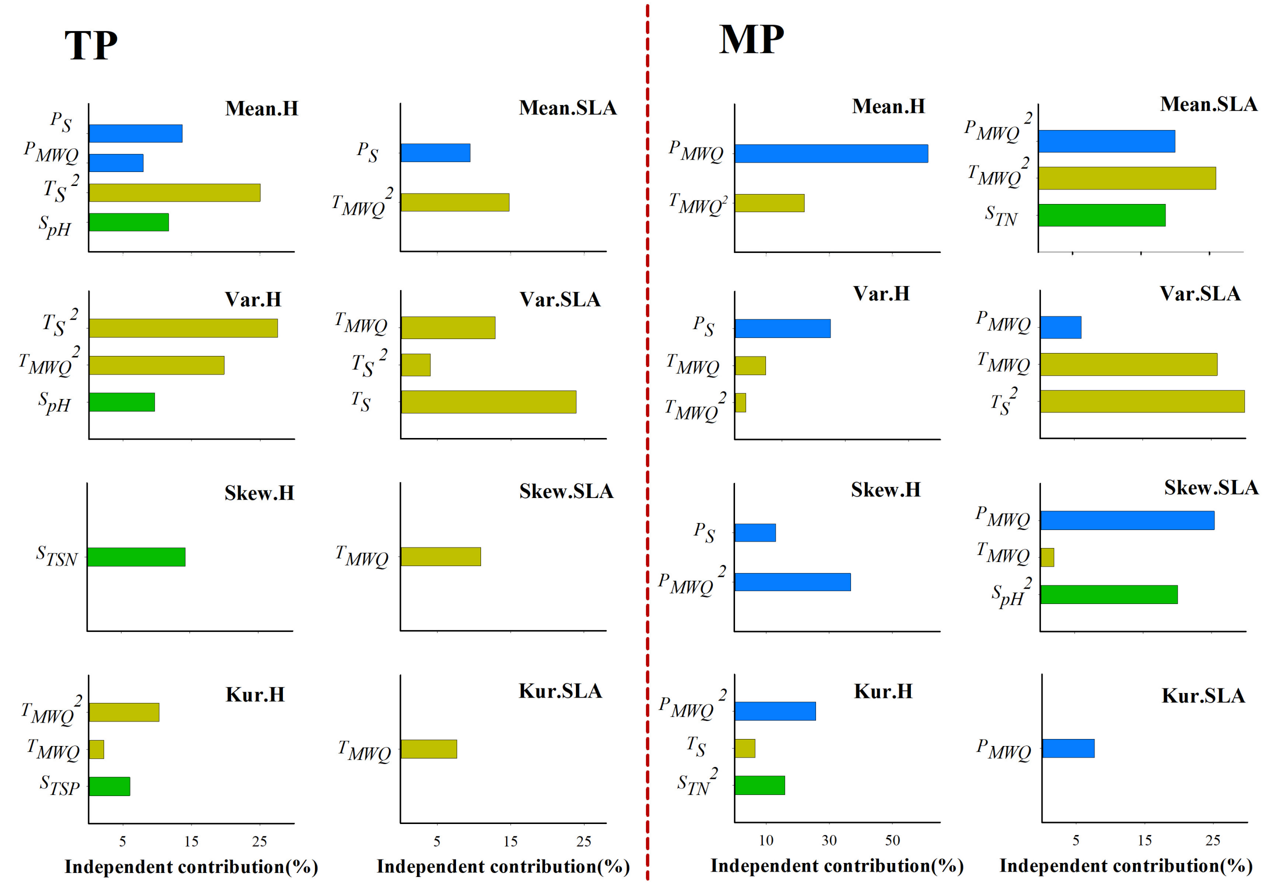


FIGURE 3 | Independent influence of abiotic variables on the variations in mean, variance, skewness, and kurtosis of the height and SLA distributions within grassland communities. Skew.H and Skew.SLA represent community-weighted skewness of height and SLA, respectively; Kur.H, and Kur.SLA represent community-weighted kurtosis of height and SLA, respectively.

less than 0. This implies that the trait-convergence assembly process decreased the functional dispersion but increased the evenness of the trait frequency distribution. Functional traits can regulate species abundance by influencing fitness and performance (McGill et al., 2006; Webb et al., 2010). Trait-convergence processes, such as abiotic filtering or weaker competitive exclusion, maintain the species coexistence and filter out species with lower resistance and competitiveness, thereby reducing functional dispersion (Mayfield and Levine, 2010; Kraft et al., 2015; Šímová et al., 2015). However, species that enter the community will have relatively high and uniform fitness (Grime, 2006; Cornwell and Ackerly, 2009), which may decrease the rarity of traits.

Response of Trait Distribution to Abiotic Stressors Between Tibetan Plateau and Mongolian Plateau

Habitat conditions may directly influence the trait distribution of plant communities (Schöb et al., 2013). Temperature and drought stresses are likely the most important macrofilters for grassland community assembly on the TP and MP, respectively (Zhang et al., 2020). Therefore, the observed shifts in different key features of trait distribution along these abiotic stress gradients can be linked to community assembly processes and how communities respond to climate change (Enquist et al., 2015). In line with the findings for global drylands (Le Bagousse-Pinguet et al., 2017), this study revealed significant shifts in four moments of the height, SLA, and LDMC frequency distributions along abiotic gradients on both the TP and MP. All evidence indicates that increasing abiotic stress may lead to different shapes of the trait frequency distributions, which in turn affects grassland community assembly (Kraft et al., 2015).

However, the responses of the four moments of height, SLA, and LDMC frequency distributions to abiotic stressors differed between the TP and MP. The mean and variance of the community height data may increase along the water availability gradient on the MP but decrease on the TP because hydraulic constraints are more prevalent on the MP than on the TP (Koch et al., 2004). Lower temperatures substantially reduced plant height on the TP, indicating that low-temperature stress strongly limits plant growth in alpine grasslands. In contrast, plant height increased at the two extremes of the temperature gradient across the MP because

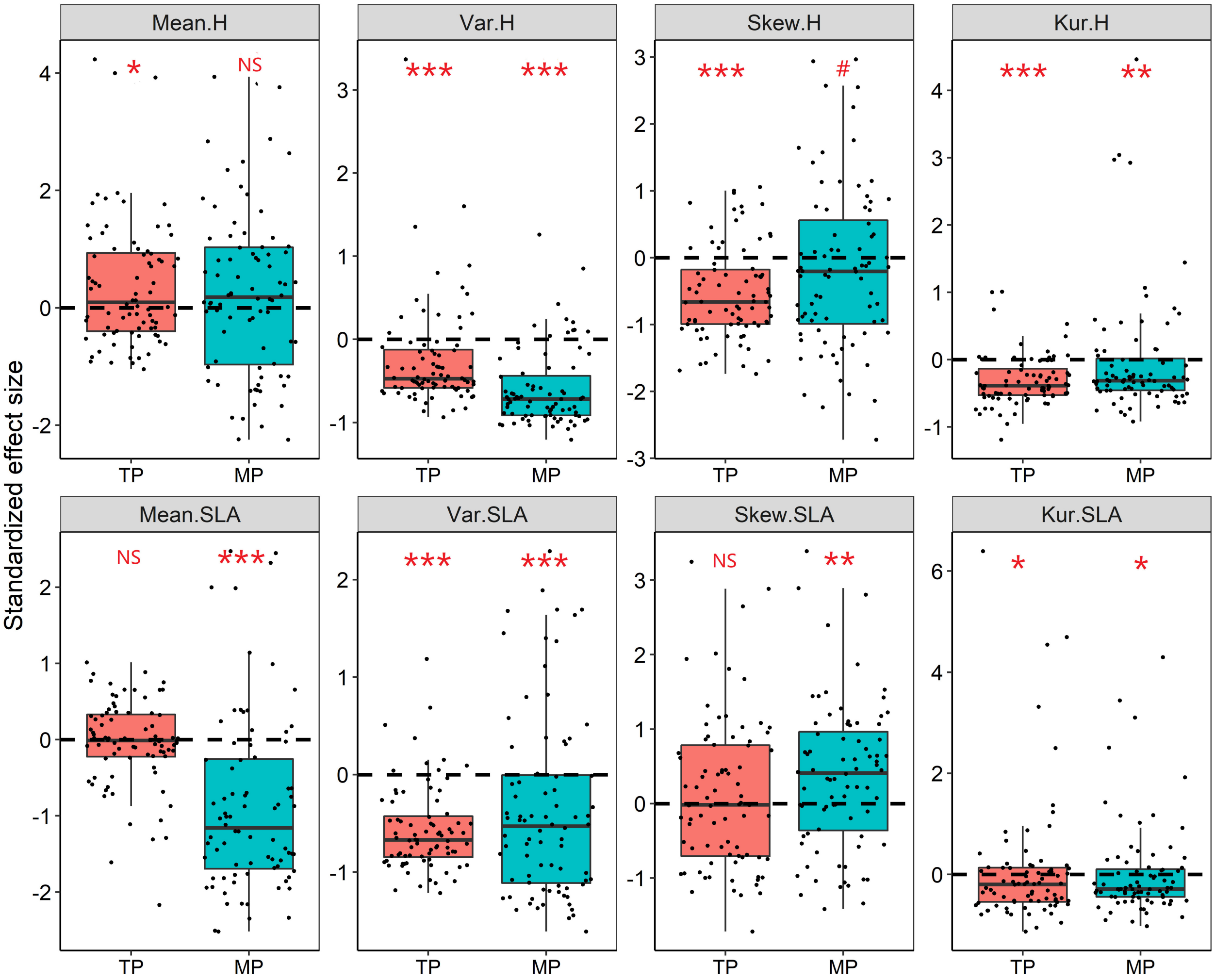


FIGURE 4 | Standardized effect size (SES) values for the mean, variance, skewness, and kurtosis of the height and SLA distributions within grassland communities. The Wilcoxon test was conducted to test significant deviations within each observed trait metric from the null expectation (SES = 0), NS, $P > 0.05$; $^{\#}P < 0.1$; $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$. The black dashed line indicates the value of zero.

of the U-shaped relationship of summer precipitation with temperature, which resulted in lighter drought stress at the two extremes of the temperature gradient. Given that the temperature on the TP was lower than that on the MP (Zhang et al., 2020), these results suggest that species strategy responses to different stressors depend on the environmental regime (Muscarella et al., 2016; Crous et al., 2018). The variance of height and SLA were more strongly related to temperature-related factors on the TP but to water availability on the MP. The kurtosis of height and SLA responded prominently to low-temperature stress on the TP but had a more powerful response to drought stress on the MP. These results demonstrate that low-temperature and drought stresses determine the functional dispersion and evenness of grassland communities on the TP and MP, respectively. Furthermore, the skewness of the LDMC frequency distribution was positively (negatively) related to precipitation seasonality on the TP (MP). These results suggest that climatic regimes may mediate community assembly responses to abiotic stressors and even climate change (**Figure 6**).

Different Abiotic Stressors Driving Community Assembly Processes on Tibetan Plateau and Mongolian Plateau

Temperature, precipitation, and their seasonality exerted the greatest individual effects on the four key moments of the height, SLA, and LDMC frequency distributions on the TP and MP, which demonstrates that climate is the dominant filter driving the trait frequency distribution (Wieczynski et al., 2019). However, we observed that soil pH and nutrient content individually explained 6.01–14.32% and 6.58–23.46% of the variation in some moments of height, SLA, and LDMC, respectively. Furthermore, these soil variables were highly correlated with climatic factors. Previous studies have reported that climate and soil interact to affect trait

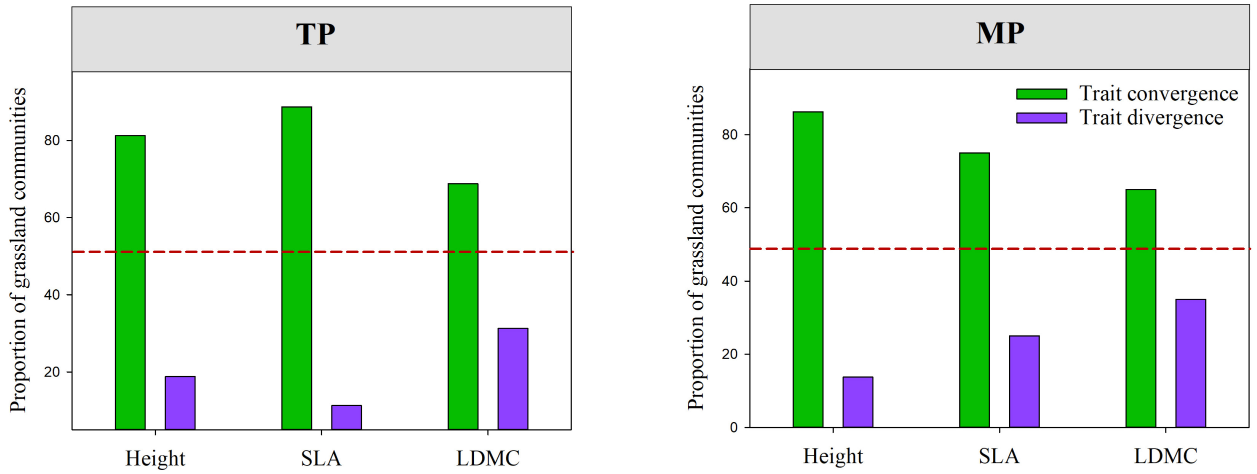


FIGURE 5 | The relative prevalence of convergence and divergence within grassland communities among different regions, which were evaluated based on standardized effect values (SES) for the variance in height, SLA and, and leaf dry matter content (LDMC) distributions within grassland communities (SES.Var.H, SES.Var.SLA, and SES.Var.LDMC). The red dashed line indicates the 50% quantile. Convergence: SES < 0; divergence: SES > 0. SES. Var.H, SES.Var.SLA, and SES.Var.LDMC represent the standardized effect size values of community-weighted variance of height, SLA, and LDMC, respectively.

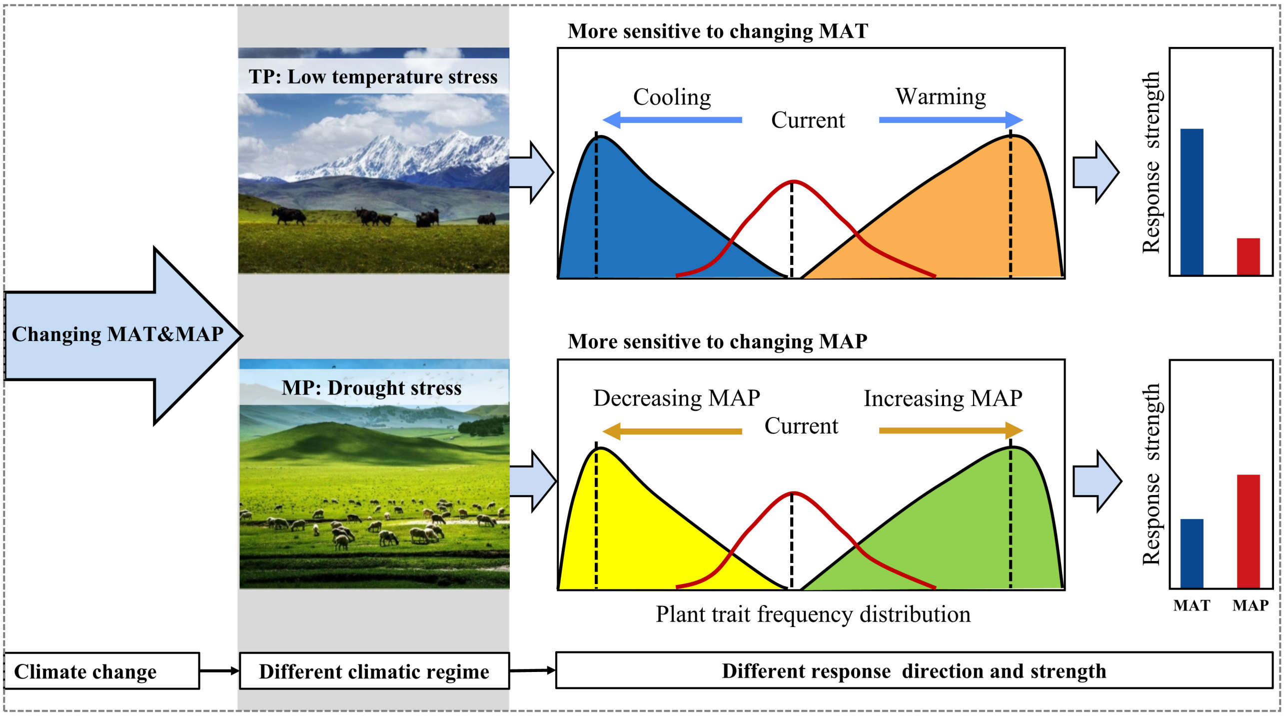


FIGURE 6 | A theoretical framework for the different responses of community assembly to climate change. The specifics of the climatic regimes will govern the direction and strength of the responses to changes in climate. MAP, mean annual precipitation; MAT, mean annual temperature.

distributions (Simpson et al., 2016; Le Bagousse-Pinguet et al., 2017). Therefore, local environmental factors, such as soil pH and nutrient availability, also play an important role in influencing the trait frequency distribution in grassland communities (Le Bagousse-Pinguet et al., 2017; Umaña et al., 2021).

Our results further showed that temperature-related factors exerted a more individual effect on the height and SLA distribution of the TP, confirming the dominant role of temperature as an abiotic filter. The skewness of the LDMC distribution was more strongly influenced by temperature on the TP, whereas the variance and kurtosis were mainly determined by drought stressors. This suggests that drought stress plays an important role in shaping the trait distribution on the TP and that multiple leaf trait strategies respond to similar abiotic stresses. The largest fraction of the variations in height, SLA, and LDMC distributions was driven by water-related factors on the MP, which implies that water availability determines the distribution of plant traits within MP communities, which is consistent with the earlier findings (Butterfield and Munson, 2016). This result suggests that divergent abiotic drivers shape functional trait frequency distributions within grassland communities among different biogeographic regions.

We demonstrated that divergent abiotic stressors regulate the strength of trait convergence and divergence on the TP and MP, respectively, which partly supports the stress-dominance hypothesis (Coyle et al., 2014; Lhotsky et al., 2016). The SESs of the variance in height and SLA were mainly affected by temperature-related variables on the TP (**Supplementary Figure 5**). On the TP, community height was more convergent and less divergent at both ends of the temperature gradient, but community SLA tended to converge at moderate levels of temperature stress. This evidence supports the hypothesis that different traits vary substantially in their roles in trait convergence and divergence in community assembly (Ding et al., 2019). In contrast, water availability plays a key role in shaping the relative strength of the trait convergence and divergence on the MP. Community height became more convergent in more drought-prone habitats, but community SLA tended to exhibit trait convergence at moderate levels of drought stress. Community LDMC became more convergent in the higher precipitation seasonality region on the TP, while it tended to exhibit trait convergence at both ends of the drought gradient on the MP. These findings provide robust evidence that different abiotic stressors determine the relative importance of abiotic filtering and biotic interactions in the grassland community assembly on the TP and MP. Overall, our findings suggest that the effect of different aspects of future climate change, such as climate warming and changing precipitation patterns, on community assembly is dependent on regional climatic regimes, especially the specific limiting factor.

CONCLUSION

Our results demonstrated that trait-convergence assembly processes, such as abiotic filtering or weaker competitive exclusion, as the dominant determinant of species coexistence in harsh environment of TP and MP, resulting in widespread coexistence of functionally similar species. However, different abiotic stressors regulated the community assembly of TP and MP. Low temperature stress acted as a strong filter determining the functional structure of alpine grassland in TP, while drought stress governed the temperate grassland assembly in MP. Our findings provide empirical evidence that regional climatic regimes govern grassland community assembly respond to environment stress. Community assembly may respond more strongly to future climate warming on the TP because of the coinciding temperature limitations and more rapid warming scenarios.

DATA AVAILABILITY STATEMENT

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

AUTHOR CONTRIBUTIONS

ML and NH conceived, designed the project, and led the data collection effort. JW, LX, ML, and PY participated in the transect investigation. JW performed the analyses and wrote the manuscript. CL helped with the data analysis. ML revised the manuscript. All authors discussed the results and contributed significantly to the final manuscript.

FUNDING

This work was supported by the National Natural Science Foundation of China (32001186, 3191101545, and 31988102), the Second Tibetan Plateau Scientific Expedition and Research Program (2019QZKK060602), and National Science and Technology Basic Resources Survey Program of China (2019FY101300).

ACKNOWLEDGMENTS

We thank the members of the field investigation team for assistance with field data collection. We confirm that the relevant data supporting the findings of this study are available within the article and its **Supplementary Material**. To request additional data, please contact NH (henp@igsnrr.ac.cn).

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpls.2021.715730/full#supplementary-material

REFERENCES

Backhaus, L., Albert, G., Cuchietti, A., Jaimes Nino, L. M., Fahs, N., Lisner, A., et al. (2021). Shift from trait convergence to divergence along old-field succession. *J. Veg. Sci.* 32:e12986.

Bernard-Verdier, M., Navas, M. L., Vellend, M., Violle, C., Fayolle, A., and Garnier, E. (2012). Community assembly along a soil depth gradient: contrasting patterns of plant trait convergence and divergence in a Mediterranean rangeland. *J. Ecol.* 100, 1422–1433.

Blanchard, G., Munoz, F., Ibanez, T., Hequet, V., Vandrot, H., Girardi, J., et al. (2019). Regional rainfall and local topography jointly drive tree community assembly in lowland tropical forests of New Caledonia. *J. Veg. Sci.* 30, 845–856. doi: 10.1111/jvs.12781

Bruelheide, 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

Butterfield, B. J., and Munson, S. M. (2016). Temperature is better than precipitation as a predictor of plant community assembly across a dryland region. *J. Veg. Sci.* 27, 938–947.

Cadotte, M. W., and Tucker, C. M. (2017). Should environmental filtering be abandoned? *Trends Ecol. Evol.* 32, 429–437. doi: 10.1016/j.tree.2017.03.004

Cooper, N., and Purvis, A. (2010). Body size evolution in mammals: complexity in tempo and mode. *Am. Nat.* 175, 727–738. doi: 10.1086/652466

Cornelissen, J. H. C., Lavorel, S., Garnier, E., Díaz, S., Buchmann, N., Gurvich, D. E., et al. (2003). A handbook of protocols for standardised and easy measurement of plant functional traits worldwide. *Aust. J. Bot.* 51, 335–380.

Cornwell, W. K., and Ackerly, D. D. (2009). Community assembly and shifts in plant trait distributions across an environmental gradient in coastal California. *Ecol. Monogr.* 79, 109–126.

Coyle, J. R., Halliday, F. W., Lopez, B. E., Palmquist, K. A., Wilfahrt, P. A., and Hurlbert, A. H. (2014). Using trait and phylogenetic diversity to evaluate the generality of the stress-dominance hypothesis in eastern North American tree communities. *Ecography* 37, 814–826. doi: 10.1111/ecog.00473

Crous, K. Y., Drake, J. E., Aspinwall, M. J., Sharwood, R. E., Tjoelker, M. G., and Ghannoum, O. (2018). Photosynthetic capacity and leaf nitrogen decline along a controlled climate gradient in provenances of two widely distributed Eucalyptus species. *Glob. Change Biol.* 24, 4626–4644. doi: 10.1111/gcb.14330

Dai, A. (2013). Increasing drought under global warming in observations and models. *Nat. Clim. Change* 3, 52–58. doi: 10.1038/nclimate1633

De Bello, F., Thuiller, W., Lepš, J., Choler, P., Clément, J. C., Macek, P., et al. (2009). Partitioning of functional diversity reveals the scale and extent of trait convergence and divergence. *J. Veg. Sci.* 20, 475–486. doi: 10.1111/j.1654-1103.2009.01042.x

Díaz, 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.

Ding, Y., Zang, R., Lu, X., Huang, J., and Xu, Y. (2019). The effect of environmental filtering on variation in functional diversity along a tropical elevational gradient. *J. Veg. Sci.* 30, 973–983. doi: 10.1111/jvs.12786

Dìaz, S., and Cabido, M. (2001). Vive la différence: plant functional diversity matters to ecosystem processes. *Trends Ecol. Evol.* 16, 646–655. doi: 10.1016/s0169-5347(01)02283-2

Easterling, D. R., Meehl, G. A., Parmesan, C., Changnon, S. A., Karl, T. R., and Mearns, L. O. (2000). Climate extremes: observations, modeling, and impacts. *Science* 289, 2068–2074. doi: 10.1126/science.289.5487.2068

Emerson, B. C., and Gillespie, R. G. (2008). Phylogenetic analysis of community assembly and structure over space and time. *Trends Ecol. Evol.* 23, 619–630. doi: 10.1016/j.tree.2008.07.005

Enquist, B. J., Norberg, J., Bonser, S. P., Violle, C., Webb, C. T., Henderson, A., et al. (2015). Scaling from traits to ecosystems: developing a general trait driver theory via integrating trait-based and metabolic scaling theories. *Adv. Ecol. Res.* 52, 249–318. doi: 10.1016/bs.aecr.2015.02.001

Fang, S., Cadotte, M. W., Yuan, Z., Lin, F., Ye, J., Hao, Z., et al. (2019). Intraspecific trait variation improves the detection of deterministic community assembly processes in early successional forests, but not in late successional forests. *J. Plant Ecol.* 12, 593–602. doi: 10.1093/jpe/rty053

Freschet, G. T., Cornelissen, J. H., Van Logtestijn, R. S., and Aerts, R. (2010). Substantial nutrient resorption from leaves, stems and roots in a subarctic flora: what is the link with other resource economics traits? *New Phytol.* 186, 879–889. doi: 10.1111/j.1469-8137.2010.03228.x

Götzenberger, L., De Bello, F., Bråthen, K. A., Davison, J., Dubuis, A., Guisan, A., et al. (2012). Ecological assembly rules in plant communities—approaches, patterns and prospects. *Biol. Rev.* 87, 111–127. doi: 10.1111/j.1469-185X.2011.00187.x

Grime, J. P. (2006). 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

Gross, N., Le Bagousse-Pinguet, Y., Liancourt, P., Berdugo, M., Gotelli, N. J., and Maestre, F. T. (2017). Functional trait diversity maximizes ecosystem multifunctionality. *Nat. Ecol. Evol.* 1:0132.

Hawkins, B. A., Field, R., Cornell, H. V., Currie, D. J., Guégan, J.-F., Kaufman, D. M., et al. (2003). Energy, water, and broad-scale geographic patterns of species richness. *Ecology* 84, 3105–3117. doi: 10.1890/03-8006

Hodgson, J., 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

Keddy, P. A. (1992). Assembly and response rules: two goals for predictive community ecology. *J. Veg. Sci.* 3, 157–164. doi: 10.2307/3235676

Koch, G. W., Sillett, S. C., Jennings, G. M., and Davis, S. D. (2004). The limits to tree height. *Nature* 428, 851–854.

Kraft, N. J. B., and Ackerly, D. D. (2010). Functional trait and phylogenetic tests of community assembly across spatial scales in an Amazonian forest. *Ecol. Monogr.* 80, 401–422. doi: 10.1890/09-1672.1

Kraft, N. J. B., Adler, P. B., Godoy, O., James, E. C., Fuller, S., and Levine, J. M. (2015). Community assembly, coexistence and the environmental filtering metaphor. *Funct. Ecol.* 29, 592–599. doi: 10.1098/rsbl.2018.0460

Kreft, H., and Jetz, W. (2007). Global patterns and determinants of vascular plant diversity. *Proc. Natl. Acad. Sci. U.S.A.* 104, 5925–5930. doi: 10.1073/pnas.0608361104

Le Bagousse-Pinguet, Y., Gross, N., Maestre, F. T., Maire, V., De Bello, F., Fonseca, C. R., et al. (2017). Testing the environmental filtering concept in global drylands. *J. Ecol.* 105, 1058–1069. doi: 10.1111/1365-2745.12735

Lhotsky, B., Kovács, B., Ónodi, G., Csecserits, A., Rédei, T., Lengyel, A., et al. (2016). Changes in assembly rules along a stress gradient from open dry grasslands to wetlands. *J. Ecol.* 104, 507–517. doi: 10.1111/1365-2745.12532

Lienin, P., and Kleyer, M. (2012). Plant trait responses to the environment and effects on ecosystem properties. *Basic Appl. Ecol.* 13, 301–311. doi: 10.1016/j.baae.2012.05.002

Liu, C., Li, Y., Zhang, J., Baird, A. S., and He, N. (2020). Optimal community assembly related to leaf economic-hydraulic-anatomical Traits. *Front. Plant Sci.* 11:341. doi: 10.3389/fpls.2020.00341

Liu, H., Mi, Z., Lin, L. I., Wang, Y., Zhang, Z., Zhang, F., et al. (2018). Shifting plant species composition in response to climate change stabilizes grassland primary production. *Proc. Natl. Acad. Sci. U.S.A.* 115, 4051–4056. doi: 10.1073/pnas.1700299114

Luo, W., Lan, R., Chen, D., Zhang, B., Xi, N., Li, Y., et al. (2021). Limiting similarity shapes the functional and phylogenetic structure of root neighborhoods in a subtropical forest. *New Phytol.* 229, 1078–1090.

Luo, Y. H., Cadotte, M. W., Burgess, K. S., Liu, J., Tan, S. L., Xu, K., et al. (2019). Forest community assembly is driven by different strata-dependent mechanisms along an elevational gradient. *J. Biogeogr.* 46, 2174–2187. doi: 10.1111/jbi.13669

Ma, Z., Liu, H., Mi, Z., Zhang, Z., Wang, Y., Xu, W., et al. (2017). Climate warming reduces the temporal stability of plant community biomass production. *Nat. Commun.* 8:15378. doi: 10.1038/ncomms15378

Macarthur, R., and Levins, R. (1967). The limiting similarity, convergence, and divergence of coexisting species. *Am. Nat.* 101, 377–385. doi: 10.1086/282505

Mayfield, M. M., and Levine, J. M. (2010). Opposing effects of competitive exclusion on the phylogenetic structure of communities. *Ecol. Lett.* 13, 1085–1093. doi: 10.1111/j.1461-0248.2010.01509.x

McGill, B. J., Enquist, B. J., Weiher, E., and Westoby, M. (2006). Rebuilding community ecology from functional traits. *Trends Ecol. Evol.* 21, 178–185. doi: 10.1016/j.tree.2006.02.002

Moeller, D. A. (2004). Facilitative interactions among plants via shared pollinators. *Ecology* 85, 3289–3301. doi: 10.1890/03-0810

Muscarella, R., Uriarte, M., Erickson, D. L., Swenson, N. G., Kress, W. J., and Zimmerman, J. K. (2016). Variation of tropical forest assembly processes across regional environmental gradients. *Perspect. Plant Ecol. Evol. Syst.* 23, 52–62. doi: 10.1016/j.ppees.2016.09.007

Pérez-Harguindeguy, N., Diaz, S., Gamier, E., Lavorel, S., Poorter, H., Jaureguiberry, P., et al. (2013). New handbook for stand-ardised measurement of plant functional traits worldwide. *Austral. J. Bot.* 61, 167–234.

Perronne, R., Munoz, F., Borgy, B., Reboud, X., and Gaba, S. (2017). How to design trait-based analyses of community assembly mechanisms: insights and guidelines from a literature review. *Perspect. Plant Ecol. Evol. Syst.* 25, 29–44. doi: 10.1016/j.ppees.2017.01.004

Pillar, V. D., Duarte, L. D. S., Sosinski, E. E., and Joner, F. (2009). Discriminating trait-convergence and trait-divergence assembly patterns in ecological community gradients. *J. Veg. Sci.* 20, 334–348. doi: 10.1111/j.1654-1103.2009.05666.x

Purschke, O., Schmid, B. C., Sykes, M. T., Poschlod, P., Michalski, S. G., Durka, W., et al. (2013). Contrasting changes in taxonomic, phylogenetic and functional diversity during a long-term succession: insights into assembly processes. *J. Ecol.* 101, 857–866. doi: 10.1111/1365-2745.12098

Rao, C. R. (1982). Diversity and dissimilarity coefficients: a unified approach. *Theor. Popul. Biol.* 21, 24–43. doi: 10.1111/1755-0998.13014

Ricklefs, R. E., and He, F. (2016). Region effects influence local tree species diversity. *Proc. Natl. Acad. Sci. U.S.A.* 113, 674–679. doi: 10.1073/pnas.1523683113

Schöb, C., Armas, C., Guler, M., Prieto, I., and Pugnaire, F. I. (2013). Variability in functional traits mediates plant interactions along stress gradients. *J. Ecol.* 101, 753–762. doi: 10.1111/1365-2745.12062

Šímová, I., Violle, C., Kraft, N. J. B., Storch, D., Svenning, J. C., Boyle, B., et al. (2015). Shifts in trait means and variances in North American tree assemblages: species richness patterns are loosely related to the functional space. *Ecography* 38, 649–658. doi: 10.1111/ecog.00867

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

Swenson, N. G. (2014). *Functional and Phylogenetic Ecology in R*. New York, NY: Springer.

Swenson, N. G., Enquist, B. J., Pither, J., Kerkhoff, A. J., Boyle, B., Weiser, M. D., et al. (2012). The biogeography and filtering of woody plant functional diversity in North and South America. *Glob. Ecol. Biogeogr.* 21, 798–808. doi: 10.1111/j.1466-8238.2011.00727.x

Umaña, M. N., Condit, R., Pérez, R., Turner, B. L., Wright, S. J., and Comita, L. S. (2021). Shifts in taxonomic and functional composition of trees along rainfall and phosphorus gradients in central Panama. *J. Ecol.* 109, 51–61. doi: 10.1111/1365-2745.13442

Vellend, M. (2010). Conceptual synthesis in community ecology. *Q. Rev. Biol.* 85, 183–206. doi: 10.1086/652373

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, J., Wang, Y., Li, M., Xu, L., He, N., Yan, P., et al. (2021). Differential response of abundant and rare bacterial subcommunities to abiotic and biotic gradients across temperate deserts. *Sci. Total Environ.* 763:142942. doi: 10.1016/j.scitotenv.2020.142942

Wang, X., Song, N. P., Yang, X. G., Chen, L., Qu, W. J., and Wang, L. (2021). Inferring community assembly mechanisms from functional and phylogenetic diversity: the relative contribution of environmental filtering decreases along a sand desertification gradient in a desert steppe community. *Land Degrad. Dev.* 32, 2360–2370. doi: 10.1002/ldr.3906

Webb, C. T., Hoeting, J. A., Ames, G. M., Pyne, M. I., and Leroy Poff, N. (2010). A structured and dynamic framework to advance traits-based theory and prediction in ecology. *Ecol. Lett.* 13, 267–283. doi: 10.1111/j.1461-0248.2010.01444.x

Weir, J. T., and Schluter, D. (2007). The latitudinal gradient in recent speciation and extinction rates of birds and mammals. *Science* 315, 1574–1576. doi: 10.1126/science.1135590

Westoby, M. (1998). A leaf-height-seed (LHS) plant ecology strategy scheme. *Plant Soil* 199, 213–227.

Wieczynski, D. J., Boyle, B., Buzzard, V., Duran, S. M., Henderson, A. N., Hulshof, C. M., et al. (2019). Climate shapes and shifts functional biodiversity in forests worldwide. *Proc. Natl. Acad. Sci. U.S.A.* 116, 587–592. doi: 10.1073/pnas.1813723116

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.

Yao, J., Huang, J., Ding, Y., Xu, Y., Xu, H., and Zang, R. (2021). Ecological uniqueness of species assemblages and their determinants in forest communities. *Divers. Distrib.* 27, 454–462. doi: 10.1111/ddi.13205

Yao, T. (2019). Tackling on environmental changes in Tibetan Plateau with focus on water, ecosystem and adaptation. *Sci. Bull.* 64:417. doi: 10.1016/j.scib.2019.03.033

Zhang, J., Nielsen, S. E., Mao, L., Chen, S., and Svenning, J. C. (2016). Regional and historical factors supplement current climate in shaping global forest canopy height. *J. Ecol.* 104, 469–478. doi: 10.1111/1365-2745.12510

Zhang, Y., Li, Y., Wang, R., Xu, L., Li, M., Liu, Z., et al. (2020). Spatial variation of leaf chlorophyll in northern hemisphere grasslands. *Front. Plant Sci.* 11:1244. doi: 10.3389/fpls.2020.01244

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.

frontiers | Frontiers in Plant Science

TYPE Original Research
PUBLISHED 08 December 2023
DOI 10.3389/fpls.2023.1303022

OPEN ACCESS

EDITED BY
Stef Bokhorst,
VU Amsterdam, Netherlands

REVIEWED BY
Jalal Kassout,
National Institute for Agricultural Research, Morocco
Maria Amélia Martins-Loução,
University of Lisbon, Portugal

*CORRESPONDENCE
Sergio de Tomás Marín
✉ dtm.sergio@gmail.com

RECEIVED 10 October 2023
ACCEPTED 17 November 2023
PUBLISHED 08 December 2023

CITATION
de Tomás Marín S, Galán Díaz J, Rodríguez-Calcerrada J, Prieto I and de la Riva EG (2023) Linking functional composition moments of the sub-Mediterranean ecotone with environmental drivers.
Front. Plant Sci. 14:1303022.
doi: 10.3389/fpls.2023.1303022

Linking functional composition moments of the sub-Mediterranean ecotone with environmental drivers

Sergio de Tomás Marín[1]*, Javier Galán Díaz[2], Jesús Rodríguez-Calcerrada[3], Iván Prieto[4] and Enrique G. de la Riva[1,4]

[1]Department of Ecology, Brandenburgische Technische Universität Cottbus-Senftenberg, Cottbus, Germany, [2]Department of Pharmacology, Pharmacognosy and Botany, Faculty of Pharmacy, Universidad Complutense de Madrid, Madrid, Spain, [3]Functioning of Forest Systems in a Changing Environment Research Group, Universidad Politécnica de Madrid, Madrid, Spain, [4]Ecology Department, Faculty of Biology and Environmental Sciences, Universidad de León, León, Spain

Introduction: Functional trait-based approaches are extensively applied to the study of mechanisms governing community assembly along environmental gradients. These approaches have been classically based on studying differences in mean values among species, but there is increasing recognition that alternative metrics of trait distributions should be considered to decipher the mechanisms determining community assembly and species coexistence. Under this framework, the main aim of this study is to unravel the effects of environmental conditions as drivers of plant community assembly in sub-Mediterranean ecotones.

Methods: We set 60 plots in six plant communities of a sub-Mediterranean forest in Central Spain, and measured key above- and belowground functional traits in 411 individuals belonging to 19 species, along with abiotic variables. We calculated community-weighted mean (CWM), skewness (CWS) and kurtosis (CWK) of three plant dimensions, and used maximum likelihood techniques to analyze how variation in these functional community traits was driven by abiotic factors. Additionally, we estimated the relative contribution of intraspecific trait variability and species turnover to variation in CWM.

Results and discussion: The first three axes of variation of the principal component analyses were related to three main plant ecological dimensions: Leaf Economics Spectrum, Root Economics Spectrum and plant hydraulic architecture, respectively. Type of community was the most important factor determining differences in the functional structure among communities, as compared to the role of abiotic variables. We found strong differences among communities in their CWMs in line with their biogeographic origin (Eurosiberian vs Mediterranean), while differences in CWS and CWK indicate different trends in the functional structure among communities and the coexistence of different functional strategies, respectively. Moreover, changes in functional composition were primarily due to intraspecific variability.

Conclusion: We observed a high number of strategies in the forest with the different communities spreading along the acquisitive-conservative axis of resource-use, partly matching their Eurosiberian-Mediterranean nature, respectively. Intraspecific trait variability, rather than species turnover, stood as the most relevant factor when analyzing functional changes and assembly patterns among communities. Altogether, our data support the notion that ecotones are ecosystems where relatively minor environmental shifts may result in changes in plant and functional composition.

KEYWORDS

community assembly, community-weighted moments, ecotone, functional structure, intraspecific variability, mixed-species forest, sub-Mediterranean community, trait-based

1 Introduction

The functional trait-based approach is a broadly-known tool applied to the study of mechanisms governing community assembly along environmental gradients (Garnier and Navas, 2012; de la Riva et al., 2018a; Dong et al., 2020; Kermavnar et al., 2022), being the initial step towards developing a more mechanistic understanding of how the environment influences ecosystem structure and function (Wieczynski et al., 2019). The classical approach for assessing the assembly processes within a given community have mostly aimed at quantifying the trait composition of plant communities, i.e., the presence of species with particular trait values within communities (e.g. Maire et al., 2012; de la Riva et al., 2016a; Hejda et al., 2019) by focusing on the first two moments of a trait distribution weighted by species abundance, i.e. community-weighted mean and variance of their trait distributions (Garnier et al., 2004; Violle et al., 2012). Mean and variance trait values are only meaningful when traits have a normal (gaussian) distribution within the community. However, the distribution of trait values within communities often deviates from the symmetric normal distribution, for example as a result of rapid environmental changes or asymmetric competition favoring a limited portion of the community with specific trait values (Enquist et al., 2015; Le Bagousse-Pinguet et al., 2017; Gross et al., 2021). Thus, there is an increasing recognition that alternative aspects of trait distributions should be considered to decipher the mechanisms determining community assembly and species coexistence, such as the third and fourth community-weighted moments (skewness and kurtosis) (Le Bagousse-Pinguet et al., 2017; Danet et al., 2018; Wieczynski et al., 2019; Gross et al., 2021). The community-weighted skewness and kurtosis bring light on the shape of trait distributions in naturally assembled communities (Figure 1) (Gross et al., 2017; Le Bagousse-Pinguet et al., 2021). The skewness quantifies the asymmetry of the trait distribution, where extreme skewness values are found when rare species within a community have infrequent trait values (Le Bagousse-Pinguet et al., 2021). The kurtosis, in turn, is a measure of the evenness of trait distributions, where extreme negative kurtosis values reflect an even distribution of trait values in the community or even bimodal trait distributions, and extreme positive kurtosis values reflect a reduced range of trait values (Gross et al., 2021; Le Bagousse-Pinguet et al., 2021). Thus, combining the skewness and kurtosis of a trait distribution with the mean value for a given trait, provides a more complete understanding of how environmental changes shape the functional diversity and species assembly of plant communities (Wieczynski et al., 2019; Gross et al., 2021). Few works have combined the study of abundance-weighted trait moments, i.e. community weighted mean, skewness and kurtosis, for a wide range of traits at the whole plant level, together with potential environmental drivers simultaneously (Wieczynski et al., 2019), despite the importance of looking simultaneously at multiple traits, since different plant organs may respond differently to different selection pressures (Carvajal et al., 2019). In fact, to the best of our knowledge, this approach has never been performed in ecotones or with belowground traits, as it is done in the present study.

The variation of the indices describing the position of the dominant trait values in the trait space and shape of the trait distribution do not reflect the source of variation along environmental gradients. That is, changes in the functional structure of plant communities may arise due to the replacement of species (species turnover) and/or intraspecific trait variability (ITV). These two sources of variation may contribute in different degrees to the overall change in the functional structure of plant communities (Albert et al., 2010; de Bello et al., 2011; Pérez-Ramos et al., 2012; de la Riva et al., 2016a). Hence, the estimation of the trait distribution moments at the whole-plant level, i.e. including both above- and belowground traits, and the role of species turnover and ITV in the variation of these distribution moments among communities, may reveal different responses of plants to environmental changes, offering higher insights into the assembly

---

Abbreviations: CWM, community-weighted mean; CWS, community-weighted skewness; CWK, community-weighted kurtosis; LES, Leaf Economics Spectrum; RES, Root Economics Spectrum; HyArq, hydraulic architecture; ITV, intraspecific trait variability; SOM, Soil organic matter.

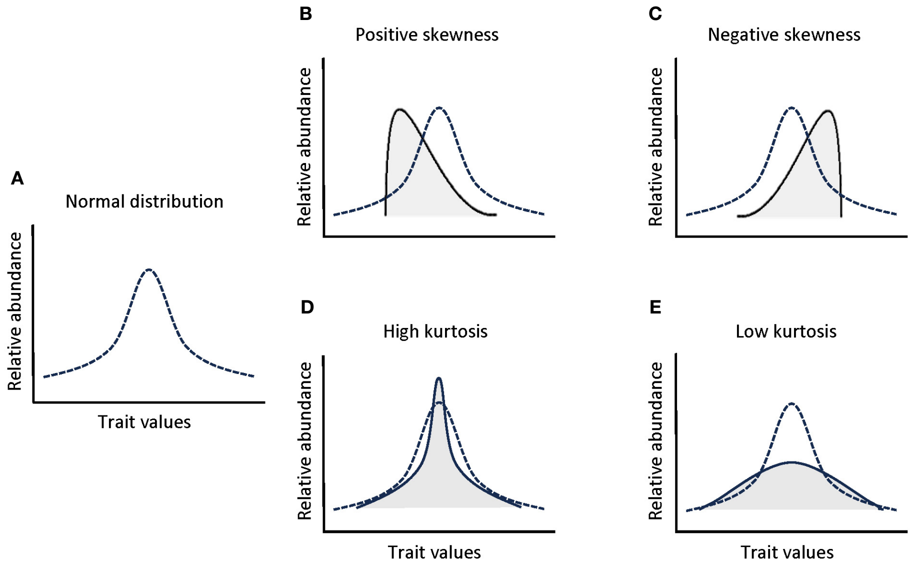


FIGURE 1
Schematic representation of the shifts in the shape of a normal trait distribution **(A)** produced by skewness (**B**: positive; **C**: negative) and kurtosis (**D**: high; **E**: low).

processes taking place in communities along environmental gradients (Lepš et al., 2011; Kichenin et al., 2013; de la Riva et al., 2016a).

Ecotones are transitional areas between adjacent biocenosis with high levels of biodiversity, as they host species from the adjacent biocenosis as well as unique species endemic to the ecotone (Hufkens et al., 2009; Vila-Viçosa et al., 2020b; Shea et al., 2022). Ecotones are of especial interest for conservation (Carlson et al., 2014; Rawal et al., 2018; Lopes et al., 2020) for their ecological uniqueness (Danso Marfo et al., 2019), and for contributing to stabilize adjacent ecosystems (Pogue and Schnell, 2001). Further, community assembly processes in ecotones are very dynamic, as slight modifications in environmental conditions lead to substantial changes in plant composition (Ruiz-Labourdette et al., 2012; Brice et al., 2020), which makes them ideal study systems to test such processes (Rahman et al., 2021). However, understanding which environmental factors govern the process of species assembly in transitional areas remains challenging (Kitagawa et al., 2020; Rahman et al., 2021) and could be key to understanding how these communities will respond to future climatic conditions.

Sub-Mediterranean areas are transition areas between Mediterranean and temperate climates. The Iberian Peninsula, in the south of Europe, represents one of the largest ecotones of this kind (Sánchez de Dios et al., 2009), being the low-latitudinal edge for the distribution of many central European species (Gil et al., 2010; Dorado-Liñán et al., 2017; Hernández-Lambraño et al., 2021). Sub-Mediterranean regions host Mediterranean and Eurosiberian species as well as many endemic species (Vila-Viçosa et al., 2020a; Box, 2021; Cantoral et al., 2023), creating a mosaic of unique plant communities with contrasting assembly processes (de la Riva et al., 2023). Sub-Mediterranean regions in the Iberian Peninsula are very sensitive to slight environmental alterations (Sánchez de Dios et al., 2009; Vila-Viçosa et al., 2020a), which makes them highly vulnerable to ongoing climatic change and thus critical areas for conservation (Benito Garzón et al., 2008; Cantoral et al., 2023). Understanding trait-based assembly rules in sub-Mediterranean regions can be critical for understanding how species coexist in these and other ecotones, which could be important to predict the trajectory of ecotone plant communities facing increasingly warmer and drier conditions (Benito Garzón et al., 2008; Frenette-Dussault et al., 2013; Pereira et al., 2021).

The broad aim of this study is to unravel the effects of environmental conditions as drivers of the assembly of plant communities in a sub-Mediterranean forest in Central Spain. To this end, we measured four leaf, two stem and three root traits in a total of 411 individuals of 19 woody plant species in six plant communities. We then calculated three trait distribution moments weighted by the relative abundance of each species in each community, i.e., the community-weighted mean (CWM), skewness (CWS) and kurtosis (CWK), of major axes of variation at the aboveground (i.e. Leaf Economics Spectrum or LES, Wright et al., 2004) and belowground levels (i.e. Root Economics Spectrum or RES; Prieto et al., 2015) across plant communities, and assessed their relationship with environmental variables to estimate which, and to what extent, environmental drivers rule the assembly of sub-Mediterranean plant communities. Additionally, we estimated the proportion of variation in CWMs attributable to species turnover and to ITV to have a detailed view of assembly processes occurring in these communities. Using this novel approach, we will gain a great understanding on the functional structure of sub-Mediterranean plant communities and, ultimately, will be able to guide conservation efforts to maintain their proper functioning (Walker, 1995).

2 Materials and methods

2.1 Study area

The study was conducted in the 'Hayedo de Montejo' (Montejo hereafter), a 125 ha. forest located in the 'Sistema Central' range in Spain (41°7'N, 3°30'W), between 1250 and 1550 m a. s. l. Orientation is predominantly northeast, although it ranges from south to north in some locations. Slope varies from nearly flat at higher elevations to ca. 58% in the steepest areas. Soil depth ranges from 30 cm in upper areas to 150 cm down in the valley. The dominant bedrock is micaceous gneiss and soils on top are acidic with a sandy-loam texture (López Santalla et al., 2003; Gil et al., 2010). The climate is sub-Mediterranean, with Mediterranean influence in the east and a continental climate in the west. Mean annual precipitation was 858.8 mm and mean annual temperature was 9.7°C for the period 1994-2021 (Montejo meteorological station). Summers are hot with a marked dry period between July and August, typical of Mediterranean climate.

The Hayedo de Montejo is a sub-Mediterranean, mixed beech-oak forest (*Fagus sylvatica-Quercus pyrenaica*), with a high diversity of plant species and communities coexisting in a relatively small area. In this forest, Eurosiberian species coexist with typically Mediterranean species in the overstory and the understory (Sánchez de Dios et al., 2009; Gil et al., 2010; Rivas-Martínez et al., 2011), which harbors some herbaceous species of special interest for conservation for their rarity, such as *Paris quadrifolia* L., *Narcissus pseudonarcissus* ssp. *pseudonarcissus* L. or *Aconitum napellus* L. (Hernández Bermejo et al., 1982; Moreno et al., 2005; Gil et al., 2010). The forest is dominated by the temperate tree species European beech (*Fagus sylvatica* L.; hereafter beech) and sessile oak (*Quercus petraea* (Matt.) Liebl.), both at the southernmost limit of their distribution range, and the Mediterranean Pyrenean oak (*Quercus pyrenaica* Willd.), at the core of its distribution range (Rubio-Cuadrado et al., 2020; Rubio-Cuadrado et al., 2021; de Tomás Marín et al., 2023).

This forest has been traditionally managed as a wooded pasture (Pardo and Gil, 2005). After centuries of forest exploitation, mainly for firewood and cattle grazing, these traditional land uses ceased in the early 1960s (López Santalla et al., 2003; Pardo and Gil, 2005) – although, today, it is still relatively easy to find a few cows grazing in the forest. These land-use changes transformed the former open woodland structure with dispersed trees and scarce tree recruitment into the dense secondary forest that we find today (Pardo and Gil, 2005; Rubio-Cuadrado et al., 2021). The Hayedo de Montejo forest is included in the 'Ancient and Primeval Beech Forests of the Carpathians and Other Regions of Europe' list of European beech forests included in the UNESCO World Heritage Site list. Furthermore, it is embedded in the 'Sierra del Rincón' (Rincón mountain range), designated as a Reserve of the Biosphere by UNESCO in 2005.

2.2 Sampling design

Three forest inventories (FI) were carried out in Montejo in the years 1994, 2005 and 2015, respectively. In each FI, 125 circular plots of 30-m diameter were systematically established in a 100 x 100 m square grid covering the entire forest. Based on the last FI, we divided the forest into six different areas (communities hereafter) according to the distribution of the three most abundant tree species, i.e., beech, Pyrenean oak and sessile oak (see also de la Riva et al., 2023). An additional community dominated by shrubs where trees are scarce and dispersed was included. These communities were classified based on the abundance of the three main species recorded in the FI of 2015 as follows: (I) *Fagus sylvatica*, (II) *Quercus pyrenaica* or (III) *Quercus petraea*, when >60% of the abundance in terms of tree density corresponded to one of these three species, respectively; (IV) mixed forest 1 (transition areas between *F. sylvatica* and *Q. pyrenaica*), (V) mixed forest 2 (transition area with co-dominance of *F. sylvatica*, *Q. pyrenaica* and *Q. petraea*) and (VI) a shrubland community (Supplementary Material, Appendix S1, Figure S1).

Ten random plots from the FIs within each plant community (I to VI) were selected for sampling. In each plot, four parallel 20-m-long transects perpendicular to the slope were randomly set, two on each side of the plot, with a minimum distance of 2 m between transects. Species composition (i.e. identity) and abundance (i.e. % cover) were recorded for each woody species intercepted by the transect line (abundance was measured as the length in meters of canopy of each woody species projecting on the transect line; total abundance may thus exceed 20 m due to overlaying vegetation layers). For leaf, stem and root trait measurements, we selected five individuals per species and community, except for Huber values, where n=3 (see below for description of this trait). Traits were measured in species that together made up at least 90% of the maximum cumulative cover within a community. With this criterion, a total of 19 species were selected across communities: *Adenocarpus hispanicus, Crataegus monogyna, Cytisus purgans, Cytisus scoparius, Erica arborea, Erica australis, Fagus sylvatica, Genista florida, Hedera helix, Ilex aquifolium, Juniperus communis, Lavandula stoechas, Prunus avium, Quercus petraea, Quercus pyrenaica, Rosa* sp., *Rubus* sp., *Sorbus aria* and *Sorbus aucuparia*. All species occurred at least in two plant communities, if not more, except *Cytisus purgans*, that was found only in the shrubland community (Supplementary Material, Appendix S2, Table S2).

2.3 Plant sampling and trait measurements

Plant sampling was carried out in June and July 2021, during the peak of the growing season. From this material, five aboveground and three belowground functional traits related to water and nutrient acquisition were measured (see functional roles in Table 1): Leaf Dry Matter Content (LDMC; leaf dry mass per unit of water-saturated fresh mass; mg g^{-1}), Specific Leaf Area (SLA; leaf area per unit of leaf dry mass; m^2 kg^{-1}), leaf carbon isotopic composition ($\delta^{13}C$; ‰), leaf carbon to nitrogen ratio (leaf C:N), Stem Dry Matter Content (SDMC; stem dry mass per unit of water-saturated fresh mass; mg g^{-1}), Huber value (Hv; sapwood cross-sectional branch area to supported leaf area ratio; cm^2 cm^{-2}), Specific Root Area (SRA; root area per unit of root dry mass; m^2 kg^{-1}), average root diameter (Rdi; mm), and root C:N ratio (root

TABLE 1 List of the nine functional traits considered in this study, their abbreviations, units and their functional roles.

Trait	Abbreviation	Unit	Functional role	References
Leaf				
Leaf dry matter content	LDMC	mg g^{-1}	Physical resistance and stress tolerance	Pérez-Harguindeguy et al., 2013; de la Riva et al., 2017
Specific leaf area	SLA	m^2 kg^{-1}	Leaf longevity, light capture and growth rate	Shipley and Vu, 2002
C:N content	Leaf C:N	unitless	Growth rate	Zhao et al., 2016; Zhang et al., 2020
Carbon isotopic composition	$\delta^{13}C$	‰	Gas exchange and water use efficiency	Prieto et al., 2018
Stem				
Stem dry matter content	SDMC	mg g^{-1}	Physical resistance	de la Riva et al., 2018b
Huber value	H_v	cm^2 cm^{-2}	Coordination of water transport and water loss	Mencuccini et al., 2019
Root				
Specific root area	SRA	m^2 kg^{-1}	Water and nutrients acquisition	de la Riva et al., 2021a
Average root diameter	Rdi	mm	Water and nutrients acquisition	Freschet et al., 2021
C:N content	Root C:N		Nutrients acquisition, root defence	Freschet et al., 2021

Modified from de Tomás Marín et al. (2023).

C:N). Leaf $\delta^{13}C$ is a proxy of intrinsic water use efficiency (iWUE, Prieto et al., 2018; Ma et al., 2023). The Huber value reflects the mass investment in xylem area with respect to leaf area (Mencuccini et al., 2019); it is thus a measure of how species adjust their leaf and sapwood areas to water supply (Carter and White, 2009; Zhang et al., 2019) and is related to groundwater depth extraction (Carter and White, 2009). Thus, we used this trait as a proxy of the hydraulic architecture of the plants.

For leaf and stem measurements, two-year-old branches with fully expanded, sun-exposed mature leaves from five healthy adult individuals per species and community were sampled. A variable number of leaves were measured depending on the average leaf size of each species. For root measurements, fine roots (<2 mm diameter) from five individuals per species and community were collected within the first 20-30 cm of soil by excavating next to the plant stem base to ensure that roots belonged to the selected individual. Roots were stored fresh in a cooler after collection and taken to the lab on the same day, where they were rinsed with distilled water to eliminate adhered soil particles, and then frozen until they were measured. The Hv was measured at regional level, i.e. selecting a total of three individuals per species for all the species, except for *F. sylvatica* and *Q. pyrenaica*, for which we measured 5 individuals per community (de Tomás Marín et al., 2023).

Leaf and root material was scanned with an EPSON® V850 PRO scanner at a resolution of 600 dpi. Leaf area was measured from the scanned images using the ImageJ software (Schneider et al., 2012). Root area and diameter were measured from the scanned images using the WinRHIZO 2009 software (Regent Instruments Inc., Quebec, Canada). After scanning, roots were oven-dried at 60°C for 48h prior to measurements. Leaf carbon isotopic composition and C and N concentrations were measured simultaneously with a THERMO/Finnigan MAT V isotope ratio mass spectrometer, coupled to a THERMO Flash EA 1112 elemental analyzer via a THERMO/Finnigan Conflo IV- interface. Leaf carbon isotopic composition is expressed in the conventional delta notation ($\delta^{13}C$) relative to VPDB (Vienna PeeDee Belemnite standard). All plant material was collected, stored and processed following the protocols detailed by Pérez-Harguindeguy et al. (2013). For a detailed protocol of sample harvesting and trait measurements see also de Tomás Marín et al. (2023), de la Riva et al. (2016a) and de la Riva et al. (2016b).

2.4 Abiotic variables

In each selected plot, soil organic matter content (SOM), total soil N, soil nitrate and soil phosphate were determined from the top 15 cm of soil. Soil samples were collected with a hand trowel in three points in the plot, one of the samples corresponding to the center of the plot and the other two approximately 5 m above and below the center, and mixed in a plastic bag to homogenize the soil. Then soils were taken to the lab where they were dried at 50°C to constant weight (~72 h) and sieved at 2 mm before analyses. Additionally, slope, orientation, altitude and soil depth were also measured in the same plots (Supplementary Material, Appendix S3, Table S3). For the description of the methods employed to measure total soil N see Rodríguez-Calcerrada et al. (2019); for the methods employed to measure the rest of abiotic variables see de Tomás Marín et al. (2023).

2.5 Data analyses

As there is a great variety of ways in which plants combine a set of traits to achieve a successful performance (Díaz et al., 2016; Iozia et al., 2023), we focused on trait syndromes rather than on individual functional traits. To this end, we conducted a Principal Component Analysis (PCA) including the nine functional traits considered in this study, measured in 411 individuals belonging to the 19 sampled species, to describe trait trade-offs or trait syndromes (Albert et al., 2011). The first three principal components (PCs) had eigenvalues greater than one and represented three key dimensions of plant ecological strategies (Supplementary Material, Appendix S4, Table S4). That is, PC1, PC2 and PC3 reflected clear spectrums related to the Leaf Economics Spectrum (PC1, LES, Wright et al., 2004), Root Economics Spectrum (PC2, RES, de la Riva et al., 2018b) and hydraulic architecture (PC3, HyArq, Tyree and Ewers, 1991), respectively. We then used the scores of each individual in these three PCA axes to estimate the community-weighted moments and in further analyses.

2.5.1 Community weighted moments along the environmental gradient

We calculated three informative moments related to the dominant trait values (mean) and the shape of the trait distribution (skewness and kurtosis) of the individual PCA scores for each of the three PC axes (LES, RES and HydArq) kept for analyses (see mathematical equations in Supplementary Material, Appendix S5). To scale up from species to community level, these moments were weighted by the relative abundance of each species in each community, resulting in the ‘community-weighted moments’ of the trait distributions. The community-weighted mean (CWM, Garnier et al., 2004) gives a representation of the dominant trait values within a community. This metric is directly related to Grime’s mass-ratio hypothesis (Grime, 1998) and considers that the traits of the most abundant species in the community have the largest influence in ecosystem processes (Bílá et al., 2014). The community-weighted skewness (CWS) quantifies the degree of asymmetry of the trait distribution within the community (Wieczynski et al., 2019; Gross et al., 2021). In our case, the CWS quantifies the degree of asymmetry of the individual values in the three spectrum axes (PC1, PC2 and PC3 scores) and reflects the presence of subordinate species within a community with trait values close to one of the extremes of the PC scores range (Le Bagousse-Pinguet et al., 2017). The community-weighted kurtosis (CWK) refers to the evenness of the distribution of individual values in the three spectrum axes (PC1, PC2 and PC3 scores) (Wieczynski et al., 2019; Gross et al., 2021), where a peaked distribution indicates low levels of diversity along the spectrums and a flat distribution indicates high levels of diversity.

To analyze the effect of the abiotic environment on the variation of the three community-weighted moments (CWM, CWS and CWK) of the different plant ecological dimensions in our communities (LES, RES and HyArq), we built multiple linear regressions models. The continuous abiotic variables (independent variables) and the discrete variable ‘community’ (factor) were introduced in the models as predictors and each of the three distribution moments of the selected PCs, separately, as response variables. The *lm* function from the base R package *stats* was used to perform the linear regression models analyses (R Core Team, 2021). To identify the best predictors, we applied the Akaike information criterion corrected for small sample size (AICc) on the full factorial model, i.e., including all the abiotic variables considered in the study (Table 2) and the communities. The selection of the best model (ΔAIC < 2) was performed using the function *dredge* from the *MuMIn* R package (Barton and Barton, 2015). The strength of the relationship between the predictors and the response variable for the selected models (lowest AICc) for each community-weighted moment and PC axis, was given by the adjusted R^2, obtained with the *summary* function, also from the *stats* package (R Core Team, 2021). To assess the % of variance explained by each predictor in each model, we obtained the sum of squares of each predictor with the *Anova* function from the R package *car* (Fox and Weisberg, 2019) and calculated the proportion that the sum of squares of each variable was relative to the total sum of squares. To determine differences among the six communities for each community-weighted moment in each of PCs spectrum (LES, RES and HydArq), we ran one-way ANOVAs followed by *post-hoc* multiple pairwise comparisons (Tukey’s test), to detect differences between pairs of communities.

2.5.2 Relative importance of intraspecific trait variability and species turnover

We determined the relative contribution of species turnover, i.e., the sum of the contributions of species occurrence and species abundance, and ITV to the overall change in the functional structure of our communities following the method developed by Lepš et al. (2011), using the function *traitflex.anova* implemented in the R software. For a detailed explanation of the method and mathematical calculations, please refer to the Appendix S6 in the Supplementary Materials and to Lepš et al. (2011). Variance partitioning was estimated on the CWMs calculated for the first two PC axes (PC1 and PC2), which represent the LES and RES, respectively. We estimated two partitions in parallel, one with “community” as factor and the other with those abiotic variables selected by AICc in the multiple regression linear models (section 2.5.1), to assess the effects of the type of community and of the environment on the variation of CWMs, respectively. We could not carry out the partition for PC3 (HyArq) because Huber values were measured at the regional level, not at the community level.

All analyses were performed in R (version 4.1.2; R Core Team, 2021) interfaced with R Studio (RStudio Team, 2021).

3 Results

3.1 Trade-offs among traits

According to the loadings on the PCA axes (Supplementary Material, Appendix S4, Table S4), the first three axes of variation (PC1, PC2 and PC3) were related to trait trade-offs within plant organs and thus to plant ecological dimensions (Figure 2). The first PCA axis accounted for 28.1% of the overall variation and was representative of the aboveground resource acquisition strategy or

TABLE 2 Best-fitted linear regression models. Models are presented for each plant ecological strategy (LES, RES and HydArq) and for each of the Community Weighted moments (CWM, CWS, CWK).

Plant ecological strategy	Community weighted moments	Altitude	Orientation	Slope	Soil depth	Soil nitrate	Soil phosphate	Total soil N	SOM	Community	Adj. R^2
LES	Mean		+ (4.3)		+ (1.7)					+ (60.4)	0.739
	Skewness		+ (4.0)							+ (61.6)	0.664
	Kurtosis									+ (48.9)	0.458
RES	Mean				+ (1.1)		- (0.9)			+ (84.5)	0.874
	Skewness				- (3.6)			+ (2.1)	+ (5.6)	+ (42.7)	0.662
	Kurtosis							+ (4.2)		+ (51.2)	0.515
HyArq	Mean	+ (4.7)	+ (3.6)		- (7.0)					+ (44.9)	0.626
	Skewness	- (4.1)			+ (8.1)					+ (23.7)	0.455
	Kurtosis									+ (52.8)	0.533

The best models were selected according to AICc values (ΔAICc<2). Slope direction ("+" or "-") and the % of variance explained by each variable (value in brackets next to slope direction) are shown for variables included in the best fitting model. Abbreviations are as follows: LES, Leaf Economics Spectrum; RES, Root Economics Spectrum; HyArq, Hydraulic Architecture; SOM, Soil Organic Matter; Adj. R^2, adjusted R^2.

Leaf Economics Spectrum (LES, Wright et al., 2004). Positive values of the PC1 represented species with high SLA, indicative of fast resource uptake strategies at aboveground level, while in the opposite end of the spectrum were species with trait values indicative of the resource conservation strategy (high LDMC, leaf C:N, leaf δ^{13}C and SDMC). The second PC axis explained 18.4% of the variability and was related to the Root Economics Spectrum (RES) (Prieto et al., 2015; de la Riva et al., 2018a). Within this axis, positive scores were associated to species with high SRA and negative scores to species with high Rdi. The third PC axis explained 13.7% of the variance and was associated to the Huber value, and thus to the plant's hydraulic architecture (Mencuccini et al., 2019). Within this axis, positive scores were associated with species with high Huber values, i.e. conservative species.

3.2 Relationships among environmental variables and functional structure (community-weighted moments) and differences in the functional structure among communities

Overall, the models showed a high predictive power (Table 2) with all adjusted R^2 values over 0.45. Our results showed that the type of community was the most important factor determining differences in the functional structure, with environmental factors having a secondary role (Table 2; Appendix S7). The effect of the different abiotic variables depended on the plant ecological dimension analyzed; altitude and orientation were relevant factors determining variation in the LES (aboveground level), while soil depth and nutrients were more important factors belowground, for RES, and HyArq (only soil depth in this case, Table 2).

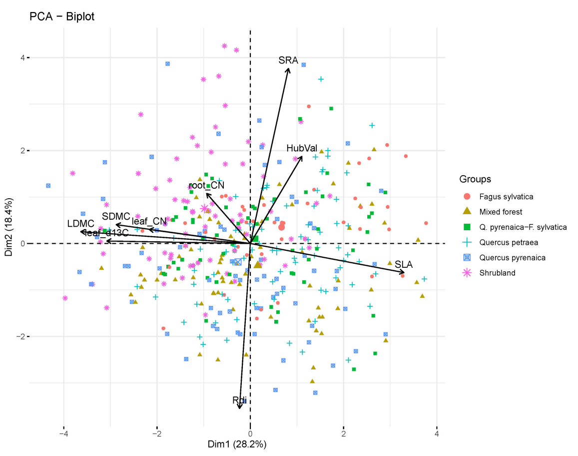


FIGURE 2
Principal Component Analysis (PCA). Results show the first two PC axes for the nine functional traits considered in this study measured on 411 individuals from 19 woody species (trait abbreviations are in Table 1; species list is in Supplementary Material, Appendix S2, Table S2). Symbols correspond to the 411 individuals. The type of community which observations belong to is shown with different symbols and colors.

Regarding variations in the functional structure among communities, we found clear differences in their community weighted moments related to the main axes of variation (Figure 3). The CWMs showed clear trends in relation to the position of the communities along the acquisitive-conservative axis of resource uptake. Mediterranean communities, i.e., *Shrubland* and *Q. pyrenaica* communities, had the lowest CWM scores in the LES (PC1 axis), i.e., these communities were composed by species with high tissue dry matter content and low SLA, and did not differ from each other, indicating a conservative resource-use strategy in both communities (Figure 3). In contrast, the Eurosiberian communities (dominated by either *Q. petraea* or *F. sylvatica*) showed the highest CWM scores in the LES axis (Figure 3); the two mixed communities showing intermediate CWM scores in the LES (Figure 3). In relation to the RES (Figure 3), the *Shrubland* community showed significantly higher CWM scores than the rest of the plant communities, indicating a more acquisitive resource use strategy belowground. *F. sylvatica* community had the second highest CWM scores, while *Q. petraea* community showed the lowest scores in the RES. Again, mixed communities (*Mixed* 1 and 2) showed intermediate CWM scores along the RES axis (Figure 3). With regard to the hydraulic strategy, we found an overall decreasing trend in CWM scores from Mediterranean to Eurosiberian communities (with the exception of *Q. petraea* community, which had similar CWM scores than Mediterranean communities; Figure 3).

We observed strong differences among communities on the community-weighted skewness (CWS) scores on the three plant ecological dimensions (LES, RES and HyArq), although we did not detect a clear pattern regarding the biogeographic origin of the communities (Mediterranean vs Eurosiberian). We found a high asymmetry in the scores' distribution (high positive CWSs) in the two *Mixed* communities and in the *Q. petraea* community for the LES, in *Shrubland* community for the RES and in *F. sylvatica* community for HyArq. By contrast, *F. sylvatica* and the *Mixed* 2 (*F.sy-Q.py-Q.pe*) communities showed a high negative asymmetry (very low values) for the RES.

Similarly, community-weighted kurtosis (CWK) varied among communities depending on the plant ecological dimension considered. CWK values greater than 0 were found in the *Mixed 2* (*F.sy-Qpy-Qpe*) community for the LES, in the *Q. pyrenaica* community for the RES, and in the *Mixed 1* (*F.sy-Qpy*) community for the HyArq. By contrast, CWK scores for the *Shrubland*, *Q. petraea* and *F. sylvatica* communities did not differ from 0 for any plant ecological strategy (except for the HyArq axis in *F. sylvatica* community).

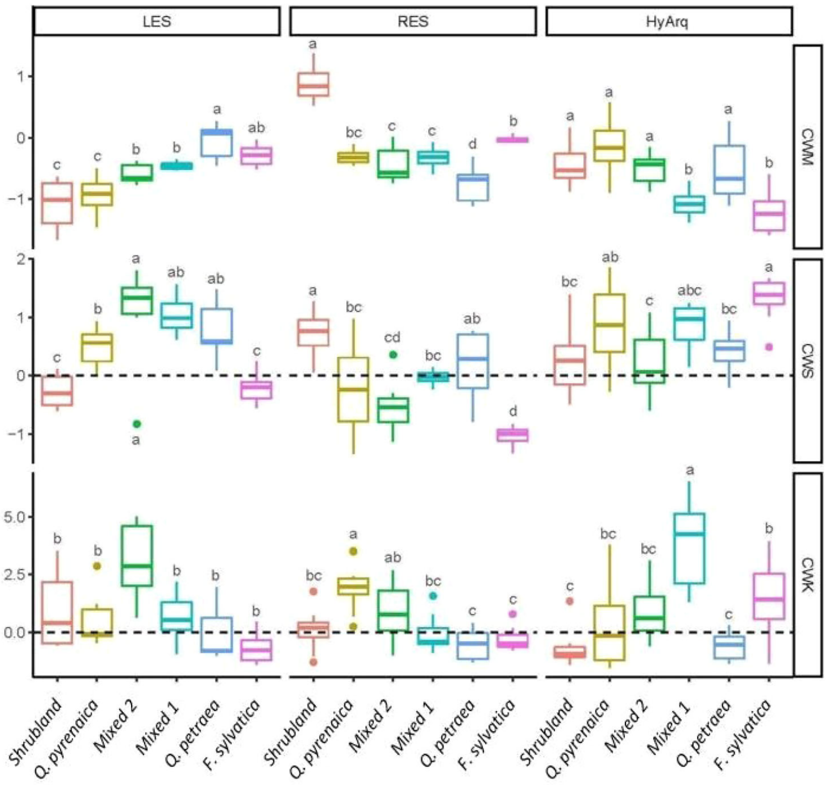


FIGURE 3
Boxplots of the community-weighted moments (CWM: community weighted mean, CWS: community weighted skewness and CWK: community weighted kurtosis) of each plant ecological strategy (LES, RES and HyArq, related to the principal components 1, 2 and 3, respectively; see materials and methods section for a detailed description of the different plant ecological strategies) for the six communities studied (see section *'2.2 Sampling design'* and Supplementary Material, Appendix S2 for the composition of each community). LES, Leaf Economics Spectrum; RES, Root Economics Spectrum; HyArq, Hydraulic Architecture. The line inside the box represents the mean value, the box limits represent the SE and the whiskers represent the SD. Different letters indicate significant differences ($p < 0.05$) among communities. Community-weighted moments are significantly different from zero when its 95%-confidence interval does not overlap with zero (dashed lines).

3.3 Contribution of ITV and species turnover to variation in the CWMs for the LES and RES ecological strategies

The percentage of variance explained by both ITV and species turnover was higher for the LES than for the RES (Figure 4; Supplementary Material, Appendix S8, Table S8.1). For both LES and RES, results showed a stronger influence of ITV than of species turnover in the CWMs' variation among communities. This was especially evident for the RES, for which the effect of species turnover was particularly small compared to that of ITV: close to 61.3% of the variation was explained by ITV against 9.5% explained by turnover (Figure 4; Supplementary Material, Appendix S8, Table S8.1).

4 Discussion

4.1 Trait distribution and environmental effects on community assembly

We found large variation in the functional trait composition considered in this study. Our results showed that functional traits at community level (functional parameters) were mainly driven by differences among plant communities, while abiotic factors exerted a minor role on community weighted trait distributions. In this sub-Mediterranean ecosystem, we observed in a recent study (de la Riva et al., 2023) that environmental suitability explained species segregation better than their functional composition, being the six woody plant communities segregated spatially and functionally partly in relation to microenvironmental conditions, mostly soil depth at aboveground level, and aspect and soil nutrients at belowground level.

Overall, the CWMs showed a trend that was in line with the acquisitive-conservative axis of resource uptake (Reich, 2014; Díaz et al., 2016; de la Riva et al., 2016b) at aboveground level. Plant communities dominated by Mediterranean species (*Q. pyrenaica*, *Shrubland* and *Mixed* 1 and 2) are dominated by species with low SLA and SRA, and high LDMC, thick root diameters (Rdi), and high leaf and root C:N. These communities are found in harsh areas which are characterized by low soil depths with stony soils, steep slopes or high insolation (de la Riva et al., 2023; de Tomás Marín et al., 2023), conditions that probably limit water availability. In fact, this water shortage promoted the prevalence of communities with a conservative hydraulic architecture (i.e. water efficient with high leaf δ^{13}C and Huber values), commonly associated with conservative water use strategies at the expense of a slower plant growth (Prieto et al., 2018; Mencuccini et al., 2019). On the other hand, communities dominated by Eurosiberian species (*Q. petraea* and *F. sylvatica* communities) are found in areas of Montejo with deeper soils —which likely increase soil water storage— and potentially lower evapotranspiration due to lower insolation. These communities had CWM values associated with faster growth and acquisitive resource-use strategies (i.e., higher SLA and SRA, and lower tissue dry matter content, Rdi, C:N ratios and Huber values).

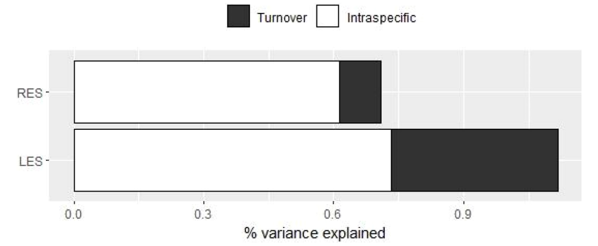


FIGURE 4
Decomposition of total variability in individual trade-offs among traits. LES corresponds to the trade-offs among the study traits related to the Leaf Economics Spectrum. The same applies for the RES in relation to the study traits related to the Root Economics Spectrum.

When belowground strategies were considered, however, *Shrubland* community was the most acquisitive (high CWM) but also showed the highest positive skewness, indicating the presence of subordinate species with conservative strategies. This community occupies flat areas with shallow soils (and thus lower water availability) and high soil nutrient contents in Montejo (de la Riva et al., 2023). In these areas, greater aridity would promote the convergence of traits towards conservative strategies (Cornwell and Ackerly, 2009; de la Riva et al., 2018b; Carrascosa et al., 2023). Although shallow soils prevent the accumulation of big amounts of water, the flat terrain favors water accumulation in the soil surface during short periods. Under these conditions, with a high-enough nutrient concentration, fast resource-uptake belowground strategies would give plants a competitive advantage to absorb the water and nutrients accumulated in shallow soils (Ryel et al., 2008; Schenk, 2008; Querejeta et al., 2021), and to grow during short wet pulses (Querejeta et al., 2018; Carvajal et al., 2019; de la Riva et al., 2021b). Shrub species from Mediterranean arid environments are able to produce cheaper roots in terms of carbon investment to structural components, resulting in roots with high SRA and high absorptive efficiency (de la Riva et al., 2021b). Further, adaptation to low soil water availability during the summer months also explains the negative kurtosis of *Shrubland* for HyArq, indicative of an evenness in the distribution of trait values related to the plant water balance, promoting the coexistence of functionally contrasting species (Gross et al., 2021). Overall, these results suggest a niche segregation and/or complementarity in water-use strategies that could minimize competition and favor coexistence (Bello et al., 2019; Illuminati et al., 2022), which could lead to a more efficient use of water in the *Shrubland* community. These results are in line with a recent study (Illuminati et al., 2022), in which the authors found a clear niche segregation and complementarity in functional traits related to water-use strategy of species coexisting in a Mediterranean shrubland community, and suggested that the presence of species with contrasting water-use strategies may represent a key driver in the assembly of dryland communities.

At the other end of the environmental gradient (see also de la Riva et al., 2023), we found the *F. sylvatica* community with predominantly acquisitive strategies at the whole plant level. In fact, species with a profligate water-use strategy, such as *F. sylvatica* (de Tomás Marín et al., 2023), dominated the lowest areas of Montejo where deeper soils are found (de la Riva et al., 2023), which could explain the shift in the hydraulic dimension along the soil depth gradient better than soil nutrients. This profligate use of water and nutrients of *F. sylvatica* makes it a strong fast-growing species that promotes strong competitive exclusion (Meyer et al., 2003; Rodríguez-Calcerrada et al., 2011; Castaño-Santamaria et al., 2021). In this regard, the highest differences in the skewness values of the RES and HyArq dimensions imply that the asymmetric competition promoted by *F. sylvatica* selects for species with very different functional profiles to that of the dominant species (Le Bagousse-Pinguet et al., 2017). Thus, sub-dominant species seem to be able to buffer competition with *F. sylvatica* by displaying contrasting morphological resource uptake strategies, and possessing different hydrological architecture. As a matter of fact, deeper soils may favor the ecohydrological niche segregation among coexisting species (Silvertown et al., 2015), allowing species to buffer competition by the uptake of water from different soil depths.

In relation to the *Mixed* communities, overall and looking at the CWM, they occupied an intermediate position along the Economics Spectrum (LES, RES and HydArq) and along the environmental gradient in Montejo. These communities, however, exhibited an overall positive asymmetric distribution (CWS = 0.62 for *Mixed 1* and CWS = 0.27 for *Mixed 2*), i.e. a higher frequency of species with traits associated to conservative strategies (CWS > 0), and especially so for the LES dimension. These mixed communities assembled following a competitive hierarchy (de la Riva et al., 2023) where species with acquisitive strategies aboveground are excluded in favor of those with more conservative traits that confer higher competitive advantage in harsher environments (Gross et al., 2013; Carmona et al., 2019). This is further supported by the high kurtosis in both mixed communities for the LES and HyArq, respectively, reflecting a selection of conservative trait values under strong competition (Le Bagousse-Pinguet et al., 2017).

Further, we found that the PC scores for the three plant ecological dimensions were normally distributed across species in the *Q. pyrenaica* community, with a predominance of conservative traits. On the other hand, *Q. petraea* community showed the most conservative traits at root level, opposite to the aboveground level, and deviated from normality for the LES (CWS significantly different from 0), which suggests contrasting resource uptake strategies at leaf level in the less dominant species with regard to the dominant aboveground acquisitive profile of the community. Thus, and similarly to the mixed communities, the departure from normality in the LES dimension in the *Q. petraea* community (indicated by its positive CWS values) suggests a process of hierarchical competition (de la Riva et al., 2023) in which species with aboveground conservative strategies are favored. Interestingly, we observed a mismatch between CWM root and leaf strategies. This mismatch can be explained by the ability of *Q. petraea* to take up water from deep soil layers to cope with drought stress during dry periods (Zapater et al., 2011; Bello et al., 2019; Staszel et al., 2022). This habit would be consistent with a vertical decoupling between water and nutrient uptake (Querejeta et al., 2021), explaining its conservative fine root traits in the topsoil (<20 cm) associated with a lower capacity for nutrient uptake.

To the best of our knowledge, our study is the first one that incorporates the use of community-weighted moments to disentangle the mechanisms and processes driving community assembly in a sub-Mediterranean ecotone. In this regard, the use of community-weighted moments of a trait distribution allowed us to get a deeper insight into the mechanisms driving community assembly in this sub-Mediterranean forest that would be otherwise overlooked, supporting the need to use metrics-based indices beyond the mean for a complete understanding of the underlying assembly patterns of plant communities (Enquist et al., 2015; Le Bagousse-Pinguet et al., 2017; Wieczynski et al., 2019; Guerin et al., 2022).

4.2 Disentangling the role of species turnover and intraspecific variability in shaping the trait distribution between communities

Our results from the analysis of variance decomposition showed that variations in community functional traits along Montejo were mainly explained by changes in intraspecific trait variability (ITV above 60%) rather than by species turnover, in line with previous findings (Jung et al., 2014; Siefert and Ritchie, 2016; Niu et al., 2020; Guo et al., 2022; or Spitzer et al., 2023) but in sharp contrast with others (de Bello et al., 2011; Pérez-Ramos et al., 2012; Kichenin et al., 2013; de la Riva et al., 2016a; Weemstra et al., 2021). The contrasting results found in the literature suggest that the relative contribution of interspecific vs intraspecific variability to the functional structure among plant communities vary with the spatial scale considered and the range of environmental variation. For instance, in this study, 17 out of the 19 species occurred in at least two communities if not more, which could explain the low contribution of species turnover. These results add further support to the hypothesis that ITV gains importance at smaller spatial scales (Lajoie and Vellend, 2015; Siefert et al., 2015; Pichon et al., 2022), when environmental conditions are less variable and interspecific trait variation is low (Cordlandwehr et al., 2013; Petruzzellis et al., 2017). Our results add up to the increasing evidence of the preponderant role that above- and belowground ITV plays on community assembly, suggesting that, in transitional areas such as ecotones, species are not solely filtered based on their mean trait values but also by their trait variability (Siefert, 2012). In fact, ITV is higher across the whole forest than at the community level in the Hayedo de Montejo (de la Riva et al., 2023), supporting the notion that adaptive shifts in traits within species allow them to establish in different communities along transitional areas.

Despite a majority of studies focusing on aboveground traits (e.g. Lepš et al., 2011; Pérez-Ramos et al., 2012; Siefert, 2012; Kichenin et al., 2013; Jung et al., 2014; Siefert et al., 2015; Siefert and Ritchie, 2016; Henn et al., 2018; Niu et al., 2020; Chelli et al., 2021; Guo et al., 2022; Pichon et al., 2022), evidence showing the contribution of belowground intraspecific trait variability to community assembly is starting to accumulate (de la Riva et al., 2016a; Navarro-Fernández et al., 2016; Weemstra et al., 2021; Hogan et al., 2023; Spitzer et al., 2023). In this study we observed

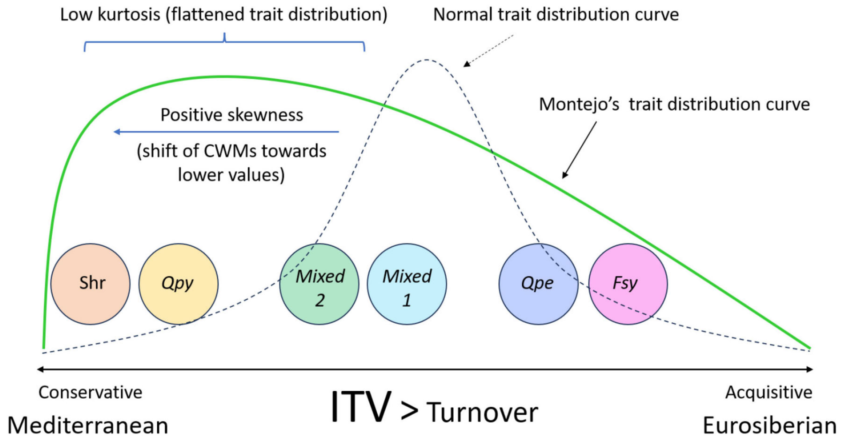


FIGURE 5

Schematic representation of the main findings of this study. Overall, the communities in Montejo spread along the conservative-acquisitive axis of resource use, partly matching their biogeographic origin (Mediterranean vs Eurosiberian). The positive skewness indicates a general shift of the Montejo forest towards more conservative trait values. The low kurtosis, shown by a flattened trait distribution curve, suggests the coexistence of different plant ecological strategies. Furhtermore, the relative contribution of ITV to the changes in the functional structure of Montejo is much higher than that of turnover, both at above- and belowground levels. Additionally, the type of community is the most relevant factor determining differences in the functinoal structure among communities, with environmental variables having a minor role. Shr, Shrubland community; Qpy, *Q. pyrenaica*-dominated community; *Mixed 1*, community co-dominated by *Fagus sylvatica* and *Q. pyrenaica*; *Mixed 2*, community co-dominated by *F.* sylvatica, *Q. pyrenaica* and *Q. petraea*; Qpe: *Q. petraea*-dominated community; Fsy, *F. sylvatica*-dominated community; ITV, Intraspecific Trait Variability.

that the extent and sources of variation of root traits followed a similar pattern to those of leaves, even with a relatively higher importance of ITV for roots than for leaves (Figure 4; Supplementary Material, Appendix S8, Table S8.1). A recent study showed that ITV explained the mean trait variation of morphological root traits rather than of chemical root traits (Spitzer et al., 2023), while previous studies carried out in Mediterranean woody communities showed higher ITV for the number of arbuscular mycorrhizas vesicles than for SRL (Navarro-Fernández et al., 2016). This indicates that ITV differs between root traits, as found at the aboveground level (Lepš et al., 2011; Kichenin et al., 2013). Considering root trait syndromes at unified dimensions help to depict the role of ITV vs species turnover in community assembly, because traits co-vary from trade-offs between different plant functions, producing syndromes that influence species' fitness (Albert et al., 2011).

5 Conclusions

In this study, we discerned the assembly patterns of woody plant communities in a sub-Mediterranean forest, using a whole-plant, trait-based approach. We observed a high number of strategies in the forest, reflecting coordination in the change of above- and belowground traits, with the different communities spreading along the acquisitive-conservative axis of resource-use, partly matching their Eurosiberian vs Mediterranean nature, respectively. Additionally, we showed that ITV is a highly relevant factor that should be incorporated when analyzing functional changes and assembly patterns in plant communities, both above- and belowground, especially at small spatial scales. We also found slight relationships between changes in trait syndromes and topographic and soil factors. This is probably due to the small spatial scale and sampling area of the study and to the potential biotic interactions occurring among the different plant species, which may prevent us from detecting stronger functional responses to environmental conditions (See Figure 5 for a conceptual illustration of the main findings of the study).

Altogether, these findings represent an important step towards a more general understanding of the processes driving the assembly and functional composition of sub-Mediterranean plant communities. Our data support the notion that ecotones are fragile regions where minor environmental shifts may result in abrupt changes in ecosystem structure and composition. Thus, understanding which and how abiotic factors produce changes in the composition and functional structure of these transitional regions (e.g. species turnover vs intraspecific variability) may help to their protection in the face of global change. Our results set the ground for future research of community assembly processes and mechanisms of plant communities in these and other ecotone regions.

Data availability statement

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

Author contributions

ST: Data curation, Formal Analysis, Writing – original draft, Writing – review & editing. JG: Data curation, Formal Analysis, Writing – review & editing. JR-C: Writing – review & editing. IP: Conceptualization, Methodology, Writing – review & editing. ER: Conceptualization, Funding acquisition, Methodology, Resources, Supervision, Writing – review & editing.

Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article. This work was financially supported by the German Research Foundation (Deutsche Forschungsgemeinschaft), being part of the project "the Functional Frontier among Mediterranean and Eurosiberian Plant Communities" (ECOFUMER, 441909701). ER and JG are respectively supported by a María Zambrano and a Margarita Salas fellowships funded by the Spanish Ministry of Universities and European Union-Next Generation Plan. IP acknowledges funding from a Ramón y Cajal contract (RYC2021-033081-I) funded by the Ministry of Science and Innovation and co-funded by the European Union-Next Generation Plan funded by European Union-NextGenerationEU.

Acknowledgments

The authors of this study would like to thank Mario Vega and all the technical staff of the "Hayedo de Montejo" for their always good willingness to facilitate our works in the forest and the interest shown in this study. We also thank Guillermo G. Gordaliza, Eva Miranda and Carlos Magro for data on FIs. Many thanks to Claudia Buchwald for her help and guidance in the lab analysis carried out for this research. Also thanks to Diego Alejandro Melo Prieto for his great help both in the field and in the lab, and to Pardis Golabvand and Marina Tsioli for their help with the lab analyses. We would also like to thank Professor Dr. Francisco Lloret for his advice on field ecological methodology and to his biology student at the time of the performance of this study Anna Ticó i Pifarré for her great help and support with the field work.

Conflict of interest

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpls.2023.1303022/full#supplementary-material

References

Albert, C. H., Grassein, F., Schurr, F. M., Vieilledent, G., and Violle, C. (2011). When and how should intraspecific variability be considered in trait-based plant ecology? *Perspect. Plant Ecol. Evol. System.* 13 (3), 217–225. doi: 10.1016/j.ppees.2011.04.003

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 (3), 604–613. doi: 10.1111/j.1365-2745.2010.01651.x

Barton, K., and Barton, M. K. (2015). *Package 'MuMIn'; Version 1.43.17*. Available at: https://cran.r-project.org/web/packages/MuMIn/MuMIn.pdf (Accessed January 5th, 2023).

Bello, J., Hasselquist, N. J., Vallet, P., Kahmen, A., Perot, T., and Korboulewsky, N. (2019). Complementary water uptake depth of *Quercus petraea* and *Pinus sylvestris* in mixed stands during an extreme drought. *Plant Soil* 437, 93–115. doi: 10.1007/s11104-019-03951-z

Benito Garzón, M., Sánchez de Dios, R., and Sainz Ollero, H. (2008). Effects of climate change on the distribution of Iberian tree species. *Appl. Vege. Sci.* 11 (2), 169–178. doi: 10.3170/2008-7-18348

Bílá, K., Moretti, M., de Bello, F., Dias, A. T., Pezzatti, G. B., Van Oosten, A. R., et al. (2014). Disentangling community functional components in a litter-macrodetritivore model system reveals the predominance of the mass ratio hypothesis. *Ecol. Evol.* 4 (4), 408–416. doi: 10.1002/ece3.941

Box, E. O. (2021). Why does Mediterranean vegetation seem so diverse? *Tools Landscape-Scale Geobot. Conserv.*, 287–313. doi: 10.1007/978-3-030-74950-7_15

Brice, M. H., Vissault, S., Vieira, W., Gravel, D., Legendre, P., and Fortin, M. J. (2020). Moderate disturbances accelerate forest transition dynamics under climate change in the temperate–boreal ecotone of eastern North America. *Global Change Biol.* 26 (8), 4418–4435. doi: 10.1111/gcb.15143

Cantoral, A. L., Alonso-Redondo, R., García-González, M. E., Penas, Á., and del Río, S. (2023). Phytosociological analysis of the endemic *Quercus faginea* forests of the Iberian Peninsula. *Plant Biosystems-An Int. J. Deal. All Aspects Plant Biol.* 1-51, 419–436. doi: 10.1080/11263504.2023.2165554

Carlson, B. Z., Renaud, J., Biron, P. E., and Choler, P. (2014). Long-term modeling of the forest–grassland ecotone in the French Alps: implications for land management and conservation. *Ecol. Appl.* 24 (5), 1213–1225. doi: 10.1890/13-0910.1

Carmona, C. P., de Bello, F., Azcárate, F. M., Mason, N. W., and Peco, B. (2019). Trait hierarchies and intraspecific variability drive competitive interactions in Mediterranean annual plants. *J. Ecol.* 107 (5), 2078–2089. doi: 10.1111/1365-2745.13248

Carrascosa, A., Silvestre, M., Morgado, L., and Azcárate, F. M. (2023). Diversity and mean specific leaf area of Mediterranean woody vegetation changes in response to summer drought across a double stress gradient: The role of phenotypic plasticity. *J. Vege. Sci.* 34 (2), e13180. doi: 10.1111/jvs.13180

Carter, J. L., and White, D. A. (2009). Plasticity in the Huber value contributes to homeostasis in leaf water relations of a mallee Eucalypt with variation to groundwater depth. *Tree Physiol.* 29 (11), 1407–1418. doi: 10.1093/treephys/tpp076

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 (3), 1079–1092. doi: 10.1111/1365-2745.13092

Castaño-Santamaria, J., López-Sánchez, C. A., Obeso, J. R., and Barrio-Anta, M. (2021). Structure, environmental patterns and impact of expected climate change in natural beech-dominated forests in the Cantabrian Range (NW Spain). *For. Ecol. Manage.* 497, 119512. doi: 10.1016/j.foreco.2021.119512

Chelli, S., Ottaviani, G., Simonetti, E., Campetella, G., Wellstein, C., Bartha, S., et al. (2021). Intraspecific variability of specific leaf area fosters the persistence of understorey specialists across a light availability gradient. *Plant Biol.* 23 (1), 212–216. doi: 10.1111/plb.13199

Cordlandwehr, V., Meredith, R. L., Ozinga, W. A., Bekker, R. M., van Groenendael, J. M., and Bakker, J. P. (2013). Do plant traits retrieved from a database accurately predict on-site measurements? *J. Ecol.* 101 (3), 662–670. doi: 10.1111/1365-2745.12091

Cornwell, W. K., and Ackerly, D. D. (2009). Community assembly and shifts in plant trait distributions across an environmental gradient in coastal California. *Ecol. Monogr.* 79 (1), 109–126. doi: 10.1890/07-1134.1

Danet, A., Anthelme, F., Gross, N., and Kéfi, S. (2018). Effects of indirect facilitation on functional diversity, dominance and niche differentiation in tropical alpine communities. *J. Vege. Sci.* 29 (5), 835–846. doi: 10.1111/jvs.12667

Danso Marfo, T., Datta, R., Vranová, V., and Ekielski, A. (2019). Ecotone dynamics and stability from soil perspective: Forest-agriculture land transition. *Agriculture* 9 (10), 228. doi: 10.3390/agriculture9100228

de Bello, F., Lavorel, S., Albert, C. H., Thuiller, W., Grigulis, K., Dolezal, J., et al. (2011). Quantifying the relevance of intraspecific trait variability for functional diversity. *Methods Ecol. Evol.* 2 (2), 163–174. doi: 10.1111/j.2041-210X.2010.00071.x

de la Riva, E. G., Marañón, T., Pérez-Ramos, I. M., Navarro-Fernández, C. M., Olmo, M., and Villar, R. (2018a). 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., Marañón, T., Violle, C., Villar, R., and Pérez-Ramos, I. M. (2017). Biogeochemical and ecomorphological niche segregation of Mediterranean woody species along a local gradient. *Front. Plant Sci.* 8. doi: 10.3389/fpls.2017.01242

de la Riva, E. G., Pérez-Ramos, I. M., Tosto, A., Navarro-Fernández, C. M., Olmo, M., Marañón, T., et al. (2016a). 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 (3), 354–363. doi: 10.1111/oik.01875

de la Riva, E. G., Prieto, I., de Tomás Marín, S., Rodríguez-Calcerrada, J., Golabvand, P., and Galán Díaz, J. (2023). Living at the edge: the functional niche occupation of woody plant communities in the submediterranean ecotone. *Ann. Bot.* 132 (3), 471–484. doi: 10.1093/aob/mcad138

de la Riva, E. G., Prieto, I., Marañón, T., Pérez-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 (4), 1873–1885. doi: 10.1111/1365-2745.13612

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, 2662. doi: 10.3389/fpls.2021.773118

de la Riva, E. G., Tosto, A., Pérez-Ramos, I. M., Navarro-Fernández, C. M., Olmo, M., Anten, N. P., et al. (2016b). A plant economics spectrum in Mediterranean forests along environmental gradients: is there coordination among leaf, stem and root traits? *J. Vege. Sci.* 27 (1), 187–199. doi: 10.1111/jvs.12341

de la Riva, E. G., Violle, C., Pérez-Ramos, I. M., Marañón, T., Navarro-Fernández, C. M., Olmo, M., et al. (2018b). A multidimensional functional trait approach reveals the imprint of environmental stress in Mediterranean woody communities. *Ecosystems* 21, 248–262. doi: 10.1007/s10021-017-0147-7

de Tomás Marín, S., Rodríguez-Calcerrada, J., Arenas-Castro, S., Prieto, I., González, G., Gil, L., et al. (2023). *Fagus sylvatica* and *Quercus pyrenaica*: Two neighbors with few things in common. *For. Ecosyst.* 10, 100097. doi: 10.1016/j.fecs.2023.100097

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 (7585), 167–171. doi: 10.1038/nature16489

Dong, N., Prentice, I. C., Wright, I. J., Evans, B. J., Togashi, H. F., Caddy-Retalic, S., et al. (2020). Components of leaf-trait variation along environmental gradients. *New Phytol.* 228 (1), 82–94. doi: 10.1111/nph.16558

Dorado-Liñán, I., Zorita, E., Martínez-Sancho, E., Gea-Izquierdo, G., Di Filippo, A., Gutiérrez, E., et al. (2017). Large-scale atmospheric circulation enhances the Mediterranean East-West tree growth contrast at rear-edge deciduous forests. *Agric. For. Meteorol.* 239, 86–95. doi: 10.1016/j.agrformet.2017.02.029

Enquist, B. J., Norberg, J., Bonser, S. P., Violle, C., Webb, C. T., Henderson, A., et al. (2015). Scaling from traits to ecosystems: developing a general trait driver theory via integrating trait-based and metabolic scaling theories. *Adv. Ecol. Res.* 52, 249–318. doi: 10.1016/bs.aecr.2015.02.001

Fox, J., and Weisberg, S. (2019). *An R Companion to Applied Regression. 3rd ed.* (Thousand Oaks CA: Sage). Available at: https://socialsciences.mcmaster.ca/jfox/Books/Companion/.

Frenette-Dussault, C., Shipley, B., Meziane, D., and Hingrat, Y. (2013). Trait-based climate change predictions of plant community structure in arid steppes. *J. Ecol.* 101 (2), 484–492. doi: 10.1111/1365-2745.12040

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 (3), 1123–1158. doi: 10.1111/nph.17072

Garnier, E., Cortez, J., Billès, G., Navas, M. L., Roumet, C., Debussche, M., et al. (2004). Plant functional markers capture ecosystem properties during secondary succession. *Ecology* 85 (9), 2630–2637. doi: 10.1890/03-0799

Garnier, E., and Navas, M. L. (2012). A trait-based approach to comparative functional plant ecology: concepts, methods and applications for agroecology. A review. *Agron. Sustain. Dev.* 32, 365–399. doi: 10.1007/s13593-011-0036-y

Gil, L., Náger, J. A., González Doncel, I., Aranda García, I., Gonzalo Jiménez, J., López de Heredia, U., et al. (2010). *El Hayedo de Montejo: Una Gestión Sostenible* (Comunidad de Madrid: Universidad Politécnica de Madrid. Ed. Madrid: 1036 Dirección General de Medio Ambiente), ISBN: .

Grime, J. P. (1998). Benefits of plant diversity to ecosystems: immediate, filter and founder effects. *J. Ecol.* 86 (6), 902–910. doi: 10.1046/j.1365-2745.1998.00306.x

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 (3), 637–649. doi: 10.1111/1365-2745.12063

Gross, N., Le Bagousse-Pinguet, Y. L., Liancourt, P., Berdugo, M., Gotelli, N. J., and Maestre, F. T. (2017). Functional trait diversity maximizes ecosystem multifunctionality. *Nat. Ecol. Evol.* 1 (5), 132. doi: 10.1038/s41559-017-0132

Gross, N., Le Bagousse-Pinguet, Y., Liancourt, P., Saiz, H., Violle, C., and Munoz, F. (2021). Unveiling ecological assembly rules from commonalities in trait distributions. *Ecol. Lett.* 24 (8), 1668–1680. doi: 10.1111/ele.13789

Guerin, G. R., Gallagher, R. V., Wright, I. J., Andrew, S. C., Falster, D. S., Wenk, E., et al. (2022). Environmental associations of abundance-weighted functional traits in Australian plant communities. *Basic Appl. Ecol.* 58, 98–109. doi: 10.1016/j.baae.2021.11.008

Guo, A., Zuo, X., Zhang, S., Hu, Y., Yue, P., Lv, P., et al. (2022). Contrasting effects of plant inter-and intraspecific variation on community trait responses to nitrogen addition and drought in typical and meadow steppes. *BMC Plant Biol.* 22 (1), 90. doi: 10.1186/s12870-022-03486-z

Hejda, M., Štajerová, K., Pergl, J., and Pyšek, P. (2019). Impacts of dominant plant species on trait composition of communities: comparison between the native and invaded ranges. *Ecosphere* 10 (10), e02880. doi: 10.1002/ecs2.2880

Henn, J. J., Buzzard, V., Enquist, B. J., Halbritter, A. H., Klanderud, K., Maitner, B. S., et al. (2018). Intraspecific trait variation and phenotypic plasticity mediate alpine plant species response to climate change. *Front. Plant Sci.* 9. doi: 10.3389/fpls.2018.01548

Hernández Bermejo, J. E., Costa Tenorio, M., Sáinz Ollero, H., and Clemente Muñoz, M. (1982). Catálogo florístico del hayedo de Montejo de la Sierra (provincia de Madrid). *Lagascalia* 11 (1), 3–65.

Hernández-Lambraño, R. E., de la Cruz, D. R., and Agudo, J.Á.S. (2021). Effects of the climate change on peripheral populations of hydrophytes: a sensitivity analysis for European plant species based on climate preferences. *Sustainability* 13 (6), 3147. doi: 10.3390/su13063147

Hogan, J. A., Xu, H., and Baraloto, C. (2023). Intraspecific trait variation and species turnover in successional tropical forests: assessing trait imputation for community-weighted means. *Plant Ecol.* 224 (5), 463–477. doi: 10.1007/s11258-023-01314-4

Hufkens, K., Scheunders, P., and Ceulemans, R. (2009). Ecotones in vegetation ecology: methodologies and definitions revisited. *Ecol. Res.* 24, 977–986. doi: 10.1007/s11284-009-0584-7

Illuminati, A., Querejeta, J. I., Pías, B., Escudero, A., and Matesanz, S. (2022). Coordination between water uptake depth and the leaf economic spectrum in a Mediterranean shrubland. *J. Ecol.* 110 (8), 1844–1856. doi: 10.1111/1365-2745.13909

Iozia, L. M., Crisafulli, V., and Varone, L. (2023). Climatic variations along an aridity gradient drive significant trait intraspecific variability in Mediterranean plant species. *J. Arid Environ.* 217, 105042. doi: 10.1016/j.jaridenv.2023.105042

Jung, V., Albert, C. H., Violle, C., Kunstler, G., Loucougaray, G., and Spiegelberger, T. (2014). Intraspecific trait variability mediates the response of subalpine grassland communities to extreme drought events. *J. Ecol.* 102 (1), 45–53. doi: 10.1111/1365-2745.12177

Kermavnar, J., Kutnar, L., and Marinšek, A. (2022). Variation in floristic and trait composition along environmental gradients in the herb layer of temperate forests in the transition zone between Central and SE Europe. *Plant Ecol.* 1-14, 229–242. doi: 10.1007/s11258-021-01203-8

Kichenin, E., Wardle, D. A., Peltzer, D. A., Morse, C. W., and Freschet, G. T. (2013). Contrasting effects of plant inter-and intraspecific variation on community-level trait measures along an environmental gradient. *Funct. Ecol.* 27 (5), 1254–1261. doi: 10.1111/1365-2435.12116

Kitagawa, R., Masumoto, S., Nishizawa, K., Kaneko, R., Osono, T., Hasegawa, M., et al. (2020). Positive interaction facilitates landscape homogenization by shrub expansion in the forest–tundra ecotone. *J. Vege. Sci.* 31 (2), 234–244. doi: 10.1111/jvs.12818

Lajoie, G., and Vellend, M. (2015). Understanding context dependence in the contribution of intraspecific variation to community trait–environment matching. *Ecology* 96 (11), 2912–2922. doi: 10.1890/15-0156.1

Le Bagousse-Pinguet, Y., Gross, N., Maestre, F. T., Maire, V., de Bello, F., Fonseca, C. R., et al. (2017). Testing the environmental filtering concept in global drylands. *J. Ecol.* 105 (4), 1058–1069. doi: 10.1111/1365-2745.12735

Le Bagousse-Pinguet, Y., Gross, N., Saiz, H., Maestre, F. T., Ruiz, S., Dacal, M., et al. (2021). Functional rarity and evenness are key facets of biodiversity to boost multifunctionality. *Proc. Natl. Acad. Sci.* 118 (7), e2019355118. doi: 10.1073/pnas.2019355118

Lepš, J., de Bello, F., Šmilauer, P., and Doležal, J. (2011). Community trait response to environment: disentangling species turnover vs intraspecific trait variability effects. *Ecography* 34 (5), 856–863. doi: 10.1111/j.1600-0587.2010.06904.x

Lopes, T. R., Moura, L. B., Nascimento, J. G., Junior, L. S. F., Zolin, C. A., Duarte, S. N., et al. (2020). Priority areas for forest restoration aiming at the maintenance of water resources in a basin in the Cerrado/Amazon ecotone, Brazil. *J. South Am. Earth Sci.* 101, 102630. doi: 10.1016/j.jsames.2020.102630

López Santalla, A., Navarro, F. P., Náger, J. A., and Sánchez, L. G. (2003). El aprovechamiento tradicional del monte y sus efectos sobre la vegetación en el "Hayedo de Montejo" (Madrid). *Cuadernos la Sociedad Española Cienc. Forestales* 16), 109–114.

Ma, W. T., Yu, Y. Z., Wang, X., and Gong, X. Y. (2023). Estimation of intrinsic water-use efficiency from δ13C signature of C3 leaves: Assumptions and uncertainty. *Front. Plant Sci.* 13. doi: 10.3389/fpls.2022.1037972

Maire, V., Gross, N., Börger, L., Proulx, R., Wirth, C., Pontes, L. D. S., et al. (2012). Habitat filtering and niche differentiation jointly explain species relative abundance within grassland communities along fertility and disturbance gradients. *New Phytol.* 196 (2), 497–509. doi: 10.1111/j.1469-8137.2012.04287.x

Mencuccini, M., Rosas, T., Rowland, L., Choat, B., Cornelissen, H., Jansen, S., et al. (2019). Leaf economics and plant hydraulics drive leaf:wood area ratios. *New Phytol.* 224 (4), 1544–1556. doi: 10.1111/nph.15998

Meyer, P., Tabaku, V., and v. Lüpke, B. (2003). Die Struktur albanischer Rotbuchen-Urwälder–Ableitungen für eine naturnahe Buchenwirtschaft: structural characteristics of Albanian Beech (*Fagus sylvatica* L.) Virgin Forests–Deductions for semi-natural forestry. *Forstwissenschaftliches Centralblatt* 122 (1), 47–58. doi: 10.1046/j.1439-0337.2003.02041.x

Moreno, J.Á.C., de las Heras Puñal, P., Estébanez, N. L., and Martín, M. J. R. (2005). *Caracterización paisajística y ecológica de la Sierra del Rincón (Madrid)* (Centro de Investigaciones Ambientales de la Comunidad de Madrid "Fernando González Bernáldez". Ed. Comunidad de Madrid). doi: 10.13140/2.1.4707.2322

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. Vege. Sci.* 27 (6), 1243–1253. doi: 10.1111/jvs.12446

Niu, K., Zhang, S., and Lechowicz, M. J. (2020). Harsh environmental regimes increase the functional significance of intraspecific variation in plant communities. *Funct. Ecol.* 34 (8), 1666–1677. doi: 10.1111/1365-2435.13582

Pardo, F., and Gil, L. (2005). The impact of traditional land use on woodlands: a case study in the Spanish Central System. *J. Historic. Geogr.* 31 (3), 390–408. doi: 10.1016/j.jhg.2004.11.002

Pereira, S. C., Carvalho, D., and Rocha, A. (2021). Temperature and precipitation extremes over the Iberian Peninsula under climate change scenarios: a review. *Climate* 9 (9), 139. doi: 10.3390/cli9090139

Pérez-Harguindeguy, N., Diaz, S., Garnier, E., Lavorel, S., Poorter, H., Jaureguiberry, P., et al. (2013). New handbook for standardized measurement of plant functional traits worldwide. *Aust. J. Bot.* 61, 167–234. doi: 10.1071/BT12225_CO

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 (6), 1315–1327. doi: 10.1111/1365-2745.12000

Petruzzellis, F., Palandrani, C., Savi, T., Alberti, R., Nardini, A., and Bacaro, G. (2017). Sampling intraspecific variability in leaf functional traits: Practical suggestions to maximize collected information. *Ecol. Evol.* 7 (24), 11236–11245. doi: 10.1002/ece3.3617

Pichon, N. A., Cappelli, S. L., and Allan, E. (2022). Intraspecific trait changes have large impacts on community functional composition but do not affect ecosystem function. *J. Ecol.* 110 (3), 644–658. doi: 10.1111/1365-2745.13827

Pogue, D. W., and Schnell, G. D. (2001). Effects of agriculture on habitat complexity in a prairie-forest ecotone in the Southern Great Plains of North America. *Agricult. Ecosyst. Environ.* 87 (3), 287–298. doi: 10.1016/S0167-8809(01)00150-5

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 (3), 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 economics spectrum. *J. Ecol.* 103 (2), 361–373. doi: 10.1111/1365-2745.12351

Querejeta, J. I., Prieto, I., Torres, P., Campoy, M., Alguacil, M. M., and Roldán, A. (2018). Water-spender strategy is linked to higher leaf nutrient concentrations across plant species colonizing a dry and nutrient-poor epiphytic habitat. *Environ. Exp. Bot.* 153, 302–310. doi: 10.1016/j.envexpbot.2018.06.007

Querejeta, J. I., Ren, W., and Prieto, I. (2021). Vertical decoupling of soil nutrients and water under climate warming reduces plant cumulative nutrient uptake, water-use efficiency and productivity. *New Phytol.* 230 (4), 1378–1393. doi: 10.1111/nph.17258

Rahman, I. U., Afzal, A., Iqbal, Z., Hashem, A., Al-Arjani, A. B. F., Alqarawi, A. A., et al. (2021). Species distribution pattern and their contribution in plant community assembly in response to ecological gradients of the ecotonal zone in the Himalayan region. *Plants* 10 (11), 2372. doi: 10.3390/plants10112372

Rawal, R. S., Rawal, R., Rawat, B., Negi, V. S., and Pathak, R. (2018). Plant species diversity and rarity patterns along altitude range covering treeline ecotone in Uttarakhand: conservation implications. *Trop. Ecol.* 59 (2), 225–239.

R Core Team (2021). *R: A Language and Environment for Statistical Computing* (Vienna, Austria: R Foundation for Statistical Computing), ISBN: .

Reich, P. B. (2014). The world-wide 'fast–slow' plant economics spectrum: a traits manifesto. *J. Ecol.* 102 (2), 275–301. doi: 10.1111/1365-2745.12211

Rivas-Martínez, S., Penas, Á., Díaz González, T. E., Ladero Álvarez, M., Asensi Marfil, A., Díez Garretas, B., et al. (2011). Mapa de series, geoseries y geopermaseries de vegetación de España (Memoria del mapa de vegetación potencial de España). Parte II. *Itinera Geobot.* 18 (2), 425–800.

Rodríguez-Calcerrada, J., Nanos, N., Del Rey, M. C., de Heredia, U. L., Escribano, R., and Gil, L. (2011). Small-scale variation of vegetation in a mixed forest understorey is partly controlled by the effect of overstory composition on litter accumulation. *J. For. Res.* 16 (6), 473–483. doi: 10.1007/s10310-010-0237-2

Rodríguez-Calcerrada, J., Salomón, R., Barba, J., Gordaliza, G. G., Curiel Yuste, J., Magro, C., et al. (2019). Regeneration in the understory of declining overstory trees contributes to soil respiration homeostasis along succession in a sub-Mediterranean beech forest. *Forests* 10 (9), 727. doi: 10.3390/f10090727

Rubio-Cuadrado, Á., Camarero, J. J., Gordaliza, G. G., Cerioni, M., Montes, F., and Gil, L. (2020). Competition overrides climate as trigger of growth decline in a mixed Fagaceae Mediterranean rear-edge forest. *Ann. For. Sci.* 77, 1–18. doi: 10.1007/s13595-020-01004-5

Rubio-Cuadrado, Á., Camarero, J. J., Rodríguez-Calcerrada, J., Perea, R., Gómez, C., Montes, F., et al. (2021). Impact of successive spring frosts on leaf phenology and radial growth in three deciduous tree species with contrasting climate requirements in central Spain. *Tree Physiol.* 41 (12), 2279–2292. doi: 10.1093/treephys/tpab076

Ruiz-Labourdette, D., Nogués-Bravo, D., Ollero, H. S., Schmitz, M. F., and Pineda, F. D. (2012). Forest 980 composition in Mediterranean mountains is projected to shift along the entire elevational gradient 981 under climate change. *J. Biogeography* 39 (1), 162–176. doi: 10.1111/j.1365- 982 2699.2011.02592

Ryel, R. J., Ivans, C. Y., Peek, M. S., and Leffler, A. J. (2008). Functional differences in soil water pools: a new perspective on plant water use in water-limited ecosystems. *Prog. Bot.* 69, 397–422. doi: 10.1007/978-3-540-72954-9_16

Sánchez de Dios, R., Benito-Garzón, M., and Sainz-Ollero, H. (2009). Present and future extension of the Iberian submediterranean territories as determined from the distribution of marcescent oaks. *Plant Ecol.* 204 (2), 189–205. doi: 10.1007/s11258-009-9584-5

Schenk, H. J. (2008). The shallowest possible water extraction profile: a null model for global root distributions. *Vadose Zone J.* 7 (3), 1119–1124. doi: 10.2136/vzj2007.0119

Schneider, C. A., Rasband, W. S., and Eliceiri, K. W. (2012). NIH Image to ImageJ: 25 years of image analysis. *Nat. Methods* 9 (7), 671–675. doi: 10.1038/nmeth.2089

Shea, M. E., Mladenoff, D. J., Clayton, M. K., Berg, S., and Elza, H. (2022). Pattern of tree species co-occurrence in an ecotone responds to spatially variable drivers. *Landscape Ecol.* 37 (9), 2327–2342. doi: 10.1007/s10980-022-01485-x

Shipley, B., and Vu, T. T. (2002). Dry matter content as a measure of dry matter concentration in plants and their parts. *New Phytol.* 153 (2), 359–364. doi: 10.1046/j.0028-646X.2001.00320.x

Siefert, A. (2012). Incorporating intraspecific variation in tests of trait-based community assembly. *Oecologia* 170 (3), 767–775. doi: 10.1007/s00442-012-2351-7

Siefert, A., and Ritchie, M. E. (2016). Intraspecific trait variation drives functional responses of old-field plant communities to nutrient enrichment. *Oecologia* 181, 245–255. doi: 10.1007/s00442-016-3563-z

Siefert, A., Violle, C., Chalmandrier, L., Albert, C. H., Taudiere, A., Fajardo, A., et al. (2015). A global meta-analysis of the relative extent of intraspecific trait variation in plant communities. *Ecol. Lett.* 18 (12), 1406–1419. doi: 10.1111/ele.12508

Silvertown, J., Araya, Y., and Gowing, D. (2015). Hydrological niches in terrestrial plant communities: a review. *J. Ecol.* 103 (1), 93–108. doi: 10.1111/1365-2745.12332

Spitzer, C. M., Sundqvist, M. K., Wardle, D. A., Gundale, M. J., and Kardol, P. (2023). Root trait variation along a sub-arctic tundra elevational gradient. *Oikos* 2023 (1), e08903. doi: 10.1111/oik.08903

Staszel, K., Lasota, J., and Błońska, E. (2022). Effect of drought on root exudates from *Quercus petraea* and enzymatic activity of soil. *Sci. Rep.* 12 (1), 7635. doi: 10.1038/s41598-022-11754-z

Team, R. (2021). *RStudio: integrated development for R* 2020 (Boston, MA: RStudio, PBC). Available at: https://posit.co/.

Tyree, M. T., and Ewers, F. W. (1991). The hydraulic architecture of trees and other woody plants. *New Phytol.* 119 (3), 345–360. doi: 10.1111/j.1469-8137.1991.tb00035.x

Vila-Viçosa, C., Arenas-Castro, S., Marcos, B., Honrado, J., García, C., Vázquez,, et al. (2020a). Combining satellite remote sensing and climate data in species distribution models to improve the conservation of Iberian White Oaks (*Quercus* L.). *ISPRS Int. J. Geo-Inform.* 9 (12), 735. doi: 10.3390/ijgi9120735

Vila-Viçosa, C., Gonçalves, J., Honrado, J., Lomba, Â., Almeida, R. S., Vázquez,, et al. (2020b). Late Quaternary range shifts of marcescent oaks unveil the dynamics of a major biogeographic transition in southern Europe. *Sci. Rep.* 10 (1), 1–12. doi: 10.1038/s41598-020-78576-9

Violle, C., Enquist, B. J., McGill, B. J., Jiang, L. I. N., Albert, C. H., Hulshof, C., et al. (2012). The return of the variance: intraspecific variability in community ecology. *Trends Ecol. Evol.* 27 (4), 244–252. doi: 10.1016/j.tree.2011.11.014

Walker, B. (1995). Conserving biological diversity through ecosystem resilience. *Conserv. Biol.* 9 (4), 747–752. doi: 10.1046/j.1523-1739.1995.09040747.x

Weemstra, M., Freschet, G. T., Stokes, A., and Roumet, C. (2021). Patterns in intraspecific variation in root traits are species-specific along an elevation gradient. *Funct. Ecol.* 35 (2), 342–356. doi: 10.1111/1365-2435.13723

Wieczynski, D. J., Boyle, B., Buzzard, V., Duran, S. M., Henderson, A. N., Hulshof, C. M., et al. (2019). Climate shapes and shifts functional biodiversity in forests worldwide. *Proc. Natl. Acad. Sci.* 116 (2), 587–592. doi: 10.1073/pnas.1813723116

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 (6985), 821–827. doi: 10.1038/nature02403

Zapater, M., Hossann, C., Bréda, N., Bréchet, C., Bonal, D., and Granier, A. (2011). Evidence of hydraulic lift in a young beech and oak mixed forest using 18O soil water labelling. *Trees* 25 (5), 885–894. doi: 10.1007/s00468-011-0563-9

Zhang, J., He, N., Liu, C., Xu, L., Chen, Z., Li, Y., et al. (2020). Variation and evolution of C:N ratio among different organs enable plants to adapt to N-limited environments. *Global Change Biol.* 26 (4), 2534–2543. doi: 10.1111/gcb.14973

Zhang, Z., Huang, M., Zhao, X., and Wu, L. (2019). Adjustments of leaf traits and whole plant leaf area for balancing water supply and demand in Robinia pseudoacacia under different precipitation conditions on the Loess Plateau. *Agric. For. Meteorol.* 279, 107733. doi: 10.1016/j.agrformet.2019.107733

Zhao, N., Yu, G., He, N., Xia, F., Wang, Q., Wang, R., et al. (2016). Invariant allometric scaling of nitrogen and phosphorus in leaves, stems, and fine roots of woody plants along an altitudinal gradient. *J. Plant Res.* 129 (4), 647–657. doi: 10.1007/s10265-016-080

frontiers | Frontiers in Plant Science

TYPE Original Research
PUBLISHED 12 December 2023
DOI 10.3389/fpls.2023.1239190

OPEN ACCESS

EDITED BY
Manuel Enrique Figueroa,
Sevilla University, Spain

REVIEWED BY
Changcheng Liu,
North University of China, China
Muhammad Fahad Sardar,
Chinese Academy of Agricultural Sciences,
China
Xudong Peng,
Guizhou University, China
Grzegorz Żurek,
Plant Breeding and Acclimatization
Institute, Poland

*CORRESPONDENCE
Kangning Xiong
✉ xiongkn@gznu.edu.cn

RECEIVED 13 June 2023
ACCEPTED 20 November 2023
PUBLISHED 12 December 2023

CITATION
Song S, Xiong K and Chi Y (2023)
Response of grassland ecosystem
function to plant functional traits
under different vegetation restoration
models in areas of karst desertification.
Front. Plant Sci. 14:1239190.
doi: 10.3389/fpls.2023.1239190

Response of grassland ecosystem function to plant functional traits under different vegetation restoration models in areas of karst desertification

Shuzhen Song[1], Kangning Xiong[2]* and Yongkuan Chi[1,2]

[1]School of Karst Science, Guizhou Normal University, Guiyang, China, [2]State Engineering Technology Institute for Karst Desertification Control, Guizhou Normal University, Guiyang, China

Plant functional traits serve as a bridge between plants, the environment, and ecosystem function, playing an important role in predicting the changes in ecosystem function that occur during ecological restoration. However, the response of grassland ecosystem function to plant functional traits in the context of ecological restoration in areas of karst desertification remains unclear. Therefore, in this study, we selected five plant functional traits [namely, plant height (H), specific leaf area (SLA), leaf dry matter content (LDMC), root length (RL), and root dry matter content (RDMC)], measured these along with community-weighted mean (CWM) and functional trait diversity, and combined these measures with 10 indexes related to ecosystem function in order to investigate the differences in plant functional traits and ecosystem function, as well as the relationship between plant functional traits and ecosystem functions, under four ecological restoration models [*Dactylis glomerata* (DG), *Lolium perenne* (LP), *Lolium perenne* + *Trifolium repens* (LT), and natural grassland (NG)]. We found that: 1) the Margalef index and Shannon–Wiener index were significantly lower for plant species in DG and LP than for those in NG ($P<0.05$), while the Simpson index was significantly higher in the former than in NG ($P<0.05$); 2) CWM_H, CWM_{LDMC}, and CWM_{RDMC} were significantly higher in DG, LP, and LT than in NG, while CWM_{SLA} was significantly lower in the former than in NG ($P<0.05$). The functional richness index (FRic) was significantly higher in DG and LP than in NG and LT, but the functional dispersion index (FDis) and Rao's quadratic entropy index (RaoQ) were significantly lower in DG and LP than in NG and LT ($P<0.05$), and there was no significant difference between DG and LP, or between NG and LT ($P>0.05$); 3) ecosystem function, including ecosystem productivity, carbon storage, water conservation and soil conservation, was highest in LT and lowest in NG; and 4) CWM_{LDMC} (F=56.7, P=0.024), CWM_{RL} (F=28.7, P=0.024), and CWM_H (F=4.5, P=0.048) were the main factors affecting ecosystem function. The results showed that the mixed pasture of perennial ryegrass and white clover was most conductive to restoration of ecosystem function. This discovery has important implications for the establishment of vegetation, optimal utilization of resources, and the sustainable development of degraded karst ecosystems.

KEYWORDS

plant diversity, functional trait, ecosystem function, ecological restoration, karst desertification

Introduction

The findings of the Millennium Ecosystem Assessment report show that, globally, approximately 60% of the services provided by nature are currently being degraded or are in an unsustainable state. Furthermore, the report reveals that 78% of the benefits that humans derive from nature are rapidly declining (MEA, 2005). The sustainability of ecosystem services has become one of the most important issues for natural resource and environmental management in recent decades (Sharafatmandrad and Mashizi, 2020; Sardar et al., 2023). Biodiversity plays a crucial role in maintaining ecosystem services and human well-being, and the relationship between biodiversity and ecosystem functioning has become a hot topic in ecological research (Cardinale et al., 2012; Gamfeldt and Roger, 2017; Plas, 2019). Biodiversity is considered to be one of the fundamental indicators of ecosystem restoration (Liu M. et al., 2022). Restoring biodiversity and ecosystem function has become the primary objective in ecological restoration (Zirbel et al., 2017; Teng et al., 2023). Not only is an understanding of the relationship between biodiversity and ecosystem function helpful in better coping with the ecological consequences of biodiversity loss under a scenario of global climate change and increasing disturbance arising from human activities, but such an understanding also has practical implications for achieving sustainable management of ecosystems, ensuring the provision of ecosystem services, and realizing the goal of ecological restoration (Hector and Bagchi, 2007; Fry et al., 2018; Gonzalez et al., 2020; Miao et al., 2023). However, the outcomes of restoring biodiversity and ecosystem function are often unpredictable, and the question of how to link changes in community composition to ecosystem function is a major challenge facing current work in ecology and environmental management (Siwicka et al., 2020; Shabaan et al., 2022). Functional trait-based approaches provide a useful framework for studying plant resource acquisition, population survival strategies, and changes in ecosystem processes and functions during restoration (McCormack et al., 2017).

The term *functional traits* refers to morphological, physiological, and life history traits that indirectly influence plant fitness by affecting growth, reproduction, and survival at the individual level; these traits can be divided into response traits (traits related to the response of organisms to environmental factors, such as resources and disturbances) and effect traits (traits that determine the impact of organisms on ecosystem functions) (Violle et al., 2007). Functional traits are considered to be measurable characteristics that determine the interaction between plants and their environment (Diaz and Cabido, 2001), and have been used to study plant adaptive strategies and their effects on ecosystem function (Garnier et al., 2015). Plant functional traits, which serve as physiological and ecological indicators of plant uptake, use, and maintenance of resources, reflect plant adaptation to different environments and the physiological or evolutionary trade-offs between different functions within plants; these traits are bridges between plants, environments, and ecosystem functions, and play an important role in determining ecosystem functions (Liu et al., 2021). A growing body of research-based evidence shows that plant functional traits are closely related to ecosystem function and can, to some extent, indicate changes in ecosystem function (Faucon et al., 2015; Hobbie, 2015; Roumet et al., 2016). For example, traits related to plant structure and physiology, such as specific leaf area (SLA), stem dry matter content, and leaf nutrient concentration, determine the quality and quantity of litter produced, which in turn indirectly affects leaf carbon storage and decomposition rates (Kazakou et al., 2006). There are also related studies showing that leaf traits (e.g., SLA or carbon-to-nitrogen (C:N) ratio) and root traits related to nutrient access (e.g., diameter, nutrient content, branching intensity) may exert different effects on resource access, carbon storage, and pathogen communities in the soil (Albert et al., 2012; McCormack et al., 2015). However, since there are numerous plant species in nature and various types and intensities of climate change and disturbances arising from human activity, it has become crucial to determine how to utilize plant functional traits to accurately reflect and predict changes in plant communities and ecosystem functions in the process of ecological restoration (Lei et al., 2016).

Two hypotheses can explain the relationship between plant functional traits and ecosystem function (Cadotte, 2017). One is the "mass-ratio hypothesis", which states that the relative biomass of dominant species in the plant community and their specific traits are the dominating factors in the dynamic changes that occur in ecosystem processes (Grime, 1998); this is often characterized using the community-weighted mean (CWM) (Garnier et al., 2004; Vile et al., 2006; Díaz et al., 2007) for measures such as primary productivity (Finegan et al., 2015; Duffy et al., 2017) and soil carbon storage (Cong et al., 2014; Lange et al., 2015). The other is the "niche differentiation hypothesis", which suggests that trait differences between species maximize the diversity of resource-use strategies, enhance ecosystem processes by reducing niche overlap, and subsequently influence ecosystem functions, which are considered to be an important component of biodiversity (Petchey and Gaston, 2002; Díaz et al., 2007). Measures of the diversity of plant functional traits, including the functional richness index, functional evenness index, and functional divergence index, are typically used to assess this hypothesis (Tilman et al., 1997). For example, Qi et al. (2022) found that species diversity, functional diversity, and biodiversity in phylogenetic space were generally positively related to productivity in their examation of the relationship between biomass and species diversity, functional diversity, and phylogenetic diversity of each community in the

---

Abbreviations: DG, Dactylis glomerata grassland; LP, Lolium perenne grassland; LT, Lolium perenne + Trifolium repens grassland; NG, natural grassland; H, plant height; SLA, specific leaf area; LDMC, leaf dry matter content; RL, root length; RDMC, root dry matter content; CWM, community-weighted mean; CWM.H, community-weighted mean of plant height; CWM.LDMC, community-weighted mean of leaf dry matter content; CWM.SLA, community-weighted mean of specific leaf area; CWM.RL, community-weighted mean of root length; CWM.RDMC, community-weighted mean of root dry matter content; FRic, functional richness index; FDis, functional dispersion index; RaoQ, Rao's quadratic entropy index; AB, aboveground biomass; BB, belowground biomass; PC, plant C content; RC, root C content; SOC, soil organic carbon; WC, water conservation; SFC, soil field capacity; SP, soil porosity; TN, total nitrogen; TP, total phosphorus; TK, total potassium.

grasslands of the Qinghai-Tibet Plateau. Zhang et al. (2023) investigated the contribution of intraspecific variability to ecosystem function and found that a community with high interspecific variation in plant height and individuals with large leaf area could exhibit improved productivity through niche complementarity and dominance effects, respectively. Because functional diversity can better quantify the trait differences that define species interactions, is more sensitive to environmental stress or disturbance, and is more indicative of changes in ecosystem function, an increasing number of studies have used functional diversity measures to examine the relationship between plant functional traits and ecosystem function (Flynn et al., 2011; Chollet et al., 2014; Gross et al., 2017; Rosenfield and Müller, 2020). However, the mass ratio hypothesis and the niche differentiation hypothesis are not mutually exclusive; they jointly explain the construction of plant communities and ecosystem functions or processes, and have different relative importance in explaining ecosystem functions under different environmental conditions and vegetation types (Schleuter et al., 2010; Mouillot et al., 2013; Kraft et al., 2015; Jiang M. et al., 2022). It is therefore crucial to screen for plant functional traits that are associated with specific ecosystem processes, taking into account regional differences and ecosystem variation.

The karst ecosystem is a significant component of the terrestrial ecosystem, covering approximately 15% of the world's land area (Yuan, 1991). Among these regions, the South China Karst, with Guizhou as its center, is one of the three major areas of concentrated karst distribution in the world, and it is also the main area of karst ecosystem in China. Due to the fragility of the karst ecosystem itself, following long-term, unsustainable levels of development and use by humans, the ecosystem function is damaged and a rocky, desertified landscape appears (Xiong et al., 2002). The degradation and alteration of the ecological environment due to karst desertification result in decreased stability, weakened resistance to disturbances, and reduced biodiversity in the karst ecosystem. Consequently, the sustainable development of this region has attracted much attention from scholars (Xiong et al., 2012; Canedoli et al., 2021; Yang et al., 2021; Gu et al., 2022). In order to control karst desertification, a large number of ecological restoration projects have been carried out in the karst areas of southern China. Ecological restoration is the core task of karst desertification control, and its goal is to restore biodiversity and ecosystem functions (Benayas et al., 2009; Xiong et al., 2022). Realizing the sustainable development of the karst ecological environment is an important issue for current karst desertification control (He et al., 2019; Wang K. et al., 2020; Xiao and Xiong, 2022). The use of grassland is a pioneer strategy in terms of plant community ecosystems for ecological restoration, and "grain for green" and the establishment of artificial grassland are important components of the project to restore degraded ecosystems; these approaches play an irreplaceable role in the ecological restoration process (Chi et al., 2020). The results of ecological restoration and control over many years have shown that grasslands involved in rocky desertification control have a significant positive effect in terms of the restoration of degraded soil, biodiversity, and ecosystem function (Song et al., 2022). Therefore, a comprehensive and in-depth study on the response of grassland ecosystem function to plant functional traits under different restoration models in karst desertification control areas can provide a better understanding of the degradation and restoration processes of the karst ecosystem. Additionally, such work can provide a theoretical basis for the practice of ecological restoration.

So far, although scientists have carried out extensive research on the functional characteristics of karst plants in the context of ecological restoration, they have mainly focused on adaptive strategies. For example, Zhang S. et al. (2020) conducted a statistical analysis of the relationships among leaf functional traits, plant characteristics, and environmental factors in order to explore the ecological strategies and driving factors of dominant plants in different succession stages of the ecosystem under karst desertification. Liu L. et al. (2022) comprehensively investigated four forests (three natural secondary forests and one artificial forest) in a trough-valley karst watershed in northern Guizhou Province, southwest China, to examine the community-level adaptation strategies of karst forests. Zhou et al. (2022) studied the differences in species composition and functional characteristics between dolomite and limestone karst natural forests to clarify the adaptability of vegetation to desertified karst environments. However, there are fewer studies on the relationship between plant functional traits and ecosystem function, and research on the response of grassland ecosystem function to plant functional traits in the context of ecological restoration in areas of karst desertification is also at an exploratory stage. Therefore, there is an urgent need to explore the response mechanism of grassland ecosystem function to plant functional traits in the context of ecological restoration in areas of karst desertification. On this basis, the objectives of this study were: (1) to investigate the differences in plant functional traits and ecosystem functions between natural and artificially restored grassland ecosystems; and (2) to evaluate the relationship between plant functional traits and grassland ecosystem function. We hypothesized that CWM and functional trait diversity in plant functional traits in grassland ecosystems under different vegetation restoration models would be found to contribute equally to changes in grassland ecosystem function. To address this hypothesis, we selected five indicators of plant functional traits, namely plant height (H), specific leaf area (SLA), leaf dry matter content (LDMC), root length (RL), and root dry matter content (RDMC). We assessed CWM and functional trait diversity, and combined these measures with 10 ecosystem function-related indicators to comparatively analyze the response of grassland ecosystem function to plant functional traits under four ecological restoration models [*Dactylis glomerata* (DG) grassland, *Lolium perenne* (LP) grassland, *Lolium perenne* + *Trifolium repens* (LT) grassland, and natural grassland (NG)] employed in areas of karst desertification, with the aim of providing a scientific basis for vegetation construction, resource maximization, and sustainable development of degraded karst ecosystems.

Materials and methods

Study area

The study area is located in Salaxi Town and Yejiao Town, Qixingguan District, Bijie City, Guizhou Province, China (105°02′01′′–105°08′09′′E, 27°11′36′′–27°16′51′′N). The area of the region is 86.27 km^2, and the area of rocky desertification is 55.931 km^2, accounting for 64.93% of the total area. This area is a typical karst plateau mountain area with light-to-moderate rocky desertification. The study area has a subtropical humid monsoon climate, which is warm and humid in the summer and autumn, and cold and dry in the winter and spring. The average annual temperature is 12°C, the average annual rainfall is 984.40 mm, and rainfall is concentrated in the period from June to September. With a maximum elevation of 2,200 m and a minimum elevation of 1,495m, the terrain in the area varies greatly. The surface is fragmented and there are many peaks and depressions. The soil is mainly zonal yellow soil. The primary vegetation in the area has essentially been destroyed and the secondary vegetation now dominates; this includes *Cyclobalanopsis glauca*, *Pyracantha fortuneana*, *Pinus massoniana*, *Rhododendron simsii*, *Juglans regia*, *Rosa roxburghii*, *Artemisia lavandulaefolia*, *Chenopodium glaucum*, *Clinopodium chinense*, *Plantago asiatica*, *Stellaria media*, *Digitaria sanguinalis*, and *Polygonum hydropiper*. In order to restore the damaged karst ecosystem, based on the previous practice of the research group, artificial grassland was planted in the study area in 2012 by selecting artificial forage that would be suitable for the climatic environment of the region and would produce better ecological and economic benefits. The established forage consists mainly of *Lolium perenne*, *Trifolium repens*, *Dactylis glomerata*, etc. The variety of *Trifolium repens* is “Haifa”, the variety of *Dactylis glomerata* is “Qiangrass No. 4”, and the variety of *Lolium perenne* is “Yaqing”; the three kinds of forage seeds were provided by Guizhou Shennong Seed Industry Co., Ltd. (Guiyang, China) and Lvyi Seed Industry Co., Ltd. (Guiyang, China) (Song et al., 2022). The established artificial grassland has both single and mixed seeding. The seeding rates for *Trifolium repens*, *Dactylis glomerata*, and *Lolium perenne* have been found to be 2.0 g/m^2, 3.0 g/m^2, and 3.0g/m^2, respectively. A base fertiliser (N-P_2O_5-K_2O) was applied before planting at 0.225 g/m^2. In addition, naturally restored grassland was also present. Therefore, the overall grassland ecosystem formed in the process of karst desertification control includes the natural grassland ecosystem and the artificial grassland ecosystem.

Sample plot design, measurement of plant functional traits, and field sampling

Dactylis glomerata monoculture, *Lolium perenne* monoculture, and a mixture of *Dactylis glomerata* + *Trifolium repens* are common establishment practices in the study area, so three grassland types with similar site conditions were selected as test plots: a *Dactylis glomerata* grassland plot (DG), a *Lolium perenne* grassland plot (LP), and a *Dactylis glomerata* + *Trifolium repens* mixed seeding grassland plot (LT). A natural grassland plot (NG) was selected as a control (Table 1). DG, LP, and LT were mowed four times a year and fertilized with N-P_2O_5-K_2O at the time of the first noticeable rainfall after the first and the third mowings, at a rate of 0.1125 g/m^2. No agricultural activities or human disturbances were carried out at NG. Six sampling plots measuring 10m × 10m were set up for each experimental plot, resulting in a total of 24 sampling plots. Due to the fragmented nature of the surface of the study area, sampling plots were spaced more than 10 meters apart. Five 1m × 1m quadrats were set up in each sampling plot for acquisition of the vegetation community and biomass. The quadrat locations within the sampling plots were chosen randomly, but the locations of each quadrat were 2 m away from each other to avoid edge effects. Vegetation survey and sampling were conducted in mid-August 2021, at the peak of biomass and species diversity, and the names of the species occurring within each quadrat and basic information on them, such as height, coverage, and density, were recorded to assess the richness and diversity of each plant community. The aboveground parts of each species in each quadrat were mowed flush with the ground in their entirety to form a mixed sample, and after removal of any dead parts (litter) adhering to the soil, stones, and other impurities, this sample was oven-dried at 75°C for 48 hours to a constant weight, to be used for calculation of the aboveground biomass.

Based on the basic characteristics of the sample plots and the results of a previous study (Brown and Anand, 2022), we selected five traits that are closely related to plant life strategies and functional trade-offs: plant height (H, cm), specific leaf area (SLA, $cm^2.g^{-1}$), leaf dry matter content (LDMC, $g{\cdot}g^{-1}$), root length (RL, cm), and root dry matter content (RDMC, $g{\cdot}g^{-1}$). In each quadrat, 3–5 dominant species were randomly selected for measurement of plant height. Leaf samples from healthy and mature plants were taken, in four directions (east, south, west, and north) from each plant, using scissors; approximately 12 leaves were randomly selected for measurement of leaf area; five small sample plots of

TABLE 1 Geographical characteristics of sample plots in the study area.

Treatment	Longitude	Latitude	Altitude/m	Slope/°	Coverage/%	Dominant species
NG	105°6′6″E	27°14′49″N	1878	24	82	*Artemisia lavandulaefolia*
DG	105°6′5″E	27°14′26″N	1829	32	84	*Dactylis glomerata*
LP	105°6′6″E	27°14′35″N	1854	21	87	*Lolium perenne*
LT	105°5′59″E	27°14′49″N	1828	20	94	*Lolium perenne, Trifolium repens*

DG, *Dactylis glomerata* grassland ecosystem; LP, *Lolium perenne* grassland ecosystem; LT, *Lolium perenne+Trifolium repens* grassland ecosystem; NG, natural grassland ecosystem.

leaves within each sampling plot were completely and uniformly mixed to form a mixed sample, and young leaves, old leaves, and leaves affected by diseases and insect pests were removed from the sampling process. After removal of the litter layer from the soil surface, soil and root samples were taken from the surface layer (0–20cm) using a soil auger and a root auger, respectively, at the same location. Five soil and root samples from each quadrat were mixed to form a composite sample. If the soil depth was less than 20 cm, samples were taken from the full depth. In total, 24 samples each of leaves, soil, and roots were taken back to the laboratory. The root samples were taken out of the root auger and the root was thoroughly washed; 3–5 root systems were then randomly selected for measurement of the root length with a tape measure. The collected leaves and roots were placed in water and stored in a dark environment at 5°C for 12 h. After the leaves and roots were removed from the water, the water on the surface of the leaves and roots was immediately absorbed with absorbent paper, and the saturated fresh weight of the leaves and roots was obtained by weighing them on an electronic balance at 1/10,000. The dry weight of the fully mixed leaf and root samples was measured by drying at 75°C for 48 h to constant weight; this measurement was used to calculate the dry matter content of the leaves and roots (belowground biomass), respectively. The collected soil samples were divided into two parts: one part was used for the determination of soil field capacity and soil porosity, and the other was used for the determination of soil nutrients. The samples used for the determination of soil nutrients were naturally air-dried indoors (Li et al., 2020). Oven-dried plant samples (leaves and roots) and air-dried soil samples were sieved through 2 mm sieves for the determination of plant and soil nutrient properties, respectively.

Leaf area was measured using a leaf area meter (LI-COR, 3100C Area Meter, USA). SLA is the ratio of leaf area to leaf dry weight; LDMC is the ratio of leaf dry weight to leaf saturated fresh weight; and RDMC is the ratio of root dry weight to root saturated fresh weight. Soil field capacity and soil porosity were determined by the oven-drying method. The potassium dichromate–external heating method was used to determine the organic carbon content of plants and soil; the sulfuric acid catalyst digestion–Kjeldahl method was used to determine the total nitrogen (TN) content of soil; the concentrated sulfuric acid digestion–Mo-Sb colorimetric method was used to determine the total phosphorus (TP) content of soil; and the flame photometric method was used to determine the total potassium (TK) content of soil (Zhang, 2011). TN was determined using a continuous flow analyzer (FLOWSYS, SYSTEA, Italy). TP was determined with an ultraviolet-visible spectrophotometer (Specord 200 PLUS, Analytik, Germany). Finally, TK was determined using an atomic absorption spectrometer (ICE3500, Thermo Fisher, USA).

Ecosystem function

In accordance with the MEA description of ecosystem function (MEA, 2005) and the ecological restoration goal of control of karst desertification, we selected indicators of grassland ecosystem function such as ecosystem productivity, carbon storage, water conservation, and soil conservation for analysis in this study. Ecosystem productivity was expressed as aboveground biomass and belowground biomass (Wu et al., 2017). Carbon storage was expressed as aboveground plant carbon content, soil carbon content, and root carbon content (Wu et al., 2017). Water conservation was expressed as soil field capacity and soil porosity (Fan et al., 2019). Finally, soil fertility conservation was expressed as TN, TP, and TK (Wu et al., 2017).

Calculation of indices and data analysis

We used the Margalef index, the Shannon–Wiener index, the Pielou index, and the Simpson index to assess species richness and diversity in the grassland ecosystems. The Margalef index (R), Shannon–Wiener index (H), Pielou index (E), and Simpson index (C) were calculated for each ecosystem using the following formulae:

$$\mathrm{R} = (\mathrm{S} - 1)/\mathrm{In}N \quad \text{(Eq. 1)}$$

$$\mathrm{H} = -\sum_{i=1}^{s} P_i \ln P_i \quad \text{(Eq. 2)}$$

$$P_i = N_i/N \quad \text{(Eq. 3)}$$

$$\mathrm{E} = \mathrm{H}/\mathrm{lnS} \quad \text{(Eq. 4)}$$

$$\mathrm{C} = 1 - \sum_{i=1}^{S} \frac{N_i(N_i - 1)}{N(N - 1)} \quad \text{(Eq. 5),}$$

where S is the total number of species in the community, P_i is the frequency of species i in the quadrat, N_i is the number of individuals of species i in the quadrat, and N is the total number of individuals of all species in the quadrat.

Plant functional traits in the grassland ecosystems were analyzed on the basis of CWM and functional trait diversity. The CWM for each trait in each sample was calculated based on the trait value for the species and the relative abundance of the species or aboveground biomass as weights (Garnier et al., 2004). The calculation formula was as follows:

$$\mathrm{CWM} = \sum_{\mathrm{i}=1}^{s} P_i \times X_i, \quad \text{(Eq. 6)}$$

where S is the total number of species in the community, P_i is the aboveground biomass or relative abundance of species i in the quadrat, and X_i is the trait value of the species i in the quadrat.

The functional richness index (FRic) indicates the degree of species use of the ecological space; the functional dispersion index (FDis) indicates the degree of spatial dispersion of plant traits; and Rao's quadratic entropy index (RaoQ) integrates information on species richness and differences in functional characteristics between species (Casanoves et al., 2011). These three indices were chosen to characterize plant functional diversity in this study. They were all calculated in R using the FD package (Mammola et al., 2021).

In order to better compare grassland ecosystem functions and assess the impact of different restoration models, we use the comprehensive index of ecosystem function to evaluate this, following the research method of Kearney et al. (2019). First, the data for each ecosystem function index were standardized to fall within a range between 0.1 and 1.

$$\text{Positive indexes}: X'_i = 0.1 + \left(\frac{X_i - \min(X_i)}{\max(X_i) - \min(X_i)}\right) * 0.9 \quad \text{(Eq. 7)}$$

$$\text{Negative indexes}: X'_i = 1.1 - 0.1 + \left(\frac{X_i - \min(X_i)}{\max(X_i) - \min(X_i)}\right) * 0.9, \quad \text{(Eq. 8)}$$

where X'_i is the change value of evaluation index i, X_i is the original observation value of evaluation index i, and $\max(X_i)$ and $\min(X_i)$ are the maximum observation value and minimum observation value of evaluation index i, respectively.

The comprehensive index of grassland ecosystem function is calculated as the weighted sum of all transformation variables in each group. The weights are determined based on the relative contribution of each variable to the variance within the ecosystem function group using principal component analysis (PCA). The formula used for this calculation was as follows:

$$CI = \sum_i^n (X'_i Y_{i,PC1} + X'_i Y_{i,PC2}), \quad \text{(Eq. 9)}$$

where CI is the comprehensive index of ecosystem function, X'_i is the value converted from Equations 7 or 8 for each evaluation index i (containing n variables), and Y_i represents the factor scores on the first and second principal component axes. Finally, each composite index of ecosystem function was again adjusted to a range of 0.1–1 using Equation 7.

One-way analysis of variance (ANOVA) and Tukey pairwise comparisons were performed using IBM SPSS Statistics (version 19.0 for Windows; SPSS, Chicago, IL, USA); these tests were used to analyze the effects of different grassland restoration models on species diversity, CWM, functional trait diversity, and ecosystem function. Tukey pairwise comparisons were considered statistically significant at $P<0.05$. Pearson correlation analysis was conducted to assess the relationships among species diversity, CWM, and functional trait diversity using Origin 2021(version 2021 for Windows; OriginLab, MAS, Hampton, USA). Redundancy analysis (RDA) based on forward selection was carried out to evaluate the effects of species diversity and plant functional traits on ecosystem function using Canoco (version 5.0 for Windows; Ithaca, NY, USA), and the Monte Carlo permutation test was performed to select explanatory factors that had significant effects on changes in ecosystem function ($P<0.05$).

Results

Characteristics of species diversity and plant functional traits in grassland ecosystems under different restoration models

We conducted ANOVA and Tukey pairwise comparisons to analyze species diversity, CWM, and functional trait diversity in grassland ecosystems under different restoration models. In terms of species diversity (Table 2), the Margalef index and the Shannon–Wiener index were significantly lower in DG and LP than in NG ($P<0.05$), while the Simpson index was significantly higher in the former two ecosystems than in NG ($P<0.05$), but there was no significant difference between DG and LP ($P>0.05$). In addition, there were no significant differences ($P>0.05$) between NG and LT in terms of the Margalef index, Shannon–Wiener index, Pielou index, or Simpson index. Furthermore, the differences in Pielou index between treatments were also not significant ($P>0.05$).

The CWM of grassland ecosystems under different restoration models varied significantly (Figure 1). CWM_H ranged from 28.02 to 101.30 cm. The distribution of CWM_H was more concentrated in LT, while the variance of CWM_H was greatest in DG. Compared to NG, CWM_H was significantly higher in DG, LP, and LT ($P<0.05$). The median variation in CWM_{LDMC} ranged from 21.49 to 66.28 $g \cdot g^{-1}$. CWM_{LDMC} was highest in LT and lowest in NG. CWM_{SLA} varied from 0.61 to 1.85 $cm^2 \cdot g^{-1}$. The distribution of CWM_{SLA} was more concentrated in LT, while the variance of CWM_{SLA} was greatest in NG. CWM_{SLA} was significantly higher in NG than in other grassland ecosystems ($P<0.05$), but there were no significant differences among DG, LP, and LT ($P>0.05$). The median variation in CWM_{RL} ranged from 10.37 to 12.71 cm. CWM_{RL} was highest in LP ($P<0.05$), but there was no significant difference in CWM_{RL} between NG and DG, or between DG and LT ($P>0.05$).

TABLE 2 Species diversity of grassland ecosystems under different restoration models.

Treatment	Margalef index	Shannon–Wiener index	Pielou index	Simpson index
NG	1.08 ± 0.17a	1.10 ± 0.19a	0.63 ± 0.12a	0.47 ± 0.12b
DG	0.68 ± 0.21b	0.71 ± 0.20b	0.51 ± 0.07a	0.64 ± 0.10a
LP	0.55 ± 0.12bc	0.67 ± 0.12bc	0.54 ± 0.07a	0.64 ± 0.05a
LT	1.19 ± 0.34a	1.16 ± 0.10a	0.63 ± 0.07a	0.46 ± 0.04bc
F value	11.143**	15.11**	3.075	9.451**

Data are presented in the form mean ± standard error. Different letters in the same column indicate significant differences ($p<0.05$) between treatments based on Tukey pairwise comparisons. ** indicates significance at the 0.01 probability level. The *F* value is the F test statistic.
DG, *Dactylis glomerata* grassland ecosystem; LP, *Lolium perenne* grassland ecosystem; LT, *Lolium perenne* + *Trifolium repens* grassland ecosystem; NG, natural grassland ecosystem.

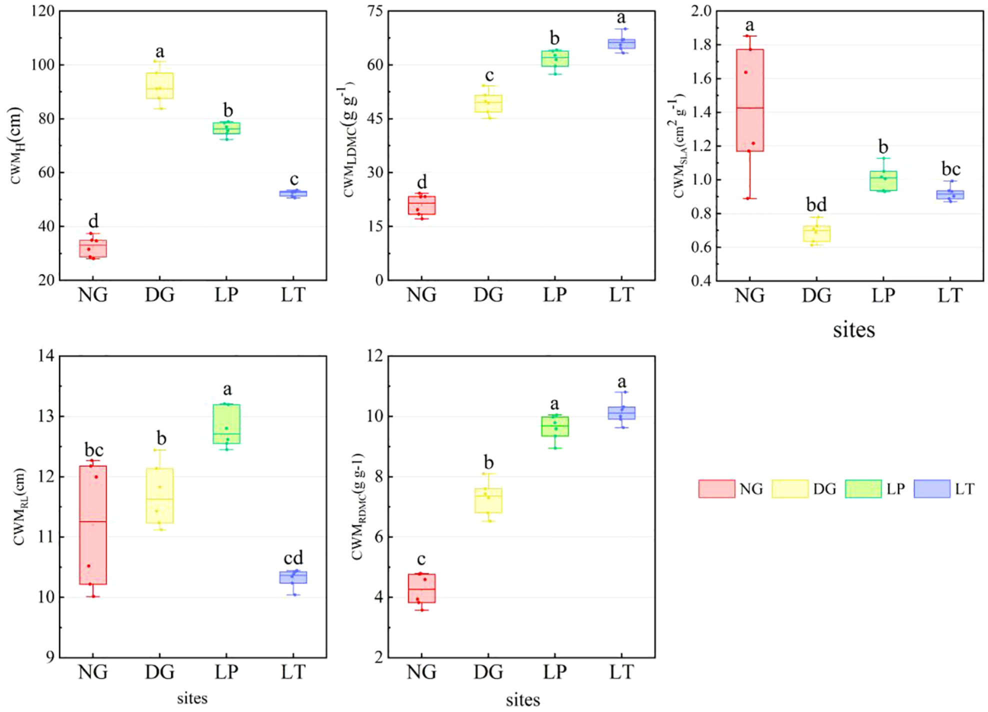


FIGURE 1

CWM of grassland ecosystems under different restoration models. Different letters in the same panel indicate significant differences between treatments based on Tukey pairwise comparisons ($p<0.05$). CWM$_H$, CWM$_{LDMC}$, CWM$_{SLA}$, CWM$_{RL}$, and CWM$_{RDMC}$ represent the CWM for plant height, leaf dry matter content, specific leaf area, root length, and root dry matter content, respectively.DG, *Dactylis glomerata* grassland ecosystem; LP, *Lolium perenne* grassland ecosystem; LT, *Lolium perenne + Trifolium repens* grassland ecosystem; NG, natural grassland ecosystem.

CWM$_{RDMC}$ ranged from 3.58 to 10.80 g·g-1. CWM$_{RDMC}$ was significantly higher in DG, LP, and LT than in NG ($P<0.05$), but there was no significant difference between LT and LP ($P>0.05$).

It was not difficult to observe that functional trait diversity in grassland ecosystems varied greatly among different restoration models (Figure 2). FRic was significantly higher in DG and LP than in NG and LT ($P<0.05$), but there was no significant difference between DG and LP, and there was also no significant difference between NG and LT ($P>0.05$). FDis and RaoQ were significantly lower in DG and LP than in NG ($P<0.05$), but there was no significant difference between DG and LP ($P>0.05$), and there was also no significant difference between NG and LT ($P>0.05$).

We performed correlation analyses of species diversity, CWM, and functional trait diversity in grassland ecosystems under different restoration models (Figure 3). Margalef index was positively correlated with Shannon–Wiener index, FDis, and RaoQ, but negatively correlated with Simpson index and CWM$_{RL}$ ($P<0.05$). Shannon–Wiener index was positively correlated with Pielou index, FDis, and RaoQ ($P<0.05$). Shannon–Wiener index and Pielou index were negatively correlated with Simpson index and CWM$_{RL}$ ($P<0.05$). Simpson index was positively correlated with CWM$_{RL}$ ($P<0.05$), but negatively correlated with FDis ($P<0.05$). CWM$_H$ was positively correlated with CWM$_{LDMC}$, CWM$_{RL}$, CWM$_{RDMC}$, and FRic, but negatively correlated with CWM$_{SLA}$, FDis, and RaoQ ($P<0.05$). CWM$_{LDMC}$ was positively correlated with CWM$_{RDMC}$, but negatively correlated with CWM$_{SLA}$ and FDis ($P<0.05$). CWM$_{SLA}$ was positively correlated with FDis but negatively correlated with CWM$_{RDMC}$ and FRic ($P<0.05$). CWM$_{RL}$ was positively correlated with FRic but negatively correlated with FDis and RaoQ ($P<0.05$). FRic was negatively correlated with FDis and RaoQ ($P<0.05$). Finally, FDis was positively correlated with RaoQ ($P<0.05$).

Characteristics of grassland ecosystem function under different restoration models

We used ANOVA and Tukey pairwise comparisons to analyze indices of grassland ecosystem function under different restoration models (Table 3). Aboveground biomass was greatest in LT, but there was no significant difference between DG and LP ($P>0.05$), and there was also no significant difference between DG and NG ($P>0.05$). Compared to NG, the aboveground biomass in DG, LP, and LT was greater by approximately 20%, 30%, and 44%, respectively. Belowground biomass was greatest in LT and lowest in NG, but there was no significant difference between DG and LP ($P>0.05$). Compared to NG, the belowground biomass in DG, LP, and LT was greater by approximately 32%, 35%, and 56%, respectively. Plant carbon content was significantly higher in DG, LP, and LT than in NG ($P<0.05$), but there were no significant differences among DG, LP, and LT ($P>0.05$). Carbon content in the roots was lowest in NG, but there was no significant difference between DG and LP, and there was also no significant difference between DG and LT ($P>0.05$). Soil organic carbon, soil field

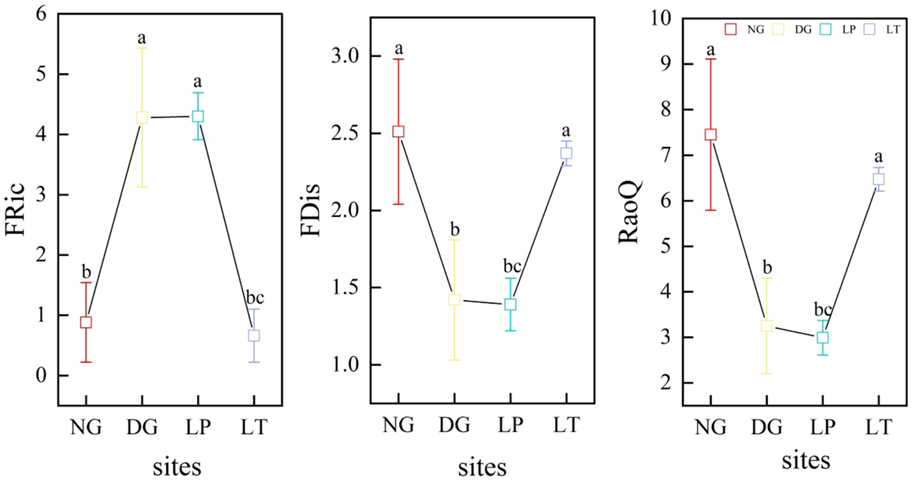


FIGURE 2
Functional trait diversity of grassland ecosystems under different restoration models. Different letters in the same panel indicate significant differences between treatments based on Tukey pairwise comparisons ($p<0.05$). FRic, FDis, and RaoQ represent the functional richness index, the functional dispersion index, and Rao's quadratic entropy index, respectively. DG, *Dactylis glomerata* grassland ecosystem; LP, *Lolium perenne* grassland ecosystem; LT, *Lolium perenne + Trifolium repens* grassland ecosystem; NG, natural grassland ecosystem.

capacity, soil porosity, TN, TP, and TK were significantly higher in LT than under the other treatments ($P<0.05$). These indices were lowest in NG, but there was no significant difference between DG and LP ($P>0.05$), except in the case of TP.

The results of PCA showed that the first and second principal components together explained 84.11% of the variance in each ecosystem function group for each evaluation indicator (Figure 4). Based on the explanatory power of the principal component and using this as a weight to calculate the comprehensive index of ecosystem function via Equation 9, we obtained values for the comprehensive index of ecosystem function for grassland ecosystems under different restoration models (Table 4). The

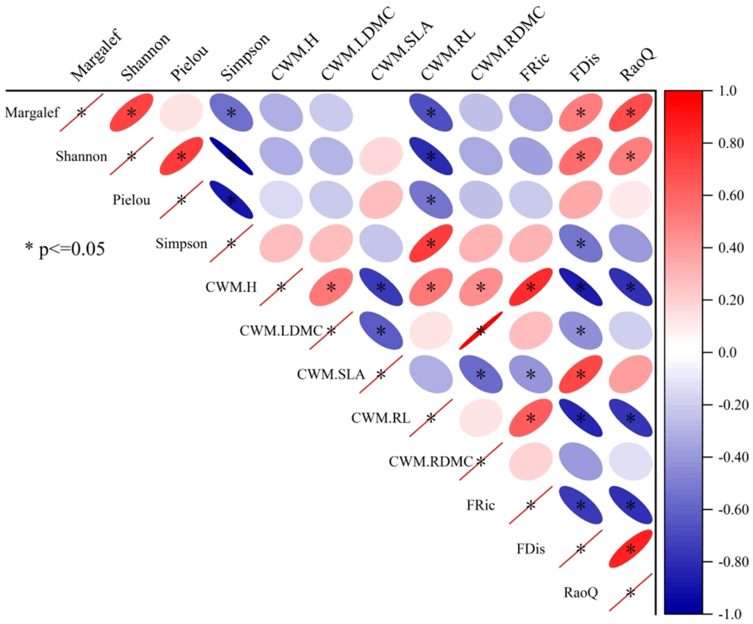


FIGURE 3
Correlation analyses of species diversity, CWM, and functional trait diversity in grassland ecosystems under different restoration models. * indicates significance at the 0.05 probability level. The labels Margalef, Shannon, Pielou, and Simpson represent the Margalef index, the Shannon–Wiener index, the Pielou index, and the Simpson index, respectively. CWM.H, CWM.LDMC, CWM.SLA, CWM.RL, and CWM.RDMC represent the CWM of plant height, leaf dry matter content, specific leaf area, root length, and root dry matter content, respectively. FRic, FDis, and RaoQ represent the functional richness index, the functional dispersion index, and Rao's quadratic entropy index, respectively.

TABLE 3 Analysis results for each evaluation index for grassland ecosystem functions under different restoration models.

EF group	NG	DG	LP	LT	*F*
Ecosystem productivity					
Aboveground biomass (dry weight, kg·m^2)	0.28 ± 0.03dc	0.35 ± 0.03bc	0.40 ± 0.05b	0.50 ± 0.04a	28.01***
Belowground biomass (dry weight, kg·m^2)	0.15 ± 0.01d	0.22 ± 0.01bc	0.23 ± 0.01b	0.34 ± 0.02a	237.51***
Carbon storage					
Plant C content (g·kg^{-1})	406.61 ± 9.37b	461.11 ± 8.64a	456.15 ± 24.69a	481.14 ± 33.26a	12.81***
Root C content (g·kg^{-1})	321.43 ± 11.08c	368.63 ± 17.93ab	350.44 ± 19.45b	380.91 ± 11.65a	16.73***
Soil organic carbon (g·kg^{-1})	12.51 ± 2.19d	17.62 ± 2.37bc	18.52 ± 3.11b	25.32 ± 3.11a	32.31***
Water conservation					
Soil field capacity (%)	27.97 ± 5.02d	36.31 ± 2.82bc	36.55 ± 1.66b	43.09 ± 2.29a	22.33***
Soil porosity (%)	41.78 ± 1.32d	47.88 ± 2.42bc	48.59 ± 0.67b	53.92 ± 3.30a	31.32***
Soil fertility conservation					
Total nitrogen (g·kg^{-1})	0.79 ± 0.05d	1.53 ± 0.24bc	1.66 ± 0.46b	2.14 ± 0.28a	21.63***
Total phosphorus (g·kg^{-1})	0.81 ± 0.05cd	0.95 ± 0.03c	1.11 ± 0.13b	1.38 ± 0.11a	44.60***
Total potassium (g·kg^{-1})	15.06 ± 0.91d	16.82 ± 0.39bc	17.23 ± 0.71b	22.21 ± 1.09a	84.73***

Data are presented in the form mean ± standard error. Different letters in the same column indicate significant differences ($p<0.05$) between treatments based on Tukey pairwise comparisons. *** indicates significance at the 0.001 probability level. The *F* value is the F test statistic.
DG, *Dactylis glomerata* grassland ecosystem; LP, *Lolium perenne* grassland ecosystem; LT, *Lolium perenne* + *Trifolium repens* grassland ecosystem; NG, natural grassland ecosystem.

same trends in terms of differences between restoration models were observed across multiple components of the comprehensive index of grassland ecosystem function: that is, ecosystem productivity, carbon storage, water conservation, and soil fertility were highest in LT and lowest in NG, and there was no significant difference between DG and LP ($P>0.05$).

Relationship between plant functional traits and grassland ecosystem function under different restoration models

Redundancy analysis of plant functional traits and ecosystem function showed that the first and second axes explained 90.68% and 2.74% of the variance in ecosystem function, respectively (Figure 5). CWM_{LDMC}, CWM_{RDMC}, CWM_H, the Margalef index, and the Shannon-Wiener index were positively correlated with ecosystem productivity, carbon storage, water conservation, and soil fertility conservation. CWM_{SLA} and CWM_{RL} were negatively correlated with ecosystem productivity, carbon storage, water conservation, and soil fertility conservation. FRic, FDis, and RaoQ were not correlated with ecosystem productivity, carbon storage, water conservation, or soil fertility conservation. Further analysis via the Monte Carlo permutation test showed that CWM_{LDMC}, CWM_{RL}, and CWM_H had larger effects on ecosystem function than other plant functional traits, explaining 76.20% (F=56.7, P=0.024), 17.7% (F=28.7, P=0.024), and 2.2% (F=4.5, P=0.048) of the variance, respectively, indicating that CWM_{LDMC}, CWM_{RL}, and CWM_H were the main factors affecting ecosystem function.

Discussion

Effects of different restoration models on species diversity and plant functional traits in grassland ecosystems

Species diversity represents the expression of biodiversity at the species level. As a fundamental characteristic of community functional structure, this measure plays a crucial role in indicating community composition, the dynamics of community change, and strategies for vegetation restoration (Crawley and Harral, 2001). Under various restoration models, variation has been observed in species composition and structure among plant communities, resulting in significant disparities in species diversity (Wang et al., 2022a). In this study, plant species diversity under the three artificial restoration models varied greatly. The Margalef index and Shannon index were highest in LT, and the species diversity of the LT ecosystem was closest to that of NG, which is consistent with the findings of Wu et al. (2019) in the degraded grassland of the Three Rivers source area and those of Zhang H. et al. (2020) in the degraded grassland of the Yellow River headwaters. This indicates that, for degraded ecosystems, planting artificial grassland by mixed sowing can promote the vegetation succession of plant communities and restore species diversity in the degraded ecosystem, which is an important way to restore degraded ecosystems. However, there was no significant difference in Margalef index, Shannon–Wiener index, or Pielou index between the DG and LP ecosystems; this finding was similar to the results of Hou et al. (2015) in the *Poa pratensis* grassland of Qinghai, but

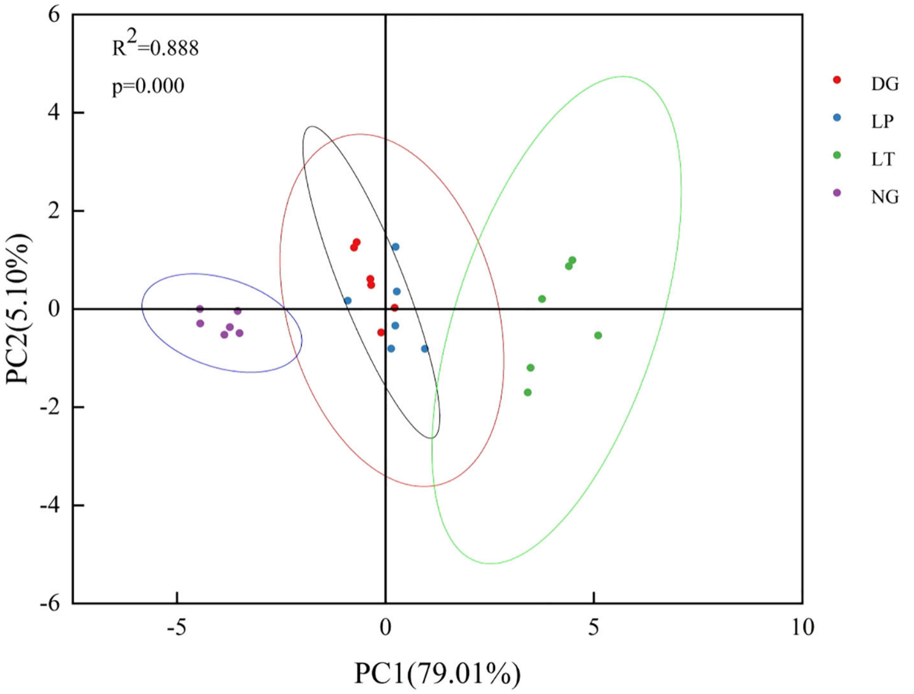


FIGURE 4
Principal component analysis of each indicator for evaluation of grassland ecosystem function under different restoration models.

different from the results of Hao et al. (2023) in the sandy grassland of Hulunbeir. This phenomenon could potentially be attributed to the similarity in dominant species between DG and LP, as well as the nature of the study conducted by Hou et al. (2015), in which both areas featured perennial grasses. Perennial grasses share commonalities in terms of morphology, physiology, and life history, which may explain the observed similarities.

Plant functional traits determine plant survival, growth, and reproduction; not only can these characteristics strongly influence ecosystem processes, but they also reflect the response process of ecosystems to environmental changes (Wright et al., 2004; Kraft et al., 2015; Li et al., 2021). The adaptability of plants to the environment and their own plasticity leads to major differences in functional traits among plants, with species themselves often acting as indicators and predictors of environmental change through their adoption of morphological and physiological traits to adapt to environmental changes and access to limited resources (Freschet et al., 2018; Hu L. et al., 2021). We found that the five CWM measures responded very differently to different restoration models during the ecosystem restoration process for management of karst desertification. CWM_H, CWM_{LDMC}, and CWM_{RDMC} were significantly higher in artificial grassland ecosystems than in the natural grassland ecosystem, which can be explained by the fact that artificial planting promotes forage with rapid growth strategies as dominant species, and that local dominance of relatively tall plants can lead to higher plant biomass and higher resource storage capacity (Conti and Díaz, 2013; Zuo et al., 2017). However, the CWM_{SLA} of plants in artificial grassland ecosystems was lower, which may be due to the fact that the karst ecosystem in southwest China has specific habitat conditions consisting of karst aridity and high soil calcium, and the plants have acquired resilience to this by altering their own structure and their physiological and biochemical processes to adapt to the environment in the course of survival (Xiong et al., 2002; Xi et al., 2011; Zhong et al., 2018). While SLA is related to stress tolerance and growth rate, species with lower SLA can compete for limited nutrients and have higher stress tolerance

TABLE 4 Comprehensive index of grassland ecosystem function under different restoration models.

Comprehensive index of EF	NG	DG	LP	LT	*F*
Ecosystem productivity	0.21 ± 0.05d	0.46 ± 0.05bc	0.55 ± 0.07b	0.94 ± 0.06a	152.947***
Carbon storage	0.25 ± 0.09d	0.64 ± 0.04b	0.58 ± 0.08bc	0.87 ± 0.09a	63.356***
Water conservation	0.18 ± 0.09d	0.53 ± 0.13bc	0.56 ± 0.04b	0.86 ± 0.01a	48.161***
Soil fertility conservation	0.16 ± 0.04d	0.42 ± 0.06bc	0.53 ± 0.12b	0.90 ± 0.07a	102.52***

Data are presented in the form mean ± standard error. Different letters in the same column indicate significant differences ($p<0.05$) between treatments based on Tukey pairwise comparisons. The *F* value is the F test statistic. *** indicates significance at the 0.001 probability level.
DG, *Dactylis glomerata* grassland ecosystem; LP, *Lolium perenne* grassland ecosystem; LT, *Lolium perenne* + *Trifolium repens* grassland ecosystem; NG, natural grassland ecosystem.

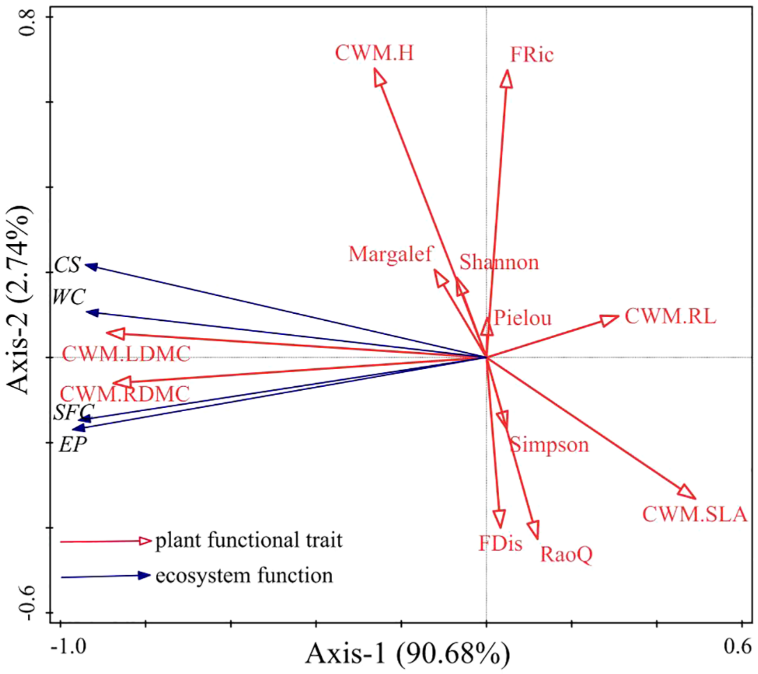


FIGURE 5
Redundancy analysis of plant functional traits and grassland ecosystem function. The labels Margalef, Shannon, Pielou, and Simpson represent the Margalef index, the Shannon–Wiener index, the Pielou index, and the Simpson index, respectively. CWM.H, CWM.LDMC, CWM.SLA, CWM.RL, and CWM.RDMC represent the CWM of plant height, leaf dry matter content, specific leaf area, root length, and root dry matter content, respectively. FRic, FDis, and RaoQ represent the functional richness index, the functional dispersion index, and Rao's quadratic entropy index, respectively. EP, CS, WC, and SFC represent ecosystem productivity, carbon storage, water conservation, and soil fertility conservation, respectively.

(Wright et al., 2004), which also proves that artificially planted grass is better adapted to the particular nature of the karst environment. This result was similar to those of Mason et al. (2016) in grazed grassland in New Zealand. Plants obtain nutrients and water from the soil, mainly through the root system, to support their growth and development, and the root system is the area where the most intense plant–soil interactions take place, largely controlling nutrient activation, water use efficiency, and soil health (Kong et al., 2014; Plaza-Bonilla et al., 2014). In this study, CWM_{RL} was significantly higher in DG and LP than in LT, which is consistent with the results of Li et al. (2022) in grassland in Inner Mongolia. This may be attributable to the fact that gramineous grasses have well-developed fibrous roots with a strong ability to compete for water, and water competition intensifies the growth of gramineous grasses roots in monoculture (Bessler et al., 2012).

The concept of functional trait diversity is intricately linked to the functions of species and ecosystems, as it involves the complementary utilization of resources (Mason et al., 2005). This approach provides a clear and intuitive means of characterizing the magnitude, distribution range, and degree of niche differentiation of functional traits within an ecosystem community (Villéger et al., 2008). The FRic reflects the utilization rate of resources by vegetation by quantifying the niche space occupied by existing species in the community (Jäschke et al., 2020). This study showed that FRic was significantly higher in DG and LP than in NG and LT, indicating that the dominant species of perennial gramineous grasses in the community occupied a larger niche space, the niche differentiation of each species was higher, and resource utilization was more efficient (Petchey and Gaston, 2006; Garcia-Cervigon et al., 2021). This result is inconsistent with the findings of Perring et al. (2018) in European temperate grassland and Freitag et al. (2021) in the Eurasian steppe, which may be caused by the particular nature of the habitat environment of karst (Huang et al., 2022). FDis and RaoQ can be used to measure the degree of niche differentiation and resource competition among plants within a community (Carmona et al., 2016), with higher index values indicating stronger ecological niche complementarity among species, weaker competition, and more efficient resource use (Dong et al., 2019). In this study, FDis and RaoQ were significantly higher in NG and LT than in DG and LP, which is consistent with the results of a related study by Huang et al. (2020) in Chongqing City in China. This may be due to the fact that natural and mixed-seeding grassland ecosystems have a great variety and number of species, producing differences in niches between species, and leading to less niche overlap in resource utilization by individuals in natural and mixed-seeding grassland ecosystems compared to single-seeding grassland ecosystems (Yang et al., 2022).

In this study, the Margalef index and Shannon index were higher in NG and LT than in DG and LP, but FRic did not show the same trend. This may be due to the fact that, in degraded karst ecosystems where species are under environmental stress, species trait show convergence in their functional traits, species trait composition is limited to traits adapted to the selective pressures of that environment, and continued increases in species richness only lead to further spatial differentiation of ecological niches, resulting in reduced interspecific trait variability and no further increase in functional diversity (Sasaki et al., 2009; Khasanova et al., 2019), as well as weakened competition between species. These findings are consistent with the results of Dong et al. (2019) on the adaptive strategies of plants in grassland ecosystems of the Tibetan Plateau and with those of Zhang Z. et al. (2021) in nine forest ecosystems from the tropical to boreal zones in China. The results of this study confirmed the following findings: 1) FDis and RaoQ were significantly higher in NG and LT compared to DG and LP; 2) the Margalef index and Shannon index were significantly positively correlated with FDis and RaoQ, but not significantly correlated with FRic; 3) CWM_H and CWM_{RL} were significantly negatively correlated with FDis and RaoQ. In addition, the relationship between the Margalef index and Shannon index, as well as functional trait diversity, varied across different types of plant communities. This was confirmed by Weng et al. (2017) through their study of forest communities, suggesting that the relationship between species diversity and functional diversity has originally varied across environmental contexts, even when these are unaffected by external disturbance.

In this study, CWM_H was significantly and positively correlated with CWM_{LDMC}, CWM_{RL}, CWM_{RDMC}, and FRic. This may be due to the fact that plant height affects the ability of plants to acquire light and to photosynthesize; specifically, the greater a plant's height, the greater its ability to so, which contributes to an increase in leaf dry matter content, root length, and root dry matter content. In turn, these also contribute to an increase in the diversity of plant functional traits (Angassa, 2014). These results are similar to those obtained by Maire et al. (2015) in their study of global terrestrial ecosystems. SLA is an important indicator for measurement of the growth status and light energy utilization efficiency of species, and LDMC mainly reflects the ability to retain plant nutrient elements (Hao et al., 2019). Related studies have shown that SLA can reflect the ability of plants to obtain resources, and SLA is usually negatively correlated with LDMC (Shen et al., 2019), which may explain the findings of a significant negative correlation between CWM_{LDMC} and CWM_{SLA} in this study. This is consistent with the results of Niu et al. (2016) in Tibetan alpine meadows, those of Zhang et al. (2018) in typical grassland of Horqin sandy land, China, and those of Wang Q. et al. (2022) in degraded woody plants of a karst area. This finding also indicates that the artificial grass in our study area was better adapted to the karst environment, and thus their LDMC was higher. Furthermore, FRic was negatively correlated with FDis and RaoQ, but FDis was positively correlated with RaoQ in our study. This is inconsistent with the results of Jiang X. et al. (2022) in the Mu Us sandy grassland of China and with those of Wang et al. (2022b) in the Maolan National Karst Forest Nature Reserve, Guizhou, China; however, it is consistent with the results of Petchey and Gaston (2002) on five typical cases of simulated plant functional traits and ecosystem relationships, and with those of Hao et al. (2019) on the functional traits of communities at different stages of succession in the temperate forests of the Changbai Mountains, Northeast China. The reason for this difference may be inconsistencies in species diversity.

Effects of different restoration models on grassland ecosystem function

The aim of ecological restoration is not only to increase vegetation coverage but also to restore ecosystem quality and function (Zhang M. et al., 2018; Zhang S. et al., 2023). Artificial grassland is an effective way to mitigate the degradation of natural grassland, improve grassland productivity, and ensure ecological security (He et al., 2020; Xu et al., 2020). In this study, DG, LP, and LT were found to have significantly increased aboveground biomass and belowground biomass in the grassland ecosystem compared to NG, with the highest levels being observed in LT. These results are consistent with those of Fox et al. (2020) in mixed seeding grassland in Zurich and those of Li C. et al. (2023) in artificial grassland in Qinghai Lake Basin; this may be because artificial pasture planting has some advantages in increasing forage yield compared to natural grassland, but the advantages of mixed seeding are greater than those of monoculture (Husse et al., 2016). The same trend was also observed in plant, root, and soil total carbon, which indicates that the process of accumulation of soil nutrients was slow during the natural process of recovery of the karst ecosystem, while the mixed-seeding grassland could significantly increase soil organic carbon; the effect of mixed leguminous forage has been found to be especially clear (Bai and Cotrufo, 2022). Furthermore, there is a positive correlation between plant and root carbon and soil carbon, with increases in soil carbon promoting higher plant and root carbon (Su et al., 2019). The results of Peng et al. (2020), in a comparative analysis of alpine meadow and alpine steppe, have also confirmed this viewpoint; this may be due to the fact that the soil environment of artificial grassland tends to be stable, with an increase in surface litter and in the underground root system, which is capable of sustainably recharging litter into the soil, increasing the source of soil organic carbon and promoting organic carbon accumulation (Li X. et al., 2023). Soil water is an important source of plant water and a carrier for nutrient cycling and material transformation (Yang et al., 2016). Soil porosity is an important indicator for characterization of soil aeration and water permeability, and soil with high porosity is more likely to expel water (Zhang et al., 2019). Not only can soil organic carbon enhance the soil's ability to hold and release fertilizer, but it can also promote the formation of granular structure and improve soil's water permeability, water-holding capacity, and aeration (Zhang J. et al., 2021). This can explain the findings of this study in which soil field capacity and soil porosity were the highest in LT and the lowest in NG, which is consistent with the results of Yang et al. (2017) study of ecological restoration projects affecting hydrological function in the karst region of southwest China.

Soil plays a crucial role in the formation of grassland ecosystem function and the provision of ecosystem services, as it carries out nutrient cycling (Eekeren et al., 2010; Bell et al., 2012). Relevant studies have shown that restoration of vegetation can effectively improve soil fertility within a relatively short period of time (15 years) (Hu et al., 2020). Soil nitrogen supply plays an important role in determining ecosystem structure and function (Hu P. et al., 2021), and the availability of soil nitrogen is often a key limiting factor for productivity (Wang D. et al., 2020). In this study, DG, LP, and LT significantly increased soil total nitrogen content compared to NG, and this was at its highest in LT. This may be due to the relative stability of the mixed-seeding grassland, which enhances photosynthesis, root activity, and soil microbial activity, and promotes nitrogen accumulation capacity; additionally, the biological nitrogen fixation of legume forage in LT also increased nitrogen content, meaning that the presence of mixed-seeding grassland was more conducive to the accumulation of soil total nitrogen (Nyfeler et al., 2011; Frankow-Lindberg and Dahlin, 2013). This is consistent with results on the planting years of different grasslands obtained in the Sanjiangyuan region of China by Xing et al. (2020), but inconsistent with the results of Hu et al. (2021), who studied the effects of vegetation restoration methods on soil N supply in artificial and natural forests in the karst region of southwest China; this might be related to the different vegetation types studied (Song et al., 2022). Soil phosphorus and potassium are the main nutrients affecting plant growth, and higher levels of phosphorus and potassium imply good soil fertility status and higher system productivity (Cheng et al., 2016; Wang et al., 2019; Zhang Y. et al., 2021). In this study, DG, LP, and LT significantly increased the total phosphorus content compared to NG, and this was at its highest in LT. This may be because the biomass, soil organic carbon, and total nitrogen content were significantly higher in artificial grassland than in natural grassland, providing a favorable soil environment for the accumulation of phosphorus and potassium; because mixed-seeding grassland creates inter-root space for the use of phosphorus and potassium nutrients; and because the advantage of the difference in the utilization of phosphorus and potassium sources contributes to a more pronounced increase in soil phosphorus and potassium content (Wagg et al., 2014; Crème et al., 2016). Although the species diversity in the natural grassland ecosystem was higher than that observed in the single-seeding grassland ecosystem in this study, N is a limiting factor affecting the productivity of natural grassland at present (Song et al., 2022). In contrast, the local dominance of high-productivity species in artificial grassland ecosystems promotes the improvement of overall primary productivity (Duchini et al., 2016), and the ecosystem function of artificial grassland is significantly enhanced under plant and soil interaction. Therefore, the interaction of the above factors resulted in the highest ecosystem productivity, carbon storage, water conservation, and soil fertility conservation occurring in LT, with the lowest occurring in NG.

Although the importance of species diversity and plant functional traits in maintaining ecological functions is still debated, numerous studies have shown that both factors have positive effects on ecosystem function (Cadotte, 2017; Armstrong et al., 2021; Chaves et al., 2021; Gao et al., 2022; Dendoncker et al., 2023; Yan et al., 2023). In this study, CWM_{LDMC}, CWM_{RDMC}, CWM_H, the Margalef index, and the Shannon index were positively correlated with ecosystem productivity, carbon storage, water conservation, and soil fertility conservation. This indicates that the functioning of the grassland ecosystem in this region is influenced by both species richness and plant functional traits. CWM can be used to assess the effect of dominant traits on ecosystem function, while the functional trait diversity index can quantify the effect of variation in the trait on ecosystem function (Choudhury et al., 2022). The results of RDA in this study showed that the CWM explained the effects of grassland ecosystem function to a greater extent than the functional trait diversity index and the species diversity index, indicating that the dominant species in the community had a greater influence on ecosystem productivity, carbon storage, water conservation, and soil fertility conservation. This suggests that ecosystem function may depend on the functional traits of the dominant species in the community. Specifically, CWM_H, CWM_{LDMC}, and CWM_{RL} were the main factors affecting grassland ecosystem function. This result has also been obtained in studies of degraded grassland ecosystems in the Loess Plateau of China (Jing et al., 2019), Selside meadows in the UK (Sweeney et al., 2020), and subtropical forest ecosystems in the central Himalayas (Sigdel et al., 2022). Therefore, in this study, the mass-ratio hypothesis was found to better explain the response of grassland ecosystem function to plant functional traits under different vegetation restoration models in this region in comparison to the niche differentiation hypothesis.

Conclusions

This research focuses on the desertification control in the karst ecosystem and examines the impact of plant functional traits on the functioning of grassland ecosystems under various vegetation restoration models in an area of karst desertification. In this study, species diversity (assessed via the Margalef index and Shannon index), FDis, and RaoQ were higher in the grassland ecosystem under the natural restoration model than in ecosystems under artificial restoration models, but the differences between the NG and LT were not significant. Furthermore, grassland ecosystem function, including ecosystem productivity, carbon storage, water conservation, and soil fertility conservation, was found to be at its lowest in NG and highest in LT. This is because the CWM explains a larger proportion of the variance in grassland ecosystem function than the functional trait diversity index, and changes in ecosystem function depend largely on the functional traits of the dominant species in the community. Therefore, we conclude that the mass-ratio hypothesis can better explain the response of grassland ecosystem function to plant functional traits under different vegetation restoration models in this region in comparison to the niche differentiation hypothesis. This may be due to the relatively short monitoring period; in order to study changes in the underlying mechanism, we will need to carry out long-term monitoring. In addition, while plant functional traits have a significant impact on grassland ecosystem function in the area of karst desertification, it is important to consider the effects of climate, soil, and anthropogenic interference on ecosystem recovery in future research. This study proved that, among the different vegetation restoration models employed in the area of karst desertification, ecosystem function was the best in the case of mixed-seeding grassland (perennial ryegrass + white clover), which indicates that degraded karst ecosystem function can be restored by the method of mixed-seeding grassland.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: Data available via the figshare https://doi.org/10.6084/m9.figshare.22758158.v1.

Author contributions

SS and KX performed the sampling and contributed to the experimental design, data analysis, and writing of the manuscript. KX provided the study site and funding. KX and YC contributed to the sampling, experimental design, data analysis, and writing of the manuscript. All authors contributed to the article and approved the submitted version.

Funding

This research was funded by the Key Science and Technology Program of Guizhou Province: Poverty Alleviation Model and Technology Demonstration for Ecoindustries Derived from Karst Desertification Control (No.5411 2017 QianKehe Pingtai Rencai); the China Overseas Expertise Introduction Program for Discipline Innovation: Overseas Expertise Introduction Center for South China Karst Eco-environment Discipline Innovation (D17016); the Natural Science Research Project of the Education Department of Guizhou Province (Qianjiaohe KY Zi (2022) 157); and the Academic New Seedling Fund Project of Guizhou Normal University (Qianshi Xinmiao B15).

Conflict of interest

Publisher's note

References

Albert, C. H., Bello, F. D., Boulangeat, I., Pellet, G., Lavorel, S., and Thuiller, W. (2012). On the importance of intraspecific variability for the quantification of functional diversity. *Oikos* 121, 116–126. doi: 10.1111/j.1600-0706.2011.19672.x

Angassa, A. (2014). Effects of grazing intensity and bush encroachment on herbaceous species and rangeland condition in Southern Ethiopia. *Land Degrad. Dev.* 25, 438–451. doi: 10.1002/ldr.2160

Armstrong, C. G., Miller, J. E. D., McAlvay, A. C., Ritchie, P. M., and Lepofsky, D. (2021). Historical indigenous land-use explains plant functional trait diversity. *Ecol. Soc.* 26, 6. doi: 10.5751/ES-12322-260206

Bai, Y. F., and Cotrufo, M. F. (2022). Grassland soil carbon sequestration: Current understanding, challenges, and solutions. *Science* 377, 603–608. doi: 10.1126/science.abo238

Bell, L. W., Sparling, B., Tenuta, M., and Entz, M. H. (2012). Soil profile carbon and nutrient stocks under long-term conventional and organic crop and alfalfa-crop rotations and re-established grassland. *Agriculture Ecosyst. Environ.* 158, 156–163. doi: 10.1016/j.agee.2012.06.006

Benayas, J. M. R., Newton, A. C., Diaz, A., and Bullock, J. M. (2009). Enhancement of biodiversity and ecosystem services by ecological restoration: A meta-analysis. *Science* 325, 1121–1124. doi: 10.1126/science.1172460

Bessler, H., Oelmann, Y., Roscher, C., Buchmann, N., Scherer-Lorenzen, M., Schulze, E. D., et al. (2012). Nitrogen uptake by grassland communities: contribution of N2 fixation, facilitation, complementarity, and species dominance. *Plant Soil* 358, 301–322. doi: 10.1007/s11104-012-1181-z

Brown, L. M., and Anand, M. P. (2022). Plant functional traits as measures of ecosystem service provision. *Ecosphere* 13, e3930. doi: 10.1002/ecs2.3930

Cadotte, M. W. (2017). Functional traits explain ecosystem function through opposing mechanisms. *Ecol. Lett.* 20, 989–996. doi: 10.1111/ele.12796

Canedoli, C., Ficetola, G. F., Corengia, D., Tognini, P., Ferrario, A., and Padoa-Schioppa, E. (2021). Integrating landscape ecology and the assessment of ecosystem services in the study of karst areas. *Landscape Ecol.* 37, 347–365. doi: 10.1007/s10980-021-01351-2

Cardinale, B. J., Duffy, J. E., Gonzalez, A., Hooper, D. U., Perrings, C., Venail, P., et al. (2012). Biodiversity loss and its impact on humanity. *Nature* 486, 59–67. doi: 10.1038/nature1114

Carmona, C. P., Bello, D. F., Mason, N. W. H., and Lepš, J. (2016). Traits without borders: Integrating functional diversity across scales. *Trends Ecol. Evol.* 31, 382–394. doi: 10.1016/j.tree.2016.02.003

Casanoves, F., Pla, L., Di Rienzo, J. A., and Díaz, S. (2011). FDiversity: a software package for the integrated analysis of functional diversity. *Methods Ecol. Evol.* 2, 233–237. doi: 10.1890/0012-9623-92.2.147

Chaves, J. E., Aranibar, J. N., and Gatica, G. (2021). Species and functional plant diversity enhance ecosystem functions in the central Monte desert. *J. Vegetation Sci.* 32, e12952. doi: 10.1111/jvs.12952

Cheng, Y. T., Li, P., Xu, G. C., Li, Z. B., Cheng, S. D., and Gao, H. D. (2016). Spatial distribution of soil total phosphorus in Yingwugou watershed of the Dan River, China. *Catena* 136, 175–181. doi: 10.1016/j.catena.2015.02.015

Chi, Y. K., Xiong, K. N., Xiao, H., Chen, H., Song, S. Z., and Shen, X. Y. (2020). Study on the relationship between disposition models of forest and grass and soil properties in karst rocky desertification areas of southwest China. *Fresenius Environ. Bull.* 29, 5424–5431.

Chollet, S., Rambal, S., Fayolle, A., Hubert, D., Foulquié, D., and Garnier, E. (2014). Combined effects of climate, resource availability, and plant traits on biomass produced in a Mediterranean rangeland. *Ecology* 95, 737–748. doi: 10.1890/13-0751.1

Choudhury, M. I., Hallin, S., Ecke, F., Hubalek, V., Juhanson, J., Frainer, A., et al. (2022). Disentangling the roles of plant functional diversity and plaint traits in regulating plant nitrogen accumulation and denitrification in freshwaters. *Funct. Ecol.* 36, 921–932. doi: 10.1111/1365-2435.14001

Cong, W. F., Ruijven, J. V., Mommer, L., Deyn, G. B. D., Berendse, F., and Hoffland, E. (2014). Plant species richness promotes soil carbon and nitrogen stocks in grasslands without legumes. *J. Ecol.* 102, 1163–1170. doi: 10.1111/1365-2745.12280

Conti, G., and Díaz, S. (2013). Plant functional diversity and carbon storage - an empirical test in semi-arid forest ecosystems. *J. Ecol.* 101, 18–28. doi: 10.1111/1365-2745.12012

Crawley, M. J., and Harral, J. E. (2001). Scale dependence in plant biodiversity. *Science* 291, 864–868. doi: 10.3390/f13091505

Crème, A., Rumpel, C., Gastal, F., Gil, M. D. L. M., and Chabbi, A. (2016). Effects of grasses and a legume grown in monoculture or mixture on soil organic matter and phosphorus forms. *Plant Soil* 402, 117–128. doi: 10.1007/s11104-015-2740-x

Dendoncker, M., Taugourdeau, S., Messier, C., and Vincke, C. (2023). A functional trait-based approach to evaluate the resilience of key ecosystem functions of tropical savannas. *Forests* 14, 291. doi: 10.3390/f14020291

Diaz, S., and Cabido, M. (2001). Vive la différence: plant functional diversity matters to ecosystem processes. *Trends Ecol. Evol.* 16, 646–655. doi: 10.1016/S0169-5347(01)02283-2

Díaz, S., Lavorel, S., Bello, F. D., Quétier, F., Grigulis, K., and Robson, T. M. (2007). Incorporating plant functional diversity effects in ecosystem service assessments. *Proc. Natl. Acad. Sci. United States America* 104, 20684–20689. doi: 10.1016/j.baae.2010.06.009

Dong, S. K., Tang, L., Zhang, X. F., Liu, S. L., Liu, Q. R., Su, X. K., et al. (2019). Relationship between plant species diversity and functional diversity in alpine grasslands. *Acta Ecologica Sin.* 37, 1472–1483. doi: 10.5846/stxb201509281981

Duchini, P. G., Guzatti, G. C., Ribeiro-Filho, H. M. N., and Sbrissia, A. F. (2016). Intercropping black oat (Avena strigosa) and annual ryegrass (*Lolium multiflorum*) can increase pasture leaf production compared with their monocultures. *Crop Pasture Sci.* 67, 574–581. doi: 10.1071/CP15170

Duffy, J. E., Godwin, C. M., and Cardinale, B. J. (2017). Biodiversity effects in the wild are common and as strong as key drivers of productivity. *Nature* 549, 261. doi: 10.1038/nature23886

Eekeren, N. V., Boer, H. D., Hanegraaf, M., Bokhorst, J., Nierop, D., Bloem, J., et al. (2010). Ecosystem services in grassland associated with biotic and abiotic soil parameters. *Soil Biol. Biochem.* 42, 1491–1504. doi: 10.1016/j.soilbio.2010.05.016

Fan, F., Liang, C. Z., Tang, Y. K., Harker-Schuch, I., and Porter, J. R. (2019). Effects and relationships of grazing intensity on multiple ecosystem services in the Inner Mongolian steppe. *Sci. Total Environ.* 675, 642–650. doi: 10.1016/j.scitotenv.2019.04.279

Faucon, M. P., Houben, D., Reynoird, J. P., Mercadal-Dulaurent, A. M., Armand, R., and Hans, L. (2015). Advances and perspectives to improve the phosphorus availability in cropping systems for agroecological phosphorus management. *Adv. Agron.* 134, 51–79. doi: 10.1016/bs.agron.2015.06.003

Finegan, B., Peña-Claros, M., de Oliveira, A., Ascarrunz, N., Bret-Harte, M. S., Carreño Rocabado, G., et al. (2015). Does functional trait diversity predict above-ground biomass and productivity of tropical forests? Testing three alternative hypotheses. *J. Ecol.* 103, 191–201. doi: 10.1111/1365-2745.12346

Flynn, D., Mirotchnick, N., Jain, M., Palmer, M., and Naeem, S. (2011). Functional and phylogenetic diversity as predictors of biodiversity-ecosystem-function relationships. *Ecology* 92, 1573–1581. doi: 10.1890/10-1245.1

Fox, A., Suter, M., Widmer, F., and Lüscher, A. (2020). Positive legacy effect of previous legume proportion in a ley on the performance of a following crop of *Lolium multiflorum*. *Plant Soil* 447, 497–506. doi: 10.1007/s11104-019-04403-4

Frankow-Lindberg, B. E., and Dahlin, A. S. (2013). N2 fixation, N transfer,and yield in grassland communities including a deep-rooted legume or non-legume species. *Plant Soil* 370, 567–581. doi: 10.1007/s11104-013-1650-z

Freitag, M., Kamp, J., Dara, A., Kuemmerle, T., Sidorova, T. V., Stirnemann, I. A., et al. (2021). Post-Soviet shifts in grazing and fire regimes changed the functional plant community composition on the Eurasian steppe. *Global Change Biol.* 27, 388–401. doi: 10.1111/GCB.15411

Freschet, G. T., Violle, C., Bourget, M. Y., Scherer-Lorenzen, M., and Fort, F. (2018). Allocation, morphology, physiology, architecture: the multiple facets of plant above- and below-ground responses to resource stress. *New Phytol.* 219, 1338–1352. doi: 10.1111/nph.15225

Fry, E. L., Savage, J., Hall, A. L., Oakley, S., Pritchard, W. J., Ostle, N. J., et al. (2018). Soil multifunctionality and drought resistance are determined by plant structural traits in restoring grassland. *Ecology* 99, 2260–2271. doi: 10.1002/ecy.2437

Gamfeldt, L., and Roger, F. (2017). Revisiting the biodiversity-ecosystem multifunctionality relationship. *Nat. Ecol. Evol.* 1, 168. doi: 10.1038/s41559-017-0168

Gao, D. X., Wang, S., Wei, F. L., Wu, X. T., Zhou, S., Wang, L. X., et al. (2022). The vulnerability of ecosystem structure in the semi-arid area revealed by the functional trait networks. *Ecol. Indic.* 139, 108894. doi: 10.1016/j.ecolind.2022.108894

Garcia-Cervigon, A. I., García-Lopez, M. A., Piston, N., Pugnaire, F. I., and Olano, J. M. (2021). Co-ordination between xylem anatomy, plant architecture and leaf functional traits in response to abiotic and biotic drivers in a nurse cushion plant. *Ann. Bot.* 127, 919–929. doi: 10.1093/aob/mcab036

Garnier, E., Cortez, J., Billès, G., Navas, M. L., Roumet, C., Debusssche, M., et al. (2004). Plant functional markers capture ecosystem properties during secondary succession. *Ecology* 85, 2630–2637. doi: 10.1890/03-0799

Garnier, E., Navas, M. L., and Karl, G. (2015). *Plant functional diversity: organism traits, community structure, and ecosystem properties* (Oxford: Oxford University Press). doi: 10.1111/aec.12498

Gonzalez, A., Germain, R. M., Srivastava, D. S., Filotas, E., Dee, L. E., Gravel, D., et al. (2020). Scaling-up biodiversity-ecosystem functioning research. *Ecol. Lett.* 23, 757–776. doi: 10.1111/ele.13456

Grime, J. P. (1998). Benefits of plant diversity to ecosystems: immediate, filter and founder effects. *J. Ecol.* 86, 902–910. doi: 10.1046/j.1365-2745.1998.00306.x

Gross, N., Bagousse-Pinguet, L. Y., Liancourt, P., Berdugo, M., Gotelli, N. J., and Maestre, F. T. (2017). Functional trait diversity maximizes ecosystem multifunctionality. *Nat. Communication Evol.* 1, 0132. doi: 10.1016/j.ecolind.2020.106415

Gu, Y. Y., Lin, N. F., Ye, X., Xu, M. J., Qiu, J., Zhang, K., et al. (2022). Assessing the impacts of human disturbance on ecosystem services under multiple scenarios in karst areas of China: Insight from ecological conservation red lines effectiveness. *Ecol. Indic.* 142, 109202. doi: 10.1016/j.ecolind.2022.109202

Hao, H. J., Guan, X., Cao, M., and Li, J. S. (2023). Study on the characteristics of plant communities in different ecological restoration stages of Hulun Buir sandy grassland. *J. Environ. Eng. Technol.*, 13: 1573–1585. doi: 10.1111/1365-2745.13250

Hao, S. J., Li, X. Y., Hou, M. M., and Zhao, X. H. (2019). Spatial variations of community functional traits at different successional stages in temperate forests of Changbai Mountains, Northeast China. *Chin. J. Plant Ecol.* 43, 208–216. doi: 10.17521/cjpe.2018.0295

He, J. S., Liu, Z. P., Yao, T., and Zhu, J. X. (2020). Analysis of the main constraints and restoration techniques of degraded grassland on the Tibetan Plateau. *Sci. Technol. Rev.* 38, 66–80. doi: 10.3981/j.issn.1000-7857.2020.17.007

He, X. J., Wang, L., Ke, B., Yue, Y. M., Wang, K. L., Cao, J. H., et al. (2019). Progress on ecological conservation and restoration for China Karst. *Acta Ecologica Sin.* 39, 6577–6585. doi: 10.5846/stxb201812292842

Hector, A., and Bagchi, R. (2007). Biodiversity and ecosystem multifunctionality. *Nature* 448, 188–190. doi: 10.1038/s41559-017-0168

Hobbie, S. E. (2015). Plant species effects on nutrient cycling: revisiting litter feedbacks. *Trends Ecol. Evol.* 30, 357–363. doi: 10.1016/j.tree.2015.03.015

Hou, X. K., Dong, Q. Q., Shi, J. J., Wang, Y. L., Chen, L. L., and Song, L. (2015). Study on the community structure characteristics and soil physico-chemical property of Poa Pratensis L.Cv.Qinghai single artificial grasslands in different ages. *Chin. J. Grassland* 37, 65–69.

Hu, L. Y., Qin, D. Y., Lu, H. Y., Li, W., Shang, K. K., Lin, D. M., et al. (2021). Urban growth drives trait composition of urban spontaneous plant communities in a mountainous city in China. *J. Environ. Manage.* 293, 112869. doi: 10.1016/J.JENVMAN.2021.112869

Hu, P. L., Xiao, J., Zhang, W., Xiao, L. M., Yang, R., Xiao, D., et al. (2020). Response of soil microbial communities to natural and managed vegetation restoration in a subtropical karst region. *Catena* 195, 104849. doi: 10.1016/j.catena.2020.104849

Hu, P. L., Zhao, Y., Xiao, D., Xu, Z. Z., Zhang, W., Xiao, J., et al. (2021). Dynamics of soil nitrogen availability following vegetation restoration along a climatic gradient of a subtropical karst region in China. *J. Soils Sediments* 21, 2167–2178. doi: 10.1007/s11368-021-02915-0

Huang, C., Wei, H., Wu, K. J., He, X. R., Wang, P., Qi, Y. C., et al. (2020). The functional diversity of understory plants during the transformation from Pinus massoniana to Cinnamomum camphora forest. *Acta Ecologica Sin.* 40, 4573–4584. doi: 10.5846/stxb201911152455

Huang, L. J., Yu, Y. M., An, X. F., Yu, L. L., and Xue, Y. G. (2022). Leaf functional traits, species diversity and functional diversity of plant community in Tiankeng forests. *Acta Ecologica Sin.* 42, 10264–10275. doi: 10.5846/stxb202110273025

Husse, S., Huguenin-Elie, O., Buchmann, N., and Lüscher, A. (2016). Larger yields of mixtures than monocultures of cultivated grassland species match with asynchrony in shoot growth among species but not with increased light interception. *Field Crops Res.* 194, 1–11. doi: 10.1016/j.fcr.2016.04.021

Jäschke, Y., Heberling, G., and Wesche, K. (2020). Environmental controls override grazing effects on plant functional traits in Tibetan rangelands. *Funct. Ecol.* 34, 747–760. doi: 10.1111/1365-2435.13492

Jiang, X. Y., Gao, S. J., Jiang, Y., Tian, B., Jia, X., and Cha, T. T. (2022). Species diversity,functional diversity,and phylogenetic diversity in plant communities at different phases of vegetation restoration in the Mu Us sandy grassland. *Biodiversity Sci.* 30, 15–25. doi: 10.17520/biods.2021387

Jiang, M., He, L. Y., Fan, B. J., Wang, T., Yang, N., Liu, Y. L., et al. (2022). Intraspecific more than interspecific diversity plays an important role on Inner Mongolia grassland ecosystem functions: A microcosm experiment. *Sci. Total Environ.* 826, 154134. doi: 10.1016/j.scitotenv.2022.154134

Jing, G. H., Cheng, J. M., Su, J. S., Wei, L., Hu, T. M., and Li, W. (2019). Community-weighted mean traits play crucial roles in driving ecosystem functioning along long-term grassland restoration gradient on the Loess Plateau of China. *J. Arid Environments* 165, 97–105. doi: 10.1016/j.jaridenv.2019.01.018

Kazakou, E., Vile, D., Shipley, B., Gallet, C., and Garnier, E. (2006). Co-variations in litter decomposition, leaf traits and plant growth in species from a Mediterranean old-field succession. *Funct. Ecol.* 20, 21–30. doi: 10.1111/j.1365-2435.2006.01080.x

Kearney, S. P., Fonte, S. J., Garcia, E., Siles, P., Chan, K. M. A., and Smukler, S. M. (2019). Evaluating ecosystem service trade-offs and synergies from slash-and-mulch agroforestry systems in El Salvador. *Ecol. Indic.* 105, 264–278. doi: 10.1016/j.ecolind.2017.08.032

Khasanova, A., Lovell, J. T., Bonnette, J., Weng, X. Y., Jenkins, J., Yoshinaga, Y., et al. (2019). The genetic architecture of shoot and root trait divergence between mesic and xeric ecotypes of a perennial grass. *Frontiersin Plant Sci.* 10. doi: 10.3389/fpls.2019.00366

Kong, X. P., Zhang, M. L., Smet, I. D., and Ding, Z. J. (2014). Designer crops: optimal root system architecture for nutrient acquisition. *Trends Biotechnol.* 32, 597–598. doi: 10.1016/j.tibtech.2014.09.008

Kraft, N. J. B., Godoy, O., and Levine, J. M. (2015). Plant functional traits and the multidimensional nature of species coexistence. *Proc. Natl. Acad. Sci. United States America* 112, 797–802. doi: 10.1073/pnas.1413650112

Lange, M., Eisenhauer, N., Sierra, C. A., Bessler, H., Engels, C., Griffiths, R. I., et al. (2015). Plant diversity increases soil microbial activity and soil carbon storage. *Nat. Commun.* 6, 6707. doi: 10.1038/ncomms7707

Lei, L. J., Kong, D. L., Li, X. M., Zhou, Z. X., and Li, G. Y. (2016). Plant functional traits, functional diversity, and ecosystem functioning: current knowledge and perspectives. *Biodiversity Sci.* 24, 922–931. doi: 10.17520/biods.2015295

Li, X. Y., Li, Y. H., Qin, W. P., Sun, Y., Ma, Z. W., and Liu, X. (2023). Impacts of artificial grasslands on soil organic carbon fractions in a degraded alpine meadow of the Three-River Source Region. *Chin. J. Ecol.*, 1–9.

Li, X. L., Png, G. K., Li, Y. H., Jimoh, S. O., Ding, Y., Li, F., et al. (2021). Leaf plasticity contributes to plant anti-herbivore defenses and indicates selective foraging: Implications for sustainable grazing. *Ecol. Indic.* 122, 107273. doi: 10.1016/j.ecolind.2020.107273

Li, H. Z., Wang, T. Q., Baoyin, T. G. T., Zhang, T. Y., Yan, W. B., Huang, N., et al. (2022). Effects of plant diversity on root morphology and leaf area index of legume-grass mixture community. *Chin. J. Grassland* 44, 50–57. doi: 10.16742/j.zgcdxb.20210144

Li, H. N., Zhang, Z. G., Duan, J. T., Li, N., Li, B. X., Song, T. T., et al. (2020). Electrochemical disinfection of secondary effluent from a wastewater treatment plant: Removal efficiency of ARGs and variation of antibiotic resistance in surviving bacteria. *Chem. Eng. J.* 392, 123674. doi: 10.1016/j.cej.2019.123674

Li, C. D., Zhang, C. P., Yang, Y., and Dong, Q. M. (2023). Effects of planting patterns on vegetation and soil characteristics of artificial pasture in the qinghai lake basin. *Acta Agrestia Sin.* 31, 471–478. doi: 10.11733/j.issn.1007-0435.2023.02.021

Liu, L. B., Hu, J., Chen, X. Y., Xu, X., Yang, Y., and Ni, J. (2022). Adaptation strategy of karst forests: Evidence from the community-weighted mean of plant functional traits. *Ecol. Evol.* 12, e8680. doi: 10.1002/ece3.8680

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. doi: 10.3389/fpls.2021.622260

Liu, M. X., Zhang, G. J., Yin, F. L., Wang, S. Y., and Li, L. (2022). Relationship between biodiversity and ecosystem multifunctionality along the elevation gradient in alpine meadows on the eastern Qinghai-Tibetan plateau. *Ecol. Indic.* 141, 109070. doi: 10.1016/j.ecolind.2022.109097

Maire, V., Wright, I. J., Prentice, I. C., Batjes, N. H., Bhaskar, R., Bodegom, P. M. V., et al. (2015). Global effects of soil and climate on leaf photosynthetic traits and rates. *Global Ecol. Biogeography* 24, 706–717. doi: 10.1111/geb.12296

Mammola, S., Carmona, C. P., Guillerme, T., and Cardoso, P. (2021). Concepts and applications in functional diversity. *Funct. Ecol.* 35, 0269–8463. doi: 10.1111/1365-2435.13882

Mason, N. W. H., Mouillot, D., Lee, W. G., and Wilson, J. B. (2005). Functional richness, functional evenness and functional divergence: the primary components of functional diversity. *Oikos* 111, 112–118. doi: 10.1111/j.0030-1299.2005.13886.x

Mason, N. W. H., Orwin, K., Lambie, S., Woodward, S. L., McCready, T., and Mudge, P. (2016). Leaf economics spectrum-productivity relationships in intensively grazed pastures depend on dominant species identity. *Ecol. Evol.* 6, 3079–3091. doi: 10.1002/ece3.1964

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

MEA (2005). *Ecosywtem and human well-beings* (Beijing: Island Press).

Miao, D. N., Peng, X. Y., Teng, Q. M., Sardar, M. F., Zhang, Q. Q., Zhu, X. T., et al. (2023). Different contributions of bacterial and fungal communities to nitrogen mineralization in Moso bamboo-invaded subtropical forests. *J. Soils Sediments* 23, 1123–1134. doi: 10.1007/s11368-022-03380-z

Mouillot, D., Graham, N. A., Villéger, S., Mason, N. W., and Bellwood, D. R. (2013). A functional approach reveals community responses to disturbances. *Trends Ecol. Evol.* 28, 167–177. doi: 10.1016/j.tree.2012.10.004

Niu, K. C., He, J. S., and Lechowicz, M. J. (2016). Grazing-induced shifts in community functional composition and soil nutrient availability in Tibetan alpine meadows. *J. Appl. Ecol.* 53, 1554–1564. doi: 10.1111/1365-2664.12727

Nyfeler, D., Huguenin-Elie, O., Suter, M., Frossard, E., and Lüscher, A. (2011). Grass-legume mixtures can yield more nitrogen than legume pure stands due to mutual stimulation of nitrogen uptake from symbiotic and non-symbiotic sources. *Agric. Ecosyst. Environ.* 140, 155–163. doi: 10.1016/j.proenv.2015.07.253

Peng, F., Xue, X., Li, C. Y., Lai, C. M., Sun, J., Tsubo, M., et al. (2020). Plant community of alpine steppe shows stronger association with soil properties than alpine meadow alongside degradation. *Sci. Total Environ.* 733, 139048. doi: 10.1016/j.scitotenv.2020.139048

Perring, M. P., Bernhardt-Romermann, M., Baeten, L., and Verheyen, K. (2018). Global environmental change effects on plant community composition trajectories depend upon management legacies. *Global Change Biol.* 24, 1722–1740. doi: 10.1111/gcb.14030

Petchey, O. L., and Gaston, K. J. (2002). Functional diversity (FD), species richness and community composition. *Ecol. Lett.* 5, 402–411. doi: 10.1046/j.1461-0248.2002.00339.x

Petchey, O. L., and Gaston, K. J. (2006). Functional diversity: back to basics and looking forward. *Ecol. Lett.* 9, 741–758. doi: 10.1111/j.1461-0248.2006.00924.x

Plas, F. V. D. (2019). Biodiversity and ecosystem functioning in naturally assembled communities. *Biol. Rev.* 94, 1220–1245. doi: 10.1111/brv.12499

Plaza-Bonilla, D., Álvaro-Fuentes, J., Hansen, N. C., Lampurlanés, J., and Cantero-Martínez, C. (2014). Winter cereal root growth and aboveground-belowground biomass ratios as affected by site and tillage system in dryland Mediterranean conditions. *Plant Soil* 374, 925–939. doi: 10.1007/s11104-013-1926-3

Qi, W., Kang, X. M., Knops, J. M. H., Jiang, J. C., Abuman, A., and Du, G. Z. (2022). The complex biodiversity-ecosystem function relationships for the Qinghai-Tibetan grassland community. *Front. Plant Sci.* 12. doi: 10.3389/fpls.2021.772503

Rosenfield, M. F., and Müller, S. C. (2020). Plant traits rather than species richness explain ecological processes in subtropical forests. *Ecosystems* 23, 52–66. doi: 10.1007/s10021-019-00386-6

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

Sardar, M. F., Younas, F., Farooqi, Z. U. R., and Li, Y. L. (2023). Soil nitrogen dynamics in natural forest ecosystem: a review. *Front. For. Glob. Change* 6. doi: 10.3389/ffgc.2023.1144930

Sasaki, T., Okubo, S., Okayasu, T., Jamsran, U., Ohkuro, T., and Takeuchi, K. (2009). Two-phase functional redundancy in plant communities along a grazing gradient in Mongolian rangelands. *Ecology* 90, 2598–2608. doi: 10.1890/08-1850.1

Schleuter, D., Daufresne, M., Massol, F., and Argillier, C. (2010). A user's guide to functional diversity indices. *Ecol. Monogr.* 80, 469–484. doi: 10.1890/08-2225.1

Shabaan, M., Asghar, H. N., Zahir, Z. A., Sardar, M. F., Parveen, R., and Ali, F. Q. (2022). Halotolerant rhizobacterial consortium confers salt tolerance to maize under naturally salt-affected soil. *Soil Sci. Soc Am. J.* 86, 1264–1279. doi: 10.1002/saj2.20438

Sharafatmandrad, M., and Mashizi, K. A. (2020). Investigating distribution of ecosystem services in rangeland landscapes: an approach based on weighted key functional traits. *Ecol. Indic.* 111, 105971. doi: 10.1016/j.ecolind.2019.105971

Shen, H., Dong, S. K., Li, S., Xiao, J. N., Han, Y. H., Yang, M. Y., et al. (2019). Grazing enhances plant photosynthetic capacity by altering soil nitrogen in alpine grasslands on the Qinghai-Tibetan plateau. *Agriculture Ecosyst. Environ.* 280, 161–168. doi: 10.1016/j.agee.2019.04.029

Sigdel, S. R., Liang, E. Y., Rokaya, M. B., Rai, S., Dyola, N., Sun, J., et al. (2022). Functional traits of a plant species fingerprint ecosystem productivity along broad elevational gradients in the Himalayas. *Funct. Ecol.* 37, 383–394. doi: 10.1111/1365-2435.14226

Siwicka, E., Thrush, S. F., and Hewitt, J. E. (2020). Linking changes in species-trait relationships and ecosystem function using a network analysis of traits. *Ecol. Appl.* 30, e02010. doi: 10.1002/eap.2010

Song, S. Z., Xiong, K. N., Chi, Y. K., He, C., Fang, J. Z., and He, S. Y. (2022). Effect of cultivated pastures on soil bacterial communities in the karst rocky desertification area. *Front. Microbiol.* 13. doi: 10.3389/fmicb.2022.922989

Su, L., Du, H., Zeng, F. P., Peng, W. X., Rizwan, M., Núñez-Delgado, A., et al. (2019). Soil and fine roots ecological stoichiometry in different vegetation restoration stages in a karst area, southwest China. *J. Environ. Manage.* 252, 109694. doi: 10.1016/j.jenvman.2019.109694

Sweeney, C. J., Vries, F. T. D., Dongen, B. E. V., and Bardgett, R. D. (2020). Root traits explain rhizosphere fungal community composition among temperate grassland plant species. *New Phytol.* 229, 1492–1507. doi: 10.1111/1365-2664.12727

Teng, Q. M., Lu, X. N., Zhang, Q. Q., Cai, L. L., Sardar, M. F., Li, Y. F., et al. (2023). Litterfall quality modulates soil ammonium and nitrate supply through altering microbial function in bamboo encroachment of broadleaf forests. *Geoderma* 437, 116592. doi: 10.1016/j.geoderma.2023.116592

Tilman, D., Knops, J., Wedin, D., Reich, P., Ritchie, M., and Siemann, E. (1997). The influence of functional diversity and composition on ecosystem processes. *Science* 277, 1300–1302. doi: 10.1126/science.277.5330.1300

Vile, D., Shiple, B., and Garnier, E. (2006). Ecosystem productivity can be predicted from potential relative growth rate and species abundance. *Ecol. Lett.* 9, 1061–1067. doi: 10.1111/j.1461-0248.2006.00958.x

Villéger, S., Mason, N. W. H., and Mouillot, D. (2008). New multidimensional functional diversity indices for a multifaceted framework in functional ecology. *Ecology* 89, 2290–2301. doi: 10.1890/07-1206.1

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

Wagg, C., Bender, S. F., Widmer, F., and Heijden, M. G. A. V. D. (2014). Soil biodiversity and soil community composition determine ecosystem multifunctionality. *Proc. Natl. Acad. Sci. United States America* 111, 5266–5270. doi: 10.1073/pnas.1320054111

Wang, Y., Chen, J., Zhang, L. M., Feng, L., Yan, L. B., Li, F. B., et al. (2022a). Relationship between diversity and stability of a karst plant community. *Ecol. Evol.* 12, e9254. doi: 10.1002/ece3.9254

Wang, Q., Rong, L., Wang, M. J., Ye, T. M., Li, T. T., Yang, W. S., et al. (2022). Response of leaf functional traits of woody plants to degraded karst soil in central Guizhou. *Earth Environ.* 50, 639–647. doi: 10.14050/j.cnki.1672-9250.2022.50.102

Wang, D. J., Xu, Z. H., Blumfield, T. J., and Zalucki, J. (2020). The potential of using 15N natural abundance in changing ammonium-N and nitrate-N pools for studying *in situ* soil N transformations. *J. Soils Sediments* 20, 1323–1331. doi: 10.1007/s11368-019-02478-1

Wang, K. L., Yue, Y. M., Chen, H. S., and Zeng, F. P. (2020). Mechanisms and realization pathways for integration of scientific poverty alleviation and ecosystem services enhancement. *Bull. Chin. Acad. Sci.* 35, 1264–1272. doi: 10.16418/j.issn.1000-3045.20200729002

Wang, Y., Zhang, L., Chen, J., Feng, L., Li, F., and Yu, L. (2022b). Functional diversity of plant communities in relationship to leaf and soil stoichiometry in karst areas of southwest China. *Forests* 13, 864. doi: 10.3390/f13060864

Wang, Y. Q., Zhang, X. C., and Huang, C. Q. (2019). Spatial variability of soil total nitrogen and soil total phosphorus under different land uses in a small watershed on the Loess Plateau, China. *Geoderma* 150, 141–149. doi: 10.1016/j.geoderma.2009.01.021

Weng, E. S., Farrior, C. E., Dybzinski, R., and Pacala, S. W. (2017). Predicting vegetation type through physiological and environmental interactions with leaf traits: Evergreen and deciduous forests in an earth system modeling framework. *Global Change Biol.* 23, 2482–2498. doi: 10.1111/gcb.13542

Wright, I. J., Reich, P. B., Westoby, M., and Villar, R. (2004). The worldwide leaf economics spectrum. *Nature* 428, 821–827. doi: 10.1038/nature02403

Wu, S. N., Zhang, X., Gao, X. X., Xu, Y. D., Wu, X. H., Shan, X. K., et al. (2019). Succession dynamics of a plant community of degraded alpine meadow during the human-induced restoration process in the Three Rivers Source region. *Acta Ecologica Sin.* 39, 2444–2453. doi: 10.5846/stxb201804070783

Wu, J. X., Zhao, Y., Yu, C. Q., Luo, L. M., and Pan, Y. (2017). Land management influences trade-offs and the total supply of ecosystem services in alpine grassland in Tibet, China. *J. Environ. Manage.* 193, 70–78. doi: 10.1016/j.jenvman.2017.02.008

Xi, X. Q., Zhao, Y. J., Liu, Y. G., Wang, X., and Gao, X. M. (2011). Variation and correlation of plant functional traits in karst area of central Guizhou Province, China. *Chin. J. Plant Ecol.* 35, 1000–1008. doi: 10.3724/SP.J.1258.2011.01000

Xiao, J., and Xiong, K. N. (2022). A review of agroforestry ecosystem services and its enlightenment on the ecosystem improvement of rocky desertification control. *Sci. Total Environ.* 856, 158538. doi: 10.1016/j.scitotenv.2022.158538

Xing, Y. F., Wang, X. L., Liu, Y. Q., Hua, R., Wang, C., Wu, J. L., et al. (2020). Characteristics of plant community and soil organic carbon and nitrogen in artificial grassland with different establishment years. *Acta Agrestia Sin.* 28, 521–528. doi: 10.11733/j.issn.1007-0435.2020.02.028

Xiong, K. N., Li, J., and Long, M. Z. (2012). Features of soil and water loss and key issues in demonstration areas for combating karst rocky desertification. *Acta Geologica Sin.* 67, 878–888.

Xiong, K. N., Li, P., Zhou, Z. F., An, Y. L., Lv, T., and Lan, A. J. (2002). *Remote sensing of karst rocky desertification: a typical GIS study—with a special reference to Guizhou Province* (Beijing Geological Press).

Xiong, K. N., Xiao, J., and Zhu, D. Y. (2022). Research progress of agroforestry ecosystem services and its implications for industrial revitalization in karst regions. *Acta Ecologica Sin.* 42, 851–861. doi: 10.5846/stxb202101080090

Xu, T. W., Zhao, X. Q., Zhang, X. L., and Xu, S. X. (2020). Sustainable development of ecological grass-based livestock husbandry in Qinghai-Tibet Plateau alpine area: principle, technology and practice. *Acta Ecologica Sin.* 40, 6324–6337. doi: 10.5846/stxb201912142705

Yan, P., He, N. P., Yu, K. L., Xu, L., and Meerbeek, K. V. (2023). Integrating multiple plant functional traits to predict ecosystem productivity. *Commun. Biol.* 6, 239. doi: 10.1038/s42003-023-04626-3

Yang, S. T., Li, C. J., Lou, H. Z., Wang, P. F., Wu, X. J., Zhang, Y. C., et al. (2021). Role of the countryside landscapes for sustaining biodiversity in karst areas at a semi centennial scale. *Ecol. Indic.* 123, 107315. doi: 10.1016/j.ecolind.2020.107315

Yang, J., Xu, X., Liu, M., Xu, C., Luo, W., Song, T., et al. (2016). Effects of Napier grass management on soil hydrologic functions in a karst landscape, southwestern China. *Soil Tillage Res.* 157, 83–92. doi: 10.1016/j.still.2015.11.012

Yang, J., Xu, X., Liu, M., Xu, C., Zhang, Y., Luo, W., et al. (2017). Effects of "Grain for Green" program on soil hydrologic functions in karst landscapes, southwestern China. *Agriculture Ecosyst. Environ.* 247, 120–129. doi: 10.1016/j.agee.2017.06.025

Yang, C., Zhang, Y. X., Zhang, H., Zheng, C. Y., and Zhu, F. (2022). Recent advances in understanding the ecosystem functioning of diverse forage mixtures. *Acta Prataculturae Sin.* 31, 206–219. doi: 10.11686/cyxb2021341

Yuan, D. X. (1991). *Karst of China* (Beijing: Geological Publishing House).

Zhang, Y. (2011). *Physicochemical analysis of soil, water and plant* (Beijing, China: Forestry Publishing House).

Zhang, L., Cappelli, S. L., Huang, M. J., Liu, X., Xiao, Y., Cardou, F., et al. (2023). Intraspecific trait variability mediates the effect of nitrogen addition and warming on aboveground productivity. *Oikos* (2023), 34: e09559. doi: 10.1111/oik.09559

Zhang, J., Chen, H. S., Fu, Z. Y., and Wang, K. L. (2021). Effects of vegetation restoration on soil properties along an elevation gradient in the karst region of southwest China. *Agriculture Ecosyst. Environ.* 320, 107572. doi: 10.1016/j.agee.2021.107572

Zhang, Z. H., Hou, J. H., and He, N. P. (2021). Predictability of functional diversity depends on the number of traits. *J. Resour. Ecol.* 12, 332–345. doi: 10.5814/j.issn.1674-764x.2021.03.003

Zhang, H. M., Li, X. L., Li, L. P., and Zhang, J. (2020). Effects of species combination on community diversity and productivity of alpine artificial grassland. *Acta Agrestia Sin.* 28, 1436–1443. doi: 10.11733/j.issn.1007-0435.2020.05.031

Zhang, M. Y., Wang, K. L., Liu, H. Y., Zhang, C. H., Yue, Y. M., and Qi, X. K. (2018). Effect of ecological engineering projects on ecosystem services in a karst region: A case study of northwest Guangxi, China. *J. Cleaner Production* 183, 831–842. doi: 10.1016/j.jclepro.2018.02.102

Zhang, S. H., Xiong, K. N., Deng, X. H., Kong, L. W., and Min, X. Y. (2023). Impact of ecological restoration on ecosystem service trade-offs: Insight from karst desertification control. *Land Degradation Dev.* 34. doi: 10.1002/ldr.4643

Zhang, Y., Xu, X., Li, Z., Liu, M., Xu, C., Zhang, R., et al. (2019). Effects of vegetation restoration on soil quality in degraded karst landscapes of southwest China. *Sci. Total Environ.* 650, 2657–2665. doi: 10.1016/j.scitotenv.2018.09.372

Zhang, Y. H., Xu, X. L., Li, Z. W., Xu, C. C., and Luo, W. (2021). Improvements in soil quality with vegetation succession in subtropical China karst. *Sci. Total Environ.* 775, 145876. doi: 10.1016/j.scitotenv.2021.145876

Zhang, S. H., Zhang, Y., Xiong, K. N., Yu, Y. H., and Min, X. Y. (2020). Changes of leaf functional traits in karst rocky desertification ecological environment and the driving factors. *Global Ecol. Conserv.* 24, e01381. doi: 10.1016/j.gecco.2020.e01381

Zhang, J., Zuo, X. A., Lv, P., Yue, X. Y., and Zhang, J. (2018). Functional traits and interrelations of dominant plant species on typical grassland in the Horqin Sandy Land, China. *Arid Zone Res.* 35, 137–143. doi: 10.13866/j.azr.2018.01.17

Zhong, Q. L., Liu, L. B., Xu, X., Yang, Y., Guo, Y. M., Xu, H. Y., et al. (2018). Variations of plant functional traits and adaptive strategy of woody species in a karst forest of central Guizhou Province, southwestern China. *Chin. J. Plant Ecol.* 42, 562–572. doi: 10.17521/cjpe.2017.0270

Zhou, H., Xu, X. L., Jiang, X., Ding, B., Wu, P., and Ding, F. J. (2022). Plant functional trait responses to dolomite and limestone karst forests in southwest China. *Forests* 13, 2187. doi: 10.3390/f13122187

Zirbel, C. R., Bassett, T., Grman, E., and Brudvig, L. A. (2017). Plant functional traits and environmental conditions shape community assembly and ecosystem functioning during restoration. *J. Appl. Ecol.* 54, 1070–1079. doi: 10.1111/1365-2664.12885

Zuo, X. A., Yue, X. Y., Lv, P., Yu, Q., Chen, M., Zhang, J., et al. (2017). Contrasting effects of plant inter- and intraspecific variation on community trait responses to restoration of a sandy grassland ecosystem. *Ecol. Evol.* 7, 1125–1134. doi: 10.1002/ece3.2711

Frontiers in Plant Science

TYPE Original Research
PUBLISHED 18 April 2024
DOI 10.3389/fpls.2024.1332192

OPEN ACCESS

EDITED BY
Claudia Bita-Nicolae,
Institute of Biology Bucharest of the Romanian Academy, Romania

REVIEWED BY
Anca Manole,
Institute of Biology Bucharest of the Romanian Academy, Romania
Marius Mirodon Fagaras,
Ovidius University, Romania

*CORRESPONDENCE
Guanghui Lv
guanghui_xju@sina.com

RECEIVED 03 November 2023
ACCEPTED 03 April 2024
PUBLISHED 18 April 2024

CITATION
Teng D, Gong X, He X, Wang J, Lv G, Wang J and Yang X (2024) Impact of meteorological variability on diurnal and seasonal net ecosystem productivity in a desert riparian forest ecosystem.
Front. Plant Sci. 15:1332192.
doi: 10.3389/fpls.2024.1332192

Impact of meteorological variability on diurnal and seasonal net ecosystem productivity in a desert riparian forest ecosystem

Dexiong Teng[1], Xuewei Gong[1], Xuemin He[2,3], Jingzhe Wang[4], Guanghui Lv[2,3]*, Jinlong Wang[2,3] and Xiaodong Yang[5]

[1]CAS Key Laboratory of Forest Ecology and Management, Institute of Applied Ecology, Chinese Academy of Sciences, Shenyang, China, [2]College of Ecology and Environment, Xinjiang University, Urumqi, China, [3]Xinjiang Jinghe Observation and Research Station of Temperate Desert Ecosystem, Ministry of Education, Urumqi, China, [4]School of Artificial Intelligence, Shenzhen Polytechnic University, Shenzhen, China, [5]Department of Geography & Spatial Information Technology, Ningbo University, Ningbo, China

The desert riparian forests are susceptible to meteorological changes and contribute significantly to the net ecosystem productivity (NEP) variations of arid ecosystems. However, the responsive patterns of their NEP variations to the meteorological variabilities remain inadequately comprehended. To address this gap, we utilized seven years of eddy covariance flux measurements in a representative desert riparian forest to investigate the NEP variations and its response to changing meteorological factors across diverse temporal scales. The results revealed significant periodic variations in half-hourly NEP, with dominant cycles spanning from five hours to one year, with a principal oscillation period of one day. Key meteorological factors including global solar radiation (Rg), relative humidity (RH), air temperature (Ta), soil temperature (Ts), and vapor pressure deficit (VPD) exhibited synchronization with NEP on daily scales. This synchronization, coupled with the observed one-day periodic NEP variations, provides robust evidence supporting the existence of a circadian rhythm in the ecosystem carbon exchange of desert riparian forest regulated by meteorological conditions. Seasonal patterns were significant in the impact of Rg phase, Ta diurnal amplitude, and VPD diurnal amplitude on NEP diurnal amplitude and phase. The NEP diurnal amplitude significantly, directly, and positively affected daily NEP in both the dormant and growing seasons, whereas its phase yielded significant negative effects ($P< 0.05$). The averages, amplitudes, and phases of diurnal meteorological conditions controlled the daily NEP by regulating NEP diurnal amplitude and phase. These findings provide evidence that the variability in circadian rhythms, caused by the increase in diurnal Ta and VPD, significantly impact the daily NEP at an ecosystem scale. This study enriches our comprehension of the meteorological mechanisms governing diurnal and seasonal carbon uptake dynamics within desert riparian forests, providing fresh insights into the direct and indirect roles of climate change in shaping patterns of ecosystem carbon exchange.

KEYWORDS

arid ecosystem, eddy covariance, carbon flux, circadian regulation, structure equation model

1 Introduction

Net ecosystem productivity (NEP) measures the equilibrium between carbon uptake through photosynthesis and carbon loss via respiration, serving as a critical indicator of ecosystem carbon sequestration and stability. Variations in NEP can reflect ecosystem responses and adaptability to ongoing climate change. Arid and semi-arid ecosystems constitute about 30% of the Earth's land area and play a substantial role in the interannual variability of the global terrestrial NEP, accounting for 39% of this process (Ahlstrom et al., 2015). The Earth's land surface has experienced significant warming and an increase in atmospheric vapor pressure deficit (VPD) over the past century, particularly noting a greater rise in daily minimum than maximum temperature in many regions (Thorne et al., 2016; Yuan et al., 2019), and including the most of arid and semi-arid lands. The pronounced trend underscores the urgency for understanding the impact of climate change on NEP in these vulnerable ecosystems. However, the mechanisms whereby climate change impacts NEP in these ecosystems have not been completely comprehended. Climate change alters meteorological patterns at regional and global scales, which may significantly impact the NEP (Reichstein et al., 2013; Franklin et al., 2016; Tang et al., 2022). To comprehensively understand and accurately predict the NEP's response to climate change, an exigency exists to investigate the impact of meteorological changes at varying time scales on the NEP.

The elucidation of NEP from a meteorological perspective begins with transformations in key driving factors governing the carbon uptake process, including solar radiation, air temperature (Ta), atmospheric humidity, VPD, and soil moisture (Law et al., 2002; van Dijk et al., 2005; Yi et al., 2010). Previous investigations have mainly focused on the direct impacts of meteorological factors on NEP. For instance, excessive light during photosynthesis can induce photoinhibition in plants (Chaves, 1991), and solar radiation determined the NEP in deserts (Yu et al., 2023). Significant depletion of humidity and soil moisture led to stomatal closure and diminished photosynthetic activity (Reichstein et al., 2007; Wolf et al., 2016). Half-hour photosynthesis and respiration exhibited a nonlinear response to temperature changes (Baldocchi et al., 2001; Way and Yamori, 2013). A synchronization mechanism governed by light and temperature regulated the timing of photosynthesis and stomatal aperture in plants (Masuda et al., 2021). Although these outstanding scientific works support the direct impact of meteorological factors, only a few studies have explored their indirect impact on NEP changes, most of which disregarded the influences of amplitude and phase of the meteorology fluctuations. In contrast, these variations in meteorology (such as light, temperature, and humidity) have been suggested to affect the circadian rhythms of tree physiology (Way and Montgomery, 2015; Singh et al., 2021). The circadian rhythms of tree physiological processes, such as leaf stomatal conductance and photoperiodic responses with a period of ~24 h, were important drivers of the photosynthesis and respiration in ecosystems, although they required the passage of a few hours (Resco de Dios and Gessler, 2018). Additionally, the amplitude of diurnal NEP changes under constant environmental conditions was 20% to 90% of that under variable environmental conditions (Dios et al., 2012). The diel and seasonal dynamics of stem growth of trees at the community level were significantly influenced by temperature and VPD (Zhou et al., 2023). The diel patterns in carbon flux were expected to be the key factors in understanding stem growth (Steppe et al., 2015). On one hand, the control and manipulation of meteorological conditions at the ecosystem level for direct tests of circadian regulation response to short-term meteorological changes pose a significant challenge. On the other hand, the variation in NEP under variable conditions may be caused by several endogenous and exogenous mechanisms. Up until now, however, there has been a knowledge gap regarding how the amplitude and phase of diurnal variations in meteorology affect the NEP of arid and semi-arid ecosystems.

Desert riparian forests played a critical role in arid and semi-arid ecosystems due to their remarkable ecosystem biodiversity and carbon sequestration (DéCamps et al., 2004; Thevs et al., 2011). Tugai forests (*Populus euphratica* forests) were widely distributed in the natural oasis of the lower inland rivers and constitute the largest types of natural desert riparian forests in arid regions (Feng and Cheng, 1998). The area of these forests in China was approximately 6.49 × 10^5 hm^2, constituting 92.3% of the entire desert riparian forest area in the country (Ding et al., 2017; Wang et al., 2017; Fan et al., 2018). Due to being subjected to the severe environmental stress such as drought and salinity, Tugai forests were particularly vulnerable to variations in the meteorological environment (Jiang et al., 2022). However, their above-ground carbon density ranged from 2.24 t/hm^2 to 30.42 t/hm^2, giving them a greater carbon sink potential than other arid ecosystems such as grasslands and sparse shrublands (Liu et al., 2016). Hence, Tugai forests are ideal for studying the effects of changing meteorological conditions on the NEP of desert riparian forest ecosystems.

Utilizing approximately seven years of eddy covariance flux measurements in a representative Tugai forest, this study introduced the amplitudes and phase of daily variations to examine the direct and indirect response mechanisms of NEP to changing meteorological conditions in both the growing and dormant seasons. The objectives of this study are to explore the variation patterns of NEP and meteorological conditions in the desert riparian forests at different time scales, analyze the relationship between NEP and meteorological conditions at various time scales, and investigate the direct and indirect response mechanisms of NEP to the changes in meteorological conditions at the daily scale. This study is expected offers insights into understanding the processes of the forest ecosystems carbon cycle, thereby enhancing predictions of carbon exchange within these ecosystems under the ongoing context of climate change.

2 Materials and methods

2.1 Site description

The study was conducted at the Ebinur Lake Wetland National Nature Reserve in northwestern Xinjiang, China, from January 1, 2012 to April 20, 2019. The study area (44°37′05″–45°10′35″N, 82° 30′47″–83°50′21″E) experiences a north temperate continental arid climate, characterized by hot summers, ample sunlight, low precipitation, and cold winters. The distribution of annual precipitation is uneven, with more in summer (~50 mm) and less in winter (<10 mm). The long-term average precipitation is 105.17 mm, and the evaporation is 1315 mm (Li et al., 2022). The flux tower was positioned approximately 100 m from the north bank of the Aqikesu River (44°37′4.8″N, 83°33′59.4″E) within the nature reserve, with a construction height of 33 m (Teng et al., 2021). There are over 30 species of plants in the study area (Zhang et al., 2015). Species such as *Populus euphratica*, *Haloxylon ammodendron* and *Phragmites australis* are dominant within investigated riparian vegetation and represent over then 60% of total vegetation coverage in the studied area. Additionally, there are a variety of halophytic shrubs, herbs, and desert-specific short-growing plants, such as *Halimodendron halodendron*, *Halocnemum strobilaceum* and *Suaeda glauca*, with an average community canopy height of approximately 8.5 m (He et al., 2014). The soil has high salinity and alkalinity, with an average electrical conductivity of 5.41 mS/cm in shallow soil layer (0–10 cm) and a pH value of 8.77. The average soil density is about 1.38 g/cm^3 (Li et al., 2022). Throughout the observation period, the flux tower was located in an area with an average annual temperature of 9°C, with maximum temperatures reaching 43°C and minimum temperatures dropping to −26°C. Groundwater served as the primary water source for the plants in the Tugai forest ecosystem, with a groundwater depth of 1.50-2.30 m (Yang et al., 2014).

2.2 Eddy covariance and meteorological measurements

The eddy covariance (EC) observation system was positioned 15 m above the ground. It comprises a three-dimensional ultrasonic anemometer (CSAT3, Campbell Scientific Ltd., Logan, UT, USA) and an infrared CO_2/H_2O analyzer (EC150, Campbell Scientific Ltd., Logan, UT, USA). Global radiation was monitored using a 4-component net radiometer (NR01, Campbell Scientific Ltd., Logan, UT, USA) installed at a height of 9 m. Additionally, the meteorological observation system includes air temperature and humidity sensors (HMP155A-L, Campbell Scientific Ltd., Logan, UT, USA), wind speed sensors (010C-1, Met One Instruments Inc., Grants Pass, OR, USA), wind direction sensors (020C-1, Met One Instruments Inc., Grants Pass, OR, USA), and an atmospheric pressure meter (CS100, Campbell Scientific Ltd., Logan, UT, USA). These instruments are programmed to automatically record routine meteorological data, such as average wind speed, temperature, and air pressure every 30 minutes.

2.3 Flux calculations and data processing

2.3.1 Flux calculation

Assuming horizontal homogeneity, the net ecosystem CO_2 exchange (NEE) in μmol m^{-2} s^{-1} can be estimated as follows (Baldocchi, 2003; Wang et al., 2016):

$$NEE = F_c + F_s = \overline{\rho_d} \cdot \overline{w'\chi_c{}'} \mid_h + \frac{\Delta\chi_c \cdot \overline{\rho_d} \cdot h}{\Delta t}. \tag{1}$$

In Equation 1, F_c represents the turbulent flux at the interface between the atmosphere and the ecosystem. F_s corresponds to the CO_2 storage flux within the control volume. w stand for the vertical wind speed. χ_c denotes the CO_2 molar mixing ratio (μmol mol^{-1}). ρ_d represents the dry air concentration (mol m^{-3}). h is the flux observation height (m); $\Delta\chi_c$ indicates the difference between the pre- and post- CO_2 molar mixing ratio measured at two adjacent moments. Δt is the time interval between the first and second measurements (30 min).

The turbulent fluxes (F_c) were calculated from high-frequency measurements. Raw data files were processed using EddyPro software (Version 6, LI-COR Inc., Lincoln, NE, USA) to calculate the F_c on a half-hourly basis. The flux processing included two-dimensional coordinate rotation, spectral corrections, frequency response correction, and Webb-Pearman-Leuning correction for the effect of air density fluctuations (Webb et al., 1980). The quality assessment for F_c data were conducted a steady-state/developed turbulence conditions test in 0-2 system (Mauder et al., 2006). The CO_2 storage (F_s) was estimated using the single-point method (Hollinger et al., 1994).

2.3.2 Data processing

The NEP is analogous to net photosynthesis in leaves, and is considered positive when the ecosystem exhibits net carbon uptake, indicating a carbon sink. It is the opposite of NEE (Lovett et al., 2006), that is $NEP = -NEE$. As ecosystem carbon exchange processes are highly dependent on diurnal rhythms and phenological laws, we identified the beginning and end of the day and the beginning and end of the growing season. The solar elevation angle was used to define the diurnal period as from sunrise to sunset. As presented in Table 1, the start and end of the growing season were determined based on the growing degree days (GDDs), and the formula was as follows (McMaster and Wilhelm, 1997):

$$GDD = \frac{1}{2}(T_{max} + T_{min}) - T_{base}, \tag{2}$$

In Equation 2, T_{base} was 6°C in this study.

The gaps in carbon flux measurements amounted to 59,906 half-hour data (approximately 46.52% of the total), and these gaps were primarily concentrated during the dormant season, especially during nighttime hours. Surprisingly, the number of data gaps during nighttime in the dormant season was more than twice as much as during nighttime in the growing season. Conversely, the high-quality data (Qc=0) accounted for 35,852 half-hour data (approximately 27.84% of the total), and were predominantly record during the daytime in the growing season. Remarkably, the amount of high-quality flux data during nighttime in the growing season was comparable to that during nighttime in the dormant season (Table 2).

In this study, distinct frictional velocity (u_*) thresholds and their corresponding confidence intervals were estimated separately for the growing and dormant seasons, considering various wind direction intervals (30°). The carbon flux data underwent rigorous screening based on the criteria outlined in Table 3, leading to the exclusion of any data that failed to meet these conditions. Finally,

TABLE 1 The starting and ending date of growing season at observation stations.

Year	Start (DOY)	End (DOY)	Duration (Day)
2012	113	283	171
2013	99	290	192
2014	120	279	160
2015	110	286	177
2016	119	283	165
2017	120	275	156
2018	99	284	186
2019	104		

the gap-filling procedure utilized the random forest model, achieving an impressive R^2 value of 0.81.

2.3.3 Amplitude and phase of the variations in NEP and meteorological conditions

To comprehensively describe the diurnal variations in NEP and meteorological factors, two key parameters were computed: amplitude and phase. Circadian regulation is known to result in time changing maximal and minimal potential values, as depicted in Figure 1. Assuming that circadian effects have an additive interaction with the mean value of the parameter of interest (Resco de Dios and Gessler, 2018), the models for circadian variations can be expressed as follows:

$$y = y_m + y_A \sin\left(\frac{2\pi t}{24} + \Phi_y\right). \tag{3}$$

In Equation 3: y represents NEP or meteorological factors; y_m denotes the mean value of y; t stands for time; and y_A and Φ_y represent the amplitude and phase of half-hourly NEP or meteorological factors variations in a daily scale, respectively. The variation in amplitude signifies the asymmetric changes in meteorological factors and the differences in diurnal and nocturnal variations in NEP (where daytime increases or decreases differ in magnitude from nighttime). On the other hand, the variation in phase denotes the changes in the timing of daily maximum or minimum values. These two parameters offered a clearer understanding of the daily patterns of carbon uptake and meteorological changes in the ecosystem.

2.4 Statistical analysis

2.4.1 Magnitude squared coherence and transfer function

The Fourier transform was applied to the time series of NEP and meteorological factors, and subsequently, the power spectrum of the time series is calculated and analyzed in terms of magnitude-squared coherence (MSC) and transfer function variation periods. MSC, a signal processing tool that yields a real value ranging from zero to one, is employed to identify significant frequency-domain correlations between two time series (Dobie and Wilson, 1989). It quantifies the degree to which two time-domain signals, $x(t)$ and $y(t)$, exhibit similarity or match each other.

To estimate the synchronization of two time series, the linear correlation in the spectral decomposition of $x(t)$ and $y(t)$ is followed by calculating the $MSC_{xy}(f)$ values at different frequencies using the equation:

$$MSC_{xy}(f) = \frac{|P_{xy}(f)|^2}{P_{xx}(f)P_{yy}(f)}, \tag{4}$$

In Equation 4, P_{xy} is the cross power spectral density of $x(t)$ and $y(t)$, and P_{xx} and P_{yy} are the associated power spectral densities.

In this study, Welch's mean-corrected periodogram method is utilized to calculate the power and mutual power spectral densities between $x(t)$ and $y(t)$. This method involves dividing the two signals into time windows with the same number of samples, calculating the power spectral density for each window, and then averaging them to obtain the final $MSC_{xy}(f)$ values at different frequencies.

A transfer function is a complex quantity whose magnitude and phase are a function of frequency. Referred to as the system function, it describes the transfer behavior of a linear system in the frequency domain, with the output being represented by $H(t)$ and the input represented by the z-transform of the impulse response $H(z)$.

The transfer function is given by the equation:

$$H(z) = \frac{Y(z)}{X(z)}, \tag{5}$$

In Equation 5, $Y(z)$ represents the output transformation and $X(z)$ represents the input transformation. The transfer function $H(z)$ captures the transfer characteristics of the system, as it multiplies the input transformation $X(z)$ to obtain the output transformation $Y(z)$. The transfer function can be derived from simple algebraic

TABLE 2 Statistics on half-hour carbon flux data.

	Growing season daytime	Growing season nighttime	Dormant season daytime	Dormant season nighttime	Total
NA	13750	8817	15449	21890	59906
Qc=0	14765	6833	8221	6033	35852
Qc=1	4286	5416	4822	7807	22331
Qc=2	1728	2554	2225	4077	10584
Total	34529	23620	30817	39807	128773

TABLE 3 Quality control process of carbon flux data.

Procedure	Volume of data		Detailed description
	Growing season	Dormant season	
Quality assessment	22567	37339	Based on the quality assessment of flux data, carbon flux data with null values and Qc of 2 were rejected.
Weather anomalies	36	152	Carbon flux data from precipitation over the observation period were excluded.
Mutation filtering	121	157	The mutation points in continuous flux data were detected and rejected.
Statistical distribution cleaning	360	468	Outliers in the flux data, counted at different times of the day, were eliminated.
u_* threshold filtering	3349	3575	Nighttime observations below the u_* thresholds were excluded.

operations that describe the differential equations of the system, or it can be determined experimentally to understand the transfer behavior of the system.

2.4.2 Structural equation model

Structural equation model (SEM), including path analysis, confirmatory factor analysis, and latent growth curve models, is primarily employed to examine multivariate interactions (McIntosh et al., 1996). As a relatively complex statistical model, SEM requires a sample size of at least 200 to yield reliable results (Fan et al., 1999). This is due to the fact that more intricate models necessitate larger samples to achieve statistical power. Furthermore, due to certain mathematical restrictions that limit the complexity of the multivariate model, a balance must be struck between model complexity, precision, and interpretability when employing SEM. We use path analysis to analyze the covariance between NEP and observed variables, with the structure of the model being driven by assumptions about causal relationships between multiple variables. For all statistical analyses, we used R version 4.2.0 (R Core Team, 2022).

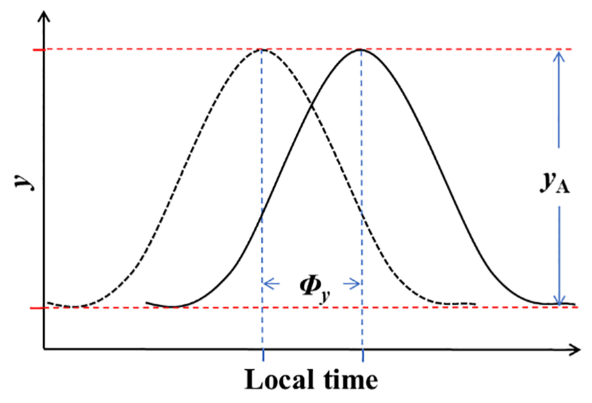


FIGURE 1
The regulation of NEP and meteorological conditions by amplitude (y_A) and phase (Φ_y) on a daily scale.

3 Results

3.1 Environmental conditions and net ecosystem production

During the study period, the dynamics of Ta_m, Ts_m, VPD_m, and Rg_m above the forest canopy exhibited a symmetrical pattern between the growing and dormant seasons (Figure 2). Notably, Rg_m, VPD_m, Ta_m, and Ts_m showed significant seasonal variations, with higher values recorded in the growing season and lower values in the dormant season. During the dormant season, the RH_m was higher than compared to the growing season. The Ta_m during the study period was 8.83°C, with a range of variation from −28.04°C to 34.13°C; VPD_m ranged from 0.02 kPa to 4.17 kPa, with the maximum and minimum values observed in July and January, respectively.

The results of the spectral analysis performed on the NEP and meteorological factor time series of the Tugai forest were presented in Figure 3. It is evident that both NEP and meteorological factors exhibit multiple periodicities, ranging from five hours to one year. Except for Rg, the amplitudes of fluctuations in both NEP and meteorological factors also increased when the period length exceeded one day but remained less than 40 days. When the periods of variability in NEP and meteorological factors were equal to or less than one day, the amplitude of fluctuations tended to become more pronounced with increasing period length. The NEP variations exhibited the strongest correlation with meteorological conditions in the one-day cycle. This observation implied that NEP in the Tugai forest followed a circadian rhythm.

3.2 Interactions between net ecosystem production and environmental variation

After conducting cross-spectral analysis of Rg, RH, Ta, Ts, and VPD with NEP, it was evident that these five meteorological factors exhibited a strong coherence with NEP (Figure 4). This coherence was particularly pronounced for long periods of 12 hours, 1 day, and 1 year, while it was relatively weaker for short periods. The cross-spectral analysis of meteorological factors with NEP partially explained the multiple periodic variation patterns of NEP. The transfer function amplitudes of these meteorological factors and NEP all displayed variation periods of 6 hours, 8 hours, 12 hours, and 1 day. Additionally, the greatest magnitude of the MSC was observed at the 1 d period. This implies that there is a strong synchronous variation of NEP with meteorological factors,

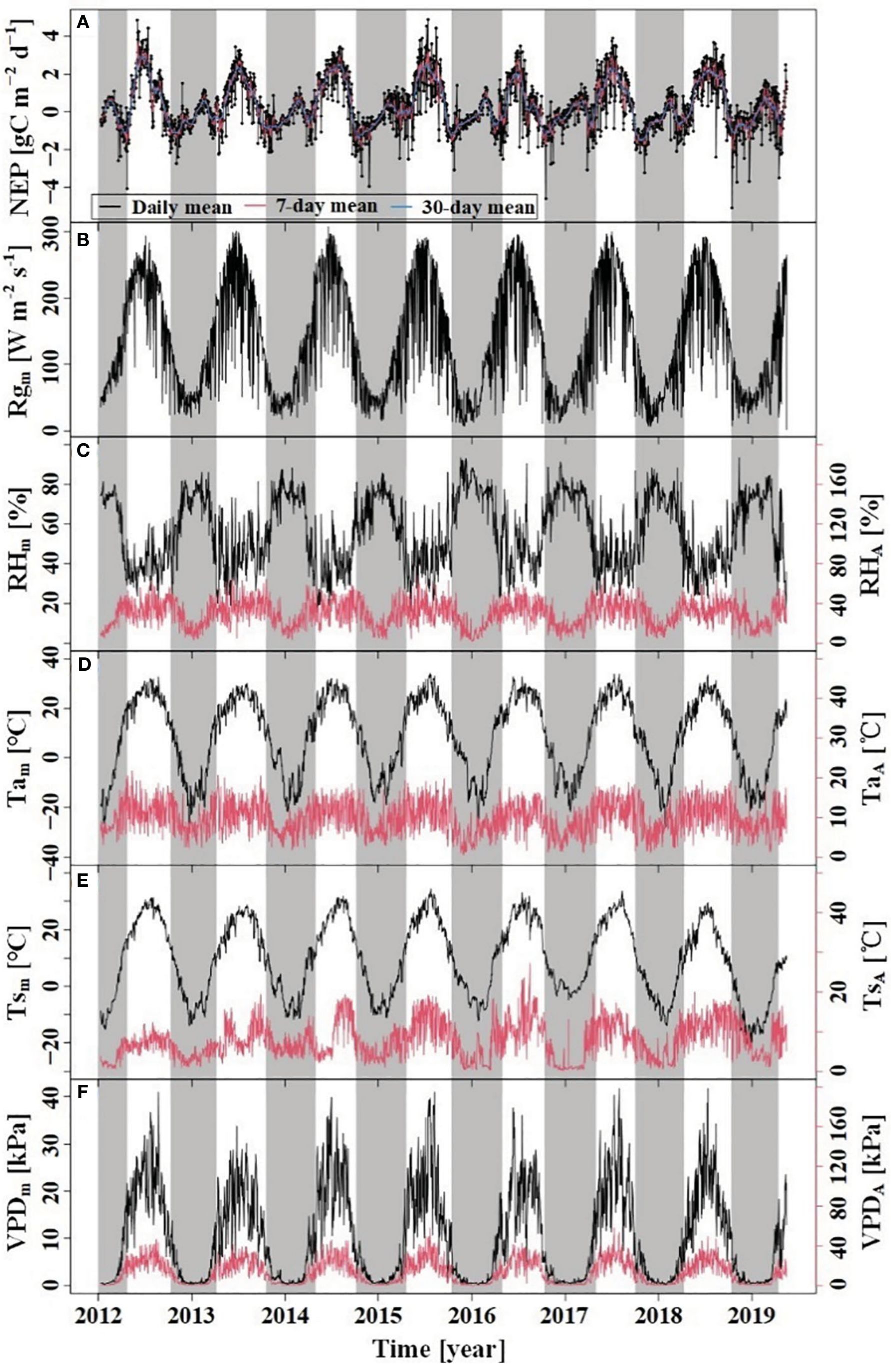


FIGURE 2
Variations of NEP and meteorological factors. **(A)** NEP: net ecosystem productivity; **(B)** Rg_m: daily mean of Rg; **(C)** RH_m: daily mean RH, RH_R: amplitude of diurnal RH; **(D)** Ta_m: daily mean Ta, TaR: amplitude of diurnal Ta; **(E)** Tsm: daily mean Ts, TsR: amplitude of diurnal Ts; **(F)** VPDm: daily mean VPD, VPD_R: amplitude of diurnal VPD. The grey region indicates the dormant season and the white indicates the growing season.

especially at the daily scale. Therefore, the circadian rhythm of NEP in the Tugai forest was regulated by meteorological conditions.

In order to investigate the response of CO_2 uptake capacity in the desert Tugai forest ecosystem to changes in the meteorological conditions, we conducted an analysis of the relationship between daily NEP and meteorological factors. Our findings suggested the Φ_{NEP} and Φ_{Rg} demonstrated negative correlations with daily NEP both during the dormant and growing season (Figures 5A, 6B). Conversely, the NEP_A and Rg_m significantly positively correlated with daily NEP (Figures 5B, 6A). This indicated that the higher the asymmetry in the diurnal changes of NEP, the higher the daily NEP value, as a result of a higher peak in diurnal NEP.

We observed a weak negative correlation between the RH_m and daily NEP in the growing season (R^2 = 0.147), but no significant correlation was found in the dormant season (Figure 7A). In contrast to RH_m, Ta_m, Ts_m, and VPD_m exhibited weak positive

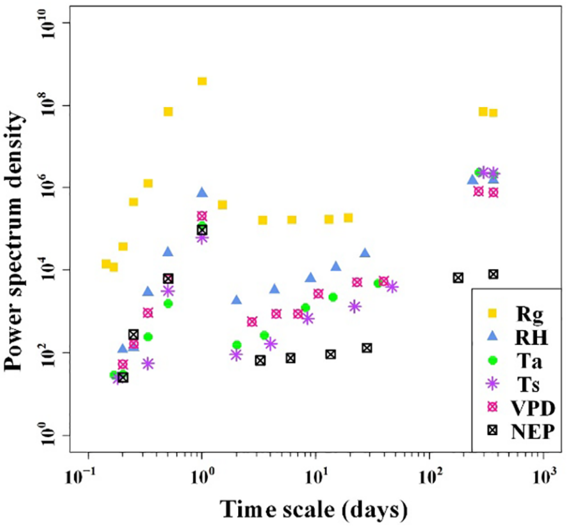


FIGURE 3
Power spectrum densities of half-hourly NEP and meteorological factors over the duration of monitoring.

correlations with NEP in the growing season, while weak negative correlations were observed in the dormant season (Figures 8A–10A). The RH_A showed no significant correlation with NEP in both the growing and dormant seasons (Figure 7B). The significant effect of the VPD_A changes on NEP during the growing season suggested that asymmetric variations in diurnal VPD significantly affect the carbon uptake of Tugai forest ecosystems (Figure 10B).

In this study, the analysis of seasonal differences in the correlation between NEP and driving factors unveiled significant seasonal variations in NEP's response to meteorological factors (likelihood ratio > 26 and $P< 0.001$, see Supplementary Material). The seasonal difference in magnitude and direction of response were observed. This implied that there were seasonal differences in the synchronization between NEP and meteorological factors at the daily scale.

3.3 The impact of meteorological variations on net ecosystem production

The results of the SEM demonstrating the influence of meteorological factors on NEP during the dormant and growing

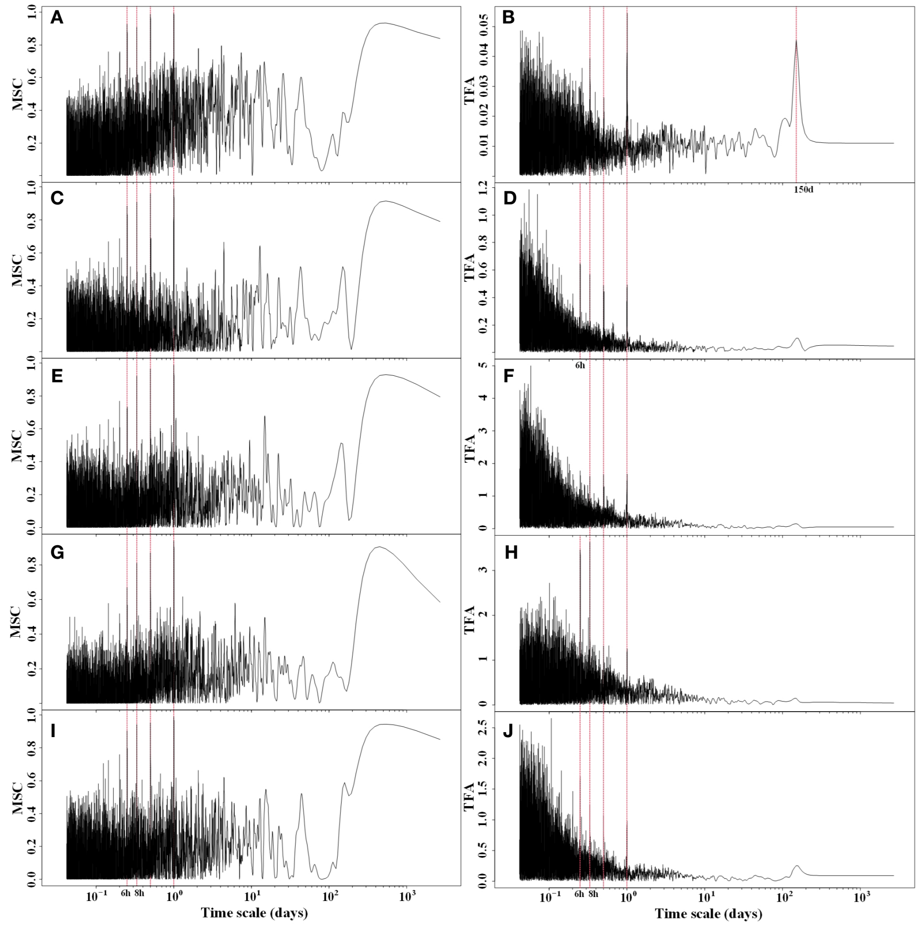


FIGURE 4
Magnitude squared coherence (MSC) and transfer function amplitude (TFA) between half-hourly NEP and **(A, B)** Rg, **(C, D)** RH, **(E, F)** Ta **(G, H)** Ts, and **(I, J)** VPD.

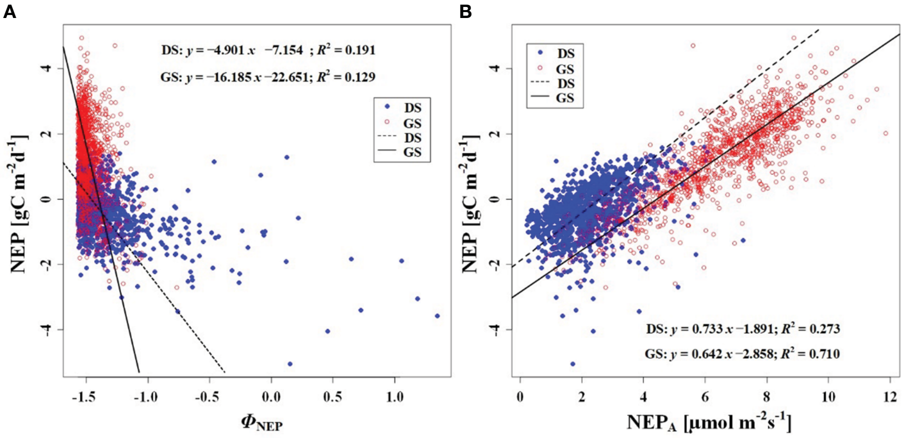


FIGURE 5
Relationship between **(A)** NEP and phase of NEP (Φ_{NEP}), and **(B)** between NEP and NEP$_A$. DS and GS indicate the dormant and growing seasons, respectively.

seasons were presented in Figure 11; Table 4. Through correlating NEP with the influencing factors, the models were meticulously fitted and tested to establish statistically significant direct and indirect effects while gradually eliminating statistically insignificant pathways. For the dormant season, the fitted R^2 was 0.551, the RMSEA was 0.029 ($P = 0.160$), and the CFI was 1.000. In the growing season, the corresponding values were 0.780, 0.021 ($P = 0.222$), and 1.000, respectively. Overall, the results indicated that both models were suitable, with the structural equation model for the growing season slightly outperforming that for the dormant season.

In the SEM, during the dormant season, Ta$_m$, Rg$_m$, RH$_m$, VPD$_m$, and Ta$_A$ had significant direct effects on Φ_{NEP}. In contrast, during the growing season, Φ_{Rg}, Rg$_m$, and RH$_m$ had significant direct effects on Φ_{NEP} ($P< 0.10$, in descending order of relative importance). For NEP$_A$ during the dormant season, Rg$_m$, Ts$_m$, VPD$_m$, VPD$_A$, and Ta$_m$ had significant direct effects, while during the growing season, Ta$_m$, Rg$_m$, VPD$_m$, Φ_{NEP}, Ta$_A$, and Φ_{Rg} had significant direct effects ($P< 0.10$, in descending order of relative importance). The Ta$_A$ significantly influenced the Φ_{NEP} in Tugai forests during the growing season, with no notable impact on the NEP$_A$. However, this pattern may be inverted during the dormant season, the Ta$_A$ could affect NEP$_A$ without influencing Φ_{NEP}. Notably, both during the dormant and growing seasons, Ts$_A$ had no statistically significant direct effect on Φ_{NEP} ($P > 0.10$), RH$_m$ had no statistically significant direct effect on NEP$_A$, and Φ_{Rg} had no statistically significant direct effect on daily NEP. Rg$_m$ had significant indirect effects on daily NEP through the NEP$_A$ and Φ_{NEP} during both the dormant and growing seasons ($P< 0.10$). This underscored the critical roles that the amplitude and phase of diurnal NEP played in regulating the light response of NEP.

In addition to the direct effects of Φ_{NEP} on daily NEP during both the dormant and growing seasons, the Φ_{NEP} impacted daily NEP by reducing the NEP$_A$ during the growing seasons, resulting in significant negative total effects on NEP ($P< 0.05$). This suggested that regulating the phase of ecosystem circadian rhythms reduced the daily NEP by lowering its peak of net carbon uptake. It's worth noting that the total effects of Φ_{Rg} on daily NEP were not

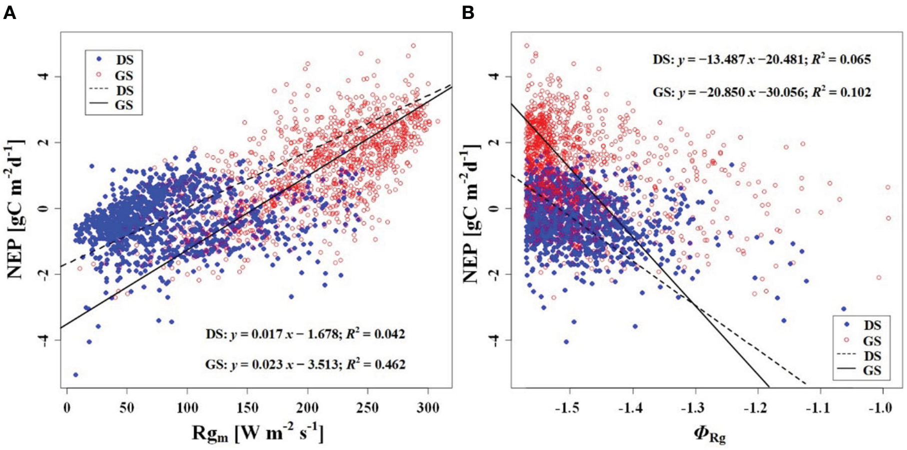


FIGURE 6
Relationship **(A)** between NEP and Rg$_m$, and **(B)** between NEP and phase of Rg (Φ_{Rg}). DS and GS indicate the dormant and growing seasons, respectively.

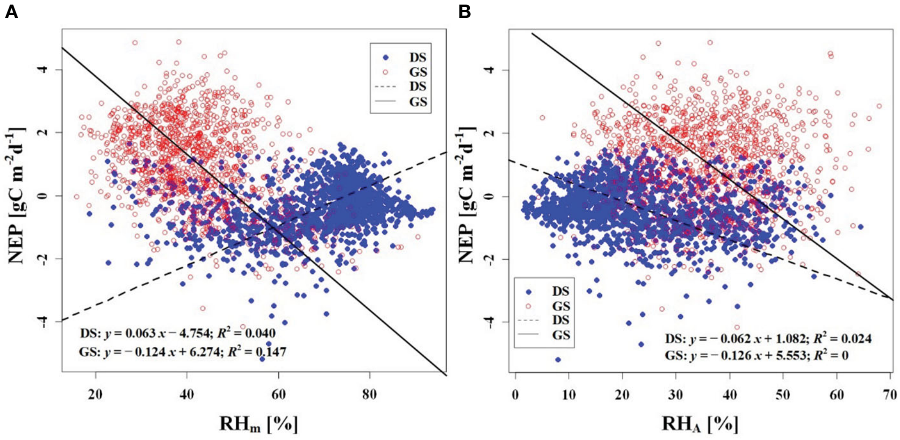


FIGURE 7
Relationship **(A)** between NEP and RH_m, and **(B)** between NEP and RH_A. DS and GS indicate the dormant and growing seasons, respectively.

statistically significant ($P > 0.1$). Seasonal differences were observed in the effect directions of RH_m on daily NEP, with direct effects being predominant during both the dormant and growing seasons. The effects of VPD_m on daily NEP were primarily indirect during the growing seasons. Reductions in the VPD_A may lead to increased daily NEP in Tugai forests during the growing season. Conversely, this relationship may be reversed during the dormant season. The VPD_A exerted a negative indirect effect on daily NEP through NEP_A during both the dormant and growing seasons. This indirect effect counterbalanced the positive direct effect of the changing amplitude in water vapor on daily NEP during the dormant season but intensified during the growing season. Daily NEP was more sensitive to the changes in VPD_A than to the variations in Ta_A. This finding suggests that the daily-scale variation in VPD has a more pronounced impact on the daily NEP within the ecosystem than the daily-scale fluctuations in air temperature.

The results of the SEM analysis comparing the dormant and growing seasons revealed that an increase in NEP_A led to an increase in daily NEP during both seasons. Conversely, an increase in the Φ_{NEP} contributed to a decrease in NEP_A, resulting in decreased daily NEP. Reductions in the Ta_A may lead to decreased daily NEP in Tugai forests during both the growing and dormant season. During the dormant season, an increase in Ta_A indirectly led to an increase in daily NEP by negatively affecting Φ_{NEP}, while during the growing season, the increase in VPD_A caused a decrease in NEP. This suggested that regulated the daily NEP during the dormant season, while water availability limited the daily NEP during the growing season. The variation in the indirect and direct effects of daily changes in meteorological factors highlighted the seasonal differences in the mechanisms by which the NEP of Tugai forests influenced meteorological conditions, as well as differences in the response to various meteorological factors. The indirect effects of daily changes in meteorological factors through NEP_A and Φ_{NEP} on daily NEP indicated that the diurnal variability in meteorological conditions influenced the daily NEP of Tugai forests by regulating the circadian rhythm of this ecosystem.

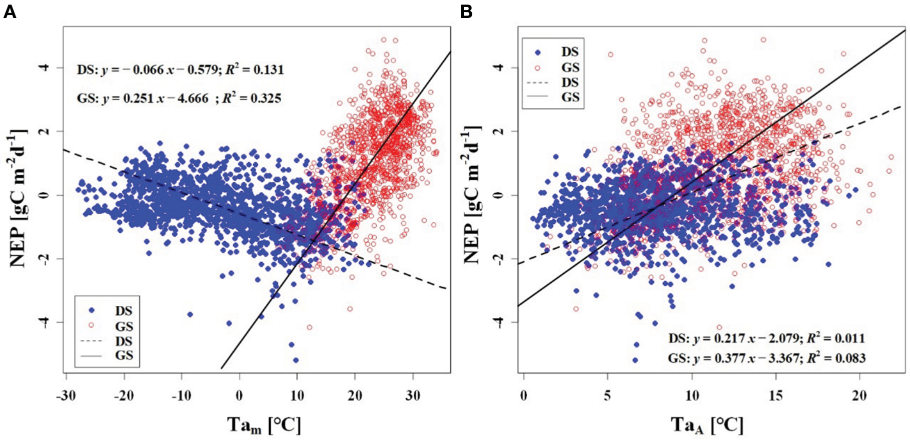


FIGURE 8
Relationship **(A)** between NEP) and Ta_m, and **(B)** between NEP and Ta_A. DS and GS indicate the dormant and growing seasons, respectively.

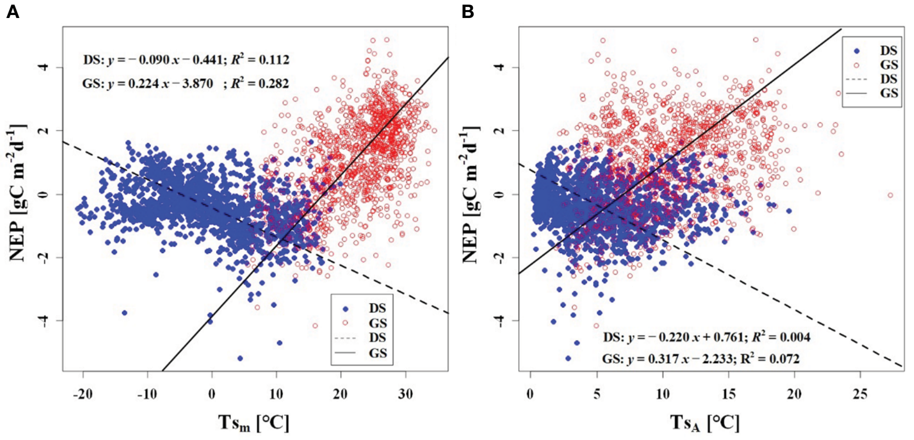


FIGURE 9
Relationship **(A)** between NEP and Ts_m, and **(B)** between NEP and Ts_A. DS and GS indicate the dormant and growing seasons, respectively.

4 Discussion

4.1 Response of NEP to variations in meteorological conditions at different time scales

Ecosystem carbon exchange has been reported to exhibit daily, weekly, and monthly cycles of variability (Baldocchi et al., 2001; Stoy et al., 2005). Consistent with these established findings, our own observations similarly revealed periodic variations in NEP (Figure 3). The diurnal NEP fluctuations are likely attributable to the photoperiod resultant from the Earth's rotation. The discerned 11-day and 14-day NEP periodic variations might potentially find explanation in the seiches of Ebinur Lake, as analogous courses of variation were evident in the meteorological factors, particularly in RH (Figure 3). The periodic variations in NEP and meteorological factors highlight the complex interactions between lake dynamics and meteorological conditions in shaping the carbon exchange dynamics of the desert riparian forest ecosystems. Remarkably, this phenomenon has been identified for the first time in our study. Consequently, we advocate for an integrated approach in investigations of ecosystem carbon exchange within regions influenced by lakes, necessitating the incorporation of the lakes' impact on local climate.

Within the Tugai forest, we have observed a striking and significant synchronization between NEP and meteorological conditions, underscoring the prominent influence of these conditions on ecosystem carbon exchange. The MSC and TFA between NEP and meteorological factors in the Tugai forest suggested that NEP has the robust synchronization with meteorological conditions on the daily scale. The findings suggested a close alignment with the light response of ecosystem carbon exchange at daily scale (Figure 4). It's worth noting that prior studies have illuminated the presence of a desert-oasis effect in the desert riparian forests (Li et al., 2016; Teng et al., 2021), which significantly influences the daily variability of meteorological conditions. This effect further bolsters the synchronization observed between NEP and meteorological factors on a daily

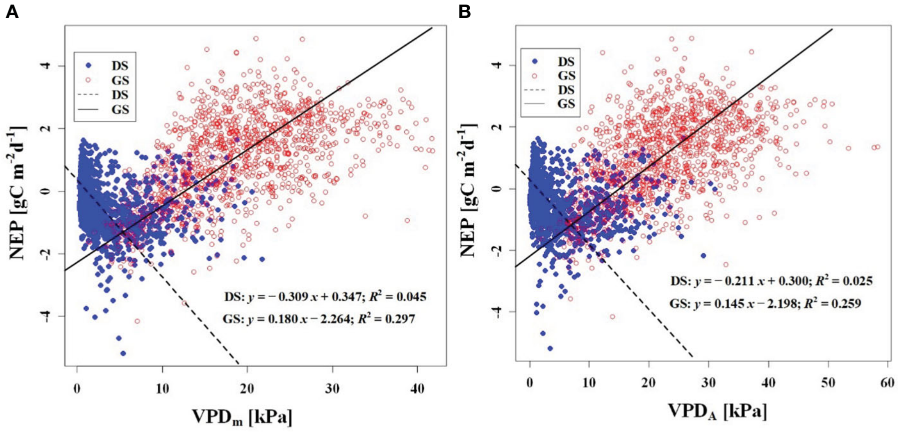


FIGURE 10
Relationship **(A)** between NEP and VPD_m, and **(B)** between NEP and VPD_A. DS and GS indicate the dormant and growing seasons, respectively.

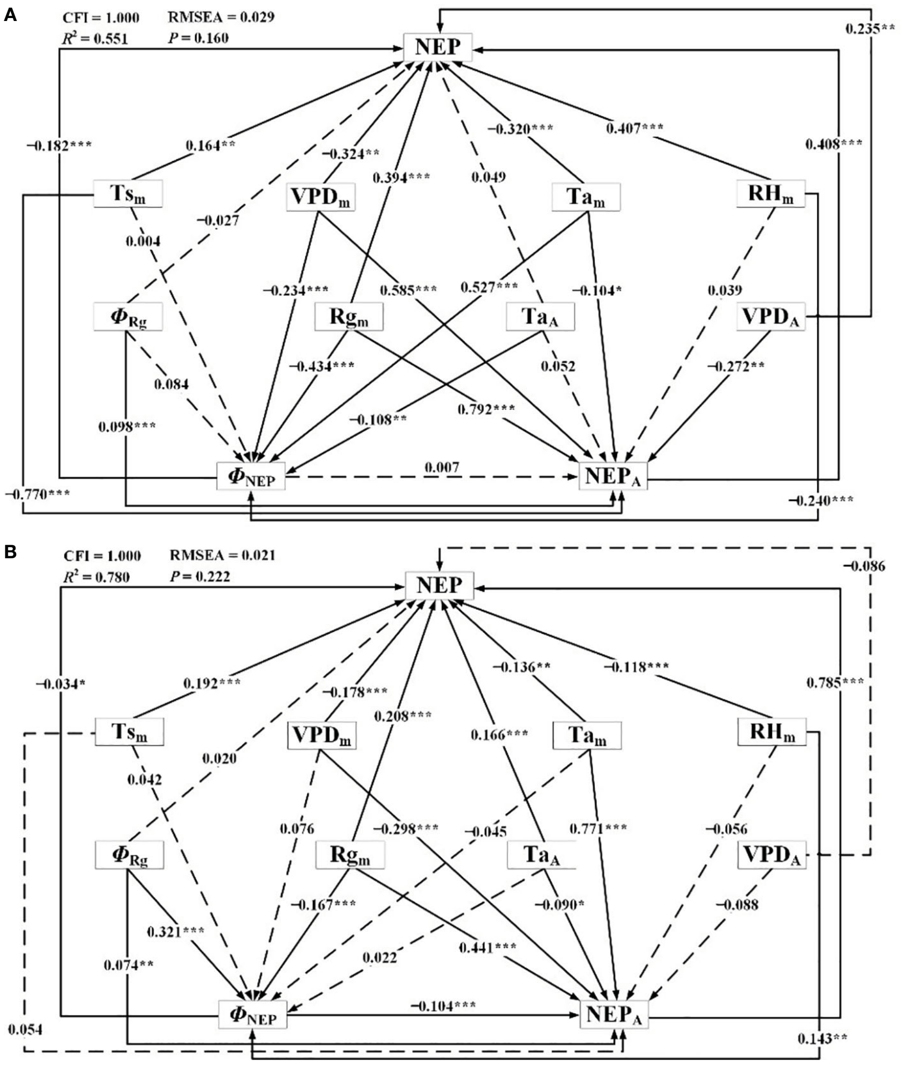


FIGURE 11
Structural equation model representing connections between net ecosystem productivity and meteorological factors during **(A)** the dormant and **(B)** growing season. Φ_{NEP}: phase of diurnal NEP, NEP_A: amplitude of diurnal NEP, Ts_m: daily mean Ts, Ta_m: daily mean Ta, Ta_A: amplitude of diurnal Ta, Rg_m: daily mean Rg, Φ_{Rg}: phase of diurnal Rg, RH_m: daily mean RH, VPD_m: daily mean VPD, VPD_A: amplitude of diurnal VPD. *$P<0.10$, **$P<0.05$, and ***$P<0.01$.

scale. Similarly, there was high synchronization between NEP and temperature at daily scale. In addition, there was a significant response of NEP in Tugai forest to mean daily temperature and its daily variation during both growing and dormant seasons. The reason is that temperature plays a crucial role in ecosystem carbon exchange as it significantly influences metabolism (Anderson-Teixeira et al., 2011). In arid and hot regions, high temperatures intensify evaporation and reduce water availability for plants, impairing carbon sequestration of trees by reducing photosynthesis and increasing respiration (Adams et al., 2009). Additionally, sustained elevation in high temperatures accelerates respiration rate and causes enzyme denaturation, further hindering the carbon sequestration capacity of plants (Banbury Morgan et al., 2021).

The response of NEP to meteorological conditions is intricate due to the differential sensitivities of plant leaves, roots, and soil microorganisms. Leaves exhibit increased photosynthesis with rising temperature, while respiration is regulated by stomatal conductance, resulting in a dynamic and adaptive response. During the dormant season, we observed weak correlations between NEP and daily average meteorological factors. However, during the growing season, NEP in the Tugai forest exhibited high sensitivity to daily variations in RH (Figure 7). This sensitivity arises from short-term changes in canopy RH that often coincide with thickening cloud cover or rainfall, leading to reduce solar radiation availability and, subsequently, decreased net CO_2 uptake. These findings suggest that there were significant differences in response mechanisms of the Tugai forest ecosystem carbon exchange to changing meteorological conditions at various time scales.

In this study, we observed significantly diurnal variations in meteorological conditions and NEP of the desert riparian forest. The diurnal variations in light, temperature, and humidity regulate the circadian rhythms of plant physiological processes and rhizosphere microbial communities, leading to synchronous changes in photosynthesis and respiration, which in turn strongly correlate with NEP at the daily scale (Gil and Park, 2019). Thus, we conclude that meteorological factors remained the primary drivers of ecosystem carbon exchange of the desert riparian forest at short time scales, both half-hourly and daily, which is consistent with previous studies (López-Blanco et al., 2017; Ma et al., 2017). It should be noted that the synchronization of meteorological factors with NEP does not follow a linear pattern across various time scales (Figure 3). Overall, the findings contribute to understanding the meteorological influences on NEP in the desert riparian forest ecosystem, highlighting the significance of light, temperature, and humidity dynamics at different time scales.

4.2 The effects of meteorological conditions on NEP at daily scale

Ecosystem carbon exchange is composed of two fundamental components: photosynthesis and ecosystem respiration. It's important to note that forest canopy photosynthesis does not adhere to a linear relationship with absorbed effective solar radiation. Moreover, its sensitivity to solar radiation is recognized to be influenced by the proportion of diffuse radiation. As a result, exercising caution becomes imperative when attempting to employ uncomplicated linear light-response models to replicate photosynthesis across both temporal and spatial scales (Running et al., 1999; Heinsch et al., 2006). Daily integration of the independent and dependent variables on a daily basis can effectively linearize the complex relationship between half-hourly photosynthesis and light (Leuning et al., 1995). This process of linearization significantly bolsters the SEM model of NEP response to meteorological factors that has been constructed within the framework of this study.

As a temperate desert riparian forest, the ecosystem respiration and photosynthesis exhibited significant variations in response to temperature fluctuations. Moreover, the NEP in the Tugai forest was dominated by gross primary productivity (GPP) during the growing season and by ecosystem respiration (R_{eco}) during the dormant season, leading to notable seasonal variations in NEP with temperature. When the daily Ta rises and the Ta_A decreases, the increase in nighttime Ta was greater than the increase in daytime Ta (Thorne et al., 2016). This asymmetric warming leads to increasing soil microbial activity, soil organic carbon decomposition, and ecosystem carbon emissions, resulting in a decrease in NEP (Xia et al., 2014). This is consistent with the response of NEP to temperature in this study. The inverse relationships between temperature (including atmospheric and soil temperature) and NEP in different seasons (Figures 8, 9) further demonstrated the seasonal variations in carbon exchange processes in the Tugai forest ecosystem. The direct effect of atmospheric temperature on NEP was seasonally distinct (dormant season was larger than growing season), but both exhibited a significant negative effect; whereas the indirect effect was not only seasonally disparate (dormant season was larger than growing season), but also displayed an opposite pattern (Table 4). The main reason for the differences in NEP response to temperature under different seasons is the different ecosystem processes that dominate CO_2 exchange. During the dormant season, CO_2 exchange in the Tugai forest ecosystem was primarily driven by carbon emissions from ecosystem respiration. During the growing season, when the temperature increases, soil respiration in the Tugai forest ecosystem was augmented along with ecosystem photosynthesis. Due to the Kok effect, the respiration rate of plant leaves decreased during the daytime, resulting in an increase in NEP (Kok, 1948, Kok, 1949; Yin et al., 2020).

Ecosystem-scale indirect meteorological forcings play a crucial role in shaping the NEP over the long term. One of the key factors contributing to this indirect effect is the sensitivity of the NEP to diurnal fluctuations in temperature and humidity, especially when plants are exposed to diverse temperature and humidity regimes. In this study, we identified temperature as the main limiting factor for NEP in the Tugai forest during the dormant season. Increasing temperatures enhanced ecosystem respiration, resulting in a decrease in ecosystem carbon uptake. Conversely, during the growing season, VPD emerged as the primary limiting factor for NEP. Although VPD had no direct significant effect on NEP in this study, it exerted an indirect significant influence (Table 4). The Tugai forest, located near riverbanks within an arid desert zone, experiences a warm growing season, often exceeding the optimal temperature for photosynthesis. Simultaneously, VPD levels typically surpass the optimal threshold, thereby regulating leaf stomata and inhibiting carbon uptake. On one hand, under favorable environmental conditions characterized by stability and less variability in meteorological factors, plants allocate more resources to growth and carbon assimilation, leading to an increase in net carbon uptake. On the other hand, unfavorable environmental conditions with unstable and highly variable meteorological factors can pose challenges to plants. In such conditions, plants may experience temperature stress and limited water availability, resulting in a higher rate of forward and reverse biochemical reactions (McDowell et al., 2008). These conditions can even lead to the denaturation of enzymes, ultimately reducing carbon uptake.

The intricate interplay between alterations in plant physiology and fluctuations in diurnal Ta and VPD contributes to the observed indirect effects on NEP. A comprehensive understanding of these complex interactions involving Ta and VPD is pivotal for comprehending the responses of ecosystem carbon exchange to environmental drivers. This study emphasizes the importance of accounting for the indirect effects of rising diurnal Ta and VPD when analyzing the seasonal variation of NEP in the desert riparian forest.

4.3 The uncertainty analysis and future prospects

In this study, we measured the NEP using the open path eddy covariance system. It is well known that the sensor-path heat exchange (SPHE) and analyzer temperature sensitivity reduce the ability of the open path eddy covariance system to characterize the response of ecosystem carbon exchange to radiative forcing (Burba et al., 2008). The effect of SPHE cannot be completely eliminated using current correction methods, such as the WPL correction,

TABLE 4 Direct and indirect effects of meteorological factors on NEP during the dormant and growing season.

Factors	Dormant season			Growing season		
	Direct effect	Indirect effect	Total effect	Direct effect	Indirect effect	Total effect
Φ_{NEP}	−0.182***	0.003	−0.179***	−0.034	−0.081***	−0.116***
NEP_A	0.408***		0.408***	0.785***		0.785***
Ts_m	0.164**	−0.315***	−0.151***	0.192***	0.047	0.239***
Ta_m	−0.320***	−0.142***	−0.457***	−0.136**	0.610***	0.474***
Ta_A	0.049	0.040*	0.090**	0.166***	−0.073*	0.093*
Rg_m	0.394***	0.401***	0.795***	0.208***	0.365***	0.573***
Φ_{Rg}	−0.027	0.025*	−0.002	0.020	0.021	0.041
RH_m	0.407***	0.059**	0.466***	−0.118***	−0.061*	−0.179***
VPD_m	−0.324**	0.281***	−0.043	−0.178***	−0.243***	−0.421***
VPD_A	0.235**	−0.111**	0.124	−0.086	−0.069	−0.155**

Φ_{NEP}, phase of diurnal NEP; NEP_A, amplitude of diurnal NEP; Ts_m, daily mean Ts; Ta_m, daily mean Ta; Ta_A, amplitude of diurnal Ta; Rg_m, daily mean Rg; Φ_{Rg}, phase of diurnal Rg; RH_m, daily mean RH; VPD_m, daily mean VPD; VPD_A, amplitude of diurnal VPD. *P<0.10; **P<0.05; and ***P<0.01.

especially during the growing season (Deventer et al., 2021). Therefore, despite the use of the WPL correction in this study, the effect of SPHE may have a potential impact on the response of forest NEP to the changing solar radiation, during both the growing and dormant seasons. Notably, due to stable atmospheric conditions or non-stationary high-frequency time series, gaps in EC- flux measurements are unavoidable and require gap filling. Current gap filling methods are all based on the changing characteristics of the time series of flux measurements and its response mechanism to meteorological factors. This study employed a fully data-based machine learning method to gap filling, preserving as much as possible the changing pattern of the flux data and its response to meteorological factors. These aspects are beyond the scope of this study and will be investigated in future studies.

Forest NEP is subject to synergistic or antagonistic effects of several factors, such as microclimate, stand age, understory species composition, phenology, and disturbance patterns. The principal factor determining the seasonal variation of all CO_2 exchange fluxes (NEP, GPP, and R_{eco}) is the phenology of the understory vegetation, and plant community structure also plays a major role in the variation of CO_2 exchange fluxes. Vegetation phenology regulates leaf development, and the larger the leaf area, the higher the light absorption capacity. Consequently, the CO_2 uptake by photosynthesis is also increased. Ecosystem respiration has a strong correlation with GPP, and studies have confirmed the strong influence of vegetation productivity on R_{eco} (Janssens et al., 2001). As ecosystems alter structurally and functionally over time, possibly in response to disturbances, predicting changes in ecosystem function will become increasingly important (Richardson et al., 2007). Forests are more productive and susceptible to natural and anthropogenic disturbances (e.g., deforestation, fire, grazing, etc.), whereas the Ebinur Lake watershed is relatively pristine and has existed for thousands of years in its present undisturbed oasis form. Tugai forests in the Ebinur Lake basin may have evolved self-regulatory mechanisms that have contributed to their endurance through various meteorological regimes over time (including seasonal and interannual variability in the area of Ebinur Lake waters) (Yang et al., 2014; Wang et al., 2019). It also benefits from the protective policy implemented within the national nature reserve, which ensures that the groundwater depth of our site in the Ebinur Lake basin is consistently maintained at less than 3 m (Yang et al., 2014). Notably, if the groundwater depth surpasses 6 m, the Tugai forests face a heightened risk of decline due to the impacts of climate change (Zhou et al., 2020). If functional changes in ecosystem responses result from adjustments to long-term exposure to specific average meteorological conditions, then in the short term such as 1–5 years, the direct effects of meteorological changes can dominate changes in CO_2 fluxes (Teklemariam et al., 2010). As the protection of the Ebinur Lake watershed is enhanced and the lake area expands, the functional type of plants across the Ebinur Lake watershed may alter in the future, with an increased prevalence of shrubs and trees, which will change the CO_2 fluxes in the area.

The potential for climate- or weather-induced changes in ecosystem response functions underscores the need to interpret measured NEP models through more than just parallel comparisons with driving meteorological conditions. This is because such an analysis would only capture transient dependencies between variables (Teklemariam et al., 2010). This point is supported by the correlation analysis between NEP and meteorological factors for different seasons, which indicates that NEP measured during the growing season exhibits the strongest correlation with the meteorological conditions at the time of flux measurements (Figure 11). Therefore, it is essential to also consider the environmental and ecological history of the site, including the adaptations that have taken place over time.

5 Conclusions

This study aimed to investigate the variations in NEP of a representative desert riparian forest ecosystem across multiple temporal scales, elucidating its relationship with meteorological conditions. Utilizing approximately seven years of eddy covariance flux measurements, we conducted a comprehensive examination of the cycles exhibited by the NEP and meteorological conditions. These cycles spanned various lengths, ranging from five hours to one year. The NEP variations exhibited a robust correlation and synchronization with meteorological conditions across diverse temporal scales, with the most significant fluctuations occurring in one-day cycle. Over the seven-year duration of our research, these findings also revealed significant seasonal differences in the direct and indirect responses of the NEP to the averages, amplitudes, and phases of diurnally changing meteorological factors at a daily scale. These variations included the differences in the magnitude of response and even a reversal of the response directions. The amplitude of diurnal air temperature significantly influenced the phase of diurnal NEP in Tugai forests during the growing season, with no notable impact on the amplitude of diurnal NEP. However, this pattern may be inverted during the dormant season. These findings underscore the substantial impact of circadian rhythms induced by meteorological conditions on the NEP of desert riparian forests at an ecosystem scale. Given the significance of both the direct and indirect effects, as well as the amplitude of periodic meteorological factors variations, careful consideration is essential when assessing the ecosystem carbon exchange of desert riparian forests. The extended time frame allows for a more comprehensive analysis of dynamics and strengthens the robustness of our conclusions. These findings provide valuable insights into the complex responses of desert riparian forests to climate change, thereby contributing to the advancement of our scientific understanding of carbon exchange pattern within arid and semi-arid 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

DT: Formal analysis, Funding acquisition, Visualization, Writing – original draft. XG: Formal analysis, Methodology, Validation, Writing – review & editing. XH: Data curation, Investigation, Methodology, Writing – review & editing. JW: Funding acquisition, Software, Validation, Writing – review & editing. GL: Conceptualization, Investigation, Supervision, Writing – review & editing. JW: Data curation, Formal analysis, Software, Validation, Writing – review & editing. XY: Formal analysis, Investigation, Resources, Supervision, Validation, Writing – review & editing.

Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article. This work was financially supported by the China Postdoctoral Science Foundation (2023M733672), the Postdoctoral Research Startup Foundation for Postdoc of Liaoning Province of China (2022-BS-022), Key R&D Program of Liaoning Province (2023JH2/101800043), the Guangdong Basic and Applied Basic Research Foundation (2023A1515011273), the Basic Research Program of Shenzhen (20220811173316001), and Specific Innovation Program of the department of Education of Guangdong Province (2023KTSCX315).

Acknowledgments

We are grateful to Xinjiang Jinghe Observation and Research Station of Temperate Desert Ecosystem, Ministry of Education, China for providing forest sites, instrument systems, and logistic supports.

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

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpls.2024.1332192/full#supplementary-material

References

Adams, H. D., Guardiola-Claramonte, M., Barron-Gafford, G. A., Villegas, J. C., Breshears, D. D., Zou, C. B., et al. (2009). Temperature sensitivity of drought-induced tree mortality portends increased regional die-off under global-change-type drought. *Proc. Natl. Acad. Sci. U.S.A.* 106, 7063–7066. doi: 10.1073/pnas.0901438106

Ahlstrom, A., Raupach, M. R., Schurgers, G., Smith, B., Arneth, A., Jung, M., et al. (2015). The dominant role of semi-arid ecosystems in the trend and variability of the land CO2 sink. *Science* 348, 895–899. doi: 10.1126/science.aaa1668

Anderson-Teixeira, K. J., Delong, J. P., Fox, A. M., Brese, D. A., and Litvak, M. E. (2011). Differential responses of production and respiration to temperature and moisture drive the carbon balance across a climatic gradient in New Mexico. *Global Change Biol.* 17, 410–424. doi: 10.1111/gcb.2010.17.issue-1

Baldocchi, D. D. (2003). Assessing the eddy covariance technique for evaluating carbon dioxide exchange rates of ecosystems: past, present and future. *Global Change Biol.* 9, 479–492. doi: 10.1046/j.1365-2486.2003.00629.x

Baldocchi, D., Falge, E., Gu, L., Olson, R., Hollinger, D., Running, S., et al. (2001). FLUXNET: A new tool to study the temporal and spatial variability of ecosystem–scale carbon dioxide, water vapor, and energy flux densities. *Bull. Am. Meteorological Soc.* 82, 2415–2434. doi: 10.1175/1520-0477(2001)082<2415:FANTTS>2.3.CO;2

Banbury Morgan, R., Herrmann, V., Kunert, N., Bond-Lamberty, B., Muller-Landau, H. C., and Anderson-Teixeira, K. J. (2021). Global patterns of forest autotrophic carbon fluxes. *Glob Chang Biol.* 27, 2840–2855. doi: 10.1111/gcb.15574

Burba, G. G., McDermitt, D. K., Grelle, A., Anderson, D. J., and Xu, L. (2008). Addressing the influence of instrument surface heat exchange on the measurements of CO2 flux from open-path gas analyzers. *Global Change Biol.* 14, 1854–1876. doi: 10.1111/j.1365-2486.2008.01606.x

Chaves, M. M. (1991). Effects of water deficits on carbon assimilation. *J. Exp. Bot.* 42, 1–16. doi: 10.1093/jxb/42.1.1

DéCamps, H., Pinay, G., Naiman, R. J., Petts, G., McClain, M., Hillbricht-Ilkowska, A., et al. (2004). Riparian zones: where biogeochemistry meets biodiversity in management practice. *Polish J. Ecol.* 52, 3–18.

Deventer, M. J., Roman, T., Bogoev, I., Kolka, R. K., Erickson, M., Lee, X. H., et al. (2021). Biases in open-path carbon dioxide flux measurements: Roles of instrument surface heat exchange and analyzer temperature sensitivity. *Agric. For. Meteorology*, 296, 108216. doi: 10.1016/j.agrformet.2020.108216

Ding, J., Zhao, W., Daryanto, S., Wang, L., Fan, H., Feng, Q., et al. (2017). The spatial distribution and temporal variation of desert riparian forests and their influencing factors in the downstream Heihe River basin, China. *Hydrology Earth System Sci.* 21, 2405–2419. doi: 10.5194/hess-21-2405-2017

Dios, V. R., Goulden, M. L., Ogle, K., Richardson, A. D., Hollinger, D. Y., Davidson, E. A., et al. (2012). Endogenous circadian regulation of carbon dioxide exchange in terrestrial ecosystems. *Global Change Biol.* 18, 1956–1970. doi: 10.1111/j.1365-2486.2012.02664.x

Dobie, R. A., and Wilson, M. J. (1989). Analysis of auditory evoked potentials by magnitude-squared coherence. *Ear Hearing* 10, 2–13. doi: 10.1097/00003446-198902000-00002

Fan, X., Thompson, B., and Wang, L. (1999). Effects of sample size, estimation methods, and model specification on structural equation modeling fit indexes. *Struct. Equation Modeling: A Multidiscip. J.* 6, 56–83. doi: 10.1080/10705519909540119

Fan, H., Zhao, W., Daryanto, S., Fu, B., Wang, S., and Wang, Y. (2018). Vertical distributions of soil organic carbon and its influencing factors under different land use types in the desert riparian zone of downstream Heihe River Basin, China. *J. Geophysical Research: Atmospheres* 123, 7741–7753. doi: 10.1029/2018JD028268

Feng, Q., and Cheng, G. (1998). Current situation, problems and rational utilization of water resources in arid north-western China. *J. Arid Environments* 40, 373–382. doi: 10.1006/jare.1998.0456

Franklin, J., Serra-Diaz, J. M., Syphard, A. D., and Regan, H. M. (2016). Global change and terrestrial plant community dynamics. *Proc. Natl. Acad. Sci. U.S.A.* 113, 3725–3734. doi: 10.1073/pnas.1519911113

Gil, K. E., and Park, C. M. (2019). Thermal adaptation and plasticity of the plant circadian clock. *New Phytol.* 221, 1215–1229. doi: 10.1111/nph.15518

He, X., Lü, G., Qin, L., Yang, J., Liu, D., and Guo, Z. (2014). Research on data characteristics during non-growing season of desert-wetland ecosystem in Ebinur Lake. *Acta Ecologica Sin.* 34, 6655–6665. doi: 10.5846/stxb201401130092

Heinsch, F. A., Maosheng, Z., Running, S. W., Kimball, J. S., Nemani, R. R., Davis, K. J., et al. (2006). Evaluation of remote sensing based terrestrial productivity from MODIS using regional tower eddy flux network observations. *IEEE Trans. Geosci. Remote Sens.* 44, 1908–1925. doi: 10.1109/TGRS.2005.853936

Hollinger, D. Y., Kelliher, F. M., Byers, J. N., Hunt, J. E., McSeveny, T. M., and Weir, P. L. (1994). Carbon dioxide exchange between an undisturbed old-growth temperate forest and the atmosphere. *Ecology* 75, 134–150. doi: 10.2307/1939390

Janssens, I. A., Lankreijer, H., Matteucci, G., Kowalski, A. S., Buchmann, N., Epron, D., et al. (2001). Productivity overshadows temperature in determining soil and ecosystem respiration across European forests. *Global Change Biol.* 7, 269–278. doi: 10.1046/j.1365-2486.2001.00412.x

Jiang, N., Zhang, Q., Zhang, S., Zhao, X., and Cheng, H. (2022). Spatial and temporal evolutions of vegetation coverage in the Tarim River Basin and their responses to phenology. *Catena* 217, 106489. doi: 10.1016/j.catena.2022.106489

Kok, B. (1948). A critical consideration of the quantum yield of Chlorella-photosynthesis. *Enzymologia* 13, 1–56.

Kok, B. (1949). On the interrelation of respiration and photosynthesis in green plants. *Biochim. Biophys. Acta* 3, 625–631. doi: 10.1016/0006-3002(49)90136-5

Law, B. E., Falge, E., Gu, L., Baldocchi, D. D., Bakwin, P., Berbigier, P., et al. (2002). Environmental controls over carbon dioxide and water vapor exchange of terrestrial vegetation. *Agric. For. Meteorology* 113, 97–120. doi: 10.1016/S0168-1923(02)00104-1

Leuning, R., Kellher, F. M., Pury, D. G. G. D., and Schulze, E. D. (1995). Leaf nitrogen, photosynthesis, conductance and transpiration–Scaling from leaves to canopies. *Plant Cell Environ.* 18, 1183–1200. doi: 10.1111/j.1365-3040.1995.tb00628.x

Li, W., Li, Y., Lv, J., He, X., Wang, J., Teng, D., et al. (2022). Rhizosphere effect alters the soil microbiome composition and C, N transformation in an arid ecosystem. *Appl. Soil Ecol.*, 170. doi: 10.1016/j.apsoil.2021.104296

Li, X., Yang, K., and Zhou, Y. Z. (2016). Progress in the study of oasis-desert interactions. *Agricultural and Forest Meteorology*, 230, 1–7. doi: 10.1016/j.agrformet.2016.08.022

Liu, M., Shi, J., Wang, X., and Ma, X. (2016). Aboveground biomass and carbon storage of Populus Euphratica plantation with different ages in Tahe River Basin. *Bull. Soil Water Conserv.* 36, 326–332. doi: 10.13961/j.cnki.stbctb.2016.05.058

López-Blanco, E., Lund, M., Williams, M., Tamstorf, M. P., Westergaard-Nielsen, A., Exbrayat, J.-F., et al. (2017). Exchange of CO2 in Arctic tundra: impacts of meteorological variations and biological disturbance. *Biogeosciences* 14, 4467–4483. doi: 10.5194/bg-14-4467-2017

Lovett, G. M., Cole, J. J., and Pace, M. L. (2006). Is net ecosystem production equal to ecosystem carbon accumulation? *Ecosystems* 9, 152–155. doi: 10.1007/s10021-005-0036-3

Ma, X., Feng, Q., Yu, T., Su, Y., and Deo, R. (2017). Carbon dioxide fluxes and their environmental controls in a riparian forest within the hyper-arid region of northwest China. *Forests* 8, 379. doi: 10.3390/f8100379

Masuda, K., Tokuda, I. T., Nakamichi, N., and Fukuda, H. (2021). The singularity response reveals entrainment properties of the plant circadian clock. *Nat. Commun.* 12, 864. doi: 10.1038/s41467-021-21167-7

Mauder, M., Liebethal, C., Göckede, M., Leps, J.-P., Beyrich, F., and Foken, T. (2006). Processing and quality control of flux data during LITFASS-2003. *Boundary-Layer Meteorology* 121, 67–88. doi: 10.1007/s10546-006-9094-0

McDowell, N., Pockman, W. T., Allen, C. D., Breshears, D. D., Cobb, N., Kolb, T., et al. (2008). Mechanisms of plant survival and mortality during drought: why do some plants survive while others succumb to drought? *New Phytol.* 178, 719–739. doi: 10.1111/j.1469-8137.2008.02436.x

McIntosh, A. R., Grady, C. L., Haxby, J. V., Ungerrleider, L. G., and Horwitz, B. (1996). Changes in limbic and prefrontal functional interactions in a working memory task for faces. *Cereb. Cortex* 6, 571–584. doi: 10.1093/cercor/6.4.571

McMaster, G. S., and Wilhelm, W. W. (1997). Growing degree-days: one equation, two interpretations. *Agric. For. Meteorology* 87, 291–300. doi: 10.1016/S0168-1923(97)00027-0

Reichstein, M., Bahn, M., Ciais, P., Frank, D., Mahecha, M. D., Seneviratne, S. I., et al. (2013). Climate extremes and the carbon cycle. *Nature* 500, 287–295. doi: 10.1038/nature12350

Reichstein, M., Ciais, P., Papale, D., Valentini, R., Running, S., Viovy, N., et al. (2007). Reduction of ecosystem productivity and respiration during the European summer 2003 climate anomaly: a joint flux tower, remote sensing and modelling analysis. *Global Change Biol.* 13, 634–651. doi: 10.1111/j.1365-2486.2006.01224.x

Resco de Dios, V., and Gessler, A. (2018). Circadian regulation of photosynthesis and transpiration from genes to ecosystems. *Environ. Exp. Bot.* 152, 37–48. doi: 10.1016/j.envexpbot.2017.09.010

Richardson, A. D., Hollinger, D. Y., Aber, J. D., Ollinger, S. V., and Braswell, B. H. (2007). Environmental variation is directly responsible for short- but not long-term variation in forest-atmosphere carbon exchange. *Global Change Biol.* 13, 788–803. doi: 10.1111/j.1365-2486.2007.01330.x

Running, S. W., Baldocchi, D. D., Turner, D. P., Gower, S. T., Bakwin, P. S., and Hibbard, K. A. (1999). A global terrestrial monitoring network integrating tower fluxes, flask sampling, ecosystem modeling and EOS satellite data. *Remote Sens. Environ.* 70, 108–127. doi: 10.1016/S0034-4257(99)00061-9

Singh, R. K., Bhalerao, R. P., and Eriksson, M. E. (2021). Growing in time: exploring the molecular mechanisms of tree growth. *Tree Physiol.* 41, 657–678. doi: 10.1093/treephys/tpaa065

Steppe, K., Sterck, F., and Deslauriers, A. (2015). Diel growth dynamics in tree stems: linking anatomy and ecophysiology. *Trends Plant Sci.* 20, 335–343. doi: 10.1016/j.tplants.2015.03.015

Stoy, P. C., Katul, G. G., Siqueira, M. B. S., Juang, J. Y., McCarthy, H. R., Kim, H. S., et al. (2005). Variability in net ecosystem exchange from hourly to inter-annual time scales at adjacent pine and hardwood forests: a wavelet analysis. *Tree Physiol.* 25, 887–902. doi: 10.1093/treephys/25.7.887

Tang, R., He, B., Chen, H. W., Chen, D., Chen, Y., Fu, Y. H., et al. (2022). Increasing terrestrial ecosystem carbon release in response to autumn cooling and warming. *Nat. Climate Change* 12, 380–385. doi: 10.1038/s41558-022-01304-w

Team, R.C (2022). *R: A language and environment for statistical computing* (Vienna, Austria: R Foundation for Statistical Computing).

Teklemariam, T. A., Lafleur, P. M., Moore, T. R., Roulet, N. T., and Humphreys, E. R. (2010). The direct and indirect effects of inter-annual meteorological variability on ecosystem carbon dioxide exchange at a temperate ombrotrophic bog. *Agric. For. Meteorology* 150, 1402–1411. doi: 10.1016/j.agrformet.2010.07.002

Teng, D., He, X., Qin, L., and Lv, G. (2021). Energy balance closure in the tugai forest in Ebinur Lake Basin, northwest China. *Forests* 12, 243. doi: 10.3390/f12020243

Thevs, N., Buras, A., Zerbe, S., Kuhnel, E., Abdusalih, N., and Ovezberdiyeva, A. (2011). Structure and wood biomass of near-natural floodplain forests along the Central Asian rivers Tarim and Amu Darya. *Forestry* 85, 193–202. doi: 10.1093/forestry/cpr056

Thorne, P. W., Menne, M. J., Williams, C. N., Rennie, J. J., Lawrimore, J. H., Vose, R. S., et al. (2016). Reassessing changes in diurnal temperature range: A new data set and characterization of data biases. *J. Geophysical Research: Atmospheres* 121, 5115–5137. doi: 10.1002/2015JD024583

van Dijk, A. I. J. M., Dolman, A. J., and Schulze, E.-D. (2005). Radiation, temperature, and leaf area explain ecosystem carbon fluxes in boreal and temperate European forests. *Global Biogeochemical Cycles* 19, GB2029. doi: 10.1029/2004GB002417

Wang, J., Ding, J., Li, G., Liang, J., Yu, D., Aishan, T., et al. (2019). Dynamic detection of water surface area of Ebinur Lake using multi-source satellite data (Landsat and Sentinel-1A) and its responses to changing environment. *Catena* 177, 189–201. doi: 10.1016/j.catena.2019.02.020

Wang, X., Shi, J., and iLiu, H. (2017). Assessment of purifying atmosphere environment function value of natural Populus euphratica in Xinjiang. *Prot. For. Sci. Technol.*, 27–30. doi: 10.13601/j.issn.1005-5215.2017.09.009

Wang, X., Wang, C., Guo, Q., and Wang, J. (2016). Improving the CO_2 storage measurements with a single profile system in a tall-dense-canopy temperate forest. *Agric. For. Meteorology* 228-229, 327–338. doi: 10.1016/j.agrformet.2016.07.020

Way, D. A., and Montgomery, R. A. (2015). Photoperiod constraints on tree phenology, performance and migration in a warming world. *Plant Cell Environ.* 38, 1725–1736. doi: 10.1111/pce.12431

Way, D. A., and Yamori, W. (2013). Thermal acclimation of photosynthesis: on the importance of adjusting our definitions and accounting for thermal acclimation of respiration. *Photosynthesis Res.* 119, 89–100. doi: 10.1007/s11120-013-9873-7

Webb, E. K., Pearman, G. I., and Leuning, R. (1980). Correction of flux measurements for density effects due to heat and water vapour transfer. *Q. J. R. Meteorological Soc.* 106, 85–100. doi: 10.1002/qj.49710644707

Wolf, S., Keenan, T. F., Fisher, J. B., Baldocchi, D. D., Desai, A. R., Richardson, A. D., et al. (2016). Warm spring reduced carbon cycle impact of the 2012 US summer drought. *Proc. Natl. Acad. Sci.* 113, 5880–5885. doi: 10.1073/pnas.1519620113

Xia, J., Chen, J., Piao, S., Ciais, P., Luo, Y., and Wan, S. (2014). Terrestrial carbon cycle affected by non-uniform climate warming. *Nat. Geosci.* 7, 173–180. doi: 10.1038/ngeo2093

Yang, X., Zhang, X., Lv, G., and Ali, A. (2014). Linking Populus euphratica hydraulic redistribution to diversity assembly in the arid desert zone of Xinjiang, China. *PloS One* 9, e109071. doi: 10.1371/journal.pone.0109071

Yi, C. X., Ricciuto, D., Li, R., Wolbeck, J., Xu, X. Y., Nilsson, M., et al. (2010). Climate control of terrestrial carbon exchange across biomes and continents. *Environ. Res. Lett.* 5, 34007. doi: 10.1088/1748-9326/5/3/034007

Yin, X., Niu, Y., Putten, P. E. L., and Struik, P. C. (2020). The Kok effect revisited. *New Phytol.* 227, 1764–1775. doi: 10.1111/nph.16638

Yu, T., Han, T., Feng, Q., Chen, W., Zhao, C., Li, H., et al. (2023). Divergent response to abiotic factor determines the decoupling of water and carbon fluxes over an artificial C4 shrub in desert. *J. Environ. Manage* 344, 118416. doi: 10.1016/j.jenvman.2023.118416

Yuan, W., Zheng, Y., Piao, S., Ciais, P., Lombardozzi, D., Wang, Y., et al. (2019). Increased atmospheric vapor pressure deficit reduces global vegetation growth. *Sci. Adv.* 5, eaax1396. doi: 10.1126/sciadv.aax1396

Zhang, X., Lv, G., Wang, T., Ma, Y., Abliz·Abdurahman,, Zhao, X., et al. (2015). Patterns and mechanisms influencing plant diversity in an arid desert region riparian zone of the Aqikesu River, Xinjiang, China. *Acta Ecologica Sin.* 35, 5966–5974. doi: 10.5846/STXB201401160128

Zhou, B., Sterck, F., Kruijt, B., Fan, Z.-X., and Zuidema, P. A. (2023). Diel and seasonal stem growth responses to climatic variation are consistent across species in a subtropical tree community. *New Phytol.* 240, 2253–2264. doi: 10.1111/nph.19275

Zhou, H., Chen, Y., Zhu, C., Li, Z., Fang, G., Li, Y., et al. (2020). Climate change may accelerate the decline of desert riparian forest in the lower Tarim River, Northwestern China: Evidence from tree-rings of Populus euphratica. *Ecol. Indic.* 111, 105997. doi: 10.1016/j.ecolind.2019.105997

Glossary

EC	eddy covariance
GDD	growing degree day
IMF	intrinsic mode function
NEE	net ecosystem CO_2 exchange
NEP	net ecosystem productivity
NEP_A	amplitude of diurnal net ecosystem productivity
MSC	magnitude-squared coherence
Rg	global solar radiation
Rg_m	daily average of global solar radiation
RH	relative humidity
RH_m	daily average of relative humidity
RH_A	amplitude of diurnal relative humidity
SEM	structural equation model
Ta	air temperature
Ta_m	daily average of air temperature
Ta_A	amplitude of diurnal air temperature
TFA	transfer function amplitude
Ts	soil temperature
Ts_m	daily average of soil temperature
Ts_A	amplitude of diurnal soil temperature
VPD	vapor pressure deficit
VPD_m	daily average of vapor pressure deficit
VPD_A	amplitude of diurnal vapor pressure deficit
Φ_{NEP}	phase of diurnal net ecosystem productivity
Φ_{Rg}	phase of diurnal Rg

frontiers | Frontiers in Plant Science

TYPE Original Research
PUBLISHED 14 June 2024
DOI 10.3389/fpls.2024.1375194

Check for updates

OPEN ACCESS

EDITED BY
Praveen Dhyani,
University of Koblenz, Germany

REVIEWED BY
Rahul Jain,
University of Amsterdam, Netherlands
Wayan Wangiyana,
University of Mataram, Indonesia
Muhammad Ahsan Asghar,
Aarhus University, Denmark

*CORRESPONDENCE
Shumin Li
✉ shuminli@neau.edu.cn
Lingbo Meng
✉ menglingbo2004@126.com

RECEIVED 23 January 2024
ACCEPTED 27 May 2024
PUBLISHED 14 June 2024

CITATION
Zhang S, Li S, Meng L, Liu X, Zhang Y, Zhao S and Zhao H (2024) Root exudation under maize/soybean intercropping system mediates the arbuscular mycorrhizal fungi diversity and improves the plant growth. *Front. Plant Sci.* 15:1375194.
doi: 10.3389/fpls.2024.1375194

Root exudation under maize/soybean intercropping system mediates the arbuscular mycorrhizal fungi diversity and improves the plant growth

Shu Zhang[1], Shumin Li[1*], Lingbo Meng[2*], Xiaodan Liu[1], Yuhang Zhang[1], Shuchang Zhao[1] and Haobing Zhao[1]

[1]Resource and Environmental College, Northeast Agricultural University, Heilongjiang, China, [2]School of Geography and Tourism, Harbin University, Harbin, Heilongjiang, China

Introduction: Maize/soybean intercropping is a common cropping practice in Chinese agriculture, known to boost crop yield and enhance soil fertility. However, the role of below-ground interactions, particularly root exudates, in maintaining intercropping advantages in soybean/maize intercropping systems remains unclear.

Methods: This study aimed to investigate the differences in root exudates between intercropping and monocropping systems through two pot experiments using metabolomics methods. Multiple omics analyses were conducted to explore correlations between differential metabolites and the community of Arbuscular Mycorrhizal Fungi (AMF), shedding light on the mechanisms underlying the dominance of intercropping from the perspective of root exudates-soil microorganism interactions.

Results and discussion: The study revealed that intercropping significantly increased the types and contents of root exudates, lowered soil pH, increased the availability of nutrients like available nitrogen (AN) and available phosphorus (AP), and enhanced AMF colonization, resulting in improving the community composition of AMF. Besides, root exudates in intercropping systems differed significantly from those in monocropping, with 41 and 39 differential metabolites identified in the root exudates of soybean/maize, predominantly amino acids and organic acids. The total amount of amino acids in the root exudates of soybean intercropping was 3.61 times higher than in monocropping. Additionally, the addition of root exudates significantly improved the growth of soybean/maize and AMF colonization, with the mycorrhizal colonization rate in intercropping increased by 105.99% and 111.18% compared to monocropping, respectively. The identified metabolic pathways associated with root exudates were closely linked to plant growth, soil fertility improvement, and the formation of AMF. Correlation analysis revealed a significant relationship ($P < 0.05$) between certain metabolites such as tartaric acid, oxalic acid, malic acid, aspartic acid, alanine, and the AMF community. Notably, the photosynthetic carbon fixation pathway involving aspartic acid showed a strong association with the function of Glomus_f_Glomerace, the dominant genus of AMF. A combined analysis of metabolomics and high throughput sequencing revealed that the root exudates of soybean/maize intercropping have direct or indirect connections with AMF and soil nutrients.

Conclusion: This suggests that the increased root exudates of the soybean/maize intercropping system mediate an improvement in AMF community composition, thereby influencing soil fertility and maintaining the advantage of intercropping.

KEYWORDS

soybean/maize intercropping, interspecific interactions, root exudates, AMF, soil nutrients

1 Introduction

Intercropping, a traditional agricultural technique, has gained global traction for its efficient resource utilization, encompassing sunlight, water, and nutrients (Brooker et al., 2015). This practice is pivotal in sustaining biodiversity and stability within farmland ecosystems while achieving high and consistent yields in agroecosystems (Hauggaard-Nielsen et al., 2008; Zhang et al., 2012). Although significant studies have shed light on the advantages concerning aboveground productivity and resource sharing in intercropping settings, below-ground interspecific interactions, present a more intricate picture that warrants further investigation. In recent years, greater emphasis has been placed on exploring the microecology of the soil, including soil nutrients, root exudates, and soil microorganisms (Li et al., 2014). Understanding plant-soil-microbe interactions is crucial in comprehending intercropping systems (Duchene et al., 2017). However, the specific role of these interactions in intercropping remains poorly understood.

The rhizosphere, recognized for its crucial role in nutrient cycling and supporting diverse microbial populations (Kuzyakov and Blagodatskaya, 2015), is the key zone of interaction between plants and the soil. It facilitates the transfer of materials between crops and their soil environment (Kuzyakov and Razavi, 2019). In this dynamic environment, root exudates serve as essential channels for the exchange of substances and signals between plants and soil (Coskun et al., 2017). Notably, plants allocate approximately 10% of their photosynthetically fixed carbon to generate root exudates, enriching the rhizosphere (Mohanram and Kumar, 2019). These secretions are instrumental in enhancing soil texture and chemistry while also attracting and nourishing specific advantageous microbial colonies. This enhances the soil conditions, promoting healthier plant growth (Pérez-Jaramillo et al., 2016). Soil microbes have the capability to directly utilize root exudates as carbon sources (Swenson et al., 2015), and this communication between plants and microbes are vital in maintaining the vitality of the rhizosphere microecosystem. Root exudates facilitate below-ground interactions among plant species and significantly contribute to the link between biodiversity and ecosystem function (Zhang et al., 2017).

Root exudates, are pivotal in augmenting the nutrient dynamics within soil, thereby optimizing nutrient acquisition and assimilation by vegetation (Nardi et al., 2002). The interaction between root exudates and plant species manifests in several ways: they can activate insoluble soil phosphorus (P) and trace elements, and can also form chelates with various metallic elements, such as calcium (Ca), thereby facilitating nutrient absorption and utilization by crops such as alfalfa, legume, etc (Dakora and Phillips, 2002; Shen et al., 2002; Landi et al., 2006). Fe-deficient cereals release iminocarboxylic acids to dissolve ferric compounds for uptake by cereal roots (Dakora and Phillips, 2002). Beyond their nutritional role, root exudates can also alleviate the adverse impacts of heavy metals on crops, modulate the rhizospheric microbial ecosystem, suppress plant diseases, and improve the physical and chemical conditions of the soil (Okubo et al., 2016; Karlen et al., 2019; Wang et al., 2020). Simultaneously, allelochemicals within these exudates can influence the root development and nutrient uptake of adjacent plants, thereby impacting their growth either positively or negatively (Liu et al., 2013; Hu et al., 2018).

Research demonstrates that root exudates selectively shape the microbial community of the rhizosphere, which varies across plant species, resulting in distinctive microbial profiles (Paterson et al., 2007). In turn, changes in this microbial community significantly affect root exudate production, soil material cycling, energy flow, and information transfer, ultimately impacting plant growth and development (Eisenhauer et al., 2012). Furthermore, a significant relationship exists between the variety and quantity of soil metabolites and the stimulation of microbial genetic activity (Chaparro et al., 2013).

AMF are key soil microbes, integral to plant-soil interactions in intercropping systems. The symbiosis between AMF and plants significantly extends the nutrient absorption capacity through a widespread mycelial network. This network enhances plant nutrient uptake efficiency (Ferrol et al., 2019). Additionally, mycorrhizal symbiosis assists in immobilizing approximately 5 billion tons of photosynthetic products annually into the soil, thereby stabilizing the carbon balance of the ecosystem (Bago et al., 2000). Moreover, mycorrhizal relationships are instrumental in enhancing the growth and productivity of host plants. This leads to higher agricultural yields and lessens the dependency on chemical fertilizers, thereby supporting both food production and ecological health (Raklami et al., 2019). AMF are also effective at reducing nutrient losses due to leaching or denitrification by optimizing nutrient uptake (Querejeta, 2017), which is vital for enhancing soil quality. However, limited research has been conducted on the interrelationship and regulation between AMF, root exudates, and their underlying mechanisms within intercropping systems. Given the crucial roles of AMF and root exudates in the soil ecosystems, our research aims to delve into the alterations in exudates and the interplay between root exudates and AMF in intercropping systems.

This investigation utilized pot experiments to assess the impacts of interspecific interactions on root exudates and AMF communities in a soybean/maize intercropping system. Metabolomics and high-throughput sequencing were employed for a comprehensive analysis. This study tried (1) to delineate the metabolic variances in root exudates between of soybean and maize in both intercropped and monocropped systems, (2) to assess the impact of root exudates on plant growth and the soil's microbial environment, and (3) to examine the relationship between root exudates and AMF communities. By uncovering the underlying mechanisms behind the advantages of intercropping from the perspectives of microorganisms and metabolites, this research seeks to bolster the theoretical underpinnings for the ecologically sustainable development of intercropping practices in agriculture.

2 Materials and methods

2.1 Experiment design

2.1.1 Experiment 1: impact of interspecific interactions on root exudates, AMF and plant growth

This study employed a one-way completely randomized experiment consisting of three treatments: soybean monocropping, maize monocropping, and soybean/maize intercropping. Each treatment had six replications that were randomly assigned. The pot experiment was implemented at the Horticultural Experiment Station of Northeast Agricultural University (45°03'N, 126°43'E), Harbin, China, utilizing soybean (Glycine max L.) seeds of the Dongnong-252 variety and maize (Zea mays L.) seeds of the Xianyu-335 variety. The experiment commenced on August 15, 2020. The pot (Diameter 23 cm, depth 8 cm) was divided into two parts from the middle, one for maize and the other for soybean, applying two distinct partition methodologies: (i) a solid root barrier to simulate sole cropping by obstructing root interactions, and (ii) a 30-μm nylon mesh allowing for mass flow and diffusion between species, mimicking intercropping scenarios. (Supplementary Figure S1). The root barriers experiment could ensure that two plant species occupied the same soil space whether monocropping or intercropping (plastic sheet barrier versus nylon mesh barrier) and were simultaneous planted and harvested, and the aboveground growth conditions are the same, so as to avoid the difference of root exudates caused by the influence of factors such as light on plant growth.

Soil was procured from an undisturbed mollisoil (0-20 cm layer) at the Acheng Experimental Site (44°04'N, 125°42'E) in Harbin City, China. The soil contained 17.2 g total organic carbon (TOC), 1.47 g total nitrogen (TN), 125 mg available nitrogen (AN), 67.8 mg available phosphorus (AP) and 123 mg available potassium (AK) per kg soil, and pH is 6.10. The soil was prepared by air- drying and sieving through a 2-mm mesh sieve. Each compartment of the pot was then loaded with 1.5 kg of this prepped soil. Basal fertilizers were mixed in soil and thoroughly mixed before sowing. The soil for maize was supplemented with 120 mg/kg N by applying 0.39 g urea (N 46%) in each compartment, while the soybean soil received 60 mg/kg N by applying 0.20 g urea (N 46%) in each compartment. Additionally, the soil was fertilized with 50 mg/kg P_2O_5 by applying 0.12 g $Ca(H_2PO_4)_2$ (P_2O_5 61%) in each compartment and 100 mg/kg K_2O by applying 0.28g K_2SO_4 (K_2O 54%) in each compartment. Basal nutrients in solution were added to soil at the following rates (mg/kg soil): $MgSO_4$(317), $FeSO_4·7H_2O$ (31), $MnSO_4·H_2O$ (20), $CuSO_4·5H_2O$ (25) and $ZnSO_4·7H_2O$ (28).

Seeds of soybean and maize were sterilized with 15% hydrogen peroxide(H_2O_2) for 20 minutes and rinsed with sterile water thrice. Subsequently, the sterilized corn seeds were soaked in sterile water for 24 hours to promote germination. After 24 hours of soaking, the corn seeds were placed on rectangular filter paper with approximately 1-2 cm spacing between the seeds, and arranged with the navel facing downward. The seeds were then pregerminated in the dark in a dish at a temperature of 18°C for 8 hours per day, taking approximately 1-2 days for the corns to grow by about 1 cm, and then transplanted them to the compartment of pot. Initially, four soybean seeds and two maize seeds were sown in separate compartments; thinning was conducted to maintain two soybeans and one maize plant per compartment 10 days post-sowing. The plants received consistent irrigation and were cultivated in a greenhouse regulated at 20°C to ensure optimal growing conditions.

After a growth period of 60 days, the plants were harvested on October 14, 2020. Samples from both the plant's aboveground tissues and the soil surrounding the roots were gathered to evaluate aboveground and belowground biomass, root morphological characteristics (such as length, surface area, and volume), soil physicochemical traits, and variations in mycorrhizal colonization and root exudation between the soybean and maize under different treatment conditions.

2.1.2 Experiment 2: root exudate addition experiment—effects of root exudates on AMF and plant growth

To further verify the impact of root exudates in intercropping systems on crops growth and AMF, the experiment 2 was designed with exogenous addition of root exudates. The soil was the same as experiment 1, and the study comprised eight distinct treatments, each replicated four times. The treatments were arranged as follows: 1) Soybean grown alone with deionized water added (S_W); 2)

Soybean grown alone with soybean intercropping root exudates added(S_RIS); 3) Soybean grown alone with maize monocropping root exudates added (S_RMM); 4) Soybean grown alone with maize intercropping root exudates added (S_RIM); 5) Maize grown alone with deionized water added (M_W); 6) Maize grown alone with maize intercropping root exudates added (M_RIM); 7) Maize grown alone with soybean monocropping root exudates added (M_RMS); and 8) Maize grown alone with soybean intercropping root exudates added (M_RIS) (Supplementary Figure S2).

The experiment commenced on August 15, 2021, with the sterilization and germination of soybean and maize seeds, following the method described in Experiment 1. The sprouted seeds were then planted in the pots (Diameter 12 cm, depth 6 cm), each containing 1.5 kg of the prepared soil, air-dried and sifted through a 2 mm mesh. Fertilizer and potting management were conducted according to the guidelines outlined in experiment1. After the crops grow for 10 days, keep 2 soybeans or 1 maize in each pot. The crops were cultivated in a greenhouse at 20°C. Additionally, separate pots were utilized for the cultivation of soybean and maize monocrops, as well as intercrops, while root exudates were collected from soybean monocrop, soybean intercrop, maize monocrops, and maize intercrops, respectively. From the 14th day after sowing (DAS), root exudates were collected (dynamic collection for 24 hours), and the collected root exudates (RIM, RMM, RIS, RMS) were added to the rhizosphere soil of soybean and maize respectively according to the experimental settings. Add 40 ml exudates to the respective treatment every 5 days, while an equal measure of deionized water served as a control in comparison treatments. On the 60th DAS, samples from plant biomass and rhizosphere soil were collected for examination. The assessments included analysis of both aboveground and belowground biomass, and evaluation of root architectural traits, such as root length, surface area, and volume. In addition, the study extended to measure soil physicochemical traits, and the levels of amino acids and organic acid, alongside appraising variations in AMF colonization under different treatment conditions.

2.2 Plant growth and soil physicochemical properties

After harvesting, the aboveground and underground biomass of soybean and maize, as well as their respective root morphological parameters were measured. The plant's aerial sections were initially sterilized at 105°C for 30 minutes and then consistently dried at 65°C until achieving a stable weight for subsequent analysis. Roots were imaged using an Epson scanner set to professional mode, 16-bit grayscale, at a resolution of 600 dpi, with the images being recorded in.jpg format. The WinRhizo 2005 software was applied to analyze these images, extracting data on total root length, diameter, volume, and surface area for both crops. Post-scanning, root samples were dried at 65°C to a constant weight for further assessments. In parallel, soil samples were collected, and a subset was air-dried and sieved through a 2 mm mesh to evaluate soil characteristics. Soil analyses encompassed measurements of pH, TN, AN, AP, AK, and TOC. Soil pH levels were gauged using a PHS-3C Meter (Toledo) in a 2.5:1 water-to-soil ratio.TN levels were determined following the Kjeldahl distillation technique (Hou et al., 2007). AP levels were gauged using the sodium bicarbonate ($NaHCO_3$) solution method at pH 8.5, following Olsen's protocol (Olsen and Sommers, 1982). AN concentrations were obtained through the diffusion adsorption method (Lu, 2000). AK extraction was performed with NH_4OAc (pH 7.0) and quantified using flame atomic absorption spectrometry (Schollenberger and Simon, 1945). TOC was determined utilizing the Multi N/C 2000 TOC analyzer (Germany), following the method of Xiao et al (Xiao et al., 2019). Finally, the mycorrhizal colonization rate was established utilizing the trypan blue staining technique (Giovannetti and Mosse, 1980).

2.3 AMF mycorrhizal infection rate and community

For determining the extent of AMF colonization in plant roots, we adapted the procedure described by Phillips and Hayman (1970). Fine roots (2 mm in diameter) were cut into segments of 1-2 cm length and subjected to clearing with 10% KOH at 90°C for 30 min, followed by triple rinsing with distilled water. The root segments were then acidified using 2% HCl and stained with a 0.05% trypan blue solution for 15 min at 90°C, followed by another series of washes with distilled water. Subsequently, the stained roots were immersed in a lacto-glycerol mixture (equal parts of lactic acid, glycerol, and water) and allowed to stand at ambient temperature. For microscopic examination, 15 root fragments were positioned on slides pre-marked for analysis (resulting in 60 fragments evaluated per treatment) (Brundrett and Kendrick 1990). Observations were conducted under 40× and 100× magnification. The degree of AMF infestation in root segments was categorized into 0, 10%, 20%…100% infestation levels, and the mycorrhizal infestation rate (%) of the samples was calculated by the weighting method of root infection rate.

The rhizosphere soil AMF community composition at genus level has been measured by high-throughput sequencing. Nested PCR was utilized to amplify fragments of the arbuscular mycorrhizal fungal 18S rRNA genes. The first PCR amplification was performed utilizing the universal primers AML1F (5'-ATCAACTTTCGATGGTAGGATAGA-3') and AML2R (5'-GAACCCAAACACTTTGGTTTCC-3'). For the second PCR procedure, we used these amplicons from the first PCR as the template, and the AMF-specific primers AMV4-5NF (5'-AAGCTCGTAGTTGAATTTCG-3') and AMDGR (5'-CCCAACTATCCCTATTAATCAT-3') were used to amplify the partial AMF 18S rRNA gene fragment (Xu et al., 2017). The resultant products from the second PCR, following verification and purification, were sequenced employing the Illumina MiSeq platform from Majorbio Bioinformatics Technology Co., Ltd. (Shanghai, China). Subsequently, sequencing outputs were consolidated and subjected to quality checks utilizing FLASH software. Clustering of the sequences into operational taxonomic units (OTUs) was implemented at a similarity threshold of 97%. Taxonomic classification of all sequences into diverse groups was executed utilizing the RDP Classifier algorithm against the

MaarjAM database with a confidence threshold of 70% (Öpik et al., 2010).

2.4 Soil metabolite analysis

2.4.1 Collection and extraction of root exudates

After 60 days of plant growth, collect soil solution from the rhizosphere of crops and use a soil solution sampler (Rhizon soil moisture samplers^{TM}, OD 2.5mm, nylon wire, Pore size of the membrane 0.12, μm PVC-PE tubing 30 cm; Rhizosphere Research Products bv Wageningen, Netherlands) as an instrument. Before use, the samplers were washed using de-ionized water and dried at 25°C. Gently insert the sampler into the rhizosphere soil 5-8 cm away, connect the outer end of the sampler to a syringe, and then vacuum the inside of the syringe. Use pressure to suck the soil solution into the syringe, Disposable syringes of 50ml were used at selected sampling dates to collect 40 ml soil solution, collecting five times in total. To collect the soil solution, soil moisture was kept at 70% capacity. During the collection process, promptly store the collected solution in a -80°C refrigerator, and mix and store the solutions in each basin after they are fully collected. Once collected, the root secretions were concentrated to a volume of 50 mL. To analyze the amino acids, 35 mL of the filtrate was vacuum freeze-dried to obtain a dry powder, which was then dissolved in 0.5 mL of deionized water. The dissolved powder was transferred to a 1.5 mL centrifuge tube and stored in a -20°C refrigerator for further analysis. Furthermore, for the analysis of low molecular weight organic acids, 15 mL of the filtrate was vacuum freeze-dried to obtain a dry powder, which was then dissolved in 1 mL of deionized water. The solution was placed in an ice bath at -20°C for analysis.

2.4.2 Nontargeted measurement of root exudates

The method for extracting soil metabolites was modified from Swenson and Northen's technique (Swenson and Northen, 2019). Briefly, one gram of soil was transferred into a 5 ml EP tube, to which 1 ml of methanol (adjusted to a 3:1 volume/volume ratio with water) and 1 ml of ethyl acetate were added, along with 10 μl of adonitol (0.5 mg/ml) serving as an internal standard. This blend was homogenized utilizing a ball mill at a frequency of 45 Hz for 4 min, followed by an ultrasonic bath in chilled water for 5 min. The mixture was then centrifuged at 12,000 rpm and 4°C for 15 min, and the supernatants were collected into fresh EP tubes. This extraction step was repeated once more with another 1 ml of methanol and 1 ml of ethyl acetate. Post a second cycle of homogenization and centrifugation, the collected supernatants were merged and subsequently dehydrated using a vacuum concentrator at ambient temperature. To finalize the sample preparation, 30 μl of methoxyamine hydrochloride (dissolved in pyridine to make a20 mg/ml solution) was introduced to the desiccated extracts, followed by a 30-minute incubation at 80°C.

Non targeted analysis of metabolites was carried out using the LC-MS system. Chromatographic resolution occurred through a UPLC system by SCIEX (UK) (Seybold et al., 2020). For separation, an ACQUITY UPLC T3 column (100 mm × 2.1 mm, 1.8 μM, Waters, UK) was utilized under reversed-phase conditions. The column temperature was set at 35°C with a consistent flow of 0.4 mL/min. Gradient elution was conducted utilizing a mixture of solvent A (water enhanced with 0.1% formic acid) and solvent B (acetonitrile enhanced with 0.1% formic acid), based on the modified approach by Yu and colleagues (Yu et al., 2018). An injection volume of 4 μL was used for the analysis. ESI-MS^n evaluations were performed with a Thermo Q Exactive mass spectrometer, scanning both positive and negative ion modes. Settings for the mass spectrometer were as follows: mass range 50–1200 Da; spray temperature 400°C; ion source temperature 120°C; nebulizer gas flow 800 L/h; and capillary voltage 40 V. Identification of soil metabolites was completed by Majorbio BioPharm Technology (Shanghai, China), and the subsequent data handling was performed employing the Majorbio Cloud Platform (www.majorbio.com).

2.4.3 Targeting measurement of specific root exudates

2.4.3.1 Amino acid analysis

Amino acids in the analyzed root exudates were quantified using high-pressure liquid chromatography with pre-column derivatization (Li et al., 2013). The amino acids were initially converted into fluorescent compounds, followed by separation on a C_{18} column. Detection and quantification were achieved using fluorescence detection methods, supported by comparative analyses with standard amino acid mixtures. The chromatographic conditions included the use of a Waters 470 fluorescence detector and a Nova2pak C_{18} column (150 × 3.9 mm, 4 μm) at ambient temperature. The mobile phase comprised two solvents: solvent A (20 mmol/L sodium acetate solution) and solvent B (a blend of 20 mmol/L sodium acetate solution, methanol, and acetonitrile in a 1:2:2 ratio, v/v/v). Fluorescence detection settings included an excitation wavelength of 338 nm, and an emission wavelength of 262 nm. The application of a gradient elution method was utilized with a flow rate set at 1.0 mL min^{-1}, and the injection volume was fixed at 10 μL. Amino acid identification was based on matching the mass-to-charge ratio (*m/z*), retention time, and ESI–MS/MS dissociation patterns to those of authentic standards.

2.4.3.2 Organic acid analysis

The type and mass concentration of organic acids were analyzed utilizing high-performance liquid chromatography (HPLC). The HPLC method employed the LC-100 high-performance liquid chromatograph, which consisted of an LC-P100 high-pressure constant flow pump, an LC-UV100 ultraviolet detector, an autosampler, and a temperature-controlled box.

The chromatographic setup utilized for analysis incorporated a reversed-phase C_{18} column (150 mm × 4.6 mm, 5 μm), employing an 18 mmol/L KH_2PO4 (pH 2.48), as the mobile phase. The operational temperature of the column was maintained at 30°C, and the mobile phase flow rate was established at 1.0 mL/min. For each run, a 10 μL sample volume was introduced, and analyte detection was conducted at 214 nm, with an overall run time of 15 min. The identification of LMWOA relied on comparing their retention times with those of standard organic acids. Quantification of the organic acids present in the samples was achieved through the external standard technique, with concentration calculations based on the comparative peak areas.

2.5 Data and statistical analysis

Principal component analysis (PCA) was implemented utilizing the SIMCA 14.1 software (Umetrics, Umeå, Sweden). For bubble diagram analysis, MetaboAnalyst was utilized (Chong et al., 2019). We analyzed the significance of differences in metabolites utilizing analysis of variance (ANOVA) and applied the least significant difference (LSD) tests for *post hoc* comparisons in SPSS V17.0, setting the significance threshold at $p < 0.05$. Orthogonal partial least squares discriminant analysis (OPLS-DA) was conducted with variable importance (VIP) values of N1. The relationships and visual representations of the differences between microbes and metabolites were explored utilizing R software v.3.2.1 and Cytoscape 3.4.0.

3 Results

3.1 Analysis of differential metabolites between monocropping and intercropping systems

Untargeted metabolomic profiling of the root exudates revealed a diverse array of compounds. A total of 13,885 mass spectrum peaks were detected from the root exudates, with 749 metabolites identified. Among these metabolites, 574 were found in public databases such as HMDB and Lipidmaps. The secreted compounds were further categorized, including 178 lipid substances, 106 organic acids, 89 organic heterocyclic compounds, 70 benzene-type compounds, 56 organic oxygen compounds, 51 phenylpropane and polyketone compounds, as well as other compounds. The proportions of these various compound classes were 31.01%, 18.47%, 15.51%, 12.20%, 9.76%, 8.89%, and 4.18% respectively (Figure 1A).

The PLS-DA plot comparing MM *vs* IM and MS *vs* IS were presented in Figures 1B, C. As observed from the figure, a clear distinction can be made between MM and IM as well as MS and IS, suggesting significant variations in the content of different compounds in the root exudates of soybean and maize under monocropping and intercropping conditions (Figures 1B, C).

Based on the ANOVA of known metabolites, significant differences ($P < 0.5$) were witnessed in the mean concentrations of 39 and 41 metabolites between IM and MM, as well as between IS and MS soil types (Figure 2). Examining the species composition of different metabolites, it was found that 38 root exudates exhibited significant up-regulated changes (Figures 2A, B). Further investigation into the root exudates with significant differences between intercropping and monocropping in maize identified eleven specific up-regulating substances, including Avocadiene, Phytal, 3,6,7-Trihydroxy-4'-methoxyflavone 7-rhamnoside, Acetyl

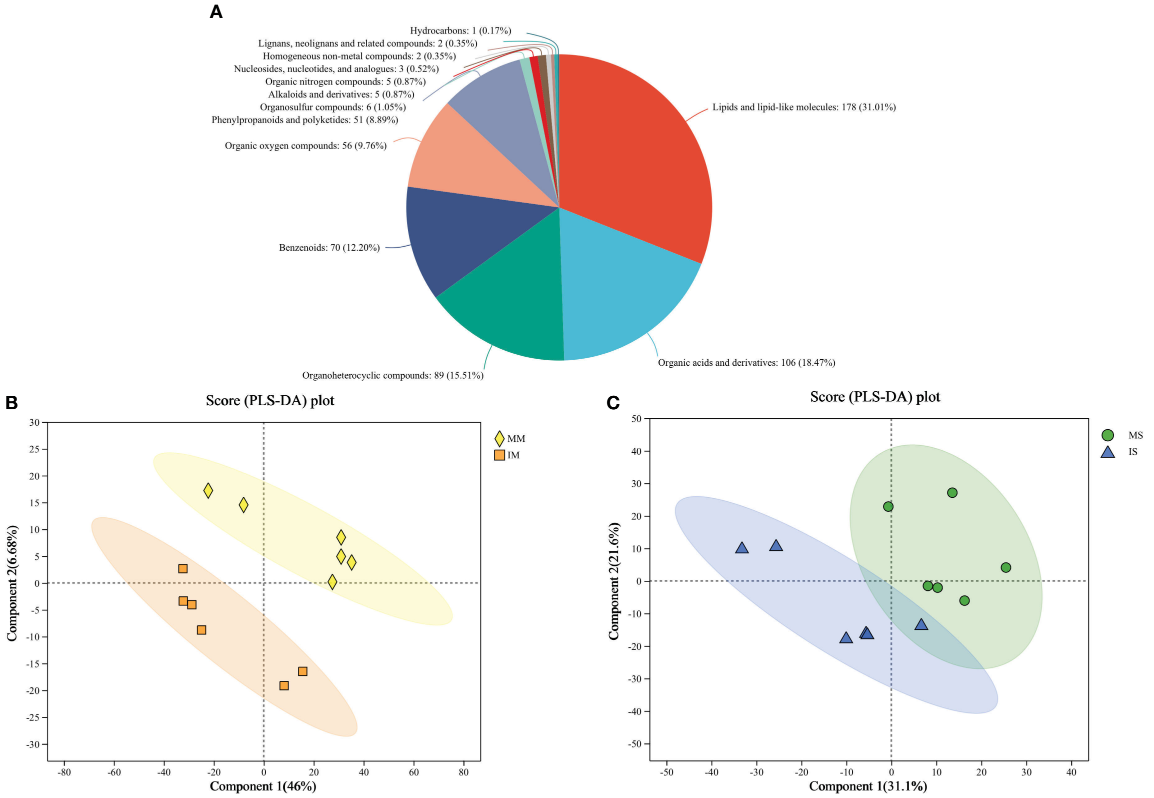


FIGURE 1
Metabolites types identified from monocropping and intercropping of soybean and maize root exudates **(A)**. Partial Least-Squares Discriminant Analysis of the root exudates in maize **(B)** and soybean **(C)** soils.

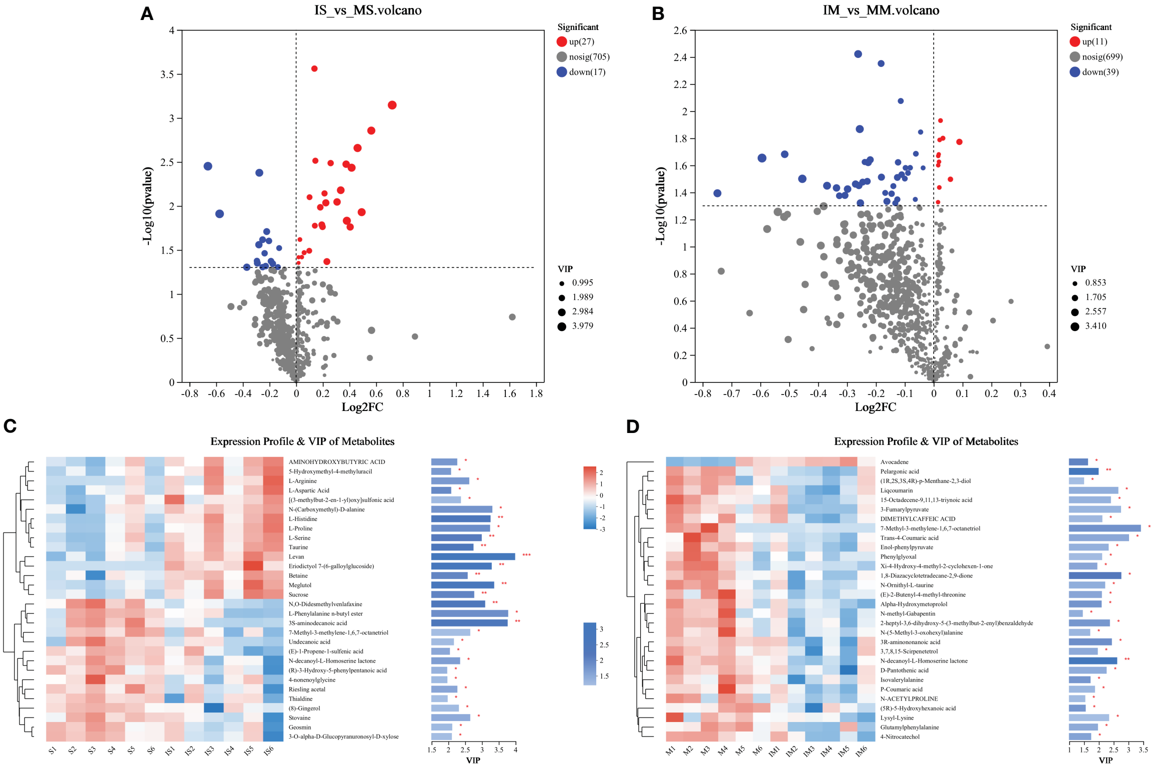


FIGURE 2
Volcano map **(A, B)** and Heatmap map (Top30) **(C, D)** of metabolites expressed differently between “IM *vs* MM” and “IS *vs* MS”.

tributyl citrate, Myristoleic acid, Shogaol, Gluten exorphin, 2-amino-14,16-dimethyloctadecan-3-ol, PE, and PG. Similarly, the analysis of common and unique root exudates in intercropping and monocropping of soybean revealed 27 specific up-regulating substances, primarily consisting of amino acids and their derivatives (9), sugars and their derivatives (3), small molecular acids (4), alcohols (2), amines (1), alkaloids and their derivatives (1), and others (7). These findings indicate that intercropping significantly alters root exudates.

To elucidate the primary metabolic pathways associated with differential metabolites, all identified metabolites in the root exudates of maize/soybean monocropping and intercropping were imported into the online analysis platform, Metaboanalyst (http://www.metaboanalyst.ca/). We identified significant pathways based on an impact value threshold above 0.1 and an enrichment pathway p-value below 0.05 (Figure 3). In the comparison between IM and MM, five distinct pathways were characterized: Phenylalanine metabolism, Tyrosine metabolism, beta-alanine metabolism, Caprolactam degradation, and alpha-linolenic acid metabolism (Figures 3A–C). Likewise, in the comparison between IS and MS, five distinct pathways were identified: Glycine, serine, and threonine metabolism, beta-alanine metabolism, Cyanoamino acid metabolism, Histidine metabolism, and Carbon fixation in photosynthetic organisms (Figures 3B–D).

3.2 The effect of intercropping on the content of amino acids and organic acids in root exudates

Quantitative targeted analysis of amino acids in the root exudates of monocropped and intercropped soybeans revealed notable findings. Glu and Arg were solely detected in the root exudates of soybean intercropping, whereas Asp, Thr, Ser, Gln, Gly, Ala, Val, Ile, Tyr, Phe, Lys, and His displayed significantly higher levels in intercropped soybeans compared to monocropped soybeans ($P < 0.05$). The total amount of amino acids in the root exudates of intercropped soybeans was 3.61 times greater than that in monocropped soybeans (Table 1). In this study, it was found that soybean/maize intercropping significantly improved the exudation of amino acids by soybean roots. Furthermore, aspartic, glutamic, alanine and other amino acids also participated in important metabolic pathways, such as alanine metabolism and glutamate metabolism, which promote plant growth.

Targeted quantitative assessments of amino acids in maize root exudates from both monocropped and intercropped systems yielded significant insights. Specifically, Arg was uniquely identified in the exudates from intercropping, whereas Cys was exclusively observed in those from monocropping. Additionally, concentrations of Glu, Gln, Ala, Val, Tyr, Lys and His were found to

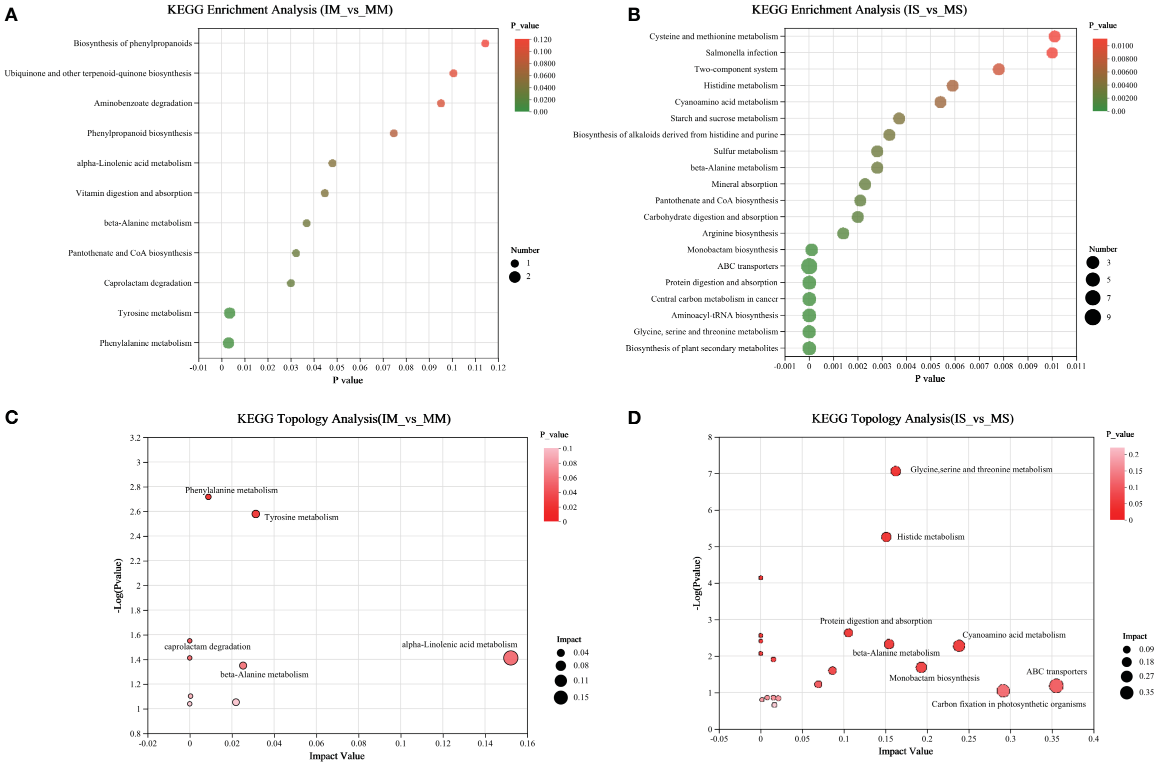


FIGURE 3
KEGG functional pathway involved in different metabolites of "IM&MM" **(A)** and "IS&MS" **(B)**. Topological diagram of important functional paths participated by differential metabolites in "IM&MM" **(C)** and "IS&MS" **(D)**.

TABLE 1 Content of amino acids and organic acids in root exudates under soybean/maize monocropped and intercropped system (unit: ug/L).

	IS	MS	IM	MM
Asp	47.70 ± 3.85a	14.10 ± 3.63b	17.99 ± 3.28a	15.73 ± 0.04a
Thr	46.87 ± 4.19a	14.52 ± 2.60b	14.82 ± 5.19a	13.88 ± 3.38a
Ser	214.17 ± 51.92a	49.99 ± 7.82b	54.61 ± 22.12a	54.16 ± 8.86a
Glu	24.60 ± 6.21	——	8.20 ± 1.44a	3.61 ± 0.01b
Gln	25.30 ± 1.60a	7.16 ± 1.30b	16.34 ± 4.19a	11.52 ± 3.65b
Gly	100.26 ± 28.80a	25.08 ± 6.50b	27.52 ± 3.19a	25.88 ± 2.41a
Ala	68.10 ± 2.36a	20.11 ± 6.62b	22.00 ± 5.47a	19.64 ± 2.43b
Val	34.91 ± 5.61a	17.40 ± 3.11b	21.27 ± 5.45a	17.50 ± 0.25b
Cys	1.53 ± 0.14a	1.74 ± 0.08a	——	0.43 ± 0.01
Met	5.15 ± 1.17a	3.49 ± 0.42a	——	——
Ile	18.74 ± 3.97a	7.28 ± 1.74b	8.99 ± 2.25a	7.37 ± 0.54a
Leu	22.98 ± 2.86a	9.73 ± 0.94b	7.53 ± 1.77a	9.79 ± 1.67a
Tyr	21.76 ± 1.74a	13.13 ± 4.06b	9.66 ± 3.08a	6.07 ± 4.60b
Phe	16.75 ± 3.68a	5.91 ± 1.57b	4.88 ± 0.71a	5.84 ± 1.18a
Lys	24.73 ± 5.82a	6.13 ± 0.90b	13.12 ± 3.53a	10.47 ± 1.70b
His	79.45 ± 13.19a	13.87 ± 2.17b	19.09 ± 5.73a	9.12 ± 0.92b
Arg	3.29 ± 1.90	——	12.48 ± 5.90	——
Total amino acid	756.27 ± 89.58a	209.64 ± 40.70b	258.47 ± 52.95a	211.00 ± 20.62b

Different lower-case letters in the same row indicate significant differences in different treatments ($P < 0.05$).

be elevated in the exudates of intercropped maize compared to those of monocropped maize ($P < 0.05$). The aggregate level of amino acids in the exudates from intercropped maize exceeded that from monocropping by 1.22-fold. Importantly, glutamine, tyrosine, and alanine are essential components participating in key metabolic pathways such as β-alanine metabolism, caprolactam degradation, and tyrosine metabolism.

Notable variations were identified in the organic acid content of both crops under monocropping and intercropping conditions (Table 2). The total amount of organic acids in soybeans from intercropped settings markedly exceeded that of those grown in isolation, reaching a magnitude 1.80 times greater. Notably, oxalic acid (442.86%, $P < 0.05$), malic acid (185.71%, $P < 0.05$), and lactic acid (31.61%, $P < 0.05$) exhibited significantly higher levels in intercropping than corresponding monocropping, and citric acid was not detected in soybean root exudates. Conversely, maize displayed an augmented total organic acid concentration under monocropping rather than under intercropping, with notable increases in oxalic (172.22%, $P < 0.05$) and malic acids (200.00%, $P < 0.05$) when grown alone. However, intercropped maize showed higher quantities of tartaric, lactic (12.59%, $P < 0.05$), and citric acids (300.00%, $P < 0.05$) than when grown singly.

3.3 Effects of root exudates on plant growth and AMF colonization in intercropping

Although not statistically significant, the biomass of soybean/maize plants separated by nylon net was higher than those separated by plastic. However, a significant difference was witnessed in the biomass of the roots, with the roots separated by nylon net displaying significantly higher biomass than those separated by plastic ($P < 0.05$). This underground interaction resulted in a remarkable elevation in the biomass of maize roots by 30.30% and soybean roots by 52.63% (Figure 4B). Compared to plastic separation, nylon net separation enhanced the exchange of root exudates and nutrients in the underground environment, facilitating biomass accumulation in both soybean and maize plants.

Throughout the development phases of soybean and maize, the incorporation of root exudates was observed to notably enhance both aboveground and belowground biomass, surpassing the results from the control group devoid of root exudate supplementation (Supplementary Figures S3A, B). The dry weight of soybean added deionized water and added root exudates of IS, MM and IM were 2.42 g, 3.10 g, 2.67 g and 3.68 g, respectively. Adding root exudates of IS, MM and IM increased the dry weight of soybean shoot by 35.22%, 23.99% and 47.29% respectively, and the dry weight of soybean root increased by 42.74%, 51.28% and 66.67%, respectively. It can be seen that the application of IS and IM root exudates has a more significant increase in aboveground biomass, and the application of IM root exudates has a more significant increase in root biomass. This effect underscores the significance of specific compounds within root exudates in fostering both vegetative and root growth. Moreover, the infusion of root exudates, particularly those derived from maize within an intercropping context, resulted in a marked increase in the formation of soybean nodules, underscoring the vital role these exudates play in augmenting soybean nodulation (Supplementary Figure S3E).

The root length of maize under nylon net separation was significantly higher than that of maize under plastic separation by 22.76% ($P < 0.05$, Figures 4D–G). When separated by plastic, crops underground growth mode is equivalent to monoculture, while under nylon mesh separation, Crops can interact through root exudates between each other. This implies that root exudates have a promoting effect on root growth under the same aboveground condition. Additionally, the adding of root exudates brings about the enhancement of root length and surface area in soybean and maize roots, as depicted in Supplementary Figures S3F–M. For example, the root length of soybean was increased by 61.60% with added root exudates of IM, and the root length of maize was increased by 34.11% with added root exudates of IS. This showed that root exudates play an important role in soybean/maize intercropping system and have a direct effect on plant root growth.

The results revealed a significantly higher AMF colonization rate in soybean/maize intercropping compared to their respective monocropping ($P < 0.05$). Specifically, the mycorrhizal colonization rate in intercropping increased by 105.99% and 111.18% when compared to monocropping conditions, as depicted in Figure 4C. Moreover, in this experiment, intercropping significantly increased the relative abundance of *Glomus_f_Glomeraceae*, the dominant genus of AMF (Figure 4A). *Glomus* plays an important roli in soil carbon fixation and nutrient cycling. So, it is suggested that

TABLE 2 Content of organic acids in root exudates under soybean/maize monocropped and intercropped system (unit: mg/L).

	IM	MM	IS	MS
tartaric acid	0.07 ± 0.00a	0.06 ± 0.00a	0.08 ± 0.00a	0.06 ± 0.00a
oxalate	0.18 ± 0.06c	0.31 ± 0.09b	1.14 ± 0.50a	0.21 ± 0.07c
malic acid	0.20 ± 0.10b	0.60 ± 0.22a	0.60 ± 0.27a	0.21 ± 0.11b
lactic acid	1.61 ± 0.30c	1.43 ± 0.21d	2.54 ± 0.21a	1.93 ± 0.68b
citric acid	0.04 ± 0.01a	0.01 ± 0.00b	——	——
Total organic acid	2.08 ± 0.48c	2.40 ± 0.07b	4.35 ± 0.57a	2.41 ± 0.84b

Different lower-case letters in the same row indicate significant differences in different treatments (P < 0.05).

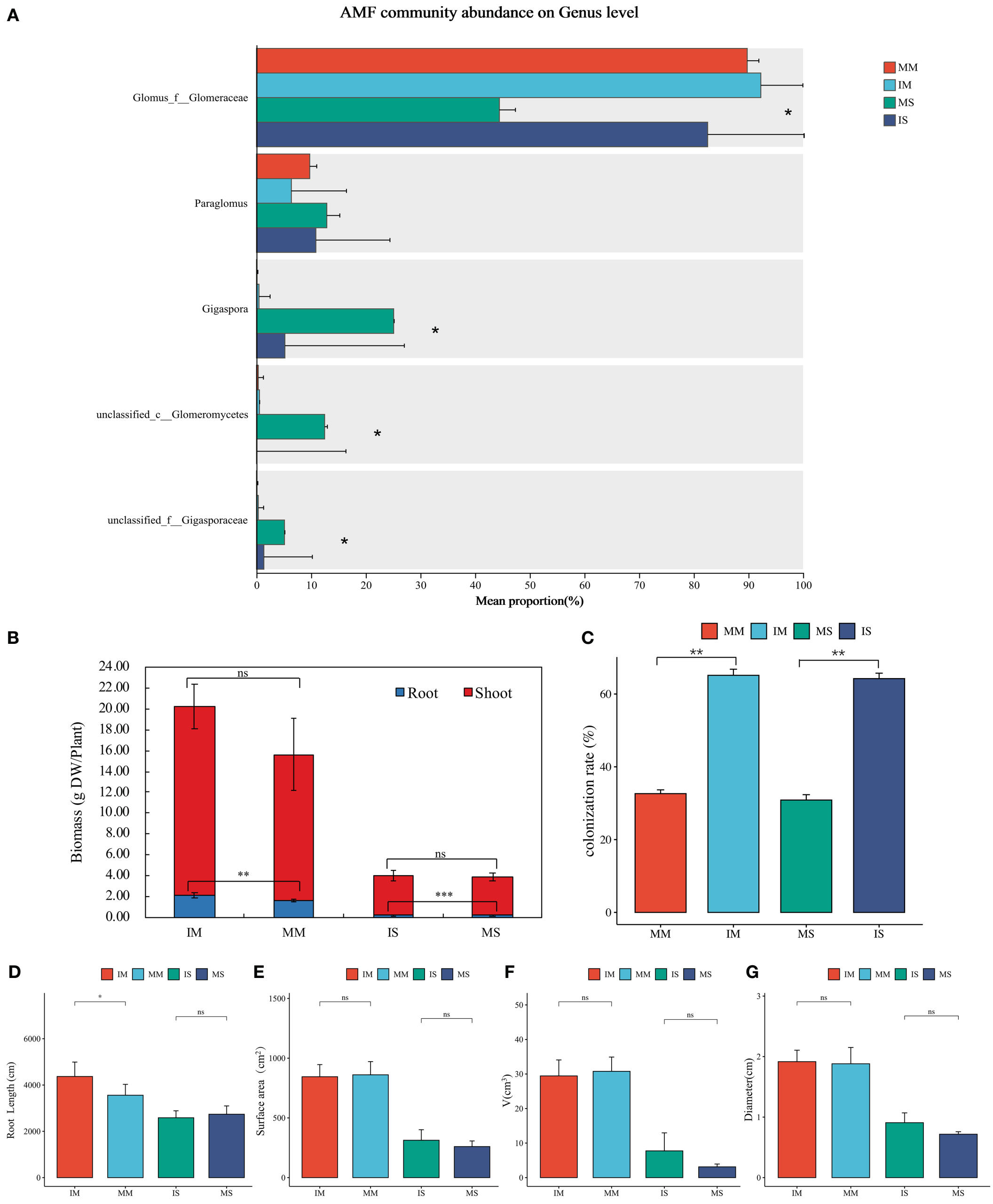


FIGURE 4
Effects of interspecific interaction on plant growth and AMF in intercropping system. Including: Relative abundances of main AMF genus in the rhizosphere soil of soybean/maize monocropping and intercropping **(A)**, The aboveground and underground biomass **(B)**, AMF colonization rate **(C)** and Root morphological parameters **(D–G)** of maize/soybean under different planting methods. *Represents the significant difference between different treatments at $P < 0.05$, ** represents the significant difference between different treatments at $P < 0.01$, *** represents the significant difference between different treatments at $P < 0.001$, ns represents no significant difference between different treatments.

intercropping not only enhances the colonization rate of arbuscular mycorrhizal fungi, but also improves the community of AMF. Moreover, the addition of root exudates from soybean or maize during the growth process significantly increased the AMF colonization rate compared to treatments without root exudates, as shown in Supplementary Figures S3C, D. In the growth of soybean, the mycorrhizal infection rate of the treatment group added root exudates of maize intercropping was the highest, which was 126.11% higher than that without root exudates. During the growth of maize, the mycorrhizal infection rate of the treatment added root exudates from soybean intercropping was the highest, which was 97.38% higher than that without root exudates. It is worth noting that adding root exudates from soybean intercropping significantly increased mycorrhizal infection in soybeans, while adding root exudates from maize intercropping did not have a significant effect on mycorrhizal infection in maize. This observation suggests that intercropped system mediated an increase in the type and quantity of root exudates, and certain substances in which may facilitate mycorrhizal colonization and community improvement.

3.4 Correlation between root exudates and AMF community

Utilizing the Mantel and Procrustes analyses, correlations were assessed between the top 20 distinct metabolites identified in the root exudates and the composition of the AMF community, as depicted in Figures 5A, B. Notable associations emerged between certain metabolites and the mycorrhizal fungal community within both monocropped and intercropped systems. Specifically, Within the differential metabolites between soybean monocropping and intercropping, several metabolites, including amino acids like L-Proline, L-Histidine, and L-Arginine, the saccharide Levan, certain organic acids such as Taurine and (R)-3-hydroxyl-5-phenylpropanoic acid, the biomolecule Choline, and the nucleic base analog 5-Hydroxymethyl-4-methyluracil were significantly correlated ($P < 0.05$) with *Glomus*, the predominant genus of the

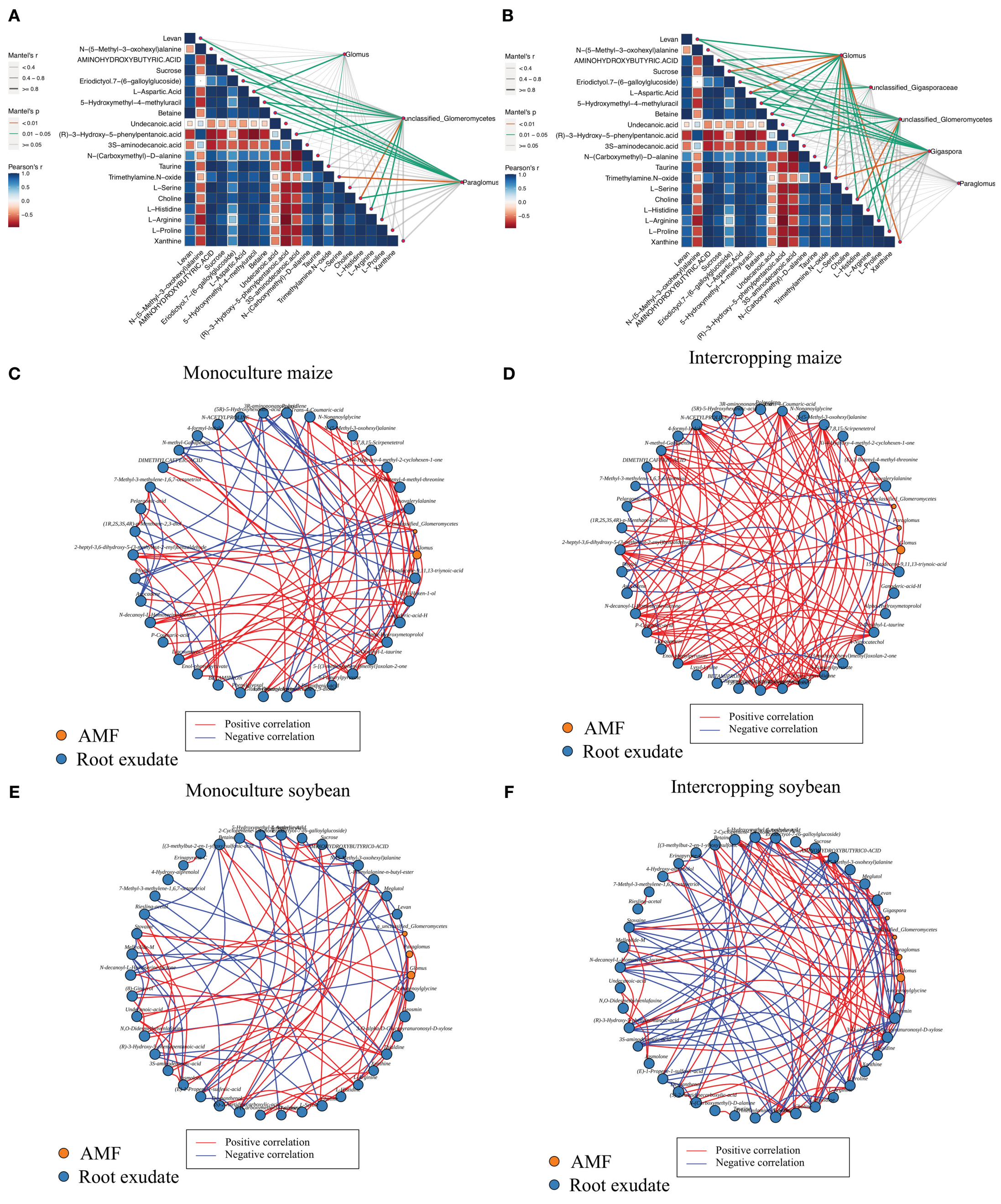


FIGURE 5

Correlation analysis between AMF and different metabolites of maize **(A)** and soybean **(B)** in monocropping and intercropping system. Analysis of the interaction network between differential metabolites and AMF (genus level) in maize monocropping **(C)** and intercropping **(D)**, and soybean monocropping **(E)** and intercropping **(F)**.

AMF community. Furthermore, compounds like Sucrose, L-Aspartic Acid, Trimethylamine N-oxide, and Xanthine were highly significant correlated ($P < 0.01$) with *Glomus* in soybean soil. Trimethylamine N-oxide also showed a positive association ($P < 0.01$) with *Gigaspora*. In contrast, within maize environments, both Sucrose and L-Aspartic Acid demonstrated notable correlations ($P < 0.05$) with *Glomus*, while Trimethylamine N-oxide was closely linked ($P < 0.05$) with the *Paraglomus* genus, and Choline was highly significant correlated ($P < 0.01$) with *unclassified_c_Glomeromycetes*.

The differential metabolites obtained from the intercropping and monocropping systems of soybean and maize were analyzed for their interaction networks with important genera of AMF. The results, illustrated in Figures 5C–F, demonstrated that the intercropping of soybean and maize led to a more intricate interplay between root exudates and AMF genera. In the interaction network specific to soybean monocropping (Figure 5E), there were 42 nodes, consisting of 39 differential metabolites and interactions with three AMF genera. This network comprised 83 pairs of interactions, with 47 pairs showing positive correlations and 36 pairs exhibiting negative correlations. The root exudates with the highest number of interactions were 3S-amino decanoic acid (amino acids) and L-Histidine (amino acids), while the genus with the most complex interactions was *Glomus*. Similarly, the soybean intercropping interaction network (Figure 5F) consisted of 43 nodes, 39 differential metabolites, and interactions involving four AMF genera. It encompassed 122 pairs of interactions, of which 63 pairs exhibited positive correlations, and 59 pairs displayed negative correlations. The root exudates that demonstrated the most complex interactions with the AMF community in this network included (R)-3-Hydroxy-5-phenylpentanoic (organic acids), Geosmin (alcohols), Histidine (amino acids), and Eriodictyol 7-(6-galloylglucoside) (sugar). Furthermore, the maize monocropping interaction network (Figure 5C) consisted of 38 nodes, representing 36 differential metabolites and interactions with two AMF genera. It comprised 94 pairs of interactions, with 59 pairs showing positive correlations and 35 pairs displaying negative correlations. The root exudate with the highest number of interactions in this network was N-decanoyl-L-Homoserine lactone (sugar). Finally, the maize intercropping interaction network (Figure 5D) consisted of 40 nodes, 37 differential metabolites, and interactions involving three AMF genera. It encompassed 120 pairs of interactions, with 99 pairs demonstrating positive correlations and 21 pairs exhibiting negative correlations. The root exudates with the most interactions in this network were N-methyl-Gabapentin (organic acid), 3,7,8. 15-Scirpenetetrol (sugar), and D-Pantothenic acid (organic acid). These findings indicated that intercropping significantly enhanced the mutualistic relationship between root exudates and the AMF community.

Phenylalanine and leucine in maize root exudates exhibited a highly significant positive correlation with *Glomus*, the prominent genus in AMF ($P < 0.01$). Additionally, serine was positively correlated with *Glomus* ($P < 0.05$), as depicted in Figure 6B. In the case of soybean root exudates, glycine, methionine, isoleucine, phenylalanine, and arginine demonstrated significant positive correlations with *Glomus* ($P < 0.01$), as shown in Figure 6A. Furthermore, aspartic acid, threonine, serine, glutamic acid, and other metabolites displayed significant positive correlations with *Glomus* as well ($P < 0.05$). Interestingly, significant or highly significant correlations were observed between tartaric acid, oxalic acid, malic acid, and Glomus in soybean root exudates ($P < 0.05$), as displayed in Figures 6C, D.

4 Discussion

4.1 Different expression of root exudates under different planting modes

Root systems release a diverse array of organic substances into the rhizosphere, playing crucial roles in nutrient and water uptake, and mediating interactions with surrounding plants (Bais et al., 2006). These exudates range from low-molecular-weight entities like sugars and amino acids to high-molecular-weight compounds including proteins and polysaccharides (Bertin et al., 2003; Cesco et al., 2010). Notably, the nature of these exudates is often altered by different agricultural practices such as intercropping. For example, pairing maize with peanuts significantly modifies the latter's root exudates, notably increasing total isoflavones by 22.4% (Gao et al., 2022). In a tea-persimmon intercropping system, intercropping was found to enhance the ability of roots to secrete amino acids (Zhu et al., 2006). Similarly, in a perilla/corn intercropping pot experiment, Qin et al. discovered that intercropping increased the concentration of oxalic acid and citric acid in the root exudates of perilla by 43.8% and 75.4%, respectively, while maize decreased them by 18.5% and 18.7%, respectively. Furthermore, the changes in root exudates were found to impact the bioavailability of soil Cd (Qin et al., 2021). Our findings echo these observations, demonstrating significant alterations in the composition and content of root exudates under intercropping conditions for both maize and soybean. Specifically, intercropped soybean and maize exhibited variations in 41 and 39 metabolites, respectively, compared to their monocropped counterparts, encompassing esters, sugars, amino acids, and organic acids. Notably, amino acid and organic acid levels in intercropped soybean were observed to be 3.61 and 1.80 times higher, respectively, than in their monocropped versions. The greater effect of intercropping on soybean may be due to the distinctive nutrient absorption dynamics between soybean and maize roots within intercropping systems (Li et al., 2007). For example, when intercropped leguminous crops with Poaceae crops, leguminous crops can produce more organic acids compared to monoculture, thereby activating insoluble phosphorus compounds. However, Poaceae did not show this reaction. This mainly because the competition of Gramineae roots for phosphorus (P) in rhizosphere soil of leguminous crops is fierce, which makes the concentration of available phosphorus in rhizosphere of leguminous crops decrease. As a result, leguminous crops produce more organic acids, activate soil insoluble phosphorus compounds, and promote the absorption of phosphorus (P) by leguminous crops (Li et al., 2007).

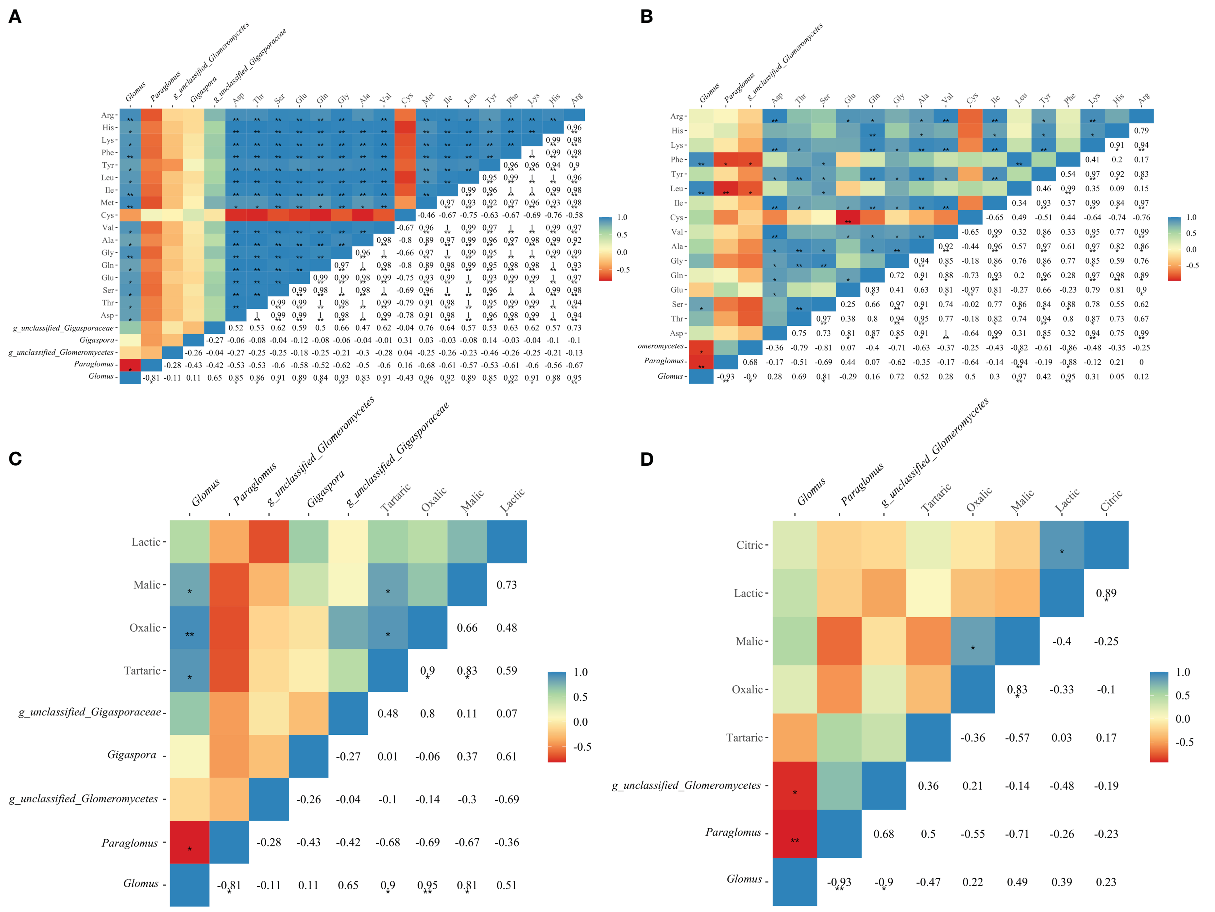


FIGURE 6
Correlation analysis between amino acids, organic acids and AMF community in soybean **(A, C)** and maize **(B, D)** soil.

This study revealed that intercropping soybean and maize notably enhanced the rate of amino acid secretion by soybean roots (Table 1). This increase in secretion could be attributed to the promotion of nitrogen absorption and utilization capacity of soybeans as a result of intercropping (Duchene et al., 2017; Lu et al., 2024). Consequently, the root's ability to secrete amino acids is enhanced. Amino acids present in root exudates serve as a vital nitrogen source for the growth of rhizosphere microorganisms (Broeckling et al., 2008). Intercropping, by enhancing amino acid secretion, provides a material foundation for increasing the abundance of rhizosphere microorganisms. Furthermore, the reciprocal influence between these microorganisms and the secretion of plant amino acids necessitates further investigation (Phillips et al., 2004).

Intercropping soybean and maize can lead to nutrient competition, potentially increasing the rate of organic acid secretion. Such secretion patterns often alter in response to nutrient deficiencies (Degryse et al., 2008). In this study, the primary influencing factors are light and nutrient stress caused by maize's shading and competition for nutrients with soybean in the intercropping system (Li et al., 2009). Consequently, the types and secretion rates of organic acids may differ between monocropping and intercropping systems. Maize-soybean intercropping not only impacts the types of root organic acid secretion but also significantly enhances the rate of secretion. This can be attributed to the promotion of crop nitrogen (N) and phosphorus (P) absorption and utilization, improvement in crop carbon and nitrogen assimilation capacities, enhancement of organic acid metabolism in plants, and ultimately, alterations in the secretion characteristics of low-molecular-weight organic acids in the roots of intercropped crops.

4.2 Effects of root exudates on plant growth in intercropping system

Root exudates serve as vital agents for material and information exchange between plants and the soil, acting as the primary regulators of root activity (Gao et al., 2022). In this experiment, the soybean and maize under nylon netting separation, exhibited increased above-ground and below-ground biomass (Figure 4B; Supplementary Figure S4) as well as root growth (Figures 4D–G) compared to plastic sheeting separation. When separated by plastic, crops underground growth mode is equivalent to monoculture, and there is no competition in water and nutrient absorption caused by mass flow diffusion between crop roots, and there is also a lack of mechanisms and motivation to promote root growth (Peng et al., 2017). While under nylon mesh separation, Crops can interact through root exudates between each other. This implies that root exudates have a promoting effect on plant and root growth under the same aboveground condition. Furthermore, the direct addition of exogenous root secretions significantly boosted biomass and root growth in both soybean and maize (Supplementary Figures S3A, B, F–M), which was consistent with the results of the current study (Li et al., 2016). And a study has shown that L-Tryptophan in root exudates can promote the growth of roots (Lu et al., 2024). This finding suggested the essential role of root exudates on plants growth in soybean/maize intercropping systems. Additionally, the addition of root exudates led to an elevation in the number of soybean nodules during crop growth (Supplementary Figure S3E). This aligns with findings from Li et al (Li et al., 2008), which highlighted the improved nodulation and nitrogen-fixing capabilities in legumes when intercropped with cereals. Additionally, the legume/cereal intercropping model has been noted for its ability to enhance phosphorus (P) utilization by inducing the secretion of organic acids from leguminous roots (Latati et al., 2014), and for boosting nitrogen uptake through increased nodulation in leguminous plants (Fang et al., 2006; Zhao et al., 2020). Thus, root exudates in intercropping systems enhance the absorption and utilization of mineral elements by plants and enable their adaptation to the changes in the external environment.

This study found that intercropping significantly increased the secretion of aspartic acid, glycine, alanine, tyrosine and glutamic acid (Table 1), which may promote the growth of crops. It has been found that soil amino acids were closely related to soil fertility and plant productivity (Moreira and Moraes, 2016), and affect the metabolism of carbon and nitrogen in the soil. A study found that glutamic acid plays a crucial role as an important component in chlorophyll synthesis, significantly promoting crop growth (El-Metwally et al., 2022). When glutamic acid is present in the free form in soil pore water, it forms a film on the surface of crop root hair (Limami et al., 2008), effectively enhancing nutrient absorption from the soil and regulating the acidity and alkalinity of the soil microbial environment (Limami et al., 2008). In this research, we found that root exudates from soybean and maize, when intercropped, play pivotal roles in essential metabolic pathways associated with amino acid metabolism, significantly influencing carbon and nitrogen dynamics within the soil (Figure 3). Among these, α-linolenic acid metabolism was critical for enhancing crop quality. Metabolism of compounds such as alanine, aspartate, and glutamate is crucial in modulating nitrogen flow, augmenting carbon availability, and altering cytosolic pH levels to support plant development (Limami et al., 2008).In maize, nitrogen metabolism stands as a critical pathway reflecting not only the plant's developmental stages but also affecting yield and quality (Zhao et al., 2018; Lu et al., 2019). Compounds present in root exudates are involved in significant pathways of amino acid and carbon and nitrogen metabolism, potentially explaining the improved mycorrhizal rate, enhanced nutrient absorption efficiency, and increased crop biomass after intercropping (Moe, 2013). Additionally, in soybean and maize intercropping systems, elements such as sugars, carboxylic acids, and amino acids act as primary carbon sources for rhizospheric microorganisms (Dong et al., 2013). Intercropping systems also increased the secretion of organic acids from plant roots, especially legumes (Table 2). Organic acids are instrumental in nutrient enrichment within the root vicinity, easing nutrient acquisition by plants and thereby enhancing their nutritional profile (Haichar et al., 2014). These acids are crucial for activating essential nutrient elements, with oxalic, citric, acetic, succinic, tartaric, malic, and malonic acids being notably prevalent in root exudates. Their presence reduces rhizospheric pH, boosting the solubility of compounds otherwise insoluble (Shen et al., 2002), and forms chelates with various metal ions (Dakora and Phillips, 2002), thus liberating nutrient in the rhizosphere soil. The augmentation in organic acid exudation within the intercropping arrangement is thought to play a crucial role in mobilizing non-soluble soil nutrients, particularly enhancing phosphorus (P) solubility (Jones, 1998), thereby boosting the accessible phosphorus (P) levels in the rhizospheric soil and fostering an improved nutritional state through natural self-adjustment mechanisms. Furthermore, these organic acids can react with toxic metal ions in the soil, forming compounds with reduced toxicity and mitigating the harmful effects of toxic ions on plant roots. Notably, this study identified two main types of root exudates, namely amino acids and organic acids, each exerting positive effects on crop growth and soil environment, aligning with the experimental results obtained.

4.3 Effects of root exudates on soil environment and microorganisms, especially AMF, under intercropping system

In the intercropping rhizosphere environment, plants primarily utilize root exudates to regulate the physical and chemical properties of the soil (Bertin et al., 2003; Okubo et al., 2016). Our study demonstrates a significant correlation between the type and content of root exudates and the levels of AN, AP, and AK in intercropped soils (Supplementary Figure S6). Root exudates in intercropping systems play a crucial role in activating otherwise inaccessible nutrients in the soil, enhancing nutrient effectiveness, and optimizing root distribution for resource utilization (Li et al., 2006, 2014). Through the secretion of root exudates, plants can also improve soil microbiological conditions, alleviate nutrient stress, and regulate microbial communities to promote their growth (Withers et al., 2020). Moreover, root exudates serve as substrates, signaling molecules, and antimicrobial agents, which influence the relative abundance of rhizosphere microorganisms and indirectly enhance the soil environment by stimulating microbial growth and activity, facilitating the production of extracellular enzymes (Li et al., 1997; Nardi et al., 2002), and promoting the degradation of soil organic matter (Razavi et al., 2016; Lopez-Sangil et al., 2017).

Root exudates not only regulate the nutrient availability of the rhizosphere soil but also influence and modulate the soil microbial community (Philippot et al., 2013; Zhalnina et al., 2018). Intercropping significantly affects the structure and diversity of the soil microbial community through root exudates (Chen et al., 2019). Our study found that intercropping increased AMF colonization and improved AMF community structure (Figure 4C; Supplementary Figure S5). These findings align with a previous field experiment that demonstrated how intercropping substantially improves the diversity of mycorrhizal fungi (Zhang et al., 2020). Moreover, our study revealed significant differences in root exudates between soybean/maize monocropping and intercropping, and correlation analysis indicated a close relationship between root exudates and AMF (Figure 5). Furthermore, the addition of soybean or maize root exudates to crop growth substantially enhances mycorrhizal fungi colonization (Supplementary Figures S3C, D). Root exudates are crucial in modulating plant-AMF interactions and shaping the soil microbial community (Haichar et al., 2008; Hugoni et al., 2018). Studies demonstrate that the addition of root exudates significantly stimulates the germination rates of AM fungal spores and promotes hyphal growth (Bücking et al., 2008; Nagata et al., 2016). This phenomenon can be attributed to the metabolic function of root exudates and the reciprocal regulation between exudates and soil microorganisms. Research shows that roots release various compounds during plant growth, such as sugars, organic acids, amino acids, phenolic acids, and other metabolites. These compounds selectively attract bacteria and fungi, promoting their aggregation and proliferation in the rhizosphere, thus forming a rhizosphere interaction (Brencic and Winans, 2005). Root exudates are key in initiating and modulating interactions between plant roots and soil microbes, crucially influencing the communication between these two entities (Bais et al., 2006; Houlden et al., 2008). Even minor variations in the composition or quantity of these exudates can significantly alter the microbial community and its structure in the rhizosphere (Ling et al., 2011). Accumulating evidence have shown that a range of compounds including vitamins, amides, terpenoids, organic acids, amino acids, derivatives, lipids, and benzoxazinoids play roles in regulating both the soil microbiota and plant growth (Faure et al., 2009; Hu et al., 2018; Zhalnina et al., 2018). Moreover, components like sugars, organic acids, amino acids and derivatives, and lipids serve as carbon and energy sources for rhizosphere microorganisms (Mavrodi et al., 2021). Our study revealed that intercropping between soybean and maize increased the secretion of sugar, amino acids, organic acids, and lipids in root exudates. This enhanced secretion provides an abundant supply of carbon sources for rhizosphere microorganisms, thereby promoting the improvement of rhizosphere microbial diversity, particularly AMF diversity in intercropping systems. Additionally, we identified important metabolic pathways in soybean/maize intercropping, which involved amino acid metabolism, carbon and nitrogen metabolism, carbon fixation by photosynthetic organisms, and other important pathways (Figure 3). The increase in soil carbon and nitrogen metabolism may be a significant factor contributing to the enhanced mycorrhizal colonization rate following intercropping. Furthermore, the carbon fixation pathway of photosynthetic organisms exhibits a functional connection with AMF (Jeewani et al., 2021; Agnihotri et al., 2022). Organic acids present in root exudates, as vital energy sources for soil microbial growth, stimulate microbial and enzyme activities in the root zone (Haichar et al., 2014). One previous study has demonstrated that organic acids, such as fumaric acid, promote the formation of biofilms in Bacteroides polymyxa SQR9 (Liu et al., 2014). In our investigation, we detected oxalic acid, malic acid, citric acid, succinic acid, and tartaric acid in the root exudates of maize/soybean intercropping, which may serve as nutritional sources for the growth and reproduction of AMF. Furthermore, organic acids can activate insoluble nutrients in the soil and improve its physical and chemical properties, thereby enhancing mycorrhizal colonization and AMF diversity. We also observed a close relationship between the relative abundance of the significant AMF genus *Glomus_f_Glomerace* and the concentration of differential metabolites (Figures 5, 6). Studies show that *Glomus*, can effectively obtain nitrogen (N) and phosphorus (P) (Jiang et al., 2020), and *Glomus* is beneficial to carbon fixation and soil organic carbon protection, therefore *Glomus* plays an important role in soil nutrient cycle. Intercropping system can increase the abundance of *Glomus* in AMF community, which is of positive significance to improve soil fertility. Correlation analysis revealed significant associations between several differential metabolites (tartaric acid, oxalic acid, malic acid, aspartic acid, and alanine) and the AMF community (Figure 6). Notably, the photosynthetic carbon fixation pathway involving aspartic acid was identified as a pathway that may be related to the formation of AMF (Agnihotri et al., 2021), and aspartic acid showed a significant connection with *Glomus_f_Glomerace* ($P < 0.05$, Figure 6). Therefore, it is demonstrated that the root exudates of soybean/maize intercropping mediate changes in AMF diversity and community structure. Moreover, the mycorrhizal symbiotic relationships between AMF and plants can enhance the nutrient absorption capacity and help to improve the growth and productivity of host plants (Ferrol et al., 2019). Therefore, root exudates in intercropping can not only directly promote crops growth, but also indirectly promote plant growth by regulating the colonization and community of AMF. Intercropping can bring higher crop productivity as well as maintain soil nutrients, which is of positive significance to improve the sustainability of soil and the diversity and stability of farmland ecosystem. Therefore, it is summarized that in the intercropping system of soybean/maize, intercropping influences AMF and soil microecology by altering root exudate patterns, ultimately establishing intercropping dominance.

5 Conclusion

In this study, the diversity of AMF communities in the maize/soybean intercropping system was distinctly influenced due to altered root exudate compositions and metabolic profiles. Notably, analyses revealed 39 unique metabolites in the exudates of intercropped versus monocropped maize, and 41 distinct metabolites between intercropped and monocropped soybean ($P < 0.05$, VIP > 1). There was a notable increase in the release of amino acids, organic acids, and other substances in intercropped system ($P < 0.05$). Such changes were associated with enhanced growth in plants and roots, increased mycorrhizal colonization, and improved soil nutrient status, which was attributed to the influence of root exudates. And the addition of root exudates significantly bolstered plant growth and AMF colonization in both soybeans and maize. Crucial metabolic pathways involved in differential metabolites, such as amino acid metabolism and carbon fixation pathways, were found to be significantly correlated to AMF functionality. The variation in root exudates and metabolic processes contributed to improved plant growth and soil microecology. Metabolites like tartaric acid, oxalic acid, malic acid, aspartic acid, and alanine showed significant correlations with AMF communities ($P < 0.05$). Notably, the pathway of photosynthetic carbon fixation involving aspartic acid demonstrated a close functional relationship with AMF, and aspartic acid was significantly associated with the dominant AMF genus, Glomus_f_Glomerace. The findings suggested that maize/soybean intercropping was more effective in increasing AMF colonization and metabolite accumulation than monocropping. Compounds such as aspartic acid in the root exudates of the intercropping system significantly altered the diversity and community structure of AMF, thus contributing to the improvement of soil quality and microecology. This research sheds light on the pivotal role of root exudates in facilitating plant-soil interactions within intercropping systems and underscores their importance in sustaining agricultural ecosystem health and promoting soil vitality. Additionally, it provides a scientific basis for diverse cropping aimed at conserving black soil and ensuring sustainable field management.

Data availability statement

The data presented in the study are deposited in the NCBI Sequence Read Archive repository, accession number Bio Project PRJNA781197.

Author contributions

SZ: Data curation, Formal analysis, Investigation, Validation, Writing – original draft. SL: Funding acquisition, Writing – review & editing, Project administration, Supervision. LM: Funding acquisition, Writing – review & editing. XL: Writing – review & editing. YZ: Visualization, Writing – review & editing. SCZ: Writing – review & editing, Software. HZ: Writing – review & editing.

Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article. The study was supported by National Natural Science Foundation of China (32271657).

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

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpls.2024.1375194/full#supplementary-material

References

Agnihotri, R., Bharti, A., Ramesh, A., Prakash, A., and Sharma, M. P. (2021). Glomalin related protein and C16: 1ω5 PLFA associated with AM fungi as potential signatures for assessing the soil C sequestration under contrasting soil management practices. *Eur. J. Soil Biol.* 103, 103286. doi: 10.1016/j.ejsobi.2021.103286

Agnihotri, R., Sharma, M. P., Prakash, A., Ramesh, A., Bhattacharjya, S., and Patra, A. K. (2022). Glycoproteins of arbuscular mycorrhiza for soil carbon sequestration: Review of mechanisms and controls. *Sci. Total Environ.* 806, 150571. doi: 10.1016/j.scitotenv.2021.150571

Bago, B., Pfeffer, P. E., and Shachar-Hill, Y. (2000). Carbon metabolism and transport in arbuscular mycorrhizas. *Plant Physiol.* 124, 949–958. doi: 10.1104/pp.124.3.949

Bais, H. P., Weir, T. L., Perry, L. G., Gilroy, S., and Vivanco, J. M. (2006). The role of root exudates in rhizosphere interactions with plants and other organisms. *Annu. Rev. Plant Biol.* 57, 233–266. doi: 10.1146/annurev.arplant.57.032905.105159

Bertin, C., Yang, X., and Weston, L. A. (2003). The role of root exudates and allelochemicals in the rhizosphere. *Plant Soil.* 256, 67–83. doi: 10.1023/A:1026290508166

Brencic, A., and Winans, S. C. (2005). Detection of and response to signals involved in host-microbe interactions by plant-associated bacteria. *Microbiol. Mol. Biol. Rev.* 69, 155–194. doi: 10.1128/MMBR.69.1.155-194.2005

Broeckling, C. D., Broz, A. K., Bergelson, J., Manter, D. K., and Vivanco, J. M. (2008). Root exudates regulate soil fungal community composition and diversity. *Appl. Environ. Microbiol.* 74, 738–744. doi: 10.1128/AEM.02188-07

Brooker, R. W., Bennett, A. E., Cong, W. F., Daniell, T. J., George, T. S., Hallett, P. D., et al. (2015). Improving intercropping: a synthesis of research in agronomy, plant physiology and ecology. *New Phytol.* 206, 107–117. doi: 10.1111/nph.13132

Brundrett, M., and Kendrick, B. (1990). The roots and mycorrhizae of herbaceous woodland plants: II. structural aspects of morphology. *New Phytol.* 114, 469–479. doi: 10.1111/j.1469-8137.1990. tb00415.x

Bücking, H., Abubaker, J., Govindarajulu, M., Tala, M., Pfeffer, P. E., Nagahashi, G., et al. (2008). Root exudates stimulate the uptake and metabolism of organic carbon in germinating spores of Glomus intraradices. *New Phytol.* 180, 684–695. doi: 10.1111/j.1469-8137.2008.02590.x

Cesco, S., Neumann, G., Tomasi, N., Pinton, R., and Weisskopf, L. (2010). Release of plant-borne flavonoids into the rhizosphere and their role in plant nutrition. *Plant Soil.* 329, 1–25. doi: 10.1007/s11104-009-0266-9

Chaparro, J. M., Badri, D. V., Bakker, M. G., Sugiyama, A., Manter, D. K., and Vivanco, J. M. (2013). Root exudation of phytochemicals in Arabidopsis follows specific patterns that are developmentally programmed and correlate with soil microbial functions. *PloS One* 8, e55731. doi: 10.1371/annotation/51142aed-2d94-4195-8a8a-9cb24b3c733b

Chen, C., Chen, H. Y. H., Chen, X., and Huang, Z. (2019). Meta-analysis shows positive effects of plant diversity on microbial biomass and respiration. *Nat. Commun.* 10, 1332. doi: 10.1038/s41467-019-09258-y

Chong, J., Yamamoto, M., and Xia, J. (2019). MetaboAnalystR 2.0: from raw spectra to biological insights. *Metabolites.c* 9, 57. doi: 10.3390/metabo9030057

Coskun, D., Britto, D. T., Shi, W., and Kronzucker, H. J. (2017). How plant root exudates shape the nitrogen cycle. *Trends Plant Sci.* 22, 661–673. doi: 10.1016/j.tplants.2017.05.004

Dakora, F. D., and Phillips, D. A. (2002). Root exudates as mediators of mineral acquisition in low-nutrient environments. *Plant Soil.* 245, 35–47. doi: 10.1023/A:1020809400075

Degryse, F., Verma, V., and Smolders, E. (2008). Mobilization of Cu and Zn by root exudates of dicotyledonous plants in resin-buffered solutions and in soil. *Plant Soil.* 306, 69–84. doi: 10.1007/s11104-007-9449-4

Dong, Y., Yang, Z.-x., Dong, K., Tang, L., Zheng, Y., and Hu, G.-b. (2013). Effects of nitrogen application rate on faba bean fusarium wilt and rhizospheric microbial metabolic functional diversity. *J. Appl. Ecol.* 24, 1101–1108. doi: 10.13287/j.1001-9332.2013.0265

Duchene, O., Vian, J.-F., and Celette, F. (2017). Intercropping with legume for agroecological cropping systems: Complementarity and facilitation processes and the importance of soil microorganisms. *Agric Ecosyst. Environ.* 240, 148–161. doi: 10.1016/j.agee.2017.02.019

Eisenhauer, N., Scheu, S., and Jousset, A. (2012). Bacterial diversity stabilizes community productivity. *PloS One* 7, e34517. doi: 10.1371/journal.pone.0034517

El-Metwally, I. M., Sadak, M. S., and Saudy, H. S. (2022). Stimulation effects of glutamic and 5-aminolevulinic acids on photosynthetic pigments, physio-biochemical constituents, antioxidant activity, and yield of peanut. *Gesunde Pflanz.* 74, 915–924. doi: 10.1007/s10343-022-00663-w

Fang, Q., Qiang, Y., Wang, E., Chen, Y., Zhang, G., Wang, J., et al. (2006). Soil nitrate accumulation, leaching and crop nitrogen use as influenced by fertilization and irrigation in an intensive wheat-maize double cropping system in the North China Plain. *Plant Soil.* 284, 335–350. doi: 10.1007/s11104-006-0055-7

Faure, D., Vereecke, D., and Leveau, J. H. J. (2009). Molecular communication in the rhizosphere. *Plant Soil.* 321, 279–303. doi: 10.1007/s11104-008-9839-2

Ferrol, N., Azcón-Aguilar, C., and Pérez-Tienda, J. (2019). Review: Arbuscular mycorrhizas as key players in sustainable plant phosphorus acquisition: An overview on the mechanisms involved. *Plant Sci.* 280, 441–447. doi: 10.1016/j.plantsci.2018.11.011

Gao, Q., Deng, H., Yang, Z., Yang, Q., Zhang, Y., Yuan, X., et al. (2022). Sodium danshensu attenuates cerebral ischemia-reperfusion injury by targeting AKT1. *Front. Pharmacol.* 13. doi: 10.3389/fphar.2022.946668

Giovannetti, M., and Mosse, B. (1980). An evaluation of techniques for measuring vesicular arbuscular mycorrhizal infection in roots. *New Phytol.* 84, 489–500. doi: 10.1111/j.1469-8137.1980.tb04556.x

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

Haichar, F. Z., Santaella, C., Heulin, T., and Achouak, W. (2014). Root exudates mediated interactions belowground. *Soil Biol. Biochem.* 77, 69–80. doi: 10.1016/j.soilbio.2014.06.017

Hauggaard-Nielsen, H., Jørnsgaard, B., Kinane, J., and Jensen, E. S. (2008). Grain legume–cereal intercropping: The practical application of diversity, competition and facilitation in arable and organic cropping systems. *Renew Agric. Food Syst.* 23, 3–12. doi: 10.1017/S1742170507002025

Hou, Z. N., Li, P. F., Li, B. G., Gong, J., and Wang, Y. N. (2007). Effects of fertigation scheme on N uptake and N use efficiency in cotton. *Plant Soil.* 290, 115–126. doi: 10.1007/s11104-006-9140-1

Houlden, A., Timms-Wilson, T. M., Day, M. J., and Bailey, M. J. (2008). Influence of plant developmental stage on microbial community structure and activity in the rhizosphere of three field crops. *FEMS Microbiol. Ecol.* 65, 193–201. doi: 10.1111/fem.2008.65.issue-2

Hu, L., Robert, C. A. M., Cadot, S., Zhang, X., Ye, M., Li, B., et al. (2018). Root exudate metabolites drive plant-soil feedbacks on growth and defense by shaping the rhizosphere microbiota. *Nat. Commun.* 9, 2738. doi: 10.1038/s41467-018-05122-7

Hugoni, M., Luis, P., Guyonnet, J., and Haichar, F. (2018). Plant host habitat and root exudates shape fungal diversity. *Mycorrhiza.* 28, 451–463. doi: 10.1007/s00572-018-0857-5

Jeewani, P. H., Luo, Y., Yu, G., Fu, Y., He, X., and Van Zwieten, L. (2021). Arbuscular mycorrhizal fungi and goethite promote carbon sequestration *via* hyphal-aggregate mineral interactions. *Soil Biol. Biochem.* 162, 108417. doi: 10.1016/j.soilbio.2021.108417

Jiang, Y., Luan, L., Hu, K., Liu, M., and Sun, B. (2020). Trophic interactions as determinants of the arbuscular mycorrhizal fungal community with cascading plant-promoting consequences. *Microbiome.* 8, 1–14. doi: 10.1186/s40168-020-00918-6

Jones, D. L. (1998). Organic acids in the rhizosphere–a critical review. *Plant Soil.* 205, 25–44. doi: 10.1023/A:1004356007312

Karlen, D. L., Veum, K. S., Sudduth, K. A., Obrycki, J. F., and Nunes, M. R. (2019). Soil health assessment: Past accomplishments, current activities, and future opportunities. *Soil Tillage Res.* 195, 104365. doi: 10.1016/j.still.2019.104365

Kuzyakov, Y., and Blagodatskaya, E. (2015). Microbial hotspots and hot moments in soil: Concept & review. *Soil Biol. Biochem.* 83, 184–199. doi: 10.1016/j.soilbio.2015.01.025

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

Landi, L., Valori, F., Ascher, J., Renella, G., Falchini, L., and Nannipieri, P. (2006). Root exudate effects on the bacterial communities, CO2 evolution, nitrogen transformations and ATP content of rhizosphere and bulk soils. *Soil Biol. Biochem.* 38, 509–516. doi: 10.1016/j.soilbio.2005.05.021

Latati, M., Blavet, D., Alkama, N., Laoufi, H., Drevon, J. J., Gérard, F., et al. (2014). The intercropping cowpea-maize improves soil phosphorus availability and maize yields in an alkaline soil. *Plant Soil.* 385, 181–191. doi: 10.1007/s11104-014-2214-6

Li, L., Li, S.-M., Sun, J.-H., Zhou, L.-L., Bao, X.-G., Zhang, H.-G., et al. (2007). Diversity enhances agricultural productivity *via* rhizosphere phosphorus facilitation on phosphorus-deficient soils. *PNAS.* 104, 11192–11196. doi: 10.1073/pnas.0704591104

Li, B., Li, Y.-Y., Wu, H.-M., Zhang, F.-F., Li, C.-J., Li, X.-X., et al. (2016). Root exudates drive interspecific facilitation by enhancing nodulation and N2 fixation. *PNAS.* 113, 6496–6501. doi: 10.1073/pnas.1523580113

Li, Y., Ran, W., Zhang, R., Sun, S., and Xu, G. (2009). Facilitated legume nodulation, phosphate uptake and nitrogen transfer by arbuscular inoculation in an upland rice and mung bean intercropping system. *Plant Soil.* 315, 285–296. doi: 10.1007/s11104-008-9751-9

Li, M., Shinano, T., and Tadano, T. (1997). Distribution of exudates of lupin roots in the rhizosphere under phosphorus deficient conditions. *Soil Sci. Plant Nutr.* 43, 237–245. doi: 10.1080/00380768.1997.10414731

Li, L., Sun, J. H., Zhang, F. S., Guo, T. W., Bao, X. G., Smith, F. A., et al. (2006). Root distribution and interactions between intercropped species. *Oecologia.* 147, 280–290. doi: 10.1007/s00442-005-0256-4

Li, L., Tilman, D., Lambers, H., and Zhang, F. S. (2014). Plant diversity and overyielding: insights from belowground facilitation of intercropping in agriculture. *New Phytol.* 203, 63–69. doi: 10.1111/nph.12778

Li, Y., Yu, C., Sun, J., Li, C., Li, L., and Cheng, X. (2008). Nitrogen environmental endurance and economically-ecologically appropriate amount of nitrogen fertilizer in faba bean/maize intercropping system. *Trans. CSAE.* 24, 223–227. doi: 10.3901/JME.2008.09.177

Li, X., Zhang, T. L., Wang, X. X., Hua, K., Zhao, L., and Han, Z. M. (2013). The composition of root exudates from two different resistant peanut cultivars and their effects on the growth of soil-borne pathogen. *Int. J. Biol. Sci.* 9, 164–173. doi: 10.7150/ijbs.5579

Limami, A. M., Glévarec, G., Ricoult, C., Cliquet, J.-B., and Planchet, E. (2008). Concerted modulation of alanine and glutamate metabolism in young Medicago truncatula seedlings under hypoxic stress. *J. Exp. Bot.* 59, 2325–2335. doi: 10.1093/jxb/ern102

Ling, N., Raza, W., Ma, J. H., Huang, Q. W., and Shen, Q. R. (2011). Identification and role of organic acids in watermelon root exudates for recruiting Paenibacillus polymyxa SQR-21 in the rhizosphere. *Eur. J. Soil Biol.* 47, 374–379. doi: 10.1016/j.ejsobi.2011.08.009

Liu, S., Wu, F., and Wen, X. (2013). Allelopathic effects of root exudates of Chinese onion on tomato growth and the pathogen Fusarium oxysporum(Sch1) f.sp. lycopersici. *Allelopathy J.* 31, 387–404.

Liu, Y., Zhang, N., Qiu, M., Feng, H., Vivanco, J. M., Shen, Q., et al. (2014). Enhanced rhizosphere colonization of beneficial Bacillus amyloliquefaciens SQR9 by pathogen infection. *FEMS Microbiol. Lett.* 353, 49–56. doi: 10.1111/fml.2014.353.issue-1

Lopez-Sangil, L., George, C., Medina-Barcenas, E., Birkett, A. J., Baxendale, C., Bréchet, L., et al. (2017). The Automated Root Exudate System (ARES): a method to apply solutes at regular intervals to soils in the field. *Methods Ecol. Evolution.* 8, 1042–1050. doi: 10.1111/2041-210X.12764

Lu, R. K. (2000). *Analysis methods of soil agricultural chemistry*. Beijing: China Agricultural Science and Technology Press. 156–159.

Lu, S., Haixing, S., Guan, C., Lepo, J., Wu, Z., He, X., et al. (2019). Long-term rice-rice-rape rotation optimizes 1,2-benzenediol concentration in rhizosphere soil and improves nitrogen-use efficiency and rice growth. *Plant Soil.* 445:23–37. doi: 10.1007/s11104-019-04177-9

Lu, J., Liu, Y., Zou, X., Zhang, X., Yu, X., Wang, Y., et al. (2024). Rotational strip peanut/cotton intercropping improves agricultural production through modulating plant growth, root exudates, and soil microbial communities. *Agric Ecosyst. Environ.* 359, 108767. doi: 10.1016/j.agee.2023.108767

Mavrodi, O. V., McWilliams, J. R., Peter, J. O., Berim, A., Hassan, K. A., Elbourne, L. D. H., et al. (2021). Root exudates alter the expression of diverse metabolic, transport, regulatory, and stress response genes in rhizosphere pseudomonas. *Front. Microbiol.* 12. doi: 10.3389/fmicb.2021.651282

Moe, L. A. (2013). Amino acids in the rhizosphere: From plants to microbes. *Am. J. Bot.* 100, 1692–1705. doi: 10.3732/ajb.1300033

Mohanram, S., and Kumar, P. (2019). Rhizosphere microbiome: revisiting the synergy of plant-microbe interactions. *Ann. Microbiol.* 69, 307–320. doi: 10.1007/s13213-019-01448-9

Moreira, A., and Moraes, L. A. C. (2016). Yield, nutritional status and soil fertility cultivated with common bean in response to amino-acids foliar application. *J. Plant Nutr.* 40, 344–351. doi: 10.1080/01904167.2016.1240194

Nagata, M., Yamamoto, N., Miyamoto, T., Shimomura, A., Arima, S., Hirsch, A. M., et al. (2016). Enhanced hyphal growth of arbuscular mycorrhizae by root exudates derived from high R/FR treated Lotus japonicus. *Plant Signaling behavior.* 11, e1187356. doi: 10.1080/15592324.2016.1187356

Nardi, S., Sessi, E., Pizzeghello, D., Sturaro, A., Rella, R., and Parvoli, G. (2002). Biological activity of soil organic matter mobilized by root exudates. *Chemosphere.* 46, 1075–1081. doi: 10.1016/S0045-6535(01)00160-6

Okubo, A., Matsusaka, M., and Sugiyama, S. (2016). Impacts of root symbiotic associations on interspecific variation in sugar exudation rates and rhizosphere microbial communities: a comparison among four plant families. *Plant Soil.* 399, 345–356. doi: 10.1007/s11104-015-2703-2

Olsen, S., and Sommers, L. (1982). "Phosphorus," in *Methods of soil analysis. part 2. chemical and microbiological properties, second ed.* Eds. A. L. Page, R. H. Miller and D. R. Keeney (Madison: SSSA).

Öpik, M., Vanatoa, A., Vanatoa, E., Moora, M., Davison, J., Kalwij, J. M., et al. (2010). The online database maarj AM reveals global and ecosystemic distribution patterns in arbuscular mycorrhizal fungi (Glomeromycota). *New Phytol.* 188, 223–241. doi: 10.1111/j.1469-8137.2010.03334.x

Paterson, E., Gebbing, T., Abel, C., Sim, A., and Telfer, G. (2007). Rhizodeposition shapes rhizosphere microbial community structure in organic soil. *New Phytol.* 173, 600–610. doi: 10.1111/j.1469-8137.2006.01931.x

Peng, Y., LI, X., and Li, C. (2017). Temporal and spatial profiling of root growth revealed novel response of maize roots under various nitrogen supplies in the field. *PloS One* 7, e37726. doi: 10.1371/journal.pone.0037726

Pérez-Jaramillo, J. E., Mendes, R., and Raaijmakers, J. M. (2016). Impact of plant domestication on rhizosphere microbiome assembly and functions. *Plant Mol. Biol.* 90, 635–644. doi: 10.1007/s11103-015-0337-7

Philippot, L., Raaijmakers, J. M., Lemanceau, P., and van der Putten, W. H. (2013). Going back to the roots: the microbial ecology of the rhizosphere. *Nat. Rev. Microbiol.* 11, 789–799. doi: 10.1038/nrmicro3109

Phillips, D. A., Fox, T. C., King, M. D., Bhuvaneswari, T., and Teuber, L. R. (2004). Microbial products trigger amino acid exudation from plant roots. *Plant Physiol.* 136, 2887–2894. doi: 10.1104/pp.104.044222

Phillips, J. M., and Hayman, D. S. (1970). Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. *Trans. Brit Mycol Soc* 55, 158–161. doi: 10.1016/S0007-1536(70)80110-3

Qin, L., Li, Z., Li, B., Wang, J., Zu, Y., Jiang, M., et al. (2021). Organic acid excretion in root exudates as a mechanism of cadmium uptake in a sonchus asper–zea mays intercropping system. *Bull. Environ. Contam Toxicol.* 107, 1059–1064. doi: 10.1007/s00128-021-03361-x

Querejeta, J. I. (2017). Soil water retention and availability as influenced by mycorrhizal symbiosis: consequences for individual plants, communities, and ecosystems - scienceDirect. *Mycorrhizal Mediation Soil.*, 299–317. doi: 10.1016/B978-0-12-804312-7.00017-6

Raklami, A., Bechtaoui, N., Tahiri, A.-i., Anli, M., Meddich, A., and Oufdou, K. (2019). Use of rhizobacteria and mycorrhizae consortium in the open field as a strategy for improving crop nutrition, productivity and soil fertility. *Front. Microbiol.* 10, 1106. doi: 10.3389/fmicb.2019.01106

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

Schollenberger, C. J., and Simon, R. H. (1945). Determination of exchange capacity and exchangeable bases in soil–ammonium acetate method. *Soil Sci.* 59, 13–24. doi: 10.1097/00010694-194501000-00004

Seybold, H., Demetrowitsch, T. J., Hassani, M. A., Szymczak, S., Reim, E., Haueisen, J., et al. (2020). A fungal pathogen induces systemic susceptibility and systemic shifts in wheat metabolome and microbiome composition. *Nat. Commun.* 11, 1910. doi: 10.1038/s41467-020-15633-x

Shen, H., Yan, X., Zhao, M., Zheng, S., and Wang, X. (2002). Exudation of organic acids in common bean as related to mobilization of aluminum-and iron-bound phosphates. *Environ. Exp. Bot.* 48, 1–9. doi: 10.1016/S0098-8472(02)00009-6

Swenson, T. L., Jenkins, S., Bowen, B. P., and Northen, T. R. (2015). Untargeted soil metabolomics methods for analysis of extractable organic matter. *Soil Biol. Biochem.* 80, 189–198. doi: 10.1016/j.soilbio.2014.10.007

Swenson, T. L., and Northen, T. R. (2019). Untargeted soil metabolomics using liquid chromatography-mass spectrometry and gas chromatography-mass spectrometry. *Methods Mol. Biol.* 1859, 97–109. doi: 10.1007/978-1-4939-8757-3_4

Wang, X., Whalley, W. R., Miller, A. J., White, P. J., Zhang, F., and Shen, J. (2020). Sustainable cropping requires adaptation to a heterogeneous rhizosphere. *Trends Plant Sci.* 25, 1194–1202. doi: 10.1016/j.tplants.2020.07.006

Withers, E., Hill, P. W., Chadwick, D. R., and Jones, D. L. (2020). Use of untargeted metabolomics for assessing soil quality and microbial function. *Soil Biol. Biochem.* 143, 107758. doi: 10.1016/j.soilbio.2020.107758

Xiao, X., Xi, B. D., He, X. S., Zhang, H., Li, D., Zhao, X. Y., et al. (2019). Hydrophobicity-dependent electron transfer capacities of dissolved organic matter derived from chicken manure compost. *Chemosphere.* 222, 757–765. doi: 10.1016/j.chemosphere.2019.01.173

Xu, X., Chen, C., Zhang, Z., Sun, Z., Chen, Y., Jiang, J., et al. (2017). The influence of environmental factors on communities of arbuscular mycorrhizal fungi associated with chenopodium ambrosioides revealed by MiSeq sequencing investigation. *Sci. Rep.* 7, 45134. doi: 10.1038/srep45134

Yu, C. N., Luo, X. J., Zhan, X. R., Hao, J., Zhang, L., Song, Y. B., et al. (2018). Comparative metabolomics reveals the metabolic variations between two endangered Taxus species (T. fuana and T. yunnanensis) in the Himalayas. *BMC Plant Biol.* .18, 197. doi: 10.1186/s12870-018-1412-4

Zhalnina, K., Louie, K. B., Hao, Z., Mansoori, N., Da Rocha, U. N., Shi, S., et al. (2018). Dynamic root exudate chemistry and microbial substrate preferences drive patterns in rhizosphere microbial community assembly. *Nat. Microbiol.* 3, 470–480. doi: 10.1038/s41564-018-0129-3

Zhang, X., Huang, G., Bian, X., Jiang, X., and Zhao, Q. (2012). Review of researches on advantages of intercropping. *Asian Agric. Res.* 4, 126–132. doi: 10.1016/j.saa.2013.05.053

Zhang, R., Mu, Y., Li, X., Li, S., Sang, P., Wang, X., et al. (2020). Response of the arbuscular mycorrhizal fungi diversity and community in maize and soybean rhizosphere soil and roots to intercropping systems with different nitrogen application rates. *Sci. Total Environ.* 740, 139810. doi: 10.1016/j.scitotenv.2020.139810

Zhang, P., Nie, M., Li, B., and Wu, J. (2017). The transfer and allocation of newly fixed C by invasive Spartina alterniflora and native Phragmites australis to soil microbiota. *Soil Biol. Biochem.* 113, 231–239. doi: 10.1016/j.soilbio.2017.06.003

Zhao, L., Liu, A., Song, T., Jin, Y., Xu, X., Gao, Y., et al. (2018). Transcriptome analysis reveals the effects of grafting on sugar and α-linolenic acid metabolisms in fruits of cucumber with two different rootstocks. *Plant Physiol. Biochem.* 130, 289–302. doi: 10.1016/j.plaphy.2018.07.008

Zhao, Y., Liu, X., Tong, C., and Wu, Y. (2020). Effect of root interaction on nodulation and nitrogen fixation ability of alfalfa in the simulated alfalfa/triticale intercropping in pots. *Sci. Rep.* 10, 4269. doi: 10.1038/s41598-020-61234-5

Zhu, H. Y., Liu, Z. D., Wang, C. R., and Zhong, Z. C. (2006). Effects of intercropping with persimmon on the rhizosphere environment of tea. *Front. Biol. China.* 1, 407–410. doi: 10.1007/s11515-006-0054-3

frontiers | Frontiers in Plant Science

TYPE Opinion
PUBLISHED 28 June 2024
DOI 10.3389/fpls.2024.1436310

OPEN ACCESS

EDITED BY
Claudia Bita-Nicolae,
Institute of Biology Bucharest of the Romanian Academy, Romania

REVIEWED BY
Maria Mihaela Antofie,
Lucian Blaga University of Sibiu, Romania

*CORRESPONDENCE
Ilias Travlos
✉ travlos@aua.gr
Ioannis Gazoulis
✉ giangazoulis@gmail.com

RECEIVED 21 May 2024
ACCEPTED 12 June 2024
PUBLISHED 28 June 2024

CITATION
Gazoulis I, Kanatas P, Zannopoulos S, Kokkini M, Kontogeorgou V, Antonopoulos N and Travlos I (2024) Agroecology and beyond: enhancing ecosystem services provided by natural vegetation and inventing “service weeds”. *Front. Plant Sci.* 15:1436310. doi: 10.3389/fpls.2024.1436310

Agroecology and beyond: enhancing ecosystem services provided by natural vegetation and inventing “service weeds”

Ioannis Gazoulis[1]*, Panagiotis Kanatas[2], Stavros Zannopoulos[3], Metaxia Kokkini[1], Vasiliki Kontogeorgou[1], Nikolaos Antonopoulos[1] and Ilias Travlos[1]*

[1]Laboratory of Agronomy, Department of Crop Science, Agricultural University of Athens, Athens, Greece, [2]Department of Crop Science, University of Patras, Mesolonghi, Greece, [3]Koniareio Citrus Institute, Ministry of Rural Development & Food, Kechries, Greece

KEYWORDS
ecosystem services, weeds, natural vegetation, service weeds, agroecology

1 Introduction

Weeds are considered serious and troublesome pests for crops, and their management had always been at the core of plant protection (Oerke, 2006; Travlos et al., 2021). Despite the negative connotation of the term “weed,” referring to plants that are spatially and temporally unwanted, weeds can sometimes have some positive impacts in agroecosystems due to their utility as food, feed, and their multiple ecosystem services (Blaix et al., 2018; Gaba et al., 2020). Numerous field studies have shown that biodiversity is a key factor in determining ecosystem services (Cardinale et al., 2012; Tilman et al., 2014; Visconti et al., 2018). Consequently, balancing biodiversity and productivity in agroecosystems is a challenging goal that can provide additional benefits due to multifunctionality (Mitchell et al., 2014). The aim of this opinion article is to present the ecosystem services potentially provided by (or related to) weeds in agroecosystems and discuss their quantification and further enhancement in an agroecological context. The keystone question is whether and how weeds could be managed and turned into “service weeds,” similar to service crops, to contribute valuable ecosystem services.

2 “Service weeds” and how to invent and promote them

2.1 Ecosystem services potentially provided by the weeds and their assessment

Service crops, like cover crops, are cultivated in agroecosystems to provide non-marketed ecosystem services, diverging from food, fiber, and fuel production (Garcia et al., 2018). In this context, we suggest the term "service weeds" to describe spontaneous plants that can provide important ecosystem services with minimal disservices, under specific pedoclimatic conditions, in various agroecosystems, as parts of wider communities and with proper management. The need for the introduction of this term comes from the necessity to highlight that weeds are not always or universally unwanted and aims to shift the paradigm to exploit weeds by means of their management, and to understand biodiversity as part of the field and the agroecosystem.

Under specific conditions, weeds are associated with several disservices, like competition with crops for water, light, and nutrients, a host of harmful pests and diseases, significant yield reduction, an increase in frost risk and damage, fungal diseases, and poisonous effects on livestock. On the contrary, weed communities can contribute to agroecosystem services, among others, by providing food for different organisms; as hosts for pollinators and natural enemies; for soil stability, nutrient cycling, and improved water infiltration and facilitation of water acquisition by hydraulic lift, runoff, and erosion prevention (Dale and Polasky, 2007; Power, 2010; Petit et al., 2011). It is notable how weeds can reduce the sensitivity of several crops to pests, either by strengthening their defense mechanisms or by making them less attractive to the pests (Capinera, 2005; Fagundez, 2014; Blaix et al., 2018). The "push-pull" strategies, which involve the diversification of tolerant and trap crops, respectively, could also be extended to natural vegetation and thus mitigate the infestation of the crops (Shelton and Badenes-Perez, 2006). Weeds also reduce N leaching due to erosion mitigation and increased N uptake and fixation and enrich soil with carbon, other nutrients, and organic matter (Moreau et al., 2020). Rahman et al. (2009) found that floor vegetation consisting of naturally occurring weed species increased beneficial nematodes and organic matter near the soil surface, while the presence of various weeds is related to increased nutrient use efficiency of the crops, increased carbon storage, improved soil functionality, and conservation of biodiversity and wildlife (Salomé et al., 2016; Garcia et al., 2018). Thus, this subset of ecosystem services that is defined for agroecosystems will further support pollinators as an already defined indicator (Balvanera et al., 2014). Furthermore, according to several qualitative and quantitative modeling studies, climate change is likely to lead to pollinator decline, soil erosion, and other negative impacts, and therefore the importance of the contributions of nature to people and quality of life, i.e., ecosystem services, gets bigger (Martín-López et al., 2018).

In general, species diversification in space and time is used to provide and enhance ecological processes that support multiple ecosystem services (Garcia et al., 2018; Nijmeijer et al., 2019). However, biodiversity does not result in better ecosystem services *per se* since pedoclimatic and socioeconomic conditions also play a role, and the invaluable functional diversity is not always closely correlated with genetic diversity (Isbell et al., 2011; Mace et al., 2012; Balvanera et al., 2014; Gaba et al., 2015). In all cases, a scientific consensus has been reached that biodiversity loss reduces the efficiency of several provisioning ecosystem services and, hence, the contributions of nature to human wellbeing (Cardinale et al., 2012; Balvanera et al., 2014; Visconti et al., 2018). Therefore, the need for enhancement, assessment, and quantification of the several ecosystem services is imperative. While there are several well-known ecosystem services provided by specific weed species (e.g., nitrogen enrichment due to Fabaceae species), what is missing is the systematic and standardized promotion of ecosystem services in a validated and broadly accepted way for the agroecosystems and the upscaling from the single species to the communities' level. Quantification of ecosystem services is the key to the development of the most suitable agroecosystems and the enhancement of ecosystem multifunctionality (Kremen and Ostfeld, 2005). Robinson et al. (2022) suggested the use of a large precision yield dataset to answer questions about how landscapes influence yield. Brooker et al. (2023) suggested that a focus on functional traits like height, rooting depth, and production of allelochemicals and associated trait complementarity will improve understanding of why combinations of cultivars, crop species, or crops and weeds seem to be more productive or resilient to climate, pests, and disease. According to them, a diversity of non-crop plants can provide resources necessary for in-field functional processes, both below and above ground (e.g., carbon input and resource continuity for pollinators and natural enemies). Furthermore, there are important interactions between ecosystem services, with these relationships being either synergistic or antagonistic (Gaba et al., 2015). This is valid for the ecosystem services potentially provided by the weed communities, with their presence often associated with increased organic matter, while at the same time, crop production could be hindered in the short term.

Comprehensive systems, methods, and approaches are needed to quantify the different ecosystem services with due consideration of the difference in the distribution of the ecosystem services provided (Johnson et al., 2010). For instance, the ecosystem services functional spatial unit (ESSU) concept proposed by Rafflegeau et al. (2023) could be a great tool and a sound base to build a protocol for ecosystem services quantification, modeling, and enhancement.

2.2 Enhancement of ecosystem services

The above-mentioned quantification of ecosystem services is not merely theoretical but rather necessary for the enhancement of these services through the identification, evaluation, and protection of weed communities that can be exploited for multiple purposes.

We hereby propose a specific sequence of steps to be taken in the identification, assessment, and promotion of service weeds and natural vegetation in agroecosystems that can provide ecosystem services (Figure 1).

In our opinion, the good knowledge of the biology and ecology of weeds and the observation of the progressive differentiation of weed flora, both within fields and along field margins and boundaries, are very important for transforming them into "service weeds" (Step 1). Field boundaries and spaces between tree rows covered by service weeds, acting as "cover weeds," that can contribute to important ecosystem services without risking crop health and productivity would be revolutionary. Field margins, fallow land, strips, and grasslands can increase biodiversity, promote diversification, and provide various ecosystem services to crops by creating better abiotic conditions for crop growth and productivity (Concepcion

FIGURE 1
Steps to be taken in the framework of identification, assessment, and promotion of service weeds and service natural vegetation in agroecosystems.

et al., 2012; Roschewitz et al., 2005; Gaba et al., 2015; Robinson et al., 2022). However, monitoring the composition of weed flora and its changes is the baseline, as these reflect specific conditions, agronomic practices, and the entire field history.

The next step (Step 2) involves the prioritization of several provisioning and regulating ecosystem services that add value to the specific agroecosystem and, in the medium and long term, would be beneficial for the farmers as well, exactly as proposed by Gaba et al. (2015) for crops. For instance, in poor soil with low fertility, the increase of soil nitrogen and soil organic matter would be of top priority, while in a sloppy orchard, the prevention of soil erosion and a good soil structure would be very important. Prioritization should be done in collaboration with farmers and other stakeholders. In this way, it will reveal their needs, raise their awareness and commitment, and help demonstrate good examples and success stories.

Step 3 refers to the identification of specific weeds and weed communities whose traits and complementarity can provide the most desired ecosystem services and consider their interaction and impact on the ecosystem. Indeed, each species is characterized by several functional traits linked to agroecosystem services (Barberi et al., 2018; Ciaccia et al., 2020). For example, depending on the priorities and the specific pedoclimatic conditions and weeds' functional traits, we can either promote Fabaceae species for nitrogen enrichment or Poaceae species for structure improvement and erosion prevention. As reviewed by Brooker et al. (2023), complementarity effects result from processes such as niche differentiation or plant facilitation. The weed trait database proposed by Barberi et al. (2018) could be further enriched and translated in a tailor-made way at the community level in order to take into account any interactions and limitations due to non-compatible, non-synchronized, or competitive species. Approaches relying on combinations of functional traits by means of multispecies ideotypes, as proposed by Gaba et al. (2015) and Lavorel and Garnier (2002), could also be applied to weed communities. Yvoz et al. (2021) identified that small weed species with a short life cycle and low competitiveness present the optimum proxy combination, i.e., high services and low harmfulness. It has to be noted also that the coexisting ability of the different species based on resource partitioning requires diverse traits and usually gives resistance to invasion by other species (Dukes, 2001; Gaba et al., 2015).

The management (manipulation) of weed communities in favor of weeds with desirable traits and the mitigation of any major disservices (Step 4) is crucial for the exploitation of weeds as ecosystem services' providers. For instance, the conservation of natural vegetation and the manipulation of weed flora by means of mowing at different heights could be some good ideas for boosting specific species instead of others and enhancing ecosystem services. Additionally, the selection as cover crops or "service weeds" of some self-seeding species that provide adequate ground cover without being competitive with the main crops is also recommended (Carpio et al., 2020). In all cases and similar to service crops, even for weeds, we have to select the combinations that increase positive effects in the agroecosystems like pest deterrence and improved water infiltration (Rafflegeau et al., 2023) and mitigate crop competition and other disservices. Within this context, protocols and decision support systems focused on weeds, ecosystem services, and agroecological management could be developed (Kanatas et al., 2020).

Step 5, which refers to the evaluation of the performance of several practices and weed communities in the growing season, should not be underestimated since this will be the basis for any required adaptation and optimization of the overall strategy. Quantification of ecosystem services is necessary and can be achieved by means of field trials in a wide range of pedoclimatic conditions and multidisciplinary research combining diverse pros and cons (Rapidel et al., 2009; Gaba et al., 2015). Any assessment could shift from the single species to the community level. Moreover, when we talk about agricultural land, the effects of any practices and approaches on crop yields or exploitation of the produced biomass as forage should not be ignored (Daryanto et al., 2019; Robinson et al., 2022).

Regarding the obstacles, the major issue is to overcome the nudges regarding weeds. Indeed, farmers and agronomists are not used or trained to systematically recognize the ecosystem services provided by plants, particularly by spontaneous vegetation, and consequently to value and promote such benefits and communities. Thus, demonstration and training go first with an eye always to the local conditions, available labor and machinery, and top priorities. Training and technical support are crucial, especially during the first steps for the dispersal of the most beneficial species and the enhancement of the ecosystem services provided by the weed flora. Moreover, ecosystem services usually rely on a limited number of spontaneous or sown species. Therefore, further research is needed to study more species and their potential roles and interactions without risking the dominance of a few noxious and competitive species in the agroecosystem and the decrease in crop yields due to competition and allelopathy. The potential need for the termination of these “service weeds” should also be taken into account, exactly as it happens with cover crops.

3 Conclusions and perspectives

The strategic integration of non-crop plant diversity at field, farm, and landscape scales is an invaluable tool for achieving sustainability (Hawes et al., 2021). More diverse communities have a higher likelihood of some species functioning well under various conditions (“insurance hypothesis”), maintaining multiple ecosystem processes across multiple places and times, and contributing to more resilient agroecosystems (Isbell et al., 2011; Visconti et al., 2018). As Gaba et al. (2015) highlighted, determining plant diversity and designing and applying management practices that could deliver a set of targeted services under given environmental and socioeconomic conditions are crucial. The question of whether weeds can play a role or should be kept only as opponents (Travlos et al., 2023) is answered here: weeds do have a role to play in the agroecosystem. Due to their short life cycles, plasticity, and adaptability, the good traits that selection pressure gave them, the art of coexistence and complementarity that they have developed, and many other advantages, they can remarkably turn into “service weeds” and contribute to the already defined main ecosystem services. The obstacles related to their disservices, inadequate training, the difficult quantification of ecosystem services, and the nudges among farmers and other stakeholders can be overcome through multidisciplinary research, training, and policy initiatives. We propose the conservation, management, and optimization of a native weed flora (diversified) in perennial crops, field margins, strips, and between crop rows and its manipulation by means of mowing, grazing, deep tillage, etc., in favor of small growth and short growing cycle annual species towards a significant enhancement of ecosystem services. It is time to “unbox” the potential value of weeds for the agroecosystems, even if it is to redefine in our minds the term “weed”.

Author contributions

IG: Conceptualization, Writing – review & editing, Writing – original draft. PK: Writing – review & editing, Writing – original draft. SZ: Writing – review & editing, Writing – original draft. MK: Writing – original draft. VK: Writing – original draft, Visualization. NA: Writing – original draft. IT: Writing – review & editing, Writing – original draft, Conceptualization.

Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article. Financed by the European Union under the grant agreement no. 101083589 (Agroecology is GOOD 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.

Publisher’s note

Author disclaimer

The views and opinions expressed are solely those of the author (s) and do not necessarily reflect those of the European Union or REA. Neither the European Union nor the grant awarding authority can be held responsible for them.

References

Balvanera, P., Siddique, I., Dee, L., Paquette, A., Isbell, F., Gonzalez, A., et al. (2014). Linking biodiversity and ecosystem services: Current uncertainties and the necessary next steps. *BioScience* 64, 49–57. doi: 10.1093/biosci/bit003

Barberi, P., Bocci, G., Carlesi, S., Armengot, L., Blanco-Moreno, J. M., and Sans, F. X. (2018). Linking species traits to agroecosystem services: a functional analysis of weed communities. *Weed Res.* 58, 76–88. doi: 10.1111/wre.12283

Blaix, C., Moonen, A. C., Dostatny, D. F., Izquierdo, J., Le Corff, J., Morrison, J., et al. (2018). Quantification of regulating ecosystem services provided by weeds in annual cropping systems using a systematic map approach. *Weed Res.* 58, 151–164. doi: 10.1111/wre.12303

Brooker, R. W., Hawes, C., Ianetta, P. P. M., Karley, A. J., and Renard, D. (2023). Plant diversity and ecological intensification in crop production systems. *J. Plant Ecol.* 16, rtad015. doi: 10.1093/jpe/rtad015

Capinera, J. L. (2005). Relationships between insect pests and weeds: an evolutionary perspective. *Weed Sci.* 53, 892–901. doi: 10.1614/WS-04-049R.1

Cardinale, B. J., Duffy, J. E., Gonzalez, A., Hooper, D. U., Perrings, C., Venail, P., et al. (2012). Biodiversity loss and its impact on humanity. *Nature* 486, 59–67. doi: 10.1038/nature11148

Carpio, A. J., Soriano, M-A., Gomez, J. A., and Tortosa, F. S. (2020). The self-seeding of Anthemis arvensis L. for cover crop in olive groves under intense rabbit grazing. *Agronomy.* 10, 1412. doi: 10.3390/agronomy10091412

Ciaccia, C., Armengot Martinez, L., Testani, E., Leteo, F., Campanelli, G., and Trinchera, A. (2020). Weed functional diversity as affected by agroecological service crops and no-till in a Mediterranean organic vegetable system. *Plants* 9, 689. doi: 10.3390/plants9060689

Concepcion, E. D., Fernandez-Gonzalez, F., and Diaz, M. (2012). Plant diversity partitioning in Mediterranean croplands: Effects of farming intensity, field edge, and landscape context. *Ecol. Appl.* 22, 972–981. doi: 10.6084/m9.figshare.c.3295409

Dale, V. H., and Polasky, S. (2007). Measures of the effects of agricultural practices on ecosystem services. *Ecol. Econ.* 61, 286–296. doi: 10.1016/j.ecolecon.2007.05.009

Daryanto, S., Jacinthe, P.-A., Fu, B., Zhao, W., and Wang, L. (2019). Valuing the ecosystem services of cover crops: barriers and pathways forward. *Agric. Ecosyst. Environ.* 270-271, 76–78. doi: 10.1016/j.agee.2018.10.021

Dukes, J. S. (2001). Biodiversity and invasibility in grassland microcosms. *Oecologia* 126, 563–568. doi: 10.1007/s004420000549

Fagundez, J. (2014). "The paradox of arable Weeds," in *Agroecology, Ecosystems, and Sustainability*. Ed. N. Benkeblia (Boca Raton, FL, USA: CRC Press), 139–150. doi: 10.1201/b17775-8

Gaba, S., Cheviron, N., Perrot, T., Piutti, S., Gautier, J.-L., and Bretagnolle, V. (2020). Weeds enhance multifunctionality in arable lands in South-West of France. *Front. Sustain. Food Syst.* 4. doi: 10.3389/fsufs.2020.00071

Gaba, S., Lescourret, F., Boudsocq, S., Enjalbert, J., Hinsinger, P., Journet, E. P., et al. (2015). Multiple cropping systems as drivers for providing multiple ecosystem services: from concepts to design. *Agron. Sustain. Dev.* 35, 607–623. doi: 10.1007/s13593-014-0272-z

Garcia, L., Celette, F., Gary, C., Ripoche, A., Valdes-Gomez, H., and Metay, A. (2018). Management of service crops for the provision of ecosystem services in vineyards: a review. *Agr. Ecosyst. Environ.* 251, 158–170. doi: 10.1016/j.agee.2017.09.030

Hawes, C., Iannetta, P. P. M., and Squire, G. R. (2021). Agroecological practices for whole-system sustainability. *CAB Rev.* 16, 5. doi: 10.1079/PAVSNNR202116005

Isbell, F., Calcagno, V., Hector, A., Connolly, J., Harpole, W., Reich, P., et al. (2011). High plant diversity is needed to maintain ecosystem services. *Nature* 477, 199–202. doi: 10.1038/nature10282

Johnson, G., Bagstad, K., Snapp, R., and Villa, F. (2010). "Service path attribution networks (SPANs): spatially quantifying the flow of ecosystem services from landscape to people," in *Int. Conf. Computat. Sci. Applic*. Eds. D. Taniar, O. Gervasi, B. Mergante, E. Pardede and B. Apduhan (Berlin, Heidelberg: Springer), 238–253.

Kanatas, P., Travlos, I. S., Gazoulis, I., Tataridas, A., Tsekoura, A., and Antonopoulos, N. (2020). Benefits and limitations of Decision Support Systems (DSS) with a special emphasis on weeds. *Agronomy* 10, 548. doi: 10.3390/agronomy10040548

Kremen, C., and Ostfeld, R. S. (2005). A call to ecologists: measuring, analyzing, and managing ecosystem services. *Front. Ecol. Environ.* 3, 540–548. doi: 10.1890/1540-9295(2005)003[0540:ACTEMA]2.0.CO;2

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

Mace, G. M., Norris, K., and Fitter, A. H. (2012). Biodiversity and ecosystem services: a multi-layered relationship. *TREE* 27, 19–26. doi: 10.1016/j.tree.2011.08.006

Martín-López, B., Church, A., Başak Dessane, E., Berry, P., Chenu, C., Christie, M., et al. (2018). "Chapter 2: Nature's contributions to people and quality of life," in *IPBES: The IPBES regional assessment report on biodiversity and ecosystem services for Europe and Central Asia*. Eds. M. Rounsevell, M. Fischer, A. Torre-Marin Rando and A. Mader (Secretariat of the Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services, Bonn, Germany), 57–185.

Mitchell, M. G. E., Bennett, E. M., and Gonzalez, A. (2014). Forest fragments modulate the provision of multiple ecosystem services. *J. Appl. Ecol.* 51, 909–918. doi: 10.1111/1365-2664.12241

Moreau, D., Pointurier, O., Nicolardot, B., Villerd, J., and Colbach, N. (2020). In which cropping systems can residual weeds reduce nitrate leaching and soil erosion? *Eur. J. Agron.* 119, 126015. doi: 10.1016/j.eja.2020.12601

Nijmeijer, A., Lauri, P. E., Harmand, J. M., Freschet, G. T., Essobo Nieboukaho, J. D., Fogang, P. K., et al. (2019). Long-term dynamics of cocoa agroforestry systems established on lands previously occupied by savannah or forests. *Agric. Ecosyst. Environ.* 275, 100–111. doi: 10.1016/j.agee.2019.02.004

Oerke, E.-C. (2006). Crop losses to pests. *J. Agric. Sci.* 144, 31–43. doi: 10.1017/S0021859605005708

Petit, S., Boursault, A., Guilloux, M., Munier-Jolain, N., and Reboud, X. (2011). Weeds in agricultural landscapes. A review. *Agron. Sustain. Devel.* 31, 309–317. doi: 10.1051/agro/2010020

Power, A. G. (2010). Ecosystem services and agriculture: tradeoffs and synergies. *Philos. Trans. R. Soc. B: Biol. Sci.* 365, 2959–2971. doi: 10.1098/rstb.2010.0143

Rafflegeau, S., Gosme, M., Barkaoui, K., Garcia, L., Allinne, C., Deheuvels, O., et al. (2023). The ESSU concept for designing, modeling and auditing ecosystem service provision in intercropping and agroforestry systems. A review. *Agron. Sustain. Dev.* 43, 43. doi: 10.1007/s13593-023-00894-9

Rahman, L., Whitelaw-Weckert, M. A., Hutton, R. J., and Orchard, B. (2009). Impact of floor vegetation on the abundance of nematode trophic groups in vineyards. *Appl. Soil Ecol.* 42, 96–106. doi: 10.1016/j.apsoil.2009.02.006

Rapidel, B., Traoré, B. S., Sissoko, F., Lançon, J., and Wery, J. (2009). Experiment-based prototyping to design and assess cotton management systems in West Africa. *Agron. Sustain. Dev.* 29, 545–556. doi: 10.1051/agro/2009016

Robinson, S. V. J., Nguyen, L. H., and Galpern, P. (2022). Livin' on the edge: Precision yield data shows evidence of ecosystem services from field boundaries. *Agric. Ecosyst. Environ.* 333, 107956. doi: 10.1016/j.agee.2022.107956

Roschewitz, I., Gabriel, D., Tscharntke, T., and Thies, C. (2005). The effects of landscape complexicity on arable weed species diversity in organic and conventional farming. *J. Appl. Ecol.* 42, 873–882. doi: 10.1111/j.1365-2664.2005.01072.x

Salomé, C., Coll, P., Lardo, E., Metay, A., Villenave, C., Marsden, C., et al. (2016). The soil quality concept as a framework to assess management practices in vulnerable agroecosystems: a case study in Mediterranean vineyards. *Ecol. Indic.* 61, 456–465. doi: 10.1016/j.ecolind.2015.09

Shelton, A. M., and Badenes-Perez, F. R. (2006). Concepts and applications of trap cropping in pest management. *Ann. Rev. Entomol.* 51, 285–308. doi: 10.1146/annurev.ento.51.110104.150959

Tilman, D., Isbell, F., and Cowles, J. M. (2014). Biodiversity and ecosystem functioning. *Annu. Rev. Ecology Evolution Systematics* 45, 471–493. doi: 10.10.1146/annurev-ecolsys-120213-091917

Travlos, I., Scavo, A., and Kanatas, P. (2023). The future of weed science: novel approaches to weed management. *Agronomy* 13, 2787. doi: 10.3390/agronomy13112787

Travlos, I., Tsekoura, A., Antonopoulos, N., Kanatas, P., and Gazoulis, I. (2021). Novel sensor-based method (quick test) for the in-season rapid evaluation of herbicide efficacy under real field conditions in durum wheat. *Weed Sci.* 69, 147–160. doi: 10.1017/wsc.2021.8

Visconti, P., Elias, V., Sousa Pinto, I., Fischer, M., Ali-Zade, V., Báldi, A., et al. (2018). "Chapter 3: Status, trends and future dynamics of biodiversity and ecosystems underpinning nature's contributions to people," in *IPBES: The IPBES regional assessment report on biodiversity and ecosystem services for Europe and Central Asia*. Eds. M. Rounsevell, M. Fischer, A. Torre-Marin Rando and A. Mader (Secretariat of the Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services, Bonn, Germany), 187–382.

Yvoz, S., Cordeau, S., Ploteau, A., and Petit, S. (2021). A framework to estimate the contribution of weeds to the delivery of ecosystem (dis)services in agricultural landscapes. *Ecol. Indic.* 132, 108321. doi: 10.1016/j.ecolind.2021.108321

Frontiers in Plant Science

TYPE Original Research
PUBLISHED 07 August 2024
DOI 10.3389/fpls.2024.1409493

OPEN ACCESS

EDITED BY
Praveen Dhyani,
University of Koblenz, Germany

REVIEWED BY
Claudia Bita-Nicolae,
Institute of Biology Bucharest of the Romanian Academy, Romania
Qicong Wu,
Shandong Agricultural University, China

*CORRESPONDENCE
Guanzhi Liu
✉ czylgz@imau.edu.cn
Runhong Gao
✉ grhzwdm@163.com

†These authors have contributed equally to this work and share first authorship

RECEIVED 30 March 2024
ACCEPTED 15 July 2024
PUBLISHED 07 August 2024

CITATION
Li Z, Qiao J, Li Z, Gao X, Hong G, Yang H, Hu E, Liu C, Wang X, Liu G and Gao R (2024) Shallow tillage mitigates plant competition by increasing diversity and altering plant community assembly process. *Front. Plant Sci.* 15:1409493. doi: 10.3389/fpls.2024.1409493

Shallow tillage mitigates plant competition by increasing diversity and altering plant community assembly process

Zihao Li[1,2†], Jirong Qiao[3†], Zhuofan Li[1], Xiaowei Gao[1], Guangyu Hong[1], Haifeng Yang[1], Ercha Hu[1,2], Chenming Liu[1], Xiaojiang Wang[1], Guanzhi Liu[3*] and Runhong Gao[2*]

[1]Institute of Forest Ecology, Inner Mongolia Academy of Forestry Sciences, Hohhot, China, [2]College of Forestry, Inner Mongolia Agricultural University, Hohhot, China, [3]College of Grassland, Resources and Environment, Inner Mongolia Agricultural University, Hohhot, China

Introduction: Understanding how human activities affect biodiversity is needed to inform systemic policies and targets for achieving sustainable development goals. Shallow tillage to remove *Artemisia ordosica* is commonly conducted in the Mu Us Desert. However, the impacts of shallow tillage on plant community species diversity, phylogenetic structure, and community assembly processes remain poorly understood.

Methods: This study explores the effects of shallow tillage on species diversity including three a-diversity and two b-diversity indicators, as well as phylogenetic structure [phylogenetic diversity (PD), net relatedness index (NRI), and nearest taxon index (NTI)]. Additionally, this research analyzes the effects of shallow tillage on the community assembly process.

Results and discussion: The results showed that the a-diversity index, b-diversity index, and PD of the shallow tillage (ST) communities were significantly higher than those of the non-shallow tillage (NT) communities, and the phylogenetic structures of both the ST and NT communities tended to be differentiated, with competitive exclusion being the main mechanism of plant assembly. However, shallow tillage increased the relative importance of the stochastic processes dominated by dispersal limitation, mitigating plant competition in the communities. This conclusion was supported by the Raup–Crick difference index-based analysis.

Conclusion: Therefore, for the ecological restoration of the Mu Us Desert, species with adaptability and low niche overlap should be selected to increase the utilization efficiency of the environmental resources. The results of this study provide a foundation for policy development for ecosystem management and restoration in the Mu Us Desert.

KEYWORDS

shallow tillage, species diversity, phylogenetic structure, community assembly, Mu Us Desert

1 Introduction

Biodiversity underpins ecosystem stability and social development, yet it is declining globally due to human activities (Zhang and Chen, 2022; Zhang et al., 2023). Desert ecosystems cover 12% of the Earth's land surface (Durant et al., 2012; Chen et al., 2023) and, compared to other ecosystems, are environmentally fragile and sensitive to disturbance, which can have disproportionately severe impacts on biodiversity. Previous studies have shown that even the smallest soil disturbance can significantly alter plant community structure and biodiversity in arid ecosystems (Valone, 2003; Erfanian et al., 2019). Shallow, a common disturbance measure in agroecosystems, is also used for desert grassland improvement, whereby local people remove vegetation that does not provide economic benefits by plowing it to utilize desert grassland resources. This practice, although tailored to local needs, significantly risks community species composition biodiversity and the natural processes supporting desert ecosystems (Randriamalala et al., 2012; Cordeau et al., 2017). Consequently, understanding the ecological consequences of anthropogenic interventions is essential for developing more sustainable management practices (Jaureguiberry et al., 2022).

Community assembly is an intrinsic mechanism of biodiversity change and maintenance and can be used to assess the impacts of external drivers, such as human activities, on biodiversity (Chase, 2010). Ecological niche theory (based on deterministic processes) and neutral theory (based on stochastic processes) are the two hypotheses for the community assembly process, and it is widely accepted that deterministic and stochastic factors work together in the community assembly process. However, assessing the relative importance of these factors is challenging, especially in arid regions. Traditionally, species α-diversity and β-diversity are often used in community assembly process analyses (Mori et al., 2018; Jin et al., 2023). However, species diversity reflects only one dimension of biodiversity and does not consider evolutionary diversity (Capmourteres and Anand, 2016; Heino and Tolonen, 2017). Therefore, the use of phylogenetic structures to analyze community assembly processes has gained prominence (Cadotte et al., 2009; Mishler et al., 2014). This methodology not only assesses the evolution of biodiversity and evolutionary distances among taxa but also reflects functional diversity, as closely related species often exhibit similar traits (Cadotte et al., 2008). Although species diversity and phylogenetic structure are often highly correlated, they may indicate different patterns of community assembly (Tucker and Cadotte, 2013). Accordingly, it is vital to integrate various measures of biodiversity to achieve a more comprehensive understanding of community assembly. Uncovering these hidden patterns can provide more targeted guidance for restoring damaged ecosystems and deepen the understanding of diversity formation and maintenance.

The studies on the effects of tillage on community diversity and assembly processes have mainly focused on agroecosystems (Travlos et al., 2018). It has been shown that the removal of native vegetation by tillage creates a large number of gap ecological niches that favor plant species colonization (Czerwiński et al., 2018). Consequently, tillage alters the species composition of weed communities, in which perennial species shifted to annual species, and increases the community evenness index (Booth and Swanton, 2002) but does not change diversity indices such as species richness and the Shannon–Weiner index (Légère et al., 2005; Randriamalala et al., 2012; Barroso et al., 2015). Tillage imposed environmental filtering, an artificial deterministic process, on the weed community and therefore altered the drivers of weed community assembly (Alarcón et al., 2018). However, the relative importance of deterministic and stochastic processes is largely influenced by community type (Cordeau et al., 2017). Although phylogenetic structure is important for understanding the mechanisms of community diversity change and formation, little is known about the effects of tillage on plant phylogenetic structure.

Artemisia ordosica plays a crucial role in wind and sand control (Wei et al., 2016). However, its strong odor leads to its unpopularity among livestock. Consequently, local herders frequently remove it through shallow tillage, which is defined as soil disturbance up to a depth of 10–15 cm (Cooper et al., 2016; Laudicina et al., 2017) to improve grasslands. This practice promotes the growth of more palatable herbaceous plants to support production and livelihoods in the Mu Us Desert but also heightens the risk of desertification (Wang et al., 2017). However, less research has been conducted on shallow tillage on plant diversity and community assembly processes in desert grasslands. This study evaluates the short-term impacts of shallow tillage on plant communities in the Mu Us Desert, focusing on changes in species diversity and phylogenetic structure, as well as community assembly processes, before and after tillage. Specifically, we aim to answer the following: (1) "Does shallow tillage affect the species diversity and phylogenetic structure of the communities and how?" and (2) "Does shallow tillage affect the ecological processes that drive community assembly?" We propose the hypothesis that shallow tillage increases community species and phylogenetic diversity but does not alter its community assembly processes because studies have shown that shallow tillage leads to a large number of blank ecological niches, while multiple tillage alters weed community assembly processes. The study of the effects of shallow tillage on multilevel of biodiversity (species diversity and phylogenetic diversity) of desert ecosystems is an important reference for conservation strategies and land management practices.

2 Materials and methods

2.1 Study area

The study was conducted in a fixed monitoring site at the Ulan Tolgoi Desertification Control Station located in Wushen Banner (38°48′N, 109°19′E, at 1,390 m), Inner Mongolia, China (Figure 1). This region in the hinterland of the Mu Us desert has a semi-arid continental monsoon climate, which is dry and windy. The average annual temperature ranges from 6.0°C to 9.0°C (Zheng et al., 2019a). The annual precipitation in the area varies greatly, ranging from 250 to 400 mm, and the precipitation is mainly concentrated in July–September, accounting for up to 75% of the annual precipitation. The precipitation in August can account for up to 54% of the whole rainy season, and the average annual evaporation ranges from 2,100 to 2,600 mm (She et al., 2017). The predominant soil type is sandy (FAO soil classification), and the geomorphological types include fixed, semi-fixed, wandering dune, and inter-dune lowlands (Chen et al., 2022). Drought-tolerant plants, such as *A. ordosica*, *Salix cheilophila*, *Caragana microphylla*, and *Corethrodendron fruticosum* var. *mongolicum*, are widely distributed in the area (Zhang et al., 2018).

2.2 Sample plots and data collection

In March 2022, shallow tillage was applied to the *A. ordosica* community (there is no history of any tillage). In mid-August of the same year, 10 shallow tillage (ST) strips and 10 non-shallow tillage (NT) strips, each approximately 8 m wide and 200 m long, were selected, in which the two were interleaved, and the distance between adjacent strips under the same treatment was greater than 200 m to include more heterogeneous habitats. Three 8 m × 20 m sample plots were randomly set up in each shallow-tillage and non-shallow-tillage strip, and a detailed survey of plant species and their abundance occurring in the plots was conducted (Supplementary Table S1) to assess species diversity and phylogenetic structure.

2.3 Species diversity and phylogenetic structure

2.3.1 α-Diversity

Based on the species matrix data that were obtained from the survey, we calculated the Shannon–Weiner index (H), Simpson's index (D), Pielou's evenness index (J), and the cumulative number of species as measures of species α-diversity, and the formulas are as follows:

Shannon diversity index:

$$H = -\sum_{i=1}^{S} P_i \ln P_i$$

Simpson's index:

$$D = 1 - \sum_{i=1}^{S} {P_i}^2$$

Pielou's evenness index:

$$J = \frac{H}{\ln S}$$

where S is the number of species and P_i is the relative abundance of species *i*. $P_i = n_i/N$, where n_i represents the number of individuals of species *i* and N represents the total number of individuals of all the plant species in a particular sample.

2.3.2 β-diversity

The Bray–Curtis dissimilarity index (β_{BC}) and the Jaccard dissimilarity index (β_J) were used to explore the β-diversity characteristics of the plant communities in the shallow and non-shallow tillage belts. The β_{BC} is based on the species multiplicity matrix to measure the compositional dissimilarity between the plots based on differences in species abundance, and it ranges from 0 (when both plots have the same species with equal abundances) to 1 (when the plots do not share any species; Catalán et al., 2023). The β_{BC} was calculated using the following equation:

$$\beta_{BC} = \frac{\sum_{i=1}^{S} \left| x_{ij} - x_{ik} \right|}{\sum_{i=1}^{S} x_{ij} + x_{ik}}$$

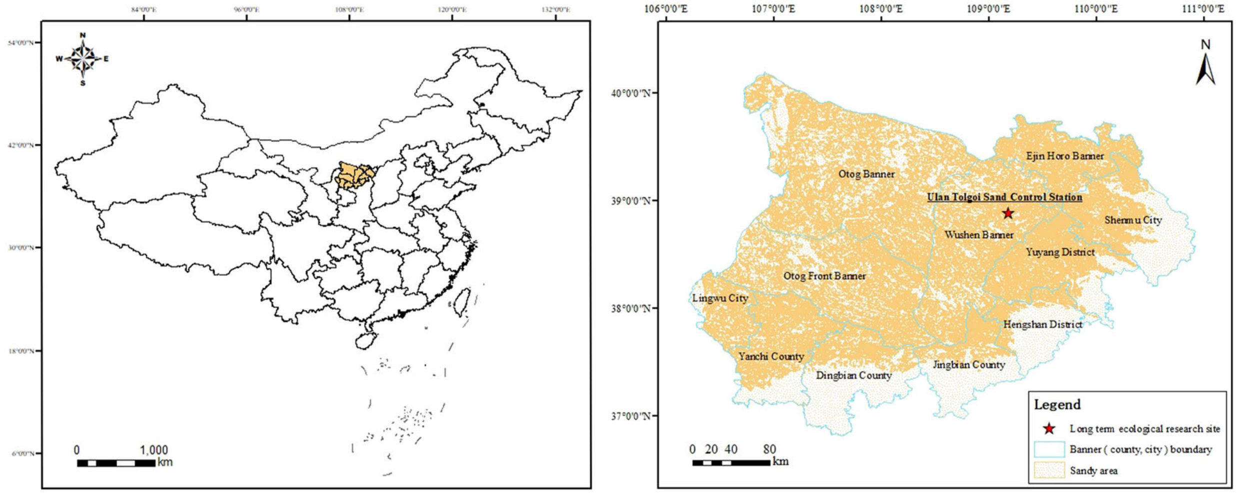


FIGURE 1
Geographic location of the study area.

where S is the number of species in the sample-species matrix, and x_{ij} and x_{ik} are the abundance of species *i* in *j* and *k* plots, respectively.

The β_J is based on the incidence to measure the compositional dissimilarity between the plots based only on the differences in species occurrence (presence/absence), and it ranges from 0 (when both plots have the same species) to 1 (when the plots do not share any species; Catalán et al., 2023). Podani and Schmera (2011) proposed that the β_J consists of two components: the first component (Rrel = 2 min(b,c)/n) accounts for species replacement, and the second (Drel = |b − c|/n) accounts for richness differences. β_J was calculated using the following equation:

$$\beta_J = \frac{b+c}{a+b+c} = \frac{\textit{number of species not shared}}{\textit{total number of species}}$$

$$= \frac{2min(b,c)}{a+b+c} + \frac{|b-c|}{a+b+c}$$

2.3.3 Phylogenetic structure

Based on the list of species from the survey, the Chinese Flora (http://www.iplant.cn/frps) and the Plant List website (http://www.theplantlist.org/) were used to standardize the descriptions (Kalwij, 2012). To construct the phylogenetic tree, the awk version of the "phylomatic" software was used, and the phylogenetic tree was visualized using the "ggtree" package in R (Zheng et al., 2019b; Figure 2).

To characterize the phylogenetic diversity (PD), the PD index was used, which represents the sum of the lengths of the evolutionary branches of all the species in the community, and the phylogenetic structure was measured using the net relatedness index (NRI) and the net nearest taxon index (NTI). The PD index and phylogenetic structure were calculated using the "picante" package in R (Chai et al., 2016; Qian and Sandel, 2017).

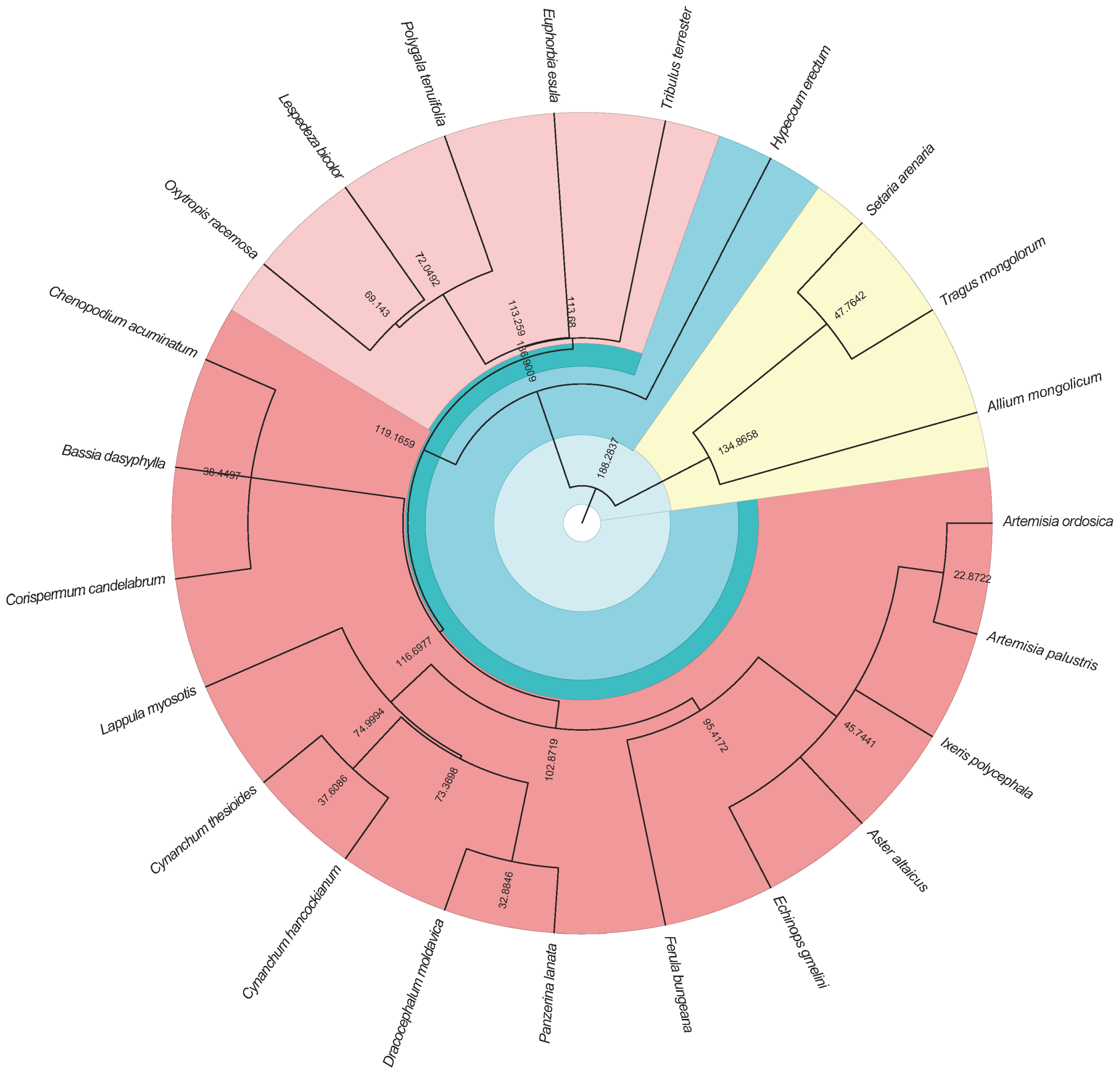


FIGURE 2
The phylogenetic tree of the plant species in the survey. The branch is the distance between the species.

The NRI and NTI were calculated using the following equations:

$$NRI = -1 \times \frac{MPD_s - MPD_{mds}}{SD(MPD_{mds})}$$

$$NTI = -1 \times \frac{MNTD_s - MNTD_r}{SD(MNTD_r)}$$

where MPD is the mean phylogenetic distance between all the species pairs in the community, and MPD_s and $MNTD_s$ denote the average observed paired phylogenetic distance and the average observed nearest neighbor phylogenetic distance, respectively. MPD_{mds} and $MNTD_{mds}$ denote the average paired phylogenetic distance and the average nearest neighbor phylogenetic distance, respectively, under 999 null model simulations. SD is the standard deviation.

2.4 Data analysis

The statistical analyses were performed in the R platform (version 3.5.3). The significant differences between the different communities for the diversity indicators were evaluated using the Kruskal–Wallis rank sum test and the "dplyr" library. The overall change in the species composition was measured using a similarity percentage analysis (SIMPER) and the "vegan" package to calculate the contribution of the individual species to the overall community dissimilarity. The β-diversity was separated into two components, species replacement [or turnover (Repl)] and richness differences [or nestedness (RichDiff)], to clarify their relative contribution using the "adespatial" package. To examine whether the ST and NT communities were spatially similar, non-metric multidimensional scaling and hierarchical clustering were performed based on the Raup–Crick distance using the "vegan" library. Then, a correlation analysis was conducted between the phylogenetic and species diversity indices using the "corrplot" package. The roles of the deterministic and stochastic processes in community assembly were assessed using the β-null model (Tucker et al., 2016).

3 Results

3.1 Species composition and diversity

The survey revealed that there were 20 species of seed plants from 12 families and 20 genera identified in the ST communities, with Asteraceae having the most species (20.0%), followed by Amaranthaceae (15.0%). The proportion of the remaining families was less than 10.0%. There were nine herbaceous annuals, nine herbaceous perennials, and two semi-shrub species, with *Setaria arenaria* and *Corispermum candelabrum* (Supplementary Table S1). A total of 15 species of seed plants from eight families and 14 genera were identified in the NT communities, among which Asteraceae had the highest number of species (33.3%), which was followed by Amaranthaceae (20.0%) and Gramineae (13.3%), and the proportion of the remaining families was less than 10.0%. There were nine herbaceous annuals, four herbaceous perennials, one semi-shrub species, and one shrub species, with *A. ordosica* being the dominant species (Supplementary Table S1).

3.1.1 α-diversity

The plant community in the shallow-tilled area had a high species diversity and evenness, with an H, D, J, and richness (S) of 1.68 ± 0.18, 0.72 ± 0.07, 0.66 ± 0.07, and 12.90 ± 2.85, respectively. In the control area, those measures were 0.98 ± 0.44, 0.46 ± 0.20, 0.54 ± 0.16, and 6.10 ± 2.38, respectively, and they were significantly higher ($p < 0.05$) in the shallow plowed area than in the control area (Table 1).

3.1.2 β-diversity

The β-diversity analysis that was based on the Bray–Curtis dissimilarity index showed that the mean values of the β-diversity of the plant communities in the ST and NT communities were 0.48 and 0.42, respectively, and there was a highly significant difference between them ($p < 0.001$, Figure 3). The β-diversity decomposition analyses showed that the species replacement process contributed more (60.82%) to the differences in the plant community composition in the ST, and the richness differences contributed less to the β-diversity (Figure 4A). The differences in the richness and species turnover processes contributed similarly to the differences in the plant community composition in the NT, being 54.68% and 45.32%, respectively (Figure 4B).

The Venn diagram (Supplementary Figure S1) shows the presence of 13 common species between the two communities. Of the reported species in this survey, eight were exclusively identified in the ST (*Ferula bungeana*, *Oxytropis racemose*, *Polygala tenuifolia*, *Allium mongolicum*, *Dracocephalum moldavica*, *Tribulus terrestris*, *Panzeria alaschanica*, and *Cynanchum thesioides*), and three were only found in the NT (*Tragus mongolorum*, *Hypecoum erectum*, and *A. ordosica*).

The results of SIMPER analysis showed that *C. candelabrum*, *S. arenaria*, and *Chenopodium acuminatum* contributed the most to the inter-community species differences and that the contribution of the three plants to the inter-community dissimilarity between the ST and the NT was more than 10% (*C. candelabrum*, 18.44%; *S. arenaria*, 17.52%; *C. acuminatum*, 10.57%). The three plants' cumulative contribution to the inter-community variation amounted to 68.13%, while the contribution of the other four plants to the inter-community differences amounted to 10.57%.

TABLE 1 Species diversity indices of the different communities.

Community type	ST	NT
Shannon–Weiner index (H)	1.68 ± 0.18a	0.98 ± 0.44b
Simpson index (D)	0.72 ± 0.07a	0.46 ± 0.20b
Pielou's evenness index (J)	0.66 ± 0.07a	0.54 ± 0.16b
Richness (S)	12.90 ± 2.85a	6.10 ± 2.38b

Different lowercase letters in the same row indicate significant differences between the two data groups ($p < 0.05$).
ST, shallow tillage area; NT, non-shallow tillage area.

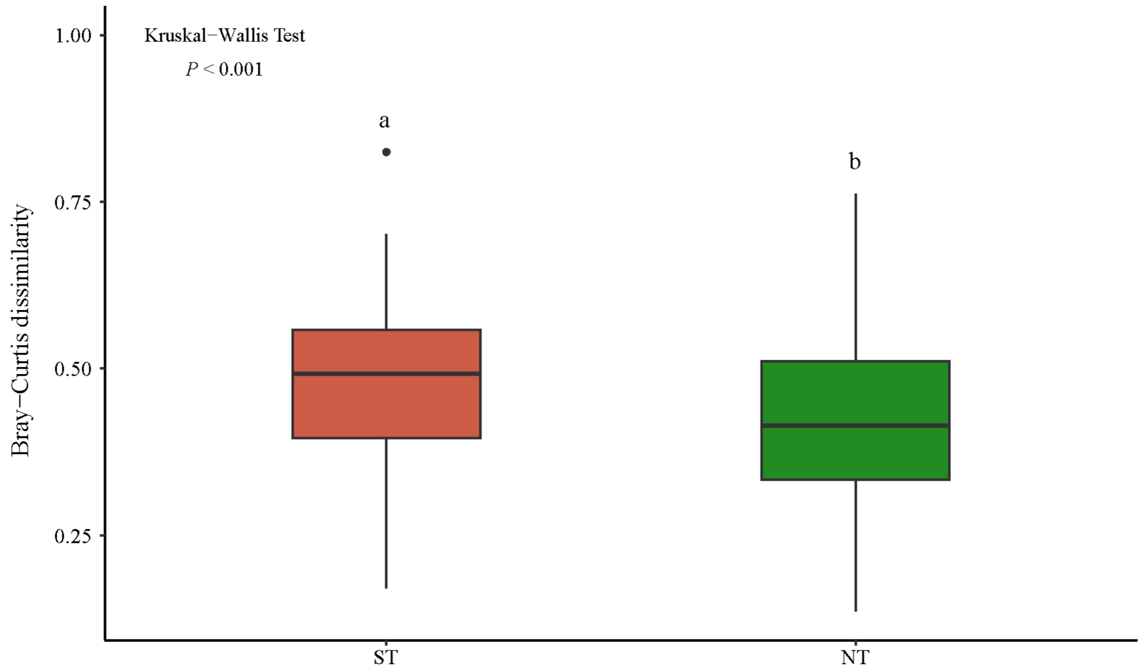


FIGURE 3
Differences in the Bray–Curtis dissimilarity index between the communities. The horizontal lines indicate the median values, the boxes indicate 25%–75% confidence intervals, the vertical lines indicate 10%–90% confidence intervals, the solid circles indicate outliers, and the different lowercase letters indicate significant differences among the communities. ST, shallow tillage area; NT, non-shallow tillage area.

The cumulative contribution of the seven plants to the inter-community variation amounted to 86.87% (Table 2).

3.2 Phylogenetic structure

The mean values of the PD, NRI, and NTI were 1,251.06 ± 70.94, −0.74 ± 0.03, and −0.06 ± 0.18 in the ST, respectively; the mean values of the PD, NRI, and NTI were 683.26 ± 62.37, −0.89 ± 0.13, and −0.84 ± 0.51 in the NT, respectively. The PD was significantly higher in the ST than in the NT, and the NRI was significantly lower in the ST than in the NT ($p < 0.001$, Supplementary Table S2). No significant correlation was found between the PD values and species diversity indices in the ST and NT communities. In the ST communities, the NTI was significantly and positively correlated with the H, D, and J ($p < 0.001$; Figure 5A).

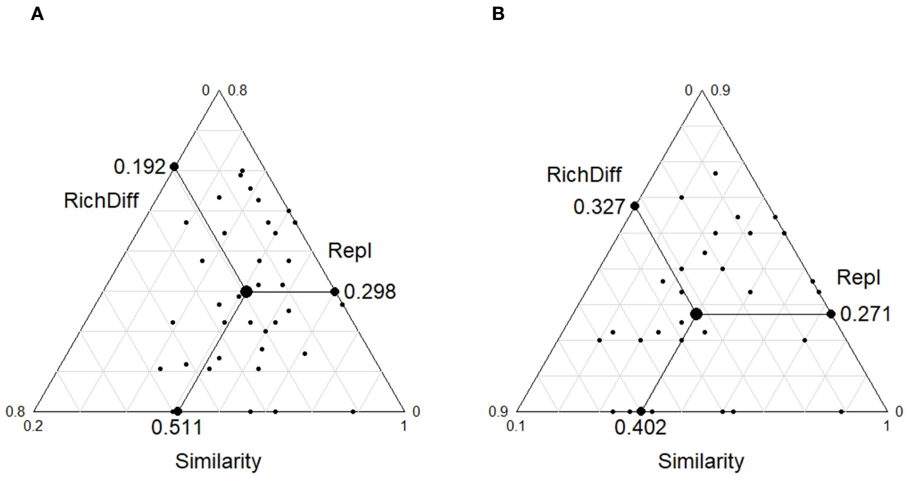


FIGURE 4
Triangular plots of the β-diversity comparisons (using the Jaccard index) for the plant communities among the **(A)** shallow tillage area (ST) and **(B)** non-shallow tillage area (NT). Each point represents a pair of sites. Its position is determined by a triplet of values, namely, the similarity (S), replacement (Repl), and richness difference (RichDiff) matrices, with each triplet summing to 1. The mean values of S, Repl, and RichDiff are shown.

TABLE 2 Contribution of the main species to community dissimilarity.

Species	Average contribution	Cumulative contribution
Corispermum candelabrum	0.1844	0.3348
Setaria arenaria	0.1752	0.5253
Chenopodium acuminatum	0.1057	0.6813
Grubovia dasyphylla	0.0749	0.7384
Aster altaicus	0.0355	0.7911
Ixeris chinensis subsp. *versicolor*	0.0235	0.8363
Euphorbia esula	0.0147	0.8687

The NTI and NRI were significantly and positively correlated with the SR in the NT communities ($p < 0.05$, Figure 5B).

3.3 Community assembly process

The results revealed that the NT communities were more similar, indicating that they deviated from the null hypothesis (Figure 6). However, the ST communities were farther apart and spread over half of the space, suggesting that the null hypothesis may be supported. These results suggest that stochastic processes play a greater role in the ST communities. A similar result was observed for the phylogenetic structure. In the ST and NT communities, the NRI and NTI were both less than 0 (Supplementary Table S2), indicating that the phylogenetic structure of the plant community diverged, there were more distantly related species in the community, the construction of the ST and NT communities was dominated by stochastic processes, and competitive exclusion was the primary mechanism of plant construction. However, when compared with the NT communities, the NRI and NTI were closer to 0 in the ST communities, indicating that the ST communities were subject to stronger stochastic processes.

4 Discussion

4.1 Species diversity and species composition

The plant community structure and function are determined by a variety of biotic and abiotic drivers, but anthropogenic environmental changes may alter these drivers and their effects on the plant communities (Brooker, 2006). The Mu Us Desert, as a typical agricultural and pastoral area, is ecologically fragile and frequently disturbed by human activities, and a full understanding of the biodiversity changes before and after disturbance is needed for effective conservation. Studies have confirmed that the SR of woody and herbaceous plants is strongly affected by environmental changes and anthropogenic disturbances (Zhou et al., 2018). Shallow tillage removes almost all the existing above-ground vegetation, increases the ecological niche space for communities (Tortorelli et al., 2022), and alters the physical properties, such as soil compactness, creating open and variable soil conditions and promoting soil seed germination and organic matter decomposition (Lu et al., 2018). Herbaceous plants, whose seeds are generally small and easy to disseminate, can quickly and effectively utilize temporary habitats for reproduction and respond to external disturbances than woody plants, which resulted in a significantly higher species diversity index in the ST communities than in the NT communities. Additionally, the allelopathic effect of *A. ordosica* significantly inhibits seed germination and seedling growth of herbaceous plants (Lopes et al., 2022). When a layer of up to 80%

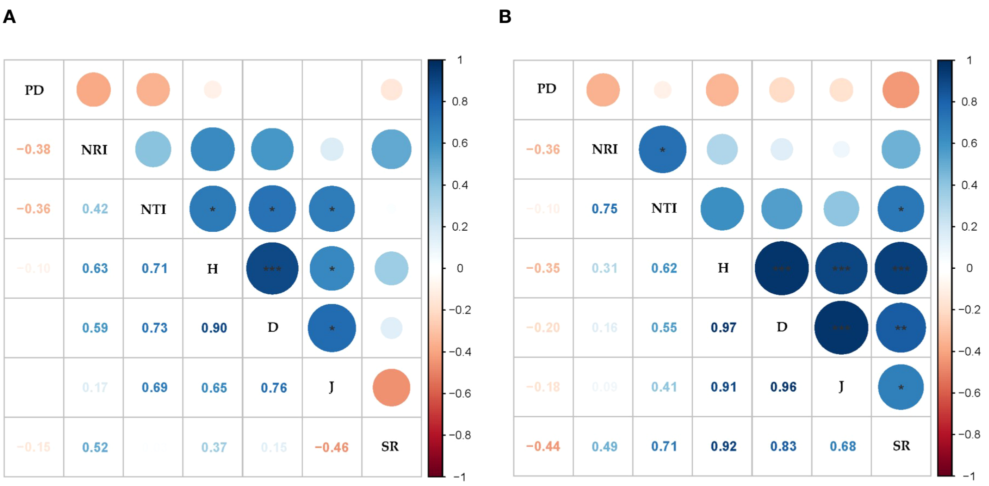


FIGURE 5
Correlation analysis between the species diversity and phylogenetic indices. **(A)** Shallow tillage area (ST). **(B)** Non-shallow tillage area (NT). Blue indicates a positive correlation, and orange indicates a negative correlation; the darker the color, the stronger the correlation. PD, phylogenetic diversity; NRI, net relatedness index; NTI, net nearest taxon index; H, Shannon–Weiner index; D, Simpson's index; J, Pielou's evenness index; SR, species richness. *p < 0.05; **p < 0.01; ***p < 0.001.

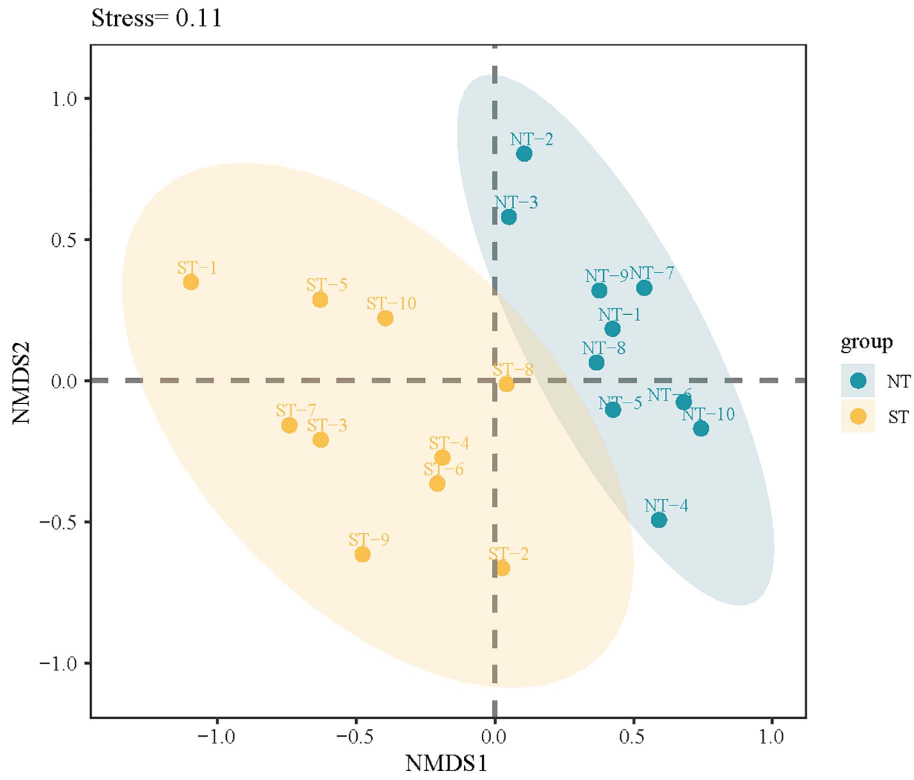


FIGURE 6
Non-metric multidimensional scaling (NMDS) analysis of the species composition in the sample plots. ST, shallow tillage area (yellow circle); NT, non-shallow tillage area (blue circle).

shrubs is formed, annual and biennial herbaceous plants are disadvantaged in resource competition and are mostly distributed in the unshaded edges of *A. ordosica* scrub. Thus, competition for available resources, such as light and soil nutrients, influences the maintenance of species diversity (Adler et al., 2018). At the same time, the stochastic nature of seed dispersal produced a preferential effect (Weidlich et al., 2021), with large differences in the species composition among the ST communities, which were clearly differentiated from the NT communities. Analyses of the β-diversity also revealed differences in the species composition between the ST and NT communities, with the β_{ST} being dominated by species replacement processes, indicating that the different communities tended to have more endemic species, and differences in abundance played a larger role than species replacement processes in the β_{NT}. This showed that a few communities contained the vast majority of the species in that state.

Several studies have demonstrated a negative correlation between the community restoration time and the SR; with the extension of the restoration time, early colonizing herbaceous plants will largely disappear (Daws et al., 2021; Standish et al., 2021). Ecological communities are always dynamic and vary in space and time, and they often develop along relatively predictable successional trajectories (Mori et al., 2018). We speculate that without re-imposed anthropogenic disturbance, the ST community will become more similar to the NT and form a zonal sub-top-level community with *A. ordosica* as a single dominant species (Webb et al., 1987; Li et al., 2015), i.e., a convergent process of community succession.

4.2 Phylogenetic structure

The low PD values shown for the NT communities suggested that fewer clades successfully colonized the NT and that the NT has a high frequency of closely related species. This could reflect the fact that phylogenetic diversity is lower in environments with greater competitive pressures for resources, as these environments may represent ecophysiological barriers that are difficult to surmount evolutionarily (Honorio Coronado et al., 2015); however, due to the small sample size and the limited extent of plots, further research is needed.

The increase in the SR may imply more complex interspecific affinities, which in turn leads to increased PD values, but no significant positive correlation between the PD values and the species diversity index was found in both the ST and NT in this study. Most studies have demonstrated a correlation between the two, but the correlation decreases with unbalanced evolutionary trees or narrow species distributions (Gascuel et al., 2015). When the ST and NT communities were viewed as a whole, the PD, NTI, and NRI were significantly positively correlated with the H, D, and S (Supplementary Figure S2). This significant relationship indicates that the species composition and distribution have some influence on the phylogenetic structure.

4.3 Community assembly

The phylogenetic structure of both the ST and NT communities tended to diverge (NTI < 0 and NRI < 0), suggesting that competitive exclusion is a dominant mechanism influencing community assembly. This pattern may be attributed to the limited availability of soil water and nutrients in the Mu Us Desert, which likely prevents closely related species with similar ecological niches from coexisting due to competition for these scarce resources, ultimately leading to the strengthening of interspecific ecological niche differentiation and the dispersion of phylogenetic structure (Webb et al., 2002; Silvertown, 2004). It is important to note that shallow tillage did not alter the major ecological processes driving community assembly, but the relative importance of deterministic and stochastic processes differed between the two treatments. The NRI and NTI of the ST communities differed significantly from those of the NT communities ($p < 0.05$, Supplementary Table S2), and analysis based on the Raup–Crick dissimilarity index also showed that the β_{RC} within the ST communities was closer to the zero expectation than in the NT communities (Supplementary Figure S4), suggesting that in addition to being dominated by competitive exclusion, the ST was also largely influenced by stochastic processes dominated by dispersal limitation, which resulted in lower species similarity within the communities. Studies have confirmed that disturbance may promote stochastic processes (Didham et al., 2005; Didham and Norton, 2006).

The desert maintains environmental consistency at large scales (Teramoto et al., 2022), and it can be assumed that the effective seed bank is the result of deterministic abiotic factors, such as temperature or precipitation, and stochastic factors, such as dispersal constraints. In contrast, habitat sieving at small scales mainly consists of biotic factors, such as interspecific interactions (Royo and Ristau, 2013; Zou et al., 2021). As *A. ordosica* requires a large amount of resources as a community-building species, including light, it results in a limited number of ecological niches being available in the community, which prevents certain species from persisting (Deng et al., 2022). In contrast, under shallow tillage conditions, where the community species are essentially removed and available ecological niches proliferate, herbaceous seeds can germinate rapidly and complete their life history cycles when the climatic conditions are favorable (Grime, 1977). Under these conditions, rapid reproductive processes may reduce the role of severe constraints on the hydrothermal conditions and inter- and intraspecific competition in shaping community membership. In future investigations, more attention should be paid to the role of annual and biennial herbaceous plants in the process of community assembly under human interference.

5 Conclusions

Shallow tillage increases species alpha, beta, and phylogenetic diversity and reduces the importance of competitive exclusion in community assembly. Therefore, we suggest that plant species with low ecological niche overlap should be selected for artificial vegetation restoration in the Mu Us Desert to reduce competition for the same resources.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding authors.

Author contributions

ZL: Conceptualization, Investigation, Data curation, Writing – original draft, Writing – review & editing. JQ: Writing – original draft, Writing – review & editing. ZL: Investigation, Writing – review & editing. XG: Investigation, Writing – review & editing. GH: Investigation, Writing – review & editing. HY: Investigation, Writing – review & editing. EH: Investigation, Writing – review & editing. CL: Investigation, Writing – review & editing. XW: Writing – review & editing. GL: Writing – review & editing, Funding acquisition. RG: Writing – review & editing, Supervision.

Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article. This work is supported by the Key Special Project for the Action of “Revitalizing Inner Mongolia through Science and Technology” (Grant No. 2022EEDSKJXM003-1), Natural Science Foundation of Inner Mongolia Autonomous Region (Grant No. 2024QN03025), Inner Mongolia Autonomous Region Key R&D and Achievement Transformation Program Project (Grant No. 2022YFHH0106), National “14th Five-Year Plan” Key R&D Program (No. 2022YFF1302503-3), Scientific Research Capacity Enhancement Project of Inner Mongolia Academy of Forestry Sciences (Grant No. 2024NLTS01 and 2024NLTS03), and National Natural Science Foundation of China (Grant No. 32360422).

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

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpls.2024.1409493/full#supplementary-material

References

Adler, P. B., Smull, D., Beard, K. H., Choi, R. T., Furniss, T., Kulmatiski, A., et al. (2018). Competition and coexistence in plant communities: intraspecific competition is stronger than interspecific competition. *Ecol. Lett.* 21, 1319–1329. doi: 10.1111/ele.13098

Alarcón, R., Hernández-Plaza, E., Navarrete, L., Sánchez, M. J., Escudero, A., Hernanz, J. L., et al. (2018). Effects of no-tillage and non-inversion tillage on weed community diversity and crop yield over nine years in a mediterranean cereal-legume cropland. *Soil Tillage Res.* 179, 54–62. doi: 10.1016/j.still.2018.01.014

Barroso, J., Miller, Z. J., Lehnhoff, E. A., Hatfield, P. G., and Menalled, F. D. (2015). Impacts of cropping system and management practices on the assembly of weed communities. *Weed Research* 55, 426–435. doi: 10.1111/wre.12155

Booth, B. D., and Swanton, C. J. (2002). Assembly theory applied to weed communities. *Weed Science* 50, 2–13. doi: 10.1614/0043-1745(2002)050[0002:AIATAT]2.0.CO;2

Brooker, R. W. (2006). Plant–plant interactions and environmental change. *New Phytol.* 171, 271–284. doi: 10.1111/j.1469-8137.2006.01752.x

Cadotte, M. W., Cardinale, B. J., and Oakley, T. H. (2008). Evolutionary history and the effect of biodiversity on plant productivity. *Proc. Natl. Acad. Sci. U.S.A.* 105, 17012–17017. doi: 10.1073/pnas.0805962105

Cadotte, M. W., Cavender-Bares, J., Tilman, D., and Oakley, T. H. (2009). Using phylogenetic, functional and trait diversity to understand patterns of plant community productivity. *PloS One* 4, e5695. doi: 10.1371/journal.pone.0005695

Capmourteres, V., and Anand, M. (2016). Conservation value": a review of the concept and its quantification. *Ecosphere* 7, e01476. doi: 10.1002/ecs2.1476

Catalán, A. M., López, D. N., Fica-Rojas, E., Broitman, B. R., Valdivia, N., and Scrosati, R. A. (2023). Foundation species canopies affect understory beta diversity differently depending on species mobility. *Ecology* 104, e3999. doi: 10.1002/ecy.3999

Chai, Y., Yue, M., Liu, X., Guo, Y., Wang, M., Xu, J., et al. (2016). Patterns of taxonomic, phylogenetic diversity during a long-term succession of forest on the Loess Plateau, China: insights into assembly process. *Sci. Rep.* 6, 27087. doi: 10.1038/srep27087

Chase, J. M. (2010). Stochastic community assembly causes higher biodiversity in more productive environments. *Science* 328, 1388–1391. doi: 10.1126/science.1187820

Chen, Y., Lu, H., Wu, H., Wang, J., and Lyu, N. (2023). Global desert variation under climatic impact during 1982–2020. *Sci. China Earth Sci.* 66, 1062–1071. doi: 10.1007/s11430-022-1052-1

Chen, Z., Huang, M., Xiao, C., Qi, S., Du, W., Zhu, D., et al. (2022). Integrating remote sensing and spatiotemporal analysis to characterize artificial vegetation restoration suitability in desert areas: A Case Study of Mu Us Sandy Land. *Remote Sens.* 14, 4736. doi: 10.3390/rs14194736

Cooper, J., Baranski, M., Stewart, G., Nobel-de Lange, M., Bàrberi, P., Fließbach, A., et al. (2016). Shallow non-inversion tillage in organic farming maintains crop yields and increases soil C stocks: a meta-analysis. *Agron. Sustain. Dev.* 36, 22. doi: 10.1007/s13593-016-0354-1

Cordeau, S., Smith, R. G., Gallandt, E. R., Brown, B., Salon, P., DiTommaso, A., et al. (2017). Disentangling the effects of tillage timing and weather on weed community assembly. *Agriculture* 7, 66. doi: 10.3390/agriculture7080066

Czerwiński, M., Woodcock, B. A., Golińska, B., and Kotowski, W. (2018). The effect of tillage management and its interaction with site conditions and plant functional traits on plant species establishment during meadow restoration. *Ecol. Eng.* 117, 28–37. doi: 10.1016/j.ecoleng.2018.03.017

Daws, M. I., Walters, S. J., Harris, R. J., Tibbett, M., Grigg, A. H., Morald, T. K., et al. (2021). Nutrient enrichment diminishes plant diversity and density, and alters long-term ecological trajectories, in a biodiverse forest restoration. *Ecol. Eng.* 165, 106222. doi: 10.1016/j.ecoleng.2021.106222

Deng, Z., Zhao, J., Wang, Z., Li, R., Guo, Y., Luo, T., et al. (2022). Stochastic processes drive plant community assembly in alpine grassland during the restoration period. *Diversity* 14, 832. doi: 10.3390/d14100832

Didham, R. K., and Norton, D. A. (2006). When are alternative stable states more likely to occur?: A reply to T. Fukami and W. G. Lee. *Oikos* 113, 357–362. doi: 10.1111/j.0030-1299.2006.13508.x

Didham, R. K., Watts, C. H., and Norton, D. A. (2005). Are systems with strong underlying abiotic regimes more likely to exhibit alternative stable states? *Oikos* 110, 409–416. doi: 10.1111/j.0030-1299.2005.13883.x

Durant, S. M., Pettorelli, N., Bashir, S., Woodroffe, R., Wacher, T., De Ornellas, P., et al. (2012). Forgotten biodiversity in desert ecosystems. *Science* 336, 1379–1380. doi: 10.1126/science.336.6087.1379

Erfanian, M. B., Ejtehadi, H., Vaezi, J., and Moazzeni, H. (2019). Plant community responses to multiple disturbances in an arid region of northeast Iran. *Land Degrad Dev.* 30, 1554–1563. doi: 10.1002/ldr.3341

Gascuel, F., Ferrière, R., Aguilée, R., and Lambert, A. (2015). How ecology and landscape dynamics shape phylogenetic trees. *Systematic Biol.* 64, 590–607. doi: 10.1093/sysbio/syv014

Grime, J. P. (1977). Evidence for the existence of three primary strategies in plants and its relevance to ecological and evolutionary theory. *Am. Nat.* 111, 1169–1194. doi: 10.1086/283244

Heino, J., and Tolonen, K. T. (2017). Untangling the assembly of littoral macroinvertebrate communities through measures of functional and phylogenetic alpha diversity. *Freshw. Biol.* 62, 1168–1179. doi: 10.1111/fwb.12934

Honorio Coronado, E. N., Dexter, K. G., Pennington, R. T., Chave, J., Lewis, S. L., Alexiades, M. N., et al. (2015). Phylogenetic diversity of Amazonian tree communities. *Diversity Distributions* 21, 1295–1307. doi: 10.1111/ddi.12357

Jaureguiberry, P., Titeux, N., Wiemers, M., Bowler, D. E., Coscieme, L., Golden, A. S., et al. (2022). The direct drivers of recent global anthropogenic biodiversity loss. *Sci. Adv.* 8, eabm9982. doi: 10.1126/sciadv.abm9982

Jin, H., Xu, J., Peng, Y., Xin, J., Peng, N., Li, Y., et al. (2023). Impacts of landscape patterns on plant species diversity at a global scale. *Sci. Total Environ.* 896, 165193. doi: 10.1016/j.scitotenv.2023.165193

Kalwij, J. M. (2012). Review of 'The Plant List, a working list of all plant species.'. *J. Vegetation Sci.* 23, 998–1002. doi: 10.1111/j.1654-1103.2012.01407.x

Laudicina, V. A., Palazzolo, E., Catania, P., Vallone, M., García, A. D., and Badalucco, L. (2017). Soil quality indicators as affected by shallow tillage in a vineyard grown in a semiarid mediterranean environment. *Land Degradation Dev.* 28, 1038–1046. doi: 10.1002/ldr.2581

Légère, A., Stevenson, F. C., and Benoit, D. L. (2005). Diversity and assembly of weed communities: contrasting responses across cropping systems. *Weed Research* 45, 303–315. doi: 10.1111/j.1365-3180.2005.00459.x

Li, S.-L., Yu, F.-H., Werger, M. J., Dong, M., During, H. J., and Zuidema, P. A. (2015). Mobile dune fixation by a fast-growing clonal plant: a full life-cycle analysis. *Sci. Rep.* 5, 8935. doi: 10.1038/srep08935

Lopes, A. D., Nunes, M. G. I. F., Francisco, J. P., and dos Santos, E. H. (2022).“Potential allelopathic effect of species of the Asteraceae family and its use in agriculture,”. In: *Vegetation dynamics, changing ecosystems and human responsibility* (IntechOpen). Available online at: https://www.intechopen.com/chapters/84835 (Accessed May 29, 2024).

Lu, D., Wang, G. G., Yu, L., Zhang, T., and Zhu, J. (2018). Seedling survival within forest gaps: the effects of gap size, within-gap position and forest type on species of contrasting shade-tolerance in Northeast China. *Forestry: Int. J. For. Res.* 91, 470–479. doi: 10.1093/forestry/cpy007

Mishler, B. D., Knerr, N., González-Orozco, C. E., Thornhill, A. H., Laffan, S. W., and Miller, J. T. (2014). Phylogenetic measures of biodiversity and neo-and paleo-endemism in Australian Acacia. *Nat. Commun.* 5, 4473. doi: 10.1038/ncomms5473

Mori, A. S., Isbell, F., and Seidl, R. (2018). β-diversity, community assembly, and ecosystem functioning. *Trends Ecol. Evol.* 33, 549–564. doi: 10.1016/j.tree.2018.04.012

Podani, J., and Schmera, D. (2011). A new conceptual and methodological framework for exploring and explaining pattern in presence–absence data. *Oikos* 120, 1625–1638. doi: 10.1111/j.1600-0706.2011.19451.x

Qian, H., and Sandel, B. (2017). Phylogenetic relatedness of native and exotic plants along climate gradients in California, USA. *Diversity Distributions* 23, 1323–1333. doi: 10.1111/ddi.12620

Randriamalala, J. R., Hervé, D., Randriamboavonjy, J.-C., and Carrière, S. M. (2012). Effects of tillage regime, cropping duration and fallow age on diversity and structure of secondary vegetation in Madagascar. *Agriculture Ecosyst. Environ.* 155, 182–193.

Royo, A. A., and Ristau, T. E. (2013). Stochastic and deterministic processes regulate spatio-temporal variation in seed bank diversity. *J. Vegetation Sci.* 24, 724–734. doi: 10.1111/jvs.12011

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.

Silvertown, J. (2004). Plant coexistence and the niche. *Trends Ecol. Evol.* 19, 605–611. doi: 10.1016/j.tree.2004.09.003

Standish, R. J., Gove, A. D., Grigg, A. H., and Daws, M. I. (2021). Beyond species richness and community composition: Using plant functional diversity to measure restoration success in jarrah forest. *Appl. Vegetation Sci.* 24, e12607. doi: 10.1111/avsc.12607

Teramoto, M., Hamamoto, T., Liang, N., Taniguchi, T., Ito, T. Y., Hu, R., et al. (2022). Abiotic and biotic factors controlling the dynamics of soil respiration in a coastal dune ecosystem in western Japan. *Sci. Rep.* 12, 14320. doi: 10.1038/s41598-022-17787-8

Tortorelli, C. M., Kerns, B. K., and Krawchuk, M. A. (2022). Community invasion resistance is influenced by interactions between plant traits and site productivity. *Ecology* 103, e3697. doi: 10.1002/ecy.3697

Travlos, I. S., Cheimona, N., Roussis, I., and Bilalis, D. J. (2018). Weed-species abundance and diversity indices in relation to tillage systems and fertilization. *Front. Environ. Sci.* 6. doi: 10.3389/fenvs.2018.00011

Tucker, C. M., and Cadotte, M. W. (2013). Unifying measures of biodiversity: understanding when richness and phylogenetic diversity should be congruent. *Diversity Distributions* 19, 845–854. doi: 10.1111/ddi.12087

Tucker, C. M., Shoemaker, L. G., Davies, K. F., Nemergut, D. R., and Melbourne, B. A. (2016). Differentiating between niche and neutral assembly in metacommunities using null models of β-diversity. *Oikos* 125, 778–789. doi: 10.1111/oik.02803

Valone, T. J. (2003). Examination of interaction effects of multiple disturbances on an arid plant community. *Southwestern Nat.* 48, 481–490. doi: 10.1894/0038-4909(2003)048<0481:eoieom>2.0.co;2

Wang, X., Lou, J., Ma, W., Jiao, L., Li, H., and Hua, T. (2017). The impact of reclamation on aeolian desertification of four species in the otindag desert, china. *CATENA* 157, 189–194. doi: 10.1016/j.catena.2017.05.024

Webb, C. O., Ackerly, D. D., McPeek, M. A., and Donoghue, M. J. (2002). Phylogenies and community ecology. *Annu. Rev. Ecol. Syst.* 33, 475–505. doi: 10.1146/annurev.ecolsys.33.010802.150448

Webb, R. H., Steiger, J. W., and Turner, R. M. (1987). Dynamics of Mojave Desert shrub assemblages in the panamint mountains, California: ecological archives E068-001. *Ecology* 68, 478–490. doi: 10.2307/1938453

Wei, H., Luo, T., and Wu, B. (2016). Optimal balance of water use efficiency and leaf construction cost with a link to the drought threshold of the desert steppe ecotone in northern China. *Ann. Bot.* 118, 541–553. doi: 10.1093/aob/mcw127

Weidlich, E. W. A., Nelson, C. R., Maron, J. L., Callaway, R. M., Delory, B. M., and Temperton, V. M. (2021). Priority effects and ecological restoration. *Restor. Ecol.* 29, e13317. doi: 10.1111/rec.13317

Zhang, L., Hong, G., Li, Z., Gao, X., Wu, Y., Wang, X., et al. (2018). Assessment of the ecosystem service function of sandy lands at different times following aerial seeding of an endemic species. *Sustainability* 10, 902. doi: 10.3390/su10040902

Zhang, T., and Chen, Y. (2022). The effects of landscape change on habitat quality in arid desert areas based on future scenarios: Tarim River Basin as a case study. *Front. Plant Sci.* 13. doi: 10.3389/fpls.2022.1031859

Zhang, T., Chen, Y., and Ali, S. (2023). Abiotic stress and human activities reduce plant diversity in desert riparian forests. *Ecol. Indic.* 152, 110340. doi: 10.1016/j.ecolind.2023.110340

Zheng, Y., Dong, L., Li, Z., Zhang, J., Li, Z., Miao, B., et al. (2019b). Phylogenetic structure and formation mechanism of shrub communities in arid and semiarid areas of the Mongolian Plateau. *Ecol. Evol.* 9, 13320–13331. doi: 10.1002/ece3.5787

Zheng, D., Wang, Y., Shao, Y., and Wang, L. (2019a). The vegetation dynamics and climate change responses by leaf area index in the Mu Us Desert. *Sustainability* 11, 3151. doi: 10.3390/su11113151

Zhou, Y., Chen, S., Hu, G., Mwachala, G., Yan, X., and Wang, Q. (2018). Species richness and phylogenetic diversity of seed plants across vegetation zones of Mount Kenya, East Africa. *Ecol. Evol.* 8, 8930–8939. doi: 10.1002/ece3.4428

Zou, C., Martini, F., Xia, S.-W., Castillo-Diaz, D., and Goodale, U. M. (2021). Elevation and micro environmental conditions directly and indirectly influence forests' soil seed bank communities. *Global Ecol. Conserv.* 26, e01443. doi: 10.1016/j.gecco.2020.e01443

Frontiers in Plant Science

TYPE Brief Research Report
PUBLISHED 24 October 2024
DOI 10.3389/fpls.2024.1468395

Strict biennial lifecycle and anthropogenic interventions affect temporal genetic differentiation in the endangered endemic plant, *Pedicularis hallaisanensis*

Seongjun Kim*, Byoung-Doo Lee, Chang Woo Lee, Hwan-Joon Park, Jung Eun Hwang, Hyeong Bin Park, Young-Joong Kim, Daeyoung Jeon and Young-Jun Yoon

Research Center for Endangered Species, National Institute of Ecology, Yeongyang, Gyeongbuk, Republic of Korea

Strict biennials are among the least known lifecycles in plant ecology due to their rarity in nature, and their population genetics still remain unknown. The present study addressed the strict biennial lifecycle and associated population genetics of *Pedicularis hallaisanensis*, an endangered endemic plant in Korea. All *P. hallaisanensis* individuals were counted in August from 2021 to 2023 in the wild population of Gayasan National Park, and lifecycle and morphological changes were monitored monthly. *A de novo* draft genome and single nucleotide polymorphism (SNP) analysis were used to study the population's genetic structure. *P. hallaisanensis* strictly requires a 2-year lifecycle per generation, including 8 and 10 months of growing periods as a first-year seedling and second-year adult, respectively. Facultative annual and perennial lifecycles were undetected, resulting in odd-year and even-year flowering cohorts. Permutational multivariate analysis of variance on the detected 3,716 SNPs demonstrated that the flowering group ($p < 0.005$), microhabitat ($p < 0.001$), and their interaction ($p < 0.01$) had a significant effect on genetic structure, which was differentiated between odd-year and even-year flowering cohorts. Other cluster analyses also showed that a microhabitat under historical anthropogenic interventions contained lowered genetic diversity due to a decreased genetic distance between odd-year and even-year flowering cohorts ($p < 0.05$). Overall, the findings suggest that excessive anthropogenic interventions should be avoided to preserve genetic diversity in the wild *P. hallaisanensis* population. Moreover, conservation programs for similar biennial plants should collect wild breeds from both odd-year and even-year flowering cohorts to improve the genetic diversity of artificially propagated individuals.

KEYWORDS

anthropogenic intervention, biennial plant, genetic diversity, genotyping-by-sequencing, hemiparasitic herb, lousewort, single nucleotide polymorphism

OPEN ACCESS

EDITED BY
Praveen Dhyani,
University of Koblenz, Germany

REVIEWED BY
Arun K. Jugran,
Govind Ballabh Pant National Institute of Himalayan Environment and Sustainable Development, India
Michele Wyler,
MWSchmid GmbH, Switzerland
César Marín,
Santo Tomás University, Chile

*CORRESPONDENCE
Seongjun Kim
✉ dao1229@nie.re.kr

RECEIVED 22 July 2024
ACCEPTED 30 September 2024
PUBLISHED 24 October 2024

CITATION
Kim S, Lee B-D, Lee CW, Park H-J, Hwang JE, Park HB, Kim Y-J, Jeon D and Yoon Y-J (2024) Strict biennial lifecycle and anthropogenic interventions affect temporal genetic differentiation in the endangered endemic plant, *Pedicularis hallaisanensis*. *Front. Plant Sci.* 15:1468395.
doi: 10.3389/fpls.2024.1468395

1 Introduction

The lifecycle of endangered plants is important for conservation programs, as it reflects the legacy of adaptations to the surrounding environment (Niklas and Kutschera, 2010). The lifecycle of any plant species results from the optimization of resource allocation and utilization to enhance its fitness to the given habitat conditions (Kenrick, 2017). Since lifecycle strategy determines population dynamics and associated genetic diversity, understanding the lifecycle of endangered plants is critical for identifying potential bottlenecks in population growth and setting conservation priorities to sustain wild populations and habitats (Aronne, 2017; Tsuzuki et al., 2022). Although classifying lifecycles is primarily based on expected lifespan (annual, biennial, and perennial) and the total number of reproductive bouts throughout the lifespan (monocarpic and polycarpic), the diversity found in nature has caused many intermediate, idiosyncratic lifecycle patterns (e.g., facultative annual) of endangered plant species (Friedman, 2020).

Strict biennials are one of the least understood lifecycles in plant ecology because of their rarity under natural conditions (Friedman, 2020; Viswanathan and Aarssen, 2000). Several studies have described the lifecycle of biennial plants, such as *Pedicularis sylvatica* (Petrů, 2005) and *Gentianella campestris* (Glav Lundin and Eriksson, 2021). Such species strictly require a 2-year growing period prior to reproduction and death, in contrast to other facultative biennials, like winter annuals and monocarpic perennials, whose lifespan and reproduction depend on climatic event or growth rate (Bradshaw, 1978; Ter Bort et al., 1980; Kelly, 1985). This age dependence for reproduction also leads to the coexistence of two age classes (first-year seedling and second-year adult) within a single habitat (Kisdi, 2012), as well as the potential differentiation of cohorts flowering in either odd or even years (Petrů, 2005).

Recent advancements in genotyping-by-sequencing (GBS) have expanded knowledge regarding the population structure and genetic diversity of endangered plant species through fast, reliable processes at a low cost. This technique has helped identify genotype cohorts within a given endangered species according to single nucleotide polymorphism (SNP) loci (Cai et al., 2021; Wang et al., 2024). Nonetheless, GBS approaches for endangered strict biennial plants have principally focused on genetic variations across multiple habitats and population sizes, without considering their unique lifecycle patterns (Cho and Choi, 2011; Reisch and Hoiß, 2019). Since the differentiation in reproduction time may decelerate gene flow between cohorts flowering in odd and even years (Petrů, 2005; Rusterholz et al., 2023), genetic comparisons between odd- and even-year flowering cohorts are necessary for totally understanding the genetic diversity and population dynamics of biennial plants.

The present study addressed the strict biennial lifecycle and associated population genetics of *Pedicularis hallaisanensis* (Orobanchaceae), an endangered endemic plant species in Korea. It was hypothesized that *P. hallaisanensis* cohorts flowering in odd-year would differ in genetic structure from those flowering in even-year because the isolated reproduction times may limit gene flow between the two cohorts. To test this hypothesis, a wild *P. hallaisanensis* population was periodically monitored to confirm that it followed a strict biennial lifecycle. Given the unavailability of a reference nuclear genome for the target species, we assembled a *de novo* draft genome of *P. hallaisanensis* to detect SNPs and compare the genetic structure between the different flowering years.

2 Methods

2.1 Target species

The target species is the root-hemiparasitic herb species, *P. hallaisanensis* (Figures 1C, D). This species is endemic to Korea and is legally protected as an endangered species (grade II) of the Ministry of Environment (Chung et al., 2023). It originally inhabited Korean mountaintop grasslands (altitude: 1,400–1,500 m) such as Hallasan and Gayasan (Kim et al., 2018), with voucher specimens stored at the herbariums of Inha University (Cho. 98454) and the Korea National Park Research Institute (Gaya_20160439), respectively (Cho and Choi, 2011; Han et al., 2022). However, the most wild populations have disappeared due to habitat loss and climate change (Kim et al., 2018). This species is known to be phylogenetically close to other hemiparasitic Orobanchaceae species, such as *Pedicularis spicata*, *Pedicularis verticillata, Pedicularis alaschanica*, and *Pedicularis longiflora*, according to previous ribosomal and chloroplast DNA studies (Cho and Choi, 2011; Cho et al., 2018; Zhang et al., 2020). Morphologically, *P. hallaisanensis* is distinguishable by its dense glandular hairs covering the entire body, a shorter galea compared to the lower lip, and three unequal calyx lobes when compared to other allied Orobanchaceae species (Cho and Choi, 2011; Kim et al., 2018). Although *P. hallaisanensis* has traditionally been speculated to be either an annual, biennial, or perennial species (Cho et al., 2018; Kim et al., 2018), our monitoring was the first to confirm that this species features a strict biennial lifecycle (see our Results section).

2.2 Study area and lifecycle monitoring

The study area was located in a mountaintop grassland within Gayasan National Park in South Korea (35°49′25″N, 128°7′10″E) (Figures 1A, B). The altitude of the study area ranges from 1,410 to 1,430 m above sea level (asl), with slopes varying between 0° and 20°. The average annual precipitation is 1,296 mm, with a relative humidity of 74.8% and an average air temperature of 7.6°C. The soil is less than 20 cm, lacking distinct evidence of horizon development (entisols), and is underlain by an impermeable bedrock layer. This was the only study area available for studying the target species, although we have investigated all known natural habitats around Hallasan, Gayasan, Seoraksan, and Bangtaesan from 2019 to 2023 in search of additional wild *P. hallaisanensis* populations. National inventory data from the National Institute of Ecology have also recorded no wild *P. hallaisanensis* population remaining in any natural habitats since 2020, except for this study area.

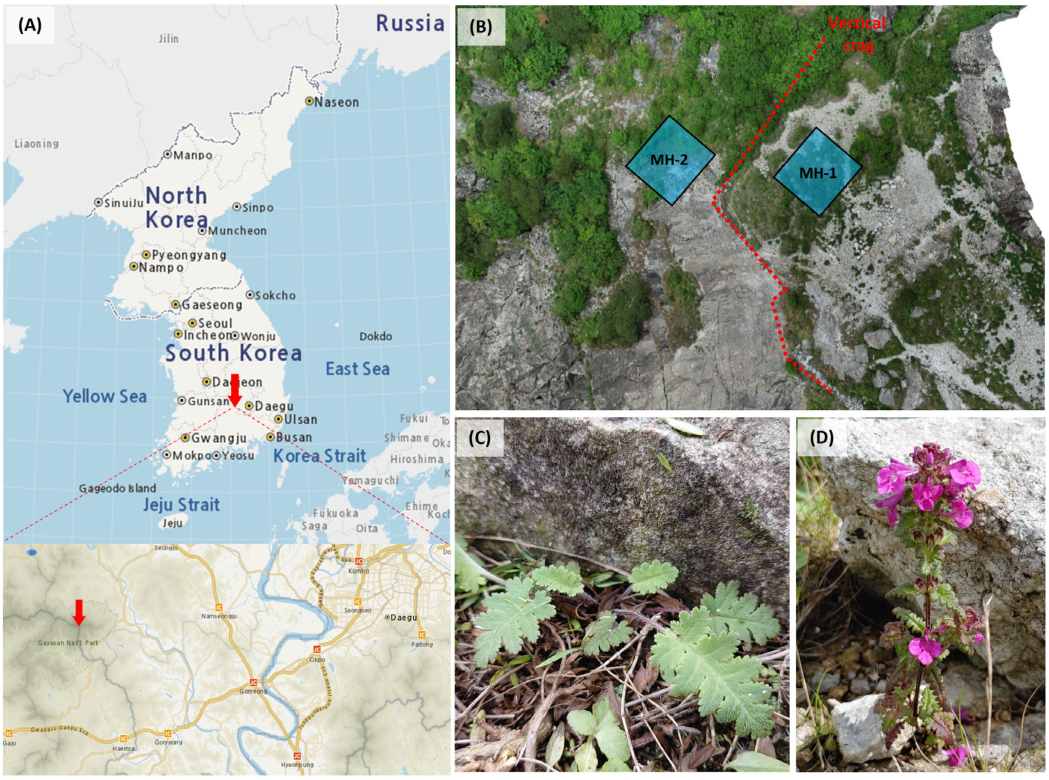


FIGURE 1
Location of the study area **(A)**, microhabitats (MH-1 and MH-2) for sampling of *Pedicularis hallaisanensis* SNP analysis in the study area **(B)**, and examples of first-year seedling **(C)** and a second-year adult **(D)** of *P. hallaisanensis*. (Source of **(A)**: https://map.ngii.go.kr/ms/map/NlipMap.do, accessed on 8 April 2024).

All *P. hallaisanensis* individuals were counted in August from 2021 to 2023 to quantify the population size in the study area. Of these, one individual was used for the draft genome analysis, and 20 were used for SNP analyses. All *P. hallaisanensis* individuals in the study area were sorted into age classes according to the elapsed time after germination (first-year seedling and second-year adult). Each *P. hallaisanensis* was labeled and monthly monitored to track any morphological changes throughout the biennial lifecycle during the growing season (April–November).

2.3 DNA extraction and draft genome assembly

Fresh leaves of a *P. hallaisanensis* individual were sampled in June 2023, stored in an icebox (4°C), and brought to the laboratory for draft genome assembly. Genomic DNA was extracted from the leaf samples using the Aprep Total DNA KIT (APBIO, Namyangju, South Korea) based on the manufacturer's instructions. The extracted DNA was then quantified with a Thermo Scientific Nanodrop 8000 spectrophotometer (Fisher Scientific, Waltham, MA, USA), digested using the ApeKI enzyme (GCWGC), and reorganized into short reads of 151 bp length, following the protocols of Elshire et al. (2011) and Oh et al. (2023). Sequencing was conducted using the Illumina Hiseq X Ten Platform (Illumina Inc., San Diego, CA, USA). Illumina raw reads were filtered using Trimmomatic v.0.39 to exclude poor-quality reads (window size: 4, mean quality: ≧ 15, leading and trailing: ≧ 3, read length: ≧ 36 bp) (Bolger et al., 2014), and *de novo* assembly was done using SOAPdenovo2 v.2.04 (K-mer = 69) (Luo et al., 2012).

2.4 SNP detection and filtration

Two 5 × 5 m plots were established for SNP analyses in two different microhabitats (MH-1 and MH-2). Although the distance between these two microhabitats was only 20 m, they differed in terms of historical anthropogenic interventions (Figure 1B). MH-1 had been artificially flattened and managed as a heliport until the early 2010s (Supplementary Figure S1), but it has recently become revegetated after such management was stopped. Conversely, MH-2 was located outside the old heliport sites and was relatively sheltered from heavy anthropogenic interventions. Both MH-1 and MH-2 contained both odd-year-flowering (OYF) and even-year-flowering (EYF) *P. hallaisanensis* cohorts, in contrast to several other microhabitats, which included either OYF or EYF cohorts only.

Fresh cauline leaves from five OYF and five EYF were randomly sampled from each plot for SNP detection in June 2023 and 2022, respectively (*n* = 20). Short DNA reads (151 bp) were obtained using the protocols of Elshire et al. (2011) and Oh et al. (2023) and sequenced with the Illumina Hiseq X Ten platform (Illumina Inc., CA, USA). Low-quality raw reads were then removed using cutadapt v.1.8.3 (Li, 2013) and Trimmomatic v.0.39 (Bolger et al., 2014). Filtered clean reads were mapped to the assembled draft genome of *P. hallaisanensis* using BWA v.0.7.17-r1188 (Li, 2013), and raw SNPs were detected using SAMtools v.0.1.16 (Li et al., 2009). To ensure SNP quality, only biallelic SNP loci without any missing values throughout all 20 samples were selected for further statistical analyses (3716 SNPs in total).

2.5 Statistical analyses

To describe the genetic diversity, Nei's genetic diversity (GD), polymorphism informative content (PIC), minor allele frequency (MAF), and observed heterozygosity (Ho) were calculated using the snpReady package in R v.4.3.2 software (Granto et al., 2018).

Permutational multivariate analysis of variance (PERMANOVA) and permutational analysis of multivariate dispersion (PERMDISP) were conducted using Bray–Curtis dissimilarity based on 9,999 permutations to test the effects of flowering group (OYF or EYF) and microhabitat (MH-1 or MH-2) on the multivariate genetic centroids and dispersions of the SNP data from 20 P. *hallaisanensis* samples ($\alpha = 0.05$). Nonmetric multidimensional ordination scaling (NMDS) was further conducted using Bray–Curtis dissimilarity to visualize the multivariate genetic variability, and a general linear model (GLM) was applied to test the relationship between NMDS axes, flowering group, and microhabitat ($n = 20$, $\alpha = 0.05$). These analyses were performed using the vegan (Oksanen et al., 2024) and agricolae (De Mendiburu and Simon, 2015) packages in R v.4.3.2 software (R Core Team, 2023).

Neighbor-joining method and k-means clustering of the silhouette width approach were implemented using the factoextra (Kassambara and Mundt, 2016) and ape (Paradis et al., 2004) packages in R v.4.3.2 software to show genetic differences among the sampled *P. hallaisanensis* ($n = 20$) (Batool and Henning, 2021). STRUCTURE 2.3.4 software was also used with 10,000 burn-in periods, 100,000 Markov chain Monte Carlo (MCMC) replications, and 10 iterations to estimate the membership probability of each *P. hallaisanensis* sample according to hypothetical ancestral genotypes (Pritchard et al., 2000).

3 Results

3.1 Lifecycle and population size of *P. hallaisanensis*

Our monitoring demonstrated that *P. hallaisanensis* strictly required 2 years of lifecycle per generation, with approximately 8 and 10 months of growing periods as first-year seedling and second-year adult, respectively. From April to May, first-year seedlings of *P. hallaisanensis* germinated and developed leaves and roots, eventually forming overwintering buds in November (Figure 2A). There were only rosette leaves without distinguishable shoots and cauline leaves in the aboveground of the first-year seedlings

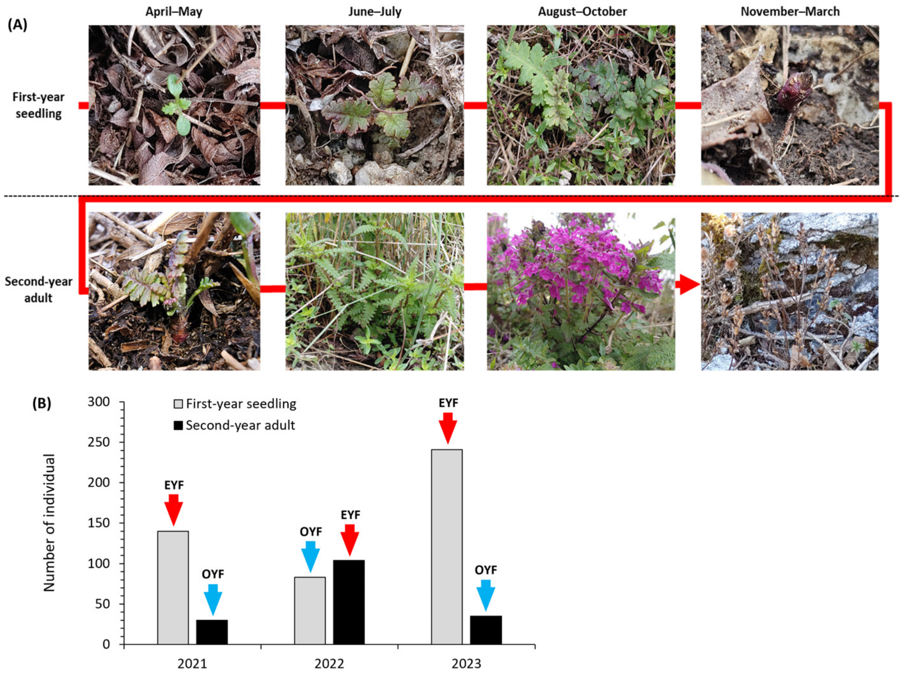


FIGURE 2
(A) Summary of the strict biennial lifecycle of *Pedicularis hallaisanensis* and **(B)** number of first-year seedling and second-year adult of *P. hallaisanensis* individuals in the study area. Arrows above the bars in **(B)** indicate odd-year-flowering (OYF) and even-year-flowering (EYF) *P. hallaisanensis*.

(Figure 2A). However, second-year adults of *P. hallaisanensis* rapidly established shoots and cauline leaves from the overwintering buds starting in April. Flowering and seed production of occurred from August to November in the second year, and all second-year adults died after producing seeds (Figure 2A). Facultative annual and perennial lifecycle patterns were undetected for *P. hallaisanensis* in the study area. Accordingly, a flowering event of *P. hallaisanensis* occurred biennially at several microhabitats (other than MH-1 and MH-2), when they contained only first-year seedlings or second-year adults.

The total number of *P. hallaisanensis* individuals in the study area was 170, 187, and 276 in 2021, 2022, and 2023, respectively (Figure 2B). First-year seedlings and second-year adults accounted for 44.4%–87.3% and 12.7%–55.6% of the *P. hallaisanensis* population, respectively. EYF consistently showed a larger number of *P. hallaisanensis* individuals than OYF regardless of age throughout the monitoring period (Figure 2B).

3.2 Draft genome and filtered SNPs

Sequencing of *P. hallaisanensis* provided 100.9 Gb of Illumina short reads, which were used to assemble the reference draft genome, involving 2.6 million contigs with a total length of 1.23 Gb and an N50 length of 0.54 Mb, for further SNP identification (Supplementary Table S1). Subsequently, a total of 123.7 Tb of Illumina short reads was obtained from 10 OYF and 10 EYF samples (20 genotypes), from which 3,716 filtered SNPs were identified to analyze the genetic structure across flowering groups and microhabitats. Genetic diversity indices for 3,716 SNPs are described in Supplementary Table S2.

3.3 Effects of flowering group and microhabitat

PERMANOVA on the 3,716 SNPs demonstrated that the flowering group ($p < 0.005$), microhabitat ($p < 0.001$), and their interaction ($p < 0.01$) had a significant effect on the multivariate centroid of 3,716 SNPs from *P. hallaisanensis* (Figure 3A). These three factors explained 44.8% of the multivariate variances in SNPs, while the remaining 55.2% of the variance were attributed to within-group variabilities. On the other hand, PERMDISP indicated that only microhabitat ($p < 0.05$) had a significant effect on the multivariate dispersion of 3,716 SNPs from *P. hallaisanensis* (Figure 3A).

NMDS ordination showed a similar pattern with PERMANOVA and PERMDISP (stress value: 0.12, Figure 3B). Additional GLM on NMDS axes found that axes 1 and 2 represented the variabilities resulting from microhabitat ($p <$ 0.001) and flowering group ($p < 0.005$), respectively (Figure 3B). Since the multivariate centroids of OYF and EYF were closer in

(A)

PERMANOVA				
Factor	Mean square error	Variance explained (%)	Pseudo-F	*p* value
Flowering group	0.1619	10.3	2.99	< 0.005
Microhabitat	0.4006	25.5	7.41	< 0.001
Flowering group × microhabitat	0.1417	9.0	2.62	< 0.01

PERMDISP			
Factor	Mean square error	Pseudo-F	*p* value
Flowering group	0.0015	1.18	0.29
Microhabitat	0.0096	5.32	< 0.05

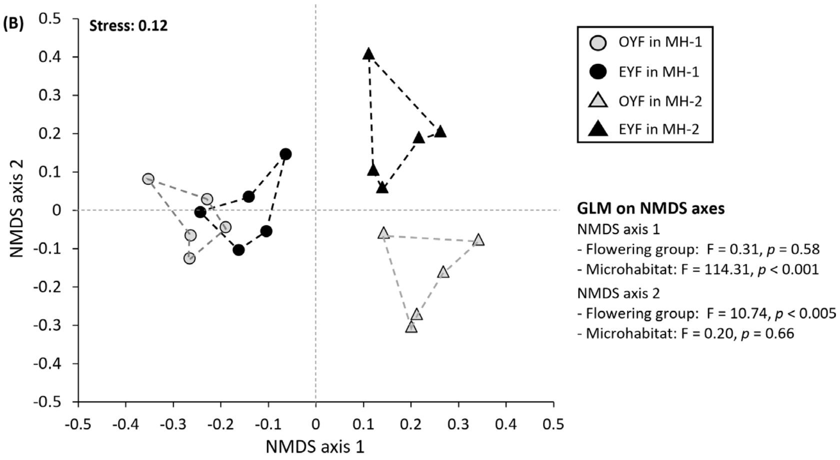


FIGURE 3
(A) Results of permutational multivariate analysis of variance (PERMANOVA) and permutational analysis of multivariate dispersion (PERMDISP). **(B)** Nonmetric multidimensional ordination scaling (NMDS; n = 20) and general linear model (GLM; n = 20) on each NMDS axis using 3,716 SNP data from odd-year-flowering (OYF) and even-year-flowering (EYF) *Pedicularis hallaisanensis* in the two studied microhabitats (MH-1 and MH-2).

MH-1 than in MH-2 (Figure 3B), the multivariate dispersion conversely became larger in MH-2 regardless of the similar genetic diversity indices among the four combinations of OYF and EYF in MH-1 and MH-2 (Supplementary Table S2).

3.4 Genetic clustering

The silhouette width approach suggested that the optimum K number was four, followed by three and two (Figure 4A). Neighbor-joining phylogenetic tree and k-means clustering revealed that the 20 genotypes of *P. hallaisanensis* were divided by microhabitat (MH-1 and MH-2) at $K = 2$. At $K = 3$, the genotypes in MH-2 were subdivided by flowering group (OYF and EYF) (Figure 4B). Only one of the genotypes of OYF in MH-2 was separated as an additional cluster at $K = 4$, while the subdivisions between OYF and EYF in MH-1 remained relatively unclear (Figure 4B). The STRUCTURE analysis exhibited a similar pattern with the neighbor-joining phylogenetic tree, including the differentiation between MH-1 and MH-2 at $K = 2$ (Figure 4C), and a clearer subdivision by the flowering group in MH-2 than in MH-1 at $K = 3$ and 4 (Figure 4C).

4 Discussion

Strict biennial plant species have a distinctive lifecycle compared to annual and facultative biennial plants, characterized

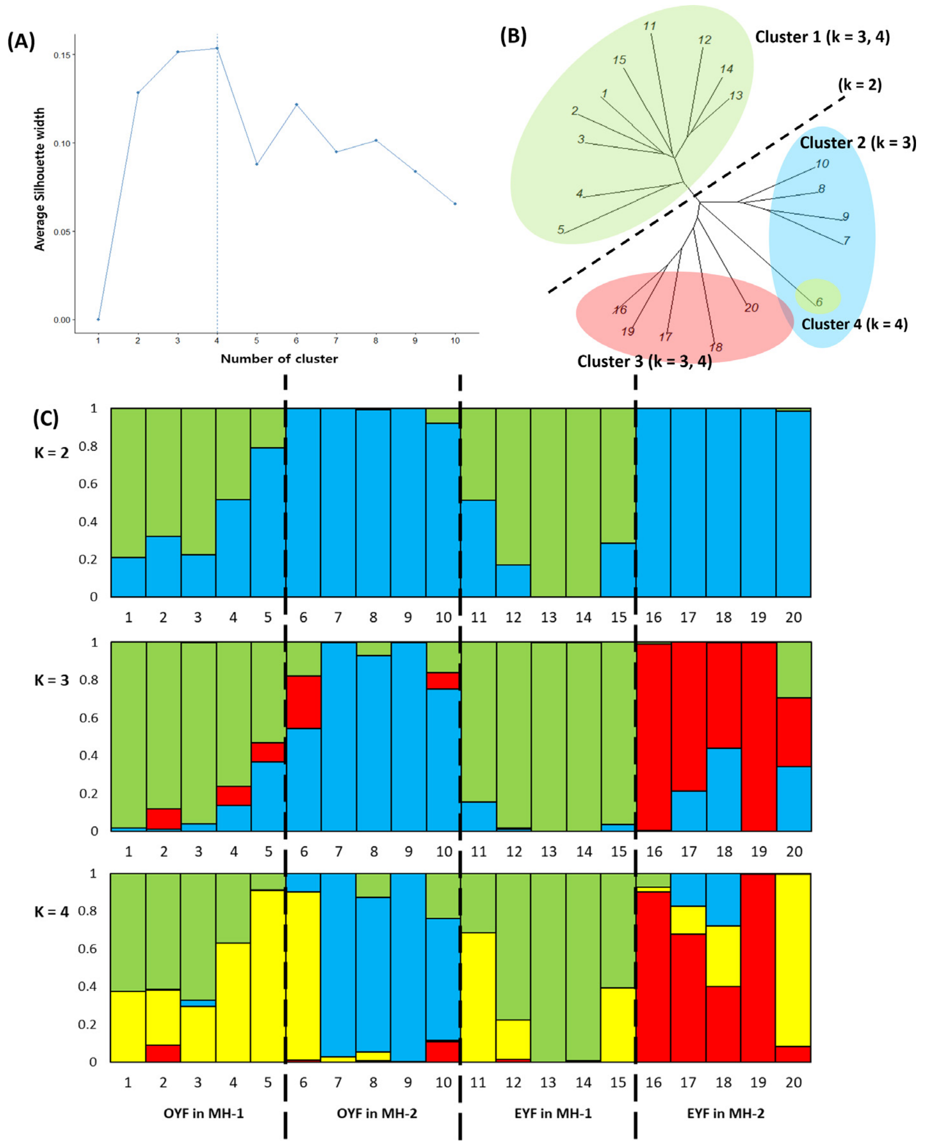


FIGURE 4
(A) Selection of the number of clusters using the silhouette width criterion (k), **(B)** neighbor-joining phylogenetic tree, and **(C)** STRUCTURE analysis results on 3,716 SNP data from odd-year-flowering (OYF; MH-1: 1–5, MH-2: 6–10) and even-year-flowering (EYF; MH-1: 11–15, MH-2: 16–20) *Pedicularis hallaisanensis* **(B)** in the two studied microhabitats (MH-1 and MH-2). The colors of the bar graphs in **(C)** imply the different clusters estimated by STRUCTURE analysis.

by an extended time period for each generation (2 years from seed germination to flowering and death) (Kelly, 1985). All the studied *P. hallaisanensis* required a 2-year period from seed germination to blooming, seed production, and death (Figure 2A), which fits into the biennial lifecycle patterns of other *Pedicularis* species in alpine meadows (Petrů, 2005; Zhang et al., 2011). Our results support several expectations on biennial lifecycle in nature. For example, flowers of *P. hallaisanensis* were detected every year within the study area (Figure 2B), confirming that strict biennial plants can establish two distinct cohorts with different flowering periods (odd or even years), sharing the same microhabitat (Kelly, 1985; Petrů, 2005). Our results are in line with the mathematical studies (Davydova et al., 2003; Kisdi, 2012), which anticipate the coexistence of first-year seedlings and second-year adults of biennial plants within a given time frame and microhabitat. The detected annual oscillations in the number of flowering second-year adults are also consistent with previous findings on other strict biennial plants by Anderson et al. (2021) and Petrů (2005) (Figure 2B).

Temporal genetic differentiation in plant ecology is traditionally considered to be the evolutionary changes in population genetics over long periods or the short-term genetic constitutions caused by natural and anthropogenic disturbances along several generations (Linhart and Grant, 1996; Stadler et al., 2010; Gómez et al., 2018). It may also reflect the genetic variability within a perennial plant population due to the complexity of its age structure and intraseasonal variations in flowering phenology (Reed et al., 2022; Tsuzuki et al., 2022). In the present study, multivariate comparisons using the detected SNPs showed that the flowering group had a significant effect on the genetic structure of *P. hallaisanensis* (Figure 3). Due to the absence of comparable studies on the population genetics of strict biennial plants, it is uncertain whether the observed genetic differentiation is consistent across plant species with similar lifecycle characteristics (Petrů, 2005; Zhang et al., 2011; Anderson et al., 2021; Glav Lundin and Eriksson, 2021). Therefore, further studies should address this uncertainty in other biennial *Pedicularis* species to totally confirm if the detected temporal genetic differentiation can be generalized. Nonetheless, the findings enable us to expect that the strict biennial lifecycle may contribute to temporal genetic differentiation by creating two different cohorts that flower in either odd or even years within a single habitat, in contrast to the interannual maintenance of genetic structure observed in facultative biennial plant species (Valverde et al., 2016).

There was a significant effect of microhabitat, which was even greater than that of the flowering group (Figure 3A). The magnitude of temporal genetic differentiation due to the strict biennial lifecycle also depended on the spatial location of the individuals, as evidenced by the closer genetic distance between OYF and EYF individuals within the same microhabitat, compared to that between OYFs (or EYFs) in different microhabitats (Figures 3B, 4B). These patterns indicate that gene flow between the flowering groups is likely to occur, at least within a single microhabitat (Rusterholz et al., 2023). The detailed mechanisms behind these patterns are currently unknown. However, eventual seed dispersal and dormancy may allow the gene flow between OYF and EYF, potentially confounding the observed temporal genetic differentiation (Valverde et al., 2016; Glav Lundin and Eriksson, 2021). This is despite the fact that most seeds of biennial *Pedicularis* plants are known to germinate in the first spring after they are produced (Kelly, 1985; Petrů, 2005; Kim et al., 2019). The significant difference between microhabitats suggests that gene flow between MH-1 and MH-2 may have been limited, despite the small habitat area, allowing small-scale spatiotemporal changes in genetic structure to become detectable (Chan et al., 2018; Zhang et al., 2021).

It is also notable that flowering group × microhabitat interaction played a marked role in the genetic diversity across the studied *P. hallaisanensis* cohorts (Figure 3A). The genetic variability between OYF and EYF was remarkable in MH-2, in contrast to the relatively unclear genetic differences in MH-1 (Figures 3B, 4). These patterns further induced the lowered multivariate dispersions in MH-1 (Figure 3), reflecting the reduction of genetic diversity in MH-1 (Anderson et al., 2006). These patterns might be related to the previous anthropogenic interventions around the heliport near MH-1 (Supplementary Figure S1), given that extreme disturbance events can reduce the genetic diversity of short-lived herb populations by increasing the self-compatible reproduction rate and confounding the species' lifecycle pattern (Quintana-Ascencio et al., 2011; González et al., 2019; Almeida-Rocha et al., 2020; Anderson et al., 2021). Given the hemiparasitic characteristics of *P. hallaisanensis*, historical disturbances to the surrounding host plants may have also impacted the health and genetic diversity of *P. hallaisanensis*, particularly in MH-1 (Kim et al., 2019). Therefore, the findings allow us to suspect that excessive anthropogenic interventions could reduce the genetic diversity of strict biennial plant populations by hindering genetic differentiation between OYF and EYF cohorts. However, moderate disturbances, such as grazing and mowing, may promote seedling recruitment in biennial *Pedicularis* species by helping to maintain grassland habitats (Petrů, 2005).

In summary, this study is the first to record the strict biennial lifecycle and associated genetic variabilities of the endangered endemic plant species, *P. hallaisanensis*, using GBS and SNPs. Our results showed temporal genetic differentiation between OYF and EYF, which may contribute to the genetic diversity of the remaining *P. hallaisanensis* population. Such genetic differentiation, driven by the biennial lifecycle, varied across microhabitats and may be related to differing levels of historical anthropogenic interventions. In this context, excessively strong anthropogenic interventions in the *P. hallaisanensis* habitat should be avoided to preserve the genetic diversity of the wild population. Instead, moderate management practices like mowing and grazing can help protect mountain grassland habitats from the invasion of competitive shrubs and trees (Petrů, 2005; Kim et al., 2018). Moreover, future conservation programs should collect original wild breeds from both OYF and EYF to ensure that genetic diversity in the wild population is passed on to artificially propagated *P. hallaisanensis*. The detected biennial lifecycle is also remarkable for *ex situ* conservation, highlighting the necessity of 2-year cultivation cycles to successfully propagate collected *P. hallaisanensis* (Kim et al., 2019).

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: https://www.ncbi.nlm.nih.gov/, PRJNA1136402.

Author contributions

SK: Conceptualization, Data curation, Formal analysis, Investigation, Writing – original draft. B-DL: Conceptualization, Investigation, Methodology, Writing – review & editing. CL: Conceptualization, Investigation, Methodology, Writing – review & editing. H-JP: Investigation, Resources, Writing – review & editing. JH: Investigation, Resources, Writing – review & editing. HP: Investigation, Resources, Writing – review & editing. Y-JK: Investigation, Resources, Writing – review & editing. DJ: Investigation, Resources, Writing – review & editing. Y-JY: Project administration, Supervision, Writing – review & editing.

Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article. This study was supported by the National Institute of Ecology (NIE-B-2024-49) of South Korea.

Acknowledgments

The authors thank the technicians of SEEDERS Inc. for their significant contributions to the draft genome and SNP experiments.

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

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpls.2024.1468395/full#supplementary-material

References

Almeida-Rocha, J. M., Soares, L. A. S. S., Andrade, E. R., Gaiotto, F. A., and Cazetta, E. (2020). The impact of anthropogenic disturbances on the genetic diversity of terrestrial species: A global meta-analysis. *Mol. Ecol.* 29, 4812–4822. doi: 10.1111/mec.15688

Anderson, M., Ellingsen, K. E., and AcArdle, B. H. (2006). Multivariate dispersion as a measure of beta diversity. *Ecol. Lett.* 9, 683–693. doi: 10.1111/j.1461-0248.2006.00926.x

Anderson, R. C., Anderson, M. R., Bauer, J. T., Loebach, C., Mullarkey, A., and Engelhardt, M. (2021). Response of the invasive *Alliaria petiolata* to extreme temperatures and drought. *Ecosphere* 12, e03510. doi: 10.1002/ecs2.3510

Aronne, G. (2017). Identification of bottlenecks in the plant life cycle for sustainable conservation of rare and endangered species. *Front. Ecol. Evol.* 5, 76. doi: 10.3389/fevo.2017.00076

Batool, F., and Henning, C. (2021). Clustering with the average Silhouette width. *Comput. Stat. Data Anal.* 158, 107190. doi: 10.1016/j.csda.2021.107190

Bolger, A. M., Lohse, M., and Usadel, B. (2014). Trimmomatic: A flexible trimmer for Illumina sequence data. *Bioinformatics* 30, 2114–2120. doi: 10.1093/bioinformatics/btu170

Bradshaw, M. D. (1978). Plant population studies and their relevance to nature conservation. *Biol. Conserv.* 14, 223–242. doi: 10.1016/0006-3207(78)90012-5

Cai, C., Xiao, J., Ci, X., Conran, J. G., and Li, J. (2021). Genetic diversity of *Horsfieldia tetratepala* (Myristicaceae), an endangered plant species with extremely small populations to China: Implications for its conservation. *Plant Syst. Evol.* 307, 50. doi: 10.1007/s00606-021-01774-z

Chan, Y. M., Tnah, L. H., Lee, S. L., Bhassu, S., Lee, C. T., and Chua, L. W. L. (2018). Limited dispersal and geographic barriers cause population differentiation and structuring in *Begonia maxwelliana* at both large and small scales. *Plant Ecol. Divers.* 11, 69–83. doi: 10.1080/17550874.2018.1471625

Cho, W.-B., and Choi, B.-H. (2011). Taxonomic position of *Pedicularis hallaisanensis* Hurusawa, an endemic plant of Mt. Halla. *Korean J. Plant Taxon.* 41, 130–137. doi: 10.11110/kjpt.2011.41.2.130

Cho, W.-B., Lee, D.-H., Choi, I.-S., and Lee, J.-H. (2018). The complete chloroplast genome of hemi-parasitic *Pedicularis hallaisanensis* (Orobanchaceae). *Mitochondrial DNA Part B: Resour.* 3, 235–236. doi: 10.1080/23802359.2018.1437820

Chung, G. Y., Jang, H. D., Chang, K. S., Choi, H. J., Kim, Y.-S., Kim, H.-J., et al. (2023). A checklist of endemic plants on the Korean Peninsula II. *Korean J. Plant Taxon.* 53, 79–101. doi: 10.11110/kjpt.2023.53.2.79

Davydova, N. V., Diekmann, O., and van Gils, S. A. (2003). Year class coexistence or competitive exclusion for strict biennials? *J. Math. Biol.* 46, 95–131. doi: 10.1007/s00285-002-0167-5

De Mendiburu, F., and Simon, R. (2015). Agricolae - ten years of an open source statistical tool for experiments in breeding, agriculture and biology. *PeerJ Preprints. Peerj.preprints.* 1404v1. doi: 10.7287/peerj.preprints.1404v1

Elshire, R. J., Glaubitz, J. C., Sun, Q., Poland, J. A., Kawamoto, K., Buckler, E. S., et al. (2011). A robust, simple gynotyping-by-sequencing (GBS) approach for high diversity species. *PloS One* 6, e19379. doi: 10.1371/journal.pone.0019379

Friedman, J. (2020). The evolution of annual and perennial plant life histories: Ecological correlates and genetic mechanisms. *Annu. Rev. Ecol. Evol. Syst.* 51, 461–481. doi: 10.1146/annurev-ecolsys-110218-024638

Glav Lundin, L., and Eriksson, O. (2021). The decline of *Gentianella campestris*: Three decades of population development of an endangered grassland plant in Sweden. *Nord. J. Bot.* 39, e03007. doi: 10.1111/njb.03007

Gómez, R., Méndez-Vigo, B., Marcer, A., Alonso-Blanco, C., and Picó, F. X. (2018). Quantifying temporal change in plant population attributes: Insights from a resurrection approach. *AOB Plants* 10, ply063. doi: 10.1093/aobpla/ply063

González, A. V., Gómez-Silva, V., Ramírez, M. J., and Fontúrbel, F. E. (2019). Meta-analysis of the differential effects of habitat fragmentation and degradation on plant genetic diversity. *Conserv. Biol.* 34, 711–720. doi: 10.1111/cobi.13422

Granto, I. S. C., Galli, G., De Oliveira Couto, E. G., E Souza, M. B., Mendonca, L. F., and Fritsche-Neto, R. (2018). snpReady: A tool to assist breeders in genomic analysis. *Mol. Breed.* 38, 102. doi: 10.1007/s11032-018-0844-8

Han, S., Leem, H., Jang, H.-D., Kim, Y.-Y., and So, S. (2022). Floristic study of Gayasan National Park in Korea. *Korean J. Plant Res.* 35, 248–288. doi: 10.7732/kjpr.2022.35.2.248

Kassambara, A., and Mundt, F. (2016). *Package 'factoextra.' Package, version 1.0.3.*

Kelly, D. (1985). On strict and facultative biennials. *Oecologia* 67, 292–294. doi: 10.1007/BF00384302

Kenrick, P. (2017). Changing expressions: A hypothesis for the origin of the vascular plant life cycle. *Phil. Trans. R. Soc B* 373, 20170149. doi: 10.1098/rstb.2017.0149

Kim, L.-K., Choi, S.-D., Choo, G.-C., Hwang, B.-Y., Gang, G.-H., So, S.-K., et al. (2018). Environmental characteristics and floristic study of endangered *Pedicularis hallaisanensis* habitats. *J. Agricul. Life Sci.* 52, 163–173. doi: 10.14397/jals.

Kim, L.-K., Park, E.-H., Gang, G., Hwang, B.-Y., Jung, H.-J., Kim, M.-Y., et al. (2019). Form and embryonic characteristics of *Pedicularis hallaisanensis* seeds as endangered wild species II-class using host plant. *J. Korean Soc For. Sci.* 108, 290–295. doi: 10.14578/jkfs.2019.108.3.290

Kisdi, É. (2012). Year-class coexistence in biennial plants. *Theor. Popul. Biol.* 82, 18–21. doi: 10.1016/j.tpb.2012.03.003

Li, H. (2013). Aligning sequence reads, clone sequences and assembly contigs with BWA-MEM. *Priprint at arXiv. arXiv* 1303, 3997v2. doi: 10.48550/arXiv.1303.3997

Li, H., Handsaker, B., Wysoker, A., Fennell, T., Ruan, J., Homer, N., et al. (2009). The sequence alignment/map format and SAMtools. *Bioinformatics* 25, 2078–2079. doi: 10.1093/bioinformatics/btp352

Linhart, Y. B., and Grant, M. C. (1996). Evolutionary significance of local genetic differentiation in plants. *Annu. Rev. Syst.* 27, 237–277. doi: 10.1146/annurev.ecolsys.27.1.237

Luo, R., Liu, B., Xie, Y., Li, Z., Huang, W., Yuan, J., et al. (2012). SOAPdenovo2: An empirically improved memory-efficient short-read *de novo* assembler. *GigaScience* 1, 18. doi: 10.1186/2047-217X-1-18

Niklas, K. J., and Kutschera, U. (2010). The evolution of the land plant life cycle. *New Phytol.* 185, 27–41. doi: 10.1111/j.1469-8137.2009.03054.x

Oh, J.-E., Kim, J.-E., Kim, J., Lee, M.-H., Lee, K., Kim, T.-H., et al. (2023). Development of an SNP marker set for marker-assisted backcrossing using genotyping-by-sequencing in tetraploid perilla. *Mol. Genet. Genomics* 298, 1435–1447. doi: 10.1007/s00438-023-02066-6

Oksanen, J., Simpson, G. L., Blanchet, F. G., Kindt, R., Legendre, P., Minchin, P. R., et al. (2024). *Package 'vegan.' Community ecology package, version 2.6-6.*

Paradis, E., Claude, J., and Strimmer, K. (2004). APE: Analysis of phylogenetics and evolution in R language. *Bioinformatics* 20, 289–290. doi: 10.1093/bioinformatics/btg412

Petrů, M. (2005). Year-to-year oscillations in demography of the strictly biennial *Pedicularis sylvatica* and effects of experimental disturbance. *Plant Ecol.* 181, 289–298. doi: 10.1007/s11258-005-7223-3

Pritchard, J. K., Stephens, M., and Donnelly, P. (2000). Inference of population structure using multilocus genotype data. *Genetics* 155, 945–959. doi: 10.1093/genetics/155.2.945

Quintana-Asencio, P. F., Menges, E. S., Weekley, C. W., Kelrick, M. I., and Pace-Aldana, B. (2011). Biennial cycling caused by demographic delays in a fire-adapted annual plant. *Popul. Ecol.* 53, 131–142. doi: 10.1007/s10144-010-0228-3

R Core Team. (2023). *R: A language and environment for statistical computing.* Vienna, Austria: R Foundation for Statistical Computing. Available online at: https://www.R-project.org/.

Reed, W. J., Ison, J. L., Waananen, A., Shaw, F. H., Wagenius, S., and Shaw, R. G. (2022). Genetic variation in reproductive timing in a long-lived herbaceous perennial. *Am. J. Bot.* 109, 1861–1874. doi: 10.1002/ajb2.16072

Reisch, C., and Hoiß, B. (2019). Genetic variation of Gentianella campestris ssp. *Campestris* in the Northern Alps: How important are population size and isolation. *Alp. Bot.* 129, 11–20. doi: 10.1007/s00035-019-00216-4

Rusterholz, H.-P., Ursenbacher, S., Weibel, U., Coray, A., and Baur, B. (2023). Genetic diversity and population structure derived from body remains of the endangered flightless longhorn beetle *Iberodorcadion fuliginator* in grassland fragments in Central Europe. *Diversity* 15, 16. doi: 10.3390/d15010016

Stadler, K., Koch, M., Bernherdt, K.-G., and Greimler, J. (2010). Spatial arrangement and genetic structure in *Gentianella aspera* in a regional, local, and temporal context. *Plant Syst. Evol.* 286, 7–19. doi: 10.1007/s00606-010-0274-5

Ter Bort, S. J., Janse, A., and Kwak, M. M. (1980). Life cycle variation in *Pedicularis palustris* L. (Scrophulariaceae). *Acta Bot. Neerl.* 29, 397–405. doi: 10.1111/plb.1980.29.issue-5-6

Tsuzuki, Y., Takada, T., and Ohara, M. (2022). Modeling temporal dynamics of genetic diversity in stage-structured plant populations with reference to demographic genetic structure. *Theor. Popul. Biol.* 148, 76–85. doi: 10.1016/j.tpb.2022.11.001

Valverde, J., Gómez, J. M., García, C., Sharbel, T. F., Jiménez, M. N., and Perectti, F. (2016). Inter-annual maintenance of the fine-scale genetic structure in a biennial plant. *Sci. Rep.* 6, 37712. doi: 10.1038/srep37712

Viswanathan, D., and Aarssen, L. W. (2000). Why biennials are so few: Habitat availability and the species pool. *Ecoscience* 7, 461–465. doi: 10.1080/11956860.2000.11682618

Wang, Y., Li, H., Yang, Z., Liu, B., Liu, Y., and Zhong, Y. (2024). Genotyping-by-sequencing study of the genetic diversity and population structure of the endangered plant *Tsoongiodendron odorum* Chun in China. *Forests* 15, 910. doi: 10.3390/f15060910

Zhang, L., Guo, H., Wang, M., and Du, G. (2011). Plasticity of reproductive traits responding to variation in light availability at the rosette stage of the first year in a strict biennial, *Pedicularis torta*, from a field on the Qinghai-Tibet Plateau, China. *Plant Species Biol.* 26, 105–110. doi: 10.1111/j.1442-1984.2010.00305.x

Zhang, R., Xu, B., Li, J., Zhao, Z., Han, J., Lei, Y., et al. (2020). Transit from autotrophism to heterotrophism: Sequence variation and evolution of chloroplast genomes in Orobanchaceae species. *Front. Genet.* 11, 542017. doi: 10.3389/fgene.2020.542017

Zhang, X., Yang, L., Liu, Y.-H., Zhou, X.-L., Zhang, L.-Q., Wang, Y.-H., et al. (2021). Genetic diversity, genetic structure, and demographic history of *Cinnamomum chago*, a plant species with extremely small populations in China. *Glob. Ecol. Conserv.* 31, e01808. doi: 10.1016/j.gecco.2021.e01808

frontiers | Frontiers in Plant Science

TYPE Original Research
PUBLISHED 14 November 2024
DOI 10.3389/fpls.2024.1474272

OPEN ACCESS

EDITED BY
Praveen Dhyani,
University of Koblenz, Germany

REVIEWED BY
Dharam C. Attri,
Islamic University of Science and Technology, India
Hao Shen,
Beijing Forestry University, China

*CORRESPONDENCE
Huakun Zhou
hkzhou@nwipb.cas.cn

RECEIVED 01 August 2024
ACCEPTED 23 October 2024
PUBLISHED 14 November 2024

CITATION
Wei J, Zhang Z, Ma L, Hu X, Ade H, Su H, Shi Z, Li H and Zhou H (2024) Effects of short- and long-term plant functional group removal on alpine meadow community niche. *Front. Plant Sci.* 15:1474272. doi: 10.3389/fpls.2024.1474272

Effects of short- and long-term plant functional group removal on alpine meadow community niche

Jingjing Wei[1], Zhonghua Zhang[1], Li Ma[1], Xue Hu[1], Haze Ade[1,2], Hongye Su[1,2], Zhengchen Shi[1,2], Honglin Li[1] and Huakun Zhou[1]*

[1]Qinghai Provincial Key Laboratory of Restoration Ecology in Cold Regions, Northwest Institute of Plateau Biology, Chinese Academy of Sciences, Xining, China, [2]College of Life Sciences, University of Chinese Academy of Sciences, Beijing, China

The rapid loss of global biodiversity affects the creation and maintenance of community biodiversity and ecosystem structure and function. Thus, it is insufficient to focus solely on the effects of biodiversity loss on community biodiversity without also considering other impacts such as community assembly, niches, interspecific relationships, community stability, and biodiversity–ecosystem function. In this study, a 3- and 10-year biodiversity manipulation experiment was conducted in an alpine meadow to examine the effects of the individual plant functional group (PFG) removal on the niches of community dominant species by removal of Gramineae, Cyperaceae, legumes, and other forbs. The results indicated that PFG removal led to variation in community niches. The long-term PFG removal led to a gradual decline in the number of Gramineae and Cyperaceae species in the community. Over time, the niche widths of dominant Gramineae and Cyperaceae species gradually narrowed, and the degree of niche overlapping decreased. The number of positively and negatively associated species tended to decrease and increase, respectively. Reduced species diversity led to significant differences in the niches of the remaining species within the community. Thus, species niche differences, resulting from variation in resource allocation, commonly determined the dynamic construction of species composition within the community.

KEYWORDS

biodiversity loss, niche, plant functional group removal, interspecific association, community stability

1 Introduction

Species extinction and community simplification threaten ecosystem productivity and services, making it crucial to understand species diversity patterns and processes for effective ecosystem management and conservation (Liang et al., 2015). Plant diversity loss can alter community vegetation characteristics, with responses generally varying based on plant species or functional groups (PFGs) (Chen et al., 2016; Zhang et al., 2017; Fanin et al., 2019). Following disturbances, secondary forbs often replace primary dominant species as new constructive elements (Wang et al., 2009; Li et al., 2013; Zhou et al., 2021). Experiments removing certain PFGs indicated that the target species loss differentially affects aboveground and belowground components in grassland ecosystems, including plant biomass, species richness, soil microorganisms, and soil animals. Studies have shown that the diversity of structural composition within plant communities is vital, as grasslands with lower diversity exhibited reduced soil nutrient retention, making them more vulnerable to changes from species loss (Chen et al., 2016, 2022). The impact of species loss on soil ecosystem functioning varies geographically, although similar contexts can differ significantly (Fanin et al., 2013, 2019). These findings indicated that belowground functions may be more sensitive to environmental changes than plant productivity (Fanin et al., 2019). Species interrelationships and ecosystem function differences are influenced by species selectivity and complementarity (Holt, 2009; Godoy et al., 2020).

A niche represents the minimal needs met by a species and its average impact on environmental conditions. This core theory explains population positions within a community's ecological space (i.e., resource use) and the dynamics of interspecific coexistence and competition (Loke and Chisholm, 2023). The prevailing hypothesis suggested that niche distinctions foster complementary effects that promote species coexistence when intraspecific competition outstrips interspecific competition (Tilman et al., 1997; Loreau and Hector, 2001; Fox, 2003). Generally, species competition or adaptation drives selection (Chesson, 2000). Community complementarity and niche divergence may arise from interspecific differences in resource use (Barry et al., 2019). Key factors facilitating these differences include selection and complementary effects, influenced by the relative abundance and density of populations, with density effects also shaped by niche differences (Maherali and Klironomos, 2007; Levine and HilleRisLambers, 2009; Godoy et al., 2014). Complementary effects occur when interspecific competition is diminished compared to intraspecific competition, illustrating niche differences (Carroll et al., 2011; Turnbull et al., 2013). When low-productivity species enhance ecosystem functions, negative selection may arise because of reduced interspecific competition, resulting in niche variances (Godoy et al., 2020). Prior studies suggested that greater niche distinctions can foster complementary effects, lessen competition, and increase community homogeneity and stability (Loke and Chisholm, 2023). Community stabilization, which promotes niche differentiation, has been a focal point of coexistence research (Tan et al., 2013). Godoy et al. (2020) found that species exhibit similar sensitivity to competition when balancing intraspecific and interspecific responses. Thus, niche differentiation may further contribute to community equilibrium, with related research indicating that niche divergence enhances resource utilization, leading to increased productivity (Cardinale et al., 2007; Weih et al., 2021).

During short-term species loss, reduced energy in the community may primarily drive changes in plant community structure, particularly leading to decreased productivity (Ward et al., 2009). A decline in community biomass over a short period can result in litter decomposition, impacting soil and species biodiversity (Chen et al., 2016). In contrast, during long-term species loss, niche complementarity likely drives changes in community structure, indicating that species-rich communities more effectively access and utilize limited resources due to their diverse ecological traits. Such ecosystems are considered functionally complete because species complement one another, optimizing resource use (Godoy et al., 2020). It is generally assumed that this complementarity arises from niche differences, which facilitate species coexistence (Buche et al., 2022).

Niche width and niche overlap are key characteristics of species niches (Pielou, 1972). Niche width quantitatively represents the resources available to species, whereas niche overlap refers to competition among different populations for the same resources (Pielou, 1972). Interspecific association denotes the spatial distribution correlations among species, with positive and negative associations indicating their interdependence (Shao and Zhang, 2021). Understanding niche changes is crucial for clarifying the spatiotemporal dynamics of species in communities and competitive exclusion (Lambers et al., 2022; Loke and Chisholm, 2023). Additionally, studying interspecific associations helps to determine the principles governing plant community niches and development, providing a theoretical foundation for species restoration and reconstruction (Huan et al., 2019).

Alpine grasslands are fragile and sensitive to climate change and human activities, making it crucial to understand how species diversity loss affects community stability. We examined the short-term and long-term impacts of specific PFG removal on the niches of remaining species and how species interrelationships change after removal. We separately analyzed data for treatment periods of 3 years (2012–2015) and 10 years (2012–2022) to compare short- and long-term effects of PFG removal. Our objectives were to determine (1) how PFG removal influenced the niches of dominant species and (2) how it affected the trends of community constructive species. Our study aims to deepen understanding of the impact of biodiversity loss on the stability of terrestrial ecosystems.

2 Materials and methods

2.1 Study area

The research site was located at the Haibei Alpine Meadow Ecosystem Research Station (37°29′–37°45′N, 101°12′–101°23′E; 3,200–3,600 m a.s.l.), Menyuan County, Qinghai Province, China (Figure 1). The station is characterized by a typical continental plateau climate, with cool, short summers and long, cold winters, a mean temperature of −1.7°C, and a mean annual precipitation of

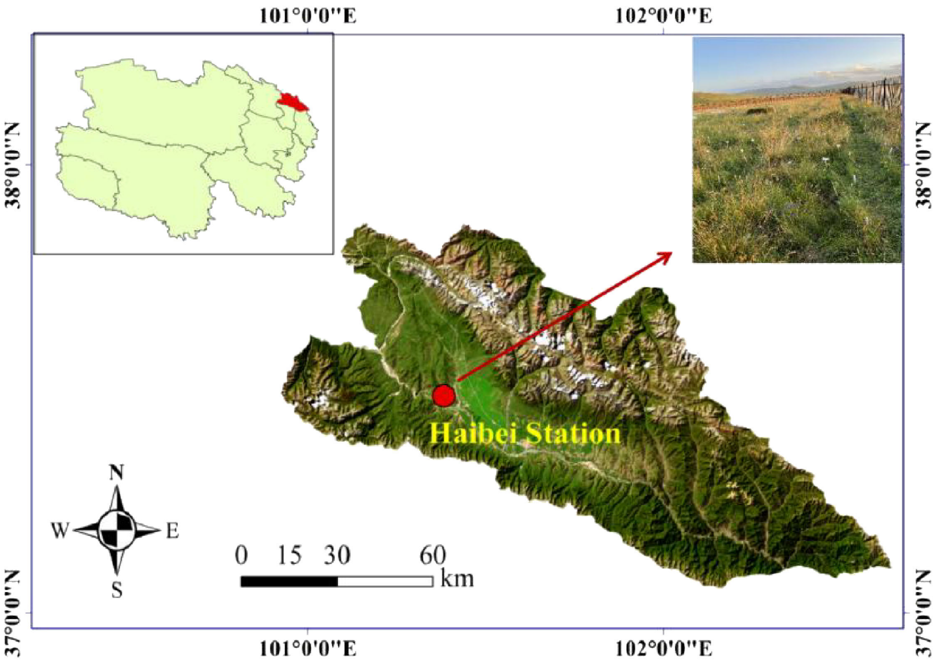


FIGURE 1
Study area location and site characteristics (satellite layer data source: http://www.locaspace.cn/).

580 mm (Chen et al., 2022). The local soil is gelic cambisol, which has relatively homogeneous physiochemical properties. The main soil body type is alpine meadow soil, which is a shallow layer of young, weakly alkaline soil formed through simple soil formation processes that is rich in stored nutrients but poor in fast-acting nutrients (Zhang et al., 2017).

2.2 PFG removal experiment

The experiment was established on a flat and undisturbed site in 2012. For conservation purposes and to enable regular experimentation, the site was fenced. The targeted species were divided into four PFGs based on ecophysiological characteristics potentially associated with response variables, such as biomass production, resource use patterns, and nitrogen (N) fixation ability (Reich et al., 2001; Gross et al., 2007; McLaren and Turkington, 2010). Cyperaceae are the dominant species in the alpine meadow. Gramineae species exhibit a resource conservation strategy due to their high C:N ratio and leaf dry matter content (Chen et al., 2022). Legumes and other forbs promote species and functional diversity, offering different plant resource utilization pathways that enhance nutrient use and release, leading to higher N and P content in alpine plant communities (Chen et al., 2022). By contrast, species and functional diversity can be fostered by the growth of forbs and legumes, which provide access strategies to improve species resource use and release, leading to higher foliar N and P contents (Chen et al., 2022). In the study area, the species are typical of subalpine meadows, dominated by Cyperaceae, with a diverse forb community. Other PFGs such as Gramineae and legume species are rare, with about 10% Gramineae and 8% of the total coverage. The most abundant plant species are *Carex alatauensis* and *Carex parva* (Cyperaceae), accounting for 40% of the total coverage. All the rest are forbs.

The establishment of the Meadow Removal Experiment (IMGRE) at the Haibei Station was described by Chen et al. (2022). The experiment followed a completely randomized design. Five treatments were established, which include the control (CK), Gramineae removal (RG), Cyperaceae removal (RC), legumes removal (RL), and other forbs removal (RF). Each of the five treatment plots (1 m × 0.75 m) was replicated five times with a total of 25 plots spaced 1 m apart. To minimize physical disturbance to soil, plants were removed by cutting aboveground sections and tiller nodes to 3 cm soil depth, twice per month from May to August (Naeem, 2002; Chen et al., 2016, 2022). At the peak of vegetative biomass (August), we recorded the species composition, frequency, cover, height, and soil nutrients within each plot in 2015 and 2022 to examine short- and long-term effects, respectively. Plant species are identified and classified with reference to “Flora Reipublieae Popularis Sinicae” (http://www.iplant.cn/frps).

2.3 Statistical analysis

The importance value is a useful measure for species location in a community and is used to indicate the population dominance. The dominant species in the community with importance values more than 0.5% were selected for niche analysis (Camargo et al., 2021). The importance value is calculated as follows (Tables 1, 2):

TABLE 1 The dominant species Levins niche width for PFGs treatments in 2015 and 2022.

PFGs	Dominant species	Treatments									
		2015					2022				
		CK	RG	RC	RL	RF	CK	RG	RC	RL	RF
Gramineae	*Stipa aliena*	4.99	/	2.99	4.98	3.99	/	/	/	/	/
	Deschampsia cespitosa	4.98	/	3.02	4.99	3.98	/	/	/	/	/
	Elymus nutans	4.99	/	2.99	4.93	3.98	/	/	/	/	/
	Poa araratica	5.02	/	2.99	4.89	3.99	1.00	/	1.56	1.00	1.93
	Helictotrichon tibeticum	5.00	/	2.98	5.00	4.00	1.99	/	2.90	1.99	2.25
Cyperaceae	*Trichophorum distigmaticum*	3.99	2.99	/	4.95	3.94	/	/	/	/	/
	Carex parva	4.99	4.91	/	4.98	3.96	4.02	3.55	/	2.90	2.82
	Carex alatauensis	3.98	4.99	/	4.90	3.98	2.85	1.76	/	2.65	3.61
Legumes	*Oxytropis arctica*	2.99	4.62	2.99	/	3.97	2.43	2.54	4.29	/	4.68
	Medicago ruthenica	2.97	3.98	2.00	/	3.94	4.79	3.81	3.73	/	3.78
	Gueldenstaedtia verna	4.89	4.77	3.00	/	2.00	/	/	/	/	/
	Tibetia himalaica	/	/	/	/	/	2.93	1.00	1.38	/	1.91
Forbs	*Saussurea pulchra*	3.00	3.45	2.99	3.99	/	3.57	2.88	4.41	3.24	/
	Gentiana aristata	4.97	2.75	3.00	3.98	/	4.69	1.99	1.41	1.84	/
	Gentiana straminea	4.97	4.89	1.99	3.96	/	3.94	2.35	3.69	3.37	/
	Thalictrum alpinum	3.96	3.82	3.00	4.99	/	4.71	3.95	3.96	3.56	/
	Ranunculus pulchellus	2.99	4.96	1.99	3.93	/	2.71	2.83	4.55	4.57	/
	Potentilla nivea	4.00	4.07	1.98	3.90	/	3.33	3.42	4.84	3.07	/
	Argentina anserina	1.98	3.39	1.00	4.00	/	/	/	/	/	/
	Lancea tibetica	4.00	3.99	1.00	3.99	/	4.37	3.13	3.85	2.93	/
	Sibbaldianthe bifurca	1.99	2.73	1.99	1.00	/	4.72	2.97	2.80	2.00	/
	Taraxacum mongolicum	/	/	/	/	/	2.87	2.46	1.35	4.22	/
	Anemone obtusiloba	/	/	/	/	/	3.46	2.38	1.00	1.00	/
	Potentilla multifida	/	/	/	/	/	3.43	3.35	3.53	3.61	/

$$\text{Importance value} = (\text{relative density} + \text{relative cover} + \text{relative frequency})/3$$

The Levins index (B_L) was used to determine niche width (Lu et al., 2020), and the Pianka index (O_{ik}) was used to calculate niche overlap (Schoener, 1974). These indices were calculated as follows:

$$B_L = 1 \Big/ \sum_{j=1}^{r} P_{ij}^2$$

$$O_{ik} = \frac{\sum_{j=1}^{r} p_{ij} p_{kj}}{\sqrt{\sum_{j=1}^{r} p_{ij}{}^2 \sum_{j=1}^{r} p_{kj}{}^2}}$$

where P_{ij} and P_{kj} are the importance values of species i and species k within the j^{th} plot, respectively; and O_{ik} is the niche overlap index between species i and k, ranging from 0 to 1.

The variance ratio (VR) test was used to determine the overall interspecific association at the community level. VR indicates whether there is a significant relationship among multiple species in the selected area, and its significance was tested using the W statistic (Schluter, 1984). VR was calculated as follows:

$$VR = \frac{\frac{1}{N} \sum_{i=1}^{N} (T_i - t)^2}{\sum_{j=1}^{S} (1 - P_j)}$$

$$P_j = \frac{n_j}{N}$$

where N is the total number of plots in the community, T_i is the total number of target species in plot i, t is the mean number of species observed in all plots, S represents the total number of species in the community, P_j represents the frequency of species j, and n_j represents the total number of plots occupied by species j (Schluter, 1984).

TABLE 2 Interspecific association of dominant species for PFGs treatments in 2015 and 2022.

Treatments	*VR*		*W*		$[\chi^2_{0.95}, \chi^2_{0.05}]$		*P*-values	
	2015	2022	2015	2022	2015	2022	2015	2022
CK	0.717	0.419	3.584	2.096	[9.39, 28.87]	[8.67, 27.59]	$P < 0.05$	$P < 0.05$
RG	1.723	0.432	8.618	2.162	[5.89, 22.36]	[7.26, 25.00]	$P > 0.05$	$P < 0.05$
RC	0.923	0.462	4.617	2.311	[7.96, 26.30]	[7.26, 25.00]	$P < 0.05$	$P < 0.05$
RL	0.389	0.503	1.948	2.514	[7.96, 26.30]	[6.57, 23.68]	$P < 0.05$	$P < 0.05$
RF	0.014	1.437	0.071	7.186	[3.33, 16.92]	[1.64, 12.59]	$P < 0.05$	$P > 0.05$

Under the null hypothesis of independence, the expected value of *VR* is 1; that is, when $VR = 1$, it means that there is no connection between the species. If $VR > 1$, then it means that there is a positive connection between the species. If $VR < 1$, then it means that there is a negative connection between the species. The statistic *W* was used to verify the significant degree of *VR* deviation from 1, $W = VR \times N$. If $W > \chi^2_{0.05}$ (N) or $W < \chi^2_{0.95}$ (N), then it means that the overall connection between the species is significant. On the contrary, the overall connection between the species is not significant (Wang et al., 2018).

Interspecific association has been widely used in interspecific ecological studies to determine the significance of interspecific association by using the χ^2 test, to determine interspecific associations with the Association coefficient (*AC*), and to analyze the interspecific associations strength based on the Ochiai index (*OI*), and this methodology has also been applied to the study of interspecific relationships in grassland plants (Zhang et al., 2018; Liu et al., 2019). Pairwise interspecific association is one of the important quantitative and structural characteristics of plant communities, which is the basis for the formation and evolution of plant community structure (Zhong et al., 2010). *OI* analysis of the strength of connectivity between species pairs (Zhang et al., 2018). The χ^2 value, *AC*, and *OI* were calculated as follows:

$$x^2 = \frac{N(|ab - bc| - 0.5N)^2}{(a+b)(c+d)(a+c)(b+d)}$$

$$\mathrm{AC} = \frac{\mathrm{ad} - \mathrm{bc}}{(\mathrm{a}+\mathrm{b})(\mathrm{b}+\mathrm{d})}\ (\mathrm{ad} > \mathrm{bc})$$

$$AC = \frac{\mathrm{ad} - \mathrm{bc}}{(\mathrm{a}+\mathrm{b})(\mathrm{a}+\mathrm{c})}\ (\mathrm{bc} > \mathrm{ad},\ \mathrm{d} \geq \mathrm{a})$$

$$AC = \frac{\mathrm{ad} - \mathrm{bc}}{(\mathrm{a}+\mathrm{b})(\mathrm{d}+\mathrm{c})}\ (\mathrm{bc} > \mathrm{ad},\ \mathrm{d} > \mathrm{a})$$

$$OI = \frac{a}{\sqrt{(a+b)(a+c)}}$$

where *N* represents the total number of quadrats, α represents the number of quadrats with two species, b and c represent the number of quadrats with only one species, whereas d represents the number of quadrats with neither of the two species. The interspecific association is not significant when $\chi^2 < 3.814$ ($P > 0.05$), at which point it is considered largely independent; when $\chi^2 > 3.814$, at which point it is considered significant ($P < 0.05$); when ad > bc, the interspecific association is considered positive, and vice versa.

All analyses were conducted in the R v4.2.1 statistical platform. The niche width, niche overlap, community-level interspecific association, *AC*, *OI*, and statistical analyses were performed using the "*spaa*" package, and semi-matrix plots were performed using the "*ggplot*" package.

3 Results

3.1 Response of dominant species niche width to PFG removal

Differences in the dominant species niche widths within the same experimental treatments at different time scales (Figures 2, 3; Table 1). In the short term (2015), there were 20 dominant species in the study area, including 5 Gramineae, 3 Cyperaceae, 3 legumes, and 9 other forbs. In the long term (2022), there were 18 dominant species in the study area, including 2 Gramineae, 2 Cyperaceae, 3 legumes, and 11 other forbs. The species with the niche width range in the short-term removal period was *Poa araratica* (5.02) in the control, *Carex alatauensis* (4.99) in the RG treatment, and *Deschampsia cespitosa* (3.02) in the RC treatment. The species with the greatest niche width in the RL treatment was *Helictotrichon tibeticum* (5.00), and, in the RF treatment, it was *Helictotrichon tibeticum* (4.00). In the long-term removal period, the species with the niche width range was *Sibbaldianthe bifurca* (4.72) in the control, *Thalictrum alpinum* (3.95) in the RG treatment, *Potentilla nivea* (4.84) in the RC treatment, *Ranunculus pulchellus* (4.57) in the RL treatment, and *Oxytropis arctica* (4.68) in the RF treatment. The maximum niche wide of the species in each treatment was lower in the short term compared to that in the control, whereas there was no regular change in the long term.

3.2 Response of dominant species niche overlap to PFG removal

Differences in the dominant species niche overlaps after PFG removal (Figure 4A; Supplementary Figure S1). The species pairs with maximum niche overlap during the short-term removal were

85 for the control, 43 species pairs for the RG treatment, 57 species pairs for the RC treatment, 93 species pairs for the RL treatment, and 36 species pairs for the RF treatment. For the long-term removal, the species pairs with maximum niche overlap were 30 for the control, 27 species pairs for the RG treatment, 26 species pairs for the RC treatment, 26 species pairs for the RL treatment, and 3 species pairs for the RF treatment. Overall, the number of species pairs with maximum niche overlap decreased as removal time proceeded.

3.3 Response of dominant species interspecific association to PFG removal

We evaluated overall interspecific associations between species within the community (Table 2). Positive correlations ($VR > 1$) were observed among 14 species pairs in the RG treatment during the short-term time. Similarly, positive correlations ($VR > 1$) were present among seven species pairs in the RF treatment during the long-term time. Conversely, negative correlations were identified among all remaining species pairs in the other treatments ($VR < 1$). The W Statistics showed that the overall associations among dominant species in the treatment with negative correlations among species pairs were all significant.

The number of species pairs showing a positive correlation in the short-term removal time varied among treatments (Table 3; Supplementary Figure S2). There were 89 species pairs (46.84%) in the control, 64 species pairs (60.95%) in the RG treatment, 101 species pairs (74.26%) in the RC treatment, 72 species pairs (52.94%) in the RL treatment, and 25 species pairs (45.45%) in the RF treatment. In the long-term removal time, the number of positively correlated species pairs were 72 (47.06%) in the control, 55 (45.83%) in the RG treatment, 61 (50.83%) in the RC treatment, 57 (54.28%) in the RL treatment, and 12 (57.14%) in the RF treatment. All other species pairs exhibited a negative correlation (Table 3; Supplementary Figure S2). The number of species pairs that were positively correlated across treatments decreased with time of removal compared to the control.

3.4 Response of dominant species association coefficient to PFG removal

In the short-term removal time, the number of species pairs within the maximum AC range among the dominant species in the treatments followed the order of CK (21) > RL (5) > RF (4) = RC (4) > RG (2) (Supplementary Figure S3). In the long-term removal time, the number of species pairs within the maximum AC range of the dominant species in the treatments followed the order of RL (13) = RG (13) > RC (6) > CK (4) > RF (1) (Supplementary Figure S3). Under the highest interspecific association coefficients, the CK and RL treatment had the highest number of species pairs within the community, and the niches between species were more complex.

3.5 Response of dominant species Ochiai index to PFG removal

The number of species pairs present in the conditions with the highest values of interspecific linkage strength varied among treatments (Figure 4B; Supplementary Figure S4). There were 63 species pairs in the control, 4 species pairs in the RG treatment, 18 species pairs in the RC treatment, 47 species pairs in the RL treatment, and 14 species pairs in the RF treatment during the short-term removal time. In the long-term removal time, there are 57 species pairs in the control, 28 species pairs in the RG treatment, 35 species pairs in the RC treatment, 38 species pairs in the RL treatment, and 5 species pairs in the RF treatment.

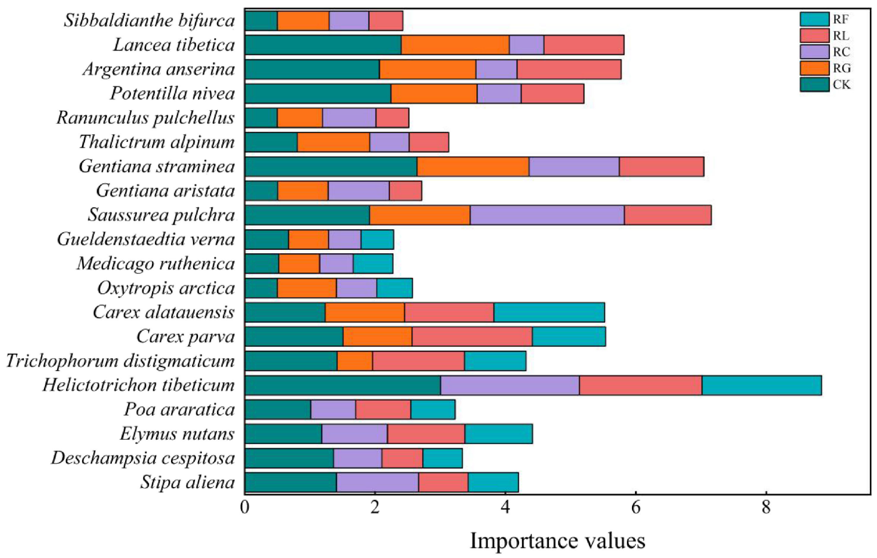


FIGURE 2
Importance values for dominant species for PFGs treatments in 2015.

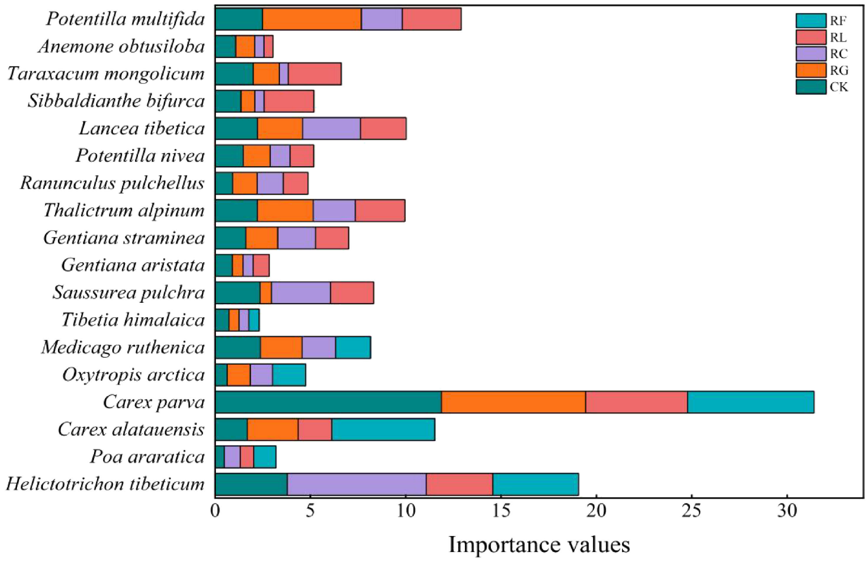


FIGURE 3
Importance values for dominant species for PFGs treatments in 2022.

4 Discussion

4.1 Effects of PFG removal on niche width and niche overlap

Niche width reflects the position and role of a population within a community (Carscadden et al., 2020). In this study, the number of dominant species differed across the experimental treatments, with a decrease in the number of Gramineae and Cyperaceae and an increase in the number of forbs other than legumes. Thus, long-term species diversity loss led to gradual reductions in the niche widths of Gramineae and Cyperaceae, slowly disadvantaging them in terms of resource competition. By contrast, the niche width of other forbs gradually increased, enhancing their competitiveness for resources. Our results also provided strong evidence of alpine meadow degradation. The present findings are consistent with a previous report that the combined dominance ratios of Gramineae, Cyperaceae, and forbs other than legumes are negatively correlated (Jiang et al., 2021). In this study, only when other forbs were removed did the remaining Gramineae and Cyperaceae attain larger niche widths. The niche widths of the remaining functional group treatments gradually shifted toward dominance by other forbs, indicating that these forbs were the most powerful competitors for environmental resources in the alpine meadow plant communities. Kong et al. (2011) indicated that PFG removal resulted in less aboveground biomass to provide the photosynthetic products needed to maintain root biomass, thereby affecting root nutrients; thus, the aboveground traits of forbs generally favor the uptake of more environmental resources, which in turn, can affect the survival of other PFGs to a greater extent. In this manner, the presence of forbs may be among the most important factors affecting the survival of other species. The niche width of the remaining PFGs after species loss varied by time scale; generally, longer intervals resulted in more realistic niche widths, as reflected by compensatory effects in the remaining species. Short-term species culling might have caused our results to be influenced by the compensatory effects of the remaining PFGs; because most of the biomass in alpine meadow is allocated to belowground plant parts, the species remaining after culling may not show significant compensation in the short term (Díaz et al., 2003; Bai et al., 2004), such that long-term monitoring would lead to more accurate results.

Niche overlap is a measure of the degree of species similarity in terms of environmental adaptation or resource use (Pastore et al., 2021). In this study, the niche overlap of the remaining dominant species in the community shifted from high to low, and the number of species pairs with the highest niche overlap shifted from high to low as the monitoring period was extended. Thus, our results indicated that species loss caused the remaining species to be less similar in terms of resource use. Previous studies have shown that species coexistence presupposes niche differentiation and that species with identical niches cannot coexist in the long term (Song, 2017); however, niche overlap does not necessarily lead to competition between species and may be necessary for exploitative competition, as competition is also related to resource amounts and population sizes (Letten et al., 2017). The high degree of niche overlap detected in this study cannot directly confirm intense competition between species but rather indicates high potential for competition.

4.2 Effects of PFG removal on interspecific association

The overall connectivity between community species is closely related to stability (Wittebolle et al., 2009). Based on previous studies,

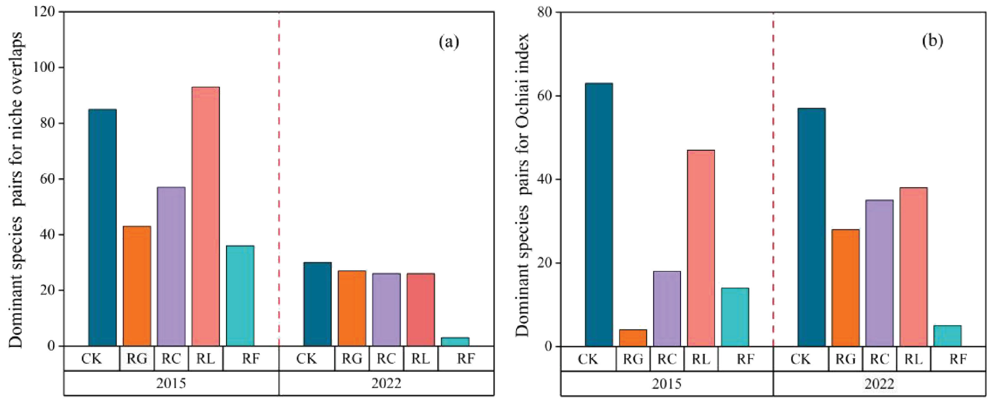


FIGURE 4
The dominant species pairs for niche overlaps **(A)** and Ochiai index **(B)** for PFGs treatments in 2015 and 2022.

during positive community succession, species maximize resource use through competition and cooperation, species composition is gradually fixed, community structure becomes more stable and eventually reaches an apex, and coexisting species tend to be positively associated; however, the association between individual species pairs decreases over the course of succession (Guo et al., 2008; Haugaasen and Peres, 2009). In this study, overall species connectivity remained negative during the 10-year monitoring trial; however, overall species connectivity was positive in the treatment in which forbs other than legumes were completely removed, probably because Gramineae and Cyperaceae are dominant species in alpine meadows and thus determine the development direction of the community to some extent. As such, the community examined in this study may have reached a relatively stable late successional stage.

Interspecific associativity reflects interrelationships between the species comprising a community over a period of time (Ru et al., 2022). It is often assumed that positively associated species pairs have mutually beneficial and complementary relationships, whereas negatively associated species pairs are more likely to experience competition and exclusion (Levine et al., 2017). As time passes, most of the treatment trials showed negative associations, suggesting that species interactions tend to be mainly competitive and exclusionary; however, the treatment in which other forbs were completely removed showed the opposite trend, toward mutualism and complementarity, indicating that species interactions under this treatment were dominant in terms of resource use (Åkesson et al., 2021). Interspecific correlations are based on multidegree data between species pairs and can provide a basis for analyzing species extinction patterns within a community (Norberg et al., 2012). These species interrelationships are consistent with interspecific associations but differ somewhat in terms of sensitivity. Generally, when two species appear or disappear simultaneously in a sample, the association between these species is positive; however, the association could be negative if the number of individuals of one species increases while that of the other species decreases (Liu et al., 2019). In our study, this difference was reflected by the higher proportion of negatively correlated species pairs detected by the Pearson correlation test than by the χ^2 test and AC values in this study.

TABLE 3 Correlated species pairs of dominant species for PFGs treatments in 2015 and 2022.

Treatments	2015		2022	
	Positive	Negative	Positive	Negative
CK	89	101	72	81
RG	64	41	55	65
RC	101	35	61	59
RL	72	64	57	48
RF	25	30	12	9

4.3 Effects of PFG removal on niche construction

It is increasingly recognized that all organisms modify their environments (i.e., niche construction or ecosystem engineering) in response to various environmental disturbances (Odling-Smee et al., 2013). Such changes include effects on the distribution and abundance of organisms, dominant species, control of energy, material flows, ecosystem resilience, and specific nutrient relationships (Chapin et al., 1997). There is evolutionary evidence that, even when key resources are independently renewed or depleted, the effects of niche construction can override selection from external sources, thereby creating new evolutionary trajectories and equilibria, generating and eliminating polymorphisms, and producing time-lagged selection as well as other unusual dynamic responses (Laland et al., 1999). The present study revealed that long-term species loss resulted in larger niche widths for forbs other than legumes and smaller niche widths for dominant species in the community. Furthermore, niche overlapping pairs among dominant species in the community tended to decrease with removal treatment time, and the number of related species pairs decreased, indicating that competition intensity between species within the community tended to weaken. Interestingly, competitive intensity between species became stronger or weaker more rapidly when forbs other than legumes were removed from the community. The removal of different PFGs in this study was considered to reflect the process of reconstructing the niche of each species within the community under temporal turnover, including resource acquisition, resource use within changing communities, and interactions between species. Further investigation is required to explore how niche reconstruction among species in communities occurs over time, the key channels through which such reconstruction occurs, and changing patterns in the construction of linkages among species.

The niche overlap pairs of dominant species in each treatment were fewer compared to the control, and the niche overlap value in the control changed with the delay. The community's species relationships exhibited a decline in positively correlated species pairs over time, with both the number of these pairs and the strength of associations in each treatment being lower than those in the control. Species interactions can both mitigate and exacerbate the effects of climate change on species and interact with other eco-evolutionary processes (Post, 2013). For example, species interactions can affect the evolutionary response to altered environmental conditions; dispersal can release species from negative interactions or increase them through migration or through invasions (Mazancourt et al., 2008; Walther et al., 2009; Van Eldijk et al., 2020). However, this phenomenon is not certainly caused by changes in the environment but is closely related to the number of potential interspecific competitors in the community or the so-called "diffuse competition," and it has been argued that the maximum tolerable overlap should be inversely proportional to the intensity of the competition, which is really the main content of the niche overlap hypothesis (Pianka, 1974). More importantly, most of the studies have been conducted under fenced conditions, and the results of the experiments have limitations. However, an increasing number of studies pointed out that the species niches are affected by space and time and that quantifying species niches is needed to explore the relationship between species distributions and environment, as well as to predict the potential risk of species invasions and future species extinction rates (Jackson et al., 2009; Liu et al., 2020). In the future, we need to further explore the effects of plant diversity loss on species niches and ecosystem functioning at broader spatial scales and longer time scales.

5 Conclusions

Our results indicated that removal of different PFGs affected plant community composition and niche dynamics. After 3 and 10 years of experiments, the species composition of the community gradually shifted from being dominated by Gramineae and Cyperaceae to other forbs. Compared to the short term, long-term PFG removal led to narrower niche widths for Gramineae and Cyperaceae, reduced community niche overlap, and a trend toward fewer positively related species. In the case of environmental resource use, the decreased niche overlap indicates interspecific competition within the community, albeit decreasing. In summary, reduced species diversity resulted in significant niche differences among the remaining species in the community, shifting resource allocation from advantaged to disadvantaged species. Therefore, we emphasize the importance of ongoing long-term PFG removal experiments and studies that investigate a wider range of domains across broader spatial and temporal scales to achieve a more accurate and refined response mechanism.

Data availability statement

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

Author contributions

JW: Validation, Writing – original draft, Writing – review & editing. HZ: Project administration, Writing – review & editing. LM: Investigation, Software, Writing – review & editing. ZZ: Data curation, Methodology, Writing – review & editing. XH: Methodology, Writing – review & editing. HA: Formal analysis, Writing – review & editing. HS: Data curation, Methodology, Writing – review & editing. ZS: Investigation, Software, Writing – review & editing. HL: Conceptualization, Investigation, Software, Writing – review & editing.

Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article. This study was supported by Qinghai Natural Science Foundation Innovation Team Project (2021-ZJ-902), the National Natural Science Foundation of China Joint Fund Project (U21A20186, U20A2006), and the Second Tibetan Plateau Comprehensive Scientific Expedition and Research Program (2019QZKK0302-02).

Conflict of interest

The authors declare 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

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpls.2024.1474272/full#supplementary-material

References

Åkesson, A., Curtsdotter, A., Eklöf, A., Ebenman, B., Norberg, J., and Barabás, G. (2021). The importance of species interactions in eco-evolutionary community dynamics under climate change. *Nat. Commun.* 12, 4759. doi: 10.1038/s41467-021-24977-x

Bai, Y., Han, X., Wu, J., Chen, Z., and Li, L. (2004). Ecosystem stability and compensatory effects in the Inner Mongolia grassland. *Nature* 431, 181–184. doi: 10.1038/nature02850

Barry, K. E., Mommer, L., van Ruijven, J., Wirth, C., Wright, A. J., Bai, Y., et al. (2019). The future of complementarity: Disentangling causes from consequences. *Trends Ecol. Evol.* 342, 167–180. doi: 10.1016/j.tree.2018.10.013

Buche, L., Spaak, J. W., Jarillo, J., and de Laender, F. (2022). Niche differences, not fitness differences, explain predicted coexistence across ecological groups. *J. Ecol.* 110, 2785–2796. doi: 10.1111/1365-2745.13992

Camargo, N. F., Reis, G. G., Camargo, A. C., Nardoto, G. B., Kneitel, J. M., and Vieira, E. M. (2021). Seasonal isotopic niche of a rodent: High between-individual variation but no changes in individual niche width during the rich-resource period. *Biotropica.* 53, 966–975. doi: 10.1111/btp.12921

Cardinale, B. J., Wright, J. P., Cadotte, M. W., Carroll, I. T., Hector, A., Srivastava, D. S., et al. (2007). Impacts of plant diversity on biomass production increase through time because of species complementarity. *Proc. Natl. Acad. Sci.* 104, 18123–18128. doi: 10.1073/pnas.0709069104

Carroll, I. T., Cardinale, B. J., and Nisbet, R. M. (2011). Niche and fitness differences relate the maintenance of diversity to ecosystem function. *Ecology* 925, 1157–1165. doi: 10.1890/10-0302.1

Carscadden, K. A., Emery, N. C., Arnillas, C. A., Cadotte, M. W., Afkhami, M. E., Gravel, D., et al. (2020). Niche breadth: Causes and consequences for ecology, evolution, and conservation. *Q. Rev. Biol.* 95, 179–214. doi: 10.1086/710388

Chapin, F. S., Walker, B. H., Hobbs, R. J., Hooper, D. U., Lawton, J. H., Sala, O. E., et al. (1997). Biotic control over the functioning of ecosystems. *Science* 277, 500–504. doi: 10.1126/science.277.5325.500

Chen, D., Pan, Q., Bai, Y., Hu, S., Huang, J., Wang, Q., et al. (2016). Effects of plant functional group loss on soil biota and net ecosystem exchange: a plant removal experiment in the Mongolian grassland. *J. Ecol.* 104, 734–743. doi: 10.1111/1365-2745.12541

Chen, W. J., Zhou, H. K., Wu, Y., Wang, J., Zhao, Z. W., Li, Y. Z., et al. (2022). Loss of plant functional groups impacts soil carbon flow by changing multitrophic interactions within soil micro-food webs. *Appl. Soil Ecol.* 178, 104566. doi: 10.1016/j.apsoil.2022.104566

Chesson, P. (2000). Mechanisms of maintenance of species diversity. *Annu. Rev. Ecology Evolution Systematics* 31, 343–366. doi: 10.1146/ANNUREV.ECOLSYS.31.1.343

Díaz, S. M., Symstad, A. J., Chapin, F. S., Wardle, D. A., and Huenneke, L. F. (2003). Functional diversity revealed by removal experiments. *Trends Ecol. Evol.* 18, 140–146. doi: 10.1016/S0169-5347(03)00007-7

Fanin, N., Fromin, N., Buatois, B., and Hättenschwiler, S. (2013). An experimental test of the hypothesis of non-homeostatic consumer stoichiometry in a plant litter-microbe system. *Ecol. Lett.* 166, 764–772. doi: 10.1111/ele.12108

Fanin, N., Kardol, P., Farrell, M., Kempel, A., Ciobanu, M., Nilsson, M., et al. (2019). Effects of plant functional group removal on structure and function of soil communities across contrasting ecosystems. *Ecol. Lett.* 227, 1095–1103. doi: 10.1111/ele.13266

Fox, J. W. (2003). The long-term relationship between plant diversity and total plant biomass depends on the mechanism maintaining diversity. *Oikos* 102, 630–640. doi: 10.1034/J.1600-0706.2003.12618.X

Godoy, Ó., Gómez-Aparicio, L., Matías, L., Pérez-Ramos, I. M., and Allan, E. (2020). An excess of niche differences maximizes ecosystem functioning. *Nat. Commun.* 11, 4180. doi: 10.1038/s41467-020-17960-5

Godoy, Ó., Kraft, N. J., and Levine, J. M. (2014). Phylogenetic relatedness and the determinants of competitive outcomes. *Ecol. Lett.* 177, 836–844. doi: 10.1111/ele.12289

Gross, N., Suding, K., Lavorel, S., and Roumet, C. (2007). Complementarity as a mechanism of coexistence between functional groups of grasses. *J. Ecol.* 95, 1296–1305. doi: 10.1111/j.1365-2745.2007.01303.x

Guo, L., Zhang, W., and Liu, G. (2008). Species diversity and interspecific association in development sequence of hippophae rhamnoides plantations in the loess hilly region, China. *Front. Biol. China* 3, 489–495. doi: 10.1007/s11515-008-0070-6

Haugaasen, T., and Peres, C. A. (2009). Interspecific primate associations in Amazonian flooded and unflooded forests. *Primates* 50, 239–251. doi: 10.1007/s10329-009-0135-4

Holt, R. D. (2009). Bringing the hutchinsonian niche into the 21st century: Ecological and evolutionary perspectives. *Proc. Natl. Acad. Sci.* 106, 19659–19665. doi: 10.1073/pnas.0905137106

Huan, J., Hui, Z., Wen, X. L., Yan, S. F., Ming, Q. F., and Kong, X. Z. (2019). Interspecific associations and niche characteristics of communities invaded by decalobanthus boisianus. *Biodiversity Sci.* 27, 388–399. doi: 10.17520/BIODS.2019007

Jackson, S. T., Betancourt, J. L., Booth, R. K., and Gray, S. T. (2009). Ecology and the ratchet of events: Climate variability, niche dimensions, and species distributions. *Proc. Natl. Acad. Sci.* 106, 19685–19692. doi: 10.1073/pnas.0901644106

Jiang, L., Hu, J., Yang, Z. A., Zan, W., Zhao, C., Zhu, D., et al. (2021). Effects of plant functional group removal on community structure, diversity and productivity in alpine meadows. *J. Ecol.* 41, 1402–1411. doi: 10.5846/stxb202004220959

Kong, D., Wu, H., Zeng, H., Lü, X., Simmons, M. E., Wang, M., et al. (2011). Plant functional group removal alters root biomass and nutrient cycling in a typical steppe in Inner Mongolia, China. *Plant Soil* 346, 133–144. doi: 10.1007/s11104-011-0803-1

Laland, K. N., Odling-Smee, F. J., and Feldman, M. W. (1999). Evolutionary consequences of niche construction and their implications for ecology. *Proc. Natl. Acad. Sci. United States America* 9618, 10242–10247. doi: 10.1073/PNAS.96.18.10242

Lambers, H., de Britto Costa, P., Cawthray, G. R., Denton, M. D., Finnegan, P. M., Hayes, P. E., et al. (2022). Strategies to acquire and use phosphorus in phosphorus-impoverished and fire-prone environments. *Plant Soil* 476, 133–160. doi: 10.1007/s11104-022-05464-8

Letten, A. D., Ke, P., and Fukami, T. (2017). Linking modern coexistence theory and contemporary niche theory. *Ecol. Monogr.* 87, 161–177. doi: 10.1002/ECM.1242

Levine, J. M., Bascompte, J., Adler, P. B., and Allesina, S. (2017). Beyond pairwise mechanisms of species coexistence in complex communities. *Nature* 546, 56–64. doi: 10.1038/nature22898

Levine, J. M., and HilleRisLambers, J. (2009). The importance of niches for the maintenance of species diversity. *Nature* 461, 254–257. doi: 10.1038/nature08251

Li, X., Gao, J., Brierley, G., and Qiao, Y. (2013). Rangeland degradation on the Qinghai-Tibet plateau: implications for rehabilitation. *Land Degrad. Dev.* 24, 72–80. doi: 10.1002/ldr.1108

Liang, J., Zhou, M., Tobin, P. C., McGuire, A. D., and Reich, P. B. (2015). Biodiversity influences plant productivity through niche-efficiency. *Proc. Natl. Acad. Sci. United States America* 112, 5738–5743. doi: 10.1073/pnas.1409853112

Liu, J. H., Wang, Z. W., and Dong, K. K. (2019). Effects of heavy grazing on interspecific relationship of major plant species and community stability in a desert steppe. *J. Ecol.* 38, 2595–2602. doi: 10.13292/j.1000-4890.201909.012

Liu, C., Wolter, C., Xian, W., and Jeschke, J. M. (2020). Most invasive species largely conserve their climatic niche. *Proc. Natl. Acad. Sci.* 117, 23643–23651. doi: 10.1073/pnas.2004289117

Loke, L. H., and Chisholm, R. A. (2023). Unveiling the transition from niche to dispersal assembly in ecology. *Nature* 618, 537–542. doi: 10.1038/s41586-023-06161-x

Loreau, M., and Hector, A. (2001). Partitioning selection and complementarity in biodiversity experiments. *Nature* 412, 72–76. doi: 10.1038/35083573

Lu, Y., Chen, W., Yao, J., Huang, Y., Zhang, Y., Liu, H., et al. (2020). Multiple spatial scale analysis of the niche characteristics of the rhododendron dauricum plant communities in northeast China. *Chin. Geographical Sci.* 30, 614–630. doi: 10.1007/s11769-020-1138-8

Maherali, H., and Klironomos, J. (2007). Influence of phylogeny on fungal community assemblyand ecosystem functioning. *Science* 316, 1746–1748. doi: 10.1126/science.1143082

Mazancourt, C. D., Johnson, E., Barraclough, T. G., and Barraclough, T. G. (2008). Biodiversity inhibits species' evolutionary responses to changing environments. *Ecol. Lett.* 114, 380–388. doi: 10.1111/j.1461-0248.2008.01152.x

McLaren, J. R., and Turkington, R. (2010). Ecosystem properties determined by plant functional group identity. *J. Ecol.* 98, 459–469. doi: 10.1111/j.1365-2745.2009.01630.x

Naeem, S. (2002). Ecosystem consequences of biodiversity loss: the evolution of a paradigm. *Ecology* 83, 1537–1552. doi: 10.1890/0012-9658(2002)083[1537:ECOBLT]2.0.CO;2

Norberg, J., Urban, M. C., Vellend, M., Klausmeier, C. A., and Loeuille, N. (2012). Eco-evolutionary responses of biodiversity to climate change. *Nat. Climate Change* 2, 747–751. doi: 10.1038/NCLIMATE1588

Odling-Smee, J. C., Erwin, D. H., Palkovacs, E. P., Feldman, M. W., and Laland, K. N. (2013). Niche construction theory: A practical guide for ecologists. *Q. Rev. Biol.* 88, 3–28. doi: 10.1086/669266

Pastore, A. I., Barabás, G., Bimler, M. D., Mayfield, M. M., and Miller, T. E. (2021). The evolution of niche overlap and competitive differences. *Nat. Ecol. Evol.* 5, 330–337. doi: 10.1038/s41559-020-01383-y

Pianka, E. R. (1974). Niche overlap and diffuse competition. *Proc. Natl. Acad. Sci. United States America* 715, 2141–2145. doi: 10.1073/PNAS.71.5.2141

Pielou, E. C. (1972). Niche width and niche overlap: a method for measuring them. *Ecology* 53, 687–692. doi: 10.2307/1934784

Post, E. (2013). *Ecology of Climate Change: the importance of Biotic Interactions* (Princeton: Princeton University Press) 52, 25–32.

Reich, P. B., Tilman, D., Craine, J. M., Ellsworth, D. S., Tjoelker, M. G., Knops, J. M., et al. (2001). Do species and functional groups differ in acquisition and use of C, N and water under varying atmospheric CO$_2$ and N availability regimes? a field test with 16 grassland species. *New Phytol.* 150, 435–448. doi: 10.1046/J.1469-8137.2001.00114.x

Ru, H., Zhong, L., Nian, W., Li, Y., Sheng, Q., and Ni, Z. (2022). Variations of trophic structure and niche space in fish community along a highly regulated subtropical large river. *Ecol. Evol.* 12, e9424. doi: 10.1002/ece3.9424

Schluter, D. (1984). A variance test for detecting species associations, with some example applications. *Ecology* 65, 998–1005. doi: 10.2307/1938071

Schoener, T. W. (1974). Resource partitioning in ecological communities. *Science* 185, 27–39. doi: 10.1126/science.185.4145.27

Shao, L., and Zhang, G. (2021). Niche and interspecific association of dominant tree populations of Zelkova schneideriana communities in eastern China. *Botanical Sci.* 99, 823–833. doi: 10.17129/botsci.2809

Song, Y. C. (2017). *Vegetation ecology (2nd ed.)* (Beijing: Higher Education Press), 74–80.

Tan, R., Li, W., Wang, J., Du, F., and Yang, Y. (2013). "Effects of Disturbance on Niche Breadth, Overlap, and differentiation of dominant plant species in Napa Plateau Wetland, Shangrila," in *2013 Third International Conference on Intelligent System Design and Engineering Applications.* (Hong Kong, China), 666–670. doi: 10.1109/ISDEA.2012.159

Tilman, D., Lehman, C. L., and Thomson, K. T. (1997). Plant diversity and ecosystem productivity: theoretical considerations. *Proc. Natl. Acad. Sci. United States America* 945, 1857–1861. doi: 10.1073/PNAS.94.5.1857

Turnbull, L. A., Levine, J. M., Loreau, M., and Hector, A. (2013). Coexistence, niches and biodiversity effects on ecosystem functioning. *Ecol. Lett.* 16 Suppl 1, 116–127. doi: 10.1111/ele.12056

Van Eldijk, T. J., Bisschop, K., and Etienne, R. S. (2020). Uniting community ecology and evolutionary rescue theory: community-wide rescue leads to a rapid loss of rare species. *Front. Ecol. Evol.* 8, 552268. doi: 10.3389/fevo.2020.552268

Walther, G. R., Roques, A., Hulme, P. E., Sykes, M. T., Pyšek, P., Kühn, I., et al. (2009). Alien species in a warmer world: risks and opportunities. *Trends Ecol. Evol.* 2412, 686–693. doi: 10.1016/j.tree.2009.06.008

Wang, C. T., Long, R. J., Wang, Q. L., Jing, Z. C., and Shi, J. J. (2009). Changes in plant diversity, biomass and soil c, in alpine meadows at different degradation stages in the headwater region of three rivers, China. *Land Degrad. Dev.* 20, 187–198. doi: 10.1002/LDR.879

Wang, Q., Zhu, R., Cheng, J., Deng, Z., Guan, W., and El-Kassaby, Y. A. (2018). Species association in Xanthoceras sorbifolium Bunge communities and selection for agroforestry establishment. *Agroforestry Syst.* 93, 1531–1543. doi: 10.1007/s10457-018-0265-z

Ward, S. E., Bardgett, R. D., Mc Namara, N. P., and Ostle, N. J. (2009). Plant functional group identity influences short-term peatland ecosystem carbon flux: evidence from a plant removal experiment. *Funct. Ecol.* 23, 454–462. doi: 10.1111/J.1365-2435.2008.01521.X

Weih, M., Nordh, N., Manzoni, S., and Hoeber, S. (2021). Functional traits of individual varieties as determinants of growth and nitrogen use patterns in mixed stands of willow (Salix spp.). *For. Ecol. Manage.* 479, 118605. doi: 10.1016/J.FORECO.2020.118605

Wittebolle, L., Marzorati, M., Verstraete, W., and Boon, N. (2009). Initial community evenness favours functionality under selective stress. *Nature* 458, 623–626. doi: 10.1038/nature07840

Zhang, X., Johnston, E. R., Barberán, A., Ren, Y., Lü, X., and Han, X. (2017). Decreased plant productivity resulting from plant group removal experiment constrains soil microbial functional diversity. *Global Change Biol.* 23, 4318–4332. doi: 10.1111/gcb.13783

Zhang, D. M., Zhao, W. Z., and Luo, W. C. (2018). Niche and interspecific association of dominant plant species in saline-alkaline soils of desert steppe zone. *Chin. J. Ecol.* 37, 1307–1315.

Zhong, Y., Zhang, J., Yang, W. Q., Wu, F. Z., Feng, M. S., and Liu, Q. B. (2010). Interspecific association of dominant shrub species under eucalyptus grandis plantation. *Ecol. Environ. Sci.* 19, 631–636.

Zhou, T., Hou, G., Sun, J., Zong, N., and Shi, P. (2021). Degradation shifts plant communities from S- to R-strategy in an alpine meadow, Tibetan Plateau. *Sci. total Environ.* 800, 149572. doi: 10.1016/j.scitotenv.2021.149572

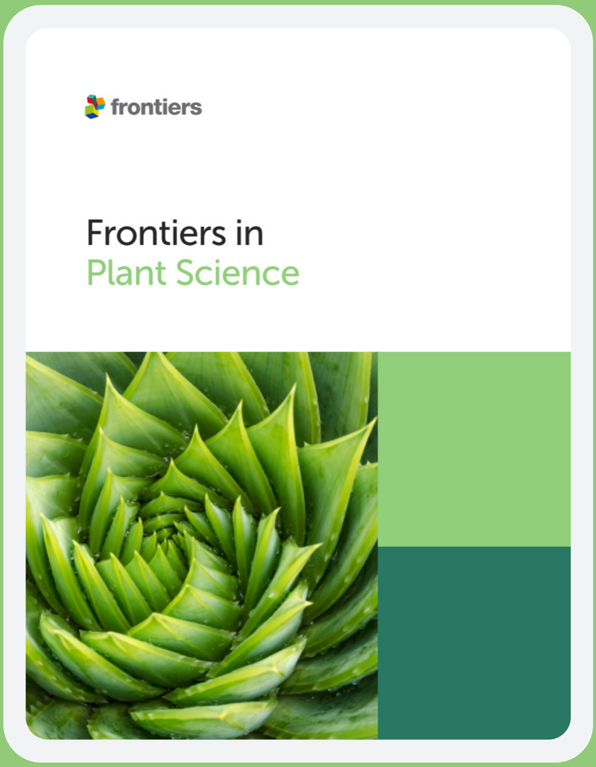
Frontiers in
Plant Science

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

Frontiers in
Plant Science

Frontiers

Avenue du Tribunal-Fédéral 34
1005 Lausanne, Switzerland
frontiersin.org

Contact us

+41 (0)21 510 17 00
frontiersin.org/about/contact