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Clonality in the Anthropocene: Adaptation, evolution, and functioning of clonal plants from individuals to ecosystems

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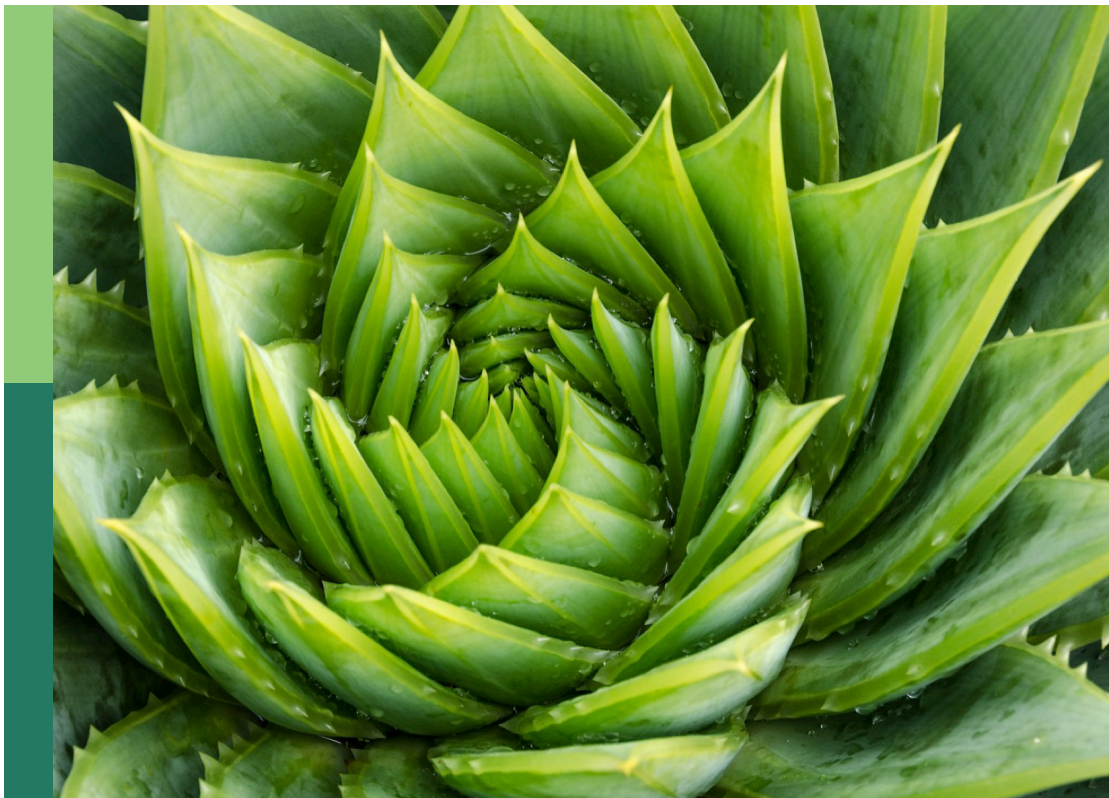
Published in
Frontiers in Plant Science



ISSN 1664-8714
ISBN 978-2-8325-5394-7
DOI 10.3389/978-2-8325-5394-7

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Clonality in the Anthropocene: Adaptation, evolution, and functioning of clonal plants from individuals to ecosystems

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Citation

Wang, Y.-J., Tao, J., Shabbir, A., Xue, W., Yu, F.-H., Luo, F.-L., eds. (2024). *Clonality in the Anthropocene: Adaptation, evolution, and functioning of clonal plants from individuals to ecosystems*. Lausanne: Frontiers Media SA.
doi: 10.3389/978-2-8325-5394-7

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frontiers | Frontiers in Plant Science

TYPE Editorial
PUBLISHED 22 August 2024
DOI 10.3389/fpls.2024.1448891

OPEN ACCESS

EDITED AND REVIEWED BY
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RECEIVED 14 June 2024
ACCEPTED 07 August 2024
PUBLISHED 22 August 2024

CITATION
Yu F-H, Tao J-P and Shabbir A (2024) Editorial: Clonality in the Anthropocene: adaptation, evolution, and functioning of clonal plants from individuals to ecosystems. *Front. Plant Sci.* 15:1448891. doi: 10.3389/fpls.2024.1448891

Editorial: Clonality in the Anthropocene: adaptation, evolution, and functioning of clonal plants from individuals to ecosystems

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KEYWORDS

biological invasions, clonal growth, ecosystem functioning, global change, human activities

Editorial on the Research Topic

Clonality in the Anthropocene: adaptation, evolution, and functioning of clonal plants from individuals to ecosystems

In the Anthropocene, plants on Earth face a number of challenges with ongoing global changes in climate, land use, acidity, and toxin concentration. Clonal plants, capable of both asexual (clonal) and sexual reproduction, exhibit a wide distribution and reveal considerable benefits in many habitats, which may be connected with their distinctive characteristics of clonality (Herben and Klimešová, 2020; Roiloa et al., 2023). Clonal traits include clonal regeneration (e.g., production of asexual individuals, called ramets), clonal integration (e.g., resource and signaling sharing between interconnected ramets of the same clone), clonal resource foraging (e.g., selective positioning of ramets), clonal storage and resource reallocation (e.g., reallocation of stored energy/nutrients between interconnected ramets), initiation of meristem bud banks, and trade-offs between clonal and sexual reproduction, which enable clonal plants to quickly adapt to environmental changes (Chen et al., 2019; Klimešová et al., 2021; Shi et al., 2021; Dong et al., 2022). The environmental conditions experienced by a parental ramet of a clonal plant can be transmitted to its offspring via clonal reproduction (i.e., clonal parental effects), thereby influencing the phenotypes of the progeny (Latzel and Klimešová, 2010; Luo et al., 2022; Quan et al., 2022). These adaptations may enhance survival, competitiveness, invasiveness, and the spread of clonal species across ecosystems, in response to global climatic change during the Anthropocene (Wang et al., 2017; 2024; Zhang et al., 2024). Numerous studies have sought to integrate clonality into the research agenda of plant ecology, thereby offering a roadmap to gain valuable insights into the evolutionary dynamics of plants (Cornelissen et al., 2014; Klimešová et al., 2021; Roiloa et al., 2023).

This Research Topic compiles research papers aimed at understanding how clonal plants respond to environmental changes and their contributions to patterns and processes at the population, community, and ecosystem levels. It contributes to enhance our understanding of the roles clonality plays in ecosystem functioning, the influence of clonal growth on the invasiveness of alien plants and the invisibility of native plant communities, as well as the mechanisms, functioning, and adaptive strategies of plant clonality in response to global change at various ecological levels.

Three papers delve into physiological adaptation and effects of exogenous hormone on clonal plants under varying environmental stress conditions. In their study on the stoloniferous herb *Centella asiatica*, Duan et al. discovered that the external application of abscisic acid (ABA) within the clonal network led to a significant increase in biomass accumulation both in the below-ground parts and across the entire clonal fragment of the plant. These findings suggest that the rapid activation and sustained resistance responses induced by local exogenous ABA application within the clonal network may enhance the fitness of clonal plants exposed to abiotic stress. By cultivating a clonal plant *Alternanthera philoxeroides* under different submergence depths, Jing et al. demonstrated that plants achieved greater growth at 2 m and 5 m submergence depths compared to control plants (un-submerged), and gibberellin (GA) induced the differential elongation of the internode as plant submerged at a depth of 2 m had the highest GA accumulation. Therefore, the effects of submergence depth and duration on stem elongation have improved our understanding of the physiological adaptation of clonal plants in deeply flooded environments. In field populations, individual rhizome systems of clonal herb *Podophyllum peltatum* were fed $^{14}CO_2$ with different times of demography, Watson and Vuorisalo showed that carbon fixed at various times was utilized differently, with assimilates preferentially moving into old rhizomes rather than supporting the formation of new ramets. These findings suggest that demography influences, integrative physiology, and physiological restrictions on within-season redistribution of assimilates limit the capacities of clonal plants to adapt to rapid environmental changes in the Anthropocene.

Four papers examine physiological integration, resource sharing, and resource utilization of clonal plants across various vegetation environments. In a pot experiment tracing the movement of ^{15}N, Zhao et al. found that ^{15}N translocation between connected ramets of moso bamboo (*Phyllostachys pubescens*) was determined by the source-sink relationship in heterogeneous environments. The allocation of ^{15}N in the fertilized ramet was higher compared to the connected unfertilized ramet. The findings suggest that physiological integration significantly improved the nitrogen use efficiency of moso bamboo, which has implications for fertilization management in moso bamboo forests. Guo et al. demonstrated that the dominant clonal grass *Leymus chinensis* benefited more from physiological integration in sexual reproduction compared to companion clonal species in an *in situ* ^{15}N labeling experiment in a grassland. Thus, differences in the ability of physiological integration between the dominant and companion species may explain the dominance of *L. chinensis* in the grassland. Yu et al. found that the performance of a clonal fern *Pyrrosia nudaa* increased with the developmental age of the ramets but decreased with an increasing number of ramets in a clonal fragment. These age-dependent impacts of clonal fragmentation provide insights into the biodiversity conservation of epiphytes and forest management in man-made plantations. Xing et al. showed that neighboring touches on parental ramets of *Glechoma longituba* influenced the performance of offspring ramets, and this effect was depended on the light environment. These results suggest that the interaction between neighboring touch and shade environment influences the growth of understory plants.

One paper investigates the successful invasions of alien clonal plants. Zhang et al. found that connection between ramets had a more pronounced effect on the biomass allocation pattern of alien invasive plants than disconnection, resulting in a greater increase in biomass for invasive plants compared to native plants. These results suggest that invasive clonal plants possess a greater capacity for resource partitioning than native plants, which may confer a competitive advantage and enable them to successfully invade in some heterogeneous habitats, such as forest edges.

Three papers focus on the role of bud banks in clonal plants. Through a chronosequence study of evergreen conifer plantations, Song et al. found that close-to-nature and gap management modes significantly facilitated the diversity of clonal plants, the overall plant diversity of the communities, and various parameters of ecosystem service functions in *Cunninghamia lanceolata* plantations. The diversity of clonal plants was significantly positively correlated with ecosystem service functions following forest management. They suggest that the link between forest management, diversity, and ecosystem functions should be emphasize to elucidate the mechanism of traits-ecosystem functioning relationships and the restoration of degraded plantations. In a bamboo ecosystem, Zou et al. found that the size of clonal fragments of *Phyllostachys bissetii* contributed more to the soil nitrogen turnover process compared to clonal integration, while it had the opposite effect on soil carbon availability. The findings are critical for understanding the nutrient turnover of *P. bissetii* under stressed conditions. Wu et al. discovered that vegetation attributes significantly affected bud banks in wetland ecosystems, while soil properties had a strong influence on bud banks in farmland and alpine meadow ecosystems. These findings suggest that changes in land use can impact the size and composition of bud banks.

Two papers focus on measurement tools, application, and prediction for restoration and conservation of clonal plants. Ohsowski et al. provided a rapid field-based clonal *Typha* identification and biomass assessment tool targeted management for regional conservation of *T. latifolia* and ecological restoration of wetlands impacted by invasive *Typha* taxa. To understand the relationship between plant phenological traits and phylogeny, Shahzad et al. found that phylogeny, growth form, and functional features influenced the diversity of flowering phenology within species in conjunction with local climate circumstances. This understanding aids in comprehending the evolutionary conservation mechanisms of plant phenological traits, including clonal traits, in relation to evolutionary processes across different geographical and climatic zones.

Adaptation, evolution, and functioning of clonal plants have drawn increasing attention in recent years (Klimešová et al., 2021; Roiloa et al., 2023). However, there is still much to explore in this field within the plant ecology agenda. The mechanisms at individual, population, and community scales and their interactions across scales in the Anthropocene, have rarely been discussed so far. We hope to improve and expand our knowledge of this crucial issue in the future.

Author contributions

J-PT: Conceptualization, Methodology, Supervision, Writing – original draft. AS: Methodology, Writing – original draft. F-HY: Conceptualization, Funding acquisition, Project administration, Supervision, Writing – review & editing.

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

Chen, D., Xiong, H., Lin, C. G., He, W., Zhang, Z. W., Wang, H., et al. (2019). Clonal integration benefits invasive alien plants under water variability in a native community. *J. Plant Ecol.* 12, 574–582. doi: 10.1093/jpe/rty050

Cornelissen, J. H. C., Song, Y. B., Yu, F. H., and Dong, M. (2014). Plant traits and ecosystem effects of clonality: a new research agenda. *Ann. Bot.* 114, 369–376. doi: 10.1093/aob/mcu113

Dong, B. C., Wang, P., and Luo, F. L. (2022). Sharing of nitrogen between connected ramets of *Alternanthera philoxeroides* in homogeneous environments. *Plant Soil* 478, 445–460. doi: 10.1007/s11104-022-05475-5

Herben, T., and Klimešová, J. (2020). Evolution of clonal growth forms in angiosperms. *New Phytol.* 225, 999–1010. doi: 10.1111/nph.16188

Klimešová, J., Ottaviani, G., Charles-Dominique, T., Campetella, G., Canullo, R., Chelli, S., et al. (2021). Incorporating clonality into the plant ecology research agenda. *Trends Plant Sci.* 26, 1236–1247. doi: 10.1016/j.tplants.2021.07.019

Latzel, V., and Klimešová, J. (2010). Transgenerational plasticity in clonal plants. *Evol. Ecol.* 24, 1537–1543. doi: 10.1007/s10682-010-9385-2

Luo, F. L., Wang, M. Z., Dong, B. C., and Chen, Y. H. (2022). Clonal and sexual parental effects and their mechanisms. *Acta Ecologica Sin.* 42, 6474–6486. doi: 10.5846/stxb202107211968

Quan, J., Munzbergova, Z., and Latzel, V. (2022). Time dynamics of stress legacy in clonal transgenerational effects: A case study on *Trifolium repens*. *Ecol. Evol.* 12, e8959. doi: 10.1002/ece3.8959

Roiloa, S. R., Xue, W., Dong, B. C., and Yu, F. H. (2023). Editorial: Ecological implications of plant clonality. *Flora* 309, 152420. doi: 10.1016/j.flora.2023.152420

Shi, X. P., Bai, Y. F., Song, P., Liu, Y. Y., Zhang, Z. W., Zheng, B., et al. (2021). Clonal integration and phosphorus management under light heterogeneity facilitate the growth and diversity of understory vegetation and soil fungal communities. *Sci. Total Environ.* 767, 144322. doi: 10.1016/j.scitotenv.2020.144322

Wang, Y. J., Liu, Y. Y., Chen, D., Du, D. L., Müller-Schärer, H., and Yu, F. H. (2024). Clonal functional traits favor the invasive success of alien plants into native communities. *Ecol. Appl.* 34, e2756. doi: 10.1002/eap.2756

Wang, Y. J., Muller-Scharer, H., van Kleunen, M., Cai, A. M., Zhang, P., Yan, R., et al. (2017). Invasive alien plants benefit more from clonal integration in heterogeneous environments than natives. *New Phytol.* 216, 1072–1078. doi: 10.1111/nph.14820

Zhang, R. F., Guo, Y. J., Lai, Y. W., Zhao, T. T., Li, G. L., Yan, Z. G., et al. (2024). Microplastics promote the invasiveness of invasive alien species under fluctuating water regime. *J. Appl. Ecol.* doi: 10.1111/1365-2664.14726

frontiers | Frontiers in Plant Science

TYPE Original Research
PUBLISHED 13 June 2023
DOI 10.3389/fpls.2023.1203881

OPEN ACCESS

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RECEIVED 11 April 2023
ACCEPTED 30 May 2023
PUBLISHED 13 June 2023

CITATION
Zhao J and Cai C (2023) Effects of physiological integration on nitrogen use efficiency of moso bamboo in homogeneous and heterogeneous environments. *Front. Plant Sci.* 14:1203881. doi: 10.3389/fpls.2023.1203881

Effects of physiological integration on nitrogen use efficiency of moso bamboo in homogeneous and heterogeneous environments

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Introduction: Moso bamboo is one of the important clonal plants with complex underground rhizome-root system. Ramets connected by rhizome can translocate and share nitrogen (N), which may affect the nitrogen use efficiency (NUE) of moso bamboo. The aims of this study were to investigate the mechanisms of N physiological integration and its relationship with NUE of moso bamboo.

Methods: A pot experiment was conducted to trace the movement of ^{15}N between the connected ramets of moso bamboo in both homogeneous and heterogeneous N environments.

Results: Results showed that N translocation within clonal fragments of moso bamboo was detected in both homogeneous and heterogeneous environments. The intensity of physiological integration (IPI) was significantly lower in homogeneous environments than that in heterogeneous environments. ^{15}N translocation between the connected ramtes of moso bamboo was determined by the source-sink relationship in heterogeneous environments, and the ^{15}N allocation of the fertilized ramet was higher than that of the connected unfertilized ramet. The NUE of connected treatment was significantly higher than that of severed treatment, which suggested that physiological integration significantly improved the NUE of moso bamboo. In addition, the NUE of moso bamboo was significantly higher in heterogeneous environments than that in homogeneous environments. The contribution rate of physiological integration (CPI) on NUE in heterogeneous environments was significantly higher than that in homogenous environments.

Discussion: These results will provide theoretical basis for precision fertilization in moso bamboo forests.

KEYWORDS

***Phyllostachys edulis*, physiological integration, clonal plant, N translocation, NUE**

1 Introduction

Bamboo, the fast-growing grass plant, is a precious treasure bestowed upon humankind by nature and has significant ecological, economic, and social benefits (Jiang, 2007). There are 1642 bamboo species in the world and the area of bamboo forest is more than 35 million ha (Vorontsova et al., 2017). Bamboo culms are connected with each other through the rhizome-root system, with strong physiological integration functions and environmental adaptability (Zhuang et al., 2011). Physiological integration is an important characteristic of clonal plants, that refers to the translocation and sharing of photosynthates, water and mineral nutrients between the connected ramets by rhizomes, stolons, or roots (He et al., 2010; Portela et al., 2021; Shi et al., 2021; Wang et al., 2021; Li et al., 2022; Shi et al., 2022).

Moso bamboo (*Phyllostachys edulis*) is an important economic bamboo species for the production of bamboo timbers and bamboo shoots, which is widely distributed in southern China (Song et al., 2011; Shi et al., 2022; Zhao et al., 2022). The 9th national forest resources inventory shows that there is 4.68 million ha of moso bamboo forest, accounting for 72.96% of the total area of bamboo forest (National Forestry and Grassland Administration, 2019). As a group of typical clonal plants, moso bamboo has many unique properties that can effectively utilize resources in heterogeneous habitats through physiological integration (Zhuang et al., 2011). The integration between connected ramets can transfer nutrients to each other and then increase the net growth rate of the population.

Moso bamboo forest is also a typical uneven-aged forest composed of individuals of different ages due to its unique growth characteristics and conventional managements (Su et al., 2019; Shi et al., 2022). The ages of bamboo culms are expressed by "du" due to the growth cycle of two years (on-year and off-year) in moso bamboo forests (Su et al., 2019; Zhao et al., 2019; Li et al., 2021). In "on-year", more than 90% of new shoots are produced, followed by a few new shoots in "off-year" (Tang et al., 2015). In addition, the growth of moso bamboo in both diameter and height is completed within two months after shoot sprouting, which is referred to as "explosive growth" (Song et al., 2016; Shi et al., 2022). During the "explosive growth" period, the nutrients for the growth of new individuals are supplied by the connected older individuals through rhizome (Sun et al., 2019), which has important ecological significance for the survival, growth, reproduction, expansion and resource utilization of moso bamboo (He et al., 2010; Portela et al., 2021; Wang et al., 2021; Li et al., 2022).

The special management measures in moso bamboo forest, such as bamboo timbers cutting and bamboo shoots harvesting, brought away large amount of nutrients, leading to an unsustainable level of long-term productivity (Guan et al., 2017; Su et al., 2019). Numerous studies showed that nitrogen (N) had the largest demand in moso bamboo forests (Su, 2012; Zhao et al., 2016). Therefore, the nitrogenous fertilizers were commonly applied in moso bamboo forests (Su et al., 2019). Mao et al. (2016) investigated the NUE of moso bamboo forests in homogeneous environments by broadcast application, and the N use efficiency (NUE) was relatively low. In order to improve the NUE of moso bamboo forests, furrow application and hole application were used to determine the appropriate fertilization placement and the target age (Su et al., 2019). What's more, cavity-injecting fertilization was conducted as heterogeneous environments, and N allocation in bamboo individuals of different ages was also conducted (Shi et al., 2022). Their results showed that the N competitive ability and NUE of I "du" (1–2 years old) bamboo were significantly higher than II "du" (3–4 years old) and III "du" (5–6 years old) bamboos (Zhao, 2016; Su et al., 2019). In addition, the unequal N translocation pattern caused by physiological integration between two connected ramets ensured necessary N supply for young moso bamboo growth, and the demand-driven source-sink relationships significantly affected N translocation in the clonal fragment of moso bamboo (Shi et al., 2022). These results mainly focused on N translocation between parent and offspring ramets, while little information was known between two connected ramets with the same age.

In this study, we sought to investigate the characteristics of N physiological integration between two connected ramets with the same age and the effect of physiological integration on the NUE of moso bamboo. To do this, we conducted a pot experiment using the clonal fragments of moso bamboo with the same age in both homogeneous and heterogeneous environments. Fertilizers (urea or ^{15}N-labeled urea) were applied, and rhizomes between two successive ramets were either connected or severed. We aimed to answer the following specific questions: (1) Is translocation of N in clonal fragments different between homogeneous and heterogeneous environments? (2) Dose physiological integration improve the NUE of moso bamboo forests in both homogeneous and heterogeneous environments?

2 Materials and methods

2.1 Materials and experimental design

The experiment was conducted in Bamboo Botanical Garden (120°03′42′′E, 30°22′25′′N), Zhejiang Academy of Forestry, China. In April 2019, the seedlings of moso bamboo were cultivated by semination technology. In May 2021, fifty healthy rhizomes were selected and cut into 0.5 m, which had more than two rhizome buds. Then, the selected rhizomes were placed on the seedbed and covered with a thin layer of cultivation substrate. The cultivation substrate was a mixture of red soil and fine sand by volume (3:1), with a pH value of 5.83, organic carbon (C) concentration of 26.47 g kg^{-1}, N concentration of 1.59 g kg^{-1}, P concentration of 0.48 g kg^{-1}, and K concentration of 14.25 g kg^{-1}. In addition, proper water was applied to keep moist, so as to promote the emergence of the rhizome buds as soon as possible. In May 2022, the clonal fragments with two successive ramets connected by rhizomes were selected and transplanted in two non-woven bags (30 cm in diameter, 30 cm in depth). The average height and ground diameter of ramets were 81.58 cm and 4.46 mm, respectively. The clonal fragments were placed under a canopy in order to avoid rainfall interference.

In June 2022, 24 clonal fragments with relatively consistent growth were selected, which were divided into two groups (connected and severed). For the severed group, rhizomes between the two connected ramets were cut at the mid-point. In each group, two contrasting treatment (homogeneous and heterogeneous environments) were applied (Figure 1). In homogeneous N environment, one ramet of each clonal fragment was applied 20 g ^{15}N-labeled urea, while the other ramet was applied 20 g urea. In heterogeneous N environment, one ramet of each clonal fragment was applied 20 g ^{15}N-labeled urea, while the other ramet was not fertilized. Six replicates for each treatment were randomly arranged. The ^{15}N-labeled urea (10.18 at%) was provided by Shanghai Research Institute of Chemical Industry, China. Fertilizers were irrigated into the soil as an aqueous solution in June 2021.

2.2 Samples collection and analysis

In October 2022, rhizomes were cut at the mid-point of the two connected ramets. Then, each ramet was harvested and separated into leaves, culms (including branches) and rhizomes (including rhizome roots). The soil particles attached to the rhizome and rhizome root were washed with running water. All the samples were dried at 65 °C in an oven (OF-12G, Lab Companion, South Korea) to a constant mass for biomass determination. Then, the dried samples were ground and sieved (100 mash). The total N concentrations and the at% ^{15}N were determined using an Isotope Ratio Mass Spectrometer (Delta V Advantage, USA) at Institute of Soil Science, Chinese Academy of Sciences.

2.3 Calculation methods

Ndff (the percentage of ^{15}N derived from ^{15}N-labeled urea, %) was calculated by the following equation (Su et al., 2019):

$$\text{Ndff}(\%) = \frac{b-a}{c-a} \times 100$$

where a is the at% ^{15}N of the unlabeled ramet in severed treatment at heterogeneous condition, b is the at% ^{15}N of all the sampling ramets, and c is the at% ^{15}N of ^{15}N-labeled urea (10.18 at %).

$$\text{Organ}^{15}\text{N concentration}(\text{g kg}^{-1}) = \text{Organ N concentration}(\text{g kg}^{-1}) \times \text{Ndff} \times 10^{-2}$$

$$\text{Organ}^{15}\text{N}(\text{g}) = \text{Organ biomass}(\text{g}) \times \text{Organ}^{15}\text{N concentration}(\text{g kg}^{-1}) \times 10^{-3}$$

$$\text{Ramet}^{15}\text{N}(\text{g}) = \text{Leaf}^{15}\text{N}(\text{g}) + \text{Culm}^{15}\text{N}(\text{g}) + \text{Rhizome}^{15}\text{N}(\text{g})$$

$$\text{NUE}(\%) = (\text{Labeled ramet}^{15}\text{N} + \text{Unlabeled ramet}^{15}\text{N})(\text{g})/\text{Fertilizer}^{15}\text{N}(\text{g}) \times 100$$

The ratio of the amount of ^{15}N translocated from labeled ramet to unlabeled ramet to the amount of ^{15}N absorbed by the clonal fragment was calculated to represent the intensity of physiological integration (IPI, %) according to the following equation (Saitoh et al., 2006):

$$\text{IPI}(\%) = \frac{AM_i}{AM_{frag}} \times 100$$

where AM_i is the amount of ^{15}N translocated from labeled ramet to unlabeled ramet, and AM_{frag} is the total amount of ^{15}N absorbed per clonal fragment.

The percentage increase of NUE in connected treatment compared with that in severed treatment was used to indicate the contribution rate of physiological integration (CPI, %).

$$\text{CPI}(\%) = \frac{NUE_{con} - NUE_{sev}}{NUE_{sev}} \times 100$$

where NUE_{con} is the NUE in connected treatment, and NUE_{sev} is the NUE in severed treatment.

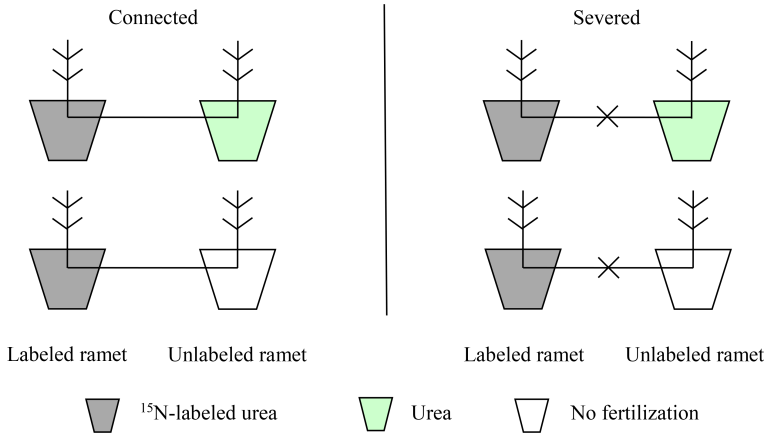

FIGURE 1
Schematic diagram of the experiment design.

2.4 Statistical analysis

Two-way analysis of variance (ANOVA) was used to investigate effects of rhizome connection status (connected vs. severed) and environment status (homogeneous vs. heterogeneous) on biomass, ^{15}N concentrations and uptakes of ramets, and biomass, ^{15}N uptakes and NUE of the whole clonal fragments. One-way analysis of variance (ANOVA) and Duncan's multiple comparisons were used to analyze the significant differences between labeled and unlabeled ramets. In addition, differences in IPI and CPI between homogeneous and heterogeneous conditions were analyzed by One-way ANOVA. All analyses were conducted using SAS 9.4 software. Figures were prepared using Origin 8.6 software.

3 Results

3.1 Biomass accumulation of the ramets and the clonal fragment

For labeled ramet, no significant effects of rhizome status, environment status and their interaction on leaf biomass, culm biomass, rhizome biomass and total biomass were observed (Table 1). No significant differences were found in leaf biomass, culm biomass, rhizome biomass and total biomass among the four treatment (Figures 2A–D). For unlabeled ramet, significant effect of environment status on total biomass was observed (Table 1). Leaf biomass, culm biomass and total biomass were not significantly different among the four treatments (Figures 2E, F, H). However, rhizome biomass of severed treatment under heterogeneous conditions was significantly lower than that of other treatments ($P< 0.05$, Figure 2G). Additionally, no significant differences were found in leaf biomass, culm biomass, rhizome biomass and total biomass between labeled and unlabeled ramets under the same treatment (Figure 2).

For the whole clonal fragment, no significant effects of rhizome status, environment status and their interaction on leaf biomass, culm biomass, rhizome biomass and total biomass were observed (Table 2). No significant differences were found in leaf biomass, culm biomass, rhizome biomass and total biomass among the four treatments (Figure 3). In heterogeneous conditions, leaf biomass, culm biomass, rhizome biomass and total biomass under the connected treatment were higher than those under the severed treatment, but no significant differences were observed (Figure 3).

3.2 ^{15}N concentrations of the ramets

For labeled ramet, no significant effects of rhizome status, environment status and their interaction on ^{15}N concentrations in leaf, culm and rhizome were observed (Table 1). ^{15}N concentrations in leaf, culm and rhizome were not significantly different among the four treatments (Figures 4A–C). For unlabeled ramet, significant effects of rhizome status, environment status and their interaction on ^{15}N concentrations in leaf, culm and rhizome were observed (Table 1). ^{15}N concentrations in leaf, culm and rhizome of connected treatment in heterogeneous conditions were significantly higher than those in homogeneous conditions ($P< 0.05$), while these variables of severed treatment had no significant difference between homogeneous and heterogeneous conditions

TABLE 1 Results of significance test for biomass, ^{15}N concentrations and uptakes of labeled and unlabeled ramets.

	Organ	Labeled Ramet			Unlabeled Ramet		
		Rhizome status (R)	Environment status (E)	R×E	Rhizome status (R)	Environment status (E)	R×E
Biomass	Leaf	0.02	0.01	0.00	0.09	3.39	0.16
	Culm	0.04	0.03	0.08	0.39	5.06	1.65
	Rhizome	0.78	0.02	0.19	4.30	5.30	3.48
	Total	0.19	0.02	0.10	1.88	7.12*	0.17
^{15}N concentration	Leaf	0.56	0.33	3.77	565.39***	220.84***	220.84***
	Culm	0.03	0.58	1.07	1071.73***	440.16***	440.16***
	Rhizome	0.22	0.01	0.02	970.86***	505.07***	505.07***
^{15}N uptake	Leaf	0.23	0.05	0.85	207.09***	75.79***	75.79***
	Culm	0.01	0.24	0.66	713.74***	281.47***	281.47***
	Rhizome	0.00	0.01	0.06	845.62***	434.88***	434.88***
	Total	0.01	0.02	1.05	606.64***	264.32***	264.32***

Values are F ratio and significance are indicated by ***(P< 0.001), *(P< 0.05).
The effects of rhizome status (connected vs. severed), environment status (homogeneous vs. heterogeneous) and their interaction were tested in two-way ANOVA.

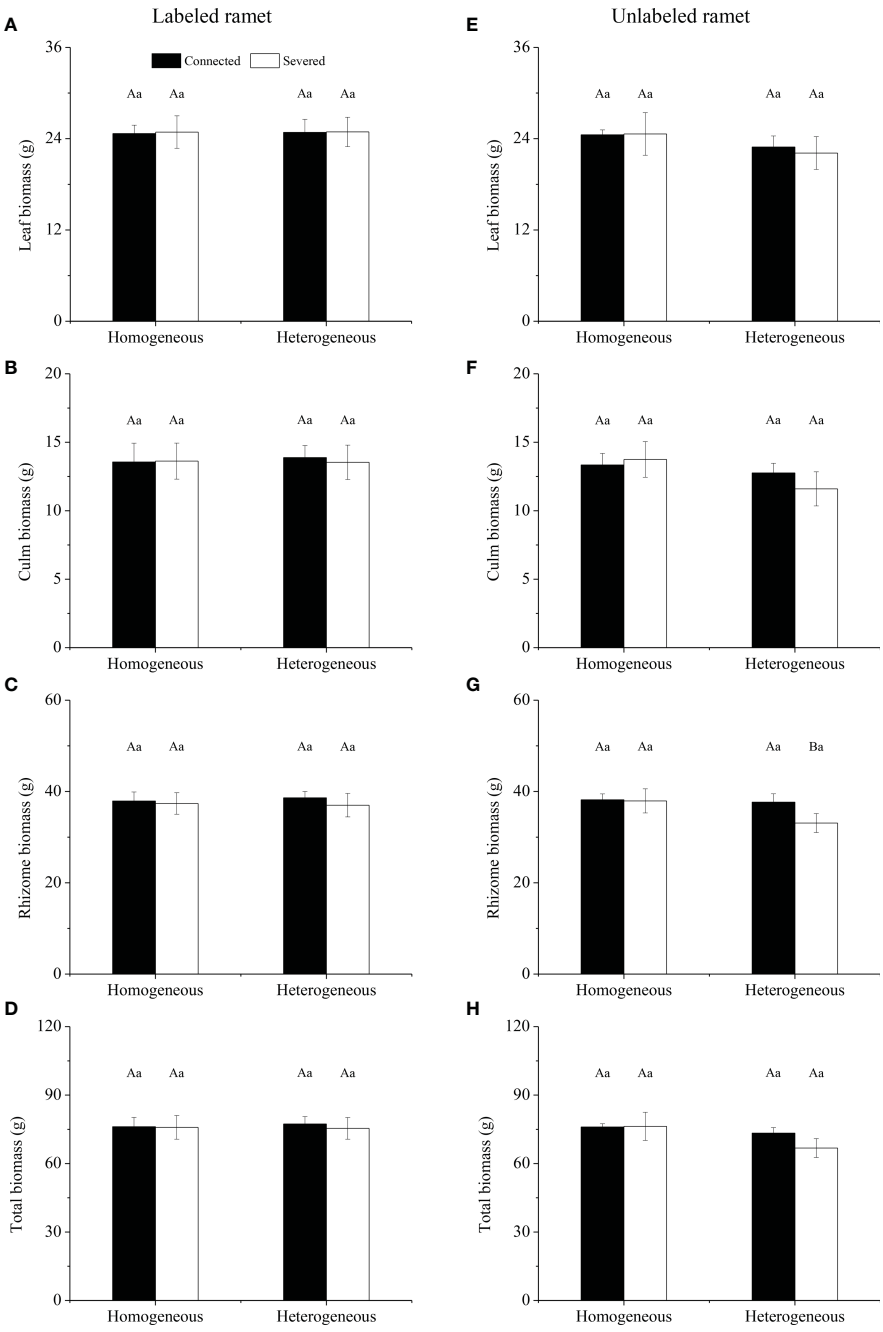

FIGURE 2
Leaf biomass **(A, E)**, culm biomass **(B, F)**, rhizome biomass **(C, G)** and total biomass **(D, H)** of the labeled **(A–D)** and the unlabeled **(E–H)** ramets in homogeneous and heterogeneous environments. The labeled and unlabeled ramets were either connected or severed. Different uppercase letters in each subgraph indicate statistically significant differences ($P< 0.05$) among the four treatments, and different lowercase letters indicate statistically significant differences ($P< 0.05$) between labeled and unlabeled ramets under the same treatment.

(Figures 4D–F). The ^{15}N concentrations in leaf, culm and rhizome of connected treatment were significantly higher than those of severed treatment in both homogeneous conditions and heterogeneous conditions ($P< 0.05$, Figures 4D–F). In addition, the ^{15}N concentrations in leaf, culm and rhizome of labeled ramet were significantly higher than those of unlabeled ramet under the same treatment ($P< 0.05$, Figure 4).

3.3 ^{15}N uptakes of the ramets and the clonal fragment

For labeled ramet, no significant effects of rhizome status, environment status and their interaction on ^{15}N uptakes in leaf, culm and rhizome were observed (Table 1). ^{15}N uptakes in leaf, culm, rhizome and the total ^{15}N uptake were not significantly

TABLE 2 Results of significance test for biomass and ^{15}N uptakes of the whole clonal fragments.

	Organ	Rhizome status (R)	Environment status (E)	R×E
Biomass	Leaf	0.01	0.93	0.06
	Culm	0.18	1.04	0.65
	Rhizome	3.27	1.69	1.97
	Total	0.90	1.66	0.90
^{15}N uptake	Leaf	3.82	1.56	0.33
	Culm	7.35*	4.78	6.33*
	Rhizome	15.97**	8.82*	9.71*
	Total	8.77*	4.43	3.30

Values are F ratio and significance are indicated by **(P< 0.01), *(P< 0.05).
The effects of rhizome status (connected vs. severed), environment status (homogeneous vs. heterogeneous) and their interaction were tested in two-way ANOVA.

different among the four treatments (Figures 5A–D). For unlabeled ramet, significant effects of rhizome status, environment status and their interaction on ^{15}N concentrations in leaf, culm and rhizome were observed (Table 1). ^{15}N uptakes in leaf, culm, rhizome and the total ^{15}N uptake of connected treatment in heterogeneous conditions were significantly higher than those in homogeneous conditions ($P< 0.05$), while these variables of severed treatment had no significant difference between homogeneous and heterogeneous conditions (Figures 5E–H). The ^{15}N uptakes in leaf, culm, rhizome and the total ^{15}N uptake of connected treatment were significantly higher than those of severed treatment in both homogeneous and heterogeneous conditions ($P< 0.05$, Figures 5E–H). In addition, the ^{15}N uptakes in leaf, culm, rhizome and the total ^{15}N uptake of labeled ramet were significantly higher than those of unlabeled ramet under the same treatment ($P< 0.05$, Figure 5).

For the whole clonal fragment, no significant effect of rhizome status, environment status and their interaction on ^{15}N uptakes in leaf was observed, whereas significant effect on ^{15}N uptakes in rhizome was observed was found (Table 2). ^{15}N uptakes in leaf was not significantly different among the four treatments (Figure 6A). However, the ^{15}N uptakes in culm, rhizome and the total ^{15}N uptake of connected treatment in heterogeneous environments were significantly higher than other treatments ($P< 0.05$, Figures 6B–D). What's more, the total ^{15}N uptake of connected fragment was

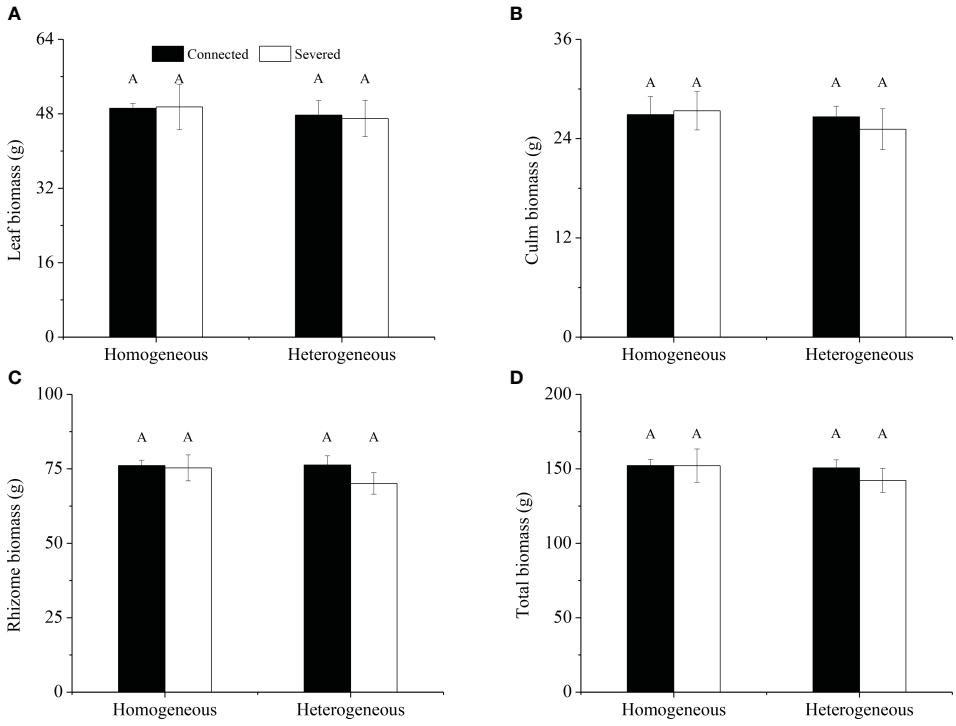

FIGURE 3
Leaf biomass **(A)**, culm biomass **(B)**, rhizome biomass **(C)** and total biomass **(D)** of the clonal fragment in homogeneous and heterogeneous environments. Different uppercase letters in each subgraph indicate statistically significant differences ($P< 0.05$) among the four treatments.

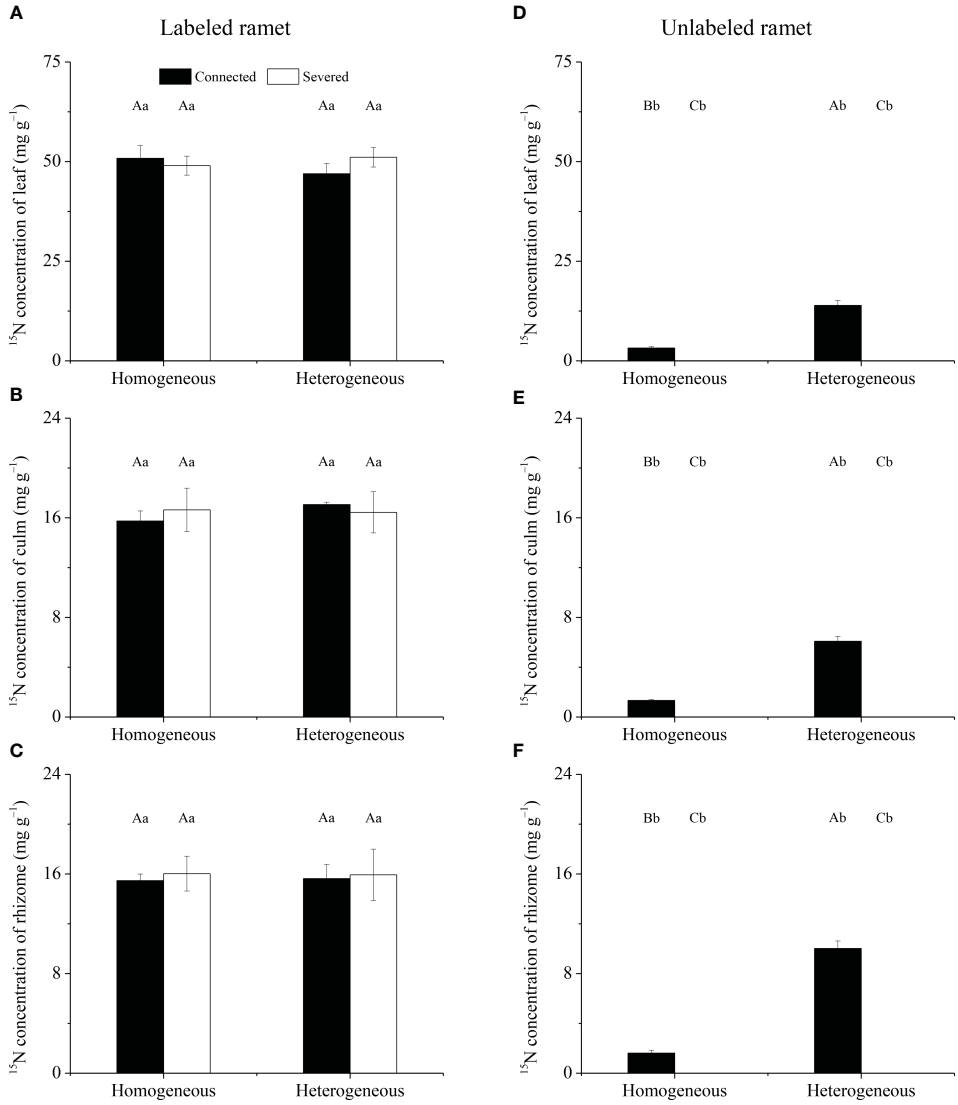

FIGURE 4
The ^{15}N concentrations in leaf **(A, D)**, culm **(B, E)** and rhizome **(C, F)** of the labeled **(A–C)** and unlabeled **(D–F)** ramets in homogeneous and heterogeneous environments. The labeled and unlabeled ramets were either connected or severed. Different uppercase letters in each subgraph indicate statistically significant differences ($P< 0.05$) among the four treatments, and different lowercase letters indicate statistically significant differences ($P< 0.05$) between labeled and unlabeled ramets under the same treatment.

higher than that of severed fragment in both homogeneous environments and heterogeneous environments (Figure 6).

3.4 Distributions of ^{15}N in the connected clonal fragment

In homogeneous environments, the percentage of ^{15}N absorption by unlabeled ramet was lower than the percentage of ^{15}N absorption by labeled ramet (Figure 7). Approximately 92.85% of the recovered ^{15}N was used by the labeled ramet, and its leaf, culm and rhizome retained 56.68%, 9.65% and 26.51% of ^{15}N, respectively. In contrast, only 7.15% of the recovered ^{15}N was moved to the unlabeled ramet, and its leaf, culm and rhizome retained 3.55%, 0.80% and 2.80% of ^{15}N, respectively. In heterogeneous environments, when ^{15}N-labeled urea was applied to one ramet, about 72.14% of the recovered ^{15}N was stored in the labeled ramet, and the distributions of ^{15}N in leaf, culm and rhizome were 41.89%, 8.51% and 21.74%, respectively. However, only 27.86% of the recovered ^{15}N was moved to the connected ramet, and the distributions of ^{15}N in leaf, culm and rhizome were 11.51%, 2.79% and 13.56%, respectively. As a result, the intensity of physiological integration (IPI) of the connected clonal fragments in heterogeneous environments was significantly higher than that in homogeneous environments ($P< 0.05$, Figure 8).

3.5 NUE of the clonal fragment

Significant effect of rhizome status on NUE was observed, while no significant effects of environment status and their interaction on

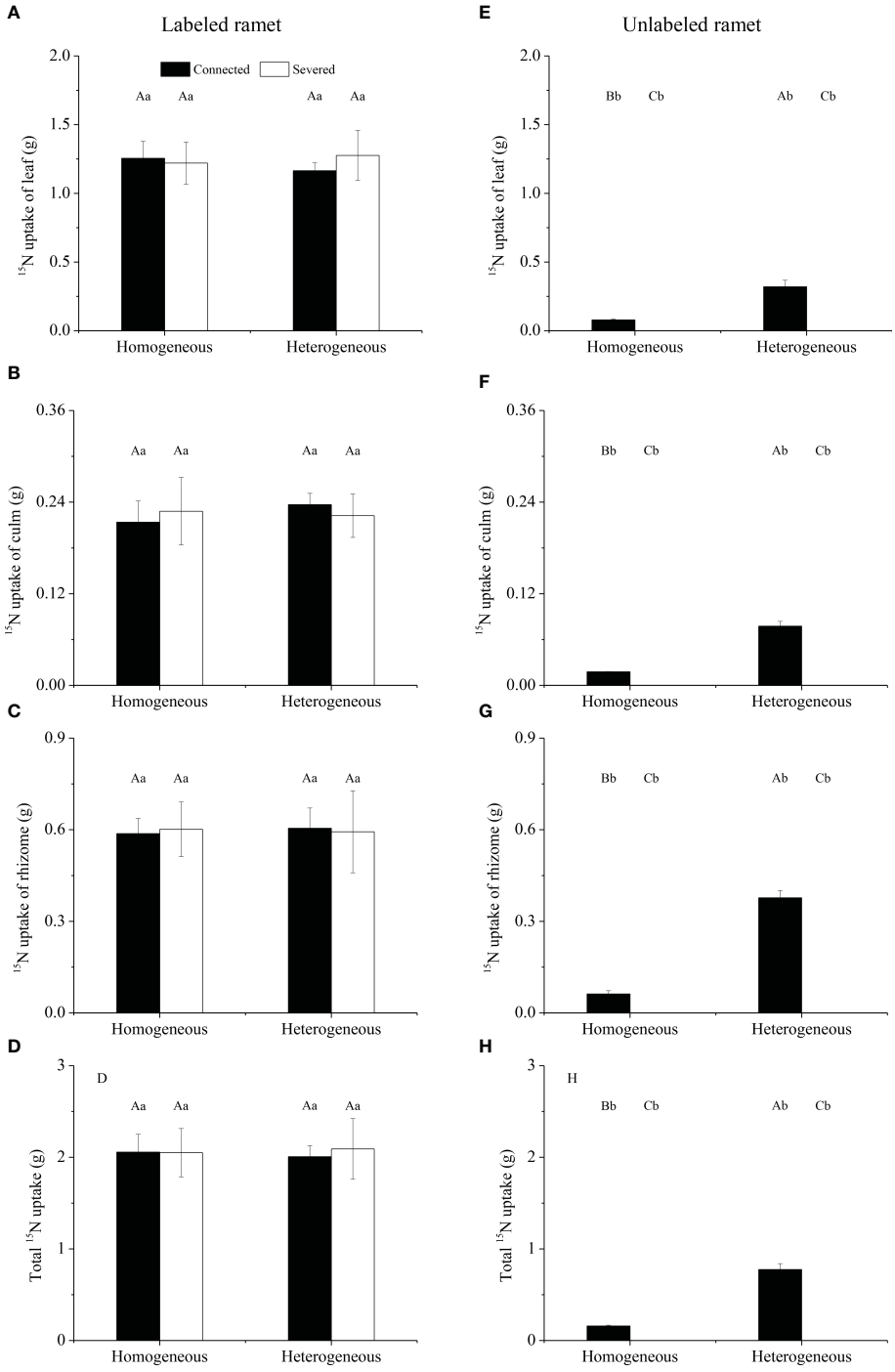

FIGURE 5
The ^{15}N uptakes in leaf **(A, E)**, culm **(B, F)**, rhizome **(C, G)** and total **(D, H)** of the labeled **(A–D)** and unlabeled **(E–H)** ramets in homogeneous and heterogeneous environments. The labeled and unlabeled ramets were either connected or severed. Different uppercase letters in each subgraph indicate statistically significant differences ($P< 0.05$) among the four treatments, and different lowercase letters indicate statistically significant differences ($P< 0.05$) between labeled and unlabeled ramets under the same treatment.

NUE were found (Table 3). The NUE of the connected clonal fragment in heterogeneous environments was significantly higher than that in homogeneous environments ($P< 0.05$), while no significant difference was found in severed clonal fragment between homogeneous and heterogeneous environments (Figure 9). The NUE of the connected clonal fragment was significantly higher than that of the severed clonal fragment in heterogeneous environments ($P< 0.05$), while no significant difference was found in homogeneous environments (Figure 9). In addition, the contribution rate of physiological integration (CPI) in heterogeneous environments was significantly higher than that in homogeneous environments ($P< 0.05$, Figure 10).

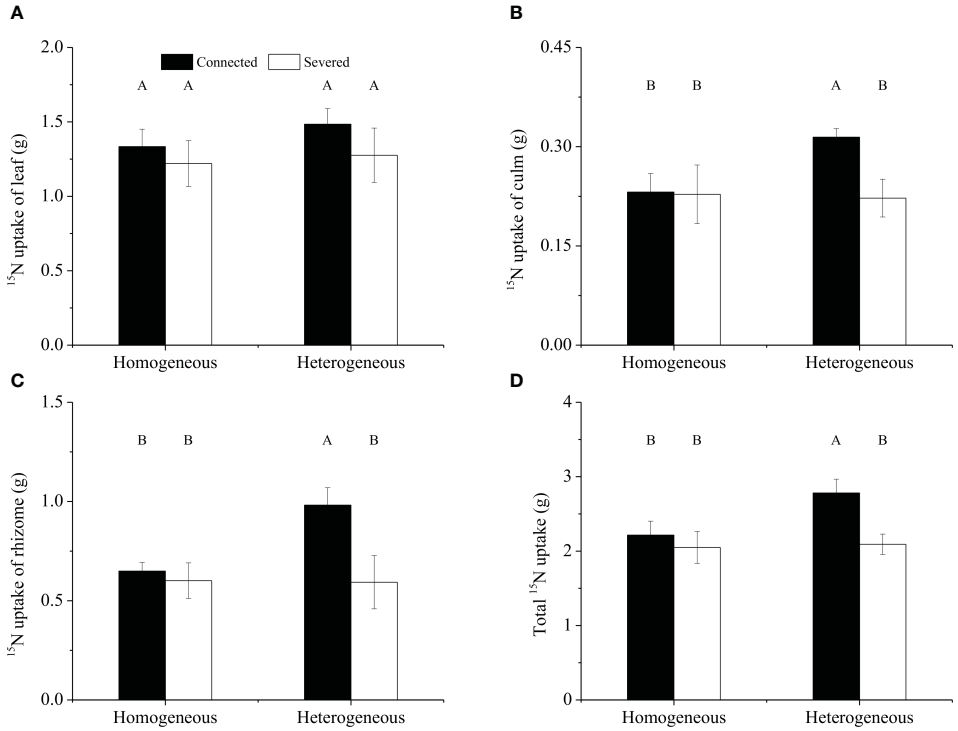

FIGURE 6
The ^{15}N uptakes in leaf **(A)**, culm **(B)**, rhizome **(C)** and total **(D)** ^{15}N uptake of the clonal fragment in homogeneous and heterogeneous environments. Different uppercase letters in each subgraph indicate statistically significant differences ($P< 0.05$) among the four treatments.

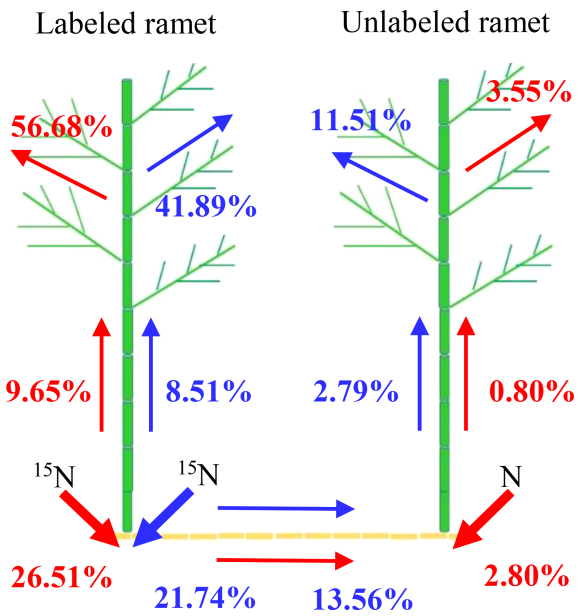

FIGURE 7
Distributions of the recovered ^{15}N in the connected clonal fragment. Red arrows indicate ^{15}N translocation and allocation in homogeneous environments, and blue arrows indicate ^{15}N translocation and allocation in heterogeneous environments. The values (%) from top to bottom represent the proportion of ^{15}N in leaf, culm and rhizome to the recovered ^{15}N, respectively.

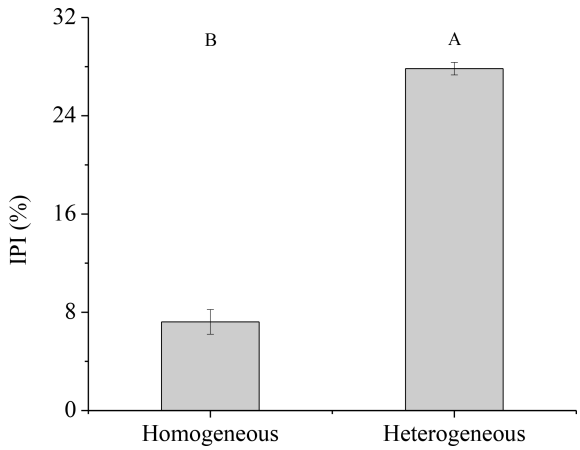

FIGURE 8
Intensity of physiological integration (IPI) of the connected clonal fragment in homogeneous and heterogeneous environments. Different uppercase letters indicate statistically significant differences ($P< 0.05$) between homogeneous and heterogeneous environments.

4 Discussion

This study investigated the effects of physiological integration on N translocation and NUE of moso bamboo in homogenous and heterogeneous environments. However, the performance of N translocation and NUE was inconsistent in homogeneous and heterogeneous environments.

Previous studies showed that habitat heterogeneity was the external driving force for spatial expansion of clonal plant, and physiological integration was the internal driving force for clonal growth in heterogeneous environments (Liu, 2011). In addition, physiological integration was affected by the source-sink relationship between the connected clonal ramets, and the resource translocation was governed by sink strength (Chen et al., 2015; Dong et al., 2015; Shi et al., 2021; Shi et al., 2022). In this study, ^{15}N translocation between labeled ramet and unlabeled ramet within clonal fragments of moso bamboo was found in heterogeneous environments (Figure 7). This discovery clearly revealed that moso bamboo was able to transfer ^{15}N between labeled ramet and unlabeled ramet through rhizome in heterogeneous environments. When ^{15}N-labeled urea was applied into one ramet (labeled ramet), approximately 27.86% of the absorbed ^{15}N was transferred to the other ramet (unlabeled ramet), and 72.14% of the absorbed ^{15}N was allocated to itself for growth. In other words, ramet grown under high-nutrient conditions could increase ^{15}N uptakes as a compensatory response to meet the demand of the other ramet grown under low-nutrient conditions (He et al., 2010). This result indicated that the ^{15}N allocation of fertilized ramet (labeled ramet) was higher than the connected unfertilized ramet (unlabeled ramet) for the growth of itself. The phenomenon with low rates of resource sharing was also found in *Fragaria chiloensis* in heterogeneous environments, which was called "selfish" (Alpert, 1999). Therefore, the fertilized ramet could transmit nutrients to the connected unfertilized ramet through physiological integration after meeting its own growth, and then improve the adaptability of the clonal fragment in heterogeneous environments.

Numerous studies showed that clonal plants rarely carried out physiological integration or refused physiological integration in homogenous environments (de Kroon et al., 1996; Alpert, 1999; Gao et al., 2013). In the present study, ^{15}N translocation between labeled ramet and unlabeled ramet within clonal fragments of moso bamboo was also found in homogenous environments (Figure 7). This discovery clearly revealed that moso bamboo was able to transfer ^{15}N between labeled ramet and unlabeled ramet through underground rhizome-root system in homogenous environments. When ^{15}N-labeled urea was applied into one ramet (labeled ramet), only 7.15% of the absorbed ^{15}N was transferred to the other ramet (unlabeled ramet), and 92.85% of the absorbed ^{15}N was allocated to itself for growth. This phenomenon suggested that N transfer occurred between the connected ramets within clonal fragment of moso bamboo in homogeneous environments, but the amount was relatively low. In other words, the unlabeled ramets did not require a lot of N supply from labeled ramets in homogeneous environments, which could decrease the cost of survival and maintain their internal balance (Gao et al., 2013).In both homogenous and heterogeneous environments, physiological integration significantly increased the ^{15}N uptakes of the unlabeled ramets (Figure 5), which was potentially able to increase the total ^{15}N uptakes and then improve the NUE of the

TABLE 3 Results of significance test for NUE of the whole clonal fragments.

	Rhizome status (R)	Environment status (E)	R×E
NUE	8.77**	4.43	3.30

Values are F ratio and significance are indicated by **($P< 0.01$).
The effects of rhizome status (connected vs. severed), environment status (homogeneous vs. heterogeneous) and their interaction were tested in two-way ANOVA.

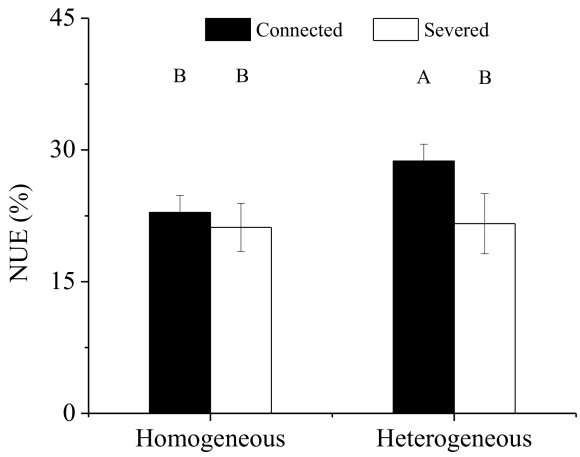

FIGURE 9
Nitrogen use efficiency (NUE) of the clonal fragment in homogeneous and heterogeneous environments. Different uppercase letters indicate statistically significant differences ($P< 0.05$) among the four treatments.

clonal fragments. Physiological integration had no significant effect on the total ^{15}N uptakes within the clonal fragments between connected and severed treatment in homogenous environments, while the total ^{15}N uptake of connected treatment was significantly higher than that of severed treatment in heterogeneous environments (Figure 6). Although the source-sink relationship was the driving force for physiological integration (Shi et al., 2021), physiological integration also occurred in homogeneous environments, with a lower intensity of physiological integration compared to heterogeneous environments (Figure 8).

Physiological integration has been considered an important factor in relation to altering nutrient use efficiency in clonal plants (He et al., 2010; Shi et al., 2022). In this study, we found that the NUE of the connected clonal fragment was significantly higher than that of the severed fragment in both homogenous and heterogeneous environments (Figure 9). This result indicated that there was a close link between NUE and physiological integration. In heterogeneous environments, ramets grown in low-nutrient conditions could obtain ^{15}N from high-nutrient conditions through physiological integration, thus improving the NUE of the whole clonal fragment. Although the ^{15}N uptakes of labeled ramets decreased, the ^{15}N uptakes of unlabeled ramets increased in both homogenous and heterogeneous environments, and the increase in the unlabeled ramet was much greater than the reduction in the labeled ramet (Figure 5). As a result, a net benefit of physiological integration to the whole clonal fragment was found (Wang et al., 2021), which improved the NUE of moso bamboo. However, the contribution rate of physiological integration (CPI) on NUE was not consistent between homogenous and heterogeneous environments, and the difference was significant. Our results also indicated that physiological integration was more likely to occur in heterogeneous environments, while it rarely occurred in homogeneous environments.

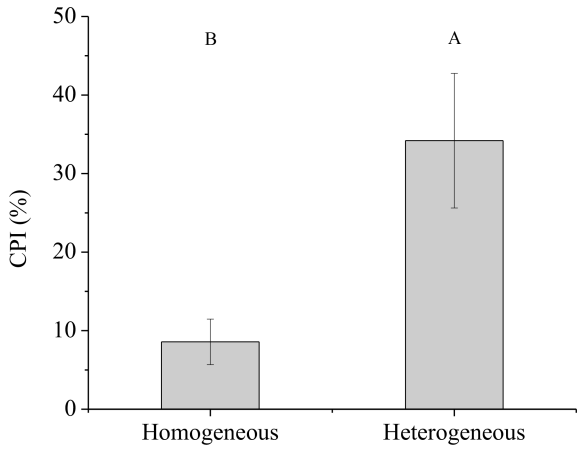

FIGURE 10
The contribution rate of physiological integration (CPI) of the connected treatments. Different uppercase letters indicate statistically significant differences ($P< 0.05$) between homogeneous and heterogeneous environments.

5 Conclusion

This study clearly revealed the relationship between physiological integration and N translocation and NUE of moso bamboo in homogenous and heterogeneous environments. The N translocation between the connected ramtes of moso bamboo was determined by the source-sink relationship in heterogeneous environments. Physiological integration was also occurred in homogeneous environments, but its intensity was relatively low. There was a close relationship between physiological integration and NUE, and physiological integration significantly improved the NUE of moso bamboo in both homogeneous and heterogeneous environments. These results will provide theoretical basis for precision fertilization in moso bamboo forests.

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

JZ: Conceptualization, Investigation, Formal analysis, Writing-original draft, Funding acquisition. CC: Conceptualization, Formal analysis, Writing-review & editing, Supervision, Funding acquisition. All authors contributed to the article and approved the submitted version.

Funding

This work was supported by the Natural Science Foundation of Zhejiang Province, China (LQ19C160007), the Special Project of Zhejiang Provincial Scientific Research Institutes, China (2021F1065-3), and the Special Research fund of the International Centre for Bamboo and Rattan, China (1632021006).

Acknowledgments

We are sincerely grateful to the staff of Bamboo Botanical Garden for their assistance. We also thank reviewers and editors for their valuable suggestions to improve the article.

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

Alpert, P. (1999). Clonal integration in *Fragaria chiloensis* differs between populations: ramets from grassland are selfish. *Oecologia* 120, 69–76. doi: 10.1007/s004420050834

Chen, B. J. W., Vermeulen, P. J., During, H. J., and Anten, N. P. R. (2015). Testing for disconnection and distance effects on physiological self-recognition within clonal fragments of *Potentilla reptans*. *Front. Plant Sci.* 6. doi: 10.3389/fpls.2015.00215

de Kroon, H., Fransen, B., van Rheenen, J. W. A., van Dijk, A., and Kreulen, R. (1996). High levels of inter–ramet water translocation in two rhizomatous *Carex species*, as quantified by deuterium labeling. *Oecologia* 106, 73–84. doi: 10.1007/BF00334409

Dong, B. C., Alpert, P., Zhang, Q., and Yu, F. H. (2015). Clonal integration in homogeneous environments increases performance of *Alternanthera philoxerodies*. *Oecologia* 179 (2), 393–403. doi: 10.1007/s00442-015-3338-y

Gao, L., Li, B., Liu, W. Y., Shen, Y. X., and Liu, W. J. (2013). Inhibition effects of daughter ramets on parent of clonal plant *Eichhornia crassipes*. *Aquat. Bot.* 107, 47–53. doi: 10.1016/j.aquabot.2013.01.010

Guan, F., Xia, M., Tang, X., and Fan, S. (2017). Spatial variability of soil nitrogen, phosphorus and potassium contents in moso bamboo forests in yong'an city, China. *Catena* 150, 161–172. doi: 10.1016/j.catena.2016.11.017

He, W. M., Yu, F. H., and Zhang, L. L. (2010). Physiological integration impacts nutrient use and stoichiometry in three clonal plants under heterogeneous habits. *Ecol. Res.* 25, 967–972. doi: 10.1007/s11284-010-0724-0

Jiang, Z. H. (2007). *Bamboo and rattan in the world* (Beijing: China Forest Publishing House).

Li, L., Li, N., Zang, Z., Lu, D., Wang, G., and Wang, N. (2021). Examining phonological variation of on-year and off-year bamboo forests based on the vegetation and environment monitoring on a new micro-satellite (VENμS) time-series data. *Int. J. Remote Sens.* 42 (6), 2203–2219. doi: 10.1080/01431161.2020.1851802

Li, Y., Ning, W., Xu, S., Yu, N., Chen, Z., and Zhang, L. (2022). Response of physiological integration in the clonal herb *Zoysia japonica* to heterogeneous water conditions. *Acta Physiol. Plant* 44, 34. doi: 10.1007/s11738-022-03367-w

Liu, W. (2011). *The strategy of spatial expansion of clonal populations leymus chinesis and carex duriuscula in heterogeneous habitats. (dissertation/master's thesis)* (Changchun: Northeast Normal University).

Mao, C., Qi, L., Liu, Q., Song, X., and Zhang, Y. (2016). The distribution and use efficiency of nitrogen in *Phyllostachys edulis* forest. *Scientia Silvae Sinicae* 52 (5), 64–70. doi: 10.11707/j.1001-7488.20160508

National Forestry and Grassland Administration (2019). *Report for Chinese forest resource, (2014–2018)* (Beijing: China Forest Publishing House).

Portela, R., Barreiro, R., and Roiloa, S. R. (2021). Effects of resource sharing directionality on physiologically integrated clones of the invasive *Carpobrotus edulis*. *J. Plant Ecol.* 14, 884–895. doi: 10.1093/jpe/rtab040

Saitoh, T., Seiwa, K., and Nishiwaki, A. (2006). Effects of resource heterogeneity on nitrogen translocation within clonal fragments of *Sasa palmate*: an isotopic (^{15}N) assessment. *Ann. Bot.* 98 (3), 657–663. doi: 10.1093/aob/mc1147

Shi, J., Mao, S., Wang, L., Ye, X., Wu, J., Wang, G., et al. (2021). Clonal integration driven source-sink relationships is constrained by rhizome branching architecture in a running bamboo species (*Phyllostachys glauca*): a ^{15}N assessment in the field. *For. Ecol. Manage.* 481, 118754. doi: 10.1016/j.foreco.2020.118754

Shi, M., Zhang, J., Sun, J., Li, Q., Lin, X., and Song, X. (2022). Unequal nitrogen translocation pattern caused by clonal integration between connected ramets ensures necessary nitrogen supply for young moso bamboo growth. *Environ. Exp. Bot.* 220, 104900. doi: 10.1016/j.envexpbot.2022.104900

Song, X., Peng, C., Zhou, G., Gu, H., Li, Q., and Zhang, C. (2016). Dynamic allocation and transfer of non-structural carbohydrates, a possible mechanism for the explosive growth of moso bamboo (*Phyllostachys heterocycla*). *Sci. Rep.* 6, 25908. doi: 10.1038/srep25908

Song, X., Zhou, G., Jiang, H., Yu, S., Fu, J., Li, W., et al. (2011). Carbon sequestration by Chinese bamboo forests and their ecology benefits: assessment of potential, problems, and future challenges. *Environ. Rev.* 19, 418–428. doi: 10.1139/a11-015

Su, W. (2012). *Fertilization theory and practice for phyllostachys edulis stand based on growth and nutrient accumulation rules. (dissertation/doctor's thesis)* (Beijing: Chinese Academy of Forestry).

Su, W., Fan, S., Zhao, J., and Cai, C. (2019). Effects of various fertilization placements on the fate of urea-^{15}N in moso bamboo forests. *For. Ecol. Manage.* 453, 117632. doi: 10.1016/j.foreco.2019.117632

Sun, H., Li, Q., Lei, Z., Zhang, J., Song, X., and Song, X. (2019). Ecological stoichiometry of nitrogen and phosphorus in moso bamboo (*Phyllostachys edulis*) during the explosive growth period of new emergent shoots. *J. Plant Res.* 132, 107–115. doi: 10.1007/s10265-018-1070-5

Tang, X., Fan, S., Qi, L., Guan, F., Liu, G., and Du, M. (2015). Effects of understory removel on root production, turnover and total belowground carbon allocation in moso bamboo forests. *iForest* 9 (2), 187–194. doi: 10.3832/ifor1674-008

Vorontsova, M. S., Clark, L. G., Dransfield, J., Govaerts, R., and Baker, W. J. (2017). *World checklist of bamboos and rattans* (Beijing: Science Press).

Wang, J., Xu, T., Wang, Y., Li, G., Abdullah, I., Zhong, Z., et al. (2021). A meta-analysis of effects of physiological integration in clonal plants under homogeneous vs. heterogeneous environments. *Funct. Ecol.* 35 (3), 578–589. doi: 10.1111/1365-2435.13732

Zhao, J. (2016). *Study on the role of nitrogen utilization and its influence factors in phyllostachys edulis forest* (Beijing: Chinese Academy of Forestry).

Zhao, J., Su, W., Fan, S., Cai, C., Su, H., and Zeng, X. (2019). Ammonia volatilization and nitrogen runoff losses from moso bamboo forests under different fertilization practices. *Can. J. For. Res.* 49 (3), 213–220. doi: 10.1139/cjfr-2018-0017

Zhao, J. C., Su, W. H., Fan, S. H., Cai, C. J., Zhu, X. W., and Peng, C. (2016). Effects of various fertilization depths on ammonia volatilization in moso bamboo (*Phyllostachys edulis*) forests. *Plant Soil Environ.* 62 (3), 128–134. doi: 10.17221/733/2015-PSE

Zhao, J., Yang, Z., Zou, J., and Li, Q. (2022). Allelopathic effects of sesame extracts on seed germination of moso bamboo and identification of potential allelochemicals. *Sci. Rep.* 12, 6661. doi: 10.1038/s41598-022-10695-x

Zhuang, M. H., Li, Y. C., and Chen, S. L. (2011). Advances in the researches of bamboo physiological integration and its ecological significance. *J. Bamboo Res.* 30 (2), 5–9. doi: 10.3969/j.issn.1000-6567.2011.02.002

frontiers | Frontiers in Plant Science

TYPE Original Research
PUBLISHED 10 July 2023
DOI 10.3389/fpls.2023.1210070

OPEN ACCESS

EDITED BY
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RECEIVED 21 April 2023
ACCEPTED 31 May 2023
PUBLISHED 10 July 2023

CITATION
Zhang J, You W-H, Li N-N and Du D-L (2023) Invasive clonal plants possess greater capacity for division of labor than natives in high patch contrast environments.
Front. Plant Sci. 14:1210070.
doi: 10.3389/fpls.2023.1210070

Invasive clonal plants possess greater capacity for division of labor than natives in high patch contrast environments

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Invasion success of clonal plants is closely related to their unique clonal life history, and clonal division of labor is a crucial clonal trait. However, so far, it is unclear whether invasive alien clonal species generally possess a greater capacity for division of labor than native species and whether this pattern is affected by environmental conditions. To test whether patch contrast affects the differences in the capacity for division of labor between invasive alien and native clonal plants, we selected five pairs of exotic invasive and native clonal plant species that are congeneric and co-occurring in China as experimental materials. We grew the clonal fragment pairs of these invasive and native plants under high, low, or no contrast of reciprocal patchiness of light and nutrient, respectively, with ramet connections either severed (division of labor prevented) or kept intact (division of labor allowed). The results showed that connection significantly decreased the proportion of biomass allocated to roots in distal (younger) ramets, whereas it increased in proximal (older) ramets of all studied plants under high -contrast treatments. This clear pattern strongly indicated the occurrence of division of labor. Furthermore, the connection had a more pronounced effect on the pattern of biomass allocation of invasive alien plants, resulting in a greater increase in biomass for invasive alien plants compared to native plants. These findings suggest that the invasive alien plants possess a greater capacity for division of labor, which may confer a competitive advantage to them over natives, thus facilitating their invasion success in some heterogeneous habitats such as forest edges where light and soil nutrients show a high negative correlation.

KEYWORDS

plant invasion, clonal plants, clonal integration, heterogeneity, root to shoot ratio

Introduction

Biological invasions are reported to be a major threat to global biodiversity and can cause serious economic and ecological damage (Diagne et al., 2021; Li et al., 2022). Many studies have been conducted to identify and understand the mechanisms underlying the process of invasion, and some inherent traits associated with clonal growth are widely recognized as critical determinants contributing to plant invasiveness (Song et al., 2013; Roiloa, 2019). The plausibility of this argument rests on the fact that many of the most problematic invasive plant species exhibit clonal propagation (Cadotte et al., 2006; Roiloa, 2019). Furthermore, recent studies have demonstrated that clonal- introduced plants decrease the native richness to a greater extent than non-clonal introduced plants worldwide (Vilà et al., 2015; Franklin et al., 2021).

A crucial clonal trait is the capacity for division of labor mediated by physiological integration and driven by source–sink relationships (Hutchings and Wijesinghe, 1997; Xi et al., 2019). It is defined as the specialization of resource uptake between independent parts of the clonal plants (Stuefer, 1998; Roiloa et al., 2016). In nature, resources essential for plant growth and survival, such as light, water, and nutrients, are typically distributed unevenly (Jackson and Caldwell, 1993; Peipoch et al., 2016). By means of vegetative growth and reproduction, clonal plants have the ability to occupy extensive areas, thus increasing their potential to encounter environmental heterogeneity (Stuefer and Hutchings, 1994, Marshall, 1999). In some environments, the availability of two resources may be negatively correlated, especially when high availability of one resource is accompanied by a decline in the other (Friedman and Alpert, 1991; Struefer et al., 1996). For instance, nitrogen-fixing shrubs could increase the effectiveness of soil N but reduce the level of light under their canopy (Friedman and Alpert, 1991; Roiloa et al., 2007; Wang et al., 2011). The division of labor allows each ramet to capture locally abundant resources, as resource uptake is more economical in more resource-rich patches, and subsequent reciprocal transfer of resources between ramets should improve the performance of the whole clone (Stuefer et al., 1996; Huang et al., 2018; Roiloa, 2019). Numerous studies have shown that the negative correlation between the spatial distribution of different basic resources induces a division of labor and that the division of labor improves the performance of clonal plants (Friedman and Alpert, 1991; Wang et al., 2016; Huang et al., 2018; Lin et al., 2018).

Contrast, which refers to the extent of the relative variation in resource availability between patches or between a patch and its surrounding matrix, constitutes one aspect of environmental heterogeneity (Struefer et al., 1996). Theoretical studies predict that greater patch contrast may lead to a stronger division of labor (Stuefer et al., 1998; Magyar et al., 2007). This is also supported by experimental evidence. For example, Wang et al. (2011) found that the division of labor occurred only when the patch contrast exceeded a threshold in an environment where soil nutrients and light were negatively correlated. Roiloa et al. (2007) showed that clones from habitats with greater patch contrast had a stronger division of labor than those from more homogeneous habitats. More recently, a study by Roiloa et al. (2019) found that the highly invasive exotic clonal species *Carpobrotus edulis* exhibited greater division of labor relative to the exotic non-invasive clonal species *Carpobrotus chilensis*. This suggests that the division of labor may be a feature of the correlation between clonal growth and plant invasion (Roiloa et al., 2019). However, to date, little is known about whether the capacity for division of labor between invasive alien clonal plants and native plants differs in certain regions and how it is affected by patch contrast.

In the present experiment, to avoid large differences between invasive and native species in their habitat preferences and phylogenetic relatedness (Felsenstein, 1985), we selected five pairs of congeneric and co-occurring invasive and native clonal plant species to serve as experimental material. We grew the clonal fragment pairs of these invasive and native plants under high, low, or no contrast of reciprocal patchiness of light and nutrient, respectively, and with ramet connections either severed (division of labor prevented) or kept intact (division of labor allowed). We predicted that invasive plants have greater capacity for division of labor than natives. Based on the theoretical studies, we further predicted that the difference in the capacity for division of labor would be greater under higher patch contrast.

Materials and methods

Species selection and cultivation

We chose five pairs of asexual clonal plants, three of which were stoloniferous and the other two were rhizomes, as described in Table 1. In each pair, one species is an invasive alien species, and the other is a common native species in China that is co-occurring with the invasive alien species in the wild. We opted for species within the same family (or genus) in order to elucidate the phylogenetic correlation between the two species within each pair. All plants used were collected from the field in Jiangsu Province or Guangdong Province (China). To enhance the probability of collecting plant material from different genotypes (genes), we obtained multiple fragments of each species from various locations separated by over 500 m. Then, the collected fragments were propagated asexually in a greenhouse at Jiangsu University in Zhenjiang, Jiangsu Province, China. In April 2022, 36 similarly sized pairs of plants of each species were selected for the following experiment, each pair consisting of two rooted, similarly sized ramets interconnected by a single stolon or rhizome internode.

Experimental design

The experiment took place in the greenhouse at Jiangsu University. In late April 2022, we transplanted each pair of ramets into two plastic pots measuring 120×88×188 mm (top bottom × bottom × height), with a small 2×2cm opening at the top of each pot for the rhizome or stolon connecting the two ramets to pass through. The substrate consisted of a blend of river sand and yellow-brown soil in a 1:1 ratio by volume, with a very low nutrient concentration (Xi et al., 2019).

10.3389/fpls.2023.1210070

TABLE 1 Clonal plant species used in the experiment.

Species	Family	Origin	Native range	Clonal organ	Typical habitat
***Sphagneticola trilobata* (L.) Pruski**	Asteraceae	Invasive alien	North and South America	Stolon	Moist grasslands, edges of canals, roadsides
***Sphagneticola calendulacea* (L.) Pruski**	Asteraceae	Native	Asia	Stolon	Moist grasslands, edges of canals, crop fields, roadsides
***Alternanthera philoxeroides* (Mart.) Griseb**	Amaranthaceae	Invasive alien	South America	Stolon	Wetlands, canals, nearby fields
***Alternanthera sessilis* (L.) DC**	Amaranthaceae	Native	Asia, Africa	Stolon	Wetlands, other moist habitats
***Hydrocotyle verticillata* Thunb.**	Araliaceae	Invasive alien	North America, Europe	Stolon	Wetlands, other moist habitats
Hydrocotyle sibthorpioides	Araliaceae	Native	Asia	Stolon	Wetlands, other moist habitats
***Paspalum notatum* Flugge**	Poaceae	Invasive alien	North and South America	Rhizome	Roadsides and grasslands
***Paspalum orbiculare* (G. Forster) Hackel**	Poaceae	Native	Asia, Oceania	Rhizome	Roadsides, other moist habitats
***Paspalum virgatum* L.**	Poaceae	Invasive alien	South America	Rhizome	Moist grasslands
***Paspalum distichum* L.**	Poaceae	Native	Tropics and subtropics of Asia, America	Rhizome	Moist grasslands

Origin and habitat information are based on the Flora of China (www.iplant.cn), Scientific Database of China Plant Species (DCP) (http://www.plants.csdb.cn/eflora), and other reference (Wang et al. (2017).

After a recovery period of approximately 1 week, we conducted the experiment to assess the impacts of species origin, intact stolon/rhizome, and patch contrast. We designated younger ramets growing in high light and low nutrient patches as distal ramets and older ramets growing in low light and high nutrient patches as proximal ramets. The connection between the two ramets was either severed in the middle (preventing division of labor) or kept intact (allowing division of labor).

The light and nutrient addition protocols for all fragments in the experiment are presented in Table 2. To create different patch contrast environments, we used polypropylene shade nets of varying shade intensities to cover the ramets, while controlling nutrient effectiveness through the use of different quality of slow-release fertilizers. For each combination of the experiment, we established six replicates, resulting in a total of 360 ramet pairs across 10 species. During the experimental period, regular watering was provided to support plant growth, and the average light intensity at noon was 1,200–1,400 μmol m^{-2} s^{-1}, with a mean air temperature of 25°C–32 °C in the greenhouse. The experiment was conducted for 9 weeks and ended in early July 2022.

Measurements

We harvested the distal and proximal ramets in each pair of containers. The clonal fragments in each container were separated into below-ground (root) and above-ground (shoot) parts. Different plant parts were dried in an oven at 80°C for 72 h and then weighed to obtain the dried biomass.

Statistical analysis

We used histograms and quantile–quantile plots to graphically check whether the residuals of all models were normally distributed. This was made using the *ggplot* function of the " ggplot2" package

TABLE 2 Light exposures and nutrient concentrations applied to the ramets in three treatments with different patch contrasts (control, low, and high).

	Proximal		Distal	
Patch contrast	Light (% full sunlight)	Nutrient (g)	Light (% full sunlight)	Nutrient (g)
Control	55	0.5	55	0.5
Low contrast	40	0.7	70	0.3
High contrast	10	0.9	100	0.1

Notes: The fertilizer used is slow-release fertilizer, Osmocote^{R}, N–P–K: 16–9–12.

(Wilkinsom, 2016) in R 4.2.0 (R Core Team, 2022). Data transformation was performed to satisfy normality if necessary. We analyzed the effect of treatments on the biomass and root to shoot ratio using a linear mixed model with the *lme* function from the R package "nlme" (Pinheiro et al., 2020). In these models, we used species origin (invasive vs. native), intact (stolon/rhizome remaining connected or severed), patch contrast (control vs. low contrast vs. high contrast), and their interaction as fixed factors. To account for the differences between species pairs and species, we included species nested within species pairs as random factors in our model. In addition, since the variance varies between species, we used the *varIdent* function of the " nlme" package to allow each species to have a different variance structure (Pinheiro et al., 2020). The significance of fixed effects was assessed using likelihood ratio tests when comparing models with and without the effects of interest (Zuur et al., 2009). All analyses were performed using the free software R (version 4.2.0; R Development Core Team, 2022).

Results

Overall, invasive plants had a greater biomass than native plants (Figure 1). Connection (intact) significantly increased the root to shoot ratio of proximal ramets, whereas it decreased in distal ramets under high contrast, as indicated by significant intact × contrast interaction (Table 3; Figure 2). The effect of connection on the root to shoot ratio of proximal ramets was more significant in invasive plants than in native plants under high contrast (significant origin × intact × contrast interactions in Table 3; Figures 2A, C). Similar results also occurred in the distal ramets (Table 3; Figures 2B, D). Moreover, the connection greatly increased the total biomass (proximal biomass + distal biomass) of the whole clone under high contrast, especially for invasive species (significant origin × intact × contrast interactions in Table 3; Figure 1).

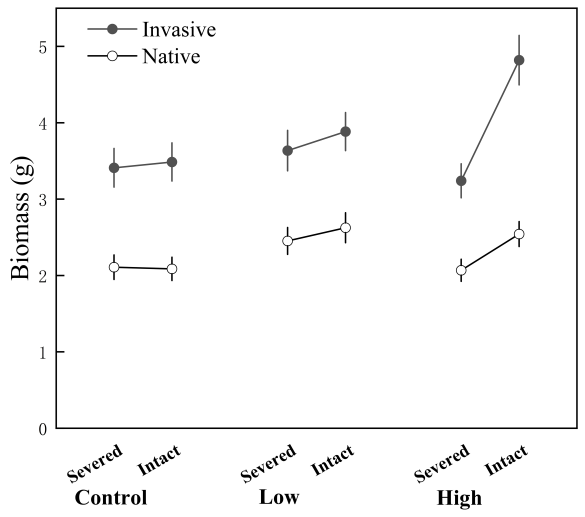

FIGURE 1
Biomass of the whole clone of the invasive alien and native clonal species when the clone was grown under high- contrast, low-contrast, and control treatments with connections between the proximal and distal ramets severed or remained intact. Values are means ± standard error (SE).

Discussion

Not entirely consistent with the conjecture, our results suggested that in a patchy environment where light and nutrients were negatively correlated, division of labor occurred only under high- contrast treatments and that invasive alien plants had a greater capacity for division of labor than native plants. We can conclude that there is a difference in the capacity for division of labor between invasive alien and native clonal plants, but this difference is environmentally dependent.

No division of labor was observed in either the control or low-contrast treatments. One possible explanation is that division of labor is more likely to occur in patchy habitats where plant functions are limited due to resource scarcity. Previous studies suggest that when ramets are cultivated under optimal conditions, they do not exhibit adaptive responses to heterogeneous environment (Zhang and He, 2009). However, this explanation appears somewhat implausible when considering the findings of other researchers, who have demonstrated that the resource settings in our control and low-contrast treatments are indeed capable of reducing plant growth performance (Guo et al., 2011). In a patchy environment with reciprocal resource distribution, division of labor emerges as a vital mechanism for enhancing the performance of clonal plants. Each ramet concentrates its efforts on locally abundant resources. However, if the patch environment undergoes changes or the connections between ramets are severed, each ramet is then confronted with a scarcity of locally resources, at which point the division of labor may become detrimental (Stuefer et al., 1998; Magyar et al., 2007; Ikegami et al., 2008). Consequently, division of labor occurs exclusively when the growth of ramets faces significant constraints and the benefits derived from resource exchange far outweigh the associated costs. This is the result of a clone-wide cost–benefit tradeoff that is important for risk aversion, especially in disturbed environments (Wang et al., 2011).

In fact, there are also studies showing that clonal plants can significantly alter their distribution balance and show division of labor even in homogeneous environments (Dong et al., 2015; Xi et al., 2019). This phenomenon, known as "developmentally programmed division of labor," is inherently governed by the plant's internal developmental processes, independent of external environmental factors (Liu et al., 2016). In addition, the division of labor can be achieved not only by adjusting biomass allocation but also by regulating certain physiological functions and may be easier to express (Roiloa et al., 2007). Since physiological characteristics are more readily reversible compared to morphological traits, the risks to entire clones or clone fragments may be relatively lower (Wang et al., 2011). For example, Wang et al. (2011) found that all-lit connected ramets displayed significantly higher photosynthetic capacity than isolated ramets in three different patch contrast treatments of high, medium, and low, suggesting that the connected ramets are specialized for photosynthesis.

Connection significantly increased the proportion of biomass allocated to roots in proximal ramets and the proportion of biomass allocated to shoots in distal ramets under high- contrast treatments. This clear pattern strongly indicated the occurrence of division of labor. Additionally, we observed a substantial increase in the overall biomass

TABLE 3 Results of linear mixed models for effects of origin (invasive vs. native), patch contrast (control vs. low vs. high), and intact (connection vs. severed) on the total biomass of the whole clone and the root to shoot ratio of distal ramets and proximal ramets.

Model terms *Fixed effects*	df	Root to shoot ratio of distal ramets χ2 p		Root to shoot ratio of proximal ramets χ^2 p		Total biomass χ^2 p	
Intact (I)	1	**18.412**	**<0.001**	**31.712**	**<0.001**	**19.067**	**<0.001**
Contrast (C)	2	**47.807**	**<0.001**	**20.319**	**<0.001**	**15.135**	**0.005**
Origin (O)	1	0.687	0.407	**5.244**	**0.022**	**10.959**	**0.009**
I × C	2	**29.779**	**<0.001**	**69.174**	**<0.001**	**23.061**	**<0.001**
I × O	1	**6.024**	**0.014**	**5.525**	**0.018**	**6.648**	**0.01**
C × O	2	4.811	0.090	**12.032**	**0.002**	2.082	0.353
I × C × O	2	3.983	0.136	**8.375**	**0.015**	**6.248**	**0.044**
Random effects	N		**SD**		**SD**		**SD**
Taxonomic pair	5		0.552		0.155		0.833
Species identity[a]	10		0.066		0.019		0.382
Residual			0.428		0.099		0.546

Significant effects ($p < 0.05$) are shown in bold.
[a]The SDs shown in the table are for the alien species Wedelia trilobata (L.) Hitchc, and the SDs for all species are shown in Supplementary Table S1.

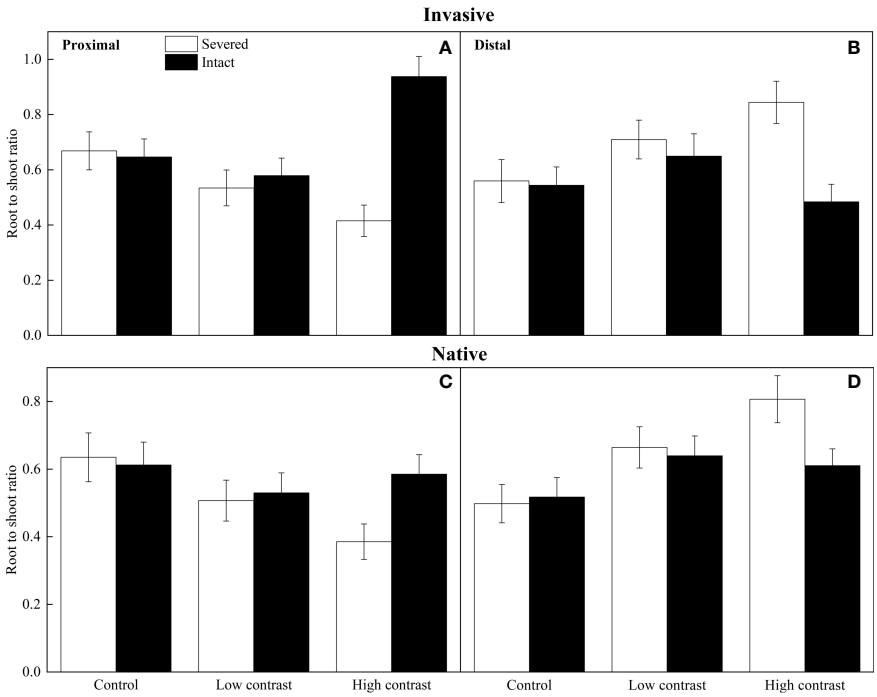

FIGURE 2
Effects of experimental treatments on the root to shoot ratio of proximal and distal ramets of the invasive alien **(A, B)** and native **(C, D)** clonal species. The data indicate the means ± SE.

of the entire clonal fragment when connected. These findings align with previous studies that have demonstrated the advantageous uptake and exchange of resources among clonal plants in heterogeneous resource environments, resulting in enhanced production efficiency, increased biomass, and improved fitness of the entire clonal system (You et al., 2013; You et al., 2014). Furthermore, our results highlighted a noteworthy distinction: the resource uptake specialization was significantly more pronounced in invasive alien plants compared to native plants. This suggests that invasive alien plants possesse a greater capacity for division of labor, which also leds to a greater increase in biomass for invasive species. The disparity in division of labor capabilities may confer a competitive advantage to invasive plants over native species, thereby facilitating their invasion.

Disregarding the division of labor, the invasive alien plants always had a greater biomass than native plants. Our results do not directly indicate that invasive plants are more competitive. In fact, a recent

study by Wang et al. (2019) revealed that invasive clonal plants exhibited increased biomass production and vegetative reproduction when grown in the presence of interspecific competition compared to intraspecific competition, while the opposite was observed for native clones. This suggests that the invasive clonal plants are competitively superior to concurrently co-occurring native plants. The high intrinsic growth rates of the invasive plants may be the main driver of its high competitive ability (Zhang and van Kleunen, 2019).

One notable aspect to consider is that our study solely focused on the spatial heterogeneity of resource availability, disregarding temporal heterogeneity. Consequently, this study may not accurately reflect real-life habitat environments (Yu et al., 2018; Wang et al., 2021). A modeling study conducted by Magyar et al. (2007) indicated that the advantage of plasticity diminishes as the rate of environmental change intensifies, suggesting that we may be overestimating the benefits of division of labor. Therefore, future studies should strive to provide additional experimental evidence to further investigate this matter. Nonetheless, our empirical research suggests that invasive clonal plants have a stronger capacity for division of labor than native plants under high contrast.

Furthermore, Roiloa et al. (2016) found that the invasive clonal plant *C. edulis* could benefit more from division of labor in the invasion site compared to the native population through common garden experiments, suggesting that the division of labor, which positively contributes to the clonal growth and reproduction of clonal plants, has evolved rapidly and adaptively in the invasion area. Nevertheless, additional research is necessary to determine whether this adaptive evolution is a common occurrence among other invasive species.

In conclusion, our results showed that in a patchy environment where light and soil nutrients were highly negatively correlated, both exotic invasive clonal plants and native clonal plants in China were able to alleviate the pressure of resource scarcity and promote their own growth through division of labor. More importantly, invasive clonal plants had significantly greater capacity for clonal division of labor than native plants, which also brought them greater biomass increase. The difference in the capacity for clonal division of labor between exotic invasive clonal plants and native clonal plants may explain the success of invasion in certain habitats such as forest edge where light and soil nutrients show a high negative correlation.

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.

Author contributions

JZ and W-HY conceived and designed the experiments. JZ and N-NL performed the experiments. W-HY and JZ analyzed the data. W-HY and D-LD contributed reagents, materials, and analysis tools. JZ, N-NL and W-HY wrote the manuscript. All authors contributed to the article and approved the submitted version.

Funding

We greatly appreciate the financial support by the National Natural Science Foundation of China (31770394 and 32071521), the carbon peak and carbon neutrality technology innovation foundation of Jiangsu Province (BK20220030), and the Jiangsu Province Engineering Research Center of Green Technology and Contigency Management for Emerging Pollutants.

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.2023.1210070/full#supplementary-material

SUPPLEMENTARY TABLE 1
Standard deviations of each invasive alien and native clonal species for each of the response variables in the models presented in Table 3.

References

Cadotte, M. W., Murray, B. R., and Lovett-Doust, J. (2006). Ecological patterns and biological invasions: using regional species inventories in macroecology. *Biol. Invasions* 8 (4), 809–821. doi: 10.1007/s10530-005-3839-4

Diagne, C., Leroy, B., Vaissière, A.-C., Gozlan, R. E., Roiz, D., Jarić, I., et al. (2021). High and rising economic costs of biological invasions worldwide. *Nature* 592 (7855), 571–576. doi: 10.1038/s41586-021-03405-6

Dong, B. C., Alpert, P., Zhang, Q., and Yu, F. H. (2015). Clonal integration in homogeneous environments increases performance of *Alternanthera philoxeroides*. *Oecologia* 179 (2), 393–403. doi: 10.1007/s00442-015-3338-y

Felsenstein, J. (1985). Phylogenies and the comparative method. *Am. Nat.* 125 (1), 1–15. doi: 10.1086/284325

Franklin, S., Alpert, P., Salguero-Gómez, R., Janovský, Z., Herben, T., Klimešová, J., et al. (2021). Next-gen plant clonal ecology. *Perspect. Plant Ecology Evol. Systematics* 49, 125601. doi: 10.1016/j.ppees.2021.125601

Friedman, D., and Alpert, P. (1991). Reciprocal transport between ramets increases growth of *Fragaria chiloensis* when light and nitrogen occur in separate patches but only if patches are rich. *Oecologia* 86 (1), 76–80. doi: 10.1007/BF00317392

Guo, W., Song, Y.-B., and Yu, F.-H. (2011). Heterogeneous light supply affects growth and biomass allocation of the understory fern *Diplopterygium glaucum* at high patch contrast. *PloS One* 6 (11), e27998. doi: 10.1371/journal.pone.0027998

Huang, Q., Li, X., Huang, F., Wang, R., Lu, B., Shen, Y., et al. (2018). Nutrient addition increases the capacity for division of labor and the benefits of clonal integration in an invasive plant. *Sci. Total Environ.* 643, 1232–1238. doi: 10.1016/j.scitotenv.2018.06.294

Hutchings, M. J., and Wijesinghe, D. K. (1997). Patchy habitats, division of labour and growth dividends in clonal plants. *Trends Ecol. Evol.* 12 (10), 390–394. doi: 10.1016/S0169-5347(97)87382-X

Ikegami, M., Whigham, D. F., and Werger, M. J. A. (2008). Optimal biomass allocation in heterogeneous environments in a clonal plant–spatial division of labor. *Ecol. Model.* 213 (2), 156–164. doi: 10.1016/j.ecolmodel.2007.11.016

Jackson, R. B., and Caldwell, M. M. (1993). Geostatistical patterns of soil heterogeneity around individual perennial plants. *J. Ecol.* 81 (4), 683–692. doi: 10.2307/2261666

Li, S.-p., Jia, P., Fan, S.-y., Wu, Y., Liu, X., Meng, Y., et al. (2022). "Functional traits explain the consistent resistance of biodiversity to plant invasion under nitrogen enrichment. *Ecol. Lett.* 25 (4), 778–789. doi: 10.1111/ele.13951

Lin, H. F., Alpert, P., Zhang, Q., and Yu, F. H. (2018). Facilitation of amphibious habit by physiological integration in the clonal, perennial, climbing herb *Ipomoea aquatica*. *Sci. Total Environ.* 618, 262–268. doi: 10.1016/j.scitotenv.2017.11.025

Liu, F., Liu, J., and Dong, M. (2016). Ecological consequences of clonal integration in plants. *Front. Plant Sci.* 7, 770. doi: 10.3389/fpls.2016.00770

Magyar, G., Kun, Á., Oborny, B., and Stuefer, J. F. (2007). Importance of plasticity and decision-making strategies for plant resource acquisition in spatio-temporally variable environments. *New Phytol.* 174 (1), 182–193. doi: 10.1111/j.1469-8137.2007.01969.x

Marshall, C. (1999). Editorial – clonal plants and environmental heterogeneity – space, time and scale. *Plant Ecol.* 141 (1), 1–1. doi: 10.1038/s41586-021-03405-6

Peipoch, M., Gacia, E., Bastias, E., Serra, A., Proia, L., Ribot, M., et al. (2016). Small-scale heterogeneity of microbial n uptake in streams and its implications at the ecosystem level. *Ecology* 97 (5), 1329–1344. doi: 10.1890/15-1210.1

Pinheiro, J. C., Bates, D. M.R-core (2020). *Linear and nonlinear mixed effects models [R package nlme version 3.1-149]*.

Roiloa, S. R. (2019). Clonal traits and plant invasiveness: the case of *Carpobrotus* N.E.Br. (Aizoaceae). *Perspect. Plant Ecology Evol. Systematics* 40, 125479. doi: 10.1016/j.ppees.2019.125479

Roiloa, S. R., Alpert, P., and Barreiro, R. (2019). Differences in physiological integration between invasive and noninvasive introduced clonal species of *Carpobrotus*. *J. Plant Ecol.* 12 (6), 972–981. doi: 10.1093/jpe/rtz035

Roiloa, S., Alpert, P., Tharayil, N., Hancock, G., and Bhowmik, P. (2007). Greater capacity for division of labour in clones of *Fragaria chiloensis* from patchier habitats. *J. Ecol.* 95, 397–405. doi: 10.1111/j.1365-2745.2007.01216.x

Roiloa, S. R., Retuerto, R., Campoy, J. G., Novoa, A., and Barreiro, R. (2016). Division of labor brings greater benefits to clones of *Carpobrotus edulis* in the non-native range: evidence for rapid adaptive evolution. *Front. Plant Sci.* 7, 349. doi: 10.3389/fpls.2016.00349

Song, Y.-B., Yu, F.-H., Keser, L. H., Dawson, W., Fischer, M., Dong, M., et al. (2013). United we stand, divided we fall: a meta-analysis of experiments on clonal integration and its relationship to invasiveness. *Oecologia* 171 (2), 317–327. doi: 10.1007/s00442-012-2430-9

Struefer, J. F., Kroon, H., and During, H. J. (1996). Exploitation of environmental heterogeneity by spatial division of labour in a clonal plant. *Funct. Ecol.* 10, 328–334. doi: 10.2307/2390280

Stuefer, J. F. (1998). Two types of division of labour in clonal plants: benefits, costs and constraints. *Perspect. Plant Ecology Evol. Systematics* 1 (1), 47–60. doi: 10.1078/1433-8319-00051

Stuefer, J. F., During, H. J., and Schieving, F. (1998). A model on optimal root–shoot allocation and water transport in clonal plants. *Ecol. Model.* 111 (2), 171–186. doi: 10.1016/S0304-3800(98)00101-X

Stuefer, J. F., and Hutchings, M. J. (1994). Environmental heterogeneity and clonal growth: a study of the capacity for reciprocal translocation in *Glechoma hederacea* l. *Oecologia* 100 (3), 302–308. doi: 10.1007/BF00316958

Stuefer, J. F., Kroon, H., and During, H. J. (1996). Exploitation of environmental heterogeneity by spatial division of labour in a clonal plant. *Funct. ecology.* 10 (3), 328–334. doi: 10.2307/2390280

Vilà, M., Rohr, R. P., Espinar, J. L., Hulme, P. E., Pergl, J., Le Roux, J. J., et al. (2015). Explaining the variation in impacts of non-native plants on local-scale species richness: the role of phylogenetic relatedness. *Global Ecol. Biogeography* 24 (2), 139–146. doi: 10.1111/geb.12249

Wang, Y.-J., Chen, D., Yan, R., Yu, F.-H., and van Kleunen, M. (2019). Invasive alien clonal plants are competitively superior over co-occurring native clonal plants. *Perspect. Plant Ecology Evol. Systematics* 40, 125484. doi: 10.1016/j.ppees.2019.125484

Wang, Z., Li, Y., During, H. J., and Li, L. (2011). Do clonal plants show greater division of labour morphologically and physiologically at higher patch contrasts? *PloS One* 6 (9), e25401. doi: 10.1371/journal.pone.0025401

Wang, Y.-J., Müller-Schärer, H., van Kleunen, M., Cai, A.-M., Zhang, P., Yan, R., et al. (2017). Invasive alien plants benefit more from clonal integration in heterogeneous environments than natives. *New Phytol.* 216 (4), 1072–1078. doi: 10.1111/nph.14820

Wang, Y. J., Shi, X. P., Meng, X. F., Wu, X. J., Luo, F. L., and Yu, F. H. (2016). Effects of spatial patch arrangement and scale of covarying resources on growth and intraspecific competition of a clonal plant. *Front. Plant Sci.* 7, 753. doi: 10.3389/fpls.2016.00753

Wang, J., Wang, Q., Hu, J., Yu, H., Liu, C., and Yu, D. (2021). The influence of small-scale resource heterogeneity caused by human activities on the growth phenotype of invasive aquatic plants. *Ecol. Indic.* 125, 107504. doi: 10.1016/j.ecolind.2021.107504

Wilkinsom, L. (2016). *ggplot2: elegant graphics for data analysis* (Springer- Verlag).

Xi, D.-G., You, W.-h., Hu, A.-A., Huang, P., and Du, D.-L. (2019). Developmentally programmed division of labor in the aquatic invader *Alternanthera philoxeroide*s under homogeneous soil nutrients. *Front. Plant Sci.* 10. doi: 10.3389/fpls.2019.00485

You, W., Fan, S., Yu, D., Xie, D., and Liu, C. (2014). An invasive clonal plant benefits from clonal integration more than a co-occurring native plant in nutrient-patchy and competitive environments. *PloS One* 9 (5), e97246. doi: 10.1371/journal.pone.0097246

You, W., Yu, D., Liu, C., Xie, D., and Xiong, W. (2013). Clonal integration facilitates invasiveness of the alien aquatic plant *Myriophyllum aquaticum* l. under heterogeneous water availability. *Hydrobiologia* 718 (1), 27–39. doi: 10.1007/s10750-013-1596-4

Yu, H., Shen, N., Yu, D., and Liu, C. (2018). Effects of temporal heterogeneity of water supply and spatial heterogeneity of soil nutrients on the growth and intraspecific competition of bolboschoenus yagara depend on plant density. *Front. Plant Sci.* 9, 1987. doi: 10.3389/fpls.2018.01987

Zhang, L.-L., and He, W.-M. (2009). Spatial covariance in resources affects photosynthetic rate and water potential, but not the growth of *Glechoma longituba* fragments. *Flora - Morphology Distribution Funct. Ecol. Plants* 204 (8), 628–634. doi: 10.1016/j.flora.2008.09.003

Zhang, Z., and van Kleunen, M. (2019). Common alien plants are more competitive than rare natives but not than common natives. *Ecol. Lett.* 22 (9), 1378–1386. doi: 10.1111/ele.13320

Zuur, A. F., Ieno, E. N., Walker, N. J., Saveliev, A. A., and Smith, G. M. (2009). *Mixed effects models and extensions in ecology with r* (Springer).

frontiers | Frontiers in Plant Science

TYPE Original Research
PUBLISHED 11 August 2023
DOI 10.3389/fpls.2023.1205166

OPEN ACCESS

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RECEIVED 13 April 2023
ACCEPTED 25 July 2023
PUBLISHED 11 August 2023

CITATION
Guo J, Li H, Yang Y and Yang X (2023) Clonal dominant grass *Leymus chinensis* benefits more from physiological integration in sexual reproduction than its main companions in a meadow. *Front. Plant Sci.* 14:1205166. doi: 10.3389/fpls.2023.1205166

Clonal dominant grass *Leymus chinensis* benefits more from physiological integration in sexual reproduction than its main companions in a meadow

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The bioecological characteristics of plants determine their status and role in the community. The advantages of dominant species in the community compared with companion species in terms of physiological and ecological characteristics remain unclear. When both dominant and companion species in grassland plant communities are clonal, these plants are able to share resources within clones (physiological integration). However, it is unclear how the clonal dominant and companion species differ in the effect of their physiological integration on sexual reproduction. We chose *Leymus chinensis*, the dominant species of the most widespread meadow plant communities in the semiarid and arid regions of northern China, and its main companion species *L. secalinus*, *Calamagrostis ripidula*, *C. pseudophragmites*, and *C. epigeios* and conducted a series of *in situ* field experiments in a homogeneous environment, including the determination of the phenotypic characteristics of reproductive ramets with connected (allowing physiological integration) and disconnected (preventing integration) tillering nodes for each species, as well as ^{15}N leaf labeling of ramet pairs at the milk-ripe stage. In the clonal populations of the five grasses, physiological integration between vegetative ramets and reproductive ramets interconnected by tillering nodes significantly increased the leaf, stem, inflorescence and ramet biomasses of reproductive ramets, and relative changes in ramet biomass were greatest in *L. chinensis*. ^{15}N labeling showed that vegetative ramets supplied nutrients to reproductive ramets through tillering nodes; the amount of translocated ^{15}N per unit of reproductive ramet biomass was highest in *L. chinensis*. Overall, our results indicate that in the five clonal grasses, physiological integration between functionally different ramets under tillering node connections had a significant positive effect on sexual reproduction, indicating interspecific consistency in the contribution of physiological integration to sexual reproduction between the dominant and companion species, but this positive effect was greater in the dominant

species *L. chinensis* than in the four main companion species. Therefore, differences in the physiological integration ability between the dominant and main companion species, identified for the first time in this study, may explain, at least partly, the dominance of *L. chinensis* in the community.

KEYWORDS

dominant species, companion species, perennial herb, resource translocation, sexual reproduction, tillering node connection, relative benefit

1 Introduction

Plants rarely grow alone and often cluster together to form communities in nature. Based on the different statuses and roles of species in the community, they can be classified as dominant species, companion species and other types. Dominant species are species that have a high abundance relative to that of other species in a community and have obvious control over the community structure and environmental conditions, and their status and development trend in the community largely influence the stability and species diversity of the community (Smith and Knapp, 2003; Avolio et al., 2019). By contrast, companion species are species that occur frequently in the community and exist in companion with the dominant species but do not have major effects on the community structure or environmental conditions (Yang and Zhu, 2011). Although it is now recognized that dominant species in any community have a significant competitive advantage over their companion species, which is closely related to their large number of individuals and high biomass, it is surprising that relatively few studies have been conducted on the physiological and ecological characteristics of dominant and companion species in communities. In forest plant communities, dominant species in the arboreal layer showed significant superiority in the maximum net photosynthetic rate per unit leaf area (Zhang et al., 2017) and specific leaf area (Wang et al., 2014) compared with those of companion species. In grassland plant communities, both dominant and companion species are capable of clonal growth. However, little is known about whether clonal dominant species have physiological and ecological characteristics superior to those of companion species.

An important and unique feature of clonal plants is physiological integration (intraclonal resource sharing), i.e., the translocation of resources such as water, mineral nutrients and carbohydrates between connected ramets of the same clone (Ashmun et al., 1982; Alpert, 1996). For dominant plant species, physiological integration has been repeatedly shown to promote the establishment of newly produced daughter ramets (Evans and Cain, 1995; Dong and Alaten, 1999; Sun et al., 2022a), to increase the growth of ramets in stressful or heterogeneous environments (Roiloa et al., 2014; Zhou et al., 2014), and to enhance the fitness of the whole clone (Song et al., 2013; Chen et al., 2015). Other studies have shown that physiological integration can also greatly increase the growth performance of cooccurring plant species (Wang et al., 2017a; Roiloa et al., 2019; Zhang et al., 2022). Most previous studies on physiological integration focused on the survival and growth of clonal plants. Sexual reproduction is an important link in the life history of clonal plants (Eriksson, 1997), an important means of their adaptation to unstable environments and the geographical migration of species (Eckert, 2001), and therefore, it is vital to population adaptation and evolution. However, it is poorly understood how physiological integration affects sexual reproduction in clonal plants and whether there are differences in the physiological integration ability between clonal dominant and companion species.

The nonzonal vegetation (i.e., meadows) in the semiarid and arid regions of northern China is an important part of the terrestrial ecosystem of the Eurasian steppe and is also the most widely distributed natural vegetation. The dominant species of meadow plant communities is perennial rhizomatous *Leymus chinensis*, and the common main companion species are *L. secalinus*, *Calamagrostis ripidula*, *C. pseudophragmites*, and *C. epigeios* (Li et al., 2001); they are all typical clonal plants of Gramineae. *L. chinensis* has high nutritional value and good palatability; therefore, different types of *L. chinensis* meadows are excellent mowing and grazing grounds (Jia, 1987; Zhu, 2004). Because of its economic and ecological significance, *L. chinensis* has received considerable attention (Guo et al., 2021; Meng et al., 2022; Sun et al., 2022b). However, its main companion species, such as *L. secalinus*, *C. ripidula*, *C. pseudophragmites* and *C. epigeios*, have received less attention. For example, in terms of physiological integration between connected ramets, Gao et al. (2014) found that physiological integration increased the ramet biomass of *L. chinensis* in environments with resource heterogeneity, and Zhou et al. (2014) reported that physiological integration increased the maximum net photosynthetic rate, apparent quantum efficiency, respiration rate, water use efficiency, and chlorophyll content of *L. chinensis* in environments with nutrient heterogeneity and confirmed that differences in the physiological integration ability between the two ecotypes resulted in their different performance levels. Sui et al. (2011) found that physiological integration enhanced the total biomass, belowground biomass, ramet number and total rhizome length of *L. secalinus* under mechanical stimulation. No studies on physiological integration have been reported thus far in *C. ripidula*, *C. pseudophragmites* and *C. epigeios*. Therefore, it is unclear how physiological integration affects sexual reproduction in *L. chinensis* and its four main

companion species as well as whether there are interspecific differences in the physiological integration ability.

In this study, we grew *L. chinensis* and its main companion species *L. secalinus*, *C. ripidula*, *C. pseudophragmites* and *C. epigeios* in a homogeneous field environment and measured the phenotypic characteristics of reproductive ramets with connected (allowing physiological integration) and disconnected (preventing integration) tillering nodes for each species. We also labeled the vegetative ramets with an isotope (^{15}N) at the milk-ripe stage to verify whether vegetative ramets translocated resources toward the connected reproductive ramets. The objectives of our study were (1) to assess the effect of physiological integration on the sexual reproductive performance of the dominant species *L. chinensis* and its four main companion species and (2) to explore the differences in the physiological integration ability among the dominant species *L. chinensis* and its four main companion species. Here, we hypothesize that (1) physiological integration will increase the sexual reproductive performance of the dominant species *L. chinensis* and its four main companion species and (2) the positive effect of physiological integration will be greater in the dominant species *L. chinensis* than in its four main companion species.

2 Materials and methods

2.1 Study area

This study was conducted at the Jilin Songnen Grassland Ecosystem National Observation and Research Station (44°38′N, 123°41′E), which is in the southern region of the Songnen Plain. This area has a semiarid, semihumid, and temperate continental monsoonal climate with rainy, hot summers and dry, cold winters. The annual mean temperature ranges from 4.6°C to 6.4°C, and the annual mean precipitation varies from 300 mm to 450 mm, with the majority concentrated from June to September. The growing season with a frost-free period is approximately 130-165 days (Li et al., 2018; Guo et al., 2020a). The meadow vegetation in this study area is dominated by *L. chinensis*, which is accompanied by *L. secalinus*, *C. ripidula*, *C. pseudophragmites*, *C. epigeios*, *Hierochloe glabra*, etc.

2.2 Study species

A total of five species were included in this study, namely, *L. chinensis*, *L. secalinus*, *C. ripidula*, *C. pseudophragmites* and *C. epigeios*, all of which are main forage grasses in natural grassland. *L. chinensis* is a perennial grass that is widely distributed in western North Korea, the People's Republic of Mongolia, northwestern Siberia, the Inner Mongolian Plateau, and the Northeast Plain of China (Kuo, 1987). *L. chinensis* has very strong adaptability and tolerance to saline-alkaline, drought and low-temperature conditions (Jin et al., 2008; Chen et al., 2013; Zhai et al., 2014); thus, it often forms *L. chinensis* steppes and meadows as a dominant species, among which *L. chinensis* meadows are the most widely distributed natural vegetation in the study area. *L. secalinus* is a perennial grass that mainly occurs in typical steppe, sandy grassland, mountain slope, farmland and roadside habitats in northern China, Korea and Japan (Dong, 1999). *L. secalinus* has strong tolerance to drought, low temperature, and light soil salinization. *C. ripidula*, *C. pseudophragmites* and *C. epigeios* are all perennial species that occur in natural grasslands, artificial forest edges and understories in temperate regions of Eurasia and are tolerant to saline-alkaline conditions and certain humidities (Jia, 1987). These five grasses are all typical clonal plants; the clonal ramets are interconnected via rhizomes or tillering nodes (which refer to the unelongated basal internodes of ramets). It is common for the five grasses to have one reproductive ramet and one vegetative ramet per tillering node. On the Songnen Plain, the five grasses usually begin turning green in April, undergo heading in May-June, and then flower and fruit in June-August (Zhu, 2004).

2.3 Experimental platform

A total of 75 experimental plots were established at the beginning of May 2017. Each plot had an area of 1 m^2 (1 m × 1 m), and adjacent plots were at least 0.5 m apart. For each species, vegetative ramets of a similar size were collected from fifteen populations that were 50 m apart from each other in the natural meadow, and thus, each species was represented by fifteen clones (genotypes). We then adopted a completely randomized experimental design and transplanted nine vegetative ramets of any one species in each plot with rows 0.25 m apart and ramets 0.25 m apart. All of the experimental plots were manually irrigated for several days after transplanting to ensure that the ramets survived. Then, the plots were weeded regularly, without any irrigation or fertilization, and the five species were not affected by any pests or diseases. The soil type is sandy loam (Li et al., 2001; Guo et al., 2020a). The soil of the top 30-cm-thick layer was homogenous, and the total N content, total organic C content and total P content were 1.05 ± 0.01 g kg^{-1}, 5.82 ± 0.09 g kg^{-1}, and 0.76 ± 0.01 g kg^{-1}, respectively. The pH was 8.34 ± 0.04, the bulk density was 1.18 ± 0.02 g cm^{-3}, and the electrical conductivity was 75.03 ± 0.80 μS cm^{-1}.

2.4 Intact and severed ramet design

To investigate the effect of physiological integration on sexual reproduction as well as its interspecific differences, at the early heading stage of each species in both 2018 and 2019, we used colored tags to mark two ramet pairs with similar sizes at the edge of each plot. Each ramet pair consisted of one reproductive ramet (mother ramet) and one vegetative ramet (daughter ramet) connected by a tillering node, and the inflorescence top of the reproductive ramet reached approximately 1 cm above the flag leaf sheath. The connection between the reproductive and vegetative ramets of one ramet pair was left intact (allowing integration), while the connection between the reproductive and vegetative ramets of the other ramet pair was severed (preventing integration) with scissors (Figure 1). We gently set aside the topsoil around the ramet

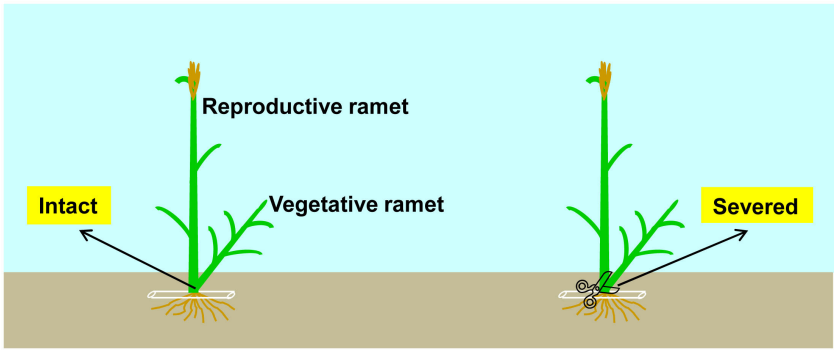

FIGURE 1
Schematic representation of the experimental design. Each clonal fragment of the five clonal plant species (*Leymus chinensis*, *L. secalinus*, *Calamagrostis ripidula*, *C. pseudophragmites* and *C. epigeios*) consisted of one reproductive ramet and one vegetative ramet. Physiological integration: connections between the reproductive ramet and vegetative ramet remained intact (allowing integration) (left) or were severed (preventing integration) (right).

pairs to expose the tillering nodes of the reproductive ramets to verify that the vegetative ramets were indeed growing on the tillering nodes of the reproductive ramets, then cut the vegetative ramets from the tillering nodes with scissors, and finally, rapidly restored the topsoil to its original position. Although the severing of physical connections between the ramets may cause physiological stress and make the plants more susceptible to disease infection (Jónsdóttir and Watson, 1997), we did not observe any signs of disease infection in the reproductive ramets throughout the experiment, so severing the vegetative ramets did not cause harm to the growth of the reproductive ramets. We transported the cutoff vegetative ramets to the laboratory. All marked ramet pairs were harvested at the seed-maturing stage of each species. The reproductive ramet height and inflorescence length were measured. Each reproductive ramet was separated into the leaves, stem and inflorescence. Leaf biomass, stem biomass, inflorescence biomass, reproductive ramet biomass, and vegetative ramet biomass were measured after drying in an oven at 65°C for 48 h.

2.5 Stable isotope labeling

To verify whether vegetative ramets translocate their own resources to the connected reproductive ramets during sexual reproduction as well as their interspecific differences, an *in situ* leaf labeling experiment was carried out at the milk-ripe stage of each species in 2019. We randomly selected four out of fifteen plots for each species to carry out the stable isotope labeling experiment. Two ramet pairs with similar sizes (one ramet pair for the control treatment and another for the ^{15}N labeling treatment) were randomly chosen at the edge of each plot, and each ramet pair consisted of one reproductive ramet (mother ramet) and one vegetative ramet (daughter ramet) connected by a tillering node. The two ramet pairs were at least 50 cm apart. The control solution was distilled water, and the ^{15}N labeling solution was a solution of urea (made at the Shanghai Research Institute of Chemical Industry, China) with a concentration of 0.02 g·mL^{-1} and a ^{15}N abundance of 5.18%. The labeling method strictly followed the scheme of Guo et al. (2020a). The aboveground reproductive ramets in both the control treatment and ^{15}N labeling treatment in each plot were harvested exactly 2 days after labeling. Each reproductive ramet was de-enzymed at 105°C for 30 min and then dried at 65°C for 48 h. We then measured the dry mass of each reproductive ramet and ground it to a fine powder with a ball mill (MM 400 Retsch, Haan, Germany). For each sample, approximately 3 mg of solid powder was loaded into a capsule, and then the isotope values ($\delta^{15}N$) were determined using a vario EL cube (Elementar, Langenselbold, Germany) interfaced with an Isoprime 100 isotope-ratio mass spectrometer (Elementar, Langenselbold, Germany), with an overall precision greater than 0.2‰. The amount of ^{15}N translocated from the labeled vegetative ramets toward the unlabeled reproductive ramets was calculated following the protocol of Guo et al. (2020a).

2.6 Statistical analysis

The statistical analysis was performed with IBM SPSS 20.0 (SPSS Inc., Chicago, IL, USA). All variables were tested for a normal distribution and homogeneity of variances. All results were reported as the means ± standard errors, and a significance level of $P \leq 0.05$ was used for all analyses.

For each species, a paired-samples *t* test was used to determine the differences in ramet height, inflorescence length, leaf biomass, stem biomass, inflorescence biomass and ramet biomass between tillering node connections that remained intact and were severed and to test for differences in the $\delta^{15}N$ of reproductive ramets between the control and ^{15}N labeling treatments. One-way analysis of variance (ANOVA) was performed to assess the effects of species identity on the absolute and relative benefits of reproductive ramets, the inflorescence biomass allocation of reproductive ramets, the vegetative ramet biomass, the total amount of translocated ^{15}N, and the amount of translocated ^{15}N per unit of reproductive ramet biomass. Duncan's multiple range test was used to test for significant differences between the means of multiple groups. The absolute benefit of reproductive ramets was

calculated as the difference in the reproductive ramet biomass of intact clones and the reproductive ramet biomass of severed clones. The relative benefit of reproductive ramets (expressed as a percentage) was calculated as the ratio of the reproductive ramet biomass difference between the two connection treatments to the reproductive ramet biomass of the severed clone. The inflorescence biomass allocation (expressed as a percentage) was calculated as the ratio of the inflorescence biomass to the reproductive ramet biomass. The amount of translocated ^{15}N per unit of reproductive ramet biomass was calculated as the ratio of the total amount of translocated ^{15}N to the reproductive ramet biomass.

3 Results

3.1 Biomass production and growth characteristics of reproductive ramets

In the five clonal plants *L. chinensis*, *L. secalinus*, *C. ripidula*, *C. pseudophragmites* and *C. epigeios*, the leaf biomass (Figures 2A-E), stem biomass (Figures 2F-J), inflorescence biomass (Figures 2K-O) and ramet biomass (Figures 2P-T) of reproductive ramets under severed tillering node connections were reduced compared with those under intact connections over the two consecutive years. Except for the leaf biomass of *L. secalinus* in 2018 and the leaf biomass of *C. epigeios* in 2018 and 2019, all differences between the two treatments reached a significant ($P < 0.05$) or extremely significant ($P < 0.01$) level. There was no significant difference ($P > 0.05$) in the height or inflorescence length of reproductive ramets between the tillering node connections that remained intact and severed over the two consecutive years. These results indicated that physiological integration had a significant effect on the biomass production characteristics rather than on the growth characteristics of the reproductive ramets in the five clonal grasses.

3.2 Benefit of reproductive ramets

The absolute change in the reproductive ramet biomass of *C. ripidula* was significantly ($P < 0.05$) larger than that of the other four clonal plants over the two consecutive years (Figures 3A1, A2). The relative change in the reproductive ramet biomass of *L. chinensis* was significantly ($P < 0.05$) larger than that of the other four clonal plants, while the relative changes in the reproductive ramet biomass of *C. pseudophragmites* and *C. epigeios* were significantly ($P < 0.05$) smaller than those of the other three clonal plants over the two consecutive years (Figures 3B1, B2).

3.3 Inflorescence biomass allocation of reproductive ramets

The inflorescence biomass allocation of the reproductive ramets of *L. chinensis* was significantly ($P < 0.05$) greater than that of the

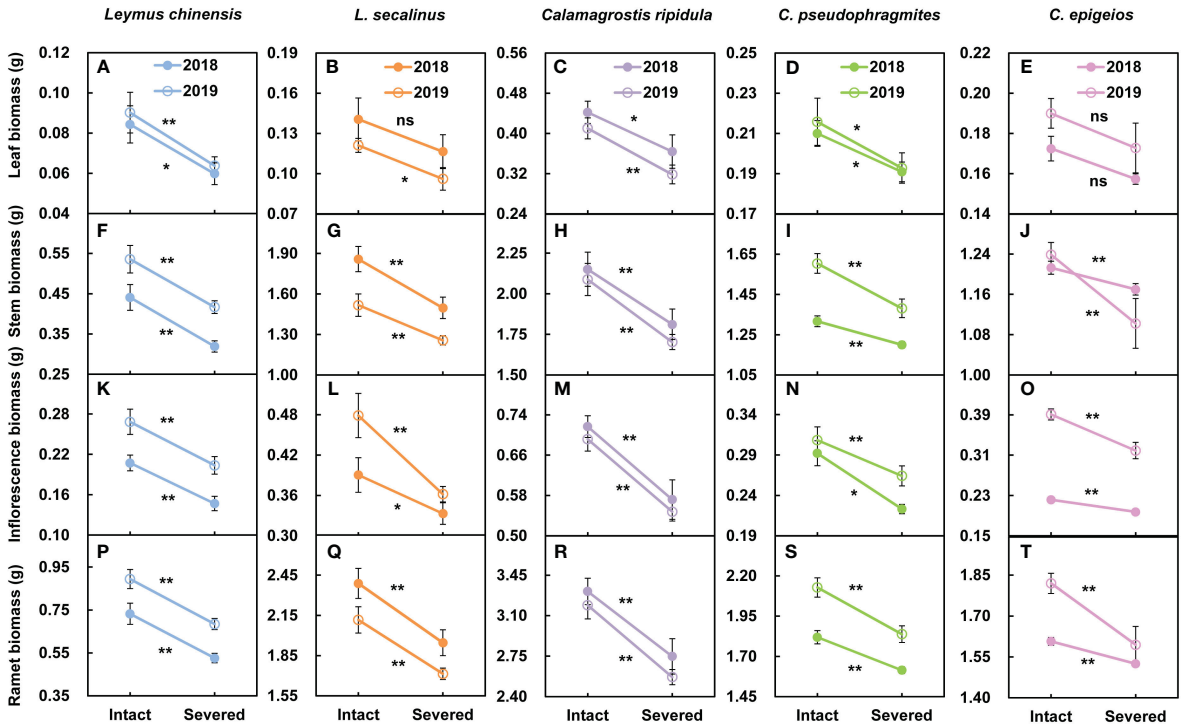

FIGURE 2
Comparison of the leaf biomass **(A-E)**, stem biomass **(F-J)**, inflorescence biomass **(K-O)** and ramet biomass **(P-T)** of reproductive ramets between the tillering node connections that remained intact and were severed in the five clonal plants grown under the same environmental conditions in 2018 and 2019. Data are means ± SEs (n = 15). The P values are expressed as follows: $^{**}P < 0.01$; $^{*}0.01 < P < 0.05$; ns, $P > 0.05$.

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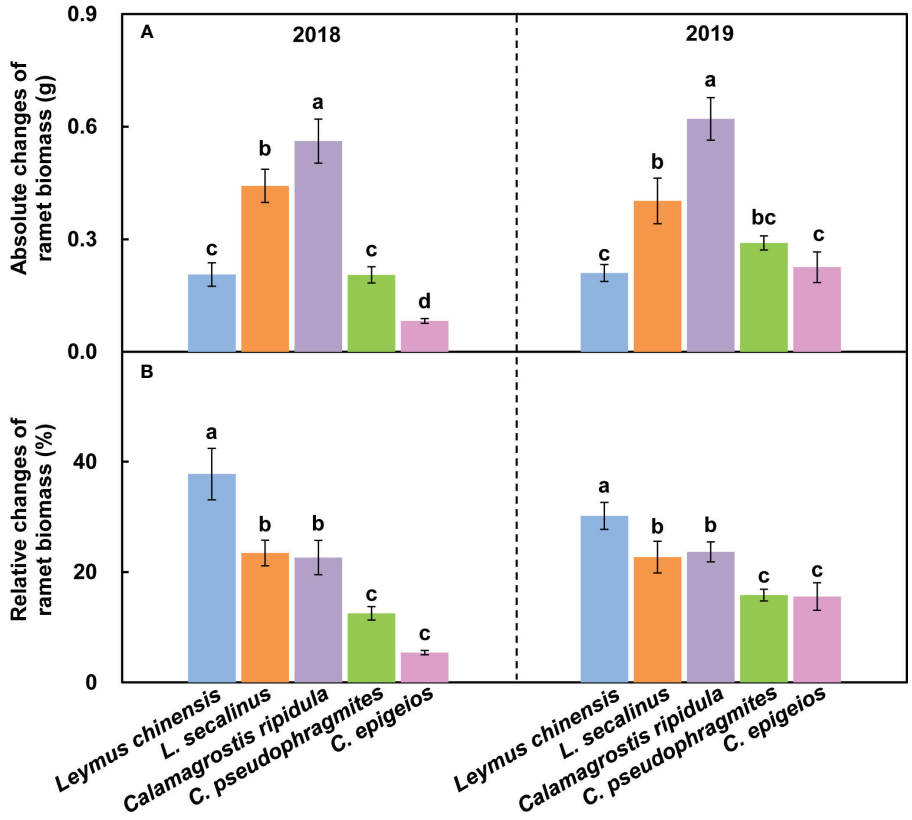

FIGURE 3
Comparison of the absolute changes in ramet biomass **(A1, A2)** and relative changes in ramet biomass **(B1, B2)** among the five clonal plants in 2018 and 2019. Data are means ± SEs (n = 15). Different lowercase letters indicate significant differences (P < 0.05) between plant species.

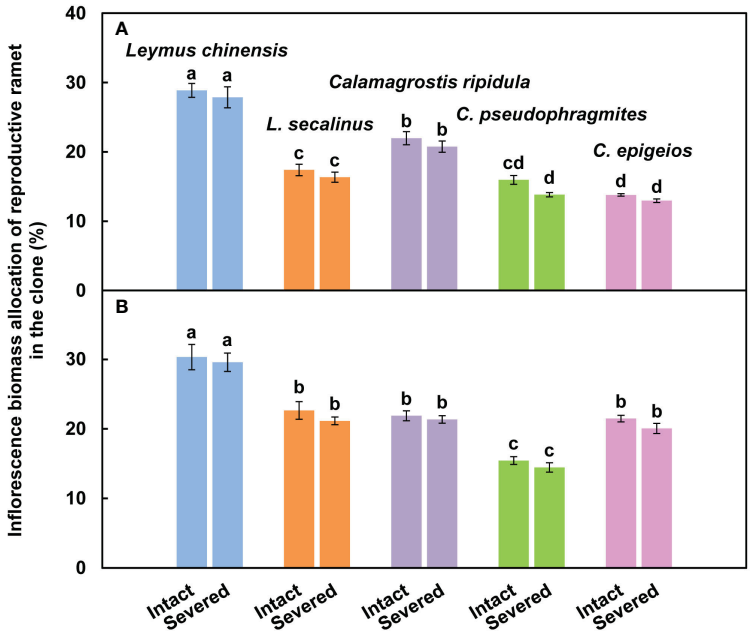

FIGURE 4
Comparison of the inflorescence biomass allocation of reproductive ramets among the five clonal plants in the intact and severed treatments in 2018 **(A)** and 2019 **(B)**. Data are means ± SEs (n = 15). Different lowercase letters indicate significant differences (P < 0.05) between plant species.

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other four clonal plants in both severed and intact clones over the two consecutive years (Figure 4).

3.4 Biomass production of vegetative ramets

The vegetative ramet biomass of *C. ripidula* was significantly ($P < 0.05$) larger than that of the other four clonal plants, while the vegetative ramet biomass of *C. pseudophragmites* and *C. epigeios* was significantly ($P < 0.05$) smaller than that of the other three clonal plants in both severed and intact clones over the two consecutive years (Figure 5).

3.5 Transfer of ^{15}N from vegetative ramets to connected reproductive ramets

In the five clonal plants *L. chinensis*, *L. secalinus*, *C. ripidula*, *C. pseudophragmites* and *C. epigeios*, reproductive ramets had a significantly higher $\delta^{15}N$ level in the ^{15}N labeling treatment than in the control treatment (Table 1). These results demonstrated that vegetative ramets connected to tillering nodes translocated their own nutrients toward reproductive ramets through physiological integration. The total amount of translocated ^{15}N in the reproductive ramets of *L. secalinus* and *C. ripidula* was larger than that of the other three clonal plants, while the total amount of translocated ^{15}N in the reproductive ramets of *C. epigeios* was smaller than that of the other four clonal plants (Table 2). The amount of translocated ^{15}N per unit of reproductive ramet biomass of *L. chinensis* was larger than that of the other four clonal plants (Table 2).

4 Discussion

4.1 Physiological integration increases the sexual reproductive performance of five clonal plants

In grassland plant communities, many plant species are clonal, and one of their unique traits is physiological integration, i.e., the translocation and sharing of resources between ramets through physical connections (Ashmun et al., 1982; Alpert, 1996). First, from the perspective of ramet function (rather than the developmental age of the ramet), two different types of aboveground ramets are widely distributed in clonal plant populations: reproductive ramets and vegetative ramets (Harper, 1977). Therefore, the so-called paired ramet system often includes the following three cases: vegetative ramet–vegetative ramet, reproductive ramet–reproductive ramet, and vegetative ramet–reproductive ramet. Obviously, the first two cases are paired ramet systems with the same function, and the third one is a paired ramet system with different functions. Second, from the perspective of physical connection, apart from rhizome or stolon connections, tillering node connection is also a very common connection form between clonal ramets. Under natural conditions, vegetative ramets may grow on the unelongated basal internodes of reproductive ramets, in which case the two types of ramets are interconnected by tillering nodes. Studies have

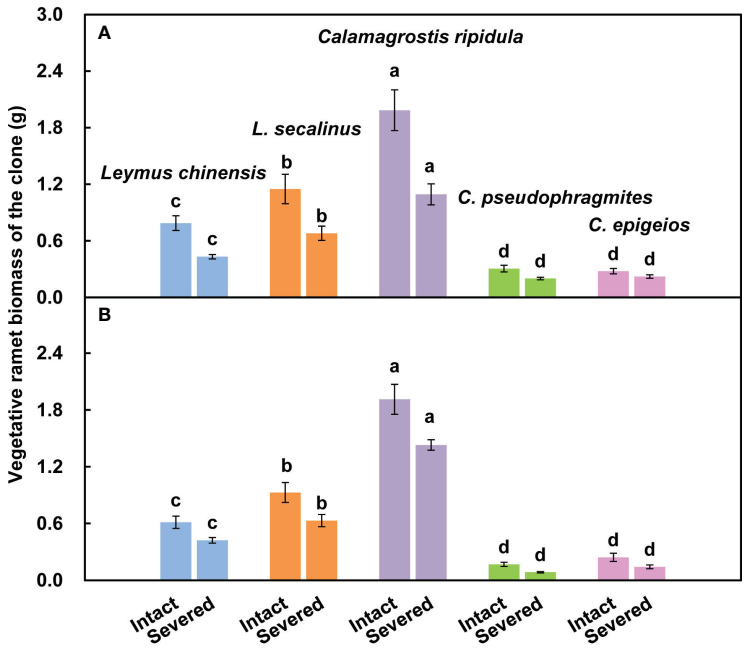

FIGURE 5
Comparison of the vegetative ramet biomass of clones among the five clonal plants in the intact and severed treatments in 2018 **(A)** and 2019 **(B)**. Data are means ± SEs ($n = 15$). Different lowercase letters indicate significant differences ($P < 0.05$) between plant species.

TABLE 1 Comparison of the $\delta^{15}N$ of reproductive ramets between the control and ^{15}N labeling treatments in the five clonal plants grown under the same environmental conditions in 2019 (means ± SEs, n = 4).

Species	CK	^{15}N labeling	t	P
Leymus chinensis	2.37 ± 0.19	125.26 ± 6.80	-17.63	<0.001
L. secalinus	3.51 ± 0.44	78.73 ± 5.38	-13.86	0.001
Calamagrostis ripidula	4.07 ± 0.63	92.06 ± 2.11	-39.44	<0.001
C. pseudophragmites	5.71 ± 0.67	122.75 ± 4.05	-30.85	<0.001
C. epigeios	3.01 ± 0.29	66.24 ± 6.69	-9.61	0.002

repeatedly revealed that physiological integration between vegetative ramets and vegetative ramets with the same function via a rhizome or stolon has a positive effect on the survival and growth of the ramets (Evans and Cain, 1995; Dong and Alaten, 1999; Song et al., 2013; Chen et al., 2015), but little research has been performed on the effect of physiological integration between vegetative ramets and reproductive ramets with different functions by tillering node connection on sexual reproductive performance. Our previous studies showed that physiological integration between vegetative and reproductive ramets connected by a tillering node increased sexual reproductive characteristics such as inflorescence biomass, floret number, seed number, seed biomass and the seed-setting rate in *H. glabra* (Guo et al., 2020b) and *L. chinensis* (Guo et al., 2020c). In the present study, by severing connections, we found that physiological integration between vegetative ramets and reproductive ramets connected by a tillering node significantly increased the leaf biomass, stem biomass, inflorescence biomass, and ramet biomass of reproductive ramets in *L. chinensis*, *L. secalinus*, *C. ripidula*, *C. pseudophragmites* and *C. epigeios* (Figure 2), and these results supported our first hypothesis. Most importantly, the consistent finding that vegetative ramets are very advantageous for sexual reproduction under tillering node connection in these five clonal plants not only indicates interspecific consistency in the contribution of physiological integration to sexual reproduction in clonal grasses but also reflects the convergent adaptation of physiological integration between the two types of ramets to the same habitat conditions in these five grasses. Our previous research revealed that compared with reproductive ramets with different numbers of connecting vegetative ramets, reproductive ramets with zero connecting vegetative ramets in *H. glabra* had poorer performance, and their inflorescence biomass was significantly lower than that of reproductive ramets with 1, 2, and 3 connecting vegetative ramets (Guo et al., 2020b). These results all imply that vegetative ramets play an important role in sexual reproduction apart from the spatial expansion of clonal plant populations.

TABLE 2 Comparison of the amount of translocated ^{15}N of reproductive ramets among the five clonal plants grown under the same environmental conditions in 2019 (means ± SEs, n = 4).

Species	Total ^{15}N amount (μg)	^{15}N amount per unit ramet biomass (μg g^{-1})
Leymus chinensis	5.50 ± 0.15 bc	7.56 ± 0.53 a
L. secalinus	16.37 ± 1.46 a	4.66 ± 0.48 b
Calamagrostis ripidula	13.50 ± 0.74 a	3.65 ± 0.33 bc
C. pseudophragmites	7.53 ± 0.46 b	3.03 ± 0.13 cd
C. epigeios	3.75 ± 0.52 c	2.14 ± 0.35 d

Different lowercase letters indicate significant differences (P < 0.05) between different plant species.

The degree to which physiological integration affects sexual reproductive performance varies with reproductive characteristics. In this study, we found that physiological integration had no effect on ramet height or inflorescence length but had significant effects on biomass production characteristics such as leaf biomass, stem biomass, inflorescence biomass and ramet biomass (Figure 2), which may be due to the different growth properties and growth times of each phenotypic characteristic. Under natural conditions, the reproductive ramets of the five clonal grasses in this study area usually stop increasing in ramet height and inflorescence length before flowering (*L. chinensis*: mid-June, *L. secalinus*: late June, and *C. ripidula*, *C. pseudophragmites* and *C. epigeios*: early July), and the increases in leaf biomass, stem biomass, inflorescence biomass and ramet biomass will continue until seed maturity (*L. chinensis*: mid-July, *L. secalinus*: late July, and *C. ripidula*, *C. pseudophragmites* and *C. epigeios*: early August). Thus, phenotypic characteristics such as ramet height and inflorescence length are less affected by physiological integration during growth because of the short growth period, whereas phenotypic characteristics related to biomass production are greatly affected by physiological integration because of the long growth period.

Previous studies concerning the effects of the physiological integration of clonal plants were mostly conducted using greenhouse pot experiments (Zhou et al., 2014; Wang et al., 2017a; Zhang et al., 2022), which enable easy manipulation but do not readily result in vegetative ramet–reproductive ramet pairs that meet the experimental requirements due to growth space, growth period and microenvironmental limitations. Field experiments can provide a more realistic test than greenhouse pot experiments but are more difficult to conduct. In this study, we grew five clonal grasses in a homogeneous field environment, and then at the early heading stage of each species, we selected similarly sized, synchronously heading ramet pairs (vegetative ramet–reproductive ramet) as experimental samples, thus minimizing or eliminating the effects of inherent differences and asynchronous heading on sexual reproductive performance (Li et al., 2018). Moreover, the selected ramet pairs were all located at the edges of the plots. Considering the low density of ramets at the edge, the large interval between ramets, and the proximity of resource supply, the other ramets had minimal influence on sexual reproductive performance compared with vegetative ramets growing on the tillering nodes. Therefore, the field experiments that we conducted in a homogeneous environment provide a more realistic test for assessing the effect of physiological integration between different functional ramets connected by tillering nodes on sexual reproductive performance. The effect of physiological integration between different functional

ramets connected through rhizomes or stolons on sexual reproductive performance in a heterogeneous environment can be explored in the future.

Isotope labeling technology is an effective means of exploring resource transfer between connected ramets of clonal plants (Zhang et al., 2002). A study using ^{15}N isotope labeling revealed that vegetative ramets of the wetland clonal plant *Iris laevigata* could translocate their resources to connected reproductive ramets (Wang et al., 2017b). In this study, when the connected vegetative ramets were labeled with ^{15}N at the milk-ripe stage in clonal populations of *L. chinensis*, *L. secalinus*, *C. ripidula*, *C. pseudophragmites* and *C. epigeios* growing in a homogeneous field environment, significantly larger amounts of ^{15}N than the background value were detected in the reproductive ramets (Table 1), indicating that vegetative ramets can transfer their own resources to the connected reproductive ramets in these five clonal grasses. The same results were obtained in our previous study on *H. glabra* (Guo et al., 2020b). Most importantly, we obtained consistent findings among these five clonal grasses, indicating commonality among them; i.e., vegetative ramets with tillering node connections can supply resources to reproductive ramets through physiological integration at the most critical stage of sexual reproduction, which is beneficial for sexual reproduction, thus further verifying the convergent adaptation of physiological integration between the two types of ramets to the same habitat conditions in the five clonal grasses.

4.2 Differences in the capacity for physiological integration between *L. chinensis* and its major companion species

Differences in physiological integration ability among clonal plants may determine their performance differences in the community. Many invasive alien plants are clonal. A comparative study on the physiological integration ability of invasive plants and congeneric co-occurring native plants showed that invasive plants had a higher physiological integration ability than native plants in heterogeneous environments, and physiological integration benefitted invasive clonal plants more than native plants and thus may confer a competitive advantage to invasive plants (Wang et al., 2017a). However, in grassland plant communities, when both dominant species and companion species are clonal, it is not clear whether the native dominant species have a higher physiological integration ability than the companions. In the present study, we found that the absolute change in reproductive ramet biomass was the largest for *C. ripidula* rather than for the dominant species *L. chinensis* (Figures 3A1, A2), indicating that the reproductive ramets of *C. ripidula* benefit more from physiological integration in absolute terms than those of the other four grasses. One potential mechanism for the higher absolute benefit of physiological integration in *C. ripidula* may be that this species has a higher capacity for resource translocation from vegetative ramets to their connected reproductive ramets than the other four grasses. We used isotope labeling to detect the amount of resource translocation from vegetative ramets and found that *C. ripidula* had a higher N translocation efficiency (Table 2). Another potential mechanism may be that the vegetative ramets of *C. ripidula* can absorb and utilize resources such as nutrients, water and light more efficiently than those of the other four grasses, so that a stronger source of nutrients, water and photosynthates could be created in the vegetative ramets of *C. ripidula* than in those of the other four grasses. We found that the vegetative ramet biomass of *C. ripidula* was significantly larger than that of the other four grasses in both severed and intact clones (Figure 5), confirming the plausibility of the second mechanism. Both a higher resource translocation capacity and a stronger source would allow for higher resource translocation from the vegetative to the reproductive ramets and thus benefit the growth of the latter.

Irrespective of the effect of physiological integration, we also found that *C. ripidula* generally produced taller and heavier reproductive ramets than the other four grasses (Figure 2). Our finding that reproductive ramets of *C. ripidula* benefitted more from physiological integration in absolute terms than those of the other four grasses in a homogeneous environment (Figures 3A1, A2) could therefore simply indicate that large plants benefit more from physiological integration in absolute terms than small plants in terms of sexual reproduction. In fact, an additional analysis showed that the relative change in reproductive ramet biomass was significantly larger in *L. chinensis* than in the other four grasses (Figures 3B1, B2). Meanwhile, isotope labeling showed that the amount of translocated ^{15}N per unit of reproductive ramet biomass was significantly larger in *L. chinensis* than in the other four grasses (Table 2). Both results indicate that the reproductive ramets of the dominant species *L. chinensis* benefit more from physiological integration in relative terms than those of the other four grasses, which supports our second hypothesis. The total amount of translocated ^{15}N or the absolute change in reproductive ramet biomass is mainly caused by inherent differences in biology between species, while the amount of translocated ^{15}N per unit of ramet biomass or the relative change in reproductive ramet biomass better reflects differences in physiological integration ability and their effects between species. Thus, the greater physiological integration ability of *L. chinensis* may confer an advantage over its four companion species in terms of sexual reproduction. This study is the first to report differences in the physiological integration ability of these five clonal grasses.

4.3 Analysis of the comprehensive reasons for *L. chinensis* being a dominant species in the meadow plant community

The bioecological characteristics of plant species determine their status and role in the community. Their tolerances to adversity, vegetative propagation characteristics, sexual reproduction characteristics, and physiological characteristics are important manifestations of their bioecological characteristics. *L. chinensis* is the dominant species of the most widely distributed natural meadow vegetation in the study area, while *L. secalinus*, *C. ripidula*, *C. pseudophragmites* and *C. epigeios* are the common companion species (Li et al., 2001). On the one hand, *L. chinensis* is more tolerant to drought and saline-alkaline conditions in representative habitats in the study area than *L. secalinus*, *C.*

ripidula, *C. pseudophragmites* and *C. epigeios* (Jia, 1987). On the other hand, the tillering nodes of *L. chinensis* ramets can propagate for four generations (Yang et al., 2003), while those of *L. secalinus* propagate for only two generations (Yang and Zhang, 2004) during the growing season in sandy soil habitats with enough growing space and no interspecies competition, implying that the tillering nodes of *L. chinensis* have a higher capacity for vegetative propagation than those of *L. secalinus*. In long-term mowed meadows, the clonal ramets of *L. chinensis* populations consisted of four age classes (Zhang et al., 2020), while the clonal ramets of *C. ripidula* (Yang et al., 1998), *C. pseudophragmites* (Yang and Zheng, 2000) and *C. epigeios* (Zhang et al., 2016) populations all consisted of two age classes, indicating that the clonal ramets of *L. chinensis* have a higher capacity for vegetative propagation than those of *C. ripidula*, *C. pseudophragmites* and *C. epigeios*. Therefore, these findings explain why *L. chinensis* became the dominant species, while *L. secalinus*, *C. ripidula*, *C. pseudophragmites* and *C. epigeios* became the companion species in the community from the perspective of vegetative propagation.

Sexual reproductive capacity affects the competitiveness of plant species in the community and their adaptability to changing environments. In this study, although the inflorescence biomass and ramet biomass of the reproductive ramets of *L. chinensis* were lower than those of the other four grasses (Figure 2), the inflorescence biomass allocation was significantly greater than that of the other four grasses (Figure 4). Allocation to sexual reproduction in *L. chinensis* is more advantageous than that in the other four grasses. Previous studies also showed that the inflorescence biomass allocation of reproductive ramets of *L. chinensis* was significantly greater than that of *L. secalinus* (Hong, 2020), *C. ripidula*, *C. pseudophragmites* and *C. epigeios* (Li, 2002) in this study area. In addition, we found that the amount of translocated ^{15}N per unit of reproductive ramet biomass was significantly larger in *L. chinensis* than in the other four grasses (Table 2), which means that the physiological integration ability and its effects were evidently stronger in *L. chinensis* than in the other four grasses. Therefore, differences in the tolerance, vegetative propagation characteristics, sexual reproduction characteristics, and physiological integration ability of the five clonal grasses together determine their status in the community.

5 Conclusions

In clonal populations of the dominant species *L. chinensis* and its main companion species *L. secalinus*, *C. ripidula*, *C. pseudophragmites* and *C. epigeios* growing in a homogeneous field environment, physiological integration between vegetative and reproductive ramets connected by tillering nodes significantly increased the leaf biomass, stem biomass, inflorescence biomass and ramet biomass of reproductive ramets. Vegetative ramets translocated their own resources to the connected reproductive ramets through tillering nodes. The physiological integration ability and its positive effect on sexual reproduction were stronger in the dominant species *L. chinensis* than in the other four main companion species. This study is the first to explain, from the perspective of physiological integration, the dominance of *L. chinensis* and the companion status of *L. secalinus*, *C. ripidula*, *C. pseudophragmites* and *C. epigeios* in the community. However, the current study was carried out in a homogeneous field environment with only one level of resource supply. Further studies that explore the effects of physiological integration between different functional ramets connected through rhizomes under different levels of resource supply or a heterogeneous resource supply will help us better understand the population adaptation and species evolution of clonal dominant species and their companion species in grassland plant communities in future environments.

Data availability statement

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

Author contributions

HL and YY designed the experiments. JG performed the experiments. JG and XY analyzed the data. JG and HL wrote the manuscript. All authors read and approved the manuscript. All authors contributed to the article.

Funding

This work was funded by the National Natural Science Foundation of China (32201477, 32171682, and 31670427), the Natural Science Foundation of Science and Technology Department of Jilin Province (20220101290JC), and the Program of Introducing Talents of Discipline to Universities (B16011).

Acknowledgments

We thank Xuenan Hong for helping with the experiments. We would like to acknowledge the editor and reviewers for their helpful comments on the manuscript.

Conflict of interest

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

The reviewer LD declared a past collaboration/shared affiliation with the author XY to the handling editor at the time of review.

Publisher's note

References

Alpert, P. (1996). Nutrient sharing in natural clonal fragments of *Fragaria chiloensis*. *J. Ecol.* 84, 395–406. doi: 10.2307/2261201

Ashmun, J. W., Thomas, R. J., and Pitelka, L. F. (1982). Translocation of photoassimilates between sister ramets in two rhizomatous forest herbs. *Ann. Bot.* 49, 403–415. doi: 10.1093/oxfordjournals.aob.a086264

Avolio, M. L., Forrestel, E. J., Chang, C. C., La Pierre, K. J., Burghardt, K. T., and Smith, M. D. (2019). Demystifying dominant species. *New Phytol.* 223, 1106–1126. doi: 10.1111/nph.15789

Chen, S. Y., Huang, X., Yan, X. Q., Liang, Y., Wang, Y. Z., Li, X. F., et al. (2013). Transcriptome analysis in sheepgrass *Leymus chinensis*: a dominant perennial grass of the Eurasian Steppe. *PloS ONE* 8, e67974. doi: 10.1371/journal.pone.0067974

Chen, J. S., Li, J., Zhang, Y., Zong, H., and Lei, N. F. (2015). Clonal integration ameliorates the carbon accumulation capacity of a stoloniferous herb, *Glechoma longituba*, growing in heterogenous light conditions by facilitating nitrogen assimilation in the rhizosphere. *Ann. Bot.* 115, 127–136. doi: 10.1093/aob/mcu207

Dong, M. (1999). Effects of severing rhizome on clonal growth in rhizomatous grass species *Psammochloa villosa* and *Leymus secalinus*. *Acta Bot. Sin.* 41, 194–198. doi: 10.3321/j.issn:1672-9072.1999.02.017

Dong, M., and Alaten, B. (1999). Clonal plasticity in response to rhizome severing and heterogeneous resource supply in the rhizomatous grass *Psammochloa villosa* in an Inner Mongolian dune, China. *Plant Ecol.* 141, 53–58. doi: 10.1023/A:1009875000149

Eckert, C. G. (2001). The loss of sex in clonal plants. *Evol. Ecol.* 15, 501–520. doi: 10.1007/978-94-017-1345-0_15

Eriksson, O. (1997). "Clonal life histories and the evolution of seed recruitment," in *The Ecology and Evolution of Clonal Plants*. Eds. H. Kroon and J. M. van Groenendael (Leiden, The Netherlands: Backhuys Publishers), 137–154.

Evans, J. P., and Cain, M. L. (1995). A spatially explicit test of foraging behavior in a clonal plant. *Ecology* 76, 1147–1155. doi: 10.2307/1940922

Gao, Y., Wang, D., Xing, F., Liu, J., and Wang, L. (2014). Combined effects of resource heterogeneity and simulated herbivory on plasticity of clonal integration in a rhizomatous perennial herb. *Plant Biol.* 16, 774–782. doi: 10.1111/plb.12122

Guo, J., Li, H., and Yang, Y. (2020a). Phenotypic plasticity in sexual reproduction based on nutrients supplied from vegetative ramets in a *Leymus chinensis* population. *Front. Plant Sci.* 10, 1681. doi: 10.3389/fpls.2019.01681

Guo, J., Li, H., and Yang, Y. (2020b). Physiological integration increases sexual reproductive performance of the rhizomatous grass *Hierochloe glabra*. *Plants-basel* 9, 1608. doi: 10.3390/plants9111608

Guo, J., Li, H., Zhou, C., and Yang, Y. (2020c). Effects of flag leaf and number of vegetative ramets on sexual reproductive performance in the clonal grass *Leymus chinensis*. *Front. Plant Sci.* 11, 534278. doi: 10.3389/fpls.2020.534278

Guo, J., Richards, C. L., Holsinger, K. E., Fox, G. A., Zhang, Z., and Zhou., C. (2021). Genetic structure in patchy populations of a candidate foundation plant: a case study of *Leymus chinensis* using genetic and clonal diversity. *Am. J. Bot.* 108, 2371–2387. doi: 10.1002/ajb2.1771

Harper, J. L. (1977). *Population Biology of Plants* (London: Academic Press).

Hong, X. (2020). *Module structure and growth analysis of Leymus secalinus clones in Songnen Plain, China* (Changchun: Northeast Normal University).

Jia, S. X. (1987). *Forage Flora of China* (Beijing: China Agricultural Press).

Jin, H., Kim, H. R., Plaha, P., Liu, S. K., Park, J. Y., Piao, Y. Z., et al. (2008). Expression profiling of the genes induced by Na_2CO_3 and NaCl stresses in leaves and roots of *Leymus chinensis*. *Plant Sci.* 175, 784–792. doi: 10.1016/j.plantsci.2008.07.016

Jónsdóttir, I., and Watson, M. (1997). "Extensive physiological integration: an adaptive trait in resource-poor environments," in *The ecology and evolution of clonal plants*. Eds. H. Kroon and J. van Groenendael (Leiden: Backhuys Publishers), 109–136.

Kuo, P. C. (1987). *Flora Reipublicae Popularis Sinicae* (Beijing: Science Press).

Li, H. (2002). *Study on the reproductive characteristics and convergent adaptation theory of rhizome grass population in Songnen Plain* (Changchun: Northeast Normal University).

Li, H., Jin, X., and Yang, Y. (2018). Consequences of asynchronous heading in a perennial bunchgrass (*Elymus excelsus*). *Sci. Rep.* 8, 3323. doi: 10.1038/s41598-018-21759-2

Li, J. D., Wu, B. H., and Sheng, L. X. (2001). *Jilin Vegetation* (Changchun: Jilin Science and Technology Press).

Meng, B., Li, J., Yao, Y., Nippert, J. B., Williams, D. G., Chai, H., et al. (2022). Soil N enrichment mediates carbon allocation through respiration in a dominant grass during drought. *Funct. Ecol.* 36, 1204–1215. doi: 10.1111/1365-2435.14033

Roiloa, S. R., Alpert, P., and Barreiro, R. (2019). Differences in physiological integration between invasive and non-invasive introduced clonal species of *Carpobrotus*. *J. Plant Ecol.* 12, 972–981. doi: 10.1093/jpe/rtz035

Roiloa, S. R., Antelo, B., and Retuerto, R. (2014). Physiological integration modifies $\delta^{15}N$ in the clonal plant *Fragaria vesca*, suggesting preferential transport of nitrogen to water-stressed offspring. *Ann. Bot.* 114, 399–411. doi: 10.1093/aob/mcu064

Smith, M. D., and Knapp, A. K. (2003). Dominant species maintain ecosystem function with non-random species loss. *Ecol. Lett.* 6, 509–517. doi: 10.1046/j.1461-0248.2003.00454.x

Song, Y. B., Yu, F. H., Keser, L. H., Dawson, W., Fischer, M., Dong, M., et al. (2013). United we stand, divided we fall: a meta-analysis of experiments on clonal integration and its relationship to invasiveness. *Oecologia* 171, 317–327. doi: 10.1007/s00442-012-2430-9

Sui, Y., He, W. M., Pan, X., and Dong, M. (2011). Partial mechanical stimulation facilitates the growth of the rhizomatous plant *Leymus secalinus*: modulation by clonal integration. *Ann. Bot.* 107, 693–697. doi: 10.1093/aob/mcr012

Sun, K., Cai, J. F., Zhang, Y., Mu, Y. N., A, S. H., Shen, Y. L., et al. (2022a). Heterogeneous nitrogen supply with high frequency and ramet damage increases the benefits of clonal integration in invasive *Hydrocotyle vulgaris*. *Front. Plant Sci.* 13, 825492. doi: 10.3389/fpls.2022.825492

Sun, Y., Zhang, X., Wang, S., Wu, X., Wang, Y., Wang, X., et al. (2022b). Phenotypic plasticity couples with transcriptomic flexibility in *Leymus chinensis* under diverse edaphic conditions. *Environ. Exp. Bot.* 197, 104838. doi: 10.1016/j.envexpbot.2022.104838

Wang, Y. J., Müller-Schärer, H., van Kleunen, M., Cai, A. M., Zhang, P., Yan, R., et al. (2017a). Invasive alien plants benefit more from clonal integration in heterogeneous environments than natives. *New Phytol.* 216, 1072–1078. doi: 10.1111/nph.14820

Wang, M., Wan, P., Chai, Y., Guo, Y., Zhang, X., and Yue, M. (2014). Adaptative strategy of leaf traits to drought conditions: *Quercus aliena* var. acuteserrata forest (The Qinling Mts. China). *Pol. J. Ecol.* 63, 77–87. doi: 10.3161/15052249pje2015.63.1.007

Wang, L., Wang, H., He, C., Sheng, L., and Tang, Z. (2017b). An irreversible division of labor through a sexually dependent system in the clonal plant *Iris laevigata* (Iridaceae). *Ecosphere* 8, e01757. doi: 10.1002/ecs2.1757

Yang, Y., and Zhang, B. (2004). Clone growth and its age structure of *Leymus secalimus* modules in Songnen Plain of China. *Chin. J. Appl. Ecol.* 15, 2109–2112. doi: 10.3321/j.issn:1000-0933.2004.02.015

Yang, Y., Zhang, B., and Li, J. (1998). Quantitative characteristics of vegetative propagation of *Calamagrostis ripidula* clonal populations in Songnen Plain of China. *Acta Pratac. Sin.* 7, 7–12. doi: 10.13287/j.1001-9332.202001.001

Yang, Y., Zhang., B., and Li., J. (2003). Module structure of *Leymus chinensis* clones under cultivated condition in Songnen Plain of China. *Chin. J. Appl. Ecol.* 14, 1847–1850. doi: 10.13287/j.1001-9332.2003.0408

Yang, Y., and Zheng, H. (2000). Age structure of clone population of *Calamagrostis pseudophragmites* in Songnen Plain of China. *Acta Pratac. Sin.* 9, 8–13. doi: 10.3321/j.issn:1004-5759.2000.03.002

Yang, Y. F., and Zhu, T. C. (2011). *Plant Ecology* (Beijing: Higher Education Press).

Zhai, J. F., Dong, Y. Y., Sun, Y. P., Wang, Q., Wang, N., Wang, F. W., et al. (2014). Discovery and analysis of microRNAs in *Leymus chinensis* under saline-alkali and drought stress using high-throughput sequencing. *PloS ONE* 9, e105417. doi: 10.1371/journal.pone.0105417

Zhang, X. M., He, L. X., Xiao, X., Lei, J. P., Tang, M., Lei, N. F., et al. (2022). Clonal integration benefits an invader in heterogeneous environments with reciprocal patchiness of resources, but not its native congener. *Front. Plant Sci.* 13, 1080674. doi: 10.3389/fpls.2022.1080674

Zhang, L., Li, H., and Yang, Y. (2016). Age structures of modules in a *Calamagrostis epigeios* population in single-species dominant patches in the Songnen Plain of China. *Acta Pratac. Sin.* 25, 20–27. doi: 10.11686/cyxb2016065

Zhang, M., Liu, F. D., An, S. Q., and Cao, X. Z. (2017). Leaf traits of the saplings of later successional stage tropical montane rain forest in Hainan Island. *Bull. Bot. Res.* 37, 907–914. doi: 10.1155/2014/649031

Zhang, J., Wang, R., Li, H., and Yang, Y. (2020). Age Structure of *Leymus chinensis* population modules in different habitats of Songnen Plain. *Chin. J. Grassl.* 42, 73–78. doi: 10.16742/j.zgcdxb.20190250

Zhang, C., Yang, C., and Dong, M. (2002). Clonal integration and its ecological significance in *Hedysarum laeve*, a rhizomatous shrub in Mu Us Sandland. *J. Plant Res.* 115, 113–118. doi: 10.1007/s102650200016

Zhou, C., Zhang, Z., Wang, Z., and Yang, Y. (2014). Difference in capacity of physiological integration between two ecotypes of *Leymus chinensis* underlies their different performance. *Plant Soil* 383, 191–202. doi: 10.1007/s11104-014-2169-7

Zhu, T. C. (2004). *Biological and Ecological Study of Leymus chinensis* (Changchun: Jilin Science and Technology Press).

frontiers | Frontiers in Plant Science

TYPE Original Research
PUBLISHED 03 November 2023
DOI 10.3389/fpls.2023.1275141

OPEN ACCESS

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RECEIVED 09 August 2023
ACCEPTED 02 October 2023
PUBLISHED 03 November 2023

CITATION
Song P, Xu Y-H, Yuan Y, Xu K-Q, Yao J-B and Chen S-Z (2023) High correlations between plant clonality and ecosystem service functions after management in a chronosequence of evergreen conifer plantations. *Front. Plant Sci.* 14:1275141. doi: 10.3389/fpls.2023.1275141

High correlations between plant clonality and ecosystem service functions after management in a chronosequence of evergreen conifer plantations

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Introduction: Climate change and mono-afforestation or mono-reforestation have continuously caused a decline in biodiversity and ecosystem services on forest plantations. Key plant functional traits in forests or plantations may affect ecosystem functions after forest management practices. Plant clonality, a key functional trait, frequently links to biodiversity and ecosystem functions and affects the biodiversity–ecosystem functioning relationship. However, little is known about how plant clonality affects ecosystem functions and services of plantations after forest management.

Methods: We conducted a field experiment to discuss the diversity and proportion of clonal plants, plant diversity of the communities, and ecosystem service functions and their relationships under 10 years of close-to-nature (CTN) management, artificial gap management, and control (i.e., without management) in the three stages of *C. Lanceolata* plantations.

Results: Our results showed that CTN and gap management modes significantly facilitated diversity of clonal plants, plant diversity of the communities, and parameters of ecosystem service functions in *C. lanceolata* plantations. Moreover, CTN management promoted plant community diversity, soil water conservation, and carbon storage the most in the earlier stand stages. Diversity of clonal plants was significantly positively correlated with ecosystem service functions after forest management. Structural equation modeling analysis indicated that forest gap or CTN management indirectly positively affected ecosystem service functions through increasing diversity of clonal woody plants and plant diversity of the communities.

Conclusion: Our results indicate a highly positive effect of gap or CTN management on diversity and proportion of clonal plants and on plant diversity of the communities, which link to improvements in ecosystem service functions

(i.e., water and soil conservation and carbon storage). The link between forest management, diversity, and ecosystem functions suggests that key functional traits or plant functional groups should be considered to underline the mechanism of traits–ecosystem functioning relationships and the restoration of degraded plantations.

KEYWORDS

artificial gap management, biodiversity-ecosystem functioning, clonal plants, close-to-nature management, plant diversity conservation, water and soil conservation, carbon storage

1 Introduction

Climate change and human activities (such as urban expansion and habitat destruction) have continuously affected global vegetation in the past several decades, leading to a substantial loss of biodiversity and a decline in ecosystem services (Montoya and Raffaelli, 2010; Díaz et al., 2019; Pan et al., 2022). Forest plantations, as the most significant component of vegetation, are a key way to restore degraded land and mitigate climate change (Canadell and Raupach, 2008; Bastin et al., 2019; Cook-Patton et al., 2021; Feng et al., 2022). Over recent decades, China has had the largest plantations consisting of fast-growing pure stands worldwide (Bai et al., 2020b). However, most pure plantations have suffered a severe decline in ecosystem services, that is, productivity, biodiversity, and carbon storage (Ming et al., 2019), which raised concerns about how to promote the functions and services of forest plantations to ensure effective strategic planning of afforestation and reforestation practices (Zhao et al., 2013; Bai et al., 2020b; Feng et al., 2022; Pan et al., 2022). Thus, understanding the effect of forest management on forest biodiversity and other ecosystem functions and services of plantations is critical to maintaining the sustainable supply of multiple ecosystem services.

Forest improvement management modes, including artificial gap regeneration, close-to-nature (CTN) transformation and selective thinning, are employed in improving the structure and functions of plantations, in which planting or increasing the growth of different tree species will create a high biodiversity–ecosystem functioning (BEF) relationship, such as production and carbon storage (Loreau et al., 2001; Tilman et al., 2014; Feng et al., 2022). The plantation and management types could alter the biotic and abiotic circumstances, such as plantation models (mixed vs. monoculture), plantation age, and management types (managed vs. unmanaged), which might facilitate stability and plant diversity (Felton et al., 2010; Li et al., 2012; Bonner et al., 2013; Liu et al., 2018; Wang et al., 2022). Furthermore, CTN and artificial gap regeneration management modes are commonly used in plantation improvement in China (Zang et al., 2005; Liu, 2013), which can improve the light interception capacity, soil moisture, and nutrients of the understory, which increased tree growth and biomass accumulation (Atauri et al., 2004; Brunet et al., 2010; Gong et al., 2021), and consequently might largely increase ecosystem services, that is, forest carbon storage and soil and water conservation (Cheng et al., 2017; Ming et al., 2019; Bai et al., 2020a; Bai et al., 2020b).

However, compared to the total plant diversity–ecosystem functions relationship, the effect of management on tree, shrub, or herb diversity ecosystem functions relationship in different development stages of plantations remains controversial (Powers et al., 2011; Grossman et al., 2018; Huang et al., 2018; Gong et al., 2021; Trogisch et al., 2021). Previous BEF forest management has shown that tree diversity commonly increases ecosystem functions and services (i.e., stand-level production) (Grossman et al., 2018; Huang et al., 2018; Trogisch et al., 2021). Some studies have indicated that managed forests are effective at maintaining carbon storage (Johnson and Curtis, 2001; Bai et al., 2020b). However, other studies suggest that long-term management decreased, and thinning had little impact on carbon storage in red pine plantations over a long period, no matter what tree or herb diversity changed (Powers et al., 2011). However, some studies exhibited an increase in water and soil conservation (such as water holding capacity and soil nitrogen and phosphorus content) after management of Chinese fir plantations, which may contribute to root and litter decomposition of diverse tree species (Cheng et al., 2017; Ming et al., 2019). Meanwhile, these relationships might alter with the developmental stages of plantations. Therefore, understanding the dynamics of plantations is crucial to quantifying the diversity–ecosystem function relationship after different management practices.

Plant functional traits including morphological and physiological characteristics that directly influence plant growth, and the efficiency of resource acquisition and utilization. These traits commonly respond to environmental changes and drive a variety of ecosystem processes such as ecosystem services (Pan et al., 2022). In comparison to monoculture plantations, mixed plantations with high plant diversity provide more diverse functional groups to support diverse habitat structure, food, and habitats to consumers and decomposers (Wang et al., 2019; Guo et al., 2021; Rutten et al., 2021), which might lead to higher tree, shrub, and herb diversity at the trophic levels. Moreover, clonal growth plants (i.e., clonality), a significant functional group, are present in the most productive ecosystems around the globe (de Kroon and van Groenendael, 1997; Cornelissen et al., 2014;

Moor et al., 2017; Dong et al., 2019; Zhao et al., 2020; Zhao et al., 2023). They could form large vegetation, promoting biodiversity and providing other ecosystem services, such as carbon sequestration, and nutrient and water cycling (Duarte et al., 2013; Cornelissen et al., 2014; Wang et al., 2016a; Wang et al., 2016b; Wang et al., 2017; Chen et al., 2019; Ruiz-Reynés and Gomila, 2019; Wang et al., 2019; Chen et al., 2023). Therefore, a series of traits for special plant functional groups, that is, clonal plants, after forest management provide an important link to ecosystem service trade-offs, and clonal plants are conducive to the successful restoration of degraded ecosystems (Duarte et al., 2013; Qi et al., 2021), stability of community structure and the maintenance of ecosystem functions (such as productivity, carbon sequestration, nitrogen cycle, etc.) (Yu et al., 2010; Cornelissen et al., 2014; Dickson et al., 2014; Klimešová et al., 2018; Klimešová et al., 2021). However, most studies focused on the ecological effects of clonal plants at the individual or population level (Wang et al., 2021), and there is little research on the role of clonality functional type at the community or ecosystem level (Cornelissen et al., 2014; Klimešová et al., 2021). Therefore, there is currently a lack of research on the impact and mechanisms of clonal plants on the diversity–ecosystem function relationship in managed plantations. Moreover, little was known about the roles of clonality in ecosystem service and functioning, including roles in nitrogen and phosphorus cycling, conservation of water and soil, and carbon storage (for aim of carbon neutral) in forest ecosystems.

Chinese fir [*Cunninghamia lanceolata* (Lamb.) Hook.] plantations are typical sub-tropical evergreen conifer vegetation with high-efficiency timber production (Yu, 1997; Farooq et al., 2019), and ecological functions (Yao et al., 2015; Cheng et al., 2017). However, it suffered the decline in productivity and soil fertility deficiencies of monoculture and continuous planting (Wu et al., 2017; Farooq et al., 2019). Thus, different plantation management modes on Chinese fir plantations have continuously been conducted. We conducted a field experiment to analyze the diversity and proportion of clonal plants, plant diversity of the communities, and ecosystem service functions under CTN management or artificial gap management or control (i.e., without management) in the three stages of *C. lanceolata* plantations. Specifically, we discussed the following questions: (1) How do forest management modes and forest stages affect diversity and proportion of clonal plants, plant diversity of the communities, and ecosystem service functions in *C. lanceolata* plantations? (2) the The relationships between diversity of clonal plants, plant diversity of the communities, and ecosystem service functions under different forest management modes, and (3) what is the key determinant and link to promoting these relationships?

2 Materials and methods

2.1 Study site

The experiment was carried out in plantations of Chinese fir [*Cunninghamia lanceolata* (Lamb.) Hook] at the Experimental Center of Subtropical Forestry of the Chinese Academy of Forestry in Fenyi, Jiangxi Province, China (114°38'–114°40'E, 27 ° 43'–27°45'N). This region is a typical location for *C. lanceolata* plantings in Southeast China's low mountains and hills. The average annual temperature is 15.8°C, and the average annual rainfall is 1590 mm, making the climate a typical humid subtropical monsoon. The mean annual relative humidity ranged from 80% to 85% [data from the Fenyi meteorological station (No. 57792) (114°41'E, 27 °43'N)]. Since 1957, *C. lanceolata* has been cultivated across the research region (Li et al., 2019). The study stands were constructed starting in 1987 from seedlings following recurrent clear cutting and burning.

Meanwhile, many management modes, including CTN improvement, artificial gaps, selective thinning, and so forth, have been employed in *C. lanceolata* plantations to meet the need for short-term timber, long-term large-diameter logs, and high-ecological benefits (Bai et al., 2020b). In our study, we selected three management modes: CTN management, artificial gap management, and control (without the above two management modes), and these management modes were conducted in the young (6 years), mid-aged (15 years) and pre-mature (24 years) phases of *C. lanceolata* plantations in 2012. Specifically, CTN improvement mode was that *Phoebe bournei* (Hemsl.) Yang and *Schima superba* Gardn. et Champ, dominant tree species of regional climax, were replanted to *C. lanceolata* after thinning (with accumulated thinning intensity of 30%–50% among stands) to promote the stand condition and the growth of *C. lanceolata*. Artificial gap improvement mode was thinning (with accumulated thinning intensity of 30%–50% among stands) to form 50 m^2 to 100 m^2 canopy gap in each plot to promote the growth of *C. lanceolata* and forest regeneration. Gap markers and borders were both *C. lanceolata*. In contrast, in the control stands CTN or gap practices were not conducted in the plots. All experimental stands had a similar initial density of 1340–1833 trees ha^{-1}.

In August 2022, we selected 36 20 m × 20 m plots for the three management modes and for the three stand stages after 10-year management based on a random design, that is, four plots for each stand stage under each management. Therefore, there was a two factorial experiment of three levels of management (control vs. gap management vs. CTN management) and three levels of stand stage [i.e., forest current stages, mid-aged (16 years) vs. pre-mature (25 years) vs. mature (34 years)].

2.2 Plant diversity investigation

In each 20 m × 20 m plot, the height, diameter at breast height (DBH), and crown width of each tree individual were measured. The average height, and coverage of each shrub species within each of five random 5 m × 5 m subplots were investigated. Average height, and coverage of each herb species, was measured in each of five random 1 m × 1 m quadrats within the above 5 m × 5 m subplot. We classified each species as clonal or non-clonal. Meanwhile, the coverage of clonal herbs and herb layer in all

quadrats was measured. Then, the importance values (IV) of species were calculated using the following formulas:

$$\text{IV tree layer} = (\text{relative height} + \text{relative dominance} + \text{relative density}) \times 100/3;$$

$$\text{IV shrub and herb layer} = (\text{relative height} + \text{relative coverage}) \times 100/2.$$

We used IV to calculate Shannon-Wiener (SW) diversity index for the following parameters.

2.2.1 Measurements of diversity indices of plant communities

Richness and SW diversity indexes of tree, shrub, and herb layers and all plants were employed to describe plant diversity conservation functions in *C. lanceolata* plantations. The formulas are as following (Li et al., 2012; Wang et al., 2012; Meng et al., 2015):

$$\text{Richness index R} = S$$

$$\text{SW diversity index}: H = -\sum P_i \ln P_i$$

Community-weighted SW diversity index: SW diversity index based on the weighted parameters of tree (0.50), shrub (0.26), and herb layers (0.24) (Wang et al., 2012), where P_i is the relative IV of the species and S is the total species.

2.2.2 Measurements of diversity and proportion of clonal plants

Diversity of clonal plants include richness, SW diversity index, and the proportion of clonal woody plants, clonal herbs, and all clonal plants, as well as the coverage and cover proportion of clonal herbs. These parameters were calculated by the above diversity indices and according to IV for all clonal plants in plots (Zhang and Wu, 2014). The proportion of clonal woody plants, clonal herbs, and all clonal plants was the ratio of the number of clonal woody plants, clonal herbs, and all clonal plants to the number of woody plants, herbs, and all plants. The cover proportion of clonal herbs was the same as in the above formula.

2.2.3 Measurements of water conservation functions

Water conservation functions include soil total porosity, capillary porosity, mass of un- and partially decomposed litter, and water holding capacity. Five random 1 m × 1 m quadrats within each plot were used to collect the entire litter, including un-decomposed and partially decomposed parts. Samples of litter were taken to the laboratory and dried at 80°C to a constant weight for the determination of dry matter. The maximum water-holding content of litter was calculated to analyze the capacity of litter retaining water based on the water soaking method (i.e., dried litter in a nylon bag immersed in tap water, after which the wet weight was recorded at 24h) (Zagyvai-Kiss et al., 2019). We used a cutting ring to measure soil capillary porosity and total porosity.

2.2.4 Measurements of soil conservation (nutrient preservation) functions

Total nitrogen (N), total phosphorus (P), available N, and available P were employed to describe soil conservation (i.e., nutrient preservation) functions in *C. lanceolata* plantations. Five typical sampling points were used to create a mixed soil sample for each plot. Chemical features of soil were measured in the laboratory after air drying. Soil total N was determined using the Kjeldahl method; soil available N in soil was measured using alkaline hydrolysis diffusion method; total P was extracted with HF-HNO_3-$HClO_4$ and then determined by molybdenum antimony blue colorimetry; soil available P was extracted with 0.5 mol L^{-1} $NaHCO_3$ (pH 8.5) and measured by Mo-Sb anti-spectrophotometry method (Brookes et al., 1982).

2.2.5 Measurements of carbon storage functions

Carbon storage functions include carbon storage for the whole forest ecosystem and different components in *C. lanceolata* plantations. In each 20 m × 20 m plot, based on the data of DBH and height (H) of *C. lanceolata*, volume models in different stand ages (Supplementary Table S1) were used to calculate tree volume according to both volume table of the ministry of forestry and previous studies on regional *C. lanceolata* plantations at different stand stages (Yu, 1997; Lin, 2016).

The biomass model, which measured carbon storage of trees using the biomass–expansion factor (*BEF*) (Fang et al., 1996), was evaluated using the Intergovernmental Panel on Climate Change method. The following is the formula:

$$B = V \cdot BEF$$

$$BEF = WD \cdot BEF_1 \cdot (1 + R)$$

in which B is biomass per unit area (t·hm^{-2}), V is volume per unit area (m^3·hm^{-2}), WD is the wood density of *C. lanceolata* per unit area (t·hm^{-2}) (value = 0.31), BEF_1 is biomass expansion factor for IPCC in 2006 (value = 1.53), R is root/shoot ratio (value = 0.246).

C storage per unit area is calculated by multiplying B and CR in different stand ages of *C. lanceolata* plantations (Bai et al., 2020b), our former study on carbon storage in *C. lanceolata* plantations). We collected all shrubs and herbs in sampled quadrats, respectively, and then biomass of understory vegetation and litter were measured by the dry combustion method. C content rate in shrubs, herbs, and litter is according to IPCC 2006. Soil samples were taken in the following three layers: 0 cm to 20 cm and 20 cm to 40 cm soil depth. soil organic carbon (SOC) was measured by extracting soil samples with $K_2Cr_2O_7$ and H_2SO_4 (Brookes et al., 1982).

2.3 Statistical analyses

We used generalized linear models to analyze the effects of forest management modes on parameters of clonality, plant

diversity of the communities, and parameters of ecosystem service functions such as water conservation, soil nutrient preservation, and carbon storage in a chronosequence of *C. lanceolata* plantations. Richness, SW diversity index, and the proportion of clonal woody plants, clonal herbs, and all clonal plants, as well as the coverage and cover proportion of clonal herbs were referred to clonality parameters in these models. Plant diversity of the communities, functions of water conservation, soil conservation, and carbon storage were evaluated by parameters in sections 2.1–2.5, respectively. In these models, we included stand stage [middle aged (i.e., mid-aged) vs. pre-mature vs. mature], forest management (control vs. gap management vs. CTN management), and their interactions as fixed factors (Bai et al., 2020b; Shi et al., 2021; Wang et al., 2022). *Post-hoc* multiple comparisons for each model were separately conducted if there were significant differences between treatments of stand stage or forest management. We tested above effects using function *Anova* type II errors in the car package in R 4.1.1 (R Core Team, 2020).

We used Bray distances for plant clonality and for plant diversity and ecosystem functions (including water conservation, soil nutrient preservation, and carbon storage) data. Given these distance matrices, we computed partial Mantel correlations between plant clonality and plant diversity and between plant clonality and ecosystem functions data using the *linkET* package in R. Partial *Mantel* tests were also performed between the above two.

Structural equation modeling (SEM) was used to evaluate the direct and indirect links between forest management, forest age, plant clonality (richness of woody clonal plants), plant diversity of the communities (tree richness), water conservation (capillary porosity and water holding capacity of litter), soil nutrient preservation (available N and available P), and carbon storage (total ecosystem, trees, and 0 cm–20 cm soil). We built an *a priori* conceptual framework model that included two main pathways. Forest management, forest age, plant clonality, and plant diversity of the communities directly affect ecosystem functions of water conservation, soil nutrient preservation, and carbon storage. In the second, forest management indirectly affects ecosystem functions via influencing plant clonality and plant diversity of the communities (Li et al., 2023). The effects of different variables on ecosystem functions were determined by the path standardized coefficient and associated *P* values. SEM was conducted using the *lavaan* packages (Rosseel, 2012). All statistical analyses were performed using the software R 4.1.1 (R Core Team, 2020).

3 Results

3.1 Plant diversity

3.1.1 Diversity and proportion of clonal plants

Averaged across all treatments, richness, SW diversity index, and the proportion of clonal woody plants, clonal herbs, and all clonal plants, as well as the coverage and cover proportion of clonal herbs, were significantly higher with gap or CTN management than without management (i.e., control), in the mid-aged or pre-mature stage than in the mature stage (except for the proportion of clonal herbs and cover parameters of clonal herbs) (Table 1 and Figure 1). Interestingly, the positive effects of gap or CTN management on all diversity parameters of clonal plants were significantly greatest in the mid-aged stage (89.7%–650% or 184.9%–466.7%) and greater in the pre-mature stage (75.9%–366.7% or 80.8%–450%) than in the mature stage (18.0%–216.7% or 8.4%–216.7%) [significant stand stage (S) × forest management (F) interaction in Table 1 and Figure 1]. Moreover, all clonality parameters were greater under gap management than under CTN management in the mid-aged stage, while there were significant differences between the two management modes in the pre-mature and mature stages (significant S × F interaction in Table 1 and Figure 1). Even the parameters of clonal woody plants were greater under CTN management than under gap management in the pre-mature stage (Figures 1C–E).

3.1.2 Plant diversity of the communities

Richness and SW diversity index of tree, shrub, and herb layers and all clonal plants showed a similar pattern to diversity of clonal plants and were significantly greater under gap or CTN management and in the mid-aged stage (Table 2 and Figure 2). Interestingly, the positive effects of gap or CTN management on total richness, richness of tree layer and herb layer were significantly greatest in the mid-aged (172%–208% or 192%–480%) stage and greater in the pre-mature (135%–191% or 164%–300%) stage than in the mature (100%–175% or 130%–300%) stage (S × F interaction in Table 2 and Figures 2A, C, G, H). Moreover, total richness and richness of tree layer showed greater differences between CTN and gap management in the mid-aged and pre-mature stages than in the mature stage (significant S × F interaction in Table 2 and Figures 2A, C).

3.2 Ecosystem service functions

3.2.1 Water conservation

All water conservation parameters were significantly greater under CTN and gap management than under control treatment (Table 3 and Figure 3). Especially, mass of un-decomposed litter and partially decomposed litter, and water holding capacity of litter were greatest under CTN management (Figures 3A, B, E). Similarly, the above three parameters in *C. lanceolata* plantations increased with stand stage (Table 3 and Figures 3A, B, E). However, there was no significant S × F interaction for all water conservation parameters (Table 3).

3.2.2 Soil conservation (nutrient preservation)

All soil conservation parameters were significantly higher under CTN and gap management than under control treatment (Table 4 and Figure 4). Interestingly, the positive effects of gap or CTN

TABLE 1 Results of generalized linear models for effects of stand stage (mid-aged vs. pre-mature vs. mature), forest management (control vs. gap vs. close-to-nature), and their interactions on diversity and proportion of clonal plants (i.e., plant clonality) of *Cunninghamia lanceolata* plantations.

Effects on function diversity (clonality)	DF	Total richness of clonal plants		Proportion of clonal plants		Richness of clonal woody plants		SW diversity of clonal woody plants		Proportion of clonal woody plants	
		F	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Stand stage (S)	2	**55.90**	**< 0.001**	**5.35**	**0.011**	**12.27**	**< 0.001**	**4.03**	**0.035**	**6.93**	**0.004**
Forest management (F)	2	**223.59**	**< 0.001**	**35.33**	**< 0.001**	**85.88**	**< 0.001**	**10.40**	**0.001**	**49.16**	**< 0.001**
S × F	4	**20.63**	**< 0.001**	**8.67**	**< 0.001**	**7.29**	**< 0.001**	**3.41**	**0.029**	**5.63**	**0.002**
Whole model	8	**80.18**	**< 0.001**	**14.50**	**< 0.001**	**28.18**	**< 0.001**	**7.13**	**< 0.001**	**16.83**	**< 0.001**
Effects on function diversity (clonality)	**DF**	**Richness of clonal herbs**		**SW diversity of clonal herbs**		**Proportion of clonal herbs**		**Coverage of clonal herbs**		**Cover proportion of clonal herbs**	
		F	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Stand stage (S)	2	**36.08**	**< 0.001**	**36.45**	**< 0.001**	3.11	0.061	**27.84**	**< 0.001**	**22.64**	**< 0.001**
Forest management (F)	2	**84.08**	**< 0.001**	**135.87**	**< 0.001**	**7.66**	**0.002**	**94.85**	**< 0.001**	**15.60**	**< 0.001**
S × F	4	**10.83**	**< 0.001**	**10.62**	**< 0.001**	**3.92**	**0.012**	**14.63**	**< 0.001**	**4.79**	**0.005**
Whole model	8	**35.46**	**< 0.001**	**48.39**	**< 0.001**	**4.65**	**0.001**	**37.99**	**< 0.001**	**11.96**	**< 0.001**

Values are in bold when P< 0.05.

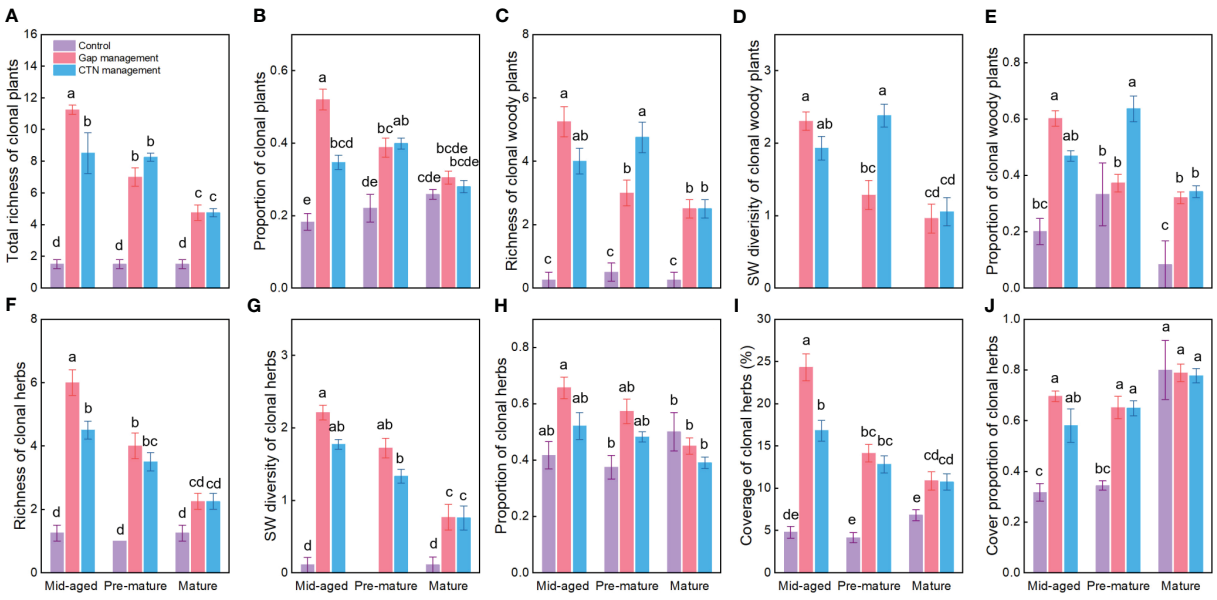

FIGURE 1
Diversity and proportion of clonal plants **(A–J)** under control, gap management, and close-to-nature (CTN) management treatments in different stand stages of *C. lanceolata* plantations. Mean ± SE are given. Different letters above the bars indicate significant difference among treatments at *P*< 0.05.

10.3389/fpls.2023.1275141

TABLE 2 Results of generalized linear models for effects of stand stage (mid-aged vs. pre-mature vs. mature), forest management (control vs. gap vs. close-to-nature), and their interactions on plant diversity of the communities of *C. lanceolata* plantations.

Effects on plant diversity of the communities	DF	Total richness		Community-weighted SW diversity		Richness of tree layer		SW diversity of tree layer	
		F	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Stand stage (S)	2	**74.54**	**< 0.001**	**18.17**	**< 0.001**	**13.23**	**< 0.001**	**3.61**	**0.041**
Forest management (F)	2	**580.57**	**< 0.001**	**301.09**	**< 0.001**	**118.02**	**< 0.001**	**96.40**	**< 0.001**
S × F	4	**6.68**	**0.001**	0.985	0.432	**5.04**	**0.004**	0.92	0.465
Whole model	8	**167.12**	**< 0.001**	**80.31**	**< 0.001**	**35.33**	**< 0.001**	**25.46**	**< 0.001**
Effects on plant diversity of the communities	**DF**	**Richness of shrub layer**		**SW diversity of shrub layer**		**Richness of herb layer**		**SW diversity of herb layer**	
		F	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Stand stage (S)	2	**4.08**	**0.028**	**6.11**	**0.006**	**20.22**	**< 0.001**	**16.76**	**< 0.001**
Forest management (F)	2	**82.04**	**< 0.001**	**124.99**	**< 0.001**	**78.84**	**< 0.001**	**95.49**	**< 0.001**
S × F	4	0.07	0.992	1.60	0.203	**3.69**	**0.016**	**4.17**	**0.009**
Whole model	8	**21.56**	**< 0.001**	**33.58**	**< 0.001**	**26.61**	**< 0.001**	**30.15**	**< 0.001**

Values are in bold when P< 0.05.

management on total N, available N, and available P were significantly greater in the pre-mature stage (41.4%–54.2% or 43.2%–61.2%) and in the mature stage (40.6%–59.4% or 48.4%–70.5%) than in the mid-aged stage (14.2%–20.6% or 14.5%–25%) (S × F interaction in Table 4 and Figures 4A, C, D). Meanwhile, under without management, total N, available N, and available P significantly decreased with the stand stages, while that is not the true under forest management modes (Figures 4C, D).

3.2.3 Carbon storage

All carbon storage parameters were significantly higher under CTN and gap management than under control treatment (expect

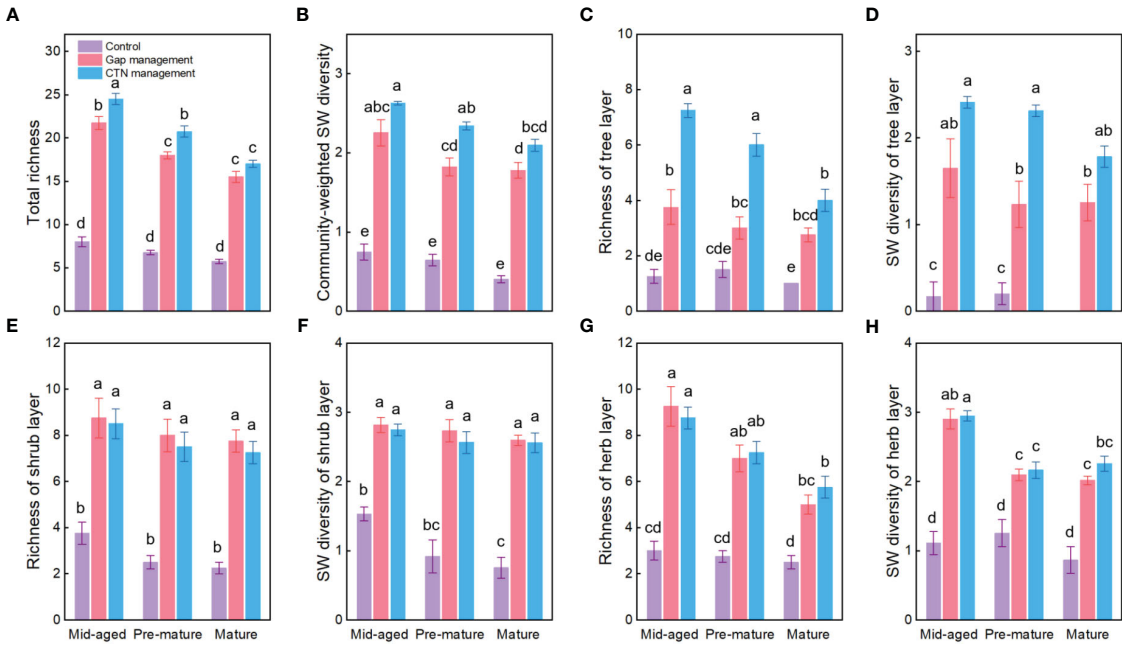

FIGURE 2
Plant diversity of the communities **(A–H)** under control, gap management, and close-to-nature (CTN) management treatments in different stand stages of *C. lanceolata* plantations. Mean ± SE are given. SW diversity: Shannon-Wiener diversity. Different letters above the bars indicate significant difference among treatments at *P*< 0.05.

TABLE 3 Results of generalized linear models for effects of stand stage (mid-aged vs. pre-mature vs. mature), forest management (control vs. gap vs. close-to-nature), and their interactions on water conservation functions of *C. lanceolata* plantations.

Effects on water conservation functions	DF	Mass of un-decomposed litter		Mass of partially decomposed litter		Total porosity		Capillary porosity		Water holding capacity of litter	
		F	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Stand stage (S)	2	**110.04**	**< 0.001**	**105.74**	**< 0.001**	0.39	0.681	0.59	0.564	**91.77**	**< 0.001**
Forest management (F)	2	**43.39**	**< 0.001**	**83.25**	**< 0.001**	**38.06**	**< 0.001**	**52.91**	**< 0.001**	**239.09**	**< 0.001**
S × F	4	1.99	0.125	0.542	0.706	1.10	0.377	1.06	0.398	0.52	0.719
Whole model	8	**39.35**	**< 0.001**	**47.517**	**< 0.001**	**10.16**	**< 0.001**	**13.90**	**< 0.001**	**82.98**	**< 0.001**

Values are in bold when P< 0.05.

for un-decomposed litter) (Table 5 and Figure 5). Especially, carbon storage in forest ecosystem, trees, and partially decomposed litter were greatest under CTN management (Figures 5A, B, E). Carbon storage of forest ecosystem, trees, un-decomposed, and partially decomposed litter, 0–20 and 20–40 cm soil increased with stand stage (Table 5 and Figures 5A, B, E–H). Interestingly, the positive effects of gap or CTN management on carbon storage of shrubs and herbs were significantly greater in the mid-aged stage (5211%–5352% or 2938%–3124%) and in the pre-mature stage (283%–718% or 232%–561%) than in the mature stage (17%–68% or 9%–72%) (S × F interaction in Table 5 and Figures 5C, D).

3.3 Relationships between clonality and plant diversity and between clonality and ecosystem service functions

Across all treatments, most clonality parameters were significantly positively related to plant diversity of the communities and ecosystem service functions (i.e., water conservation, soil nutrient preservation, and carbon storage) (Figure 6A), especially greater relationships (r > 0.4, P< 0.01) between richness and SW diversity of total, woody, and herb clonal plants, and cover proportion of clonal herbs and plant diversity of the communities, and between richness of woody and SW

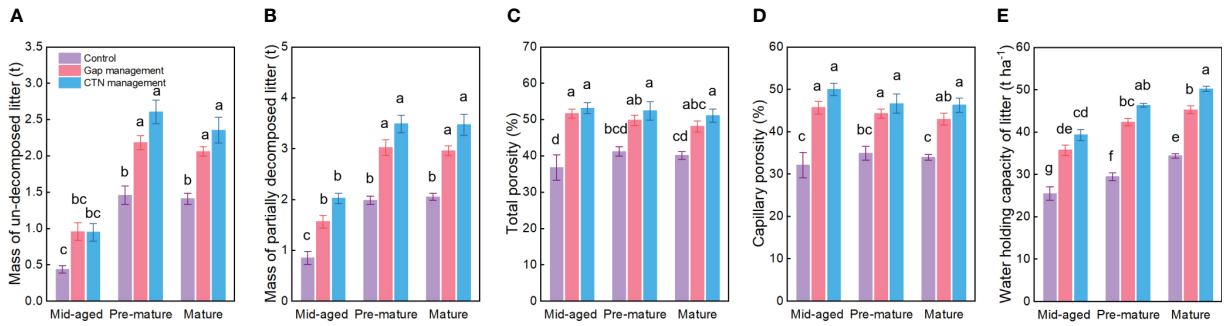

FIGURE 3
Water conservation functions **(A–E)** under control, gap management, and close-to-nature (CTN) management treatments in different stand stages of *C. lanceolata* plantations. Mean ± SE are given. Different letters above the bars indicate significant difference among treatments at P< 0.05.

TABLE 4 Results of generalized linear models for effects of stand stage (mid-aged vs. pre-mature vs. mature), forest management (control vs. gap vs. close-to-nature), and their interactions on soil nutrient preservation functions of *C. lanceolata* plantations.

Effects on soil nutrient preservation functions	DF	Total N		Total P		Available N		Available P	
		F	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Stand stage (S)	2	0.13	0.881	0.20	0.819	2.10	0.142	1.07	0.356
Forest management (F)	2	**75.89**	**< 0.001**	**3.52**	**0.044**	**122.84**	**< 0.001**	**75.07**	**< 0.001**
S × F	4	**5.34**	**0.003**	0.91	0.471	**7.90**	**< 0.001**	**2.78**	**0.047**
Whole model	8	**21.67**	**< 0.001**	**4.23**	**0.024**	**35.19**	**< 0.001**	**20.42**	**< 0.001**

Values are in bold when P< 0.05.

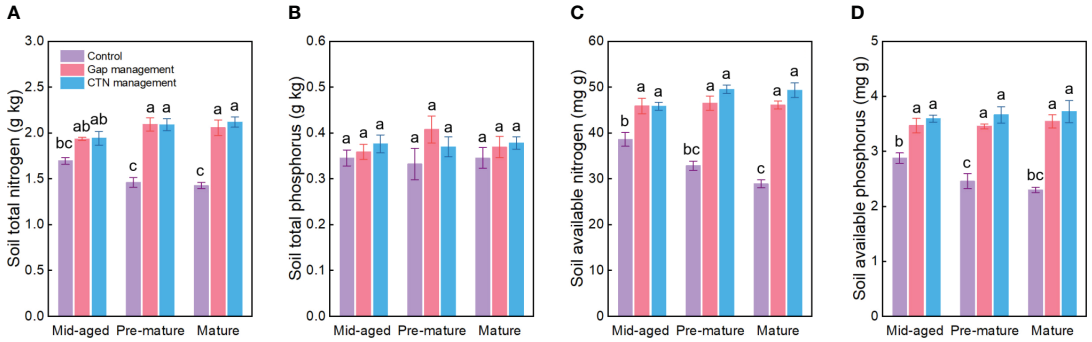

FIGURE 4
Soil nutrient preservation functions **(A–D)** under control, gap management, and close-to-nature (CTN) management treatments in different stand stages of *C. lanceolata* plantations. Mean ± SE are given. Different letters above the bars indicate significant difference among treatments at $P< 0.05$.

diversity of herb clonal plants and water conservation and soil nutrient preservation ($r > 0.4$, $P< 0.01$). All diversity and proportion parameters of clonal plants showed significantly positive correlations with carbon storage ($r > 0.2$, $P< 0.01$). However, there were fewer relationships between the proportion of total, woody, and herb clonal plants and plant diversity of the communities and ecosystem service functions of water conservation and soil nutrient preservation ($P > 0.05$). When without management, there was less correlation between clonality parameters and only a significantly positive relationship between cover proportion of clonal herbs and carbon storage herbs ($P< 0.05$) (Figure 6B).

3.4 Key path of forest management promoting ecosystem service functions

Forest gap or CTN management increased plant clonality (i.e., richness of woody clonal plants) [standardized total effect (ste) = 0.666, $P< 0.01$] and plant diversity of the communities (i.e., tree richness) (ste = 0.808, $P< 0.01$). However, plant diversity of the communities had no significantly positive effect on plant clonality (ste = 0.181, $P > 0.05$). Ecosystem service functions, that is, water conservation, soil nutrient preservation, and carbon storage, increased strongly with increasing plant clonality (ste = 0.361, $P< 0.05$; ste = 0.412, $P< 0.05$; and ste = 0.218, $P< 0.05$) and plant diversity of the communities (ste = 0.42, $P< 0.05$; ste = 0.296, $P< 0.05$; and ste = 0.271, $P > 0.05$), respectively. Forest gap or CTN management indirectly affected ecosystem service functions through increasing plant clonality and plant diversity of the communities. We observed that carbon storage increased significantly with forest age (ste = 0.982, $P< 0.01$). Relative influence of plant clonality and plant diversity of the communities on ecosystem service functions, including capillary porosity, water holding capacity of litter, available N, available P, carbon storage of total ecosystem, trees, and 0 cm–20 cm soil, across a chronosequence of *C. lanceolata* plantations (Figure 7).

TABLE 5 Results of generalized linear models for effects of stand stage (mid-aged vs. pre-mature vs. mature), forest management (control vs. gap vs. close-to-nature), and their interactions on carbon storage functions of C. *lanceolata plantations*.

Effects on carbon storage functions	DF	Total ecosystem		Trees		Shrubs		Herbs	
		F	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Stand stage (S)	2	**262.66**	**< 0.001**	**277.05**	**< 0.001**	**12.44**	**< 0.001**	**2.91**	**0.072**
Forest management (F)	2	**127.40**	**< 0.001**	**50.10**	**< 0.001**	**57.34**	**< 0.001**	**28.84**	**< 0.001**
S × F	4	1.34	0.280	1.52	0.224	**9.22**	**<0.001**	**5.59**	**0.002**
Whole model	8	**98.19**	**< 0.001**	**82.55**	**< 0.001**	**22.05**	**< 0.001**	**10.73**	**< 0.001**
Effects on carbon storage functions	DF	Un-decomposed litter		Partially decomposed litter		0 cm–20 cm soil		20 cm–40 cm soil	
		F	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Stand stage (S)	2	**13.37**	**< 0.001**	**19.96**	**< 0.001**	6.42	0.005	**7.82**	**0.002**
Forest management (F)	2	0.86	0.434	**27.28**	**< 0.001**	**93.31**	**< 0.001**	**16.04**	**< 0.001**
S × F	4	1.30	0.294	1.02	0.414	2.19	0.098	0.76	0.563
Whole model	8	4.21	0.002	**12.32**	**< 0.001**	**26.03**	**< 0.001**	**6.34**	**< 0.001**

Values are in bold when P< 0.05.

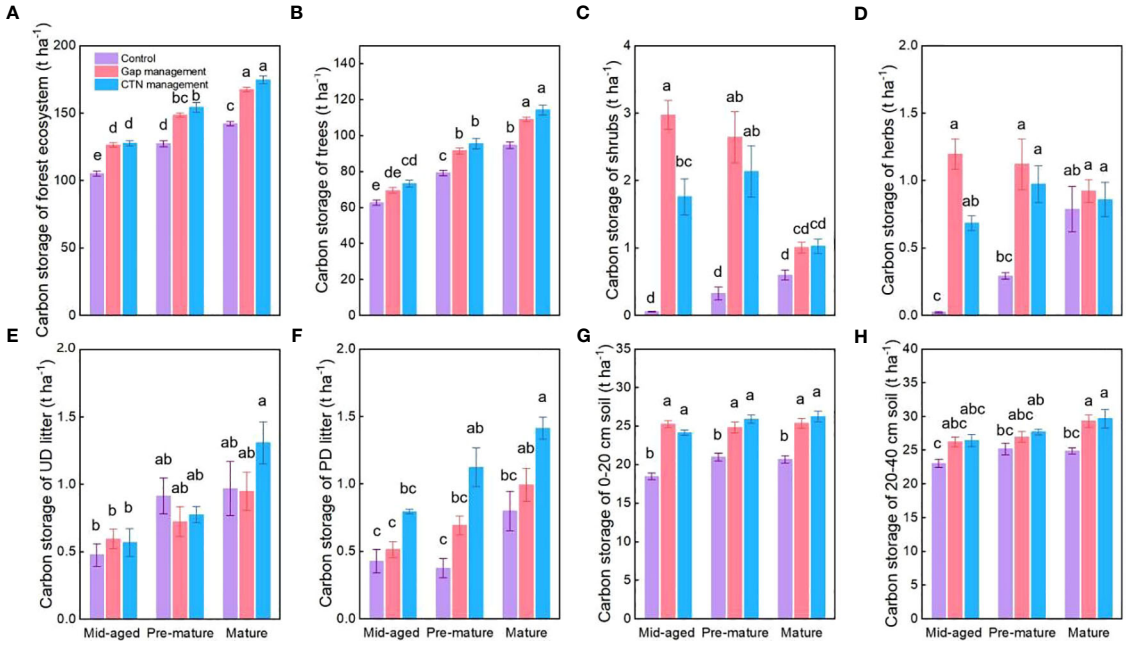

FIGURE 5
Carbon storage functions **(A–H)** under control, gap management, and close-to-nature (CTN) management treatments in different stand stages of *C. lanceolata* plantations. Mean ± SE are given. UD litter: un-decomposed litter; PD litter: partially decomposed litter. Different letters above the bars indicate significant difference among treatments at $P < 0.05$.

4 Discussion

Our results showed that CTN and gap management modes significantly facilitated diversity of clonal plants and parameters of ecosystem service functions in *C. lanceolata* plantations. Interestingly, CTN management promoted plant community diversity, soil water conservation, and carbon storage the most in the earlier stand stages. These findings imply that the link between a series of traits (plant clonality) and plant diversity and between plant clonality and ecosystem functions under forest management may be a driver of BEF relationship and the restoration of degraded plantations.

4.1 Diversity of clonal plants and ecosystem service functions under different forest management modes

Not surprisingly, we found that CTN and gap management modes significantly increase diversity and proportion of clonal plants, and plant diversity of community, water and soil conservation and carbon storage in a chronosequence of *C. lanceolata* plantations (Figures 2–5). This is consistent with findings of previous studies on forest diversity (Felton et al., 2010; Wu et al., 2013; Wang et al., 2018; Gong et al., 2021), water conservation (Zagyvai-Kiss et al., 2019), soil nutrient preservation (Jiang et al., 2019), and carbon storage (Bonner et al., 2013; Liu et al., 2018; Bai et al., 2020b). More importantly, the positive effects of CTN or gap management on diversity of clonal plants, plant diversity, and carbon storage of shrubs and herbs significantly declined with the stand stages (Figures 1, 2, 5). Generally, after CTN or gap management, the multi-species forest structure and differentiation of the niche often caused light heterogeneity of forests, leading to the coexistence of both shade-tolerant and shade-intolerant plants, and consequently might increase the diversity of woody and herbaceous plants (Gong et al., 2021), along with the diversity of clonal plants (i.e., clonal growth plants can easily and rapidly utilize light and soil water) (Shi et al., 2021). Meanwhile, multi-species or heterogeneous forest structure might affect soil moisture by modifying the re-distribution of rainfall and root characteristics, which increase hydraulic conductivity and soil water conservation by increasing the buffer and retention capacity of the multi-species forest canopy and litters (Zhao et al., 2018; Zagyvai-Kiss et al., 2019). In addition, in the forest management process, multi-species forest structure and environmental heterogeneity usually affect the growth period and the distribution of root systems, and the decomposition rate of litter and the root turnover rate, which increase woody growth, litter accumulation and soil nutrients (Jiang et al., 2019), and then increase ecosystem services such as forest carbon stock and soil nutrient conservation (Cheng et al., 2017; Ming et al., 2019; Bai et al., 2020b). Overall, the effects were consistent with a meta-analysis that found that it would take at least ten years for mixed-species plantations to significantly improve plant diversity and other ecosystem functions (Gong et al., 2021). However, our results indicated that these effects decreased in later developmental stages. This was in line with the previous results (Spake et al., 2019; Wang et al., 2022). As our study was conducted after 10-year CTN or gap management improvements, the former pre-mature stage (24 years) became mature age (34 years), meaning

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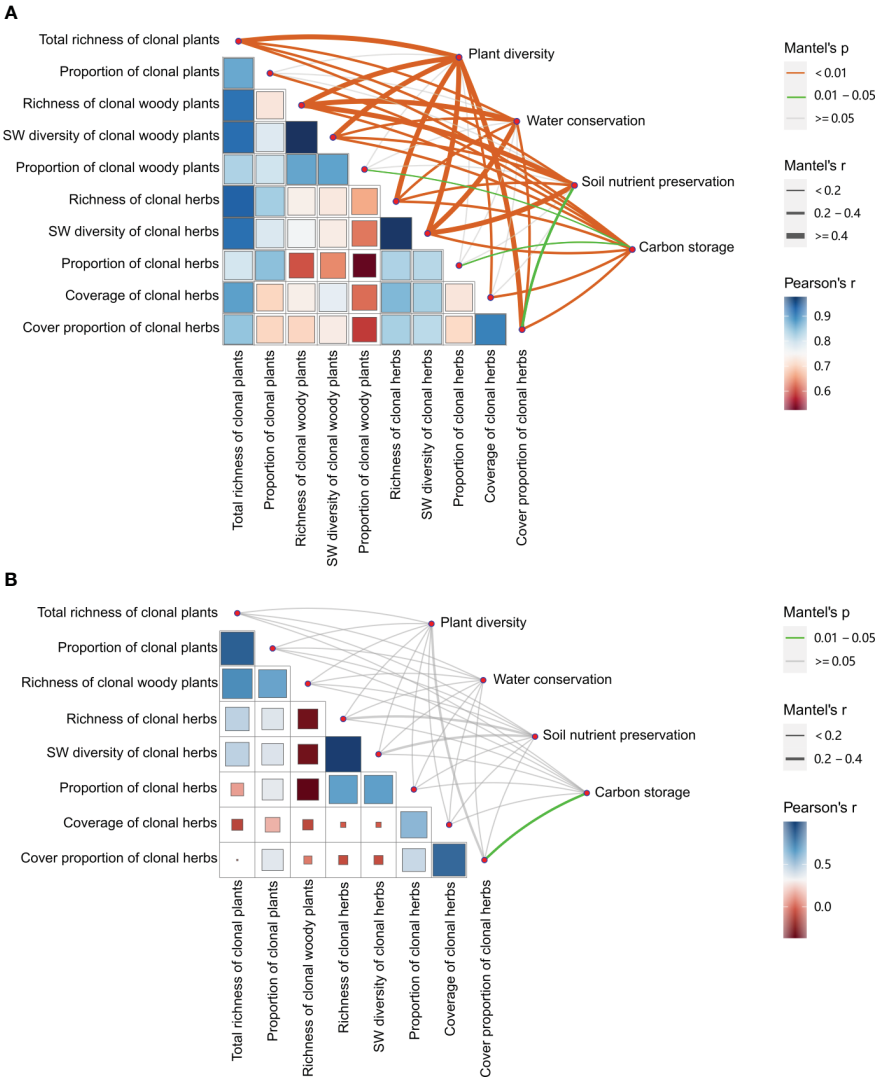

FIGURE 6
Relationships between plant clonality and plant diversity, and between plant clonality of the communities and ecosystem service functions (i.e., water conservation, soil nutrient preservation, and carbon storage). **(A)** with management; **(B)** without management.

that canopy coverage increased with plantation age, competition became intenser, and less heterogeneous habitats and shelters could support species.

4.2 Relationships between clonality and plant diversity and between clonality and ecosystem service functions

Our results indicated that there were significant links between a series of functional traits (plant clonality) and plant diversity of the communities and between plant clonality and ecosystem functions under forest management (Figure 6A). However, there was less relationship when under without management (Figure 6B). A special plant functional groups, that is, clonal plants, showed great increase after forest management (Shi et al., 2021). So, a series trait of clonality directly influences plant growth, and the efficiency of resource acquisition and utilization, which might provide an important link to parameters of ecosystem services and their trade-offs. First, as a component of plant diversity of a community, diversity of clonal plants certainly promotes the total plant diversity. Meanwhile, clonal growth can change the distribution of root and root length density, which can alter the soil structure and then improve soil porosity and soil retention capability (Cornelissen et al., 2014; Klimešová et al., 2018; Klimešová et al., 2021). Diversity of clonal woody plants and herbs not only increased growth rate of vegetation and plant community biomass but also enhance litter biomass, which in turn regulates vegetation carbon and soil carbon and water content (Yu et al., 2010; Cornelissen et al., 2014; Ruiz-Reynés and Gomila, 2019). Plant canopy structure affected diverse clonal woody plants might also determine hydrological regulating services by heterogeneous canopy structure (Shi et al., 2021).

In our study, a key path analysis (Figure 7) indicated that after CTN or gap management, an increase in clonal plants provides a series of clonal traits, such as richness and diversity, to support

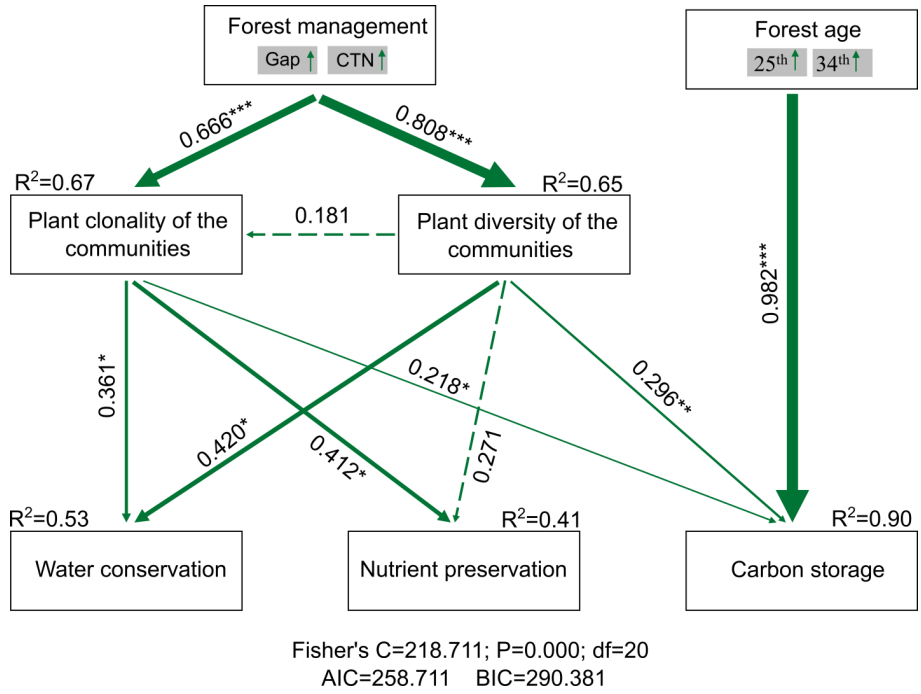

FIGURE 7
PiecewiseSEM accounting for forest management, forest age, plant clonality, and plant diversity of the communities on ecosystem service functions (water conservation, nutrient preservation, and carbon storage). The forest management and forest age were divided into composite variables. Numbers adjacent to arrows are path coefficients, which are the directly standardized effect size of the relationship. The thickness of the arrow represents the strength of the relationship. Conditional R^2 represents the proportion of variance explained by all predictors. Relationships between residual variables of measured predictors were not showed. Significance levels of each predictor are $^*P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$.

different parameters of ecosystem service functions. Moreover, clonal traits increase the relatively stable relationship with ecosystem service functions. As clonal plants are helpful for the effective restoration or improvement of many degraded ecosystems (Duarte et al., 2013; Qi et al., 2021), They also support the stability of community structure and the maintenance of ecosystem productivity, carbon sequestration (Yu et al., 2010; Cornelissen et al., 2014; Klimešová et al., 2018; Klimešová et al., 2021). Therefore, our results exhibited the effect of clonal plants ecosystem function, and relationships in managed plantations, result in the strong link between forest management, diversity (clonal plants and the communities), and ecosystem functions.

5 Conclusions

Our results indicate a highly positive influence of gap or CTN management on diversity and traits of clonal plants and on plant community diversity, which link to improvements in other ecosystem service functions (i.e., water and soil conservation and carbon storage). Forest gap or CTN management indirectly promoted ecosystem service functions through increasing diversity of clonal woody plants and plant diversity of the communities. In many forests or plantations, gap regeneration, CTN management, and other management practices may not only facilitate forest development but also change the key plant functional groups and their relationship with ecosystem functions. The link between forest management, diversity, and ecosystem functions suggests that key functional traits or plant functional groups, not only clonal plants but also groups of resource conservation or resource utilization, and so forth, under forest management should be considered to underline the mechanism of traits-ecosystem functioning relationships and the restoration of degraded plantations. Future studies on ecosystem multifunctionality should also consider the impact of clonality in forest multifunctionality and the trade-offs of ecosystem service functions.

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

PS: Data curation, Investigation, Writing – original draft. Y-HX: Formal Analysis, Methodology, Writing – original draft. YY:

Investigation, Writing – original draft. K-QX: Investigation, Writing – original draft. J-BY: Investigation, Project administration, Writing – review & editing. S-ZC: 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 research was supported by the Basic Research Fund of CAF (No. CAFYBB2020ZB002-3) and Implementation Plan for Realizing the Value of Forest and Grass Ecological Products (500102-5106).

Acknowledgments

We thank Yunduo Jin and Yangyang Shen for the practical assistance.

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.2023.1275141/full#supplementary-material

References

Atauri, J. A., De Pablo, C. L., De Agar, P. M., Schmitz, M. F., and Pineda, F. D. (2004). Effects of management on understory diversity in the forest ecosystems of Northern Spain. *Environ. Manage.* 34, 819–828. doi: 10.1007/s00267-004-0180-0

Bai, T., Liu, Y. Y., Muhammad, I., Yang, X., Yin, X. J., Bai, L., et al. (2020a). Mixed nitrogen form addition facilitates the growth adaptation of legume plant to heavy metal contamination in degraded mining areas. *Global Ecol. Conserv.* 24, e01387. doi: 10.1016/j.gecco.2020.e01387

Bai, Y. F., Shen, Y. Y., Jin, Y. D., Hong, Y., Liu, Y. Y., Li, Y. Q., et al. (2020b). Selective thinning and initial planting density management promote biomass and carbon storage in a chronosequence of evergreen conifer plantations in Southeast China. *Global Ecol. Conserv.* 24, e01216. doi: 10.1016/j.gecco.2020.e01216

Bastin, J. F., Finegold, Y., Garcia, C., Mollicone, D., Rezende, M., Routh, D., et al. (2019). The global tree restoration potential. *Science* 365, 76–79. doi: 10.1126/science.aax0848

Bonner, M. T., Schmidt, S., and Shoo, L. P. (2013). A meta-analytical global comparison of aboveground biomass accumulation between tropical secondary forests and monoculture plantations. *For. Ecol. Manage.* 291, 73–86. doi: 10.1016/j.foreco.2012.11.024

Brookes, P., Powlson, D., and Jenkinson, D. (1982). Measurement of microbial biomass phosphorus in soil. *Soil Biol. Biochem.* 14, 319–329. doi: 10.1016/0038-0717(82)90001-3

Brunet, J., Fritz, Ö., and Richnau, G. (2010). Biodiversity in European beech forests-a review with recommendations for sustainable forest management. *Ecol. Bulletins* 53, 77–94.

Canadell, J. G., and Raupach, M. R. (2008). Managing forests for climate change mitigation. *Science* 320, 1456–1457. doi: 10.1126/science.1155458

Chen, D., van Kleunen, M., Wang, Y. J., and Yu, F. H. (2023). Invasive and native plants show different root responses to feedback-mediated soil heterogeneity. *Plant Soil.* doi: 10.1007/s11104-023-06296-w

Chen, D., Xiong, H., Lin, C. G., He, W., Zhang, Z. W., Wang, H., et al. (2019). Clonal integration benefits invasive alien plants under water variability in a native community. *J. Plant Ecol.* 12, 574–582. doi: 10.1093/jpe/rty050

Cheng, X., Yu, M., and Wang, G. G. (2017). Effects of thinning on soil organic carbon fractions and soil properties in *Cunninghamia lanceolata* stands in eastern China. *Forests* 8, 198. doi: 10.3390/f8060198

Cook-Patton, S. C., Shoch, D., and Ellis, P. W. (2021). Dynamic global monitoring needed to use restoration of forest cover as a climate solution. *Nat. Climate Change* 11, 366–368. doi: 10.1038/s41558-021-01022-9

Cornelissen, J. H., Song, Y. B., Yu, F. H., and Dong, M. (2014). Plant traits and ecosystem effects of clonality: a new research agenda. *Ann. Bot.* 114, 369–376. doi: 10.1093/aob/mcu113

de Kroon, H., and van Groenendael, J. (1997). *The ecology and evolution of clonal plants* (Leiden, The Netherlands: Backhuys Publishers).

Díaz, S., Settele, J., Brondízio, E. S., Ngo, H. T., Agard, J., Arneth, A., et al. (2019). Pervasive human-driven decline of life on Earth points to the need for transformative change. *Science* 366, eaax3100. doi: 10.1126/science.aax3100

Dickson, T. L., Mittelbach, G. G., Reynolds, H. L., and Gross, K. L. (2014). Height and clonality traits determine plant community responses to fertilization. *Ecology* 95, 2443–2452. doi: 10.1890/13-1875.1

Dong, B. C., Zhang, L. M., Li, K. Y., Hu, X. T., Wang, P., Wang, Y. J., et al. (2019). Effects of clonal integration and nitrogen supply on responses of a clonal plant to short-term herbivory. *J. Plant Ecol.* 12 (4), 624–635. doi: 10.1093/jpe/rty057

Duarte, C. M., Sintes, T., and Marbà, N. (2013). Assessing the CO_2 capture potential of seagrass restoration projects. *J. Appl. Ecol.* 50, 1341–1349. doi: 10.1111/1365-2664.12155

Fang, J., Liu, G., and Xu, S. (1996). Biomass and net production of forest vegetation in China. *Acta Ecologica Sin.* 16, 497–508. doi: CNKI:SUN:STXB.0.1996-05-006

Farooq, T. H., Wu, W., Tigabu, M., Ma, X., He, Z., Rashid, M. H. U., et al. (2019). Growth, biomass production and root development of Chinese fir in relation to initial planting density. *Forests* 10, 236. doi: 10.3390/f10030236

Felton, A., Knight, E., Wood, J., Zammit, C., and Lindenmayer, D. (2010). A meta-analysis of fauna and flora species richness and abundance in plantations and pasture lands. *Biol. Conserv.* 143, 545–554. doi: 10.1016/j.biocon.2009.11.030

Feng, Y., Schmid, B., Loreau, M., Forrester, D. I., Fei, S., Zhu, J., et al. (2022). Multispecies forest plantations outyield monocultures across a broad range of conditions. *Science* 376, 865–868. doi: 10.1126/science.abm6363

Gong, C., Tan, Q., Liu, G., and Xu, M. (2021). Impacts of tree mixtures on understory plant diversity in China. *For. Ecol. Manage.* 498, 119545. doi: 10.1016/j.foreco.2021.119545

Grossman, J. J., Vanhellemont, M., Barsoum, N., Bauhus, J., Bruelheide, H., Castagneyrol, B., et al. (2018). Synthesis and future research directions linking tree diversity to growth, survival, and damage in a global network of tree diversity experiments. *Environ. Exp. Bot.* 152, 68–89. doi: 10.1016/j.envexpbot.2017.12.015

Guo, P. F., Wang, M. Q., Orr, M., Li, Y., Chen, J. T., Zhou, Q. S., et al. (2021). Tree diversity promotes predatory wasps and parasitoids but not pollinator bees in a subtropical experimental forest. *Basic Appl. Ecol.* 53, 134–142. doi: 10.1016/j.baae.2021.03.007

Huang, Y., Chen, Y., Castro-Izaguirre, N., Baruffol, M., Brezzi, M., Lang, A., et al. (2018). Impacts of species richness on productivity in a large-scale subtropical forest experiment. *Science* 362, 80–83. doi: 10.1126/science.aat6405

Jiang, J., Li, X., Jia, H., Ming, A., Chen, B., and Lu, Y. (2019). Effects of stand density on understory species diversity and soil physicochemical properties after close-to-nature transformation management of Chinese fir plantation. *J. Beijing Forestry Univ.* 41, 170–177. doi: 10.13332/j.1000-1522.20190022

Johnson, D. W., and Curtis, P. S. (2001). Effects of forest management on soil C and N storage: meta analysis. *For. Ecol. Manage.* 140, 227–238. doi: 10.1016/S0378-1127(00)00282-6

Klimešová, J., Martínková, J., and Ottaviani, G. (2018). Belowground plant functional ecology: towards an integrated perspective. *Funct. Ecol.* 32, 2115–2126. doi: 10.1111/1365-2435.13145

Klimešová, J., Mudrák, O., Martínková, J., Lisner, A., Lepš, J., Filartiga, A. L., et al. (2021). Are belowground clonal traits good predictors of ecosystem functioning in temperate grasslands? *Funct. Ecol.* 35, 787–795. doi: 10.1111/1365-2435.13755

Li, S., Huang, X., Tang, R., Li, J., Zhu, B., and Su, J. (2023). Soil microbial diversity and network complexity sustain ecosystem multifunctionality following afforestation in a dry-hot valley savanna. *CATENA* 231, 107329. doi: 10.1016/j.catena.2023.107329

Li, Z., Zhang, Y., Song, L., Hou, L., and Sun, Q. (2019). Storage of soil carbon and nitrogen in Chinese fir plantations at different ages in Dagang mountain of Jiangxi province. *J. Cent. South Univ. Forestry Technol.* 39, 116–122. doi: 10.14067/j.cnki.1673-923x.2019.10.017

Li, Z., Zhang, Z. W., Wang, Y. J., Wang, P. C., Xu, Y. R., and Zhou, Z. X. (2012). Influence of anthropogenic disturbances on understory plant diversity of urban forests in Wuhan, Central China. *Sains Malaysiana* 41 (12), 1495–1501.

Lin, Z. (2016). *The carbon accounting models, predictions and applications for chinese fir in Fujian province based on different scales* (Fuzhou: Fujian Agriculture and Forestry University).

Liu, S. R. (2013). *Principle and techniques of natural forests* (Beijing: Chinese Forestry Press).

Liu, X., Yang, T., Wang, Q., Huang, F., and Li, L. (2018). Dynamics of soil carbon and nitrogen stocks after afforestation in arid and semi-arid regions: A meta-analysis. *Sci. Total Environ.* 618, 1658–1664. doi: 10.1016/j.scitotenv.2017.10.009

Loreau, M., Naeem, S., Inchausti, P., Bengtsson, J., Grime, J. P., Hector, A., et al. (2001). Biodiversity and ecosystem functioning: current knowledge and future challenges. *Science* 294, 804–808. doi: 10.1126/science.1064088

Meng, X. F., Zhang, Z. W., Li, Z., Wu, X. J., and Wang, Y. J. (2015). The effects of city–suburb–exurb landscape context and distance to the edge on plant diversity of forests in Wuhan, China. *Plant Biosystems-An Int. J. Dealing all Aspects Plant Biol.* 149, 903–913. doi: 10.1080/11263504.2014.906510

Ming, A., Yang, Y., Liu, S., Nong, Y., Li, H., Tao, Y., et al. (2019). The impact of near natural forest management on the carbon stock and sequestration potential of Pinus massoniana (Lamb.) and *Cunninghamia lanceolata* (Lamb.) Hook. *Plantations. Forests* 10, 626. doi: 10.3390/f10080626

Montoya, J. M., and Raffaelli, D. (2010). Climate change, biotic interactions and ecosystem services. *Philos. Trans. R. Soc. B: Biol. Sci.* 365, 2013–2018. doi: 10.1098/rstb.2010.0114

Moor, H., Rydin, H., Hylander, K., Nilsson, M. B., Lindborg, R., and Norberg, J. (2017). Towards a trait-based ecology of wetland vegetation. *J. Ecol.* 105, 1623–1635. doi: 10.1111/1365-2745.12734

Pan, Q., Wen, Z., Wu, T., Zheng, T., Yang, Y., Li, R., et al. (2022). Trade-offs and synergies of forest ecosystem services from the perspective of plant functional traits: A systematic review. *Ecosystem Serv.* 58, 101484. doi: 10.1016/j.ecoser.2022.101484

Powers, M., Kolka, R., Palik, B., McDonald, R., and Jurgensen, M. (2011). Long-term management impacts on carbon storage in Lake States forests. *For. Ecol. Manage.* 262, 424–431. doi: 10.1016/j.foreco.2011.04.008

Qi, Q., Zhang, D., Tong, S., Zhang, M., Wang, X., An, Y., et al. (2021). The driving mechanisms for community expansion in a restored Carex tussock wetland. *Ecol. Indic.* 121, 107040. doi: 10.1016/j.ecolind.2020.107040

R Core Team, R., and Team, R. C. (2020). *R: a language and environment for statistical computing*. R Foundation for Statistical Computing; 2020. In.

Rosseel, Y. (2012). lavaan: An R package for structural equation modeling. *J. Stat. software* 48, 1–36. doi: 10.18637/jss.v048.i02

Ruiz-Reynés, D., and Gomila, D. (2019). Distribution of growth directions in meadows of clonal plants. *Phys. Rev. E* 100, 052208. doi: 10.1103/PhysRevE.100.052208

Rutten, G., Hönig, L., Schwaß, R., Braun, U., Saadani, M., Schuldt, A., et al. (2021). More diverse tree communities promote foliar fungal pathogen diversity, but decrease infestation rates per tree species, in a subtropical biodiversity experiment. *J. Ecol.* 109, 2068–2080. doi: 10.1111/1365-2745.13620

Shi, X. P., Bai, Y. F., Song, P., Liu, Y. Y., Zhang, Z. W., Zheng, B., et al. (2021). Clonal integration and phosphorus management under light heterogeneity facilitate the growth and diversity of understory vegetation and soil fungal communities. *Sci. Total Environ.* 767, 144322. doi: 10.1016/j.scitotenv.2020.144322

Spake, R., Yanou, S., Yamaura, Y., Kawamura, K., Kitayama, K., and Doncaster, C. P. (2019). Meta-analysis of management effects on biodiversity in plantation and secondary forests of Japan. *Conserv. Sci. Pract.* 1, e14. doi: 10.1111/csp2.14

Tilman, D., Isbell, F., and Cowles, J. M. (2014). Biodiversity and ecosystem functioning. *Annu. Rev. ecology evolution systematics* 45, 471–493. doi: 10.1146/annurev-ecolsys-120213-091917

Trogisch, S., Liu, X., Rutten, G., and Bruelheide, H. (2021). Tree diversity effects on ecosystem functioning–Introduction. *Basic Appl. Ecol.* 55, 1–5. doi: 10.1016/j.baae.2021.06.004

Wang, Y. J., Bai, Y. F., Zeng, S. Q., Yao, B., Wang, W., and Luo, F. L. (2016a). Heterogeneous water supply affects growth and benefits of clonal integration between co-existing invasive and native *Hydrocotyle* species. *Sci. Rep.* 6, 29420. doi: 10.1038/srep29420

Wang, Y. J., Chen, D., Yan, R., Yu, F. H., and van Kleunen, M. (2019). Invasive alien clonal plants are competitively superior over co-occurring native clonal plants. *Perspect. Plant Ecology Evol. Systematics* 40, 125484. doi: 10.1016/j.ppees.2019.125484

Wang, M. Q., Li, Y., Chesters, D., Anttonen, P., Bruelheide, H., Chen, J. T., et al. (2019). Multiple components of plant diversity loss determine herbivore phylogenetic diversity in a subtropical forest experiment. *J. Ecol.* 107, 2697–2712. doi: 10.1111/1365-2745.13273

Wang, Y. J., Liu, Y. Y., Chen, D., Du, D. L., Müller-Schärer, H., and Yu, F. H. (2022). Clonal functional traits favor the invasive success of alien plants into native communities. *Ecol. Appl.* doi: 10.1002/eap.2756

Wang, X., Lu, Y., Xing, H., Zeng, J., Xie, Y., Cai, D., et al. (2018). Effects of close-to-nature conversion on *Pinus massoniana* plantations at different stand developmental stages. *Trop. Conserv. Sci.* 11, 1940082918767953. doi: 10.1177/1940082918767953

Wang, Y. J., Müller-Schärer, H., van Kleunen, M., Cai, A. M., Zhang, P., Yan, R., et al. (2017). Invasive alien plants benefit more from clonal integration in heterogeneous environments than natives. *New Phytol.* 216, 1072–1078. doi: 10.1111/nph.14820

Wang, Y. J., Shi, X. P., Peng, Y., Zhong, Z. C., and Tao, J. P. (2012). Effects of fine-scale pattern of dwarf bamboo on understory species diversity in *Abies faxoniana* forest, southwest, China. *Sains Malaysiana* 41, 649–657. doi: 10.1016/j.rser.2012.02.042

Wang, Y. J., Shi, X. P., Wu, X. J., Meng, X. F., Wang, P. C., Zhou, Z. X., et al. (2016b). Effects of patch contrast and arrangement on benefits of clonal integration in a rhizomatous clonal plant. *Sci. Rep.* 6, 35459. doi: 10.1038/srep35459

Wang, J., Xu, T., Wang, Y., Li, G., Abdullah, I., Zhong, Z., et al. (2021). A meta-analysis of effects of physiological integration in clonal plants under homogeneous vs. heterogeneous environments. *Funct. Ecol.* 35, 578–589. doi: 10.1111/1365-2435.13732

Wu, X. J., Li, Q. Y., Zhang, Z. W., Meng, X. F., Li, Z., and Wang, Y. J. (2013). Dynamics of diversity, distribution patterns and interspecific associations of understory herbs in the city-suburb-exurb context of Wuhan city, China. *Arch. Biol. Sci.* 65 (4), 1619–1628. doi: 10.2298/ABS1304619W

Wu, P., Wang, G., Farooq, T. H., Li, Q., Zou, X., and Ma, X. (2017). Low phosphorus and competition affect Chinese fir cutting growth and root organic acid content: does neighboring root activity aggravate P nutrient deficiency? *J. Soils Sediments* 17, 2775–2785. doi: 10.1007/s11368-017-1852-8

Yao, L., Kang, W., Zhao, Z., and He, J. (2015). Carbon fixed characteristics of plant of Chinese fir (*Cunninghamia lanceolata*) plantation at different growth stages in Huitong. *Acta Ecologica Sin.* 35, 1187–1197. doi: 10.5846/stxb201304250814

Yu, X. (1997). *Silviculture of Chinese fir* (Fuzhou: Fujian Science & Technology Press), 1–7.

Yu, F. H., Wang, N., He, W. M., and Dong, M. (2010). Effects of clonal integration on species composition and biomass of sand dune communities. *J. Arid Environments* 74, 632–637. doi: 10.1016/j.jaridenv.2009.12.008

Zagyvai-Kiss, K. A., Kalicz, P., Szilágyi, J., and Gribovszki, Z. (2019). On the specific water holding capacity of litter for three forest ecosystems in the eastern foothills of the Alps. *Agric. For. Meteorology* 278, 107656. doi: 10.1016/j.agrformet.2019.107656

Zang, R. G., Cheng, K. W., Li, J. Q., Zhang, W. Y., Chen, X. F., Tao, J. P., et al. (2005). *Conservation and restoration of biodiversity in natural forest* (Beijing: Chinese Science and Technology Press).

Zhang, J. Y., and Wu, Y. X. (2014). Changes in diversity and importance of clonal plants during sand dune succession in northeastern China. *Ecol. Res.* 29, 393–399. doi: 10.1007/s11284-013-1124-z

Zhao, C. Y., Liu, Y. Y., Shi, X. P., and Wang, Y. J. (2020). Effects of soil nutrient variability and competitor identify on growth and co-existence among invasive alien and native clonal plants. *Environ. pollut.* 261, 113894. doi: 10.1016/j.envpol.2019.113894

Zhao, F., Wang, J., Zhang, L., Ren, C., Han, X., Yang, G., et al. (2018). Understory plants regulate soil respiration through changes in soil enzyme activity and microbial C, N, and P stoichiometry following afforestation. *Forests* 9, 436. doi: 10.3390/f9070436

Zhao, M., Xiang, W., Tian, D., Deng, X., Huang, Z., Zhou, X., et al. (2013). Effects of increased nitrogen deposition and rotation length on long-term productivity of *Cunninghamia lanceolata* plantation in southern China. *PloS One* 8, e55376. doi: 10.1371/journal.pone.0055376

Zhao, Y., Zhao, C. Y., Liu, Y. Y., Yan, Z. G., and Wang, Y. J. (2023). Clonal integration facilitates higher resistance to potentially toxic element stress in invasive alien plants than in natives. *Plant Soil* 488, 589–601. doi: 10.1007/s11104-023-05992-x

frontiers | Frontiers in Plant Science

TYPE Original Research
PUBLISHED 23 November 2023
DOI 10.3389/fpls.2023.1308072

OPEN ACCESS

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RECEIVED 05 October 2023
ACCEPTED 08 November 2023
PUBLISHED 23 November 2023

CITATION
Zou Z, Li Y and Song H (2023) Influence of the size of clonal fragment on the nitrogen turnover processes in a bamboo ecosystem.
Front. Plant Sci. 14:1308072.
doi: 10.3389/fpls.2023.1308072

Influence of the size of clonal fragment on the nitrogen turnover processes in a bamboo ecosystem

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Different sizes of clonal fragments contain various number of ramets with different spacer lengths, which strongly affects the redistribution of photosynthetic assimilates. Although clonal integration significantly affects rhizosphere processes via microbial enzymes under heterogeneous conditions, the effects of clonal fragment size (ramet number and spacer length) on rhizosphere N turnover processes remain poorly understood. Here, we sampled clonal fragments of *Phyllostachys bissetii* with different ramet numbers and spacer lengths to determine the relative effects of clonal integration and fragment size on rhizosphere processes and resource availability. We found that clonal integration had positive effects on the C and N availability of shaded ramets in clonal fragments with different ramet numbers, owing to the large resource storage in the fragment. However, it only promoted the dissolved organic carbon of the shaded ramets in clonal fragments with different spacer lengths. Results of regression analyses indicated that the response ratios of the soil variables of the shaded ramets first increased when the spacer length was about less than 30 cm and then decreased when the spacer became longer (about >30 cm), suggesting a cost–benefit tradeoff in the fragment. The contribution of the size of clonal fragment to the soil N turnover process was higher than that of clonal integration, whereas its contribution to soil C availability had the opposite effect. These results further revealed the mechanism of the size of clonal fragment in affecting the rhizosphere processes of stressed ramets, which is critical for the adaptation of *P. bissetii* to stressed habitats and further bamboo ecosystem N turnover under climate change.

KEYWORDS

ramet number, spacer length, nitrogen turnover, heterogeneous light, clonal integration

1 Introduction

Clonal plant ramets are characterized by resource translocation between connected ramets through stolons or rhizomes, including photosynthetic products (Wang et al., 2004; Chen J. S. et al., 2015), water (Hu et al., 2015), and organic matter nutrients (Li et al., 2018). Such traits are crucial for clonal plants to adapt to the heterogeneous light environments that are common in nature (Guan et al., 2023). In heterogeneous environments, the uneven distribution of essential resources increases the difficulty in plant absorption (Wang et al., 2017; Chen et al., 2019; Liang et al., 2020). The source–sink gradient in a clonal fragment enables ramets in resource-poor patches to receive resource support from other ramets in resource-rich patches (You et al., 2023; Zhang et al., 2023). Clonal plants always form different sizes of fragments containing multiple ramets with different spacer length. However, the combined effects of fragment size and clonal integration on nitrogen turnover processes in the rhizosphere remains poorly understood.

The size of clonal fragment may affect resource uptake and translocation (Zheng et al., 2023), plasticity (Ganie et al., 2016), and asexual reproduction (Alpert, 1999; Latzel and Klimesova, 2010). Both spacer length and ramet number can affect the size of a clonal fragment. Differences in spacer length may affect the energy required for resource translocation and limit the intensity of clonal integration (Liu et al., 2004; Li et al., 2008; Yu et al., 2020). Previous studies have shown that the benefits of the physiological integration of water in a clonal fragment of *Indocalamus decorus* and *Populus euphratica* decrease when the spacer length increases (Hu et al., 2015; Zhu et al., 2018). In addition, the ramet number can also lead to differences in the sizes of clonal fragments. Each ramet can serve as a source or sink for the entire clonal fragment, where a very complex process of nutrient translocation occurs (Sheng et al., 2007). As the source of nutrients may vary, the output of nutrients that these ramets can provide and the input of resources from connected ramets may also differ owing to the influence of different generations and developmental stages (Wang et al., 2004). Thus, resource redistribution among ramets and physiological integration into clonal fragments are both influenced by the number of ramets (Hutchings and Wijesinghe, 1997; Chen et al., 2010; Zhai et al., 2022). However, to the best of our knowledge, no previous study has tested the relative importance of spacer length and ramet number in clonal integration or rhizosphere processes.

Nitrogen (N) is a limiting resource for clonal plants (Saitoh et al., 2006; Tian et al., 2023), and is regulated by soil microbial community and extracellular enzyme activity (Kuzyakov, 2002; Jones et al., 2004; Sun et al., 2014). Previous studies have shown that clonal integration leads to an increase in soil carbon (C) availability in the rhizosphere of stressed ramets (Lei et al., 2014; Chen J.S. et al., 2015), which could enhance extracellular enzymes and further prime the decomposition of soil organic matter and the transformation of nitrogen (Li et al., 2019). Numerous previous studies have indicated that fragment size can strongly affect the survival, regrowth, and biomass of clonal ramets based on the tradeoff between costs and benefits (Dong et al., 2012; Lin et al., 2012; Huber et al., 2014). Several studies have tried to reveal the influence of the size of clonal fragments on water integration (Hu et al., 2015; Zhu et al., 2018). However, the effect of differences in the sizes of clonal fragments on rhizosphere processes, such as nitrogen turnover, remains largely unknown.

Bamboo, a typical rhizome clonal plant, can undergo clonal integration through connected spacers (Song et al., 2016), resulting in a higher ecological adaptability to heterogeneous light environments (Saitoh et al., 2002; Zheng and Lv, 2023). *Phyllostachys bissetii* is a dominant species in the middle and lower canopy layers of forests and strongly influence ecosystem functions of forest (Kang et al., 2019). In this study, we sampled clonal fragments of *P. bissetii* of different sizes, including different spacer lengths and ramet numbers, to determine the effect of the size of clonal fragment on clonal integration and rhizosphere processes. In this study, we aimed to 1) determine the effects of clonal integration on rhizosphere C and N availability, 2) investigate the effects of the size of clonal fragment on rhizosphere N turnover processes, and 3) estimate the relative contributions of clonal integration and size of clonal fragment to rhizosphere processes. We hypothesize that 1) connected ramets promote rhizosphere processes of shaded ramets through clonal integration, and 2) clonal integration and size of clonal fragment affect rhizosphere processes differently.

2 Materials and methods

2.1 Experimental design

P. bissetii is a perennial and monopodial bamboo species, which is a woody clonal plant that propagates vegetatively by extending its rhizome. *P. bissetii* forms dense rhizome networks in belowground, and new ramets develop from active nodal buds on the rhizome. *P. bissetii* has significant economic value and is one of the main food sources for giant pandas.

The *in-situ* field experiment was conducted at Nanbaoshan Town in Sichuan Province, China (103.019° E, 30.45° N, Figure 1A), with an elevation of 1217 m. Mean annual precipitation and temperature of this site is 1117.3 mm and 16.3°C, respectively (Li et al., 2019). In 2017, clonal fragments of different sizes were selected at this site, including clonal fragments with various spacer lengths and numbers of ramets (Figures 1B, C). Clonal fragments with different spacer lengths contained one exposed ramet and one shaded ramet, whereas clonal fragments with different ramet numbers included to 3–5 ramets. We recorded all spacer lengths between any two connected ramets in all fragments. Distant ramets were identified based on the direction of rhizome growth (Chen B. J. W. et al., 2015; Zou et al., 2018). All distant ramets at the end of each rhizome were shaded using black-shading netting that transmitted only about 20% of the ambient photosynthetically active photon flux density (PPFD). The remaining ramets in the clonal fragments were placed under natural light. Control treatments were established by severing the rhizomes of connecting shaded ramets and others in each fragment (Figure 1D). This experiment aimed to explore the effect of clonal

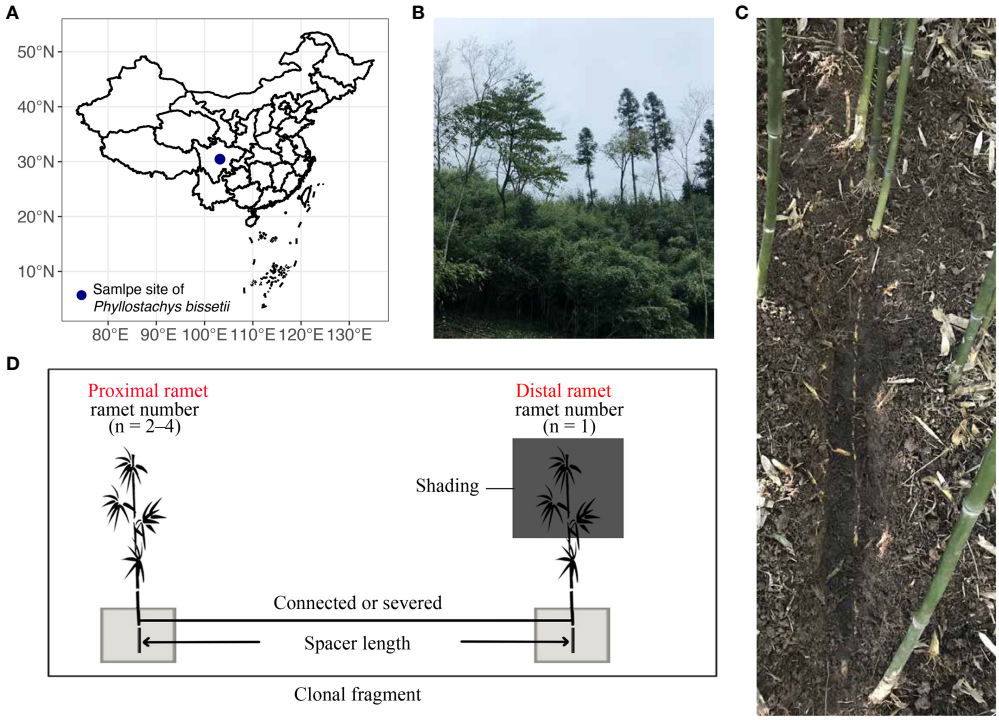

FIGURE 1
Field design for this study. **(A)** Geographical locations of the experimental areas. **(B)** habitat of *P. bissetii*. **(C)** The rhizome of a clonal fragment of *P. bissetii*. **(D)** The design of the field experiment included clonal fragments with different spacer lengths and ramet numbers. Shading treatment was applied to the distal ramets. The soil blocks were wrapped in a double-layer plastic film.

integration on the rhizosphere process of shaded ramets. Therefore, only the soil block ($50 \times 50 \times 50$ cm^3) of shaded ramet was wrapped to isolate the ramet from the environment and exclude external influences. The top surface of the block was covered with a mesh to get rid of any potential effects of leaf litter. Double-layered plastic films were used to wrap the surfaces around the block and the bottom. The length of clonal fragments with different spacer lengths ranging from 6 to 40 cm. There were 20 clonal fragments in this spacer length experiment, including 10 connected clonal fragments and 10 severed ones. Each clonal fragment with different ramet numbers was replicated three times, constituting 18 ramet pairs. The control experiment was conducted in the autumn of 2017.

2.2 Soil sampling

In June 2018, the rhizosphere soil of shaded ramets was sampled using the shaking root method (Riley and Barber, 1970). Specifically, we removed non-adherent non-rhizosphere soil by gently shaking it off the roots. The soil that strongly adhered to the root was considered the rhizosphere soil and it was gently brushed off with a sterile brush. Plant debris and gravel were removed manually. The samples were then sieved (< 2 mm) and stored at −20°C in the laboratory for chemical analyses.

2.3 Rhizosphere soil properties

Total organic carbon (TOC) and total nitrogen (TN) were measured using an element analyzer (Elementar vario MACRO cube, Frankfurt, Germany). Dissolved organic carbon (DOC) and dissolved organic nitrogen (DON) were extracted using KCl solution and then measured using a TOC/TN analyzer (TOC-L analyzer, Shimadzu, Kyoto, Japan). Microbiomass carbon (MBC) and microbiomass nitrogen (MBN) were extracted using the chloroform-fumigation extraction method at atmospheric pressure (CFAP) (Witt et al., 2000; Setia et al., 2012), and their DOC and DON levels with and without CFAP treatment were determined using a TOC/TN analyzer. Soil microbial biomass was measured using the chloroform-fumigation method reported by Vance et al. (1987) and Wu et al. (1990).

The inorganic nitrogen (NH_4^+-N and NO_3^--N) content of soil samples (5 g/sample) was extracted using KCl solution and then determined using indophenol-blue colorimetry and dual-wavelength colorimetry (Carter and Gregorich, 2007). Equal amounts of soil samples (5 g/sample) were incubated at 40°C for seven days, and the contents of NH_4^+-N and NO_3^--N were measured after incubation. N mineralization (N_{min}) and nitrification (N_{nitri}) rates were calculated based on the method reported by Zhou et al. (2011).

2.4 Soil enzyme activity

The activity of N-acetyl-β-D-glucosaminidase (NAGase) was examined using the method developed by Parham and Deng (2000), where 4-nitrophenyl-N-acetyl-β-D-glucosaminide (ρNP-NAG) was the substrate and the enzyme activity was expressed as μg ρNP g^{-1} soil h^{-1}. Urease assay was performed as reported by Kandeler and Gerber (1988) with urea as the substrate and reported in μg NH_4^+-N soil h^{-1}. The activity of polyphenol oxidase (POXase) was determined using catechol as the substrate (Perucci et al., 2000), with the activity reported in μmol oxidized catechol g^{-1} min^{-1}.

2.5 Statistical analyses

All statistical analyses were performed using R (v.4.3.1; http://www.r-project.org/), unless otherwise stated. The natural log-transformed response ratio (log-RR) was used to evaluate the effects of clonal integration on measured variables (Hedges et al., 1999).

$$log_e RR = log_e \bar{X}_{connected} - log_e \bar{X}_{severed}$$

where $\bar{X}_{connected}$ and $\bar{X}_{severed}$ are the mean values of a given variable in the connected and severed shaded ramets, respectively. Random-effect models were used to test whether the effects of clonal integration on measured variables differed from zero with the function "rma" in the package "metafor" (Viechtbauer, 2010). Linear relationships were tested between spacer length and log-RR values of each variable. A linear mixed model was adopted to test the effects of clonal integration and ramet number on the measured variables. The linear mixed model included spacer length as a random effect. The linear mixed models was implemented using the function "lmer" in the package "lme4" (Bates et al., 2015). Finally, variation partitioning was used to test the relative contributions of clonal integration and size of clonal fragment (spacer length and ramet number) on rhizosphere soil carbon related processes, nitrogen pools, and nitrogen processes with the function "varpart" in the package "vegan" (Oksanen et al., 2022).

3 Results

3.1 Effects of clonal integration on measured variables

For clonal fragments with different ramet numbers, urease, POXase, NAGase, NO_3^--N, DON, and N_{nitri} were significantly affected by connected or severed rhizomes. All variables of the shaded ramets were significantly higher when the rhizomes were connected ($p< 0.05$; Figure 2A). However, MBN, MBC, NH_4^+-N, DOC, TN, TOC, and N_{min} were not significantly affected by the connected or severe treatments ($p > 0.05$, Figure 2A). For clonal fragments with different spacer lengths, a significant effect was observed only for DOC ($p< 0.05$; Figure 2B). By combining all clonal fragments with different ramet numbers and spacer lengths, POXase, NAGase, NO_3^--N, DON, and DOC of connected ramets were significantly higher than those of severed ramets ($p< 0.05$; Figure 2C).

3.2 Relationships between RR and spacer length

Regression analysis indicated that the response ratios of NAGase, POXase, DOC, DON, N_{min} and N_{nitri} of the shaded ramets were significantly correlated with spacer length ($p< 0.05$; Figures 3A, B, E, F, I, J). Results showed that the response ratios of these soil variables first increased and then decreased with the size of clonal fragment, indicating unimodal relationships between all response ratios of these soil variables and spacer length with maximum values. However, no significant differences were observed among the other soil traits, such as urease, MCN, NH_4^+-N and NO_3^--N ($p > 0.05$, Figures 3C, D, G, H).

3.3 Contributions of clonal integration and size of clonal fragment to soil variables

Based on linear mixed models, we disentangled the relative effects of clonal integration and size of clonal fragment (combining the contributions of ramet number and spacer length) on variations in soil variables. The contribution of the size of clonal fragment to MBN, MBC, NO_3^--N, NH_4^+-N, DON, DOC, TN, TOC, and N_{min} was higher than that of clonal integration. Conversely, the contribution of the size of clonal fragment to urease, POXase, NAGase and N_{nitri} expression was lower than that to clonal integration. Furthermore, ramet number mainly contributed to DOC, TOC, and N_{min}, whereas spacer length mainly contributed to MBN, MBC, NO_3^--N, and DON (Figure 4).

Clonal integration and size of clonal fragments together explained 60, 27, and 80% of the total variation in C availability, N availability, and N turnover processes, respectively (Figure 5). For the rhizosphere soil C availability of the shaded *P. bissetii* ramets, the contribution of clonal integration was higher than that of the size of clonal fragment (0.54 > 0.11, Figure 5A). However, for the soil N turnover process, the contribution of clonal integration was lower than that of the size of clonal fragment (0.14< 0.74, Figure 5C). Similar contributions were observed between clonal integration, size of clonal fragment, and rhizosphere N availability (Figure 5B).

4 Discussion

We found that clonal integration significantly increased the soil enzyme activity and resource availability in the rhizosphere of shaded ramets, supporting our first hypothesis. Substrates translocate among rhizome-connected ramets in heterogeneous environments, significantly increasing C availability in shaded ramets (Zou et al., 2018). On the one hand, increased C can serve

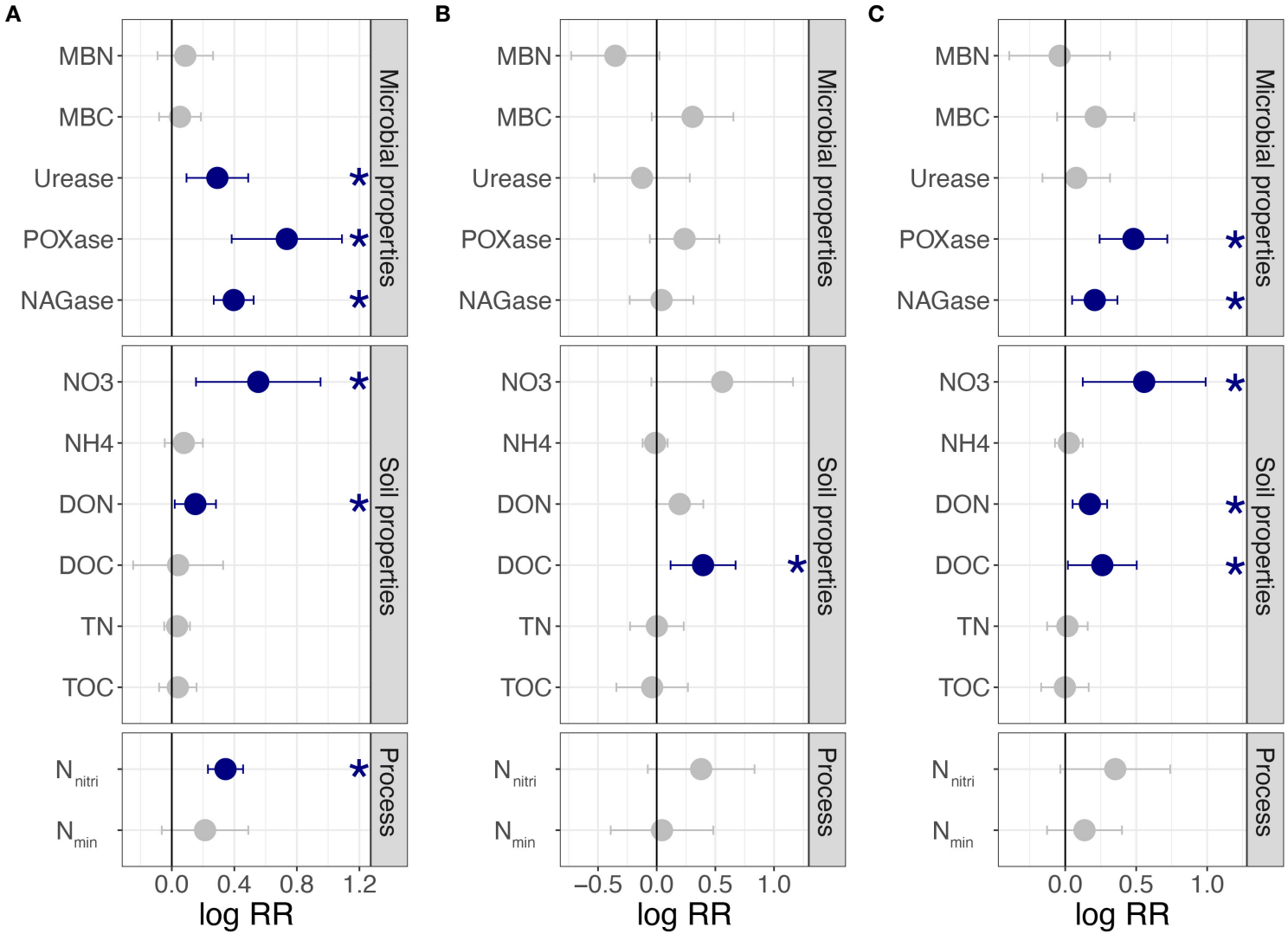

FIGURE 2
Effects of clonal integration on soil variables in the rhizosphere soil of shaded *P. bissetii* ramets **(A)** from clonal fragments with different ramet numbers, **(B)** from clonal fragments with different spacer lengths, and **(C)** from all clonal fragments. Solid lines indicate the boundary lines of the difference between connected and severed ramets. Blue points indicate the values of the connected treatments are significantly higher than that of the severed treatment. Significance is indicated by *($p < 0.05$). RR, response ratio; MBN, microbiomass nitrogen; MBC, microbiomass carbon; POXase, polyphenol oxidase; NAGase, N-acetyl-β-D-glucosaminidase; NO_3, NO_3^--N; NH4, NH_4^+-N; DON, dissolved organic nitrogen; DOC, dissolved organic carbon; TN, total nitrogen; TOC, total organic carbon; N_{nitri}, N nitrification rate; N_{min}, N mineralization rate.

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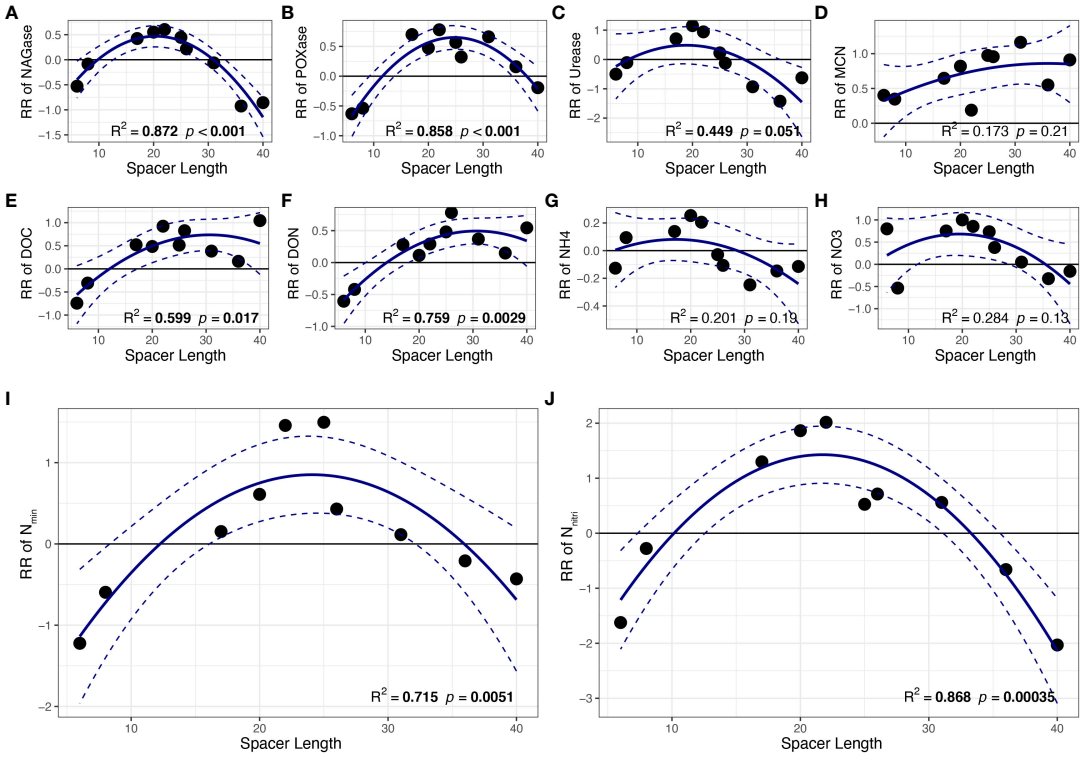

FIGURE 3
The relationships between spacer length and response ratio of soil **(A)** NAGase, **(B)** POXase, **(C)** Urease, **(D)** MCN, **(E)** DOC, **(F)** DON, **(G)** NH_4^+-N, **(H)** NO_3^--N, **(I)** N_{min}, **(J)** N_{nitri}. Mean regression lines (solid lines) and associated 95% confidence intervals (between two dashed lines) were estimated from the regression analysis with the best goodness-of-fit. Bold values denote the relationship with $R^2 > 0.5$ and $p < 0.05$. MCN, microbiomass carbon: nitrogen ratio. All other abbreviations are as in Figure 2.

as a convenient substrate for microbes and stimulate microbial activities in the rhizosphere (Kaiser et al., 2010), especially extracellular enzyme activities, which play pivotal roles in soil organic matter degradation and N cycling processes (Hu et al., 2023). However, nitrogen uptake and utilization by shaded ramets may also be enhanced by clonal integration with increased C availability (Li et al., 2018). Thus, clonal integration enables ramets in resource-poor patches to receive support from ramets in resource-rich patches, thereby enhancing the adaptability of the entire clone fragment to heterogeneous habitats (Song et al., 2013; Lu et al., 2020; You et al., 2023).

Our results showed that clonal fragments with different ramet numbers had greater promoting effects on rhizosphere soil properties than clonal fragments with different spacer lengths. This phenomenon can be attributed to the following factors. Each connected ramet serves as a source or sink for an entire clonal fragment (Sheng et al., 2007). Thus, entire clonal fragments with large ramet numbers are more likely to acquire, store, and allocate resources (Dong et al., 2010; Luo and Zhao, 2015), leading to stronger physiological integration (Zhou et al., 2017). This implies that larger clonal fragments have the potential to produce more biomass and exhibited greater competitive ability, especially under low-nutrient conditions (Roiloa and Retuerto, 2005; Zhang et al., 2019). By contrast, a large number of ramets in clonal fragments may also lead to intense intraspecific competition for space and nutrients between sibling ramets (Hellstrom et al., 2006; Zhang et al., 2020). Therefore, the positive effects of clonal fragments with different numbers of ramets are context-dependent and require further attention.

In addition, spacer length influences the effects of clonal integration on the shaded *P. bissetii* ramet. It is noteworthy that these resources are not transmitted indefinitely. According to the results, the response ratio gradually decreased with increasing spacer length in the later stages, indicating a tradeoff strategy between the cost and benefit of clonal integration. This tradeoff is due to the fact that resources translocate to distant ramets in the energy-consumption process. All clonal fragments must adopt a cost–benefit tradeoff strategy when the costs outweigh the benefits of resource translocation (Wang et al., 2014; Zhang et al., 2020). Results showed that this cost–benefit tradeoff strategy occurred when the spacer length was approximately 30 cm (Figure 3), which was different from the results for *Populus euphratica* (20–30 m) (Zhu et al., 2018), *Halophila stipulacea* (2.7 *cm*), and *Cymodocea nodosa* (81 *cm*) (Marba et al., 2002). This may be due to differences in species of clonal plants (D'Hertefeldt et al., 2014; You et al., 2014). Furthermore, to balance the cost and benefit, clonal fragments may even generate new ramets instead of resource translocation (Zhai et al., 2022). Therefore, spacer length strongly affected the clonal integration intensity of *P. bissetii* and had a negative effect over a longer distance.

Clonal integration and fragment size had different effects on rhizosphere processes. The results showed that clonal integration

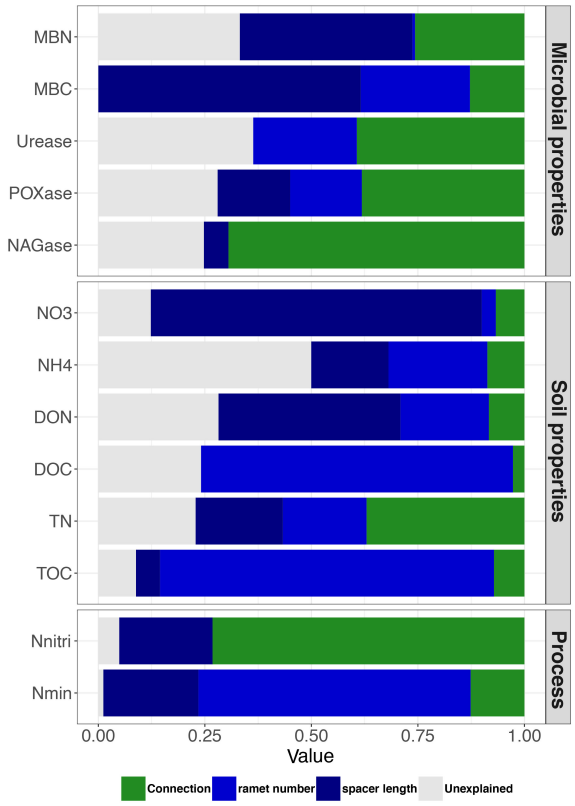

FIGURE 4
Variation partitioning showing the contribution of clonal integration (connection) and size of clonal fragment (ramet number + spacer length) to soil variables. Green, light blue, dark blue and gray columns represent connection, ramet number, spacer length, and unexplained experiment result, respectively. Abbreviations are as in Figure 2.

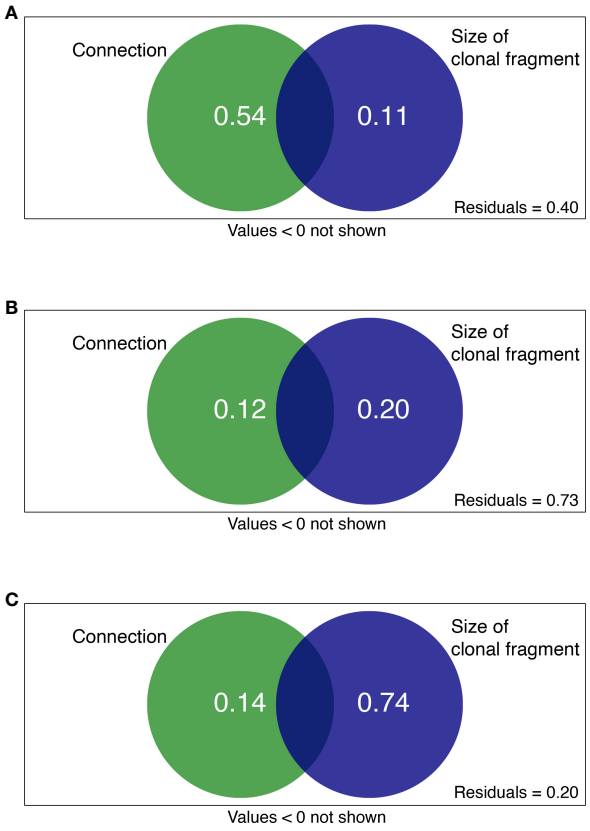

FIGURE 5
Variation partitioning showing the contribution of clonal integration (connection) and size of clonal fragment (ramet number + spacer length) to soil **(A)** C availability, **(B)** N availability, and **(C)** N turnover process. Green and blue circles represent connection and size of clonal fragment treatments, respectively.

mainly affected the rhizosphere soil enzyme activity related to soil organic matter decomposition (Kaiser et al., 2010; Chen J. S. et al., 2015; Xue et al., 2018). It has been suggested that C assimilates are the major substrates translocated between connected ramets under heterogeneous light conditions (Li et al., 2019). Photosynthetic carbon is an indispensable part of the carbon cycle in the plant–soil system, which is translocated from exposed ramets and compensates for the decreased C inputs to the soil in shaded ramets (Lei et al., 2014; Wang et al., 2019). Thus, clonal integration could supply substrates for microbes and facilitate enzyme activity and further organic matter decomposition in the rhizosphere of the shaded ramets. By contrast, the size of clonal fragment strongly contributed to rhizosphere N turnover. Nitrogen is always heterogeneously distributed in the soil matrix, which increases the difficulty in the accessibility of N to plants. Therefore, clonal fragments may change the placement of ramets, ramet generation, and spacer length to acquire essential nutrients, leading to a strong contribution to rhizosphere N processes (Ma et al., 2023). In addition, the size of clonal fragment also contributes more greatly to the soil variables (such as inorganic N, DOC and DON) than clonal integration. This further implies the potential role of fragment size and structure in adapting to heterogeneous habitats. In total, through resource translocation and regulation of the size of clonal fragment, clonal plants can strongly affect rhizosphere C and N availability and other processes, which eventually enhance the adaptability of the entire clonal fragment to heterogeneous habitats.

5 Conclusions

Through field experiments, our study revealed the relative effects of clonal integration and fragment size on rhizosphere processes and resource availability in bamboo ecosystems. We found that clonal integration significantly modified the soil properties of the shaded ramets, whereas the positive effects differed across clonal fragments with different ramet numbers and spacer lengths. Clonal fragments with more ramets could provide a better support to stressed ramets owing to higher resource storage in the fragment. The contribution of spacer length to clonal integration indicated a cost–benefit tradeoff in the fragment. These results advance our knowledge on the mechanism of influence of the size of clonal fragment on rhizosphere processes of stressed ramets, which is critical for the adaptation of *P. bissetii* to stressed habitats and further C cycling through the bamboo ecosystem under climate change.

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

ZZ: Conceptualization, Data curation, Investigation, Methodology, Software, Writing – original draft. YL: Supervision, Writing – review and editing. HS: Supervision, Writing – review and 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 Natural Science Foundation of China (Grant number 31470636).

Acknowledgments

We thank the people who participated in the field surveys.

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

Alpert, P. (1999). Effects of clonal integration on plant plasticity in *Fragaria chiloensis*. *Plant Ecol.* 141, 99–106. doi: 10.1023/A:1009823015170

Bates, D., Machler, M., Bolker, B. M., and Walker, S. (2015). Fitting linear mixed-effects models using lme4. *J. Stat. Software* 67, 1–48. doi: 10.18637/jss.v067.i0

Carter, M. R., and Gregorich, E. G. (2007). *Soil Sampling and Methods of Analysis. 2nd ed* (Boca Raton: CRC Press). doi: 10.1201/9781420005271

Chen, B. J. W., Vermeulen, P. J., During, H. J., and Anten, N. P. R. (2015). Testing for disconnection and distance effects on physiological self-recognition within clonal fragments of *Potentilla reptans*. *Front. Plant Sci.* 6. doi: 10.3389/fpls.2015.00215

Chen, D., Ali, A., Yong, X. H., Lin, C. G., Niu, X. H., Cai, A. M., et al. (2019). A multi-species comparison of selective placement patterns of ramets in invasive alien and native clonal plants to light, soil nutrient and water heterogeneity. *Sci. Total Environ.* 657, 1568–1577. doi: 10.1016/j.scitotenv.2018.12.099

Chen, J. S., Lei, N. F., and Dong, M. (2010). Clonal integration improves the tolerance of *Carex praeclara* to sand burial by compensatory response. *Acta Oecol.* 36, 23–28. doi: 10.1016/j.actao.2009.09.006

Chen, J. S., Li, J., Zhang, Y., Zong, H., and Lei, N. F. (2015). Clonal integration ameliorates the carbon accumulation capacity of a stoloniferous herb, *Glechoma longituba*, growing in heterogenous light conditions by facilitating nitrogen assimilation in the rhizosphere. *Ann. Bot.* 115, 127–136. doi: 10.1093/aob/mcu207

D'Hertefeldt, T., Enestrom, J. M., and Pettersson, L. B. (2014). Geographic and habitat origin influence biomass production and storage translocation in the clonal plant *Aegopodium podagraria*. *PloS One* 9, e85407. doi: 10.1371/journal.pone.0085407

Dong, B. C., Alpert, P., Guo, W., and Yu, F. H. (2012). Effects of fragmentation on the survival and growth of the invasive, clonal plant *Alternanthera philoxeroides*. *Biol. Invasions.* 14, 1101–1110. doi: 10.1007/s10530-011-0141-5

Dong, B. C., Yu, G. L., Guo, W., Zhang, M. X., Dong, M., and Yu, F. H. (2010). How internode length, position and presence of leaves affect survival and growth of *Alternanthera philoxeroides* after fragmentation? *Evol. Ecol.* 24, 1447–1461. doi: 10.1007/s10682-010-9390-5

Ganie, A. H., Reshi, Z. A., Wafai, B. A., and Puijalon, S. (2016). Clonal growth architecture and spatial dynamics of 10 species of the genus *Potamogeton* across different habitats in Kashmir Valley, India. *Hydrobiologia.* 767, 289–299. doi: 10.1007/s10750-015-2509-5

Guan, B., Yu, J., Wu, M., Liu, X., Wang, X., Yang, J., et al. (2023). Clonal integration promotes the growth of *Phragmites australis* populations in saline wetlands of the Yellow River Delta. *Front. Plant Sci.* 14. doi: 10.3389/fpls.2023.1162923

Hedges, L. V., Gurevitch, J., and Curtis, P. S. (1999). The meta-analysis of response ratios in experimental ecology. *Ecology.* 80, 1150–1156. doi: 10.1890/0012-9658(1999)080[1150:TMAORR]2.0.CO;2

Hellstrom, K., Kytoviita, M. M., Tuomi, J., and Rautio, P. (2006). Plasticity of clonal integration in the perennial herb *Linaria vulgaris* after damage. *Funct. Ecol.* 20, 413–420. doi: 10.1111/j.1365-2435.2006.01115.x

Hu, J., Chen, S., Guo, Z., Yang, Q., and Li, Y. (2015). Effects of spacer length on water physiological integration of *Indocalamus decorus* ramets under heterogeneous water supply. *Acta Bot. Boreal.-Occid. Sin.* 35, 2532–2541. doi: 10.7606/j.issn.1000-4025.2015.12.2532

Hu, M. J., Wang, J. L., Lu, L. L., Shao, P., Zhou, Z., Wang, D., et al. (2023). Post-fire soil extracellular enzyme activities in subtropical-warm temperate climate transitional forests. *Land Degrad. Dev.* 34, 1973–1983. doi: 10.1002/ldr.4582

Huber, H., Visser, E. J. W., Clements, G., and Peters, J. L. (2014). Flooding and fragment size interact to determine survival and regrowth after fragmentation in two stoloniferous *Trifolium* species. *AoB Plants.* 6, plu024. doi: 10.1093/aobpla/plu024

Hutchings, M. J., and Wijesinghe, D. K. (1997). Patchy habitats, division of labour and growth dividends in clonal plants. *Trends Ecol. Evol.* 12, 390–394. doi: 10.1016/s0169-5347(97)87382-x

Jones, D. L., Hodge, A., and Kuzyakov, Y. (2004). Plant and mycorrhizal regulation of rhizodeposition. *New Phytol.* 163, 459–480. doi: 10.1111/j.1469-8137.2004.01130.x

Kaiser, C., Koranda, M., Kitzler, B., Fuchslueger, L., Schnecker, J., Schweiger, P., et al. (2010). Belowground carbon allocation by trees drives seasonal patterns of extracellular enzyme activities by altering microbial community composition in a beech forest soil. *New Phytol.* 187, 843–858. doi: 10.1111/j.1469-8137.2010.03321.x

Kandeler, E., and Gerber, H. (1988). Short-term assay of soil urease activity using colorimetric determination of ammonium. *Biol. Fertil. Soils* 6, 68–72. doi: 10.1007/BF00257924

Kang, D. W., Lv, J., Li, S., Chen, X., Wang, X., and Li, J. (2019). Relationship between bamboo growth status and woody plants in a giant panda habitat. *Ecol. Indic.* 98, 840–843. doi: 10.1016/j.ecolind.2018.12.006

Kuzyakov, Y. (2002). Review: Factors affecting rhizosphere priming effects. *J. Plant Nutr. Soil Sci.* 165, 382–396. doi: 10.1002/1522-2624(200208)165:4<382::AID-JPLN382>3.0.CO;2-%23

Latzel, V., and Klimesova, J. (2010). Transgenerational plasticity in clonal plants. *Evol. Ecol.* 24, 1537–1543. doi: 10.1007/s10682-010-9385-2

Lei, N., Li, J., Ni, S., and Chen, J. (2014). Effects of clonal integration on microbial community composition and processes in the rhizosphere of the stoloniferous herb *Glechoma longituba* (Nakai) Kuprian. *PloS One* 9, e108259. doi: 10.1371/journal.pone.0108259

Li, Y., Chen, J. S., Xue, G., Peng., Y. Y., and Song., H. X. (2018). Effect of clonal integration on nitrogen cycling in rhizosphere of rhizomatous clonal plant, *Phyllostachys bissetii*, under heterogeneous light. *Sci. Total Environ.* 628–629, 594–602. doi: 10.1016/j.scitotenv.2018.02.002

Li, Y., Chen, J. S., Xue, G., Song, H. X., and Liu, C. H. (2019). Disappearing rhizosphere effect of shaded ramet re-occurs through support of carbon assimilates from unshaded one in a clonal fragment. *Rhizosphere.* 11, 100166. doi: 10.1016/j.rhisph.2019.100166

Li, Y. H., Liu, X., and Yue, M. (2008). Nutrient contents in *Kingdonia uniflora* ramet and their relations to heterogeneous environment on Taibai Mountains. *Chin. J. Appl. Ecol.* 19, 1676–1681. doi: 10.13287/j.1001-9332.2008.0298

Liang, J. F., Yuan, W. Y., Gao, J. Q., Roiloa, S. R., Song, M. H., Zhang, X. Y., et al. (2020). Soil resource heterogeneity competitively favors an invasive clonal plant over a native one. *Oecologia.* 193, 155–165. doi: 10.1007/s00442-020-04660-6

Lin, H. F., Alpert, P., and Yu, F. H. (2012). Effects of fragment size and water depth on performance of stem fragments of the invasive, amphibious, clonal plant Ipomoea aquatica. *Aquat. Bot.* 99, 34–40. doi: 10.1016/j.aquabot.2012.01.004

Liu, Q., Li, Y. X., and Zhong, Z. C. (2004). Effects of moisture availability on clonal growth in bamboo *Pleioblastus maculata*. *Plant Ecol.* 173, 107–113. doi: 10.1023/B:VEGE.0000026334.40661.06

Lu, H. Z., Brooker, R., Song, L., Liu, W. Y., Sack, L., Zhang, J. L., et al. (2020). When facilitation meets clonal integration in forest canopies. *New Phytol.* 225, 135–142. doi: 10.1111/nph.16228

Luo, W. C., and Zhao, W. Z. (2015). Burial depth and diameter of the rhizome fragments affect the regenerative capacity of a clonal shrub. *Ecol. Complex.* 23, 34–40. doi: 10.1016/j.ecocom.2015.05.004

Ma, X. W., Yu, W. C., Tao, M., Zhang, C., Zhang, Z., Yu, D., et al. (2023). Clonal integration in *Vallisneria natans* alters growth and the rhizosphere microbial community of neighboring plants under heterogeneous conditions. *Plant Soil.* 482, 297–311. doi: 10.1007/s11104-022-05690-0

Marba, N., Hemminga, M. A., Mateo, M. A., and Duarte, C. M. (2002). Carbon and nitrogen translocation between seagrass ramets. *Mar. Ecol. Prog. Ser.* 226, 287–300. doi: 10.3354/meps226287

Oksanen, J., Simpson, G. L., Blanchet, F. G., Kindt, R., Legendre, P., Minchin, P. R., et al. (2022) *Vegan: Community Ecology Package*. Available at: https://CRAN.R-project.org/package=vegan.

Parham, J. A., and Deng, S. P. (2000). Detection, quantification and characterization of β-glucosaminidase activity in soil. *Soil Biol. Biochem.* 32, 1183–1190. doi: 10.1016/S0038-0717(00)00034-1

Perucci, P., Casucci, C., and Dumontet, S. (2000). An improved method to evaluate the *o*-diphenol oxidase activity of soil. *Soil Biol. Biochem.* 32, 1927–1933. doi: 10.1016/S0038-0717(00)00168-1

Riley, D., and Barber, S. A. (1970). Salt accumulation at the soybean (Glycine Max. (L.) Merr.) root-soil interface. *Soil Sci. Soc Am. J.* 34, 154–155. doi: 10.2136/sssaj1970.03615995003400010042x

Roiloa, S. R., and Retuerto, R. (2005). Presence of developing ramets of *Fragaria vesca* L. increases photochemical efficiency in parent ramets. *Int. J. Plant Sci.* 166, 795–803. doi: 10.1086/431804

Saitoh, T., Seiwa, K., and Nishiwaki, A. (2002). Importance of physiological integration of dwarf bamboo to persistence in forest understorey: A field experiment. *J. Ecol.* 90, 78–85. doi: 10.1046/j.0022-0477.2001.00631.x

Saitoh, T., Seiwa, K., and Nishiwaki, A. (2006). Effects of resource heterogeneity on nitrogen translocation within clonal fragments of Sasa palmata: An isotopic (^{15}N) assessment. *Ann. Bot.* 98, 657–663. doi: 10.1093/aob/mcl147

Setia, R., Verma, S. L., and Marschner, P. (2012). Measuring microbial biomass carbon by direct extraction - Comparison with chloroform fumigation-extraction. *Eur. J. Soil Biol.* 53, 103–106. doi: 10.1016/j.ejsobi.2012.09.005

Sheng, L., Dezhi, L. I., Zhiling, Z. H. U., and Wang, X. (2007). Carbon physiological integration in clonal plants and its ecological effects. *Chin. J. Appl. Environ. Biol.* 13, 888–894. doi: 10.3321/j.issn:1006-687x.2007.06.028

Song, X., Peng, C., Zhou, G., Gu, H., Li, Q., and Zhang, C. (2016). Dynamic allocation and transfer of non-structural carbohydrates, a possible mechanism for the explosive growth of Moso bamboo (*Phyllostachys heterocycla*). *Sci. Rep.* 6, 25908. doi: 10.1038/srep25908

Song, Y. B., Yu, F. H., Keser, L. H., Dawson, W., Fischer, M., Dong, M., et al. (2013). United we stand, divided we fall: A meta-analysis of experiments on clonal integration and its relationship to invasiveness. *Oecologia.* 171, 317–327. doi: 10.1007/s00442-012-2430-9

Sun, Y., Xu, X., and Kuzyakov, Y. (2014). Mechanisms of rhizosphere priming effects and their ecological significance. *Chin. J. Plant Ecol.* 38, 62–75. doi: 10.3724/SP.J.1258.2014.00007

Tian, Y. Q., Cui, Y., Wen, S. H., Li, X., Song, M., Chen, X., et al. (2023). Clonal integration under heterogeneous water environment increases plant biomass and nitrogen uptake in a temperate steppe. *Plant Soil.* 491, 145–159. doi: 10.1007/s11104-023-06163-8

Vance, E. D., Brookes, P. C., and Jenkinson, D. S. (1987). An extraction method for measuring soil microbial biomass C. *Soil Biol. Biochem.* 19, 703–707. doi: 10.1016/0038-0717(87)90052-6

Viechtbauer, W. (2010). Conducting meta-analyses in {R} with the {metafor} package. *J. Stat. Software* 36, 1–48. doi: 10.18637/jss.v036.i03

Wang, Y., Hong, R., and Huang, D. (2004). The translocation of photosynthate between clonal ramets of *Leymus chinensis* population. *Acta Ecol. Sin.* 24, 900–907. doi: 10.3321/j.issn:1000-0933.2004.05.006

Wang, Y. J., Müller-Schärer, H., Van Kleunen, M., Cai, A. M., Zhang, P., Yan, R., et al. (2017). Invasive alien plants benefit more from clonal integration in heterogeneous environments than natives. *New Phytol.* 216, 1072–1078. doi: 10.1111/nph.14820

Wang, C., Xiao, R., Cui, Y., Ma, Z., Guo, Y., Wang, W., et al. (2019). Photosynthate-^{13}C allocation in the plant-soil system after ^{13}C-pulse labeling of Phragmites australis in different salt marshes. *Geoderma.* 347, 252–261. doi: 10.1016/j.geoderma.2019.03.045

Wang, P., Xu, Y. S., Dong, B. C., Xue, W., and Yu, F. H. (2014). Effects of clonal fragmentation on intraspecific competition of a stoloniferous floating plant. *Plant Biol.* 16, 1121–1126. doi: 10.1111/plb.12170

Witt, C., Gaunt, J. L., Galicia, C. C., Ottow, J. C. G., and Neue, H. U. (2000). A rapid chloroform-fumigation extraction method for measuring soil microbial biomass carbon and nitrogen in flooded rice soils. *Biol. Fertil. Soils.* 30, 510–519. doi: 10.1007/s003740050030

Wu, J., Joergensen, R. G., Pommerening, B., Chaussod, R., and Brookes, P. C. (1990). Measurement of soil microbial biomass C by fumigation-extraction—An automated procedure. *Soil Biol. Biochem.* 22, 1167–1169. doi: 10.1016/0038-0717(90)90046-3

Xue, G., Li, Y., Chen, J., and Song, H. (2018). Effect of clonal integration on soil microbial properties in the rhizosphere of *Phyllostachys bissetii*, subjected to heterogeneous light. *Acta Ecol. Sin.* 38, 3132–3144. doi: 10.5846/stxb201704030572

You, W. H., Fan, S. F., Yu, D., Xie, D., and Liu, C. (2014). An invasive clonal plant benefits from clonal integration more than a co-occurring native plant in nutrient-patchy and competitive environments. *PloS One* 9, e97246. doi: 10.1371/journal.pone.0097246

You, W., Li, N. N., Zhang, J., Song, A., and Du, D. (2023). The plant invader *Alternanthera philoxeroides* benefits from clonal integration more than its native co-genus in response to patch contrast. *Plants.* 12, 2371. doi: 10.3390/plants12122371

Yu, H. W., Wang, L. G., Liu, C. H., Yu, D., and Qu, J. (2020). Effects of a spatially heterogeneous nutrient distribution on the growth of clonal wetland plants. *BMC Ecol.* 20, 59. doi: 10.1186/s12898-020-00327-1

Zhai, S. S., Qian, J. Q., Ma, Q., Liu, Z. M., Ba, C. Q., Xin, Z. M., et al. (2022). Effect of rhizome severing on survival and growth of rhizomatous herb Phragmites communis is regulated by sand burial depth. *Plants.* 11, 3191. doi: 10.3390/plants11233191

Zhang, L. M., Alpert, P., Si, C., and Yu, F. H. (2019). Interactive effects of fragment size, nutrients, and interspecific competition on growth of the floating, clonal plant *Salvinia natans*. *Aquat. Bot.* 153, 81–87. doi: 10.1016/j.aquabot.2018.12.001

Zhang, L. M., Jin, Y., Yao, S. M., Lei, N. F., Chen, J. S., Zhang, Q., et al. (2020). Growth and morphological responses of duckweed to clonal fragmentation, nutrient availability, and population density. *Front. Plant Sci.* 11. doi: 10.3389/fpls.2020.00618

Zhang, J., You, W. H., Li, N., and Du, D. L. (2023). Invasive clonal plants possess greater capacity for division of labor than natives in high patch contrast environments. *Front. Plant Sci.* 14. doi: 10.3389/fpls.2023.1210070

Zheng, A., and Lv, J. (2023). Spatial patterns of bamboo expansion across scales: How does Moso bamboo interact with competing trees? *Landsc. Ecol.* 27, 1–9. doi: 10.1007/s10980-023-01669-z

Zheng, L. L., Yao, S. M., Xue, W., and Yu, F. (2023). Small islands of safety promote the performance of a clonal plant in cadmium-contaminated soil. *Plant Soil.* 489, 453–464. doi: 10.1007/s11104-023-06031-5

Zhou, W. M., Chen, H., Zhou, L., Lewis, B. J., Ye, Y., Tian, J., et al. (2011). Effect of freezing-thawing on nitrogen mineralization in vegetation soils of four landscape zones of Changbai Mountain. *Ann. For. Sci.* 68, 943–951. doi: 10.1007/s13595-011-0100-4

Zhou, J., Li, H.-L., Alpert, P., Zhang, M. X., and Yu, F. H. (2017). Fragmentation of the invasive, clonal plant *Alternanthera philoxeroides* decreases its growth but not its competitive effect. *Flora.* 228, 17–23. doi: 10.1016/j.flora.2017.01.007

Zhu, C. G., Li, W. H., Chen, Y. N., and Chen, Y. P. (2018). Characteristics of water physiological integration and its ecological significance for *Populus euphratica* young ramets in an extremely drought environment. *J. Geophys. Res. Atmos.* 123, 5657–5666. doi: 10.1029/2018JD028396

Zou, Z., Chen, J., Li, Y., and Song, H. (2018). Effects of transportation direction of photosynthate on soil microbial processes in the rhizosphere of *Phyllostachys bissetii*. *Chin. J. Plant Ecol.* 42, 863–872. doi: 10.17521/cjpe.2018.0078

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TYPE Original Research
PUBLISHED 05 January 2024
DOI 10.3389/fpls.2023.1330664

OPEN ACCESS

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RECEIVED 31 October 2023
ACCEPTED 12 December 2023
PUBLISHED 05 January 2024

CITATION
Wu J, Hou X, Xu L, Zhou Q, Wang Y, Guo Z, Adomako MO and Ma Q (2024) Belowground bud banks and land use change: roles of vegetation and soil properties in mediating the composition of bud banks in different ecosystems. *Front. Plant Sci.* 14:1330664. doi: 10.3389/fpls.2023.1330664

Belowground bud banks and land use change: roles of vegetation and soil properties in mediating the composition of bud banks in different ecosystems

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Introduction: Belowground bud banks play integral roles in vegetation regeneration and ecological succession of plant communities; however, human-caused changes in land use severely threaten their resilience and regrowth. Although vegetation attributes and soil properties mediate such anthropogenic effects, their influence on bud bank size and composition and its regulatory mechanisms under land use change have not been explored.

Methods: We conducted a field investigation to examine impacts of land use change on bud bank size and composition, vegetation attributes, and soil properties in wetlands (WL), farmlands (FL), and alpine meadow (AM) ecosystems in Zhejiang Province, China.

Results: Overall, 63 soil samples in close proximity to the vegetation quadrats were excavated using a shovel, and samples of the excavated soil were placed in plastic bags for onward laboratory soil analysis. The total bud density (1514.727 ± 296.666) and tiller bud density (1229.090 ± 279.002) in wetland ecosystems were significantly higher than in farmland and alpine meadow ecosystems [i.e., total (149.333 ± 21.490 and 573.647 ± 91.518) and tiller bud density (24.666 ± 8.504 and 204.235 ± 50.550), respectively]. While vegetation attributes critically affected bud banks in WL ecosystems, soil properties strongly influenced bud banks in farmland and alpine meadow ecosystems. In wetland ecosystems, total and tiller buds were predominantly dependent on soil properties, but vegetation density played a significant role in farmlands and alpine meadow ecosystems. Root sprouting and rhizome buds significantly correlated with total C in the top 0 – 10 cm layer of

farmland and alpine meadow ecosystems, respectively, and depended mainly on soil properties.

Discussion: Our results demonstrate that land use change alters bud bank size and composition; however, such responses differed among bud types in wetland, farmland, and alpine meadow ecosystems.

KEYWORDS

bud density, clonal organs, land use change, storage organs, vegetation density

Introduction

Human-derived modifications of terrestrial ecosystems, including land use changes, underlie the altered belowground bud bank densities, vegetation regeneration, and ecological succession, leading to global biodiversity loss and ecosystem services (Allan et al., 2015; Newbold et al., 2015; Chang and Turner, 2019; Winkler et al., 2021; Simkin et al., 2022). Agricultural activities (e.g., pesticides and herbicide application) and expansion of arable croplands have predominantly shifted many crucial attributes of the natural vegetation (Zhao et al., 2023), with substantial implications on belowground bud banks (storage organs e.g., rhizomes, corm, and ramets) that are vital for vegetation regrowth, aboveground recruitment, productivity (Li et al., 2018; Qian et al., 2021; Hou et al., 2022). Although expanding the arable cropland greatly affects many plant species and bud banks, their responses may differ across ecosystems due to differences in the vegetation cover and soil characteristics that support plant growth (Newbold et al., 2015; Semenchuk et al., 2022). Likewise, the land use intensity and impacts on belowground bud banks may also differ between such ecosystems. Despite these differential responses to land use change, the role of vegetation and soil attributes in mediating bud bank responses in different ecosystems has not been explored. In light of the ongoing environmental change on a worldwide scale, it is imperative to comprehend the role of vegetation and soil characteristics to develop appropriate management techniques that will increase their ecological relevance.

Soil characteristics (e.g., moisture, nutrients, and particle sizes) constitute an essential component driving the structure and vegetation cover, as well as nutrient availability of terrestrial ecosystems (Wu et al., 2020; Adomako et al., 2021; Inoue et al., 2022), and play critical roles in regeneration, growth, and productivity of plants (Zuo et al., 2009; Hoover et al., 2014). Soil properties are important ecological parameters that determine the magnitude, distribution pattern, and vegetation succession, which are a function of soil aggregate particles, belowground bud bank density, and resprouting ability (Clarke et al., 2013; Wu et al., 2020). For example, in a soil substrate heterogeneity study, Adomako et al. (2021) reported that a ceramsite-quartz mixture with larger aggregate particle sizes significantly decreased ramets growth of *Leymus chinensis* compared to plants grown in field soil with relatively smaller particle sizes, as the former substrates have greater mechanical resistance to seed or vegetative sprouting than the latter substrates (Semchenko et al., 2008). Moreover, higher clay and low nutrient content in wetland soils may decrease resprouting and growth of tiller buds than resprouting and growth of tillers in farmlands and alpine meadows (Reddy et al., 2013; Mobilian and Craft, 2021). The soil physicochemical properties strongly influence the aboveground vegetation recruitment from seeds or belowground bud banks (Adomako et al., 2021; Inoue et al., 2022; Wu and Yu, 2022). Such driving force underpins aboveground vegetation recruitment dynamics, vegetation density, and productivity of plant species in natural ecosystems (Peng et al., 2015; Adomako et al., 2021). However, information regarding interaction effects of land use change and soil characteristics on bud banks across varying ecosystem types is limited in our current understanding.

In natural systems, belowground bud banks play a pivotal role in maintaining plant biodiversity against anthropogenic and natural disturbances, such as farming and climate change (Plue and Cousins, 2018; Ott et al., 2019). Particularly in vegetations dominated by perennial species, belowground bud banks serve as ecological insurance against short- and long-term disturbances such as drought, wildfire, and grazing (Deng et al., 2014; Hoover et al., 2014; Ott et al., 2019). Although long-term effects of some ecological perturbations can adversely affect bud bank density (VanderWeide and Hartnett, 2015; Qian et al., 2023), they may likely differ between ecosystems owing to differences in vegetation cover, species composition, and soil characteristics. These differential responses of bud banks across varying ecosystems may explain variations in resilience and resprouting capacity after disturbances (Xu et al., 2021). For instance, plants adapted to grow in drier conditions (i.e., alpine meadow in our study) showed strong resistance to chronic drought stress compared to wetland plants growing under the same stressful condition (Luo et al., 2023; Ren et al., 2023). Likewise, the abundance and density of belowground organs (e.g., rhizomes and tillers) of species may also differ in their responses to disturbance in their respective ecosystems (Xu et al., 2021; Qian et al., 2022; Klimešová et al., 2023). Despite the critical roles of belowground bud banks to plant community stability and productivity, how vegetation and soil characteristics mediate their responses to land-use changes are poorly understood.

Bud types (e.g., rhizome, tiller, and root sprouting buds) play different functional roles and determine diverse adaptive strategies under variable ecosystems (Zhang et al., 2019). Belowground bud banks are widely sourced from rhizome and tiller buds (Zhang et al., 2009; Ott and Hartnett, 2015). In addition to adventitious root buds, tiller buds derived from the base of the parent shoots for hemicryptophyte, rhizome buds, and root sprouting buds mainly initiate from underground roots and rhizomes for geophyte. Previous studies reported that tiller buds are more closely related to vegetation attributes, while rhizome and root sprouting buds are sensitive to water or nutrient concentrations in surrounding habitats (Passioura, 1988). However, how vegetation attributes and soil properties interact to determine the responses of different bud bank types under the ongoing land use changes is unknown.

To explore the changes in bud bank size and composition and its regulatory mechanism under land use change, we selected two plots in Linhai and Taizhou sampling sites for wetlands (WL), farmlands (FL), and alpine meadows (AM), respectively. We took 63 sampling points in total for the bud demographic. We also measured biotic (vegetation density, aboveground biomass, and Shannon-Weiner diversity index) and abiotic parameters (soil moisture content, total carbon (C), and total N) relevant to the bud bank density for different plant functional groups. Specifically, we aim to explore (1) changes in/patterns of the bud bank traits among different land use types and (2) the role of vegetation attributes and soil properties in determining bud demographic and bud densities of different bud bank types under land use change.

Materials and methods

Study sites

The study was conducted in Linhai and Taizhou City (120°17′-121°56′E, 28°01′-29°20″N), southeastern Zhejiang Province, China (Figure 1A). In response to rapid modern development and urbanization of China, Linhai and Taizhou Cities located along the coast are undergoing massive expansion of industries and settlement, cascading potential impacts on its vegetation structure and succession. Therefore, we conducted this field study to examine how the rapid land use change may influence belowground bud banks to highlight the long-term effects on vegetation structure and dynamics in this area and beyond. The region has a typical subtropical monsoon climate with moderate annual temperatures, abundant sunshine, and precipitation; thus, the growing season lasts from late April to late September before the yearly winter commences. The landscape consists of a mosaic of forests, arable lands, and wetlands. Six sample sites in total were selected in Linhai and Taizhou City: two alpine meadows sites located in Kuocang Mountain and Lantian Mountain, two farmland sites located next to residential quarters at the foot of mountain, and two wetland sites situated next to Xiaozhi reservoir and Jiaojiang river, respectively (Figure 1B). The dominant vegetation at the wetland site comprises Phragmites communis, Arundo donax, Imperata cylindrica, Solidago canadensis, and other herbaceous plants. Some perennial and annual herbs, such as Juncus effusus, Imperata cylindrica,

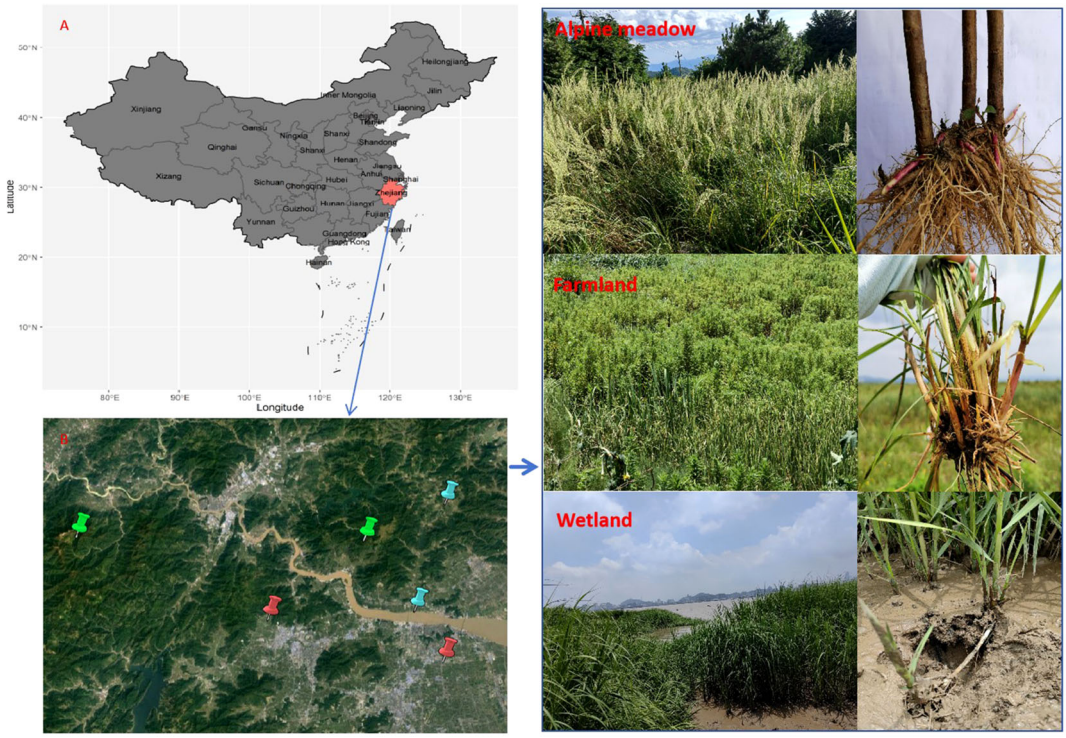

FIGURE 1
Location of Zhejiang Province **(A)** and the six sampling sites **(B)**, including two sites for farmland (red label) in Taizhou City, two sites for Alpine meadow (green label) in Linhai City, one site for wetland (blue label) in Taizhou city and one site for wetland (blue label) in Linhai city, respectively.

Lysimachia fortune, and Rubus phoenicolosius, dominate the alpine meadows' vegetation. The vegetation in WL, FL, and AM ecosystems experience the same climatic conditions, i.e., similar rainfall patterns, temperature, and humidity.

Field observations and bud bank sampling

In August 2022, we selected two plots for WL, FL, and AM in each sampling site and established transects 10 m apart between each chosen plot. In each plot, 22 sampling points were selected for wetlands, 24 for farmlands, and 17 for alpine meadows, making 63 sampling points in total. In each sampling point, 1 × 1 m quadrats were established to record the vegetation composition, identify all species within each community, and record the number of each species identified and their abundance, as well as their average height and plant density. With grasses and sedges, we counted and recorded the number of ramets (e.g., *Phragmites communis*) and calculated the number of individuals for discrete species. We used the number of all ramets/individuals/m^2 in all quadrats to estimate the vegetation density. Additionally, we sampled soils between the top 0 – 10 cm to measure soil water and nutrient content from each ecosystem using a ring cutter and soil drill with a diameter of 7 cm.

We dug 25 × 25 × 25 cm quadrats in each vegetation quadrant to record the belowground bud bank composition at each sampling point, totaling 63 quadrats in the three ecosystems. All samples were processed within two weeks, and no rotting was observed during this period. Only turgid bud tissue was counted, and tissues with necrotic signs or visibly dead tissues were discarded. We defined three types of bud banks according to the morphological characteristics of bud-bearing organs: rhizome buds (axillary buds and apical buds on hypogeogenous rhizomes), tiller buds (axillary buds at the shoot bases of caespitose species and rhizomatous grasses); and root-sprouting bud (adventitious bud formed mainly endogenously on roots of forb or shrub). In contrast to counting buds directly on rhizomes, stems, and roots, dissecting those at the base of shoots was necessary to estimate the number of tillers and root buds.

Biomass and diversity calculation

The aboveground biomass per quadrat was measured by clipping the plants at ground level. The respective biomass was dried at 70°C to constant weight. We measured species diversity indices for each quadrat using the following equations (Li et al., 2014):

$$\text{Importance value (IV)} = (\text{relative height} + \text{relative density})/2$$

$$\text{Shannon} - \text{Wiener Index (H)} = -\sum(P_i \ln P_i)$$

P_i is the relative importance value of the i_{th} species in the community.

Data analysis

The original dataset of bud densities was converted into numbers of buds per square meter. The average bud density of each sampling position was then calculated for further analysis. One-way ANOVA was applied to analyze differences in belowground bud bank density, vegetation characteristics (vegetation density, aboveground biomass, and Shannon-Weiner diversity index), and soil properties (soil water content, total C, total N) among WL, FL, and AM ecosystems. One-way ANOVA and Tukey's honestly significant difference *post hoc* test were performed using SPSS 18.0 (SPSS Inc., USA). Redundancy Analysis (RDA) was used to examine the correlations between belowground bud bank and aboveground vegetation, soil, and properties in the WL, FL, and AM ecosystems. Aboveground vegetation information included vegetation density, Shannon-Weiner diversity index, and aboveground biomass, soil properties embraced soil water content of 0 – 10 cm layer, total C of 0–10 cm layer, and total N of 0–10 cm layer. The original data was log-transformed and normalized before RDA. Some environmental factors were deleted by Monte Carlo selection under $P< 0.05$. We selected variables with high canonical loading factors, confirmed by a cutoff value of 0.35, and parameters highly correlated with canonical variables detected by high standardized coefficients (r > 0.4). RDA was performed using CANOCO *v. 4.5*.

Results

Change in bud bank traits among different land use types

We found rhizome, tiller, and root sprouting buds in WL, FL, and AM ecosystems; however, land use change affected the bud bank structure, with tiller buds accounting for the majority (81.14%) of total buds in WL ecosystems and sprouting buds (60.93%) showed dominance in FL ecosystems. In contrast, rhizome buds accounted for the highest proportion in the AM ecosystem (Figure 2). Bud bank density and soil properties differed significantly among WL, FL, and AM ecosystems (Table 1). The total bud density (1514.727 ± 296.666) and tiller bud density (1229.090 ± 279.002) in WL were significantly higher than that in FL and AM ($P< 0.01$, Figure 3). Additionally, rhizome bud density (220.235 ± 53.516) and root sprouting bud density (149.294 ± 46.496) in AM were significantly higher than that in WL and FL ($P< 0.01$; Figure 3). The bud densities of all bud types showed relatively lower in FL ecosystems compared WL and AM. The soil moisture content at the 0 – 10 cm layers in WL was significantly higher than that in FL and AM, while total C at the 0 – 10 cm layers and total N at 0 – 10 cm layers in AM were markedly higher than that in WL and FL ($P< 0.05$; Table 1).

Effects of vegetation attributes and soil properties on bud banks

In WL ecosystems, all factors combined explained 83.2% of the total variation in bud banks. The soil water content at the 0 – 10 cm layer, vegetation density, and aboveground biomass were significantly correlated with bud banks ($P< 0.05$). Vegetation density was the most

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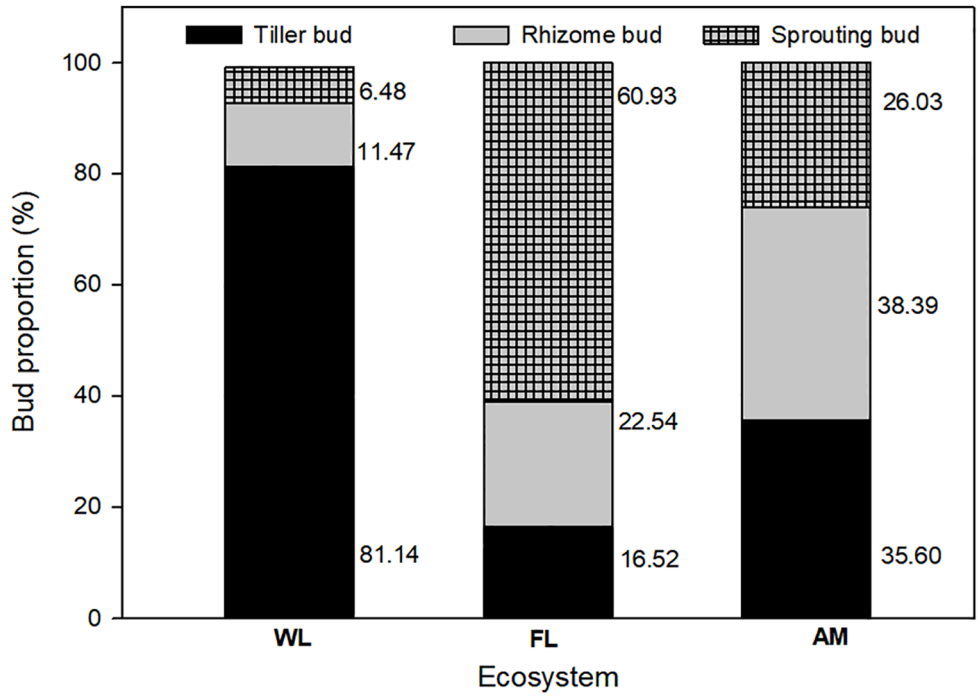

FIGURE 2
Percentage of belowground bud bank density of different bud types in wetland (WL), farmland (FL), and Alpine meadow (AM) ecosystems.

critical factor affecting bud banks, explaining 62.2% of the variation of bud banks. Moreover, soil water content at the 0 – 10 cm layer explained 11.9% of the variation of bud banks, whereas the total C at the 0 – 10 cm layer, total N at the 0 – 10 cm layer, and plant diversity had no significant effect on bud banks. The contribution of vegetation attributes and soil properties to the variation in bud bank were 59.1%, 84.5%, and 15.5%, respectively (Table 2; Figure 4).

All factors in the FL ecosystem explained 35.1% of the total variation in the bud bank. The total N at the 0 – 10 cm layer was significantly correlated with the bud bank ($P< 0.05$), and it was the decisive factor in affecting the bud bank, explaining 12.6% of the variation of the bud bank. The contribution of vegetation attributes and soil properties to the variation in bud bank was 31.5% and 68.5%, respectively (Table 2; Figure 5).

TABLE 1 One-way ANOVA of soil characteristics, vegetation characteristics, and bud bank density of different bud types in wetland (WL), farmland (FL), and Alpine meadow (AM) ecosystems.

Soil characteristics	FL	WL	AM	F	*P*
Soil moisture content (0 – 10cm) (%)	15.458 ± 1.442	36.529 ± 2.764	35.899 ± 2.907	27.634	.000
Total carbon (0 – 10cm) (g/kg)	1.634 ± 0.123	1.605 ± 0.051	3.252 ± 0.351	22.928	.000
Total nitrogen (0 – 10cm) (g/kg)	0.223 ± 0.043	0.159 ± 0.006	0.304 ± 0.028	4.622	.014
Vegetation characteristics	**FL**	**WL**	**AM**	**F**	**P**
Vegetation density (No./m^2)	407.000 ± 32.157	943.272 ± 151.363	544.941 ± 32.335	9.114	.000
Shannon-Weiner diversity index	1.234 ± 0.080	0.894 ± 0.118	1.055 ± 0.031	3.863	.026
Aboveground biomass (g)	123.371 ± 9.591	218.081 ± 57.535	93.882 ± 8.887	3.229	.047
Bud density	**FL**	**WL**	**AM**	**F**	**P**
Total bud (No./m^2)	149.333 ± 21.490	1514.727 ± 296.666	573.647 ± 91.518	15.313	.000
Rhizome bud (No./m^2)	33.666 ± 10.959	173.818 ± 53.100	220.235 ± 53.516	5.626	.006
Tiller bud (No./m^2)	24.666 ± 8.504	1229.090 ± 279.002	204.235 ± 50.550	15.207	.000
Root sprouting bud (No./m^2)	91.000 ± 19.091	98.181 ± 58.992	149.294 ± 46.496	0.475	.642

The samples were 22 for AM, 24 for WL, and 17 for FL ecosystems. Values are mean ± SE, and the difference was considered significant if $P< 0.05$.

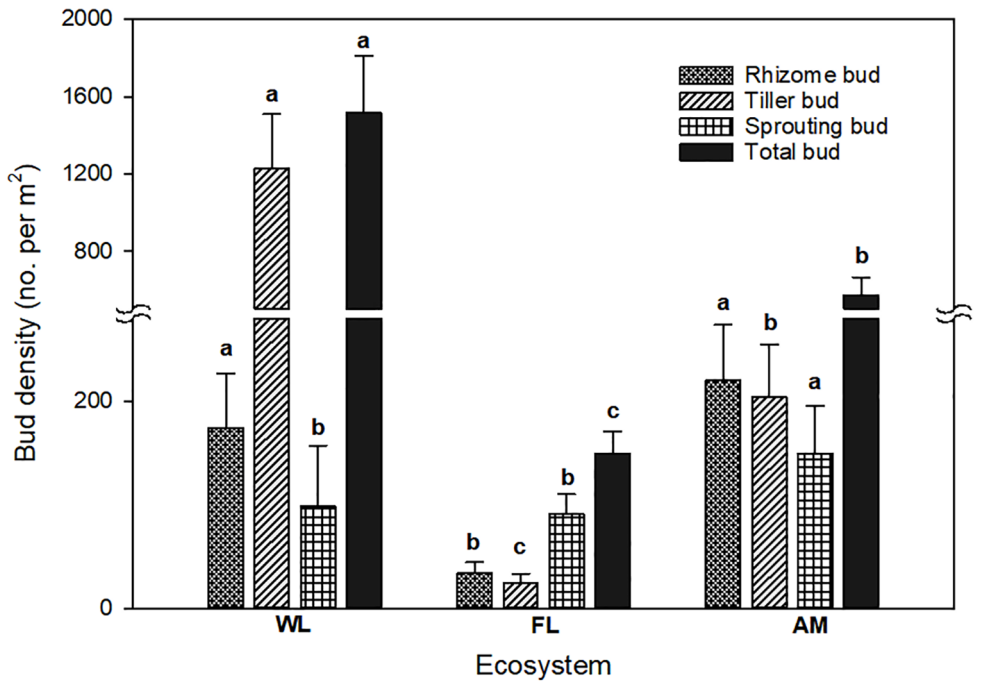

FIGURE 3
Difference in bud density of different bud types in WL, FL, and AM ecosystems. Lowercase letters indicate significant differences (*P< 0.05*) in bud density among land use types. Bars represent error bars.

All factors in the AM ecosystem explained 64.5% of the total variation in the bud bank. The soil water content at the 0 – 10 cm layer, total C at the 0 – 10 cm layer, and plant diversity were significantly correlated with bud banks (*P< 0.05*). The soil water content at the 0 – 10 cm layer was the most critical factor affecting bud banks, explaining 28.3% of the variation of bud bank, followed by total C at the 0 – 10cm layer, plant diversity explained 14.8% and 15.0% of the variation of bud bank, respectively. The contribution of vegetation attributes and soil properties to the variation in bud bank were 29.6% and 70.4%, respectively (Table 2; Figure 6).

Effect of aboveground vegetation and soil properties on bud bank densities of different types

In the WL ecosystem, the densities of total bud and tiller bud were significantly positively correlated with the soil water content (0 – 10 cm layer), vegetation density, and aboveground biomass (*P< 0.05*), and vegetation density was the most critical factor to explain the density variation of total bud and tiller bud. Still, the rhizome bud and root sprouting bud density had no significant relationship with all factors (Table 3; Figure 4).

In the FL ecosystem, the density of the total bud was significantly positively correlated with the total N at the 0 – 10 cm layer, and the density of the root sprouting bud was significantly positively correlated with the total C at the 0 – 10 cm layer. However, rhizome bud and tiller bud density had no significant relationship with all factors (Table 4; Figure 5).

In the AM ecosystem, the densities of total bud and tiller bud were significantly positively correlated with the soil water content (0 – 10 cm layer) and vegetation density (*P< 0.05*), and the soil water content at the 0 – 10 cm layer was the most critical factor that explains the density variation of total bud and tiller bud. The rhizome bud density was significantly positively correlated with total C at the 0 –10 cm layer (*P< 0.05*). Still, the density of root sprouting bud had no significant relationship with all factors (Table 5; Figure 6).

Discussion

Land use change alters the characteristics of bud bank composition and size

Land use change significantly altered the total bud bank density and the bud densities of all types (rhizome bud, tiller bud, and root sprouting bud) across all ecosystems; however, such impacts were higher in WL and AM than in the FL ecosystems. While total bud density, tiller buds, and moisture content were significantly higher in wetland ecosystems, bud densities of all bud types were relatively lower in FL. These results are consistent with previous findings that human-caused disturbances have remarkable adverse effects on belowground bud banks, which have substantial implications on bud regrowth, productivity, and ecological succession (Dalgleish and Hartnett, 2009; Collins and Calabrese, 2012; Deng et al., 2014; Chen et al., 2020). Similarly, results provide empirical evidence that various ecosystems differ in their responses to land use effects,

TABLE 2 Explanations and contributions of impact factors to the total variation in bud bank for wetland (WL), farmland (FL), and Alpine meadow (AM) ecosystems.

	Controlling Factors	Parameters	Explanations (%)	Contributions (%)	F	*P*
WL	Environment	**M10**	**11.9****	**14.3**	**8.766**	**0.006**
		C10	0.3	0.36	0.654	0.494
		N10	0.7	0.84	0.246	0.766
	Vegetation	**VD**	**62.2****	**74.7**	**32.943**	**0.002**
		BM	**5.8***	**6.9**	**5.264**	**0.048**
		SH	2.2	2.6	2.131	0.138
		Total	83.2	100		
FL	Environment	**N10**	**12.6***	**35.8**	**3.332**	**0.040**
		C10	8.3	23.6	1.998	0.140
		M10	3.2	9.1	0.871	0.400
	Vegetation	VD	5.8	16.5	1.573	0.212
		BM	5.2	14.8	1.441	0.248
		SH	0.1	0.2	0.021	0.992
		Total	35.1	100		
AM	Environment	**M10**	**28.3****	**43.8**	**6.971**	**0.004**
		C10	**14.8***	**22.9**	**2.641**	**0.070**
		N10	2.4	3.7	0.717	0.572
	Vegetation	VD	3.3	5.1	1.037	0.374
		BM	0.7	1.0	0.193	0.894
		SH	**15.0***	**23.2**	**4.651**	**0.010**
		Total	64.5	100		

M10, soil moisture content (0 – 10cm) (%); C10, total carbon (C, 0 – 10cm) (g/kg); N10, total nitrogen (0 – 10cm) (g/kg); VD, vegetation density (No./m^2); SH, Shannon-Weiner diversity index; BM, aboveground biomass (g). Values are bold if significant.
* $P< 0.05$. ** $P< 0.01$.

suggesting that variation in species composition and soil characteristics across diverse terrestrial ecosystems underlie their differential responses during ecological perturbation, as observed by previous studies (Lavorel et al., 1997; Boer and Stafford Smith, 2003).

The differential responses of the studied ecosystems (i.e., WL, FL, and AM) can be explained on the following account: firstly, tiller buds in WL accounted for approximately 80% of the total bud densities because of high levels of soil moisture content, total C, and total N at the top 0 –10 cm layer, facilitating plant establishment and population reproduction (Li et al., 2014; Adomako et al., 2020; Ma et al., 2021). These growth and productivity drivers (moisture, total C, and N) primarily promote the build-up of bud density in WL vegetation compared to AM and FL ecosystems (Dalgleish and Hartnett, 2006; Ding et al., 2019). Secondly, higher rhizome and sprouting bud densities in FL and AM compared to WL ecosystems suggest that rhizomes and sprouting buds are highly sensitive to water and nutrient availability (Hiiesalu et al., 2021; Adomako et al., 2022), conditions that are ubiquitously higher in the WL ecosystem and favor tiller buds as grass functional groups represent the dominant populations in wetland vegetation (Williams et al., 2017). Thirdly, FL had relatively the lowest bud densities of all bud types, and this can be attributed to high levels of human-caused disturbances (e.g., grazing, plowing, bush fire, herbicide application) or land use intensification in farmland vegetation compared to low farming activities in wetland zones (Allan et al., 2015). Our results suggest that plant species composition (functional groups) and soil physicochemical parameters determine the resilience and variation in response of ecosystems to anthropogenically mediated disturbances.

Land use change alters the relative contributions of vegetation attributes and soil properties on bud bank

Vegetation attributes and soil properties strongly correlated with bud banks; however, bud bank demography in the three ecosystems (WL FL, and AM) with different vegetation attributes and soil properties, we found that vegetation density, soil moisture

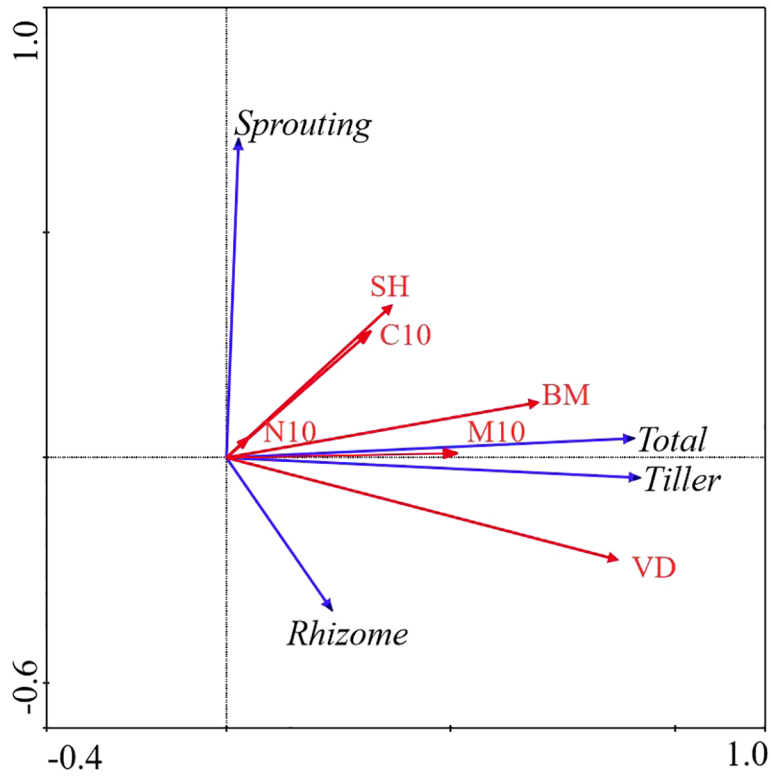

FIGURE 4
Redundancy Analysis (RDA) of the relationship between bud bank density of different bud types and environmental factors in WL ecosystem. M10, soil moisture content (0 – 10cm) (%); C10, total carbon (0 – 10cm) (g/kg); N10, total nitrogen (0 – 10cm) (g/kg); VD, vegetation density (No./m^2); SH, Shannon-Weiner diversity index; BM, aboveground biomass (g); Total, total bud density; rhizome, rhizome bud density; Tiller, tiller bud density; Sprouting, root sprouting bud density.

content, and total N was the most critical factors in WL, AM, and FL ecosystems, explaining 62.2, 28.3 and 12.6% accordingly. Higher resource (i.e., soil moisture, total N, and C) availability or growth drivers promoted plant growth, biomass accumulation, and increased vegetation cover and stability (Adomako et al., 2021; Liu et al., 2021; Ma et al., 2021). It is, therefore, plausible to suggest that higher levels of these growth resources invariably increase the vegetation density of the wetland ecosystems, which can also be a function of a greater abundance of belowground bud banks (Liu et al., 2021; Ma et al., 2021), particularly tiller buds that accounted for about 81.14%. Previous studies have indicated that belowground bud banks positively correlate with the net aboveground primary productivity (Qian et al., 2021; Wu and Yu, 2022). Notably, results suggest that wetlands' maintenance, stability, and productivity are tightly linked with a balance of soil characteristics, bud type, and vegetation attributes (Ma et al., 2021).

Furthermore, studies have indicated that soil moisture is an essential driving force for vegetation succession in the alpine meadow (Heisler-White et al., 2008; An et al., 2019; Zhang et al., 2020; Zhang et al., 2022). In the present study, our analysis indicated that soil water content at the top 0 – 10 cm soil layer significantly influences bud banks, explaining 28.3% of variation of bud banks in the alpine ecosystem. Consistent with most previous studies that reported similar patterns at the top 0 – 10 cm of the soil layer, our results suggest that hydrological regimes can potentially modulate and constrain plant growth, community structure, and stability of alpine meadow ecosystems (Heisler-White et al., 2008; An et al., 2019; Ren et al., 2023), as soil moisture is critical for resprouting and growth of belowground bud banks of all bud types. Additionally, total C, plant diversity, and soil moisture significantly correlated with bud banks, indicating that vegetation and soil physicochemical attributes play crucial roles in ecological succession and productivity output of meadow ecosystems (Hong et al., 2012; Xie et al., 2018; Świerszcz et al., 2019; Plue et al., 2021).

Lastly, total N strongly correlated with and was the most crucial factor influencing bud banks, explaining 12.6% of bud bank variation in farmlands and provides empirical evidence of the extent and magnitude of human-derived disturbances via nutrient enrichments in agrosystems (Isbell et al., 2013; Ren et al., 2019; Adomako et al., 2022). Although N limitation substantially limits plant growth (De Tezanos Pinto and Litchman, 2010; Bracken et al., 2015; Fay et al., 2015; Du et al., 2020; Adomako et al., 2022) and increased fertilization aimed at increasing agricultural output in agrosystems may promote the proliferation of bud banks in short-term period (Liu et al., 2021; Qian et al., 2021; Adomako and Yu, 2023), the long-term effects of N influxes in farmland ecosystems can trigger land degradation (Hamilton et al., 2020; Qian et al., 2021; Owusu et al., 2024). Notably, the FL ecosystem had the least attributes of all bud types measured, which can likely be linked to land use intensification. Our results confirm previous and current findings that N enrichment

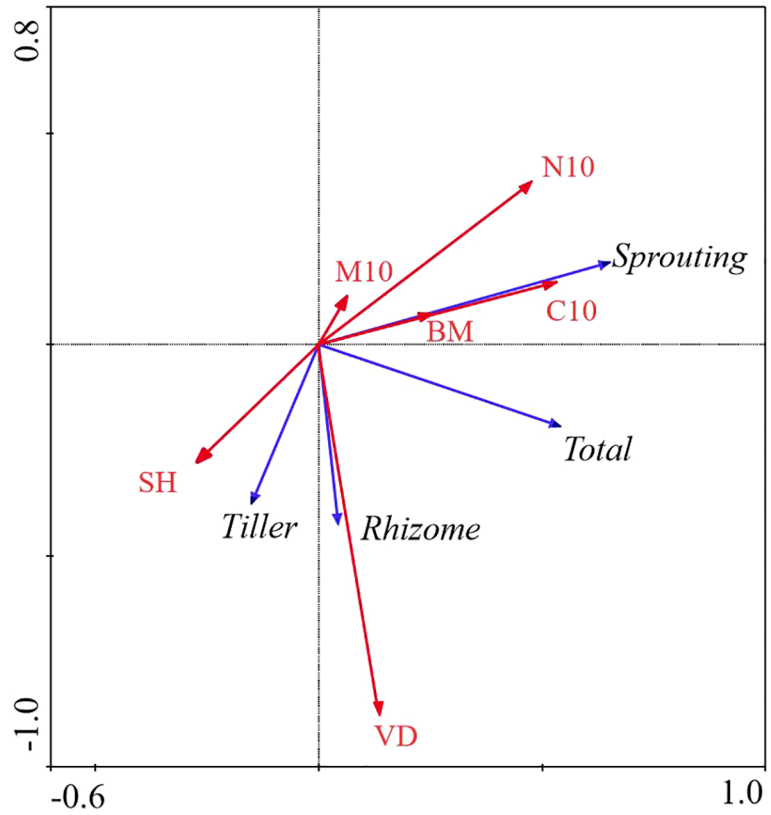

FIGURE 5
Redundancy Analysis (RDA) of the relationship between bud bank density of different bud types and environmental factors in FL ecosystem. M10, soil moisture content (0 – 10cm) (%); C10, total carbon (0 – 10cm) (g/kg); N10, total nitrogen (0 – 10cm) (g/kg); VD, vegetation density (No./m^2); SH, Shannon-Weiner diversity index; BM, aboveground biomass (g); Total, total bud density; rhizome, rhizome bud density; Tiller, tiller bud density; Sprouting, root sprouting bud density.

disrupts plant community composition and plant-microbial interactions, promoting loss of global biodiversity and soil multifunctionality (Galloway et al., 2008; Qian et al., 2021).

Mechanisms underlying factors affecting bud banks of wetland, alpine meadow, and farmland ecosystems

Overall, soil properties significantly influenced root sprouting and rhizome buds of FL and AM, consistent with previous studies in dune ecosystems (Wu et al., 2021). The measured soil properties (soil moisture, total C and N) represent important factors that plays a pivotal role in many ecosystem processes, such as organic matter mineralization, litter decomposition, and biogeochemical cyclings (Wu et al., 2020; Inoue et al., 2022), which influence nutrient availability and the spatial distribution of resprouting buds and rhizome buds (Xie et al., 2018; Xiao et al., 2021). For example, in a recent study, Xiao et al. (2021) reported a significant influence of soil properties on the spatial distribution of Moso bamboo rhizomes.

Total buds and tiller buds were more strongly correlated with soil qualities in FL and AM ecosystems than aboveground vegetation parameters in WL ecosystems. Strong aboveground recruitment and productivity are tightly linked with the abundance of belowground bud banks, especially tiller buds. Tiller buds constitute hemicryptophytes, and buds emanate from the shoot bases of the mother plant and are protected by leaf sheaths. Therefore, connected tiller buds can receive substantial growth resources from the connected mother plant, facilitating its expansion within plant communities (Ott and Hartnett, 2012). Conversely, in geophytes, root-sprouting and rhizome buds are primarily initiated from subterranean roots and rhizomes (Ott and Hartnett, 2012). These belowground structures are sensitive to water and nutrient availability in their ambient environment (Passioura, 1988; Wu et al., 2020). Therefore, bud banks can be a major driving force limiting productivity in wetland ecosystems. Unlike in FL and AM ecosystems, many wetland species are adapted to modifications in soil conditions and human-caused disturbances resulting from land use intensification. Thus, tiller buds connected to parent plants can obtain parental resource nourishments and withstand land use pressures in their ecosystem (Ott and Hartnett, 2012; Wu et al., 2020).

In contrast, as rhizome buds and root sprouting buds mainly initiate from underground roots and rhizomes, they could directly forage water or nutrients in the surrounding soil (Vesk and Westoby, 2004; Deng et al., 2013). Previous studies demonstrated that plants tend to produce more rhizome buds to increase foraging for favorable patches for persistence and regeneration in a resource-poor region, while in a relatively low resources environment, more tiller buds are

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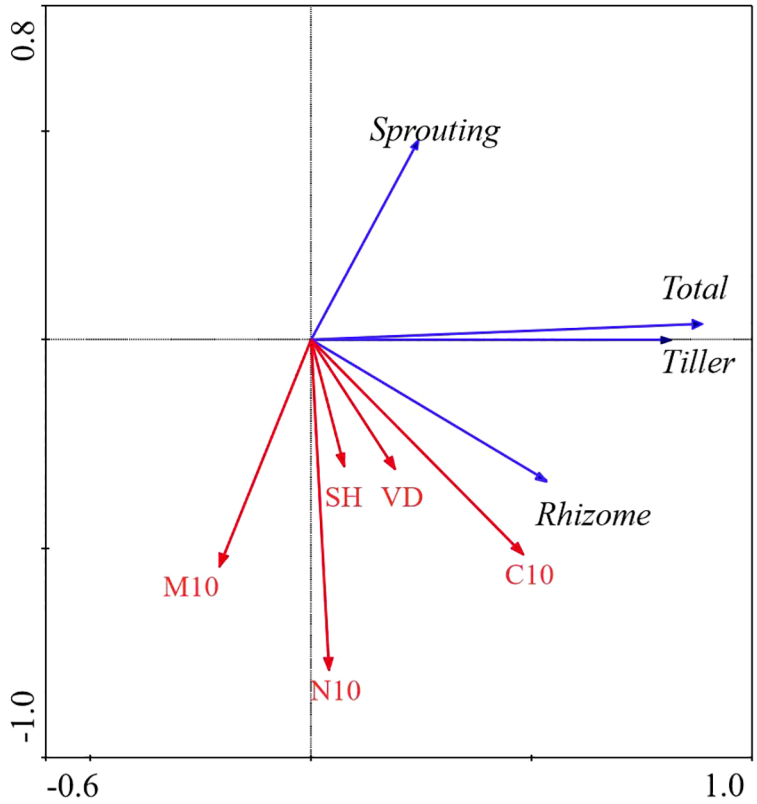

FIGURE 6
Redundancy Analysis (RDA) of the relationship between bud bank density of different bud types and environmental factors in AM ecosystem. M10, soil moisture content (0 – 10cm) (%); C10, total carbon (0 – 10cm) (g/kg); N10, total nitrogen (0 –10cm) (g/kg); VD, vegetation density (No./m^2); SH, Shannon-Weiner diversity index; BM, aboveground biomass (g); Total, total bud density; rhizome, rhizome bud density; Tiller, tiller bud density; Sprouting, root sprouting bud density.

TABLE 3 Explanations and contributions of impact factors to the total variation in bank density of different wetland types (WL).

Bud type	Controlling Factors	Parameters	Explanations (%)	Contributions (%)	F	*P*
Rhizome	Environment	C10	10.5†	20.7	3.380	0.080
		M10	13.2†	26.1	3.475	0.090
		N10	14.7†	29.1	3.441	0.084
	Vegetation	VD	5.9	11.6	1.675	0.224
		BM	5.9	11.6	1.602	0.214
		SH	0.3	0.5	0.105	0.746
		Total	50.6	100		
Tiller	Environment	**M10**	**12.5****	**14.6**	**11.001**	**0.004**
		C10	0.10	0.1	0.084	0.774
		N10	0.40	0.4	0.493	0.518
	Vegetation	**VD**	**65.8****	**76.7**	**38.493**	**0.002**
		BM	**6.6***	**7.6**	**7.812**	**0.016**
		SH	0.4	0.4	0.482	0.548

(Continued)

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TABLE 3 Continued

Bud type	Controlling Factors	Parameters	Explanations (%)	Contributions (%)	F	*P*
		Total	85.8	100		
Root sprouting	Environment	**M10**	**9.8†**	**17.7**	**3.075**	**0.092**
		C10	5.7	10.2	0.282	0.574
		N10	3.6	6.4	1.097	0.326
	Vegetation	VD	13.4†	24.2	3.217	0.082
		BM	15.2†	27.4	4.304	0.070
		SH	7.7	13.8	1.669	0.256
		Total	55.4	100		
Total	Environment	**M10**	**13.7***	**17.0**	**8.490**	**0.014**
		C10	0.1	0.1	0.091	0.774
		N10	0.5	0.5	0.407	0.538
	Vegetation	**VD**	**55.8****	**69.3**	**25.244**	**0.002**
		BM	**7.7***	**9.5**	**0.031**	**0.040**
		SH	2.7	3.4	2.292	0.134
		Total	80.5	100		

M10, soil moisture content (0 – 10cm) (%); C10, total carbon (0 – 10cm) (g/kg); N10, total nitrogen (0 – 10cm) (g/kg); VD, vegetation density (No./m^2); SH, Shannon-Weiner diversity index; BM, aboveground biomass (g). Values are bold if significant. * $P< 0.05$. ** $P< 0.01$. † $P< 0.10$.

TABLE 4 Explanations and contributions of impact factors to the total variation inbank density of different types of farmland (FL).

Bud type	Controlling Factors	Parameters	Explanations (%)	Contributions (%)	F	*P*
Rhizome	Environment	C10	0.3	1.5	0.057	0.826
		M10	5.0	26.0	0.115	0.754
		N10	<0.1	<0.1	0.002	0.976
	Vegetation	VD	12.0†	62.5	3.004	0.086
		BM	5.6	29.1	1.426	0.232
		SH	0.8	4.1	0.195	0.626
		Total	19.2	100		
Tiller	Environment	M10	0.80	4.3	0.194	0.636
		C10	0.4	2.2	1.000	0.318
		N10	0.3	1.6	0.075	0.780
	Vegetation	VD	12.7†	69.0	3.213	0.081
		BM	4.0	21.7	0.103	0.738
		SH	0.1	0.5	0.018	0.896
		Total	18.4	100		
Root sprouting	Environment	M10	9.5	20.5	3.187	0.102
		C10	**14.2***	**30.8**	**3.651**	**0.042**
		N10	10.0†	21.5	2.785	0.088
	Vegetation	VD	10.6†	22.9	3.272	0.092

(Continued)

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TABLE 4 Continued

Bud type	Controlling Factors	Parameters	Explanations (%)	Contributions (%)	F	*P*
		BM	1.90	4.1	0.570	0.440
		SH	<0.1	<0.1	0.008	0.912
		Total	46.3	100		
Total	Environment	M10	6.6	19.8	1.776	0.215
		C10	6.2	18.7	1.450	0.230
		N10	**13.4***	**40.4**	**3.503**	**0.044**
	Vegetation	VD	2.5	7.5	0.674	0.428
		BM	4.2	12.7	1.136	0.322
		SH	0.4	1.2	0.093	0.774
		Total	33.2	100		

M10, soil moisture content (0 – 10cm) (%); C10, total carbon (0 – 10cm) (g/kg); N10, total nitrogen (0 – 10cm) (g/kg); VD, vegetation density (No./m^2); SH, Shannon-Weiner diversity index; BM, aboveground biomass (g). Values are bold if significant. * $P< 0.05$. † $P< 0.10$.

TABLE 5 Explanations and contributions of impact factors to the total variation in bank density of different types for Alpine meadow (AM).

Bud type	Controlling Factors	Parameters	Explanations (%)	Contributions (%)	F	*P*
Rhizome	Environment	**C10**	**25.0***	**54.74.990**		**0.042**
		M10	8.5	18.6	3.246	0.170
		N10	5.7	12.5	1.168	0.278
	Vegetation	VD	1.5	3.2	0.276	0.628
		BM	2.6	5.7	0.512	0.166
		SH	2.5	5.5	0.513	0.438
		Total	45.7	100		
Tiller	Environment	**M10**	**25.7***	**35.3**	**6.996**	**0.016**
		C10	9.6	13.2	1.825	0.184
		N10	0.1	0.13	0.059	0.794
	Vegetation	VD	16.9	23.2	3.053	0.126
		BM	0.1	0.14	0.052	0.838
		SH	**20.3***	**27.9**	**8.848**	**0.020**
		Total	72.8	100		
Root sprouting	Environment	M10	6.9	22.3	1.118	0.280
		C10	9.7	31.3	1.634	0.204
		N10	4.4	14.2	0.714	0.396
	Vegetation	VD	8.1	26.1	1.340	0.256
		BM	1.8	0.1	0.287	0.610
		SH	<0.1	<0.1	0.003	0.968
		Total	31.0	100		
Total	Environment	**M10**	**39.4****	**51.1**	**12.706**	**0.004**

(Continued)

TABLE 5 Continued

Bud type	Controlling Factors	Parameters	Explanations (%)	Contributions (%)	F	*P*
		C10	17.2†	22.3	3.122	0.100
		N10	<0.1	<0.1	0.051	0.546
	Vegetation	VD	0.40	0.50	0.189	0.668
		BM	<0.1	<0.1	0.101	0.940
		SH	**20.1****	**26.1**	**11.179**	**0.008**
		Total	77.1	100		

M10, soil moisture content (0 – 10cm) (%); C10, total carbon (0 – 10cm) (g/kg); N10, total nitrogen (0 – 10cm) (g/kg); VD, vegetation density (No./m^2); SH, Shannon-Weiner diversity index; BM, aboveground biomass (g). Values are bold if significant. * $P< 0.05$. ** $P< 0.01$. † $P< 0.10$.

produced to increase dominance and resource capture (Qian et al., 2017; Wu et al., 2021). Our study further illustrates that root sprouting buds are related to the soil nutrient content of the top (0 – 10 cm) layer because of direct roots sensitivity to soil nutrients (Klimeš and Klimešová, 1999; Ma et al., 2019).

Conclusions

Exploring belowground bud bank responses in different ecosystems is essential for understanding the adaptive strategies and vegetation restoration under the ongoing land use changes. We found that land-use change alters bud bank composition and size characteristics and alters the relative contributions of vegetation attributes and soil properties on bud banks. In WL environments, vegetation density is a crucial determinant; soil conditions are the most important factor affecting bud banks in FL and AM habitats. For different bud types, total buds and tiller buds rely more on vegetation density in WL ecosystems, but total buds and tiller buds are more related to soil properties in ecosystems of FL and AM. Rhizome and root sprouting buds could buffer vegetation restoration under land use change. Results indicate that vegetation and soil attributes play critical roles, underly the differential responses and the composition of bud banks of different ecosystems. Given the predicted climate change impacts and rapid expansion of industrialization and settlements, similar studies involving more climate change factors under varying climatic conditions may be highly informative and insightful.

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: Conceptualization, Funding acquisition, Investigation, Methodology, Writing – original draft. XH: Investigation, Writing – original draft. LX: Methodology, Writing – original draft. QZ: Methodology, Writing – original draft. YW: Investigation, Writing – original draft. ZG: Writing – review & editing. MOA: Writing – review & editing. QM: 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 financially supported by the National Natural Science Youth Foundation of China (31600443) and the General Research Project of the Education Department of Zhejiang Province (Y202248466).

Acknowledgments

We thank Li Zhang and Mingying Tao for field assistance, processing soil samples, and analysis.

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.

The reviewer JZ declared a shared affiliation with the author ZG to the handling editor at the time of review.

Publisher's note

References

Adomako, M. O., Xue, W., Du, D.-L., and Yu, F.-H. (2021). Soil biota and soil substrates influence responses of the rhizomatous clonal grass *Leymus chinensis* to nutrient heterogeneity. *Plant Soil* 465, 19–29. doi: 10.1007/s11104-021-04967-0

Adomako, M. O., Xue, W., Du, D.-L., and Yu, F.-H. (2022). Soil microbe-mediated N: P stoichiometric effects on *Solidago canadensis* performance depend on nutrient levels. *Microbial Ecol.* 83, 960–970. doi: 10.1007/s00248-021-01814-8

Adomako, M. O., Xue, W., Tang, M., Du, D.-L., and Yu, F.-H. (2020). Synergistic effects of soil microbes on *Solidago canadensis* depend on water and nutrient availability. *Microbial Ecol.* 80, 837–845. doi: 10.1007/s00248-020-01537-2

Adomako, M. O., and Yu, F.-H. (2023). Functional group dominance-mediated N:P effects on community productivity depend on soil nutrient levels. *Rhizosphere* 26, 100692. doi: 10.1016/j.rhisph.2023.100692

Allan, E., Manning, P., Alt, F., Binkenstein, J., Blaser, S., Blüthgen, N., et al. (2015). Land use intensification alters ecosystem multifunctionality via loss of biodiversity and changes to functional composition. *Ecol. Lett.* 18, 834–843. doi: 10.1111/ele.12469

An, Y., Gao, Y., Liu, X. H., and Tong, S. Z. (2019). Interactions of soil moisture and plant community properties in meadows restored from abandoned farmlands on the Sanjiang Plain, China. *Community Ecol.* 20, 20–27. doi: 10.1556/168.2019.20.1.3

Boer, M., and Stafford Smith, M. (2003). A plant functional approach to the prediction of changes in Australian rangeland vegetation under grazing and fire. *J. Vegetation Sci.* 14, 333–344. doi: 10.1111/j.1654-1103.2003.tb02159.x

Bracken, M. E. S., Hillebrand, H., Borer, E. T., Seabloom, E. W., Cebrian, J., Cleland, E. E., et al. (2015). Signatures of nutrient limitation and co-limitation: responses of autotroph internal nutrient concentrations to nitrogen and phosphorus additions. *Oikos* 124, 113–121. doi: 10.1111/oik.01215

Chang, C. C., and Turner, B. L. (2019). Ecological succession in a changing world. *J. Ecol.* 107, 503–509. doi: 10.1111/1365-2745.13132

Chen, X., Zhu, L., Deng, Z., Xie, Y., Chen, X., Li, F., et al. (2020). Effects of clipping on bud bank and population regeneration of *Triarrhena lutarioriparia* in Dongting Lake wetland, China. *Wetlands* 40, 2635–2642. doi: 10.1007/s13157-020-01311-7

Clarke, P. J., Lawes, M. J., Midgley, J. J., Lamont, B. B., Ojeda, F., Burrows, G. E., et al. (2013). Resprouting as a key functional trait: how buds, protection and resources drive persistence after fire. *New Phytol.* 197, 19–35. doi: 10.1111/nph.12001

Collins, S. L., and Calabrese, L. B. (2012). Effects of fire, grazing and topographic variation on vegetation structure in tallgrass prairie. *J. Vegetation Sci.* 23, 563–575. doi: 10.1111/j.1654-1103.2011.01369.x

Dalgleish, H. J., and Hartnett, D. C. (2006). Below-ground bud banks increase along a precipitation gradient of the North American Great Plains: a test of the meristem limitation hypothesis. *New Phytol.* 171, 81–89. doi: 10.1111/j.1469-8137.2006.01739.x

Dalgleish, H. J., and Hartnett, D. C. (2009). The effects of fire frequency and grazing on tallgrass prairie productivity and plant composition are mediated through bud bank demography. *Plant Ecol.* 201, 411–420. doi: 10.1007/s11258-008-9562-3

Deng, L., Sweeney, S., and Shangguan, Z. P. (2014). Grassland responses to grazing disturbance: plant diversity changes with grazing intensity in a desert steppe. *Grass Forage Sci.* 69, 524–533. doi: 10.1111/gfs.12065

Deng, Z. M., Chen, X. S., Xie, Y. H., Li, X., Pan, Y., and Li, F. (2013). Effects of size and vertical distribution of buds on sprouting and plant growth of the clonal emergent macrophyte Miscanthus sacchariflorus (Poaceae). *Aquat Bot.* 104, 12–14. doi: 10.1016/j.aquabot.2012.08.004

De Tezanos Pinto, P., and Litchman, E. (2010). Interactive effects of N:P ratios and light on nitrogen-fixer abundance. *Oikos* 119, 567–575. doi: 10.1111/j.1600-0706.2009.17924.x

Ding, X., Su, P., Zhou, Z., and Shi, R. (2019). Belowground bud bank distribution and aboveground community characteristics along different moisture gradients of alpine meadow in the Zoige Plateau, China. *Sustainability* 11, 2602. doi: 10.3390/su11092602

Du, E., Terrer, C., Pellegrini, A. F. A., Ahlström, A., Van Lissa, C. J., Zhao, X., et al. (2020). Global patterns of terrestrial nitrogen and phosphorus limitation. *Nat. Geosci.* 13, 221–226. doi: 10.1038/s41561-019-0530-4

Fay, P. A., Prober, S. M., Harpole, W. S., Knops, J. M. H., Bakker, J. D., Borer, E. T., et al. (2015). Grassland productivity limited by multiple nutrients. *Nat. Plants* 1, 15080. doi: 10.1038/nplants.2015.80

Galloway, J. N., Townsend, A. R., Erisman, J. W., Bekunda, M., Cai, Z., Freney, J. R., et al. (2008). Transformation of the nitrogen cycle: Recent trends, questions, and potential solutions. *Science* 320, 889–892. doi: 10.1126/science.1136674

Hamilton, D. J., Bulmer, R. H., Schwendenmann, L., and Lundquist, C. J. (2020). Nitrogen enrichment increases greenhouse gas emissions from emerged intertidal sandflats. *Sci. Rep.* 10, 6686. doi: 10.1038/s41598-020-62215-4

Heisler-White, J. L., Knapp, A. K., and Kelly, E. F. (2008). Increasing precipitation event size increases aboveground net primary productivity in a semi-arid grassland. *Oecologia* 158, 129–140. doi: 10.1007/s00442-008-1116-9

Hiiesalu, I., Klimešová, J., Doležal, J., Mudrák, O., Götzenberger, L., Horník, J., et al. (2021). Hidden below-ground plant diversity buffers against species loss during land-use change in species-rich grasslands. *J. Vegetation Sci.* 32, e12971. doi: 10.1111/jvs.12971

Hong, J., Liu, S., Shi, G., and Zhang, Y. (2012). Soil seed bank techniques for restoring wetland vegetation diversity in Yeyahu Wetland, Beijing. *Ecol. Eng.* 42, 192–202. doi: 10.1016/j.ecoleng.2012.01.004

Hoover, D. L., Knapp, A. K., and Smith, M. D. (2014). Resistance and resilience of a grassland ecosystem to climate extremes. *Ecology* 95, 2646–2656. doi: 10.1890/13-2186.1

Hou, L., Kong, W., Qiu, Q., Yao, Y., Bao, K., Zhang, L., et al. (2022). Dynamics of soil N cycling and its response to vegetation presence in an eroding watershed of the Chinese Loess Plateau. *Agriculture Ecosyst. Environ.* 336, 108020. doi: 10.1016/j.agee.2022.108020

Inoue, T., Akaji, Y., Kohzu, A., Hinokidani, K., Adachi, H., Kezuka, M., et al. (2022). Relationship between plant growth and soil chemical properties in a mangrove afforestation stand, Kiribati. *Plant Soil* 479, 559–571. doi: 10.1007/s11104-022-05545-8

Isbell, F., Reich, P. B., Tilman, D., Hobbie, S. E., Polasky, S., and Binder, S. (2013). Nutrient enrichment, biodiversity loss, and consequent declines in ecosystem productivity. *Proc. Natl. Acad. Sci.* 110, 11911–11916. doi: 10.1073/pnas.1310880110

Klimeš, L., and Klimešová, J. (1999). Root sprouting in Rumex acetosella under different nutrient levels. *Plant Ecol.* 141, 33–39. doi: 10.1023/A:1009877923773

Klimešová, J., Martínková, J., Bartušková, A., and Ott, J. P. (2023). Belowground plant traits and their ecosystem functions along aridity gradients in grasslands. *Plant Soil* 487, 39–48. doi: 10.1007/s11104-023-05964-1

Lavorel, S., Mcintyre, S., Landsberg, J., and Forbes, T. D. A. (1997). Plant functional classifications: from general groups to specific groups based on response to disturbance. *Trends Ecol. Evol.* 12, 474–478. doi: 10.1016/S0169-5347(97)01219-6

Li, Y.-Y., Dong, S.-K., Wen, L., Wang, X.-X., and Wu, Y. (2014). Soil carbon and nitrogen pools and their relationship to plant and soil dynamics of degraded and artificially restored grasslands of the Qinghai–Tibetan Plateau. *Geoderma* 213, 178–184. doi: 10.1016/j.geoderma.2013.08.022

Li, F.-R., Liu, J.-L., Ren, W., and Liu, L.-L. (2018). Land-use change alters patterns of soil biodiversity in arid lands of northwestern China. *Plant Soil* 428, 371–388. doi: 10.1007/s11104-018-3673-y

Liu, H., Wu, Y., Xu, H., Ai, Z., Zhang, J., Liu, G., et al. (2021). N enrichment affects the arbuscular mycorrhizal fungi-mediated relationship between a C4 grass and a legume. *Plant Physiol.* 187, 1519–1533. doi: 10.1093/plphys/kiab328

Luo, W., Muraina, T. O., Griffin-Nolan, R. J., Te, N., Qian, J., Yu, Q., et al. (2023). High below-ground bud abundance increases ecosystem recovery from drought across arid and semiarid grasslands. *J. Ecol* 111, 2038–2048. doi: 10.1111/1365-2745.14160

Ma, Q., Qian, J. Q., T, L., and Liu, Z. M. (2019). Responses of belowground bud bank to disturbance and stress in the sand dune ecosystem. *Ecol Indic.* 106, 1–7. doi: 10.1016/j.ecolind.2019.105521

Ma, M., Zhu, Y., Wei, Y., and Zhao, N. (2021). Soil nutrient and vegetation diversity patterns of alpine wetlands on the Qinghai-Tibetan Plateau. *Sustainability* (Elsevier) 13, 6221. doi: 10.3390/su13116221

Mobilian, C., and Craft, C. (2021). *Wetland soils: Physical and chemical properties and biogeochemical processes* (Elsevier). doi: 10.1016/B978-0-12-819166-8.00049-9

Newbold, T., Hudson, L. N., Hill, S. L. L., Contu, S., Lysenko, I., Senior, R. A., et al. (2015). Global effects of land use on local terrestrial biodiversity. *Nature* 520, 45–50. doi: 10.1038/nature14324

Ott, J. P., and Hartnett, D. C. (2012). Contrasting bud bank dynamics of two co-occurring grasses in tallgrass prairie: implications for grassland dynamics. *Plant Ecol.* 213, 1437–1448. doi: 10.1007/s11258-012-0102-9

Ott, J. P., and Hartnett, D. C. (2015). Bud-bank and tiller dynamics of co-occurring C3 caespitose grasses in mixed-grass prairie. *Am. J. Bot.* 102, 1462–1471. doi: 10.3732/ajb.1500039

Ott, J. P., Klimešová, J., and Hartnett, D. C. (2019). The ecology and significance of below-ground bud banks in plants. *Ann. Bot.* 123, 1099–1118. doi: 10.1093/aob/mcz051

Owusu, S. M., Adomako, M. O., and Qiao, H. (2024). Organic amendment in climate change mitigation: Challenges in an era of micro- and nanoplastics. *Sci. Total Environ.* 907, 168035. doi: 10.1016/j.scitotenv.2023.168035

Passioura, J. B. (1988). Water transport in and to roots. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 39, 245–265. doi: 10.1146/annurev.pp.39.060188.001333

Peng, J., Li, Y., Tian, L., Liu, Y., and Wang, Y. (2015). Vegetation dynamics and associated driving forces in eastern China during 1999–2008. *Remote Sens.* 7, 13641–13663. doi: 10.3390/rs71013641

Plue, J., and Cousins, S. A. O. (2018). Seed dispersal in both space and time is necessary for plant diversity maintenance in fragmented landscapes. *Oikos* 127, 780–791. doi: 10.1111/oik.04813

Plue, J., Van Calster, H., Auestad, I., Basto, S., Bekker, R. M., Bruun, H. H., et al. (2021). Buffering effects of soil seed banks on plant community composition in response to land use and climate. *Global Ecol. Biogeography* 30, 128–139. doi: 10.1111/geb.13201

Qian, J., Guo, Z., Muraina, T. O., Te, N., Griffin-Nolan, R. J., Song, L., et al. (2022). Legacy effects of a multi-year extreme drought on belowground bud banks in

rhizomatous vs bunchgrass-dominated grasslands. *Oecologia* 198, 763–771. doi: 10.1007/s00442-022-05133-8

Qian, J., Wang, Z., Klimešová, J., Lü, X., Kuang, W., Liu, Z., et al. (2017). Differences in below-ground bud bank density and composition along a climatic gradient in the temperate steppe of northern China. *Ann. Bot.* 120, 755–764. doi: 10.1093/aob/mcx072

Qian, J., Wang, Z., Klimešová, J., Lü, X., and Zhang, C. (2021). Belowground bud bank and its relationship with aboveground vegetation under watering and nitrogen addition in temperate semiarid steppe. *Ecol. Indic.* 125, 107520. doi: 10.1016/j.ecolind.2021.107520

Qian, J., Zhang, Z., Dong, Y., Ma, Q., Yu, Q., Zhu, J., et al. (2023). Responses of bud banks and shoot density to experimental drought along an aridity gradient in temperate grasslands. *Funct. Ecol.* 37, 1211–1220. doi: 10.1111/1365-2435.14301

Reddy, K. R., Clark, M. W., Delaune, R. D., and Kongchum, M. (2013). Physicochemical characterization of wetland soils. *Methods Biogeochemistry Wetlands* 10, 41–54. doi: 10.2136/sssabookser10.c3

Ren, G., Du, Y., Yang, B., Wang, J., Cui, M., Dai, Z., et al. (2023). Influence of precipitation dynamics on plant invasions: response of alligator weed (Alternanthera philoxeroides) and co-occurring native species to varying water availability across plant communities. *Biol. Invasions* 25, 519–532. doi: 10.1007/s10530-022-02931-2

Ren, G.-Q., Li, Q., Li, Y., Li, J., Opoku Adomako, M., Dai, Z.-C., et al. (2019). The enhancement of root biomass increases the competitiveness of an invasive plant against a co-occurring native plant under elevated nitrogen deposition. *Flora* 261, 151486. doi: 10.1016/j.flora.2019.151486

Semchenko, M., Zobel, K., Heinemeyer, A., and Hutchings, M. J. (2008). Foraging for space and avoidance of physical obstructions by plant roots: a comparative study of grasses from contrasting habitats. *New Phytol.* 179, 1162–1170. doi: 10.1111/j.1469-8137.2008.02543.x

Semenchuk, P., Plutzar, C., Kastner, T., Matej, S., Bidoglio, G., Erb, K.-H., et al. (2022). Relative effects of land conversion and land-use intensity on terrestrial vertebrate diversity. *Nat. Commun.* 13, 615. doi: 10.1038/s41467-022-28245-4

Simkin, R. D., Seto, K. C., Mcdonald, R. I., and Jetz, W. (2022). Biodiversity impacts and conservation implications of urban land expansion projected to 2050. *Proc. Natl. Acad. Sci.* 119, e2117297119. doi: 10.1073/pnas.2117297119

Świerszcz, S., Nobis, M., Maślak, M., Smieja, A., Kojs, P., Nowak, S., et al. (2019). Varied response of underground and aboveground plant matter: functional diversity of three different vegetational types after translocation to reclaimed postindustrial land. *Land Degradation Dev.* 30, 2287–2297. doi: 10.1002/ldr.3419

Vanderweide, B. L., and Hartnett, D. C. (2015). Belowground bud bank response to grazing under severe, short-term drought. *Oecologia* 178, 795–806. doi: 10.1007/s00442-015-3249-y

Vesk, P. A., and Westoby, M. (2004). Funding the bud bank: a review of the costs of buds. *Oikos* 106, 200–208. doi: 10.1111/j.0030-1299.2004.13204.x

Williams, A. S., Kiniry, J. R., Mushet, D., Smith, L. M., Mcmurry, S., Attebury, K., et al. (2017). Model parameters for representative wetland plant functional groups. *Ecosphere* 8, e01958. doi: 10.1002/ecs2.1958

Winkler, K., Fuchs, R., Rounsevell, M., and Herold, M. (2021). Global land use changes are four times greater than previously estimated. *Nat. Commun.* 12, 2501. doi: 10.1038/s41467-021-22702-2

Wu, J., Chen, X., Xu, L., Qian, J., and Liu, Z. (2021). The spatial pattern of the belowground bud bank and its responses to soil water status in the interdune lowlands of active sand dunes of Inner Mongolia, China. *Restor. Ecol.* 29, e13223. doi: 10.1111/rec.13223

Wu, J., Wang, Y., Ma, Q., and Liu, Z. (2020). Roles of aboveground vegetation, soil properties, and disturbance in determining belowground bud bank in sand dune ecosystems. *Environ. Exp. Bot.* 178, 104155. doi: 10.1016/j.envexpbot.2020.104155

Wu, J., and Yu, F.-H. (2022). Belowground bud bank of invasive plants contributes to their successful invasion in coastal wetlands. *Restor. Ecol.* 31, e13821. doi: 10.1111/rec.13821

Xiao, L., Li, C., Cai, Y., Zhou, T., Zhou, M., Gao, X., et al. (2021). Interactions between soil properties and the rhizome-root distribution in a 12-year Moso bamboo reforested region: Combining ground-penetrating radar and soil coring in the field. *Sci. Total Environ.* 800, 149467. doi: 10.1016/j.scitotenv.2021.149467

Xie, H. H., Wu, Q. G., Hu, J. Y., Yu, L. F., Bie, P. F., Wang, H., et al. (2018). Changes in soil physical and chemical properties during the process of alpine meadow degradation along the Eastern Qinghai-Tibet Plateau. *Eurasian Soil Sci.* 51, 1440–1446. doi: 10.1134/S1064229318130045

Xu, C., Ke, Y., Zhou, W., Luo, W., Ma, W., Song, L., et al. (2021). Resistance and resilience of a semi-arid grassland to multi-year extreme drought. *Ecol. Indic.* 131, 108139. doi: 10.1016/j.ecolind.2021.108139

Zhang, Q.-P., Fang, R.-Y., Deng, C.-Y., Zhao, H.-J., Shen, M.-H., and Wang, Q. (2022). Slope aspect effects on plant community characteristics and soil properties of alpine meadows on Eastern Qinghai-Tibetan plateau. *Ecol. Indic.* 143, 109400. doi: 10.1016/j.ecolind.2022.109400

Zhang, J.-T., Mu, C.-S., Wang, D.-L., Wang, J.-F., and Chen, G.-X. (2009). Shoot population recruitment from a bud bank over two seasons of undisturbed growth of Leymus chinensis. *Botany* 87, 1242–1249. doi: 10.1139/B09-080

Zhang, W., Yi, S., Qin, Y., Sun, Y., Shangguan, D. , Meng, B., et al. (2020). Effects of patchiness on surface soil moisture of alpine meadow on the Northeastern Qinghai-Tibetan Plateau: Implications for grassland restoration. *Remote Sens.* 12, 4121. doi: 10.3390/rs12244121

Zhang, D. M., Zhao, W. Z., and Luo, W. C. (2019). Effect of the population density on belowground bud bank of a rhizomatous clonal plant Leymus secalinus in Mu Us sandy land. *J. Plant Res.* 132, 69–80. doi: 10.1007/s10265-018-01080-9

Zhao, G., Dong, J., Yang, J., Wang, H., Dai, J., Zhou, Y., et al. (2023). Cropland expansion delays vegetation spring phenology according to satellite and *in-situ* observations. *Agriculture Ecosyst. Environ.* 356, 108651. doi: 10.1016/j.agee.2023.108651

Zuo, X., Zhao, X., Zhao, H., Zhang, T., Guo, Y., Li, Y., et al. (2009). Spatial heterogeneity of soil properties and vegetation–soil relationships following vegetation restoration of mobile dunes in Horqin Sandy Land, Northern China. *Plant Soil* 318, 153–167. doi: 10.1007/s11104-008-9826-7

frontiers | Frontiers in Plant Science

TYPE Original Research
PUBLISHED 10 January 2024
DOI 10.3389/fpls.2023.1324460

OPEN ACCESS

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RECEIVED 19 October 2023
ACCEPTED 21 December 2023
PUBLISHED 10 January 2024

CITATION
Duan S-J, Sun G-J, Dan Y, Deng J, Yu D-W, Wei Q, Chen C-F, Jiang J, Wang X-M, Ren T-J, Liao Y-M and Chen J-S (2024) Timing of systemic resistance induced by local exogenous ABA application within clonal network of stoloniferous herb *Centella asiatica* subjected to low water availability. *Front. Plant Sci.* 14:1324460. doi: 10.3389/fpls.2023.1324460

Timing of systemic resistance induced by local exogenous ABA application within clonal network of stoloniferous herb *Centella asiatica* subjected to low water availability

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Resistance traits of plants can be activated both at the damaged site and undamaged parts. Systemic resistance induced by local exogenous abscisic acid (ABA) application alleviated negative effect of low water availability on growth performance of clonal plant. However, timing of systemic resistance was poorly understood. Timing of systemic resistance refers to its activation and decay time within clonal network. Clonal fragment of *Centella asiatica* with four successive ramets (including first-oldest, second-older, third-old and fourth-young ramets) subjected to low water availability (20% soil moisture content) was used to explore effects of local exogenous ABA application on the timing of resistance activation and decay. Systemic resistance activated by local exogenous ABA application after 4 days remained at least 28 days. Compared with control, biomass accumulation of whole clonal fragment, root biomass and ratio of belowground to aboveground biomass significantly increased by local exogenous ABA application after 28 days. It is suggested that rapid activation and delay of resistance response induced by local exogenous ABA application within clonal network may improve fitness of clonal plant subjected to abiotic stress.

KEYWORDS

clonal integration, resistance activation, resistance delay, chlorophyll fluorescence, photosynthetic parameters

1 Introduction

Non-resource substances (such as defense or stress signal) can be transmitted or shared between interconnected ramets of clonal plant as well as resource substances (Stuefer et al., 2005; Jelinkova et al., 2012; Liu et al., 2015). With the increase of foliar tannin content, growth performance of interconnected young ramets was improved by local herbivory on old ramets of stoloniferous herb *Trifolium repens* (Gomez et al., 2008). Similarly, damage of caterpillar *Gynaephora rnenyuanensis* herbivory on young ramets of rhizomatous sedge *Carex alrofusca* was significantly alleviated by local application of jasmonic-acid to interconnected old ramets (Chen et al., 2011). Systemic defense or resistance within clonal networks induced by transportation or sharing of non-resource substances (such as defense or stress signal) may be very important for improving fitness of clonal plant subjected to biotic or abiotic stress (Gomez et al., 2007; Koubek and Herben, 2007; Sharifi and Ryu, 2021).

Systemic defense of soybean (*Glycine max*) induced by Mexican bean beetle (*Epilachnavarivestis*) herbivory after damage by 3 days gradually decayed by 15 days after damage (Underwood, 1998). With enhanced expression of defense-related genes, phytohormone concentration of leaf tissue (such as jasmonic acid and linolenic acid) significantly increased when leaf of hybrid poplar saplings was exposed to volatile compounds (*cis*-3-hexenyl acetate) for 72-96 hours (Frost et al., 2008). Foliar palatability of stoloniferous herb *T. repens* decreased local herbivory attack of *Mamestra brassicae* larvae after damage by 38-51 hours, which lasted for 28 days at least among interconnected undamaged ramets (Gomez et al., 2010). Therefore, rapid activation and delay of systemic defense induced by local herbivory within clonal network may improve fitness of clonal plant subjected to abiotic stress.

Exposure to volatile organic compounds (bacterial volatile blends from *Bacillus subtilis* GB03 and *Bacillus amyloliquefaciens* IN937a) from rhizobacteria for as little as 4 days was sufficient to activate induced systemic resistance in *Arabidopsis* seedlings (Ryu et al., 2004). Melatonin application improved the activity of antioxidant enzymes [APX (ascorbate peroxidase), CAT (catalase), DHAR (dehydroascorbate reductase), GST (glutathione S-transferase), GR (glutathione reductase), MDHAR (monodehydroascorbate reductase), POD (peroxidase), and SOD (superoxide dismutase)] and their relative genes expression when tomato seedlings were subjected to drought stress (Altaf et al., 2022). With systemic resistance activation, oxidative stress ($O_2^{\bullet-}$ production rate and MDA content) in the leaf of the old, mature and young ramets was significantly alleviated by exogenous ABA application to the oldest ramets of stoloniferous herb *C. asiatica* subjected to low water availability (Wei et al., 2019). However, timing of systemic resistance (activation and decay time) induced among interconnected ramets was poorly understood.

Production of highly oxidizing ROS immediately affected photosynthesis when the plant was subjected to biotic or abiotic stress (Singh and Thakur, 2018; Qamer et al., 2021; Sachdev et al., 2021). Activation of MPK3/MPK6 can rapidly alter the expression of photosynthesis-related genes and inhibit photosynthesis when *Arabidopsis thaliana* was subjected to *Pseudomonas syringae* infection (Su et al., 2018). Young leaves of *A. thaliana* acclimate better to the onset of water deficit by dissipating the excess excitation energy by NPQ (Sperdouli and Moustakas, 2011). Therefore, plant subject to biotic or abiotic stress can also be evaluated by measuring photosynthetic efficiency such as maximum quantum yield of PSII (F_v/F_m), effective PSII quantum yield (ΦPSII), photochemical quenching (qP) and non-photochemical quenching (NPQ)(Corcuera et al., 2011; Lucas et al., 2014; Chen et al., 2016; He et al., 2018).

The phytohormone abscisic acid (ABA) is a key endogenous messenger in plants' responses to biotic and abiotic stresses such as various pathogens, heat, drought and high salinity (Yoshida et al., 2010; Osakabe et al., 2014; Lievens et al., 2017; Hu et al., 2018). It is rapid accumulation in response to stresses and mediation of many stress responses that help plant survival over the stresses (Sreenivasulu et al., 2012). Abscisic acid (ABA) as a stress hormone in plant responses to water shortage were well documented (Zhang et al., 2006; Zou et al., 2010; Yoshida et al., 2019). A greenhouse experiment with local application of exogenous ABA was conducted to investigate the timing of systematic resistance within clonal networks (Figure 1). We focused on (1) activation time of systemic resistance by local exogenous ABA application within clonal network of *C. asiatica*; (2) delay time of systemic resistance within clonal network after local exogenous ABA application. This research will help us to realize the mechanism for growth and fitness of clonal plant subjected to environmental stress.

2 Materials and methods

2.1 Plant material

As a perennial stoloniferous herb, *C. asiatica* was widely distributed in woodlands, forests edge, damp grass and roadsides

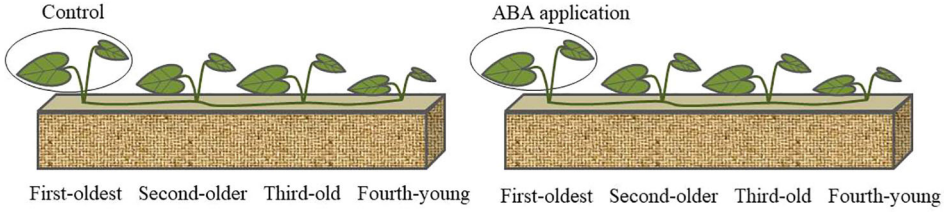

FIGURE 1
Schematic representation of the experimental design. 5 mL ABA solution (0.1mM) was applied to the first-oldest ramets; Same volume distilled water was employed as control.

or creeks. It usually takes root on each node of stolon when in contact with a moist substratum, forming a sympodial network above the ground (Li et al., 2018).

Clonal fragments of *C. asiatica* were collected from a forest edge, located in Chengdu, Sichuan province, China (30°05′~31°26′ N; 102°54′~104°53′E). Each clonal fragment comprises four rooted ramets with different age (first-oldest, second-older, third-old and fourth-young ramets).

2.2 Experimental design

The container (dimensions: 10 cm × 8.5 cm × 15 cm) separated into 4 equal pots was used for the experiment. On 18 October 2021, four successive ramets of each clonal fragment were planted into the pots respectively. The pots were filled with substrate in a 3:1 mixture of humus soil and sand. 0.2 g Peters Professional (20% N, 20% P, 20% K; The Scotts Company, LLC., Marysville, OH, USA) was added to each pot at the beginning of experiment. The volumetric soil moisture content of each pot was maintained at 20% (volume of water present/the total volume). In the everyday morning (9:00-11:00 h), all pots were measured with a portable soil moisture meter (TDR-300, Spectrum, USA) and watered to maintain corresponding soil moisture. During the experiment, the mean temperature was 28.5 ± 1.4°C, and light intensity was equivalent to approx. 90% of full daylight outside the greenhouse (minimum and maximum photosynthetic photon flux density in the greenhouse was 136.2 and 325.1 μmol m^{-2} s^{-1} respectively).

In the experiment, 5 ml of 0.1 mM ABA solution was applied to fully unfolded leaves of the first-oldest ramets and the same volume of distilled water was used as control. ABA dosage was based on a previous study (Wei et al., 2019). Neighboring sibling ramets were shielded from spray with a piece of plastic. Then, the first-oldest ramets were sealed in a transparent plastic bag until dry. The chlorophyll fluorescence parameters and photosynthetic parameters were measured at 1, 4, 7 and14 days after ABA application. The experiment lasted for 28 days. There were seven replicates for per treatment.

2.3 Measurement of chlorophyll fluorescence parameters

Chlorophyll fluorescence measurements were carried out using a portable, modulated fluorescence monitoring system (FMS-2, Hansatech Instruments Ltd., UK) on fully expanded leaves. The minimum fluorescence (F_0) was determined using a measuring beam of 0.2 μmol m^{-2} s^{-1} intensity after 30 min of dark adaptation. Following a dark-adapted state, a saturation pulse (1 s white light of 7,500 μmol m^{-2} s^{-1} intensity) was used to obtain the maximum fluorescence (F_m). Light-induced changes in chlorophyll fluorescence following actinic illumination (300 μmol m^{-2} s^{-1}) were recorded prior to the measurement of F'_o (minimum fluorescence in light-saturated state), F'_m (maximum fluorescence in light-saturated state) and F_s (steady-state fluorescence in the light-saturated state). The maximum quantum yield of PSII (F_v/F_m), the effective PSII quantum yield (ΦPSII), the photochemical quenching (qP) and non-photochemical quenching (NPQ) were calculated using $(F_m-F_0)/F_m$, $(F'_m-F_s)/F'_m$ (Genty et al., 1989), $(F'_m-F_s)/(F'_m-F'_0)$ and $(F_m-F'_m)/F'_m$ respectively (Turan and Ekmekçi, 2010).

2.4 Measurement of photosynthetic parameters

Photosynthetic parameters were made by a portable photosynthesis system GFS-3000 (Heinz Walz GmbH, Effeltrich, Germany). The measurement was conducted on the fully expanded mature leaves at a temperature of 25°C, photosynthetic photon flux density of 400 μmol·m^{-2}·s^{-1} and CO_2 concentration of 400 μmol·mol^{-1}. Net photosynthetic rate (P_n) and stomatal conductance (G_s) were recorded when gas exchange had equilibrated (taken to be when the coefficient of variation for external CO_2 partial pressure between the sample and reference analysis was below 0.3%).

2.5 Measurement of biomass characteristics of whole clonal fragment

Clonal fragments were separated into root, leaf and stolon and oven-dried to constant weight at 70°C for 72 h. Leaf and stolon biomass, root biomass, and biomass accumulation of whole clonal fragment were determined. Ratio of belowground to aboveground biomass was counted in whole clonal fragments (He et al., 2021).

2.6 Statistical analysis

The chlorophyll fluorescence parameters and photosynthetic parameters were analyzed by two-way repeated-measures (ANOVA). Two-way analysis of variance (ANOVA) was employed to investigate the leaf and stolon biomass, root biomass, ratio of belowground to aboveground biomass and biomass accumulation of whole clonal fragment. All analyses were conducted with SPSS 24.0 software (SPSS Inc.).

3 Result

3.1 Chlorophyll fluorescence parameters

Compared with control, NPQ of four interconnected ramets was decreased by local exogenous ABA application after 1 day (Table 1, Figure 2). Opposite pattern was observed in ΦPSII, F_v/F_m and qP (Table 1, Figure 2). After 4 days, significant difference was not observed between ΦPSII, F_v/F_m, qP and NPQ of four interconnected ramets subjected to local exogenous ABA application and those of control (Table 1, Figure 2). After 7 and 14 days, ΦPSII, F_v/F_m, and qP of four interconnected ramets subjected to local exogenous ABA application were significantly greater than those of control as well as significant decrease of NPQ (Table 1, Figure 2). From 7 to 14 days, significant effects of local exogenous ABA application on ΦPSII of four interconnected ramets

were detected. However, significant effects of local exogenous ABA application on F_v/F_m, qP and NPQ of four interconnected ramets were not detected (Table 1, Figure 2).

3.2 Photosynthetic parameters

Compared with control, P_n and G_s of four interconnected ramets were significantly decreased by local exogenous ABA application after 1 day (Table 1, Figure 3). After 4 days, significant difference was not observed between P_n and G_s of four interconnected ramets subjected to local exogenous ABA application and those of control (Table 1, Figure 3). After 7and 14 days, P_n and G_s of four interconnected ramets subjected to local exogenous ABA application were significantly greater than those of control (Table 1, Figure 3). G_s of fourth-young ramets was significantly greater than second-older ramets by local exogenous ABA applications after 14 days (Table 1, Figure 3). Meanwhile, P_n of fourth-young ramets was significantly greater than those of the first-oldest and third-old ramets (Table 1, Figure 3). From 7 to 14 days, significant effects of local exogenous ABA application on P_n and G_s of four interconnected ramets were detected (Table 1, Figure 3).

TABLE 1 Results of two-way repeated-measures analysis of variance, with 'exogenous ABA application' and 'ramet age' as between-subject effects for differences in chlorophyll fluorescence parameters and photosynthetic parameters among interconnected ramets.

Effects	df	ΦPSII		qP		NPQ		F_v/F_m		Stomatal conductance		Photosynthesis	
		F	*P*	F	*P*	F	*P*	F	*P*	F	*P*	F	*P*
Between-subject effects													
Exogenous ABA application (A)	1	738.284	**0.001**	171.218	**0.001**	208.048	**0.001**	171.218	**0.001**	342.657	**0.001**	398.312	**0.001**
Ramet age (R)	3	4.11	**0.017**	3.479	**0.032**	0.644	0.582	3.479	**0.032**	1.596	0.23	3.455	**0.042**
A × R	3	4.964	**0.008**	0.667	0.58	2.726	*0.066*	0.667	0.58	2.273	0.119	2.622	*0.068*
Within subject effects													
Time (T)	3	9.438	**0.001**	12.033	**0.001**	21.315	**0.001**	12.033	**0.001**	116.92	**0.001**	317.862	**0.001**
T × A	3	542.905	**0.001**	37.357	**0.001**	204.263	**0.001**	37.357	**0.001**	228.454	**0.001**	612.769	**0.001**
T × R	9	2.391	**0.02**	2.807	*0.07*	2.55	**0.013**	2.807	**0.007**	0.905	*0.0529*	3.77	**0.001**
T × A × R	9	1.446	0.185	3.73	**0.001**	2.634	**0.011**	3.73	**0.001**	1.198	**0.0318**	2.534	**0.018**

Values are in bold when $P < 0.05$, and in italic when $0.05 < P < 0.1$.

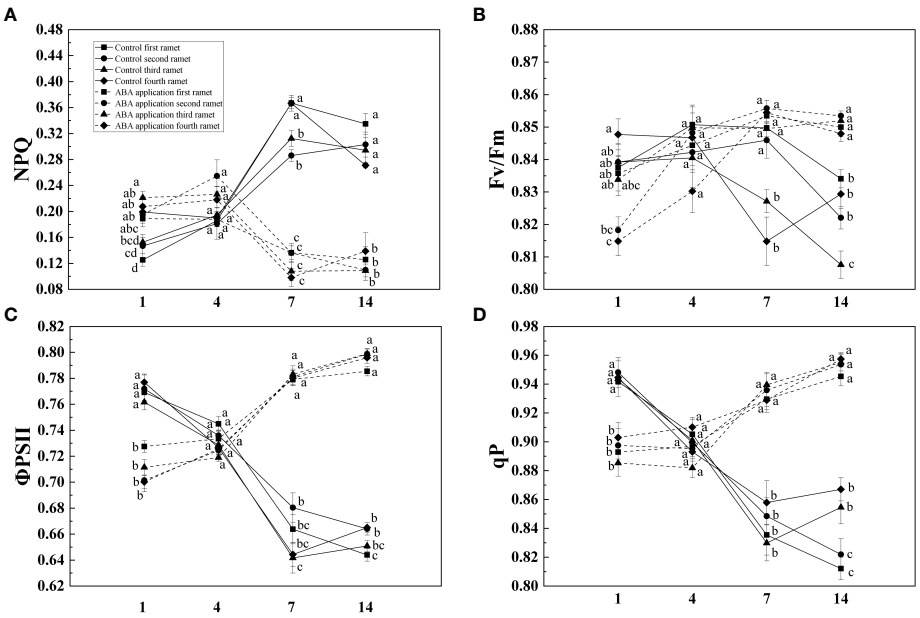

FIGURE 2
NPQ **(A)**, F_v/F_m **(B)**, ΦPSII **(C)** and qP **(D)** of interconnected ramets (including first-oldest, second-older, third-old and fourth-young ramet) 1, 4, 7 and 14 days after local exogenous ABA application. Same letters (at the same day) mean that they are not significantly different ($P > 0.05$).

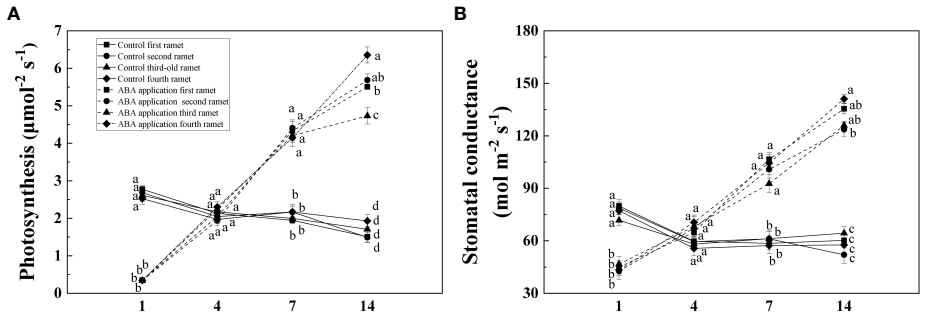

FIGURE 3
Net photosynthetic rate **(A)** and stomatal conductance **(B)** of interconnected ramets (including first-oldest, second-older, third-old and fourth-young ramet) 1, 4, 7 and 14 days after local exogenous ABA application. Same letters (at the same day) mean that they are not significantly different ($P > 0.05$).

3.3 Biomass accumulation

Root biomass, ratio of belowground to aboveground biomass and biomass accumulation of whole clonal fragments were significantly increased by local exogenous ABA application after 28 days (Table 2, Figures 4B, 5A, B). However, similar patterns were not observed in leaf and stolon biomass (Table 2, Figure 4A).

4 Discussion

Stomatal closure resulting from exogenous ABA application reduced water loss of wheat (Travaglia et al., 2010). In this study, with the stomatal closure, foliar net photosynthetic rate of four interconnected ramets significantly decreased by local exogenous ABA application after 1 day. By altering the kinetics of de-epoxidation of the xanthophyll cycle, exogenous ABA application incurred increase of NPQ in cabbage (*Brassica campestris*) and rice (*Oryza sativa L*) (Zhu et al., 2011). With its inhibition on photochemical activity, increase of NPQ implied that more light energy was used for heat dissipation to avoid damage to photosystem II of four interconnected ramets (Wilson and Ruban, 2020). Meanwhile, photoinhibition (decrease of F_v/F_m, ΦPSII and qP) was induced by abscisic acid (ABA) application after 1 day when clonal fragments of *C. asiatica* subjected to low water availability. Similar patterns were observed in the study that exogenous ABA application resulted in decrease of F_v/F_m, ΦPSII and qP of maize subjected high light intensity (1500 μmol m $^{-2}$ s $^{-1}$) (Jia and Lu, 2003).

ΦPSII and qP of sugarcane subjected to drought treatment were increased by the exogenous ABA application after 3 days and remained at least 7 days (Srivastava et al., 2009). Selenium (Se) application can alleviate oxidative stress in the chloroplasts to increase F_v/F_m when potato (*Solanum tuberosum* L.) was subjected to photooxidative stress (Turakainen et al., 2008). In this study, chlorophyll fluorescence and photosynthesis of four interconnected ramets were restored by local exogenous ABA application after 4 days. Altogether, the recovery of chlorophyll fluorescence and photosynthesis capacity are interpreted as activation of systemic resistance. With the exogenous ABA application, root growth was improved when *Arabidopsis* seedlings was subjected to low water availability (Miao et al., 2021). Exogenous ABA application significantly increased root/shoot ratio of *Malus sieversii* and *Malus hupehensis* seedlings subjected to low water availability. Similar pattern was observed in our experiment (Ma et al., 2008). Biomass accumulation significantly increased by local exogenous ABA application when wheat was subjected to low water availability (Kaur and Asthir, 2020). The positive effects on growth performance of whole clonal fragments were observed by local exogenous ABA application after 28 days. We tentatively suggested that defense induction persisted for at least 28 days.

Systemic resistance may give priority to protection of youngest tissues (Chen et al., 2011). Young ramets were the most valuable

TABLE 2 Two-way analysis of variance (ANOVA) for effects of ABA application, ramet age and their interaction on leaf and stolon biomass, root biomass, biomass accumulation of whole clonal fragments and ratio of belowground to aboveground biomass.

Effects	df	leaf and stolon biomass		root biomass		biomass accumulation of whole clonal fragments		ratio of belowground to aboveground biomass	
		F	*P*	F	*P*	F	*P*	F	*P*
Exogenous ABA application (A)	1	1.829	0.189	38.731	**0.000**	11.914	**0.002**	53.017	**0.000**
Ramet age (R)	3	1.461	0.250	1.205	0.329	0.206	0.891	8.628	**0.000**
A×R	3	0.623	0.607	1.710	0.192	1.117	0.362	0.792	0.511

Values are in bold when $P < 0.05$, and in italic when $0.05 < P < 0.1$.

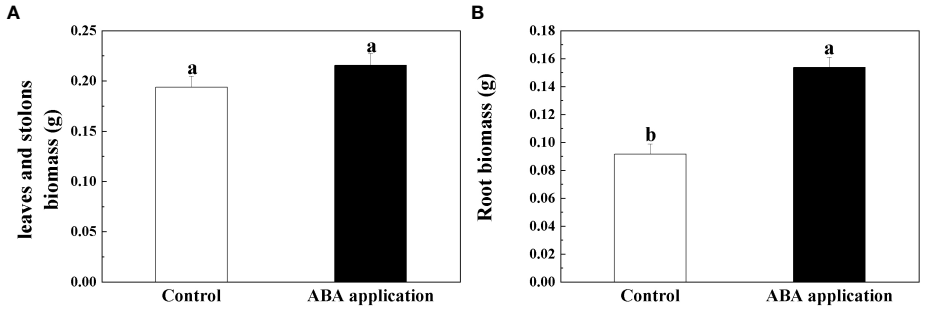

FIGURE 4
Leaf and stolon biomass **(A)** and Root biomass **(B)** of interconnected ramets after 28 days; Open bars, control; closed bars, exogenous ABA application. Error bars indicate ± s.e. for 10 replicates. Bars with the same lower case letters are not significantly different ($P > 0.05$).

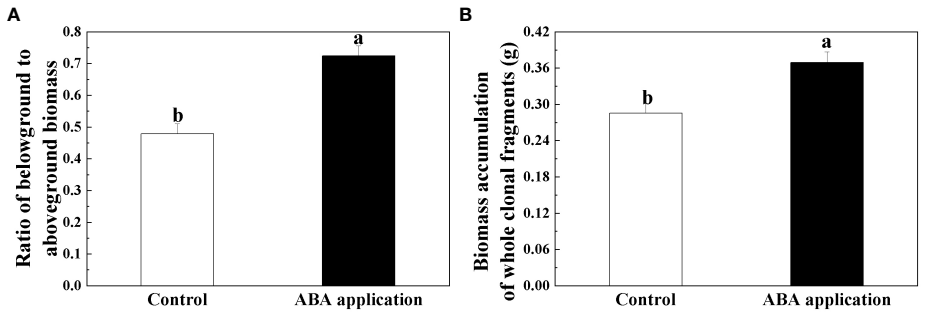

FIGURE 5
Ratio of belowground to aboveground biomass **(A)** and Biomass accumulation of whole clonal fragments **(B)** after 28 days; Open bars, control; closed bars, exogenous ABA application. Error bars indicate ± s.e. for 10 replicates. Bars with the same lower case letters are not significantly different ($P > 0.05$).

tissues for growth and fitness within clonal networks (Stuefer et al., 2005; Gomez and Stuefer, 2006). The optimal defense theory predicts that plant tissues with a high contribution to fitness should be better protected than other plant tissues (Hunziker et al., 2021). In the experiment, our study was consistent with previous study that compared with the old and mature ramets, foliar antioxidant capacity of young ramets was significantly higher and oxidative stress was significantly lower when exogenous ABA application to the oldest ramets (Wei et al., 2019). It is suggested that the protection of young ramets may confer clonal plants with considerable benefits in adapting to environmental stress.

Our study implies that rapid activation and delay of resistance response induced by local exogenous ABA application within clonal network may improve fitness of clonal plant subjected to abiotic stress. Benefit of systemic resistance will depend on the absence or presence of subsequent environmental stress (van Hulten et al., 2006). In the future, more studies are needed to understand the generality and ecological advantages afforded by systematic resistance within clonal network.

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

S-JD: Writing – original draft, Data curation, Investigation, Methodology, Conceptualization, Formal Analysis, Software, Validation, Visualization. G-JS: Data curation, Methodology, Investigation, Formal Analysis, Writing – original draft. YD: Investigation, Methodology, Data curation, Writing – original draft. JD: Methodology, Data curation, Investigation, Writing – original draft. D-WY: Methodology, Data curation, Writing – original draft. QW: Methodology, Data curation, Writing – original draft. C-FC: Investigation, Writing – original draft. JJ: Investigation, Writing – original draft. X-MW: Investigation, Writing – original draft. T-JR: Investigation, Writing – original draft. Y-ML: Writing – review & editing, Supervision, Validation. J-SC: Writing – review & editing, Supervision, Validation.

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 Sichuan Natural Science Foundation (2023NSFSC0202) and Foundation of Key Laboratory of Southwest China Wildlife Resources Conservation (Ministry of Education), China West Normal University (XNYB19-03).

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.

The reviewer ZY declared a shared affiliation with the author(s) GS and YL to the handling editor at the time of review.

Publisher's note

References

Altaf, M. A., Shahid, R., Ren, M. X., Naz, S., Altaf, M. M., Khan, L. U., et al. (2022). Melatonin improves drought stress tolerance of tomato by modulating plant growth, root architecture, photosynthesis, and antioxidant defense system. *Antioxidants* 11 (2), 309. doi: 10.3390/antiox11020309

Chen, J. S., Lei, N. F., and Liu, Q. (2011). Defense signaling among interconnected ramets of a rhizomatous clonal plant, induced by jasmonic-acid application. *Acta Oecologica* 37 (4), 355–360. doi: 10.1016/j.actao.2011.04.002

Chen, Y. E., Cui, J. M., Li, G. X., Yuan, M., Zhang, Z. W., Yuan, S., et al. (2016). Effect of salicylic acid on the antioxidant system and photosystem II in wheat seedlings. *Biol. Plantrum* 60 (1), 139–147. doi: 10.1007/s10535-015-0564-4

Corcuera, L., Gil-Pelegrin, E., and Notivol, E. (2011). Intraspecific variation in *Pinus Pinaster* PSII photochemical efficiency in response to winter stress and freezing temperatures. *PloS One* 6 (12), e28772. doi: 10.1371/journal.pone.0028772

Frost, C. J., Mescher, M. C., Dervinis, C., Davis, J. M., Carlson, J. E., and De Moraes, C. M. (2008). Priming defense genes and metabolites in hybrid poplar by the green leaf volatile cis-3-hexenyl acetate. *New Phytol.* 180 (3), 722–734. doi: 10.1111/j.1469-8137.2008.02599.x

Genty, B., Briantais, J. M., and Baker, N. R. (1989). The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll flourescence. *Biochimica et Biophysica Acta(BBA)-General Subjects.* 990 (1), 87–92. doi: 10.1015/s0304-4165(89)80016-9

Gomez, S., Latzel, V., Verhulst, Y. M., and Stuefer, J. F. (2007). Costs and benefits of induced resistance in a clonal plant network. *Oecologia* 153 (4), 921–930. doi: 10.1007/s00442-007-0792-1

Gomez, S., Onoda, Y., Ossipov, V., and Stuefer, J. F. (2008). Systemic induced resistance: a risk-spreading strategy in clonal plant networks? *New Phytol.* 179 (4), 1142–1153. doi: 10.1111/j.1469-8137.2008.02542.x

Gomez, S., and Stuefer, J. F. (2006). Members only: induced systemic resistance to herbivory in a clonal plant network. *Oecologia* 147 (3), 461–468. doi: 10.1007/s00442-005-0293-z

Gomez, S., Van Dijk, W., and Stuefer, J. F. (2010). Timing of induced resistance in a clonal plant network. *Plant Biol.* 12 (3), 512–517. doi: 10.1111/j.1438-8677.2009.00234.x

He, L. X., Xiao, X., Zhang, X. M., Jin, Y., Pu, Z. H., Lei, N. F., et al. (2021). Clonal fragments of stoloniferous invasive plants benefit more from stolon storage than their congeneric native species. *Flora* 281, 151877. doi: 10.1016/j.flora.2021.151877

He, L., Yu, L., Li, B., Du, N., and Guo, S. (2018). The effect of exogenous calcium on cucumber fruit quality, photosynthesis, chlorophyll fluorescence, and fast chlorophyll fluorescence during the fruiting period under hypoxic stress. *BMC Plant Biol.* 18 (1), 180. doi: 10.1186/s12870-018-1393-3

Hu, X. J., Chen, D., Lynne Mclntyre, C., Fernanda Dreccer, M., Zhang, Z. B., Drenth, J., et al. (2018). Heat shock factor C2a serves as a proactive mechanism for heat protection in developing grains in wheat *via* an ABA-mediated regulatory pathway. *Plant Cell Environ.* 41 (1), 79–98. doi: 10.1111/pce.12957

Hunziker, P., Lambertz, S. K., Weber, K., Crocoll, C., Halkier, B. A., and Schulz, A. (2021). Herbivore feeding preference corroborates optimal defense theory for specialized metabolites within plants. *Proc. Natl. Acad. Sci. United States America* 118 (47), 6. doi: 10.1073/pnas.2111977118

Jelinkova, H., Tremblay, F., and Desrochers, A. (2012). Herbivore-simulated induction of defenses in clonal networks of trembling aspen (*Populus tremuloides*). *Tree Physiol.* 32 (11), 1348–1356. doi: 10.1093/treephys/tps094

Jia, H., and Lu, C. (2003). Effects of abscisic acid on photoinhibition in maize plants. *Plant Sci.* 165 (6), 1403–1410. doi: 10.1016/j.plantsci.2003.08.004

Kaur, G., and Asthir, B. (2020). Impact of exogenously applied ABA on proline metabolism conferring drought and salinity stress tolerance in wheat genotypes. *Cereal Res. Commun.* 48 (3), 309–315. doi: 10.1007/s42976-020-00041-0

Koubek, T., and Herben, T. (2007). Effect of systemic diseases on clonal integration: modelling approach. *Evolutionary Ecol.* 22 (3), 449–460. doi: 10.1007/s10682-007-9219-z

Li, K. N., Chen, J. S., Wei, Q., Li, Q., and Lei, N. F. (2018). Effects of transgenerational plasticity on morphological and physiological properties of stoloniferous herb *centella asiatica* subjected to high/low light. *Front. Plant Sci.* 9. doi: 10.3389/fpls.2018.01640

Lievens, L., Pollier, J., Goossens, A., Beyaert, R., and Staal, J. (2017). Abscisic acid as pathogen effector and immune regulator. *Front. Plant Sci.* 8. doi: 10.3389/fpls.2017.00587

Liu, X., Li, Q., Yue, M., Zhang, X., Zhang, R., Zhang, B., et al. (2015). Nitric oxide is involved in integration of UV-B absorbing compounds among parts of clonal plants under a heterogeneous UV-B environment. *Physiologia Plantarum* 155 (2), 180–191. doi: 10.1111/ppl.12313

Lucas, J. A., Garcia-Cristobal, J., Bonilla, A., Ramos, B., and Gutierrez-Manero, J. (2014). Beneficial rhizobacteria from rice rhizosphere confers high protection against biotic and abiotic stress inducing systemic resistance in rice seedlings. *Plant Physiol. Biochem.* 82, 44–53. doi: 10.1016/j.plaphy.2014.05.007

Ma, X., Ma, F., Mi, Y., Ma, Y., and Shu, H. (2008). Morphological and physiological responses of two contrasting Malus species to exogenous abscisic acid application. *Plant Growth Regul.* 56 (1), 77–87. doi: 10.1007/s10725-008-9287-2

Miao, R., Yuan, W., Wang, Y., Garcia-Maquilon, I., Dang, X., Li, Y., et al. (2021). Low ABA concentration promotes root growth and hydrotropism through relief of ABA INSENSITIVE 1-mediated inhibition of plasma membrane H^+ -ATPase 2. *Sci. Adv.* 7 (12), 4113. doi: 10.1126/sciadv.abd4113

Osakabe, Y., Yamaguchi-Shinozaki, K., Shinozaki, K., and Tran, L. P. (2014). ABA control of plant macroelement membrane transport systems in response to water deficit and high salinity. *New Phytol.* 202 (1), 35–49. doi: 10.1111/nph.12613

Qamer, Z., Chaudhary, M. T., Du, X., Hinze, L., and Azhar, M. T. (2021). Review of oxidative stress and antioxidative defense mechanisms in *Gossypium hirsutum* L. @ in response to extreme abiotic conditions. *J. Cotton Res.* 4 (1), 1–9. doi: 10.1186/s42397-021-00086-4

Ryu, C. M., Farag, M. A., Hu, C. H., Reddy, M. S., Kloepper, J. W., and Pare, P. W. (2004). Bacterial volatiles induce systemic resistance in *Arabidopsis*. *Plant Physiol.* 134 (3), 1017–1026. doi: 10.1104/pp.103.026583

Sachdev, S., Ansari, S. A., Ansari, M. I., Fujita, M., and Hasanuzzaman, M. (2021). Abiotic stress and reactive oxygen species: generation, signaling, and defense mechanisms. *Antioxidants (Basel)* 10 (2), 277. doi: 10.3390/antiox10020277

Sharifi, R., and Ryu, C. M. (2021). Social networking in crop plants: Wired and wireless cross-plant communications. *Plant Cell Environ.* 44 (4), 1095–1110. doi: 10.1111/pce.13966

Singh, J., and Thakur, J. K. (2018). Photosynthesis and abiotic stress in plants. *Biotic Abiotic Stress Tolerance Plants* 27–46. doi: 10.1007/978-981-10-9029-5_2

Sperdouli, I., and Moustakas, M. (2011). Differential response of photosystem II photochemistry in young and mature leaves of *Arabidopsis thaliana* to the onset of drought stress. *Acta Physiologiae Plantarum* 34 (4), 1267–1276. doi: 10.1007/s11738-011-0920-8

Sreenivasulu, N., Harshavardhan, V. T., Govind, G., Seiler, C., and Kohli, A. (2012). Contrapuntal role of ABA: does it mediate stress tolerance or plant growth retardation under long-term drought stress? *Gene* 506 (2), 265–273. doi: 10.1016/j.gene.2012.06.076

Srivastava, M. K., Li, C., Nong, Q., and Li, Y. (2009). Effect of exogenous ABA application on chlorophyll fluorescence in sugarcane under water stress conditions. Guangxi Agric Sci. *Guangxi Agric. Sci.* 40 (11), 1411–1417.

Stuefer, J. F., Gómez, S., and Mölken, T. V. (2005). Clonal integration beyond resource sharing: implications for defence signalling and disease transmission in clonal plant networks. *Evolutionary Ecol.* 18 (5-6), 647–667. doi: 10.1007/s10682-004-5148-2

Su, J., Yang, L., Zhu, Q., Wu, H., He, Y., Liu, Y., et al. (2018). Active photosynthetic inhibition mediated by MPK3/MPK6 is critical to effector-triggered immunity. *PloS Biol.* 16 (5), e2004122. doi: 10.1371/journal.pbio.2004122

Travaglia, C., Reinoso, H., Cohen, A., Luna, C., Tommasino, E., Castillo, C., et al. (2010). Exogenous ABA increases yield in field-grown wheat with moderate water restriction. *J. Plant Growth Regul.* 29 (3), 366–374. doi: 10.1007/s00344-010-9147-y

Turakainen, M., Hartikainen, H., Sarjala, T., and Seppänen, M. M. (2008). Impact of selenium enrichment on seed potato tubers. *Agric. Food Sci.* 17 (3), 278–288. doi: 10.2137/145960608786118802

Turan, Ö., and Ekmekçi, Y. (2010). Activities of photosystem II and antioxidant enzymes in chickpea (*Cicer arietinum* L.) cultivars exposed to chilling temperatures. *Acta Physiologiae Plantarum* 33 (1), 67–78. doi: 10.1007/s11738-010-0517-7

Underwood, N. C. (1998). The timing of induced resistance and induced susceptibility in the soybean-Mexican bean beetle system. *Oecologia* 114, 376–381. doi: 10.1007/s004420050460

van Hulten, M., Pelser, M., Van Loon, L. C., Pieterse, C. M., and Ton, J. (2006). Costs and benefits of priming for defense in *Arabidopsis*. *Proc. Natl. Acad. Sci. United States America* 14 (103), 5602–5607. doi: 10.1073/pnas.0510213103

Wei, Q., Li, Q., Jin, Y., Wu, S. L., Fan, L. H., Lei, N. F., et al. (2019). Transportation or sharing of stress signals among interconnected ramets improves systemic resistance of clonal networks to water stress. *Funct. Plant Biol.* 46 (7), 613–623. doi: 10.1071/FP18232

Wilson, S., and Ruban, A. V. (2020). Enhanced NPQ affects long-term acclimation in the spring ephemeral Berteroa incana. *Biochim. Biophys. Acta Bioenergetics* 1861 (4), 148014. doi: 10.1016/j.bbabio.2019.03.005

Yoshida, T., Christmann, A., Yamaguchi-Shinozaki, K., Grill, E., and Fernie, A. R. (2019). Revisiting the basal role of ABA - roles outside of stress. *Trends Plant Sci.* 24 (7), 625–635. doi: 10.1016/j.tplants.2019.04.008

Yoshida, T., Fujita, Y., Sayama, H., Kidokoro, S., Maruyama, K., Mizoi, J., et al. (2010). AREB1, AREB2, and ABF3 are master transcription factors that cooperatively regulate ABRE-dependent ABA signaling involved in drought stress tolerance and require ABA for full activation. *Plant J.* 61 (4), 672–685. doi: 10.1111/j.1365-313X.2009.04092.x

Zhang, J., Jia, W., Yang, J., and Ismail, A. M. (2006). Role of ABA in integrating plant responses to drought and salt stresses. *Field Crops Res.* 97 (1), 111–119. doi: 10.1016/j.fcr.2005.08.018

Zhu, S. Q., Chen, M. W., Ji, B. H., Jiao, D. M., and Liang, J. S. (2011). Roles of xanthophylls and exogenous ABA in protection against NaCl-induced photodamage in rice (*Oryza sativa* L) and cabbage (*Brassica campestris*). *J. Exp. Bot.* 62 (13), 4617–4625. doi: 10.1093/jxb/err170

Zou, J. J., Wei, F. J., Wang, C., Wu, J. J., Ratnasekera, D., Liu, W. X., et al. (2010). *Arabidopsis* calcium-dependent protein kinase CPK10 functions in abscisic acid- and Ca^{2+}-mediated stomatal regulation in response to drought stress. *Plant Physiol.* 154 (3), 1232–1243. doi: 10.1104/pp.110.157545

frontiers | Frontiers in Plant Science

TYPE Original Research
PUBLISHED 12 March 2024
DOI 10.3389/fpls.2024.1348144

OPEN ACCESS

EDITED BY
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RECEIVED 01 December 2023
ACCEPTED 27 February 2024
PUBLISHED 12 March 2024

CITATION
Ohsowski BM, Redding C, Geddes P and Lishawa SC (2024) Field-based measurement tools to distinguish clonal *Typha* taxa and estimate biomass: a resource for conservation and restoration.
Front. Plant Sci. 15:1348144.
doi: 10.3389/fpls.2024.1348144

Field-based measurement tools to distinguish clonal *Typha* taxa and estimate biomass: a resource for conservation and restoration

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Two species of clonal *Typha* [*T. latifolia* (native) and *T. angustifolia* (exotic)] hybridize to form the highly invasive, heterotic (high vigor) *T.* × *glauca* in North American wetlands leading to increased primary production, litter accumulation, and biodiversity loss. Conservation of *T. latifolia* has become critical as invasive *Typha* has overwhelmed wetlands. In the field, *Typha* taxa identification is difficult due to subtle differences in morphology, and molecular identification is often unfeasible for managers. Furthermore, improved methods to non-destructively estimate *Typha* biomass is imperative to enhance ecological impact assessments. To address field-based *Typha* ID limitations, our study developed a predictive model from 14 *Typha* characters in 7 northern Michigan wetlands to accurately distinguish *Typha* taxa (n = 33) via linear discriminant analysis (LDA) of molecularly identified specimens. In addition, our study developed a partial least squares regression (PLS) model to predict *Typha* biomass from field collected measurements (n = 75). Results indicate that two field measurements [*Leaf Counts*, *Longest Leaf*] can accurately differentiate the three *Typha* taxa and advanced-generation hybrids. The LDA model had a 100% correct prediction rate of *T. latifolia*. The selected PLS biomass prediction model (*sqrt[Typha Dry Mass] ~ log[Ramet Area at 30 cm] + Inflorescence Presence + Total Ramet Height + sqrt [Organic Matter Depth]*) improved upon existing simple linear regression (SLR) height-to-biomass predictions. The rapid field-based *Typha* identification and biomass assessment tools presented in this study advance targeted management for regional conservation of *T. latifolia* and ecological restoration of wetlands impacted by invasive *Typha* taxa.

KEYWORDS

***Typha* identification, biomass prediction, field assessment, *Typha latifolia*, ecological restoration, *Typha* × *glauca*, conservation**

1 Introduction

Hybridization is a common and important evolutionary mechanism that drives phenotypic diversity, environmental adaptation capacity, and speciation (Mallet, 2005; Goulet et al., 2017). In some cases, plants exhibit heterosis (i.e., hybrid vigor) where hybrid offspring show increased fitness resulting in increased biomass, yield, and root density compared to parental counterparts (Hochholdinger and Baldauf, 2018). Path analysis models suggest plant taxa hybridization propensity at the genus level is significantly correlated with a perennial life cycle, woodiness, and reliance on vegetative reproduction systems (Mitchell et al., 2019). An invasive plant case study also documented that hybridization is often associated with perennial plants exhibiting clonal growth habits as a mechanism leading to fixed heterotic genotypes (Ellstrand and Schierenbeck, 2000). Thus, plant introductions exhibiting hybridization potential with closely related endemic plant populations and clonal growth habits may serve as a precursor to stimulate invasiveness (Ellstrand and Schierenbeck, 2000). In wetland systems, herbaceous wetland plants with clonal growth habits are common among the most highly invasive taxa (Galatowitsch et al., 1999; Zedler and Kercher, 2004).

In North America, two species within the clonal *Typha* (cattail) genus [native *T. latifolia*, non-native *T. angustifolia* (Ciotir et al., 2013)] have hybridized to form *T.* × *glauca* (Godron) (Smith, 1967). In wetlands, *T.* × *glauca* exhibits heterosis which typically results in more productive, taller, and faster growing clones that become more dominant compared to either parent species (Zapfe and Freeland, 2015; Bansal et al., 2019). Backcrossing and advanced-generation hybrids are also common (Travis et al., 2010; Kirk et al., 2011; Freeland et al., 2013; Geddes et al., 2021), complicating *Typha* genetic identity in the region (hereafter we will refer to all taxa as *Typha* unless otherwise specified).

The complicated genetics of *Typha* presents a problem for both the management of invasive *Typha* and the conservation of native *T. latifolia*. In the Great Lakes, *Typha* taxa classified as invasive [*T.* × *glauca* and *T. angustifolia*; hereafter, "invasive *Typha*"] are dominant in more than 13% of the total area of ecologically critical coastal wetland ecosystems (Carson et al., 2018). Along with its continued spread, management of invasive *Typha* has increasingly become a restoration priority (Bansal et al., 2019). Conservation of *T. latifolia* has simultaneously become imperative, due to increased dominance by invasive taxa, hybridization, and backcrossing of hybrids to *T. latifolia* (Pieper et al., 2017; Bansal et al., 2019; Geddes et al., 2021), which could result in extinction by demographic or genetic swamping (Todesco et al., 2016).

Unfortunately, field identification of the three taxa and advanced-generation hybrids using standard morphological characters (e.g., leaf width, gap between inflorescences) can be unreliable due to wide trait variability (Geddes et al., 2021). Molecular methods to identify *Typha* taxa may be impractical, if not entirely unfeasible, for many field practitioners managing invasive species populations and practicing conservation. Although the cost of molecular methods has been decreasing due to technological advancements, application of these techniques is still unrealistic for many practitioners (Hauser and Seeb, 2008; Sagarin et al., 2009). Thus, identifying field morphological characteristics that allow for the accurate differentiation of *Typha* is critical to advance the conservation of *T. latifolia* and the continued management of invasive *Typha*.

Invasive *Typha* taxa are associated with a range of ecological impacts to wetlands. They tend to thrive in wetlands with anthropogenically disturbed hydrology (Boers and Zedler, 2008; Hall and Zedler, 2010; Bunbury-Blanchette et al., 2015). They also outcompete native sedge species (i.e., *Carex stricta*, *C. lacustris*, *C. lasiocarpa*) in wetlands experiencing nutrient enrichment (Woo and Zedler, 2002). Furthermore, invasive *Typha* magnifies nutrient availability by increasing sediment retention (Horppila and Nurminen, 2001) and enhancing internal nutrient cycling (Currie et al., 2014), thus compounding the effects of nutrient enrichment. Invasive *Typha* forms monodominant stands in wetlands by outcompeting native plants and creating a thick layer of slowly decomposing leaf litter (Larkin et al., 2012). Further, they can more than double annual productivity in invaded wetlands (Woo and Zedler, 2002; Angeloni et al., 2006). Once established, invasive *Typha* reduces biodiversity and productivity of native plants (Tuchman et al., 2009), fishes (Schrank and Lishawa, 2019), and aquatic macroinvertebrates (Lawrence et al., 2016).

Accurate and stable methods to estimate productivity are necessary when quantifying metrics of plant dominance, population change, and drivers of biodiversity loss in invasion research (Crystal-Ornelas and Lockwood, 2020). Increased prediction accuracy is also highly desirable when integrating plot-level results (e.g., plant stock concentrations of nutrients, carbon, and heavy metals) across biological scales. Furthermore, plant biomass analyses can confirm and calibrate remote sensing estimates to improve model development for ecological management (Vaz et al., 2018).

Simple linear regression (SLR) standard curves of height-to-biomass have been used to non-destructively predict *Typha* biomass from field traits (Lishawa et al., 2015). Allometric equations are commonly used to non-destructively estimate biomass from forest systems (Henry et al., 2013), but tend to be less robust for herbaceous species with varied morphology and large environmental gradients (Niklas and Enquist, 2002; Pottier and Jabot, 2017). A two-step approach employing Bayesian information criterion (BIC) model selection of plant traits followed by multivariate partial least squares regression (PLS) modeling can produce highly accurate biomass predictions (Ohsowski et al., 2016). This PLS method avoids the pitfalls of excessive destructive sampling, accounts for collinearity among predictor variables, and can employ categorical and continuous data (Ohsowski et al., 2016).

Our study developed new methods that use easily quantified field measurements to accurately identify *Typha* taxa and accurately quantify *Typha* biomass to the benefit of conservation and ecological restoration. Our specific objectives developed prediction models that selected simple field measurements to: 1) accurately classify *Typha* taxa determined by diagnostic microsatellite markers using linear discriminant analysis, and 2) improve *Typha* taxa biomass assessment using BIC model selection and PLS. Additionally, we compared historically employed height-to-biomass SLR model predictions with PLS prediction.

2 Materials and methods

2.1 Study site selection and experimental design

In July 2021, we identified seven wetlands in northern Michigan (U.S.A.) to conduct the study [eastern Upper Peninsula (3 sites); northern Lower Peninsula (4 sites)]. Six of the seven wetland sites are classified as Great Lakes coastal wetlands (Munuscong Marsh, Mackinaw Bay, St. Ignace Marsh, Cecil Bay, Cheboygan Marsh, and Duncan Bay) and the remaining site was an inland emergent marsh (Alpena Wildlife Sanctuary) (Figure 1). All wetlands in the study had *Typha* stands within emergent vegetation zones.

In each wetland, we established a minimum of one continuous transect through the geographic center of established *Typha* stands. In two expansive wetlands with varied water levels and lake exposures (Cheboygan Marsh and St. Ignace Marsh), we increased the number of transects to 3 to capture environmental heterogeneity, resulting in 11 transects total. Nine of 11 transects had a standardized design to include 7 plots (1 m^2 quadrats) equidistant along a varied transect length depending upon stand extent. The remaining 2 stands were small (< 10 m diameter) but included in the study because we identified the plants as likely *T. latifolia* based on morphology (wide leaves and no separation between staminate and pistillate inflorescences) (Voss and Reznicek, 2012).

2.2 Field data collection: morphological characteristics

At each plot, we visually estimated areal coverage (< 1 – 100%) for plant community living vegetation, *Typha* living vegetation, and *Typha* standing-detritus above the water surface at all plots (Figures 2I–IV). We also collected total *Typha* ramet count in each plot to estimate ramet density. Water depth was estimated from the organic matter surface to water surface (Figure 2G). Organic matter depth was collected by firmly pushing a graduated PVC pole (1.9 cm diameter) through the decomposed organic layer until contacting mineral sediment (Figure 2H).

Following plot-level estimations, we unbiasedly selected the centermost *Typha* ramet to measure variables that potentially predict *Typha* biomass and discriminate *Typha* taxa. Adapted from Ohsowski et al. (2016), we collected the following measurements (Figures 2A–F): 1) inflorescence presence (yes/no), 2) total ramet height from organic matter surface (including inflorescence if present), 3) longest leaf length from organic matter surface, 4) maximum leaf width on the identified longest leaf (or longest leaf width), 5) ramet green leaf count, 6) widest ramet diameter at 30 cm, and 7) narrowest ramet diameter at 30 cm. We calculated ramet cross-section area at 30 cm assuming an oval: Area = pi ∗ widest cross-section/2 ∗ narrowest cross-section/2. When present, we measured the gap between pistillate and staminate inflorescences. Following all field measurements, each selected ramet was collected, dried at 60° C, and weighed.

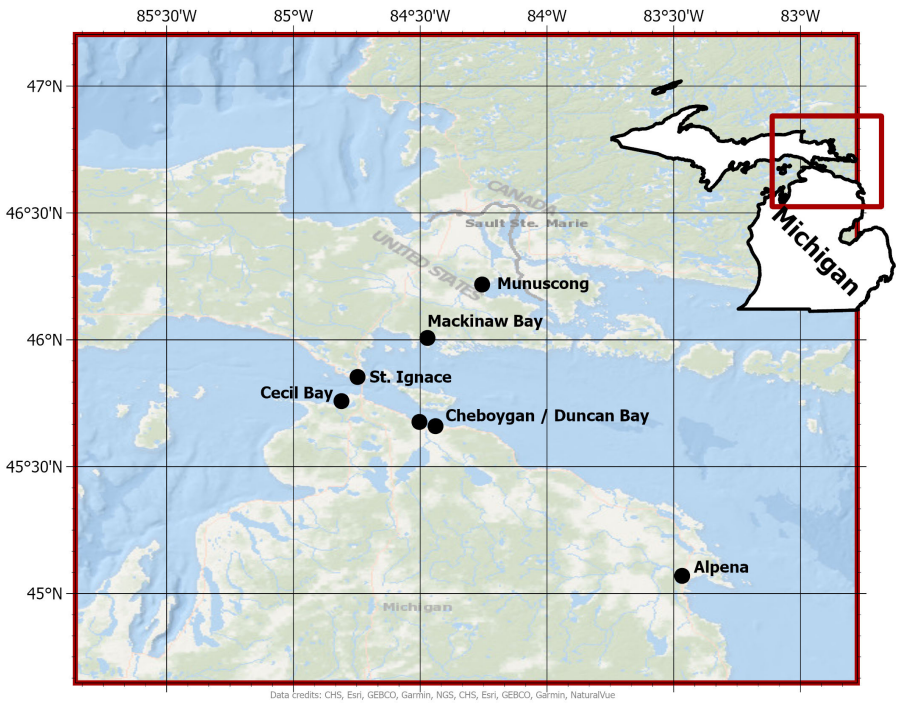

FIGURE 1
Wetland sites where *Typha* were sampled in northern Michigan (U.S.A.). Black dots represent the geographic center of each wetland site selected for the study.

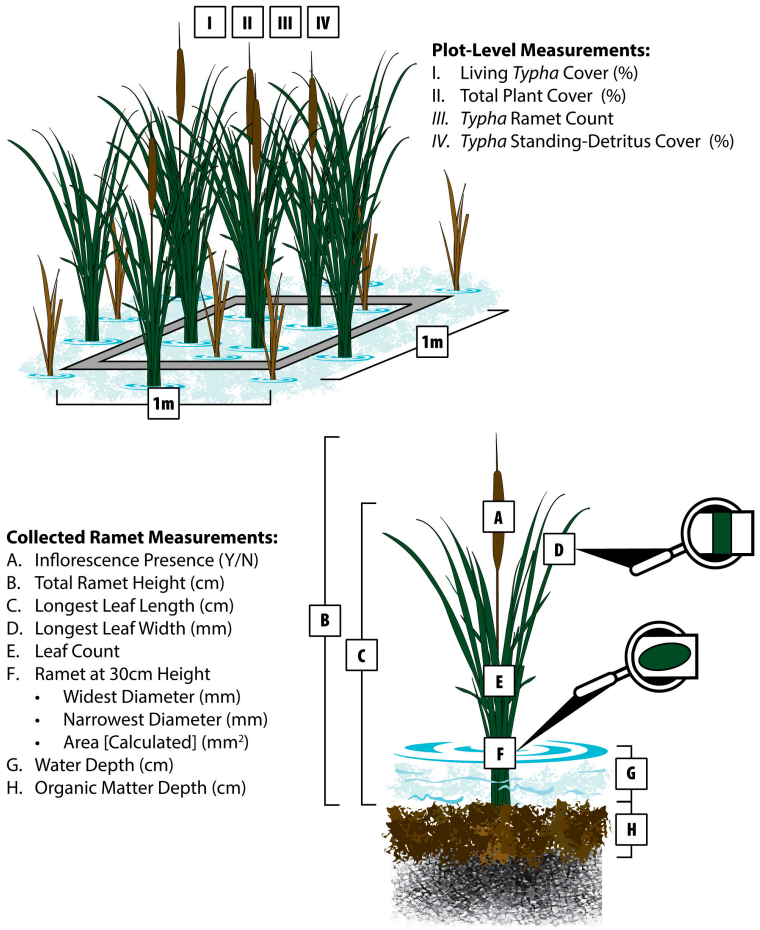

FIGURE 2
Field measurements collected at each plot (1.0 m^2 quadrat) and the centermost identified *Typha* ramet. Units are given in parentheses next to each variable listed. Variables (I.-IV.) were collected at the plot scale to assess areal cover and *Typha* ramet density. Plant morphological measurements **(A–F)** and environmental variables **(G, H)** were collected from the centermost *Typha* ramet that was subsequently collected for dry mass and genetic analysis.

2.3 Field data collection: molecular analysis

We established *a priori Typha* leaf tissue collection for molecular analysis from three non-adjacent plots per transect to minimize the probability of collecting clones; for the 2 small stands, tissue was collected from each plot (total replication = 33). Green leaf tissue (length = 10 cm) from the centermost ramet was clipped, bagged, and stored on ice (Geddes et al., 2021). Each leaf sample was then flash frozen in liquid nitrogen in the lab and stored in a cryogenic freezer (-80° C) until molecular analysis. The collected leaf tissue sample wet mass was converted to dry mass and added to total *Typha* dry mass described in Section 2.2.

2.4 Molecular analysis

Frozen *Typha* leaf tissue samples were ground with dry ice followed by DNA extraction using Qiagen DNEasy Plant kits. We selected six diagnostic microsatellite markers [TA 3, TA 5, TA 8, and TA 16 (Tsyusko-Omeltchenko et al., 2003), and TM 4 and TM 11 (Csencsics et al., 2010)], shown to be accurate in distinguishing *Typha* species, backcrosses, and advanced-generation hybrids (Geddes et al., 2021 and references therein). PCR amplification of microsatellite primers was accomplished following established protocols (Geddes et al., 2021) using 2-step PCR (Schuelke, 2000), after which a 1.4% agarose gel electrophoresis confirmed successful microsatellite amplification.

We performed microsatellite analyses on a Beckman Coulter gene sequencer with fragment sizing (400 bp ladder), scoring, and microsatellite interpretation analyzed using Beckman Coulter software. Following microsatellite scoring, each of the six microsatellite markers per tissue sample were separated into one of four molecular ID classes: *T. latifolia, T. angustifolia, T.* × *glauca*, or advanced-generation hybrid (AGH). A sample was categorized as an F1 hybrid (i.e., *T.* × *glauca*) if one allele from each parental species (*T. latifolia* and *T. angustifolia*) was present. The final taxonomic classification used for statistical analyses was determined when at least 5 of 6 diagnostic microsatellite markers agreed in the molecular ID classification. If diagnostic microsatellite markers did not meet the minimum 5 of 6 consensus among hybrid and/or both parental loci,

the sample molecular ID was classified as AGH (Snow et al., 2010; Travis et al., 2010; Travis et al., 2011).

2.5 Statistical analysis: *Typha* molecular ID classification

Plot-level *Typha* ramet measurements (Figure 2) were used to separate *Typha* molecular ID classes (*T. latifolia, T. angustifolia*, *T.* × *glauca*, AGH) using linear discriminant analysis (LDA) (replication = 33). *Typha* dry mass and all predictor variables in Figure 2 were transformed (when required), centered, and scaled to meet statistical assumptions. We used Spearman's rank correlation coefficients to determine highly collinear variables ($r_s > 0.90$) and remove one of the variable pairs from the analysis. We employed recursive feature elimination with 10-fold cross-validation to select the most relevant class separation variables using the *rfe()* function in R's *caret* package (Kuhn, 2008; Chen et al., 2020). The selected LDA model was built with the *lda()* function in R's *MASS* package (Venables and Ripley, 2013). Model and class prediction performance metrics were generated using a confusion matrix in R's *caret* package (Kuhn, 2008).

We developed an LDA permutation model to estimate test data prediction performance for *Typha* molecular ID classes. For each permutation (n = 1,000), the full dataset (replication = 33) was randomly split into a training data set (replication = 26) to establish an LDA model. The remaining test data (replication = 7) were classified into molecular ID classes by the trained LDA model. We calculated an agreement percentage for predicted within-model training data and external model test data for each iteration as % correctly predicted cases/total cases predicted.

2.6 Statistical analysis: biomass prediction

We used plot-level and *Typha* ramet measurements (Figure 2) to develop *Typha* dry mass prediction models (replication = 75). Variable standardization, Bayesian Information Criterion (BIC) model selection, and model dry mass prediction workflow followed Ohsowski et al. (2016) and references within. All predictor variable combinations and associated 2nd order polynomial terms were scored with BIC model selection with the *dredge* function in R's *MuMin* package (Bartoń, 2023). Equivalent multi-variate prediction models ($\Delta BIC \leq 2$) were averaged using the *model.avg()* function in *MuMin* to provide our selected statistical model employed for *Typha* dry mass prediction.

We trained the selected multi-variate model using partial least squares regression (PLS) (replication = 75) in R's *pls* package (Liland et al., 2023). Four model components were retained as determined by lowest root mean squared error of cross-validation (RMSECV) calculated from 10-fold cross-validation. For comparison, we developed a simple linear regression (SLR) model for *sqrt[Typha Dry Mass] ~ Total Ramet Height* (replication = 75) using the *lm* function in R's *base* package (R Core Team, 2023). RMSECV estimates for the SLR model were calculated with 10-fold cross-validation with the *errorest()* function in R's *ipred* package (Peters and Hothorn, 2023). Similar to Ohsowski et al. (2016), we calculated a simple DIFF term for all *Typha* dry mass predictions with the following formula to assess model prediction performance: DIFF = predicted *Typha* dry mass – reference *Typha* dry mass.

We developed a permutation model for the PLS and SLR models to estimate test data (i.e., external data) prediction performance for *Typha* dry mass, thus assessing model robustness and real-world model applicability. For each permutation (n = 1,000), the full dataset (replication = 75) was randomly split into training data (replication = 63) to develop the PLS and SLR models. The remaining test data (replication = 12) were predicted by the trained PLS and SLR models, back-transformed, and DIFF term was calculated for the resulting permutation model.

3 Results

3.1 Variable selection: molecular ID classification

We developed a linear discriminant analysis (LDA) to predict *Typha* molecular ID classes: *T. latifolia, T. angustifolia*, *T.* × *glauca*, and AGH. To meet test assumptions, we dropped three collinear variables from the analysis using Spearman's rank correlation coefficients: *Longest Leaf Length* (collinear with *Total Ramet Height*, $r_s = 0.996$), *Widest Ramet Diameter at 30 cm* (collinear with *Ramet Area at 30 cm*, $r_s = 0.974$), and *Narrowest Ramet Diameter at 30 cm* (collinear with *Ramet Area at 30 cm*, $r_s = 0.928$). Total ramet height was selected over longest leaf length as this character is a very simple field measurement. We also dropped the widest and narrowest ramet diameter measurements as they were highly collinear with the calculated ramet areas due to formula inclusion. In total, ten variables were used to determine the most parsimonious LDA model via recursive feature elimination with 10-fold cross-validation: *sqrt[Organic Matter Depth], sqrt[Water Depth], sqrt[Living Typha Cover], log[Typha Detritus Cover], sqrt [Leaf Count], sqrt[Longest Leaf Width], sqrt[Longest Leaf Length], sqrt[Typha Ramet Count], Typha Height, log[Ramet Area at 30 cm]*.

We determined the most relevant LDA class separation variables for the final LDA model: *Molecular ID Class ~ sqrt[Leaf Count] + sqrt[Longest Leaf Width]*. Note that *sqrt[Water Depth]* was retained in the initial selected recursive feature elimination model but removed from this analysis. We determined that including water level measurements may lead to unreliability for future application of the presented model by increasing uncertainty (*see Discussion*).

3.2 Diagnostic microsatellite markers: molecular ID classification

Agreement among the six microsatellite markers resulted in four molecular ID classes: *T. latifolia, T. angustifolia*, *T.* × *glauca*, and AGH. Out of the 33 molecular samples, 66.3% had complete diagnostic microsatellite agreement among all six molecular markers [count]: *T. angustifolia* [3], *T.* × *glauca* [15], *T. latifolia*

[3]. Additionally, 30.3% of the samples had consensus in 5 out of 6 markers resulting in molecular ID classification [count] of *T. angustifolia* [5], *T.* × *glauca* [3], and *T. latifolia* [2]. Two samples were classified as AGH because the six microsatellite markers were split in the molecular ID: one of the samples had 3 markers identifying it as *T.* × *glauca* and 3 markers as *T. angustifolia*, while the other sample had 2 markers identifying it as *T.* × *glauca* and 4 markers as *T. angustifolia*. These two latter samples likely represent backcrosses to one of the parental species (in this case *T. angustifolia*). Given our relatively low sample size for molecular analyses (n = 33), we categorized all hybrids beyond the F1 hybrid as advanced-generation hybrids. Overall, molecular ID analyses prevalence revealed that 15.2% of our samples were classified as *T. latifolia*, 24.2% as *T. angustifolia*, 54.6% as *T.* × *glauca*, and 6.1% as AGH (Table 1).

3.3 Molecular ID class separation

The overall linear discriminant analysis model had high statistical accuracy when predicting the four *Typha* molecular classes. LDA training data confusion matrix statistics revealed high confidence for internal prediction model accuracy [correct % prediction (± 95% CI): 78.8% (61.1%, 91.0%), Kappa = 66.8%]. The LDA model significantly outperformed the no information rate (i.e., null) model (p = 0.003) (Table 2). The two most descriptive linear discriminant functions (LD1: explained variance 96.8%; LD2: explained variance 3.2%) successfully separated the molecular ID classes driven by the leaf count and longest leaf width variables (Figure 3). The 95% *T. angustifolia* confidence intervals more strongly overlapped with *T.* × *glauca* compared to the clear class separation between *T.* × *glauca* and *T. latifolia* (Figure 3). *T. latifolia* and *T. angustifolia* had no 95% confidence interval overlap indicating clear molecular ID class separation between the two taxa (Figure 3). AGH had only two classification instances (6.1% of data set) leading to instability in predicting the class (Table 1) and unresolved 95% confidence intervals (Figure 3) due to low replication. LDA models revealed 100% accurate classification of *T. latifolia*, 85.6% for *T.* × *glauca*, and 83.8% for *T. angustifolia* (Table 1). Molecular class gravity centered mean measurement values were back-transformed and presented for leaf count and longest leaf width in Table 1.

The permutation LDA model result confirmed high prediction agreement for training data and test data of molecular ID classes. For each permutation iteration (n = 1,000), 78.8% of the full data set trained the LDA model to externally predict 21.2% of the test data. The average % correct molecular ID prediction for each permutation iteration confirmed model accuracy for internal training data (mean ± 1 sd % correct: 87.9% ± 3.6%) and test data (mean ± 1 sd % correct: 78.7% ± 15.3%) (Table 2).

3.4 Variable selection: *Typha* dry mass prediction

We used 14 predictor variables (and associated polynomial terms) for BIC model selection. Model selection resulted in 11 equivalent models that predicted *Typha* dry mass in the study (Table 3). Following BIC model averaging, the final PLS prediction model was reduced to 4 predictor variables: *sqrt[Typha Dry Mass] ~ log[Ramet Area at 30 cm] + Inflorescence Presence + Total Ramet Height + sqrt[Organic Matter Depth]*.

Six of 14 potential predictor variables (and associated polynomial terms) in Figure 2 were not selected in any equivalent ΔBIC ≤ 2 models: *Typha* standing-detritus cover, widest ramet diameter at 30 cm, narrowest ramet diameter at 30 cm, maximum

TABLE 1 Linear discriminant analysis (LDA) model performance metrics by class for training data to separate the four *Typha* molecular ID classes: *Typha angustifolia* [A], Advanced Generation Hybrid [AGH], *Typha* × *glauca* [G], and *Typha latifolia* [L].

LDA *Typha* Molecular ID by Class				
	A	AGH	G	L
Sensitivity (True Positive Rate)	87.5%	0.0%	77.8%	100.0%
Specificity (True Negative Rate)	80.0%	96.8%	93.3%	100.0%
Prevalence	24.2%	6.1%	54.6%	15.2%
Balanced Accuracy	83.8%	48.4%	85.6%	100.0%
LDA Gravity Centered Means				
Longest Leaf Width (mm)	7.06	8.02	10.56	15.05
Leaf Count	6.35	4.94	7.89	12.98
Raw *Typha* Measurements by Class				
Class Replication	8	2	18	5
Longest Leaf Width (mm) (mean ± 1 sd)	7.09 ± 0.91	8.03 ± 0.04	10.69 ± 2.28	15.10 ± 1.85
Leaf Count (mean ± 1 sd)	6.38 ± 0.92	5.00 ± 1.41	7.94 ± 1.35	13.00 ± 1.00

For the training data statistics, class-based sensitivity, specificity, prevalence, and balanced accuracy are given. LDA derived molecular ID group mean centers of gravity are given on the original measurement scale. In addition, raw data summary statistics for *Typha* morphological measurements and replication are given for comparison to LDA's mean center of gravity predictions.

TABLE 2 Overall linear discriminant analysis (LDA) model performance metrics to separate the four *Typha* molecular ID classes.

LDA *Typha* Molecular ID Training Data	
Response Variable:	*Typha* Molecular ID
Predictor Variables:	sqrt[Leaf Count] + sqrt[Longest Leaf Width]
Correctly Predicted:	78.8%
Correctly Predicted 95% CI:	(61.1%, 91.0%)
P-Value [Acc>NIR]:	0.003
Kappa:	66.8%
LDA *Typha* Molecular ID Permutation Model	
Number of Permutations:	1,000
Training / Test Replication:	n = 26 / n = 7
Training Data (% correct ± 1 sd):	87.9% ± 3.6%
Test Data (% correct ± 1 sd):	78.7% ± 15.3%

LDA training data statistics were extracted from a generated confusion matrix in R's *caret* package. Permutation model statistics were developed to estimate test data prediction performance for *Typha* molecular ID classes from both training data and test data. An agreement percentage for predicted data for each iteration was calculated as % correctly predicted cases/total cases predicted and permutation results subsequently averaged.

leaf width on the longest leaf (i.e., longest leaf width), ramet green leaf count, and water depth (Table 3). Prevalent variables selected within ΔBIC ≤ 2 models (% occurrence across ΔBIC ≤ 2 models, n = 11) were: ramet area at 30 cm (100%), inflorescence presence (100%), and organic matter depth (81.8%). Longest leaf length (54.5%) and total ramet height (36.3%) were never selected for the same ΔBIC ≤ 2 equivalent model. Although longest leaf length was selected more frequently, total ramet height (36.3%) was chosen as a preferred PLS predictive variable because of the relative ease of collecting ramet height data in the field without compromised predictive power. Curvilinear relationships (i.e., 2nd order polynomial terms) were infrequently included to predict *Typha* dry mass for ramet area at 30 cm and living *Typha* cover (Table 3). Polynomial terms were not influential after BIC model averaging.

3.5 PLS and SLR *Typha* dry mass prediction

Typha dry mass descriptive statistics (min: 4.78 g; max: 102.62 g; mean ± 1 sd: 34.52 g ± 19.22 g) successfully characterized plant population size class ranges encountered in the study's wetlands. To this end, the multi-variate partial least squares regression (PLS) *Typha* dry mass prediction model vastly outperformed the simple linear regression (SLR) prediction model developed for *sqrt[Typha Dry Mass] ~ Total Ramet Height*. The trained PLS prediction model [Root mean squared error of cross validation (RMSECV): 0.47 g, explained variance: 85.01%, replication = 75] had higher accuracy and precision when validating model performance compared to the trained SLR model [RMSECV: 2.27 g, explained variance: 18.38%, replication = 75] (Table 4). Thus, utilizing the selected PLS model instead of the simple SLR resulted in greater accuracy in predicting *Typha* dry mass (Figure 4). For clarity in Figure 4, the slope = 1 reference line indicates a perfect prediction between predicted and reference dry mass. In Figure 4A, the linear regression of predicted PLS *Typha* dry mass ~ reference *Typha* dry mass ($p < 0.001$, $R^2 = 0.832$) had high agreement and low unexplained variation with slope = 1 and the regression fit. Compared to slope = 1, the PLS model slightly underpredicted the reference dry mass of *Typha* within the higher biomass ranges. In contrast (Figure 4B), the linear regression of predicted SLR *Typha* dry mass ~ reference *Typha* dry mass ($p < 0.001$, $R^2 = 0.180$) displayed strong skew, low agreement, and high unexplained variation when compared to slope = 1 and the regression fit. The SLR vastly underpredicted *Typha* dry mass as reference dry mass increased, resulting in lower model confidence compared to the PLS model.

The permutation model results further confirmed superior PLS model performance compared to SLR model performance. For each permutation iteration (n = 1,000), 84.0% of the full data set trained the respective PLS and SLR model to externally predict 16% of the test

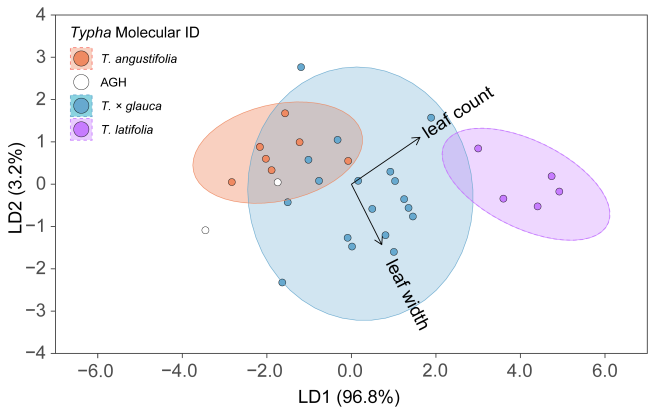

FIGURE 3
Linear discriminant analysis (LDA) results displaying linear discriminant function 1 (LD1) vs. linear discriminant function 2 (LD2) to separate the four *Typha* molecular ID classes: *Typha angustifolia*, advanced generation hybrid [AGH], *Typha* × *glauca*, and *Typha latifolia*. LD1 vs. LD2 maximized class separation given with parenthetic values for *Proportion of Trace* describing discriminant function explained variation. Respective ellipses represent 95% confidence intervals for each predicted class. Arrows represent the contribution direction and magnitude of each predictor variable. Final LDA model: *sqrt[Typha Dry Mass] ~ sqrt[Leaf Count] + sqrt[Longest Leaf Width]*. AGH had insufficient data to provide model confidence intervals.

10.3389/fpls.2024.1348144

TABLE 3 Equivalent BIC selected models (ΔBIC ≤ 2) and associated degrees of freedom (df) generated to predict *Typha* dry mass.

Equivalent *Typha* Dry Mass Prediction Models	df	Δ BIC
Response Variable: sqrt [*Typha* Dry Mass]		
Predictor Variables:		
log[Ramet Area at 30 cm] + Inflorescence Presence + Leaf Count + Longest Leaf Length + sqrt[Organic Matter Depth]	7	0.00
log[Ramet Area at 30 cm + Inflorescence Presence + Longest Leaf Length + sqrt[Organic Matter Depth]	6	0.18
log[Ramet Area at 30 cm + Inflorescence Presence + sqrt [Organic Matter Depth] + Total Ramet Height	6	0.43
log[Ramet Area at 30 cm] + Inflorescence Presence + Leaf Count + sqrt[Organic Matter Depth] + Total Ramet Height	7	0.56
log[Ramet Area at 30 cm] + (log[Ramet Area at 30 cm])^2 + Inflores- cence Presence + Longest Leaf Length + sqrt[Living *Typha* Cover] + (sqrt Living *Typha* Cover])^2	8	0.88
log[Ramet Area at 30 cm] + (log[Ramet Area at 30 cm])^2 + Inflorescence Presence + Longest Leaf Length + sqrt[Organic Matter Depth]	7	1.04
log[Ramet Area at 30 cm + (log[Ramet Area at 30 cm])^2 + Inflorescence Presence + sqrt[Organic Matter Depth] + Total Ramet Height	7	1.49
log[Ramet Area at 30 cm] + (log[Ramet Area at 30 cm])^2 + Inflores- cence Presence + Total Ramet Height + sqrt Living *Typha* Cover + (sqrt Living *Typha* Cover])2	8	1.55
log[Ramet Area at 30 cm + Inflorescence Presence + sqrt [Organic Matter Depth]	5	1.81
log[Ramet Area at 30 cm] + (log[Ramet Area at 30 cm])^2 + Inflorescence Presence + Leaf Count + Longest Leaf Length + sqrt Organic Matter Depth]	8	1.98
log[Ramet Area at 30 cm + Inflorescence Presence + Leaf Count + Longest Leaf Length + sqrt Organic Matter Depth + sqrt [Living *Typha* Cover + (sqrt[Living *Typha* Cover])^2	9	1.98

Variables were transformed (where indicated) and subsequently centered and scaled (variable mean = 0, variance = 1) prior to BIC selection.

TABLE 4 Partial least squares regression (PLS) and simple linear regression (SLR) model performance metrics for *Typha* dry mass predictions.

PLS *Typha* Dry Mass Training Data Statistics	
Response Variable:	sqrt [*Typha* Dry Mass]
Predictor Variables:	log[Ramet Area at 30 cm] + Inflorescence Presence + sqrt[Organic Matter Depth] + Total Ramet Height
PLS Components:	4
Cross-Validation Segments:	10
RMSECV *Typha* Dry Mass:	0.47 g
Explained Variance:	85.01%
SLR *Typha* Dry Mass Training Data Statistics	
Response Variable:	sqrt [*Typha* Dry Mass)
Predictor Variable:	Total Ramet Height
Cross-Validation Segments:	10
RMSECV *Typha* Dry Mass:	2.27 g
Explained Variance:	18.38%
***Typha* Dry Mass Test Data Statistics**	
Number of Permutations:	1,000
Training/Test Replication:	n = 63 / n = 12
PLS DIFF (mean ± 1 sd):	-0.53 g ± 8.70 g
SLR DIFF (mean ± 1 sd):	-2.37 g ± 18.0 g

Training data statistics for PLS and SLR models present predictor variable(s), components selection (PLS only), *k*-fold cross-validation segments, root mean square error of cross-validation (RMSECV), and explained model variance. Test data statistics are given for permutation models results to estimate accuracy of external data predictions. Permutation results are given by mean ± 1 standard deviation of: DIFF = [*predicted Typha dry mass – reference Typha dry mass]*. All presented *Typha* dry mass results were back-transformed to show original data collection scale.

data. Calculated DIFF confirmed high PLS model accuracy for test data (mean DIFF ± 1 sd: -0.53 g ± 8.70 g) compared to the more highly variable SLR model test data (mean DIFF ± 1 sd: -2.37 g ± 18.00 g).

4 Discussion

Our study emphasized field applicability from simple aboveground *Typha* morphological measurements to support rapid ecological management decisions for wetland plant conservation and restoration. Contextually, field data collected in this study occurred during peak growing season in northern Michigan (July-August). Thus, our predictive models will have widest applicability to fully mature *Typha* ramets prior to senescence. Overall, we are confident that both of our developed techniques can be employed with high precision and accuracy to generate reliable data for researchers and land managers combatting invasive *Typha* populations and implementing conservation strategies to protect *T. latifolia* in North America.

Molecular marker results indicate that hybridization is common across the study region and that introgression (e.g., hybridization beyond the F1 hybrids) may not be as prevalent in this study area compared with others (e.g., Geddes et al., 2021 and references therein). Specifically, we only identified advanced-generation hybrids twice across all samples. However, we contend that the discrepancy in classified molecular cases was not necessarily a major limitation of this study. This study's design did not prioritize quantification of occurrence frequency of *Typha* in the region as we did not specifically target equal population sizes with the intent for balanced replication or a comprehensive wetland selection protocol of all regional extant *Typha* stands. Thus, direct comparisons with prior studies that address prevalence or occurrence frequency of *Typha* taxa in North America should be avoided.

In our sampled wetlands, LDA model selection revealed that simple field measurements exhibited good taxa separation. Morphological measurements of *T.* × *glauca* fell in between *T. angustifolia* and *T. latifolia* in both LDA mean center of gravity and raw data summary statistics (Table 1). These results agree with Snow et al. (2010) who found that microsatellite markers sorted

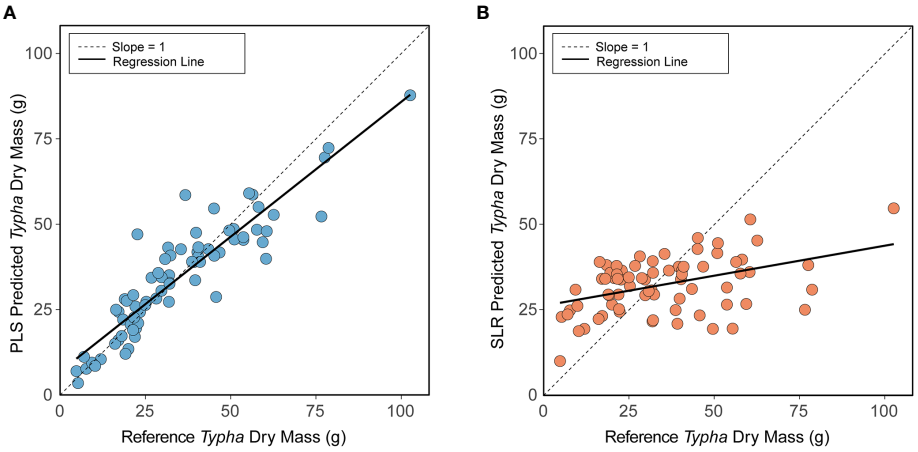

FIGURE 4
Partial least squares regression (PLS) **(A)** and simple linear regression (SLR) **(B)** model results for predicted *Typha* dry mass (training data) vs. reference *Typha* dry mass. Panel **(A)** Training data predicted from final PLS model generated from BIC model selection: *sqrt[Typha Dry Mass] ~ log[Ramet Area at 30 cm] + Inflorescence Presence + Total Ramet Height + sqrt[Organic Sediment Depth]*. Panel **(B)** Training data predicted from final SLR prediction model: *sqrt[Typha Dry Mass] ~ Total Ramet Height*. In both panels, the dashed line (—) represents a slope = 1 indicating a perfect prediction between reference *Typha* dry mass and predicted *Typha* dry mass. The solid black line (—) represents the actual best fit regression line between predicted *Typha* dry mass and reference *Typha* dry mass.

samples by measured traits into three distinct clusters represented by *T. latifolia*, *T.* × *glauca*, and *T. angustifolia*. Similar to our study, the results of Snow et al. (2010) located *T.* × *glauca* in the middle of the parental species clusters. Furthermore, our results agree with those of Kirk et al. (2011) and those of Kuehn and White (1999) who found that principal component analysis (PCA) of microsatellite markers and discriminant analyses of randomly amplified polymorphic DNA (RAPD) markers, respectively, categorized *Typha* samples into three distinct clusters (*T. latifolia*, *T.* × *glauca*, and *T. angustifolia*) by measured plant traits.

We assert that our capacity to identify *T. latifolia* is timely and crucial when detecting and distinguishing the increasingly rare *T. latifolia* from invasive *Typha*. Our study successfully showed that the molecular ID of *T. latifolia* was strongly separated from the remaining *Typha* taxa with slight 95% confidence interval overlap with *T.* × *glauca* and no overlap with *T. angustifolia* (Figure 3). Our method to accurately identify *T. latifolia* with two measurements will allow field biologists to differentiate populations of the native species quickly and accurately from the invasive taxa to improve conservation efforts.

In our study, LDA longest leaf width mean center of gravity for *T. latifolia* (15.05 mm) was reliably distinguished from longest leaf width for *T. angustifolia* (6.35 mm) and *T.* × *glauca* (10.56 mm) (Figure 2; Table 1). In Snow et al. (2010), cluster classification corresponded well with several plant field measurements that included: log(leaf length/leaf width), length of gap between inflorescences, inflorescence length, and stem diameter. In confirmation with our results, a measurement metric including leaf width [i.e., log(leaf length/leaf width)] was most useful in distinguishing between parental species and the F1 hybrid (Snow et al., 2010). In contrast, our LDA model did not select a diagnostic stem measurement (e.g., ramet area at 30 cm) as a strong predictor of class separation. In Kirk et al. (2011), *Typha* taxa clustering was also significantly driven by leaf width measurements from a random subset of selected leaves. Given the high variability of leaf width within a particular ramet based on position, size, or age, our selection of a ramet's longest leaf width will increase measurement consistency to yield a more robust metric. Lastly, in Kuehn and White (1999), cluster classification of the three *Typha* taxa corresponded with stigma width, length of inflorescence spike, gap between inflorescences, leaf width, and inflorescence width. However, they concluded that no single character or sets of characters were diagnostic due to considerable overlap among parental species and the hybrid. In addition, 4 of 5 characters used by Kuehn and White (1999) relied on the presence of inflorescences and included more complex, microscope-assisted measurements of stigma widths.

Our second LDA selected character (leaf count), is, to our knowledge, a novel measurement not previously identified as a *Typha* taxa classification trait. In our study, LDA leaf count mean center of gravity for *T. latifolia* (12.98 leaves) far surpassed leaf count for *T. angustifolia* (6.35 leaves) and *T.* × *glauca* (7.89 leaves) suggesting a simple measurement metric can be used in combination with longest leaf width to improve classification prediction (Figure 2; Table 1). Wasko et al. (2022) employed mean leaf-apex angle measured for *Typha* ramet leaves (range: 2-9 leaves; mean: 5.3 leaves per ramet) to successfully match *Typha* ID to microsatellite markers. In contrast to leaf count, the mean leaf-apex angle metric in Wasko et al. (2022) requires a labor investment in the field. As our study did not include mean leaf-apex angle, future models could include this trait to determine if its contribution greatly improves predictive class separation.

We still argue that field-based characters related to *Typha* inflorescence measurements are extremely helpful in taxa differentiation, specifically the gap between the staminate and pistillate inflorescences. Yet, we caution that relying on

inflorescence trait measurements may be problematic. *Typha* clonal vegetative growth, which allows spread via rhizomes, can be highly plastic regarding inflorescence production (Grace and Wetzel, 1982). In our current study, this was evidenced by the fact that 73% (55 of 75) of the randomly collected centermost ramets lacked an inflorescence. Wasko et al. (2022) also found that less than 50% (22 of 45) of their sampled *Typha* ramets had inflorescences. Furthermore, large-scale management focused on aboveground biomass removal via harvesting has resulted in stark reduction in inflorescence frequency in subsequent years post-harvest. For instance, following two consecutive years of invasive *Typha* harvest at Shiawassee National Wildlife Refuge (MI, USA), 0.22% of *Typha* ramets produced an inflorescence, compared with 16.66% of *Typha* ramets in unharvested control plots (Lishawa et al., 2020). Our field-based *Typha* measurements investigated in this study relied solely upon vegetative growth characteristics, thus providing a wider applicability for land managers and researchers in the field.

As noted in the results, the water depth variable in the LDA model improved *Typha* class separation accuracy by approximately 9.1%. We removed this variable as a potential predictor variable as 6 of the 7 sampled wetlands were classified as Great Lakes coastal wetlands. As water levels fluctuate considerably in the Great Lakes (Gronewold and Rood, 2019), small predictive gains for variable retention were determined to not outweigh the potentially erroneous predictive conclusions. In Great Lakes coastal wetland systems, daily water level ranges exceeding 20 cm are common due to seiche events (Trebitz, 2006). The static LDA predictive model assumes stability in water levels to separate the molecular ID classes. Future model improvement may consider including water level measurement in less dynamic, inland wetlands and/or with greater sampling breadth of *T. latifolia* populations. As *T. latifolia* populations are increasingly rare in the region, population sampling was limited to two small *T. latifolia* stands (< 10 m diameter) in this study. Summary statistics suggest that water level for *T. latifolia* (mean ± 1 sd: 7.90 cm ± 5.81 cm) may be a viable indicator in future studies but remains unreliable in this study due to high variation in *T.* × *glauca* and *T. angustifolia* (mean ± 1 sd: 31.03 cm ± 16.93 cm; 63.31 cm ± 40.05 cm, respectively). Furthermore, evidence from Lake Ontario wetlands with co-occurring *Typha* taxa suggests that the three dominant taxa do not tend to sort along a water depth gradient, but instead occupy similar habitats (McKenzie-Gopsill et al., 2012). Taken together, this evidence provided additional justification for dropping water depth from our model.

Variables selected for our PLS equations incorporated total ramet height, organic matter depth, inflorescence presence, and ramet area at 30 cm. The resulting PLS model was 85.01% accurate at predicting *Typha* dry mass training data, thus improving upon published allometric equations for *Typha* (Lishawa et al., 2015). Furthermore, our PLS model was robust to test (i.e., external) data predictions leading to high confidence in our model prediction applicability (Table 4, PLS DIFF: 0.53 g ± 8.70 g). Comparatively, SLR models solely using total ramet height performed poorly when predicting test data *Typha* dry mass (Table 4, PLS DIFF: -2.37 g ± 18.0 g). Similar to Ohsowski et al. (2016), model predictive performance with multi-variate traits vastly improved both precision and accuracy for training and test data predictions. Unsurprisingly, plant height has been used as a variable to create plant biomass predictive standard curves or as a proxy for plant biomass (Catchpole and Wheeler, 1992). Our PLS model highlights that the sole use of plant height measurements contributes to a high level of model uncertainty, especially at higher biomass values for *Typha* specifically. Our study further affirms that plant height does provide predictive power when used in conjunction with multi-variate model predictors.

Interestingly, our proposed PLS model provides researchers with additional novel morphological measurements to accurately predict *Typha* biomass. In this context, ramet area at 30 cm was a consistently selected variable to improve *Typha* dry mass prediction. Ramet area at 30 cm alludes to the thickness and shape of the culm calculated from widest ramet diameter and narrowest ramet diameter. In their molecular ID study, Snow et al. (2010) found that stem diameter helped explain the separation of the parental species and the F1 hybrid. Here, we provide evidence that ramet area is also useful in explaining predicted *Typha* dry mass. At first glance, this trait may seem challenging to measure in the field. In our experience, integrating ramet area measurement can be accomplished with common fieldwork tools such as calipers or a flexible measuring tape. We assert that including this variable is essential despite minor increases in time and labor since the multi-variate PLS model substantially increased explained variance and test data prediction precision.

Another unexpected, but reasonable, predictor of *Typha* dry mass was organic matter depth. Organic depth has been correlated with measures of *Typha* dominance. For example, in 14 Great Lakes coastal wetlands in our project region organic matter depth was more than 3-times greater and sediment ammonium was over 10-times greater where *Typha* was present (Lishawa et al., 2010). Further, *Typha* ramet density was positively correlated with organic matter depth (Lishawa et al., 2010). Organic sediments in these freshwater coastal systems are likely a strong proxy for sediment nutrient availability. *Typha* has been shown to increase sediment retention (Horppila and Nurminen, 2001), thereby creating a nutrient retention positive feedback. Corroborating these results, reviewed research indicates that roots of stoloniferous and rhizomatous species clones proliferate rapidly under conditions of increased nutrient resource availability (de Kroons and Hutchings, 1995). Thus, organic matter depth should be expected to drive plant vigor.

In conclusion, our results will benefit the work of land managers and conservation biologists by enabling the rapid identification of *Typha* taxa with minimal effort in the field. Furthermore, our biomass prediction models will lend greater confidence in non-destructive field-based measurements to improve scaled-up plot level data to the landscape level. As intended, we are confident that this study will help North American land managers parse subtle morphological trait variation in *Typha*, enhancing wetland conservation and ecological restoration efforts.

Data availability statement

The original contributions presented in the study are publicly available. This data can be found here: https://osf.io/74yvr.

Author contributions

BO: Conceptualization, Data curation, Formal analysis, Funding acquisition, Methodology, Project administration, Writing – original draft, Writing – review & editing. CR: Data curation, Methodology, Writing – original draft. PG: Conceptualization, Data curation, Formal analysis, Funding acquisition, Methodology, Writing – original draft, Writing – review & editing. SL: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Writing – original draft, Writing – review & editing.

Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article. Funding was provided by Loyola University Chicago: The Loyola Undergraduate Research Opportunities Program and the Lee Botts Great Lakes Research and Advocacy Fellowship provided field research support for CR's undergraduate research project. Loyola's School of Environmental Sustainability and the Office of Research Services provided publication fee support for the manuscript.

Conflict of interest

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

Publisher's note

References

Angeloni, N. L., Jankowski, K. J., Tuchman, N. C., and Kelly, J. J. (2006). Effects of an invasive cattail species (*Typha* × *glauca*) on sediment nitrogen and microbial community composition in a freshwater wetland. *FEMS Microbiol. Lett.* 263, 86–92. doi: 10.1111/fml.2006.263.issue-1

Bansal, S., Lishawa, S. C., Newman, S., Tangen, B. A., Wilcox, D., Albert, D., et al. (2019). *Typha* (Cattail) invasion in North American wetlands: Biology, regional problems, impacts, ecosystem services, and management. *Wetlands* 39, 645–684. doi: 10.1007/s13157-019-01174-7

Bartoń, K. (2023) *MuMIn: Multi-Model Inference. R package version 1.47.5*. Available online at: https://CRAN.R-project.org/package=MuMIn.

Boers, A. M., and Zedler, J. B. (2008). Stabilized water levels and *Typha* invasiveness. *Wetlands* 28, 676–685. doi: 10.1672/07-223.1

Bunbury-Blanchette, A. L., Freeland, J. R., and Dorken, M. E. (2015). Hybrid *Typha*×*glauca* outperforms native *T. latifolia* under contrasting water depths in a common garden. *Basic Appl. Ecol.* 16, 394–402. doi: 10.1016/j.baae.2015.04.006

Carson, B. D., Lishawa, S. C., Tuchman, N. C., Monks, A. M., Lawrence, B. A., and Albert, D. A. (2018). Harvesting invasive plants to reduce nutrient loads and produce bioenergy: An assessment of Great Lakes coastal wetlands. *Ecosphere* 9, e02320. doi: 10.1002/ecs2.2320

Catchpole, W. R., and Wheeler, C. J. (1992). Estimating plant biomass: A review of techniques. *Aust. J. Ecol.* 17, 121–131. doi: 10.1111/j.1442-9993.1992.tb00790.x

Chen, R.-C., Dewi, C., Huang, S.-W., and Caraka, R. E. (2020). Selecting critical features for data classification based on machine learning methods. *J. Big Data* 7, 52. doi: 10.1186/s40537-020-00327-4

Ciotir, C., Kirk, H., Row, J. R., and Freeland, J. R. (2013). Intercontinental dispersal of *Typha angustifolia* and *T. latifolia* between Europe and North America has implications for *Typha* invasions. *Biol. Invasions* 15, 1377–1390. doi: 10.1007/s10530-012-0377-8

Crystal-Ornelas, R., and Lockwood, J. L. (2020). The 'known unknowns' of invasive species impact measurement. *Biol. Invasions* 22, 1513–1525. doi: 10.1007/s10530-020-02200-0

Csencsics, D., Brodbeck, S., and Holderegger, R. (2010). Cost-effective, species-specific microsatellite development for the endangered dwarf bulrush (*Typha minima*) using Next-Generation Sequencing Technology. *J. Heredity* 101, 789–793. doi: 10.1093/jhered/esq069

Currie, W. S., Goldberg, D. E., Martina, J., Wildova, R., Farrer, E., and Elgersma, K. J. (2014). Emergence of nutrient-cycling feedbacks related to plant size and invasion success in a wetland community–ecosystem model. *Ecol. Model.* 282, 69–82. doi: 10.1016/j.ecolmodel.2014.01.010

de Kroons, H., and Hutchings, M. J. (1995). Morphological Plasticity in clonal plants: The foraging concept reconsidered. *J. Ecol.* 83, 143–152. doi: 10.2307/2261158

Ellstrand, N. C., and Schierenbeck, K. A. (2000). Hybridization as a stimulus for the evolution of invasiveness in plants? *Proc. Natl. Acad. Sci.* 97, 7043–7050. doi: 10.1073/pnas.97.13.7043

Freeland, J., Ciotir, C., and Kirk, H. (2013). Regional differences in the abundance of native, introduced, and hybrid Typha spp. In northeastern North America influence wetland invasions. *Biol. Invasions* 15, 2651–2665. doi: 10.1007/s10530-013-0481-4

Galatowitsch, S. M., Anderson, N. O., and Ascher, P. D. (1999). Invasiveness in wetland plants in temperate North America. *Wetlands* 19, 733–755. doi: 10.1007/BF03161781

Geddes, P., Murphy, L., Astudillo-Scalia, Y., Blasini, D., Nugent, S., Ríos, M. J., et al. (2021). Microsatellite markers reveal unprecedented high frequencies of hybridization among *Typha* species in the Midwestern US. *Wetlands* 41, 24. doi: 10.1007/s13157-021-01429-2

Goulet, B. E., Roda, F., and Hopkins, R. (2017). Hybridization in plants: Old ideas, new techniques. *Plant Physiol.* 173, 65–78. doi: 10.1104/pp.16.01340

Grace, J. B., and Wetzel, R. G. (1982). Niche differentiation between two rhizomatous plant species: *Typha latifolia* and *Typha angustifolia*. *Can. J. Bot.* 60, 46–57. doi: 10.1139/b82-007

Gronewold, A. D., and Rood, R. B. (2019). Recent water level changes across Earth's largest lake system and implications for future variability. *J. Great Lakes Res.* 45, 1–3. doi: 10.1016/j.jglr.2018.10.012

Hall, S. J., and Zedler, J. B. (2010). Constraints on sedge meadow self-restoration in urban wetlands. *Restor. Ecol.* 18, 671–680. doi: 10.1111/j.1526-100X.2008.00498.x

Hauser, L., and Seeb, J. E. (2008). Advances in molecular technology and their impact on fisheries genetics. *Fish Fisheries* 9, 473–486. doi: 10.1111/j.1467-2979.2008.00306.x

Henry, M., Bombelli, A., Trotta, C., Alessandrini, A., Birigazzi, L., Sola, G., et al. (2013). GlobAllomeTree: International platform for tree allometric equations to support volume, biomass and carbon assessment. *iForest - Biogeosciences Forestry* 6, 326. doi: 10.3832/ifor0901-006

Hochholdinger, F., and Baldauf, J. A. (2018). Heterosis in plants. *Curr. Biol.* 28, R1089–R1092. doi: 10.1016/j.cub.2018.06.041

Horppila, J., and Nurminen, L. (2001). The effect of an emergent macrophyte (*Typha angustifolia*) on sediment resuspension in a shallow north temperate lake. *Freshw. Biol.* 46, 1447–1455. doi: 10.1046/j.1365-2427.2001.00765.x

Kirk, H., Connolly, C., and Freeland, J. R. (2011). Molecular genetic data reveal hybridization between *Typha angustifolia* and *Typha latifolia* across a broad spatial scale in eastern North America. *Aquat. Bot.* 95, 189–193. doi: 10.1016/j.aquabot.2011.05.007

Kuehn, M. M., and White, B. N. (1999). Morphological analysis of genetically identified cattails *Typha latifolia*, *Typha angustifolia*, and *Typha* ×*glauca*. *Can. J. Bot.* 77, 906–912. doi: 10.1139/b99-037

Kuhn, M. (2008). Building predictive models in R using the caret package. *J. Stat. Software* 28, 1–26. doi: 10.18637/jss.v028.i05

Larkin, D. J., Freyman, M. J., Lishawa, S. C., Geddes, P., and Tuchman, N. C. (2012). Mechanisms of dominance by the invasive hybrid cattail *Typha* × *glauca*. *Biol. Invasions* 14, 65–77. doi: 10.1007/s10530-011-0059-y

Lawrence, B. A., Bourke, K., Lishawa, S. C., and Tuchman, N. C. (2016). *Typha* invasion associated with reduced aquatic macroinvertebrate abundance in northern Lake Huron coastal wetlands. *J. Great Lakes Res.* 42, 1412–1419. doi: 10.1016/j.jglr.2016.08.009

Liland, K. H., Mevik, B.-H., and Wehrens, R. (2023) *Pls: Partial Least Squares and Principal Component Regression. R package version 2.8-2*. Available online at: https://CRAN.R-project.org/package=pls.

Lishawa, S. C., Albert, D. A., and Tuchman, N. C. (2010). Water level decline promotes *Typha X glauca* establishment and vegetation change in Great Lakes coastal wetlands. *Wetlands* 30, 1085–1096. doi: 10.1007/s13157-010-0113-z

Lishawa, S. C., Dunton, E. M., Pearsall, D. R., Monks, A. M., Himmler, K. B., Carson, B. D., et al. (2020). Wetland waterbird food resources increased by harvesting invasive cattails. *J. Wildlife Manage.* 84, 1326–1337. doi: 10.1002/jwmg.21912

Lishawa, S. C., Lawrence, B. A., Albert, D. A., and Tuchman, N. C. (2015). Biomass harvest of invasive *Typha* promotes plant diversity in a Great Lakes coastal wetland. *Restor. Ecol.* 23, 228–237. doi: 10.1111/rec.12167

Mallet, J. (2005). Hybridization as an invasion of the genome. *Trends Ecol. Evol.* 20, 229–237. doi: 10.1016/j.tree.2005.02.010

McKenzie-Gopsill, A., Kirk, H., Drunen, W. V., Freeland, J. R., and Dorken, M. E. (2012). No evidence for niche segregation in a North American Cattail (*Typha*) species complex. *Ecol. Evol.* 2, 952–961. doi: 10.1002/ece3.225

Mitchell, N., Campbell, L. G., Ahern, J. R., Paine, K. C., Giroldo, A. B., and Whitney, K. D. (2019). Correlates of hybridization in plants. *Evol. Lett.* 3, 570–585. doi: 10.1002/evl3.146

Niklas, K. J., and Enquist, B. J. (2002). On the vegetative biomass partitioning of seed plant leaves, stems, and roots. *Am. Nat.* 159, 482–497. doi: 10.1086/339459

Ohsowski, B. M., Dunfield, K. E., Klironomos, J. N., and Hart, M. M. (2016). Improving plant biomass estimation in the field using partial least squares regression and ridge regression. *Botany* 94, 501–508. doi: 10.1139/cjb-2016-0009

Peters, A., and Hothorn, T. (2023) *Ipred: Improved Predictors. R package version 0.9-14*. Available online at: https://CRAN.R-project.org/package=ipred.

Pieper, S. J., Nicholls, A. A., Freeland, J. R., and Dorken, M. E. (2017). Asymmetric hybridization in cattails (*Typha* spp.) and its implications for the evolutionary maintenance of native *Typha latifolia*. *J. Heredity* 108, 479–487. doi: 10.1093/jhered/esx036

Pottier, J., and Jabot, F. (2017). Non-destructive biomass estimation of herbaceous plant individuals: A transferable method between contrasted environments. *Ecol. Indic.* 72, 769–776. doi: 10.1016/j.ecolind.2016.08.030

R Core Team (2023). *R: A Language and Environment for Statistical Computing* (Vienna: R Foundation for Statistical Computing). Available at: https://www.R-project.org/.

Sagarin, R., Carlsson, J., Duval, M., Freshwater, W., Godfrey, M. H., Litaker, W., et al. (2009). Bringing molecular tools into environmental resource management: Untangling the molecules to policy pathway. *PloS Biol.* 7, e1000069. doi: 10.1371/journal.pbio.1000069

Schrank, A. J., and Lishawa, S. C. (2019). Invasive cattail reduces fish diversity and abundance in the emergent marsh of a Great Lakes coastal wetland. *J. Great Lakes Res.* 45, 1251–1259. doi: 10.1016/j.jglr.2019.09.013

Schuelke, M. (2000). An economic method for the fluorescent labeling of PCR fragments. *Nat. Biotechnol.* 18, 233–234. doi: 10.1038/72708

Smith, S. G. (1967). Experimental and natural hybrids in North American *Typha* (Typhaceae). *Am. Midland Nat.* 78, 257–287. doi: 10.2307/2485231

Snow, A. A., Travis, S. E., Wildová, R., Fér, T., Sweeney, P. M., Marburger, J. E., et al. (2010). Species-specific SSR alleles for studies of hybrid cattails (*Typha latifolia* × *T. angustifolia*; Typhaceae) in North America. *Am. J. Bot.* 97, 2061–2067. doi: 10.3732/ajb.1000187

Todesco, M., Pascual, M. A., Owens, G. L., Ostevik, K. L., Moyers, B. T., Hübner, S., et al. (2016). Hybridization and extinction. *Evolutionary Appl.* 9, 892–908. doi: 10.1111/eva.12367

Travis, S. E., Marburger, J. E., Windels, S., and Kubátová, B. (2010). Hybridization dynamics of invasive cattail (Typhaceae) stands in the Western Great Lakes Region of North America: A molecular analysis. *J. Ecol.* 98, 7–16. doi: 10.1111/j.1365-2745.2009.01596.x

Travis, S. E., Marburger, J. E., Windels, S. K., and Kubátová, B. (2011). Clonal structure of invasive cattail (Typhaceae) stands in the Upper Midwest region of the US. *Wetlands* 31, 221–228. doi: 10.1007/s13157-010-0142-7

Trebitz, A. S. (2006). Characterizing seiche and tide-driven daily water level fluctuations affecting coastal ecosystems of the Great Lakes. *J. Great Lakes Res.* 32, 102–116. doi: 10.3394/0380-1330(2006)32[102:CSATDW]2.0.CO;2

Tsyusko-Omeltchenko, O. V., Schable, N. A., Smith, M. H., and Glenn, T. C. (2003). Microsatellite loci isolated from narrow-leaved cattail *Typha angustifolia*. *Mol. Ecol. Notes* 3, 535–538. doi: 10.1046/j.1471-8286.2003.00502.x

Tuchman, N. C., Larkin, D. J., Geddes, P., Wildova, R., Jankowski, K., and Goldberg, D. E. (2009). Patterns of environmental change associated with *Typha x glauca* invasion in a Great Lakes coastal wetland. *Wetlands* 29, 964–975. doi: 10.1672/08-71.1

Vaz, A. S., Alcaraz-Segura, D., Campos, J. C., Vicente, J. R., and Honrado, J. P. (2018). Managing plant invasions through the lens of remote sensing: A review of progress and the way forward. *Science of The Total Environment*. 642, 1328–1339. doi: 10.1016/j.scitotenv.2018.06.134

Venables, W. N., and Ripley, B. D. (2013). *Modern Applied Statistics with S-PLUS* (New York: Springer Science & Business Media).

Voss, E. G., and Reznicek, A. A. (2012). *Field Manual of Michigan Flora* (Ann Arbor: University of Michigan Press). doi: 10.3998/mpub.345399

Wasko, J. D., McGonigle, T. P., Goldsborough, L. G., Wrubleski, D. A., Badiou, P. H., and Armstrong, L. M. (2022). Use of shoot dimensions and microscopic analysis of leaves to distinguish Typha latifolia, Typha angustifolia, and their invasive hybrid Typha xglauca. *Wetlands Ecol. Manage.* 30, 19–33. doi: 10.1007/s11273-021-09836-2

Woo, I., and Zedler, J. B. (2002). Can nutrients alone shift a sedge meadow towards dominance by the invasive *Typha* × *glauca*. *Wetlands* 22, 509–521. doi: 10.1672/0277-5212(2002)022[0509:CNASAS]2.0.CO;2

Zapfe, L., and Freeland, J. R. (2015). Heterosis in invasive F1 cattail hybrids (*Typha* × *glauca*). *Aquat. Bot.* 125, 44–47. doi: 10.1016/j.aquabot.2015.05.004

Zedler, J. B., and Kercher, S. (2004). Causes and consequences of invasive plants in wetlands: Opportunities, opportunists, and outcomes. *Crit. Rev. Plant Sci.* 23, 431–452. doi: 10.1080/07352680490514673

frontiers | Frontiers in Plant Science

TYPE Original Research
PUBLISHED 10 April 2024
DOI 10.3389/fpls.2024.1367152

OPEN ACCESS

EDITED BY
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RECEIVED 08 January 2024
ACCEPTED 26 March 2024
PUBLISHED 10 April 2024

CITATION
Shahzad K, Zhu M, Cao L, Hao Y, Zhou Y, Liu W and Dai J (2024) Phylogenetic conservation in plant phenological traits varies between temperate and subtropical climates in China.
Front. Plant Sci. 15:1367152.
doi: 10.3389/fpls.2024.1367152

Phylogenetic conservation in plant phenological traits varies between temperate and subtropical climates in China

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Phenological traits, such as leaf and flowering dates, are proven to be phylogenetically conserved. The relationship between phylogenetic conservation, plant phenology, and climatic factors remains unknown. Here, we assessed phenological features among flowering plants as evidence for phylogenetic conservatism, the tendency for closely related species to share similar ecological and biological attributes. We use spring phenological traits data from 1968-2018 of 65 trees and 49 shrubs in Xi'an (temperate climate) and Guiyang (subtropical climate) to understand plant phenological traits' relationship with phylogeny. Molecular datasets are employed in evolutionary models to test the phylogenetic conservatism in spring phenological characteristics in response to climate-sensitive phenological features. Significant phylogenetic conservation was found in the Xi'an plant's phenological traits, while there was a non-significant conservation in the Guiyang plant species. Phylogenetic generalized least squares (PGLS) models correlate with phenological features significantly in Xi'an while non-significantly in Guiyang. Based on the findings of molecular dating, it was suggested that the Guiyang species split off from their relatives around 46.0 mya during the middle Eocene of the Tertiary Cenozoic Era, while Xi'an species showed a long evolutionary history and diverged from their relatives around 95 mya during the late Cretaceous Mesozoic Era. First leaf dates (FLD) indicative of spring phenology, show that Xi'an adjourned the case later than Guiyang. Unlike FLD, first flower dates (FFD) yield different results as Guiyang flowers appear later than Xi'an's. Our research revealed that various factors, including phylogeny, growth form, and functional features, influenced the diversity of flowering phenology within species in conjunction with local climate circumstances. These results are conducive to understanding evolutionary conservation mechanisms in plant phenology concerning evolutionary processes in different geographical and climate zones.

KEYWORDS

geographical regions, plant phenology, plant functional traits, phylogenetic conservation, temperate and subtropical climates

1 Introduction

Phylogenetic conservatism (closely related species tend to show similar traits) might be the biological basis for specific phenological events in plants or sensitivity to abiotic environmental factors (Davies et al., 2013; CaraDonna et al., 2014; Yang et al., 2021). Still, it is poorly understood how evolutionary mechanisms and geological and climatic conditions influence plant phenology and phylogeny relationships. Numerous studies focus more on the interannual variation of phenology that is affected by climatic factors (such as temperature, precipitation, and daylight) (Ge et al., 2015; Piao et al., 2019). In contrast, many studies demonstrated that plants are now leafing out earlier and flowering earlier in response to a warming climate (Wolkovich et al., 2012; Bucher et al., 2018; Menzel et al., 2020; Rosbakh et al., 2021). Climate change is due to geological changes such as latitudinal range (Hickling et al., 2006; Mason et al., 2015) and elevational extent (Chen et al., 2011). However, recent research suggested that the biological foundation for phenological occurrences in some plants or sensitivity to abiotic environmental stimuli may be related to plant phylogeny, which states that closely related species tend to exhibit comparable phenological properties (Caradonna and Inouye, 2015; Yang et al., 2021). This might undermine evolutionary conservatism by causing variations in phenological timing or temperature sensitivity among closely related species. Phylogenetic conservation may be obscure because the same biological groups may encounter various environmental limitations and have followed different evolutionary pathways (Davies et al., 2013; Du et al., 2015). Therefore, it is essential to accurately improve the future forecast of how geographical and environmental factors combined with phylogenetic conservatism impact plant phenophases.

Changes in plant phenology are not uniform across the globe due to the variance in plant phenological sensitivity to climate change (Menzel et al., 2006; Gao et al., 2019). Spatial variance in plant phenology change rate can modify well-established phenological patterns along geographical gradients (Ma et al., 2018; Vandvik et al., 2018; Liu et al., 2019). There are substantial spatial variations because of regional geographical conditions affecting climate (van der Wiel and Bintanja, 2021). For example, surprising regional differences with local hotspots have been identified in temperature changes across the United States (Eilperin et al., 2020). The constraint of phylogenetic conservation change in plant phenological features due to geographical and climatic zones must be understood at a regional scale (i.e., climate differences and geographical variables) (Alice Boyle and Bronstein, 2012). Relatively little attention has been paid to the potential impacts of geographical variables (such as latitude and altitude) that influence climatic variability, which will have consequences in phenological and phylogenetic conservation. By studying these variables at different spatial and temporal scales, the effects of climate and geography on the evolutionary mechanisms influencing phylogenetic conservatism in plant phenophases will be more correctly predicted.

Phylogenetic conservation contributes to plants by clarifying taxonomic status, identifying unique evolutionary lineages, determining relictual and recently derived species, and investigating the phylogenetic value for conservation priority between regional and widespread species (Coates, 2000; Ryder, 1986). The phylogenetic conservatism in plant phenophases in response to climatic sensitivity has been well-reported (Davies et al., 2013; Caradonna and Inouye, 2015; Du et al., 2015). However, the mechanism behind how climatic factors would add considerable uncertainty and affect the relationship between plant phenology and phylogeny is still unknown. For a meaningful prediction of plant phylogeny and phenology correlations with regional climatic differences, an ability to consider attributes of shared evolutionary history is essential (Cleland et al., 2012; Wolkovich and Cleland, 2014).

The phylogenetic conservatism of phenological features has since been evaluated using various techniques, such as Blomberg's K and Pagel's lambda methods (Blomberg et al., 2003; Münkemüller et al., 2012; Davies et al., 2013; Li et al., 2016). Significant efforts were made to gather phenological records and plant characteristic data from national flora books to know the relationship between plant phenology and phylogeny. However, the accuracy of these studies' phenological and genetic data is not very high (Du et al., 2015, 2017). The extent of these studies ranged from three years (Basnett et al., 2019) to a few decades (Davies et al., 2013; Du et al., 2017; Yang et al., 2021), which varied greatly in these studies. Using incomplete genetic and phenological datasets can cause incongruence in the phylogenetic signals in plant phenophases. The comparison of chloroplast genome (cpDNA) sequences among different plant species is an essential source of plant molecular phylogenetic data, making it an ideal molecule for tracing the evolutionary history of plant species (Chen et al., 2022). Here, we use the complete chloroplast genome (cpDNA) dataset to reconstruct the phylogenetic gene genealogies of plant species to better understand the phylogenetic conservation between plant phenological and climatic sensitive phenological traits.

Using plant functional traits (the characteristics of plants that determine responses in the surrounding environment, other species, and trophic levels) has become an efficient and accurate way to investigate the effects of large-scale land and climate change (Díaz and Cabido, 2001; Suding et al., 2008; Pérez-Harguindeguy et al., 2016). The functional traits of plants are essential biological characteristics that most likely reflect the adaptation strategies of plants to the environment (Funk et al., 2017). However, our understanding of the confounding influences of plant functional traits (i.e., life forms, pollination style, deciduous, and evergreen) affect plant phenology and phylogenetic conservation remains unclear. There is evidence that differences in plant functional traits, such as life form, and biotic and abiotic pollination mode, may also be associated with interspecific variation in plant phenology (Wolkovich and Cleland, 2014; Du et al., 2017; Bucher et al., 2018; Liu et al., 2021). However, some studies also found there were no significant differences in the flowering time of the entomophilous plants (Janeček et al., 2021). Therefore, the relationship between functional traits and plant phenology still deserves further exploration. This study analyzed the relationship between plant functional traits and the spring phenological characteristics of species in two different geographical regions.

Climate determines the reproductive phenology of plants, such as temperature and precipitation cause various reproductive structures to grow and mature (Rathcke and Lacey, 1985; Høye et al., 2007; Wang et al., 2023). Ting et al. (2008) concluded that the fruiting period is progressively shorter with increasing latitude because the climate varies with the geographical gradient. On the other hand, our analysis may become highly ambiguous if we ignore the long-term variations in geography and climate that affect the length of reproductive phenology. Recent studies on the phenology of common alder are consistent with the idea that local geographical climate variation affects phenology (Ziello et al., 2009; Wang et al., 2023). For instance, China's average annual rainfall gradually drops as northern latitude rises and east longitude falls (Xu and Zhang, 2020), shortening the time that photosynthesis (Huxman et al., 2004). Another illustration is when a species cannot break the dormant state of its seeds due to an environment that is too warm for it. On the other hand, frost damage can occur to plants that flower early or prematurely (Morin et al., 2007). Thus, the length of reproductive phenology may be shortened. Consequently, a thorough account of the global distribution and spatial patterns of the duration of reproductive phenology is needed. Therefore, it's equally essential to comprehend how plants might react to climate change.

This study investigates the phylogenetic conservatism in spring phenological characteristics and the functional traits correlations with phenological elements such as life forms (trees and shrubs) and evergreen or deciduous species in two different climatic conditions zones, i.e., Xi'an (temperate climatic) and Guiyang (subtropical climatic) in China. The phylogenetic signal and evolutionary models analyzed phylogenetic conservation in plant phenological traits. It should be noted that because a small sample size may reduce the predicted accuracy of the phenological model, we excluded species with less than 50 years of flowering and leaf-out data. To accomplish our objective, we address the following questions: (i) To explore the phylogenetic conservation between plant spring phenophases and climatic-sensitive spring phenophases of two different climatic zones, i.e., Xi'an and Guiyang in China. (ii) How do temperate and subtropical climatic conditions influence the phylogenetic signals in plant phenological traits of Xi'an and Guiyang species? (iii) To investigate different geographical and climatic conditions that are directly connected to the area's evolutionary processes, which strongly impact plant spring phenology and phylogeny relationships.

2 Materials and methods

2.1 Study sites

Xi'an and Guiyang are two historical regions of China with different geographical and climatic conditions. Xi'an (34°12'N, 108° 57'E) is the capital of the Shaanxi province located in north-central China (Bai et al., 2010). Xi'an (400m a.s.l) has a temperate semi-humid climate with an average temperature of 13°C and average precipitation of 578 mm annually (Bai et al., 2010). The vegetation in Xi'an is sharply differentiated into northern and southern zones with mixed deciduous broad-leaved and evergreen forests. Guiyang (26°38'N and 106°37'E) is the city of Guizhou Province, located in southwestern China. Guiyang is a humid subtropical climatic region at 1,050-1,275m a.s.l (Figure 1). Due to its high altitude, the annual temperature is 15.3°C, and rainfall is about 11,000mm (Figure 2). Guiyang City typically has harsh climatic conditions (subtropical climate), such as high relative humidity, long, cloudy, rainy days, and little sunshine. Natural wealth lies in its forests; about one-tenth of the land is under natural forest. It has rich and valuable woodlands of wild plants, among which several highly valued herbs are used in traditional Chinese medicine.

2.2 Phenological and meteorological data observation

The phenological observation records of the first leaf dates (FLD) and first flower dates (FFD) of 77 and 37 plant species from Xi'an and Guiyang respectively have been collected from the China Phenological Observation Network (CPON). The details of plant species and functional traits (life forms and evergreen or deciduous species) are given in Supplementary Table 1. The phenological observation data at each site (Xi'an and Guiyang) were collected from 1968-2018, followed by defined observation criteria and procedures (Wan and Liu, 1979). According to observational standards, the first leaf out and flowering dates are determined as a fixed individual plant of a specific species starts generating the first leaf and the first flower, respectively (Dai et al., 2013; Wang et al., 2015). We then compared differences in phenophases (FLD and FFD) and their deviations in the period 1968-2018 between Xi'an and Guiyang species.

The meteorological data is extracted from the China Meteorological Data Service Center website (https://data.cma.cn/) to acquire daily mean temperatures and precipitation from 1963 to 2018 for each station. We used the daily mean temperatures and precipitation data to calculate spring phenology's temperature and precipitation sensitivity.

2.3 Molecular phylogenetics and divergence time analysis

The available complete chloroplast genomes (cpDNA) of selected Xi'an and Guiyang station plants were downloaded from GenBank (NCBI accession numbers and species details presented in Supplementary Table 1). *Agaricus bisporus* and *Cantharellus cibarius* complete genomes are added as outgroups. Before phylogenetic analysis, each station dataset is aligned separately using Genious v 12.0 software and the multiple alignment application MAFFT (Darling et al., 2004). We directly generated an alternate phylogeny from DNA sequence data to test the sensitivity of our findings to the tree topology. Further details of tree reconstruction are provided as supplementary information (Supplementary Information Index II). We refer to the final topology as the ML tree.

In BEAST v1.8.0, the multiple fossil calibrations (Supplementary Information Index I) were used to estimate the

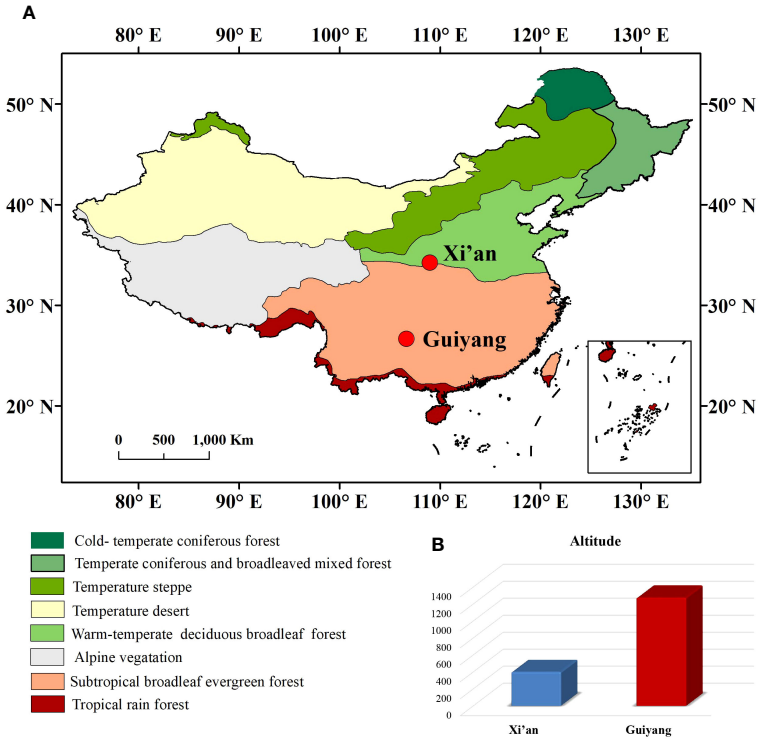

FIGURE 1
(A) Representation of geographical map with all climatic zones of China and the location of Xi'an and Guiyang. **(B)** The altitude difference between Xi'an and Guiyang.

divergence periods between lineages with a relaxed clock and Yule process speciation prior (Near et al., 2005; Marshall, 2008; Drummond et al., 2012; Lukoschek et al., 2012). MrModeltest 2.3 was used to choose the GTRAGMMA nucleotide substitution model (Nylander, 2004). In this case, we considered the uncertainty of prior knowledge using a normal prior probability distribution. The parameters were sampled every 5,000 generations, while the analysis was run for 20,000,000 generations. Using Tracer v. 1.6 (Drummond et al., 2012), the appropriate sample size (>200) was established, and the first 10% of the samples were eliminated as burn-ins. To construct a maximum clade credibility chronogram depicting the mean divergence time estimates with 95% highest posterior density (HPD) intervals, we used Tree Annotator v.1.8.0 (Drummond et al., 2012) to compile the collection of post-burn-in trees and associated parameters. FigTree V1.3.1 (Drummond et al., 2012) displayed the resulting divergence times.

2.4 Phylogenetic conservatism in spring phenology

For phylogenetic conservatism, phylogenetic topology was the resort. The following analyses were carried out using the "ape" (Paradis et al., 2004) and "picante" (Kembel et al., 2010) libraries in R (http://www.R-project.org; R Development Core Team). Blomberg's K technique was used to analyze each station's phylogenetic signal using spring phenological variables (Blomberg et al., 2003; Gao et al., 2022). A trait's evolution is influenced by phylogeny if K=1 shows that the inter-species correlation equals the Brownian expectation. According to Brownian motion (BM), K>1 demonstrates that trait similarity is more significant than expected (Blomberg et al., 2003). In contrast, K<1 denotes either stability (i.e., the characteristic is phylogenetically conserved) or absence of phylogenetic structure (i.e., the trait is not phylogenetically conserved) (Wiens et al., 2010). Using the *phytools* library in the R, we calculated the K parameter for each phenological characteristic. To determine whether the observed values significantly deviate from the randomized arrangement. The P-value might alternatively be derived by 1000 interactions in the computation of K (Revell, 2012).

We approached the close fitting of evolutionary models for evaluating phylogenetic conservatism as a random variation and evolutionary stasis shaped by selection can be directly captured by the white noise (WN) model and Ornstein-Uhlenbeck (OU) model, respectively (Felsenstein, 1985; Blomberg et al., 2003; Butler and King, 2004; Kozak and Wiens, 2010; Diniz-Filho et al., 2015). Phylogenetic signal representation (PSR) curves to investigate the evolutionary patterns of trait development (Diniz-Filho et al., 2012). We assessed each trait's evolutionary processes by comparing the close fitting of the three most popular evolutionary models (BM, OU, and WN) with the highest value of weight Akaike Information Criterion model to make up for the limitations of the phylogenetic signal approach (wAIC) (Butler and King, 2004; Diniz-Filho et al., 2012). Phylogenetically independent trait variation was modeled using the WN model with random variation, the BM model of progressive drift, and the OU model for stasis or stable selection. It

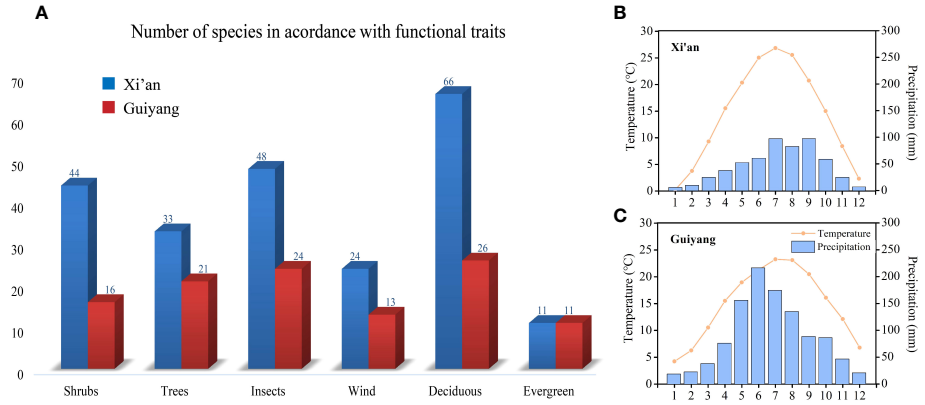

FIGURE 2
(A) Graphical overview of shrubs and trees; wind and insect-pollinated species; deciduous and evergreen species in Xi'an and Guiyang **(B)** Temperature and precipitation graph of Xi'an **(C)** Temperature and precipitation graph of Guiyang.

is predicated on the phylogenetic eigenvector regression (PVR) model, which employs eigenvectors obtained and chosen from a pairwise phylogenetic distance matrix to describe trait variation. To construct PSR curves, we followed Staggemeier et al. (2015) methods to know the evolution and conservation of phenological traits. Additionally, the PSR curve's shape reflects the pace of trait evolution in the phylogenetic tree (Diniz-Filho et al., 2012).

2.5 Sensitivity of plant phenology and phylogenetic conservation

The spring phenological traits sensitivity analysis to temperature and precipitation was performed differently between Guiyang and Xi'an stations. To identify the preseason, we determined the Pearson's correlation coefficient (r) between FLD/FFD and temperature during the 1 day, 2 days,…, and 120 days before the average FLD/FFD of the study period, respectively. The preseason was then identified as the period with the highest "r" (Dai et al., 2019). The regression slope between the FLD/FFD and the daily mean temperature averaged throughout the preseason determined the temperature and precipitation sensitivity. The above method (section 2.4) was applied to know the phylogenetic conservation in the sensitivity of plant phenology.

We performed the frequency distribution analysis to compare the temperature sensitivity as days and precipitation sensitivity as days/mm to check the advancement of the Xi'an and Guiyang species in spring phenology (FLD and FFD).

2.6 Statistical analysis

This paper mainly analyzes the influence of three functional traits, i.e., life form (shrubs and trees), pollination style (biotic and abiotic), and distinct plant groups (deciduous and evergreen), on spring phenological traits (Figure 2). Refer to "Flora of China" for functional traits classification: life form; trees whose maximum height exceeds six meters are classified as trees, and those whose size does not exceed six meters are grouped as shrubs. Pollination methods: gymnosperms and angiosperms with small, odorless flowers and many stamens are classified as anemophilous plants; angiosperms with large, fragrant flowers, conspicuous petals, and brightly colored flowers are grouped as insect-borne plants (Du et al., 2017). Deciduous plants are considered a group of plants that shed their leaves seasonally, while evergreen plants are considered a group of plants that keep their leaves throughout the entire year. We used phylogenetic generalized least squares (PGLS) models to compensate for phylogenetic autocorrelation. The '*pgls*' function from the Caper R package fits *PGLS* models (Orme et al., 2014). This allowed us to study the impacts of various plant function features on spring phenological traits in temperature and precipitation variations. Figure 2 represents the functional traits and numbers of species in Guiyang and Xi'an. We performed the mean and median range analysis to compare the plant functional features such as tree and shrub species, biotic and abiotic pollination, and deciduous and evergreen species between the spring phenological traits (FLD and FFD, temperature and precipitation sensitivities of FLD and FFD) of Guiyang and Xi'an.

3 Results

3.1 Plant phenological characteristics in Guiyang and Xi'an areas

Our results compare the spring phenological characteristics and the differences between each Guiyang and Xi'an species. Guiyang's first leaf dates (FLD) indicate that leafing begins at 40 days and lasts until 100, while Xi'an FLD findings suggest that later leafing starts after 60 days and lasts until 120 (Figure 3). Overall, Xi'an adjourned the case later than Guiyang. The first flower dates (FFD) produce distinct outcomes compared to FLD. While Xi'an's FFD results show later leaves beginning before 50 days and ending at 250 days, Guiyang's results show blooming flowers starting at 50 days and lasting more than 300 days. Typically, Guiyang flowers are later than that of Xi'an.

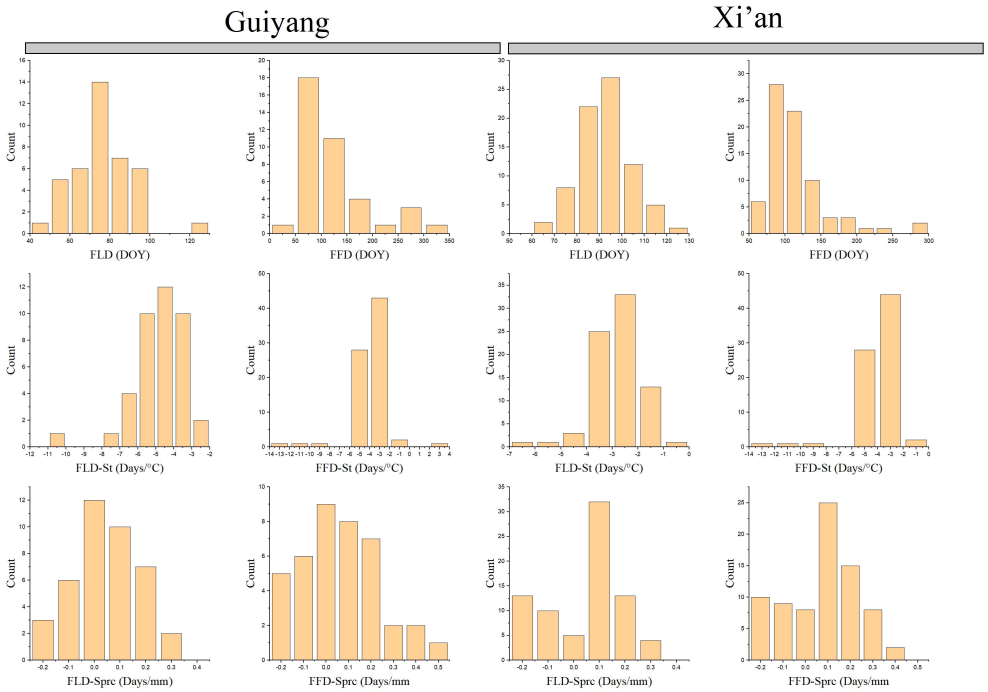

FIGURE 3
Spring phenophases of plant species collected from 1968 to 2018 in Guiyang and Xi'an. FLD, first leaf date; FFD, first flower date; FLD-S_T, first leaf date temperature sensitivity; FFD-S_T, first flower date temperature sensitivity; FLD-S_{Prc}, first leaf date precipitation sensitivity; FFD-S_{Prc}, first flower date precipitation sensitivity, respectively. DOY, Day of the year.

The spring phenological traits sensitive to temperature and precipitation reveal that Guiyang's temperature sensitivity leaf phenology (FLD-S_T) begins leafing when the temperature is between -8 and -2 °C. However, according to FLD-S_T data, plants from Xi'an begin to leaf at temperatures between -5 and -1 °C (Figure 3). In general, Xi'an has a lower leaf-out temperature than Guiyang. On the other hand, species from Guiyang and Xi'an commence flowering with an average temperature of -14 to -2 °C according to the temperature sensitivity of the first flower date (FLD-S_T) (Figure 3). In Guiyang and Xi'an species, the first leaf date (FLD-S_{prc}) precipitation sensitivity exhibits the same pattern, with an average precipitation of -0.2 to 0.3mm before leafing begins. In contrast, first flower date (FFD-S_{prc}) precipitation sensitivity analysis indicated that average rainfall starts flowering at -0.2 to 0.5mm in Guiyang. The FFD-S_{prc} findings for the Xi'an species show that flowering begins at -0.2 to 0.4mm in precipitation. Overall, Xi'an experiences less blooming than Guiyang.

3.2 Phylogenetic conservation in plant phenology and sensitivity of plant phenology

The K-value was below one for all the spring phenological characteristics in Xi'an and Guiyang, predicted by Brownian motion. However, the signal intensity was different between the two locations. The leaf and flower phenological characteristics show weaker and non-significant phylogenetic signals (K values) in Guiyang. Therefore, the phylogenetic conservation signals in the spring phenological characteristics (FLD, FLD-S_T, FLD-S_{prc}, FFD, FFD-S*T*, FFD-S_{prc}) of Xi'an species were solid and significant (Table 1). The WN model had the lowest wAIC value among the three evolutionary models, demonstrating that all the spring phenological traits were not preserved phylogenetically in Guiyang (Table 1). The OU model's lower wAIC number for all Xi'an phenological traits indicates that the evolution of spring phenological qualities was slower than predicted by the Brownian model. However, the findings from three evolutionary models of Xi'an show a similar pattern with substantial conservation, demonstrating that the degree of phylogenetic conservatism in phenological traits has recently changed.

3.3 Phylogenetic signals of each species (tree topology) in plant spring phenophases

Our molecular phylogenetic tree results demonstrate that closely related genera and species are located nearby in the topology (Figures 4, 5). However, the sizes of the circles in front of each species reveal the traits' values, i.e., a more significant size denotes later FLD or FFD, strong temperature sensitivity, or a greater need for precipitation. We addressed the outcomes of substantial phylogenetic conservation in Xi'an species here. Our findings show that the values of FFD, FFD-S_T, and FFD-S_{Prc} of

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TABLE 1 Phylogenetic conservation signals in spring phenophases and response to climatic sensitivity (temperature and precipitation).

No.	Name of Phenophases	Blomberg's K value	P Value	BM	OU	WN
Guiyang						
1	FLD	0.13	0.367	370.7142	335.716	333.3738*
2	FFD	0.12	0.463	478.10	462.64	461.87*
3	FLD-S_T	0.11	0.547	186.47	151.43	149.15*
4	FFD-S_T	0.09	0.646	241.212	218.061	215.7854*
5	FLD-S_{Prc}	0.08	0.83	291.75	278.93	274.89*
6	FFD-S_{Prc}	0.10	0.66	321.42	277.96	275.34*
Xi'an						
1	FLD	0.228	0.165	615.6328	598.8631*	603.7633
2	FFD	0.209	0.165	829.7437	807.9478*	808.2966
3	$FLDS_T$	0.207	0.217	232.9588	210.1808*	213.7730
4	$FFDS_T$	0.195	0.169	405.8885	382.4478*	383.6406
5	FLD-S_{Prc}	0.09	0.641	214.503	191.2332*	265.2623
6	FFD-S_{Prc}	0.133	0.128	224.303	199.1742*	214.5212

FLD, first leaf date; FFD, first flower date; FLD-S_T, first leaf date temperature sensitivity; FFD-S_T, first flower date temperature sensitivity; FLD-S_{Prc}, first leaf date precipitation sensitivity; FFD-S_{Prc}, first flower date precipitation sensitivity; WN, white noise model; OU, Ornstein-Uhlenbeck model; and BM, Brownian motion. * Means significant conservative value.

Xi'an species are more robust (Figure 5). High K values illustrate phylogenetic conservatism in FFD, FFD-S_T, and FFD-S_{Prc} in Xi'an. However, phylogenetic signals in the timing of spring phenology were not significantly different from these findings. These findings indicated that the phylogenetic conservatism is more significant and stable in the Xi'an species FFD, FFD-S_T, and FFD-S_{Prc} (Table 1; Figure 5). In contrast, the findings of the Guiyang species are identical, except that FLD-S_{Prc} results demonstrate stronger precipitation sensitivity (Table 1; Figure 4). According to different site ecologies, species sample sizes, and the accuracy of the underlying site-level phylogenetic trees, significance differed between sites. This variation is most likely due to these factors.

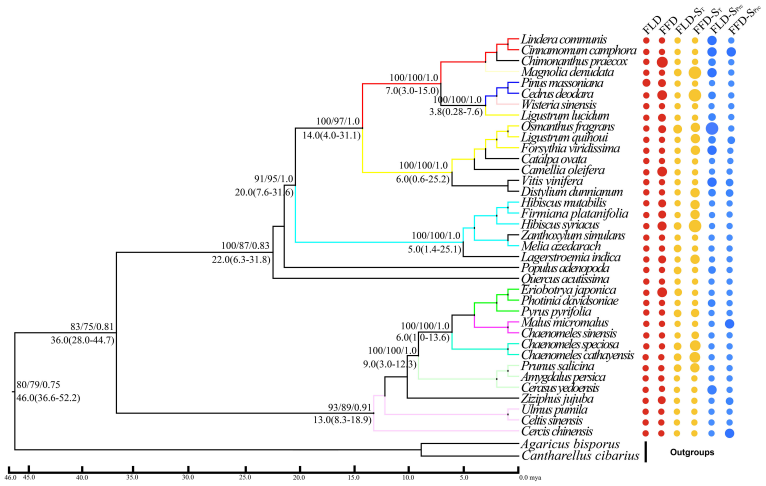

FIGURE 4
Phylogenetic signals of phenological traits in Guiyang station. A plot of spring phenological attributes at the tips of the phylogenetic tree of 37 species in Guiyang. FLD; first leaf date, FFD; first flower date, FLD-ST; first leaf date temperature sensitivity, FFD-ST; first flower date temperature sensitivity, FLD-SPrc; first leaf date precipitation sensitivity, FFD-SPrc; first flower date precipitation sensitivity, respectively. The sizes of the circles are proportional to the values of the traits, i.e., a larger size indicates later FLD or FFD, a larger size indicates stronger temperature sensitivity, and a larger size means FLD-SPrc is more sensitive with precipitation. Bootstrap values of Neighbor Joining, Maximum Likelihood and Bayesian Interference are written above each branch respectively. Molecular divergence time and 95% HPD values are written below each branch.

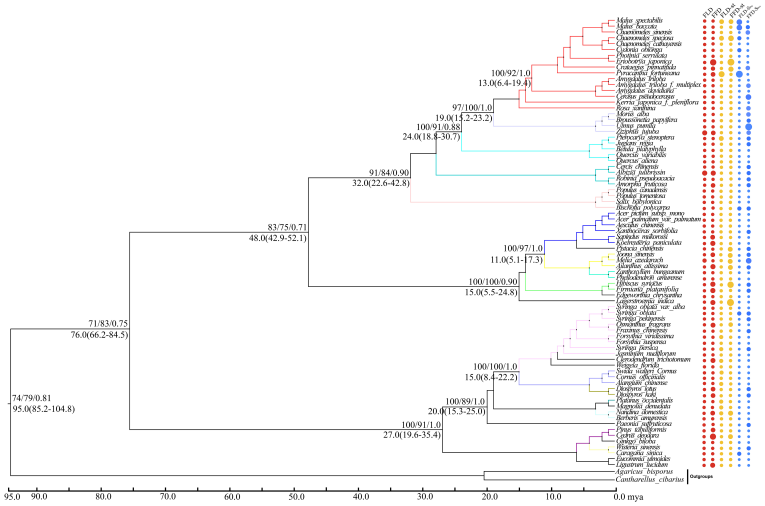

FIGURE 5
Topology diagram and phylogenetic signals of phenological traits in Xi'an station. A plot of spring phenological attributes at the tips of the phylogenetic tree of 77 species in Xi'an. FLD; first leaf date, FFD; first flower date, FLD-S_T; first leaf date temperature sensitivity, FFD-S_T; first flower date temperature sensitivity, FLD-S_{Prc}; first leaf date precipitation sensitivity, FFD-S_{Prc}; first flower date precipitation sensitivity, respectively. The sizes of the circles are proportional to the values of the characteristics, i.e., a larger size indicates later FLD or FFD, stronger temperature sensitivity (S_T with higher absolute value), and FLD-S_{Prc} more precipitation requirement. Bootstrap values of Neighbor Joining, Maximum Likelihood and Bayesian Interference are written above each branch respectively. Molecular divergence time and 95% HPD values are written below each branch.

3.4 The evolutionary history of Xi'an and Guiyang species

With strong bootstrap support values, both phylogenetic trees developed larger bi-phyletic clades. We calculated the divergence periods for the biphyletic tree lineages in Guiyang to be 36 mya based on the cpDNA data. The molecular dating results suggested that the species in each clade appeared to have separated from their relatives during the middle Eocene in the Tertiary Cenozoic Era at 46.0 mya (Figure 4). According to the cpDNA findings, the divergence periods for Xi'an's species' significant biphyletic tree lineages were 76 mya. The molecular dating findings also indicated that the Xi'an species in each clade separated from its relatives during the late Cretaceous Mesozoic epoch (Figure 5).

3.5 Evolutionary patterns of phenological traits

The spring phenological trait evolution rate was not as steady as predicted by the Brownian model. PSR curves were higher than the null model but lower than the 45-degree reference line for all phenological characteristics. The findings indicated that the black line in Guiyang was closer to the yellow line in each stage than in Xi'an, indicating that the development of spring phenological traits in Guiyang was more consistent with random shift and that phylogeny had a less significant impact than in Xi'an (Figure 6). Additionally, the pace of evolution is changing quickly. Although evolution progresses slowly at first, it accelerates quickly toward the conclusion of the curve. This demonstrated that younger species are evolving more rapidly in Xi'an and Guiyang than elder species.

3.6 Plant functional trait relationship with spring phenology

We investigated the functional characteristics (trees and shrubs; biotic and abiotic pollination; deciduous and evergreen) that influenced the spring phenology of plants in Guiyang and Xi'an. Our findings indicated that the dominant tree species in Xi'an exhibit later leafing and early flowering. In Guiyang, tree species had leafed out (15.55 days) later than shrubs with a significant correlation, while the tree's flowers bloomed (45 days) earlier than shrubs (Table 2). Trees predominate in Guiyang, suggesting leaf out and flowering would occur later. Similarly, the tree species of Xi'an also exhibit the same pattern, leaf out emerging (9.2 days) later and significantly correlated with shrubs. In comparison, flowering occurs (0.988 days) sooner in the trees than in shrubs in Xi'an (Table 2). The temperature and precipitation sensitivity of leaf out and flowering (FLD-S_T, FFD-S_T, FLD-S_{Prc}, and FFD-S_{Prc}) in Guiyang species indicate non-significant results. While temperature sensitivity results of leaf out (FLD-S_T) in Xi'an species show significant correlations, with a rise of 1°C, trees' reaction to temperature is 0.657 days/°C later than shrubs. On the other hand, the temperature sensitivity of flowering (FFD-S_T) and precipitation sensitivity of leaf out and flowering (FLD-S_{Prc} and FFD-S_{Prc}) in Xi'an species reveal a non-significant correlation.

Regarding pollination types (biotic and abiotic pollination of plants), we discovered that flowering phenology (FFD) and temperature-sensitive flower phenology (FFD-ST) interacted with a non-significant but strong relationship with biotic and abiotic pollination in Xi'an and Guiyang. At the same time, leaf-out phenology (FLD) and temperature-sensitive leaf phenology (FLD-ST) show a non-significant and weak relationship with abiotic and

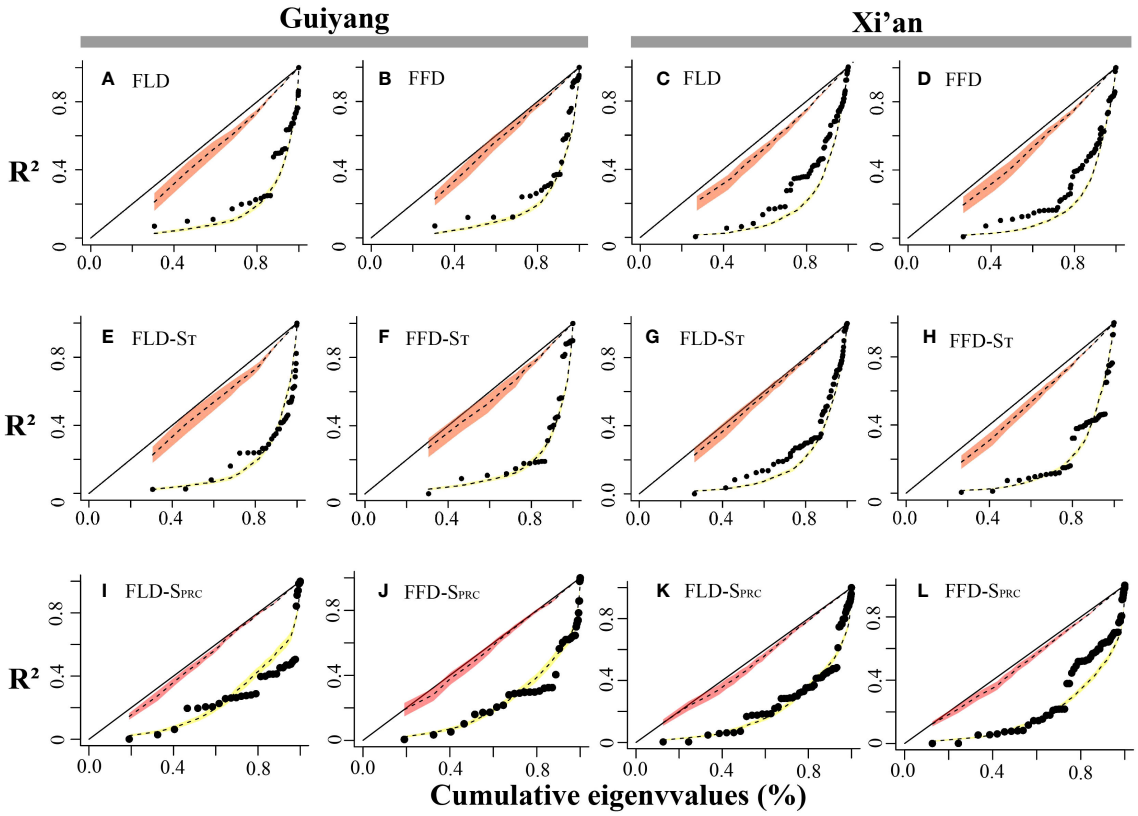

FIGURE 6
Spring phenological traits' phylogenetic signal representation (PSR) curves. The red and yellow bands show the confidence intervals for the BM model and WN random expectations. We can find the 1:1 line in black. The black dots represent the phylogenetic eigenvectors that were consecutively added. The x-axes reflect the cumulative total of the eigenvalues, while the y-axes represent the R^2 values of the successive PVR models. **(A)** FLD; first leaf date, **(B)** FFD; first flower date, **(C)** FLD; first leaf date, **(D)** FFD; first flower date, **(E)** FLD-S_T; first leaf date temperature sensitivity, **(F)** FFD-S_T; first flower date temperature sensitivity, **(G)** FLD-S_T; first leaf date temperature sensitivity, **(H)** FFD-S_T; first flower date temperature sensitivity, **(I)** FLD-S_{Prc}; first leaf date precipitation sensitivity, **(J)** FFD-S_{Prc}; first flower date precipitation sensitivity, **(K)** FLD-S_{Prc}; first leaf date precipitation sensitivity, **(L)** FFD-S_{Prc}; first flower date precipitation sensitivity, respectively.

biotic pollination in Xi'an and Guiyang species. The wind strongly correlated with flowering phenology under climatic sensitivities (FFD-S_T and FFD-S_{Prc}) (Table 2). These findings indicated that various pollination methods offer distinct correlation patterns with spring phenological features for the Guiyang and Xi'an species. Plants that receive both biotic and abiotic pollination produce leaves later but flowers earlier, with non-significant correlations (Table 2). The sensitivity of temperature and precipitation to leaf phenology reveals a weak relationship with biotic and abiotic pollination.

Our analysis of the distinct groups of plant species revealed a significant interaction between the flowering phenology in Xi'an and the mechanisms governing the species of evergreen and deciduous plants. Findings suggested that Guiyang species exhibit non-significant and weak correlations in all phenological traits with low values of the coefficient of PGLS. Contrarily, plant species in Xi'an show a different pattern with earlier leaf out in evergreen and deciduous plant species (2.046 days) and earlier flowering with significant and robust responses to evergreen and deciduous plant species (57.721* days) (Table 2). The temperature sensitivity findings of flowering (FFD-S_T) in Xi'an species are (4.626* days/°C) stronger and significant reaction (Table 2). The results of the precipitation sensitivity test for leaf out and flowering in Guiyang and Xi'an species indicate a non-significant and stronger response with evergreen and deciduous plant species (Table 2).

The mean and median range analysis revealed that similar phenological traits show a similar pattern in each functional attribute in both study areas, e.g., first leaf out dates (FLD) show a similar trend of mean and median range in each functional trait of Xi'an and Guiyang (Figure 7). The leaf out and flowering dates were counted as Days of Year (DOY), and the temperature sensitivity (days/°C) and precipitation sensitivity were shown as (days/mm) in Figure 6. The range of leaf-out phenology offers more than other phenophases in all functional traits, revealing that leaves take more time to bloom. Flowering phenology shows the minor range of mean and median in all available features except evergreen species. This represents more range, indicating that flowers take more time to bloom in the evergreen species group. The results also revealed that the mean and median of temperature sensitivity in all functional traits show the same pattern, indicating that temperature has equal importance in leaf and flowering phenology in all plant's functional characteristics. On the other side, the mean and median of the precipitation sensitivity range show a different pattern in all functional features, representing that

10.3389/fpls.2024.1367152

TABLE 2 The relationship between plant spring phenophases and sensitivity of spring phenophases with plant functional trait based on phylogenetic generalized least squares (PGLS) models.

Area	Functional Traits	Phenological Traits	Coefficient	P Value
Xi'an	Life Form (Trees and Shrubs)	FLD	9.2	<0.05*
		FFD	-0.988	0.925
		FLD-S_T	0.657	0.002*
		FFD-S_T	-0.367	0.602
		FLD-S_{Prc}	0.029	0.1739
		FFD-S_{Prc}	0.014	0.549
Guiyang	Life Form (Trees and Shrubs)	FLD	15.5476	<0.01*
		FFD	-45	0.214
		FLD-S_T	0.322	0.688
		FFD-S_T	-1.2833	0.6125
		FLD-S_{Prc}	-0.001	0.98
		FFD-S_{Prc}	0.015	0.763
Xi'an	Pollination Form (Biotic Vs Abiotic)	FLD	3.786	0.179
		FFD	-17.319	0.09
		FLD-S_T	0.178	0.417
		FFD-S_T	-0.402	0.5608
		FLD-S_{Prc}	0.0274	0.156
		FFD-S_{Prc}	-0.084	0.001
Guiyang	Pollination Form (Biotic Vs Abiotic)	FLD	7.19	0.169
		FFD	-22.821	0.453
		FLD-S_T	0.473	0.477
		FFD-S_T	-0.706	0.738
		FLD-S_{Prc}	0.030	0.187
		FFD-S_{Prc}	0.054	0.034
Xi'an	Species Group (Evergreen Vs Deciduous)	FLD	2.046	0.214
		FFD	57.721	<0.02*
		FLD-S_T	-0.53	0.688
		FFD-S_T	4.626	<0.01*
		FLD-S_{Prc}	-0.009	0.98
		FFD-S_{Prc}	-0.016	0.763
Guiyang	Species Group (Evergreen Vs Deciduous)	FLD	-5.31	0.321
		FFD	48.48	0.925
		FLD-S_T	-0.006	0.652
		FFD-S_T	0.304	0.602
		FLD-S_{Prc}	0.038	0.1739
		FFD-S_{Prc}	-0.046	0.549

FLD, first leaf date; FFD, first flower date; FLD-S_T, first leaf date temperature sensitivity; FFD-S_T, first flower date temperature sensitivity; FLD-S_{Prc}, first leaf date precipitation sensitivity; FFD-S_{Prc}, first flower date precipitation sensitivity. * Means significant value.

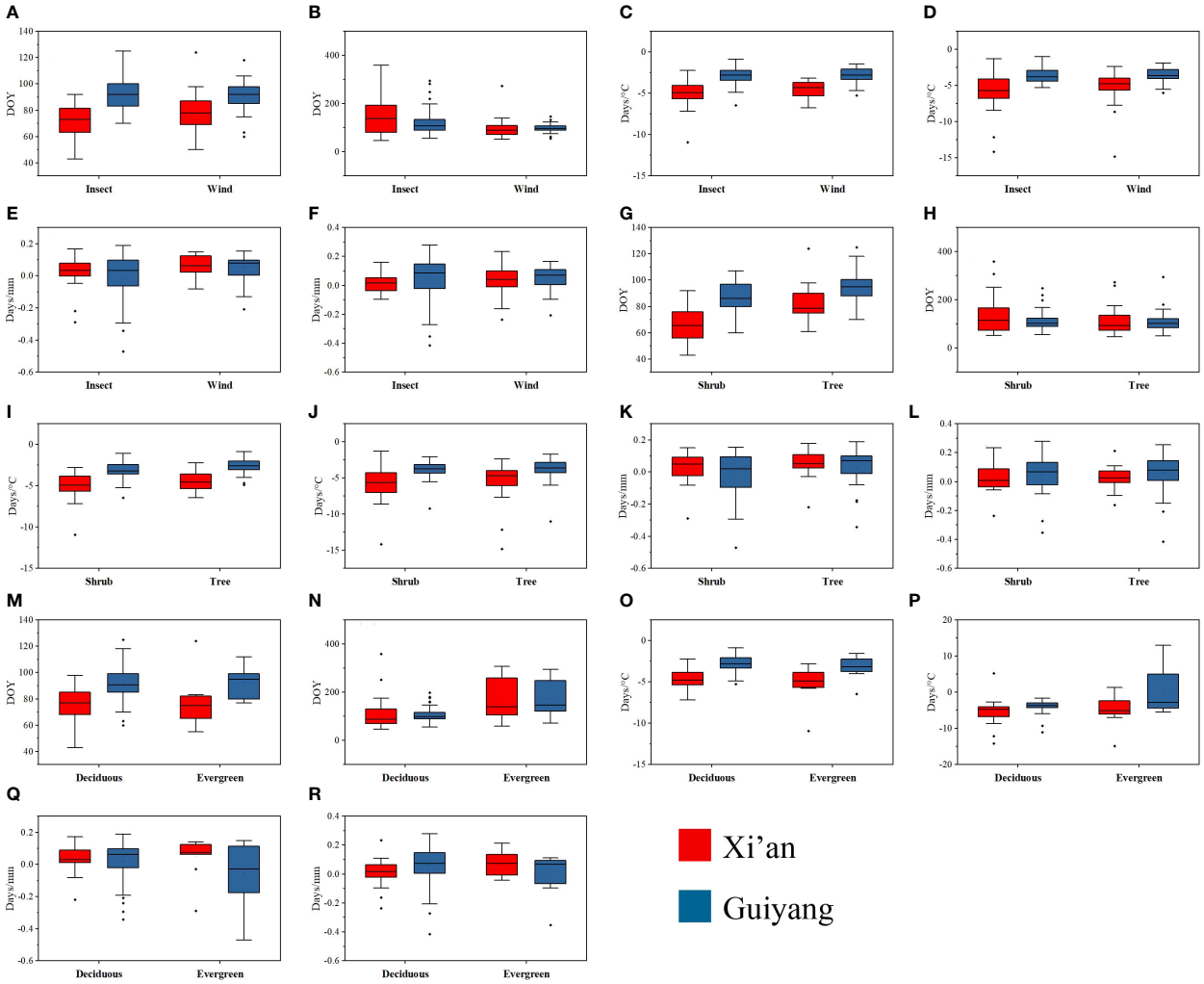

FIGURE 7
Mean and median range of spring phenological traits of tree and shrub species, biotic and abiotic pollination, and deciduous and evergreen species in response to first leaf dates, first flowering dates, temperature and precipitation sensitivity in Guiyang and Xi'an. **(A)** FLD of biotic and abiotic pollination species **(B)** FFD of biotic and abiotic pollination species **(C)** FLD-S_T of biotic and abiotic pollination species **(D)** FFD-S_T of biotic and abiotic pollination species **(E)** FLD-S_{Prc} of biotic and abiotic pollination species **(F)** FFD-S_{Prc} of biotic and abiotic pollination species **(G)** the FLD of tree and shrubs **(H)** FFD of tree and shrubs **(I)** FLD-S_T of tree and shrubs **(J)** FFD-S_T of tree and shrubs **(K)** FLD-S_{PRC} of tree and shrubs **(L)** FFD-S_{PRC} of tree and shrubs **(M)** FLD of evergreen and deciduous species **(N)** FFD of evergreen and deciduous species **(O)** FLD-S_T of evergreen and deciduous species **(P)** FFD-S_T of evergreen and deciduous species **(Q)** FLD-S_{Prc} of evergreen and deciduous species **(R)** FFD-S_{Prc} of evergreen and deciduous species. DOY: Day of the year, FLD; first leaf date, FFD; first flower date, FLD-S_T; first leaf date temperature sensitivity, FFD-S_T; first flower date temperature sensitivity, FLD-S_{Prc}; first leaf date precipitation sensitivity, FFD-S_{Prc}; first flower date precipitation sensitivity, respectively.

precipitation has different effects on each phenophases in each functional trait.

4 Discussion

4.1 Phylogenetic conservatism in plant phenophases

There was an association between phylogeny and the strength of the phenological shifts (Cohen et al., 2018; Inouye, 2022). The phylogenetic conservation in plant phenophases varies in different geographical and climatic conditions. In this study, we examined the correlations between plant spring phenology and the phylogeny of plant species in response to climatic and functional characteristics in Xi'an and Guiyang, China. Results revealed that phylogenetic signals in spring phenological traits were significantly conserved in Xi'an but non-conservative in Guiyang (Table 1). Significant phylogenetic signs in the Xi'an plant's phenological characteristics indicate that closely related species typically have similar climatic adaptability for phenology (Davies et al., 2013). However, our findings demonstrated that the spring phenological features (FLD and FFD) showed non-conservative phylogenetic signals in Guiyang (Table 1), which was comparable with the results of some studies across the Tibetan Plateau, demonstrating the absence of phylogenetic signals in leaf unfolding (Yang et al., 2021). Our finding also indicated that the Guiyang species' phylogenetic signs of blooming features were considerably more

significant than those of leaf-out traits. Contrarily, leaf unfolding, a photosynthetic characteristic, may maximize environmental resources for promoting reproductive development (Gougherty and Gougherty, 2018). Consequently, it may be more responsive to environmental changes than blooming characteristics. These correlations between structural and functional variables during evolution may have stabilized environmental circumstances and shaped flowering traits (Memmott et al., 2007).

4.2 Phylogenetic conservation in climatic sensitive spring phenological traits

An emerging result of phylogeography and plastic responses to environmental cues specific to a particular area, like temperature, precipitation, and photoperiod, were phylogenetic signals. Each site exposes its species to the same set of environmental stimuli. We observed considerable variation in strength of conservatism between temperature and precipitation-sensitive spring phenophases (FLD-ST, FFD-ST, FLD-SPrc, and FFD-SPrc), possibly reflecting variation in climatic differentiation. Significant phylogenetic signals were found in Xi'an species in temperature and precipitation-sensitive spring phenology (FLD-ST, FFD-ST, FLD-SPrc, and FFD-SPrc), indicating that species were consistent with the climate change factors. Nonetheless, in certain instances, the degree of phylogenetic conservatism for mean FLD and FFD was higher within sites than seen worldwide. Phylogenetic conservatism was frequently weaker within an area than across locations. Our findings point to a significant intrinsic evolutionary conservatism in the Xi'an species' phenological features, particularly noticeable when the species are subjected to similar external environmental stimuli. Recently, another study also reported the evolutionary signal of the temperature response of flowering time in Northeast China (Du et al., 2017). However, our findings demonstrated that the climatic sensitive spring phenological features (FLD-S_T, FFD-S_T, FLD-S_{Prc}, and FFD-S_{Prc}) showed non-conservative phylogenetic signals in Guiyang, which was similar to the results of spring phenological elements (FLD and FFD) (Table 1). Some studies in the Colorado Rocky Mountains (Caradonna and Inouye, 2015) demonstrated the absence of a phylogenetic signal (i.e., non-conservative phylogenetic signal) in the temperature sensitivity of spring phenological time. In comparison, the fact that the Rocky Mountains in Colorado have a harsher environment as the area under study and the solid abiotic selection pressures may restrict species growth and reproduction (Cavender-Bares et al., 2009; Lessard-Therrien et al., 2014). Their evolution is restricted in response to temperature and insignificant signals in temperature sensitivity species (Du et al., 2015; Basnett et al., 2019). The temperature and precipitation susceptibility of spring phenology in Guiyang species also showed no evidence supporting a phylogenetic signal, the same as spring phenology results. These findings demonstrated that the environmental (temperature and precipitation) and geographical circumstances (altitude) of the two regions (Xi'an and Guiyang) are distinct from one another and contribute to the variation in phylogenetic conservation in the spring phenology of plants.

Our findings suggest significant phylogenetic evidence in temperature and precipitation sensitivity phenological traits of Xi'an species while non-conservative in Guiyang plant species (Table 1), indicating species consistent with the studies for subtropical research (Li et al., 2020). Results revealed that the temperature of both areas is the same, but the precipitation difference is double that of Guiyang and Xi'an. Körner and Basler (Körner and Basler, 2010) previously reported that in most temperate tree species, phenological events such as flowering and autumnal cessation of growth are not primarily controlled by temperature. It was also suggested that phenology might be less sensitive to temperature and photoperiod and more tuned to seasonal shifts in precipitation (Reich, 1995; Morellato, 2003; Sanchez-Azofeifa et al., 2013). Such modifications are expected to occur in concert with rising global temperatures, but the direction and magnitude of change vary regionally (Cubasch et al., 2001). A recent study demonstrated that early flowering advanced under warming plus precipitation addition compared to warm, dry, mild, and very wet species (Ganjurjav et al., 2020). The advancement of spring phenology due to temperature and precipitation leads to evolutionary progress in phenological parameters that disturb the phylogenetic conservation of Guiyang species. The observations of weak conservatism have at least three plausible explanations. First, there is a chance that inaccurate evolutionary reconstructions or measured attributes will muddle the potential signal. Second, phenological cycles could evolve in a way that is poorly anticipated by phylogeny, for instance, when local adaptation or a comparable directional selection force is powerful and controls evolutionary trajectories. Third, phenology may show a flexible response to the environment independent of taxonomic membership (i.e., phenological plasticity), such that the environmental conditions largely determine the phenological schedules for species and populations.

4.3 Climatic conditions impacted phylogenetic conservatism in plant phenophases

Our molecular dating results revealed that Xi'an plant species diverged from their ancestors during the late Cretaceous period (95 Mya), while the Guiyang species diverged during the middle Eocene Era (46 Mya) (Figures 4, 5). During the Cretaceous, the study area experienced large-scale magmatic intrusion under the influence of the late Yunshan movement, which was characterized by a significant global greenhouse climate, with a significant decrease in temperature and an increase in sea level (Wang et al., 2022). Previously it was also found that the Eocene–Oligocene transition in south-eastern Tibet changed the climate from sub-tropical/warm temperate to cool temperate, likely reflective of both uplift and secular climate (Su et al., 2019). We proposed that the recent elevation of the Tibet Plateau influences the climatic shift that led to the phylogenetic divergence of Guiyang species from their parents and to non-conservative phylogenetic signals. In contrast, Xi'an species have a long evolutionary history, making them more climate-adaptive and exhibiting notable phylogenetic conservation.

However, our findings revealed significant phylogenetic signals in spring phenology (FLD and FFD) and climate sensitivity (FLD-S_T, FFD-S_T, FLD-S_{Prc}, and FFD-S_{Prc}) in Xi'an species, while non-conservative in Guiyang. The long evolutionary history of Xi'an species suggested that plants are comparatively more stable and conserved than Guiyang species. For example, it has long been assumed, and sometimes demonstrated, that within a habitat type, the amount of ecological differentiation among species is proportional to the amount of evolutionary and genetic divergence (Stephens and Wiens, 2004). Ecological differentiation can result in reduced resource use overlap between species, allowing species to stabilize in new habitats slowly. Hence, the phylogenetic conservation in spring phenological traits is significant and more substantial in Xi'an after ecological differentiation in new habitats. Guiyang species have recently diverged and show non-significant conservation in plant spring phenology.

Another reason for non-conservative Guiyang species is that they typically have harsh climatic and geographical conditions such as higher altitude, high precipitation, high relative humidity, long, cloudy, rainy days, and little sunshine (Figure 2). Previously, it was reported that harsher settings might cause features among lineages not closely related to converging, diminishing the phylogenetic signal (Lessard-Therrien et al., 2014; Du et al., 2015). In contrast, a report suggested that some tree species showed a significant relationship between phylogenetic conservatism and plant phenology at high altitudes under harsh climates (Li et al., 2016). These phylogenetic linkages may be the basis for species-specific phenological sensitivity to abiotic variation and may aid in predicting these responses to climate change (Caradonna and Inouye, 2015). We also assume that natural selection and their genetic link were strongly correlated with species spread.

The phylogenetic signals of spring phenology were significantly conserved in Xi'an, which was highly consistent with the findings of other studies in Europe and North America (Davies et al., 2013), and China (Du et al., 2015, 2017; Li et al., 2016). This finding further supports the phylogenetic constraint theory in temperature and precipitation sensitivity for flowering. A similar phenomenon is observed in northern Europe, where tree species richness is low during glaciations and postglacial dispersal limitations (Svenning and Skov, 2007). Finally, it is worth mentioning that the combined effect of both the contemporary environment and historical contingencies is significant (Hawkins et al., 2003; Montoya et al., 2007). This is mainly because environmental conditions vary across biogeographical regions. The different combinations of local climatic and geographical variables can result in remarkable changes in plant phenological features. These findings support our hypothesis that local environmental adaptation (LEA) changes with geographical variations directly related to regional climatic conditions and affect the relationship between plant spring phenology and phylogeny. Furthermore, considerable conservation regimes in these regions should consider species diversity and their unique ecological and evolutionary history (Forest et al., 2007). The effects of historical contingencies are partly complicated by the contemporary environment (especially climate). We believe that the impact of the modern environment on plant phenology is fundamental across the globe, dominating general trends and that historical contingency may only cause deviations in phylogenetic conservation in both regions of Xi'an and Guiyang.

4.4 Interaction between plant functional traits and spring phenophases

This work examined the spring phenology in Xi'an (temperate) and Guiyang (subtropical) for species concerning phylogeny and functional features. For instance, our study discovered a strong and significant correlation between spring phenology and plant functional traits, such as life forms and distinct groups of plants (deciduous and evergreen) species in Xi'an (Table 2). The time of leaf expansion is significant in shrubs and earlier than that of trees in Xi'an, which is consistent with the research conclusion of Panchen et al (Panchen et al., 2015). in 8 botanical gardens in temperate regions of the northern hemisphere. The probable cause is that earlier leaf expansion allows shrubs to take full advantage of light for photosynthesis before the tree canopy is fully formed (Rollinson and Kaye, 2012; Panchen et al., 2015). The study also found a significant relationship between trees and earlier flowering onset in Xi'an, similar to the research conclusion (Du et al., 2017). Our results also revealed a significant relationship between deciduous and evergreen plant species with Xi'an flowering phenology, as suggested in previous research (Alice Boyle and Bronstein, 2012; Wang et al., 2020). Our pollination analysis results are not significant (Table 2), which show two possible explanations: First, wind-pollinated tree species need to bloom before the canopy closes, thereby reducing the blocking of leaves on the wind-borne pollen (Fenner, 1998); Second, the Guiyang and Xi'an regions early spring is relatively dry, cold and windy, which restricts the activities of pollinators, resulting in rather a late flowering in plants (Griz and MaChado, 2001). Additionally, our findings showed that trees have an advantage over shrubs in light interception and wind pollination for enhancing reproductive success (Alice Boyle and Bronstein, 2012).

Climatic conditions considerably impacted species' flowering phenology differences (Chang-Yang et al., 2013; Qi et al., 2015). Functional features in this situation might operate as a steppingstone variable in the interaction between climatic conditions and spring phenology. Trees are more sensitive to temperature change than shrubs because of long-term environmental selection (Chávez-Pesqueira and Núñez-Farfán, 2016; Yang et al., 2018). However, our results support the association between growth type and spring phenology in Xi'an temperate climate zones. Temperature-sensitive results with life forms (trees and shrubs) and distinct groups (deciduous and evergreen) of plant species show a significant relationship with leaf and flower phenology in Xi'an, respectively. The above research conclusions reflect plant species' different functional trade-off strategies that respond to the external environment and reflect the differentiation of plant niches and species to a certain extent. This suggests that more specific traits better suited for one particular region be used in the future. We consider identifying the most adaptative characteristics for surviving plant species in different biomes a vital goal. Finally, this study also has certain uncertainties, such as being limited by observation records. These plants usually span multiple families, resulting in insufficient resolution of the phylogenetic tree, which affects the accuracy of research conclusions to a certain extent. In the

future, it is necessary to strengthen the phenological observation of more species records in the Xi'an and Guiyang area and refine the analysis to a few families to explore the plants more deeply and accurately.

5 Conclusions

This study examines the phylogenetic conservation between plant phenological traits and their relationship with biological characteristics. It uses first leaf and flower date data from the China Phenological Observation Network (CPON) for 77 and 40 plant species from Xi'an and Guiyang, respectively. We have shown that the initial leaf unfolding and blooming dates of plants in X'ian exhibit significant phylogenetic signals and are compatible with the OU evolutionary process; however, plant species show non-significant phylogenetic conservation in Guiyang. Similar to this, Xi'an species were significantly phylogenetic conserved with temperature and precipitation sensitivity of phenological traits (FLD-ST, FFD-ST, FLD-SPrc, and FFD-SPrc) but non-conservative in Guiyang. Our results suggested a strong relationship between FFD and FFD-S_T with plant functional traits of distinct plant groups (evergreen and deciduous) and life forms (trees and shrubs). These findings indicated that ecological and evolutionary processes under climate change and natural selection forces affect the phylogenetic conservation of the above phenological characteristics. Our results extended the basic phenology theory, providing a new perspective for correctly evaluating the relationship between climatic conditions and phylogenetic conservation with plant phenology.

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

KS: Conceptualization, Formal analysis, Investigation, Methodology, Software, Writing – original draft, Writing – review & editing. MZ: Conceptualization, Data curation, Formal analysis, Software, Writing – review & editing. LC: Data curation, Formal analysis, Investigation, Software, Writing – review & editing. YH: Data curation, Investigation, Methodology, Software, Writing – review & editing. YZ: Data curation, Formal analysis, Methodology, Software, Writing – review & editing. WL: Data curation, Methodology, Software, Writing – review & editing. JD: Conceptualization, Funding acquisition, Project administration, Supervision, Writing – original draft, Writing – review & editing.

Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article. This research has been supported by the National Key Research and Development Program of China (grant no. 2023YFF1303804), the Strategic Priority Research Program of the Chinese Academy of Sciences (grant no. XDA26010202), and the National Natural Science Foundation of China (grant no. 42271062).

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.2024.1367152/full#supplementary-material

References

Alice Boyle, W., and Bronstein, J. L. (2012). Phenology of tropical understory trees: Patterns and correlates. *Rev. Biol. Trop.* 60 (4), 1415-1430. doi: 10.15517/rbt.v60i4.2050

Bai, X. M., Voter, A. F., Hoagland, R. G., Nastasi, M., and Uberuaga, B. P. (2010). Efficient annealing of radiation damage near grain boundaries via interstitial emission. *Science* 327 (5973), 1631–1634. doi: 10.1126/science.1183723

Basnett, S., Nagaraju, S. K., Ravikanth, G., and Devy, S. M. (2019). Influence of phylogeny and abiotic factors varies across early and late reproductive phenology of Himalayan Rhododendrons. *Ecosphere* 10 (1), e02581. doi: 10.1002/ecs2.2581

Blomberg, S. P., Garland, T., and Ives, A. R. (2003). Testing for phylogenetic signal in comparative data: Behavioral traits are more labile. *Evol. (N Y)* 57 (4), 717–745. doi: 10.1111/j.0014-3820.2003.tb00285.x

Bucher, S. F., König, P., Menzel, A., Migliavacca, M., Ewald, J., and Römermann, C. (2018). Traits and climate are associated with first flowering day in herbaceous species along elevational gradients. *Ecol. Evol.* 8 (2), 1147–1158. doi: 10.1002/ece3.3720

Butler, M. A., and King, A. A. (2004). Phylogenetic comparative analysis: A modeling approach for adaptive evolution. *Am. Nat.* 164 (6), 683–695. doi: 10.1086/426002

CaraDonna, P. J., Iler, A. M., and Inouye, D. W. (2014). Shifts in flowering phenology reshape a subalpine plant community. *Proc. Natl. Acad. Sci. U.S.A.* 111 (13), 4916–4921. doi: 10.1073/pnas.1323073111

Caradonna, P. J., and Inouye, D. W. (2015). Phenological responses to climate change do not exhibit phylogenetic signal in a subalpine plant community. *Ecology* 96 (2), 355–361. doi: 10.1890/14-1536.1

Cavender-Bares, J., Kozak, K. H., Fine, P. V. A., and Kembel, S. W. (2009). The merging of community ecology and phylogenetic biology. *Ecol. Lett.* 12 (7), 693–715. doi: 10.1111/j.1461-0248.2009.01314.x

Chang-Yang, C. H., Lu, C. L., Sun, I. F., and Hsieh, C. F. (2013). Flowering and fruiting patterns in a subtropical rain forest, Taiwan. *Biotropica* 45 (2), 165–174. doi: 10.1111/j.1744-7429.2012.00911.x

Chávez-Pesqueira, M., and Núñez-Farfán, J. (2016). Genetic diversity and structure of wild populations of Carica papaya in Northern Mesoamerica inferred by nuclear microsatellites and chloroplast markers. *Ann. Bot.* 118 (7), 1293–1306. doi: 10.1093/aob/mcw183

Chen, J., Guo, Y., Hu, X., and Zhou, K. (2022). Comparison of the chloroplast genome sequences of 13 oil-tea camellia samples and identification of an undetermined oil-tea camellia species from hainan province. *Front. Plant Sci.* 12 (6045), 1024–1026. doi: 10.3389/fpls.2021.798581

Chen, I. C., Hill, J. K., Ohlemüller, R., Roy, D. B., and Thomas, C. D. (2011). Rapid range shifts of species associated with high levels of climate warming. *Science* 333. doi: 10.1126/science.1206432

Cleland, E. E., Allen, J. M., Crimmins, T. M., Dunne, J. A., Pau, S., Travers, S. E., et al. (2012). Phenological tracking enables positive species responses to climate change. *Ecology* 93 (8), 1765–1771. doi: 10.1890/11-1912.1

Coates, D. J. (2000). Defining conservation units in a rich and fragmented flora: Implications for the management of genetic resources and evolutionary processes in south-west Australian plants. *Aust. J. Bot.* 48 (3), 329–339. doi: 10.1071/BT99018

Cohen, J. M., Lajeunesse, M. J., and Rohr, J. R. (2018). Publisher Correction: A global synthesis of animal phenological responses to climate change. *Nat. Clim. Chang.* 8 (3), 258–258. doi: 10.1038/s41558-018-0099-8

Cubasch, U., Meehl, G. A., Boer, G. J., Stouffer, R. J., Dix, M., Noda, A., et al. (2001). Projections of future climate change. *In Climate Change 2001: The scientific basis. Contribution of WG1 to the Third Assessment Report of the IPCC (TAR)*. Cambridge University Press, pp. 525–582.

Dai, J., Wang, H., and Ge, Q. (2013). Multiple phenological responses to climate change among 42 plant species in Xi'an, China. *Int. J. Biometeorol.* 57, 749–758. doi: 10.1007/s00484-012-0602-2

Dai, J., Xu, Y., Wang, H., Alatalo, J., Tao, Z., and Ge, Q. (2019). Variations in the temperature sensitivity of spring leaf phenology from 1978 to 2014 in Mudanjiang, China. *Int. J. Biometeorol.* 63, 569–577. doi: 10.1007/s00484-017-1489-8

Darling, A. C. E., Mau, B., Blattner, F. R., and Perna, N. T. (2004). Mauve: Multiple alignment of conserved genomic sequence with rearrangements. *Genome Res.* 14 (7), 1394–1403. doi: 10.1101/gr.2289704

Davies, T. J., Wolkovich, E. M., Kraft, N. J. B., Salamin, N., Allen, J. M., Ault, T. R., et al. (2013). Phylogenetic conservatism in plant phenology. *J. Ecol.* 101 (6), 1520–1530. doi: 10.1111/1365-2745.12154

Díaz, S., and Cabido, M. (2001). Vive la différence: Plant functional diversity matters to ecosystem processes. *Trends Ecol. Evol.* 16 (11), 646–655. doi: 10.1016/S0169-5347(01)02283-2

Diniz-Filho, J. A. F., Alves, D. M. C. C., Villalobos, F., Sakamoto, M., Brusatte, S. L., and Bini, L. M. (2015). Phylogenetic eigenvectors and nonstationarity in the evolution of theropod dinosaur skulls. *J. Evol. Biol.* 28 (7), 1410–1416. doi: 10.1111/jeb.12660

Diniz-Filho, J. A. F., Bini, L. M., Rangel, T. F., Morales-Castilla, I., Olalla-Tárraga, M.Á., Rodríguez, M.Á., et al. (2012). On the selection of phylogenetic eigenvectors for ecological analyses. *Ecography* 35 (3), 239–249. doi: 10.1111/j.1600-0587.2011.06949.x

Drummond, A. J., Suchard, M. A., Xie, D., and Rambaut, A. (2012). Bayesian phylogenetics with BEAUti and the BEAST 1.7. *Mol. Biol. Evol.* 29 (8), 1969–1973. doi: 10.1093/molbev/mss075

Du, Y., Chen, J., Willis, C. G., Zhou, Z., Liu, T., Dai, W., et al. (2017). Phylogenetic conservatism and trait correlates of spring phenological responses to climate change in northeast China. *Ecol. Evol.* 7 (17), 6747–6757. doi: 10.1002/ece3.3207

Du, Y., Mao, L., Queenborough, S. A., Freckleton, R. P., Chen, B., and Ma, K. (2015). Phylogenetic constraints and trait correlates of flowering phenology in the angiosperm flora of China. *Global Ecol. Biogeography* 24 (8), 928–938. doi: 10.1111/geb.12303

Eilperin, J., Van Houten, C., and Muyskens, J. (2020). This giant climate hot spot is robbing the West of its water. *Washington Post*.

Felsenstein, J. (1985). Phylogenies and the comparative method. *Am. Nat.* 125 (1), 1–15. doi: 10.1086/284325

Fenner, M. (1998). The phenology of growth and reproduction in plants. *Perspect. Plant Ecol. Evol. Syst.* 1 (1), 78–91. doi: 10.1078/1433-8319-00053

Forest, F., Grenyer, R., Rouget, M., Davies, T. J., Cowling, R. M., Faith, D. P., et al. (2007). Preserving the evolutionary potential of floras in biodiversity hotspots. *Nature* 445 (7129), 757–760. doi: 10.1038/nature05587

Funk, J. L., Larson, J. E., Ames, G. M., Butterfield, B. J., Cavender-Bares, J., Firn, J., et al. (2017). Revisiting the Holy Grail: Using plant functional traits to understand ecological processes. *Biol. Rev.* 92 (2), 1156–1173. doi: 10.1111/brv.12275

Ganjurjav, H., Gornish, E. S., Hu, G., Schwartz, M. W., Wan, Y., Li, Y., et al. (2020). Warming and precipitation addition interact to affect plant spring phenology in alpine meadows on the central Qinghai-Tibetan Plateau. *Agric. For. Meteorol.* 287, 107943. doi: 10.1016/j.agrformet.2020.107943

Gao, X., Dai, J., Shahzad, K., Wang, H., Tao, Z., and Alatalo, J. M. (2022). Association of spring phenological traits with phylogeny and adaptation to native climate in temperate plant species in Northeast China. *Ecol. Indic.* 143. doi: 10.1016/j.ecolind.2022.109381

Gao, M., Piao, S., Chen, A., Yang, H., Liu, Q., Fu, Y. H., et al. (2019). Divergent changes in the elevational gradient of vegetation activities over the last 30 years. *Nat. Commun.* 10 (1), 2970. doi: 10.1038/s41467-019-11035-w

Ge, Q., Wang, H., Rutishauser, T., and Dai, J. (2015). Phenological response to climate change in China: A meta-analysis. *Glob. Chang. Biol.* 21 (1), 265–274. doi: 10.1111/gcb.12648

Gougherty, A. V., and Gougherty, S. W. (2018). Sequence of flower and leaf emergence in deciduous trees is linked to ecological traits, phylogenetics, and climate. *New Phytol.* 220 (1), 121–131. doi: 10.1111/nph.15270

Griz, L. M. S., and MaChado, I. C. S. (2001). Fruiting phenology and seed dispersal syndromes in caatinga, a tropical dry forest in the northeast of Brazil. *J. Trop. Ecol.* 17 (2), 303–321. doi: 10.1017/S0266467401001201

Hawkins, B. A., Porter, E. E., and Diniz-Filho, J. A. F. (2003). Productivity and history as predictors of the latitudinal diversity gradient of terrestrial birds. *Ecology* 84 (6), 1608–1623. doi: 10.1890/0012-9658(2003)084[1608:PAHAPO]2.0.CO;2

Hickling, R., Roy, D. B., Hill, J. K., Fox, R., and Thomas, C. D. (2006). The distributions of a wide range of taxonomic groups are expanding polewards. *Glob. Chang. Biol.* 12 (3), 450–455. doi: 10.1111/j.1365-2486.2006.01116.x

Høye, T. T., Post, E., Meltofte, H., Schmidt, N. M., and Forchhammer, M. C. (2007). Rapid advancement of spring in the High Arctic. *Curr. Biol.* 17 (12), R449–R451. doi: 10.1016/j.cub.2007.04.047

Huxman, T. E., Snyder, K. A., Tissue, D., Leffler, A. J., Ogle, K., Pockman, W. T., et al. (2004). Precipitation pulses and carbon fluxes in semiarid and arid ecosystems. *Oecologia* 141 , 254–268. doi: 10.1007/s00442-004-1682-4

Inouye, D. W. (2022). Climate change and phenology. *Wiley Interdiscip Rev. Clim Change* 13 (3), e764. doi: 10.1002/wcc.764

Janeček, Š., Chmel, K., Ewome, F. L., Hrubá, K., Klomberg, Y., Kobe, I. N., et al. (2021). Differences in nectar traits between ornithophilous and entomophilous plants on mount Cameroon. *Plants* 10 (6), 1161. doi: 10.3390/plants10061161

Kembel, S. W., Cowan, P. D., Helmus, M. R., Cornwell, W. K., Morlon, H., Ackerly, D. D., et al. (2010). Picante: R tools for integrating phylogenies and ecology. *Bioinformatics* 26 (11), 1463–1464. doi: 10.1093/bioinformatics/btq166

Körner, C., and Basler, D. (2010). Plant science. Phenology under global warming. *Science* 327 (5972), 1461–1462. doi: 10.1126/science.1186473

Kozak, K. H., and Wiens, J. J. (2010). Niche conservatism drives elevational diversity patterns in appalachian salamanders. *Am. Nat.* 176 (1), 40–54. doi: 10.1086/653031

Lessard-Therrien, M., Davies, T. J., and Bolmgren, K. (2014). A phylogenetic comparative study of flowering phenology along an elevational gradient in the Canadian subarctic. *Int. J. Biometeorol.* 58, 455–462. doi: 10.1007/s00484-013-0672-9

Li, L., Li, Z., Cadotte, M. W., Jia, P., Chen, G., Jin, L. S., et al. (2016). Phylogenetic conservatism and climate factors shape flowering phenology in alpine meadows. *Oecologia* 182, 419–428. doi: 10.1007/s00442-016-3666-6

Li, F., Shao, L., and Li, S. (2020). Tropical niche conservatism explains the eocene migration from India to southeast asia in ochyroceratid spiders. *Syst. Biol.* 69 (5), 987–998. doi: 10.1093/sysbio/syaa006

Liu, Y., Li, G., Wu, X., Niklas, K. J., Yang, Z., and Sun, S. (2021). Linkage between species traits and plant phenology in an alpine meadow. *Oecologia* 195, 409–419. doi: 10.1007/s00442-020-04846-y

Liu, Q., Piao, S., Fu, Y. H., Gao, M., Peñuelas, J., and Janssens, I. A. (2019). Climatic warming increases spatial synchrony in spring vegetation phenology across the northern hemisphere. *Geophys. Res. Lett.* 46 (3), 1641–1650. doi: 10.1029/2018GL081370

Lukoschek, V., Scott Keogh, J., and Avise, J. C. (2012). Evaluating fossil calibrations for dating phylogenies in light of rates of molecular evolution: A comparison of three approaches. *Syst. Biol.* 61 (1), 22. doi: 10.1093/sysbio/syr075

Ma, Y., Zhao, Y., Zhou, J., Jiang, Y., Yang, S., and Yu, Z. (2018). The relationship between diurnal temperature range and COPD hospital admissions in Changchun, China. *Environ. Sci. Pollut. Res.* 25, 17942–17949. doi: 10.1007/s11356-018-2013-3

Marshall, C. R. (2008). A simple method for bracketing absolute divergence times on molecular phylogenies using multiple fossil calibration points. *Am. Nat.* 171 (6), 726–742. doi: 10.1086/587523

Mason, S. C., Palmer, G., Fox, R., Gillings, S., Hill, J. K., Thomas, C. D., et al. (2015). Geographical range margins of many taxonomic groups continue to shift polewards. *Biol. J. Linn. Soc.* 115 (3), 586–597. doi: 10.1111/bij.12574

Memmott, J., Craze, P. G., Waser, N. M., and Price, M. V. (2007). Global warming and the disruption of plant-pollinator interactions. *Ecol. Lett.* 10 (8), 710–717. doi: 10.1111/j.1461-0248.2007.01061.x

Menzel, A., Sparks, T. H., Estrella, N., Koch, E., Aaasa, A., Ahas, R., et al. (2006). European phenological response to climate change matches the warming pattern. *Glob. Chang. Biol.* 12 (10), 1969–1976. doi: 10.1111/j.1365-2486.2006.01193.x

Menzel, A., Yuan, Y., Matiu, M., Sparks, T., Scheifinger, H., Gehrig, R., et al. (2020). Climate change fingerprints in recent European plant phenology. *Glob. Chang. Biol.* 26 (4), 2599–2612. doi: 10.1111/gcb.15000

Montoya, D., Rodríguez, M. A., Zavala, M. A., and Hawkins, B. A. (2007). Contemporary richness of holarctic trees and the historical pattern of glacial retreat. *Ecography* 30 (2), 173–182. doi: 10.1111/j.0906-7590.2007.04873.x

Morellato, L. P. C. (2003). South America. *Phenology: an integrative environmental science*, pp. 75–92.

Morin, X., Augspurger, C., and Chuine, I. (2007). Process-based modeling of species'distributions: what limits temperate tree species'range boundaries? *Ecology* 88, 2280–2291. doi: 10.1890/06-1591.1

Münkemüller, T., Lavergne, S., Bzeznik, B., Dray, S., Jombart, T., Schiffers, K., et al. (2012). How to measure and test phylogenetic signal. *Methods Ecol. Evol.* 3 (4), 743–756. doi: 10.1111/j.2041-210X.2012.00196.x

Near, T. J., Meylan, P. A., and Shaffer, H. B. (2005). Assessing concordance of fossil calibration points in molecular clock studies: An example using turtles. *Am. Nat.* 165 (2), 137–146. doi: 10.1086/427734

Nylander, J. (2004). Bayesian phylogenetics and the evolution of gall wasps. *(Doctoral dissertation, Acta Universitatis Upsaliensis)*..

Orme, D., Freckleton, R., Thomas, G., Petzoldt, T., Fritz, S., Isaac, N., et al. (2014). Caper: Comparative analyses of phylogenetics and evolution in R. *R package version 0.5. 2012*.

Panchen, Z. A., Primack, R. B., Gallinat, A. S., Nordt, B., Stevens, A. D., Du, Y., et al. (2015). Substantial variation in leaf senescence times among 1360 temperate woody plant species: Implications for phenology and ecosystem processes. *Ann. Bot.* 116 (6), 865–873. doi: 10.1093/aob/mcv015

Paradis, E., Claude, J., and Strimmer, K. (2004). APE: analyses of phylogenetics and evolution in R language. *Bioinformatics* 20 (2), 289–290.

Pérez-Harguindeguy, N., Díaz, S., Garnier, E., Lavorel, S., Poorter, H., Jaureguiberry, P., et al. (2016). Corrigendum to: New handbook for standardised measurement of plant functional traits worldwide. *Aust. J. Bot.* 64 (8), 715–716. doi: 10.1071/BT12225_CO

Piao, S., Liu, Q., Chen, A., Janssens, I. A., Fu, Y., Dai, J., et al. (2019). Plant phenology and global climate change: Current progresses and challenges. *Glob. Chang. Biol.* 25 (6), 1922–1940. doi: 10.1111/gcb.14619

Qi, L., Bravo-Ureta, B. E., and Cabrera, V. E. (2015). From cold to hot: Climatic effects and productivity in Wisconsin dairy farms. *J. Dairy Sci.* 98 (12), 8664–8677. doi: 10.3168/jds.2015-9536

Rathcke, B., and Lacey, E. P. (1985). Phenological patterns of terrestrial plants. *Annu. Rev. Ecol. Syst.* 16 (1), 179–214. doi: 10.1146/annurev.es.16.110185.001143

Reich, Y. (1995). The study of design research methodology. *J. Mechanical Design Trans. ASME* 117. doi: 10.1115/1.2826124

Revell, L. J. (2012). phytools: An R package for phylogenetic comparative biology (and other things). *Methods Ecol. Evol.* 3 (2), 217–223. doi: 10.1111/j.2041-210X.2011.00169.x

Rollinson, C. R., and Kaye, M. W. (2012). Experimental warming alters spring phenology of certain plant functional groups in an early successional forest community. *Glob. Chang. Biol.* 18 (3), 1108–1116. doi: 10.1111/j.1365-2486.2011.02612.x

Rosbakh, S., Hartig, F., Sandanov, D. V., Bukharova, E. V., Miller, T. K., and Primack, R. B. (2021). Siberian plants shift their phenology in response to climate change. *Glob. Chang. Biol.* 27 (18), 4435–4448. doi: 10.1111/gcb.15744

Ryder, O. A. (1986). Species conservation and systematics: the dilemma of subspecies. *Trends Ecol. Evol.* 1, 9–10.

Sanchez-Azofeifa, A., Kalacska, M. E., Quesada, M., Stoner, K. E., Lobo, J. A., and Arroyo-Mora, P. (2013). "Tropical dry climates," in *Phenology: An Integrative Environmental Science*, 121–137. doi: 10.1007/978-94-007-6925-0_9

Staggemeier, V. G., Diniz-Filho, J. A. F., Zipparro, V. B., Gressler, E., de Castro, E. R., Mazine, F., et al. (2015). Clade-specific responses regulate phenological patterns in Neotropical Myrtaceae. *Perspect. Plant Ecol. Evol. Syst.* 17 (6), 476–490. doi: 10.1016/j.ppees.2015.07.004

Stephens, P. R., and Wiens, J. J. (2004). Convergence, divergence, and homogenization in the ecological structure of emydid turtle communities: The effects of phylogeny and dispersal. *Am. Nat.* 164 (2), 244–254. doi: 10.1086/422342

Su, T., Spicer, R. A., Li, S. H., Xu, H., Huang, J., Sherlock, S., et al. (2019). Uplift, climate and biotic changes at the Eocene–Oligocene transition in south-eastern Tibet. *Natl. Sci. Rev.* 6, 495–504. doi: 10.1093/nsr/nwy062

Suding, K. N., Lavorel, S., Chapin, F. S., Cornelissen, J. H. C., Díaz, S., Garnier, E., et al. (2008). Scaling environmental change through the community-level: A trait-based response-and-effect framework for plants. *Glob. Chang. Biol.* 14 (5), 1125–1140. doi: 10.1111/j.1365-2486.2008.01557.x

Svenning, J. C., and Skov, F. (2007). Could the tree diversity pattern in Europe be generated by postglacial dispersal limitation? *Ecol. Lett.* 10 (6), 453–460. doi: 10.1111/j.1461-0248.2007.01038.x

Ting, S., Hartley, S., and Burns, K. C. (2008). Global patterns in fruiting seasons. *Global Ecol. Biogeography* 17 (5), 648–657. doi: 10.1111/j.1466-8238.2008.00408.x

van der Wiel, K., and Bintanja, R. (2021). Contribution of climatic changes in mean and variability to monthly temperature and precipitation extremes. *Commun. Earth Environ.* 2 (1), 1. doi: 10.1038/s43247-020-00077-4

Vandvik, V., Halbritter, A. H., and Telford, R. J. (2018). Greening up the mountain. *Proc. Natl. Acad. Sci. U.S.A.* 115 (5), 833–835. doi: 10.1073/pnas.1721285115

Wan, M., and Liu, X. (1979). Method of Chinese phenological observation.

Wang, C., Cao, R., Chen, J., Rao, Y., and Tang, Y. (2015). Temperature sensitivity of spring vegetation phenology correlates to within-spring warming speed over the Northern Hemisphere. *Ecol. Indic.* 50, 62–68. doi: 10.1016/j.ecolind.2014.11.004

Wang, T., Mao, L., Zou, C., You, H., and Mo, D. (2022). Facies variations in response to tectonic evolution, climate and sea-level changes since the Late Cretaceous in Wuhu region, Eastern China. *Front. Mar. Sci.* 9, 1035447. doi: 10.3389/fmars.2022.1035447

Wang, X., Morin, X., Zhang, J., Chen, G., Mao, L., Chen, Y., et al. (2023). Geographical patterns and determinants in plant reproductive phenology duration. *Front. Plant Sci.* 14, 1199316. doi: 10.3389/fpls.2023.1199316

Wang, Y., Yang, X. D., Ali, A., Lv, G. H., Long, Y. X., Wang, Y. Y., et al. (2020). Flowering phenology shifts in response to functional traits, growth form, and phylogeny of woody species in a desert area. *Front. Plant Sci.* 11, 531690. doi: 10.3389/fpls.2020.00536

Wiens, J. J., Ackerly, D. D., Allen, A. P., Anacker, B. L., Buckley, L. B., Cornell, H. V., et al. (2010). Niche conservatism as an emerging principle in ecology and conservation biology. *Ecol. Lett.* 13 (10), 1310–1324. doi: 10.1111/j.1461-0248.2010.01515.x

Wolkovich, E. M., and Cleland, E. E. (2014). Phenological niches and the future of invaded ecosystems with climate change. *AoB Plants* 6, plu013. doi: 10.1093/aobpla/plu013

Wolkovich, E. M., Cook, B. I., Allen, J. M., Crimmins, T. M., Betancourt, J. L., Travers, S. E., et al. (2012). Warming experiments underpredict plant phenological responses to climate change. *Nature* 485 (7399), 494–497. doi: 10.1038/nature11014

Xu, E., and Zhang, H. (2020). Change pathway and intersection of rainfall, soil, and land use influencing water-related soil erosion. *Ecol. Indic.* 113, 106281.

Yang, Z., Du, Y., Shen, M., Jiang, N., Liang, E., Zhu, W., et al. (2021). Phylogenetic conservatism in heat requirement of leaf-out phenology, rather than temperature sensitivity, in Tibetan Plateau. *Agric. For. Meteorol.* 304, 108413. doi: 10.1016/j.agrformet.2021.108413

Yang, X. D., Ji, P. P., Re, Y. S., and Li, H. X. (2018). Relationship between flowering phenology and phylogeny in 31 woody plants of Urumqi, Xinjiang. *Acta Ecol. Sin.* 38, 1003–1015. doi: 10.5846/stxb201612082532

Ziello, C., Estrella, N., Kostova, M., Koch, E., and Menzel, A. (2009). Influence of altitude on phenology of selected plant species in the Alpine region, (1971-2000). *Clim Res.* 39 (3), 227–234. doi: 10.3354/cr00822

frontiers | Frontiers in Plant Science

TYPE Original Research
PUBLISHED 29 April 2024
DOI 10.3389/fpls.2024.1371040

OPEN ACCESS

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RECEIVED 15 January 2024
ACCEPTED 10 April 2024
PUBLISHED 29 April 2024

CITATION
Yu X, Jin N, Bai R, Mo Y, Pu X, Li J and Lu H-Z (2024) Effects of clonal fragmentation on *Pyrrosia nuda* depend on growth stages in a rubber plantation.
Front. Plant Sci. 15:1371040.
doi: 10.3389/fpls.2024.1371040

Effects of clonal fragmentation on *Pyrrosia nuda* depend on growth stages in a rubber plantation

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Introduction: Clonal fragmentation helps to assess clonal plants' growth resilience to human and environmental disturbance. Although clonal integration in epiphytes in tropical rubber plantations is important to understand their role in enhancing biodiversity and ecosystem services, research on this subject is limited. These plantations are typically monospecific economic forests that face increased anthropogenic disturbances.

Methods: In this study, we selected the clonal fern *Pyrrosia nuda* to study its survival status, biomass, maximum quantum yield of photosystem II (F_v/F_m), and frond length in response to the level of clonal fragmentation in a tropical rubber plantation.

Results and discussion: The results showed that (1) clonal fragmentation significantly negatively affected the survival rate, biomass, and frond length of clonal plants, but with minimal effects on F_v/F_m at different growth stages; (2) the performance of a ramet (e.g., biomass or frond length) increased with ramet developmental ages and decreased with the number of ramets in a clonal fragment. The age-dependent impacts of clonal fragmentation provide insights into the biodiversity conservation of epiphytes and forest management in man-made plantations. Therefore, to better conserve the biodiversity in tropical forests, especially in environment-friendly rubber plantations, there is a need to reduce anthropogenic disturbances and alleviate the level of fragmentation.

KEYWORDS

clonal fragmentation, rubber plantation, physiological integration, restoration potential, epiphytic ferns

1 Introduction

Land-use change by human activities has brought extensive deforestation and alterations in various land types worldwide. This has not only severely damaged the species composition and biodiversity of tropical rainforests but also led to drastic changes in temperature and precipitation patterns in the tropics (Choat et al., 2012; Polson et al., 2016). These natural and anthropogenic disturbances have impacted the growth, flowering, and reproduction of plants (Willmer, 2012; Zambrano and Salguero-Gomez, 2014; Ruiz et al., 2018). The impacts of disturbances have directly or indirectly limited the availability of biotic resources crucial for human sustenance. To meet human resource demands, tropical rainforests are often exploited for agricultural, livestock, and forestry activities such as timber production. Unfortunately, such activities have undermined the structural and functional sustainability of tropical forests, resulting in insufficient ecological services to meet human needs. This exacerbates deforestation in biodiversity hotspots, destroying habitats and disrupting ecosystem equilibrium, and ultimately leading to the degradation and fragmentation of tropical forests (Jha, 2006; Morton et al., 2013). As a paramount type of monoculture artificial forest arising from land-use changes in tropical forests and as a strategic resource for many tropical nations, rubber plantation possesses indispensable economic and social value. However, because the environmental heterogeneity and microhabitat diversity in rubber plantations are lower compared to tropical rainforests, which makes epiphytes in forest canopies in these artificial plantations are more fragile. They even face the risk of species extinction (Baumbach et al., 2021) due to anthropogenic disturbances and fragmentation (Laurance et al., 2000; Malta et al., 2003; Barlow et al., 2007).

As a crucial refuge for endangered species in the tropics, forest canopies provide heterogeneous and diverse microhabitats for various plants and animals, playing a key role in maintaining flora and fauna diversity, community assembly, and ecosystem stability in tropical forests (Gargallo-Garriga et al., 2021; Roa-Fuentes et al., 2022). Nevertheless, extreme weather and human activities may exert influence on the structure and function (e.g., breaking branches, leaves and treetops, etc.) of the forest canopy, which subsequently reduces individual survival, the spread of species, and biotic interactions of host trees and epiphytes (Barlow et al., 2007; Zellweger et al., 2020). Epiphytes, growing in the tropical canopy for more light and fewer competitors, are essential components of tropical forest flora, contributing to species richness, and structural functionality (Kreft et al., 2004; Ellis, 2012). As epiphytes rely on the supportive structures of their hosts, the habitat fragmentation of tropical forests and clonal fragmentation of clonal epiphytes as a result of natural and anthropogenic disturbances pose direct and indirect threats to the performance and microhabitats of epiphytes. Fortunately, most nonvascular and vascular epiphytes can adapt to canopies with stressful resources and an unstable microenvironment through clonal reproduction (Lu et al., 2016, Lu et al., 2020).

Although the severe impacts of various disturbances on biodiversity and functionality can be mitigated by forest canopies and clonal reproduction, they could still negatively affect the productivity and lifespan of sensitive epiphytes, leading to cascading effects (Foster, 2001; Nadkarni and Solano, 2002; De Frenne et al., 2019). Though one of the most natures of clonal plants is physiological integration, which means sharing resources and information within interconnected fragments of a clone (Suzuki and Stuefer, 1999; Brezina et al., 2006). However, in previous studies, the whole clonal epiphytes decreased the growth and performance, while they relied on resource sharing within a clone and interspecific facilitation within canopy communities to mitigate severe stresses and negative effects (Lu et al., 2016, Lu et al., 2020; Dai et al., 2023). In particular, the growth and performance of two clonal plants, *Pyrrosia nummulariifolia* and *Lemmaphyllum microphyllum*, have been found to decline when they rely on clonal integration to deal with the negative effects of clonal fragmentation in a natural limestone forest (Dai et al., 2023). However, our understanding of the impact of clonal fragmentation resulting from extreme weather and human activities on epiphytes in artificial forests and/or plantations remains limited.

With land use changes and human activities, large areas of tropical forests have changed to tropical artificial plantations for decades. Rubber plantations are one of the main man-made forests in the tropics, especially in southeast Asia. The area of rubber plantation is about 573,333 ha in Xishuangbanna, southwest China (Liu et al., 2023; Qi et al., 2023). To improve the quality and efficiency of rubber plantations, researchers have focused on how to structure an environment friendly rubber plantation with higher biodiversity and interspecific facilitation to balance economic efficiency and ecological efficiency. In fact, epiphytes in artificial plantations play an important role in biodiversity maintenance in the tropics (Zheng et al., 2015; Bohnert et al., 2016). However, in rubber plantations, in addition to the disturbance and damage of clonal epiphytes by extreme weather and animals, rubber tapping will cut the clonal organs of epiphytes into several parts on rubber trees, and general management may also destroy and clear the whole cluster of epiphytes. The frequent occurrences of clonal fragmentation in clonal plants can lead to the splitting of intact clonal plants into potentially clonal individuals connected by one or a few ramets (Song et al., 2013b). Different degrees and/or levels of clonal fragmentation could be a function of the survival rate and individual performance of epiphytes in artificial plantations. For understanding the ecological strategies of clonal plants and maintaining biodiversity in man-made forests, it is crucial to study how clonal epiphytes respond to fragmentation in artificial plantations with various disturbances. It also holds practical significance for managing fragmented artificial forests and mitigating habitat disruption caused by human activities (Dong et al., 2012; Wang et al., 2018).

Clonal integration plays a crucial ecological role in mitigating the effects of clonal fragmentation caused by nature and humans. To improve the health of plant systems and the conservation of canopy biodiversity, it may be pertinent to reduce the level of fragmentation and promote system connectivity (Zotz and Hietz, 2001; Grimshaw et al., 2005; Zhang et al., 2009). Numerous studies have found that native plants and invasive plants in both wetland and terrestrial habitats exhibit excellent clonal growth capabilities under clonal fragmentation with severe disturbances, allowing rapid growth and reproduction not for native species but for invasive

plants (Dong et al., 2012; Li et al., 2013; Zhang et al., 2023). It means that the physiological integration of resources could be varied in distances or extents for different native and invasive species. Thus, clonal fragments with different numbers of ramets (levels of fragmentation) of various plants in varied forests (e.g., natural and artificial forests) will respond to disturbances differently. Furthermore, various plant species or plants with different developmental stages may rely on clonal integration to different degrees and in terms of resource sharing and resilience of diseases. In general, younger stage individuals depend on resource sharing much more for survival and growth than older ones, for the distinction of resource absorption capability and resilience of stresses. However, little is known about the dependence degree of physiological integration on developmental ages for clonal epiphytes in artificial plantations.

To investigate the impact of clonal fragmentation (i.e. the extents of physiological integration) and its dependence on the development ages of a clonal epiphyte in an artificial forest, this study was carried out as a field in-situ experiment of *P. nuda* in the rubber plantation in Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences. This study aims to address the following research questions: 1) Does increasing levels of clonal fragmentation correspond to greater negative impacts on *P. nuda*? 2) What is the effect of developmental stage on the survival and performance of *P. nuda*? 3) Do the effects of clonal fragmentation depend on the relative age of *P. nuda* in rubber plantation?

2 Materials and methods

2.1 Study site

The study site is located in Xishuangbanna Tropical Botanical Garden (XTBG) (21°55′39″N, 101°15′55″E, 580 m a.s.l.) in MengLun, Xishuangbanna Prefecture, Yunnan Province, China. As a biodiverse hotspot, the topography of Xishuangbanna features mountains and-valleys, with the Hengduan Mountains running north-south, covering approximately 95% of the region's covered by mountains and hills. This region has a typical tropical monsoon climate and is characterized by a half-year dry season (November-April) and a half-year rainy season (May-October) (Dai et al., 2023). According to the observation data from Xishuangbanna Station for Tropical Rainforest Ecosystem Studies (XSTRES), the monthly mean air temperature is approximately 22.5°C, and the mean annual precipitation is approximately 1500 mm (from 2005 to 2018), with more than 80% occurring during the rainy season. The soil in the study site is Ferralic Cambisol with a pH of around 5.0 developed from sandstone-derived alluvial deposits, which has a clayey texture with 23% coarse sand (2.0 - 0.05 mm), 30% silt (0.05 - 0.002 mm), and 47% clay (< 0.002 mm) and (Yang et al., 2020). The rubber plantation is a predominantly monoculture artificial community, and the rubber trees were cultivated at a spacing of 2.0 m × 4.5 m along the terraced slope following the clearance of primary vegetation in the late 1980s, and the most dominant epiphytic fern species is *P. nuda* in Xishuangbanna.

2.2 Experimental design

We selected *Pyrrosia nuda*, the most dominant clonal epiphytic fern, as the target species for *in situ* field experiments in the canopy of a rubber plantation. *P. nuda* has long creeping rhizomes of 1.2-2.1 mm in diameter and in cross section usually with a single, central sclerenchyma strand (State Key Laboratory of Systematic and Evolutionary Botany, 2023). Most *Pyrrosia* species are drought-tolerant (Wei et al., 2017; Derzhavina, 2020).

The experiment involved ramets at three developmental stages/ages (1st, 2nd, and 3rd ramet closest to the rhizome apex) with fiddlehead frond ramets, just-extended frond ramets, and mature frond ramets and included four levels of fragmentation as the treatment—single-, double-, triple- and intact-ramet treatments (see Figure 1). Based on previous experiments on clonal integration of epiphytic ferns, it is noted that water stress during the dry season may lead to the death of most single ramets; therefore, this study was focused on the influence of clonal fragmentation on the survival and performance in the rainy season with less water stress (Lu et al., 2015; Zhang et al., 2019; Lu et al., 2020; Zhang et al., 2020b; Dai et al., 2023).

At the beginning of the rainy season, we selected 20 host trees with similar diameter at breast height (DBH) and tree height, which had *P. nuda* as the dominant epiphyte species in the rubber plantation. These rubber trees were planted at equal intervals, and the epiphytes were located in the same canopy height with consistent light conditions and humidity (Liu et al., 2018, 2020; Zeng et al., 2018; Lu et al., 2020). First, we selected a clone of interconnected ramets, then we chose four rhizomes. For each rhizome, we chose the three youngest ramets closest to the rhizome apex as our target materials (labeled as the 1st, 2nd, and 3rd ramet). We conducted in-situ experiments on epiphytic ferns on each host rubber tree at the height of 2 to 4 m on the trunk below the first branches, with 20 replications for each treatment as below. In the single-ramet treatment, we severed the rhizome midway between any two adjacent ramets of the first four ramets closest to the rhizome apex so that each of the three target ramets (1st, 2nd, and 3rd) was isolated from the rest of the clone. In the double-ramet treatment, we severed the rhizome midway between the 2nd and the 3rd ramet closest to the rhizome apex so that the first two ramets were still connected but isolated from the rest of the clone. In the triple-ramet treatment, we severed the rhizome midway between the 3rd and the 4th ramet closest to the rhizome apex so that the three target ramets were still connected but isolated from the rest of the clone. In the intact-ramet treatment, we left the rhizome intact so that the three target ramets remained connected and connected to the rest of the clone (Figure 1).

After 150 days, we assessed the survival status of each of the three target ramets (1st, 2nd, and 3rd ramet) of *P. nuda*, and harvested each survived ramet *in situ* experiment after measuring its frond length. A ramet was considered dead once all of its fronds were shed, dried, or withered. Biomass was determined after drying at 70 $^{°C}$C for 48 h. Before harvesting, we assessed the maximum quantum yield of photosystem II (F_v/F_m) as a measure of photosynthetic capacity in *P. nuda* using a portable fluorometer (FMS-2, Hansatech, UK). The dark adaptation process involved securing healthy leaves with a Dark Leaf Clip (DLC-8) for a minimum of 20 minutes, avoiding the main

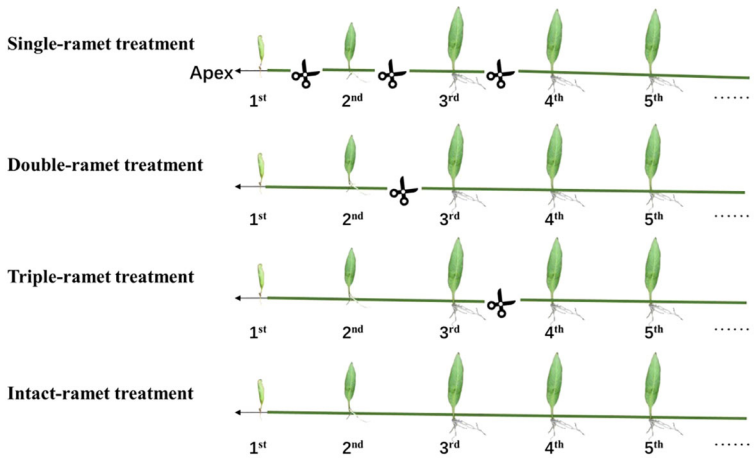

FIGURE 1
Experimental design. For each of the three epiphytic ferns and each of the 20 host trees, we selected four rhizomes with interconnected ramets. Within each rhizome, we identified the three adjacent ramets closest to the rhizome apex (labeled as 1st, 2nd, and 3rd). In the single-ramet treatment, we severed the rhizome midway between any two adjacent ramets among the first four ramets closest to the rhizome apex. For the double-ramet treatment, we severed the rhizome midway between the 2nd and 3rd ramet closest to the rhizome apex. In the triple-ramet treatment, the rhizome was severed midway between the 3rd and the 4th ramet closest to the rhizome apex. The intact-ramet treatment involved leaving the rhizome intact.

veins. Subsequently, we measured the initial fluorescence (F_0) by exposing the leaves to measuring light (< 0.5 μmol·m^{-2}·s^{-1}), followed by measuring the maximum fluorescence (F_m) using a saturating pulse (2800 μmol·m^{-2}·s^{-1}). This process was repeated, and the variable fluorescence (F_v) was calculated as $F_v = F_m - F_0$. The maximum photochemical quantum yield of photosystem II (F_v/F_m) was then determined using the formula $F_v/F_m = (F_m - F_0)/F_m$, as described in previous studies (Bolharnordenkampe et al., 1989; Heidbüchel and Hussner, 2020). After allowing fluorescence values to stabilize (approximately three to five minutes), we recorded the actual quantum yield and other fluorescence parameters. This standardized methodology ensures a precise assessment of the photosynthetic capacity in *P. nuda* fronds. F_v/F_m was calculated with the equation $F_v/F_m = (F_m - F_0)/F_m$, where F_0 and F_m are the minimum and maximum fluorescence yield of a dark-adapted sample after a saturation pulse (>5000 μmol photons m^{-2}s^{-1} of actinic white light), respectively (Bolharnordenkampe et al., 1989; Lu et al., 2020). F_v/F_m reflects the photosynthetic performance and stress resistance of plants (Butler and Kitajima, 1975; Wei et al., 2019; Li et al., 2020), which indirectly indicates the ability of plants to absorb and the strength of plants to utilize light energy for photosynthesis. This value is positively correlated with plants' growth and regeneration ability. Therefore, higher F_v/F_m can indirectly indicate greater survival rate, biomass, and frond length of epiphytic ferns, serving as an indicator to evaluate the growth ability and status of epiphytes (Gauslaa et al., 2001; Malta et al., 2003).

2.3 Data analysis

The data were analyzed using (generalized) linear mixed models with R version 4.2.0 (R Core Team, 2022). The "glmer" function in the "lme4" package and the "lmer" function in the "lmerTest" package were employed (Bates et al., 2015; Kuznetsova et al., 2017). Initially, the fixed factors included fragmentation level (single-, double-, triple- and intact-ramet treatments), developmental stage/age (1st, 2nd, and 3rd ramet closest to the rhizome apex), and their interactions. For individual survival data with binomial error distributions, a generalized linear mixed model was then applied ("glmer" function), with the level of clonal fragmentation and age as fixed factors, the initial individual height (leaf size) as covariate and the host tree in the sampling site as a random variable. For the biomass, F_v/F_m, and frond length of surviving ramets, a linear mixed model was used ("lmer" function), with the level of clonal fragmentation and age as fixed factors, the initial individual height (leaf size) as covariate and the host tree in the sampling site as a random variable. The data were log-transformed if needed before analysis to improve the normality of the residuals.

In the (generalized) linear mixed models, log-likelihood ratio tests were conducted to assess the significance of the fixed factors (Bedogni, 2010). During the post-hoc analyses of significant differences among treatments, a ramet was considered dead and its data was treated as missing when all of its fronds were shed, dried, or withered. In performing the log-likelihood ratio test, the null data were excluded in the analysis. These tests involve comparing a model with the term of interest to a model without the term of interest. The calculated log-likelihood ratios approximated a χ^2 distribution (Platt et al., 2004). Specifically, we sequentially removed the two-way interactions, each of the two key factors and the covariate, and then compared the fit of the simplified model to the more complex model. The statistical difference in model fit indicates that the effect of the removed factor is significant.

3 Results

Compared to the intact-ramet treatment, clonal fragmentation significantly reduced the clonal ramet survival, surviving ramet biomass, F_v/F_m, and frond length of *P. nuda*. With the increasing levels of clonal fragmentation, the negative effects of fragmentation

increased (Table 1, Figures 2–4). In *P. nuda*, the fragmentation led to a marked decrease in ramet biomass, with single- (0.145 ± 0.010), double- (0.167 ± 0.011), and triple-ramet (0.159 ± 0.007) treatments had lower biomass than the intact-ramet (0.224 ± 0.008). The F_v/F_m, although reduced across all fragmentation levels (single- (0.785 ± 0.012), double- (0.785 ± 0.012), triple-ramet (0.789 ± 0.005)) relative to the intact-ramet (0.811 ± 0.003), showed no difference among the three severed treatments. Notably, frond length experienced a sharp decline from the intact-ramet (145.55 ± 3.73) to single- (93.06 ± 4.41), double- (93.06 ± 4.41), and triple-ramet (101.10 ± 3.20) treatments (Figure 4).

The growth stage significantly influenced biomass and frond length of surviving ramet. Specifically, as ramets aged, both biomass and frond length increased. However, it did not affect the clonal ramet survival and F_v/F_m of surviving ramets of *P. nuda*, indicating higher survival and biomass in plants at the mature leaf stage (Table 1, Figures 2, 4). The older the ramet, the better the ramet grew during clonal fragmentation (Figures 2, 4).

There were significant interaction effects of clonal fragmentation and developmental stage on the clonal ramet survival, surviving ramet biomass and frond length of *P. nuda*. However, no such effects were observed for F_v/F_m of surviving ramets (Table 1, Figures 2-4). It meant that the negative influences of clonal fragmentation depend on growth stages, i.e. the survival rate and growth of younger ramets in small fragments (higher fragmentation levels) was lower than that in large fragments (lower fragmentation levels) and the difference was much lower for older ramets.

4 Discussion

4.1 Response of *P. nuda* to clonal fragmentation

As shown in the results, clonal fragmentation had a significantly negative effect on clonal ramet survival, biomass, F_v/F_m and frond length of surviving ramets of *P. nuda*, especially in the fiddlehead fern stage ($P < 0.001$, Figures 2-4). This result is consistent with previous findings. There were negative effects of clonal fragmentation on the survival rate and biomass of two clonal ferns *Pyrrosia nummulariifolia* and *Lemmaphyllum microphyllum* with different ecotypes of epiphytic and lithophytic clonal ramets (Dai et al., 2023). This confirmed our initial research hypothesis in that the higher the fragmentation level, the more severe the negative influence. This result suggests that epiphytes in both limestone forests and artificial forests would decrease the survival rate and performance by clonal fragmentation resulting from natural and anthropogenic disturbances. Similar to fern species, the negative affects of clonal fragmentation also decreased the competitive ability and the number of ramets of seed plants. As clonal fragmentation significantly inhibits the growth of *S. natans*, it will result in a notable reduction in its competitive fitness. This underscores the detrimental impact of clonal fragmentation on the overall competitive capacity of the species (Dong et al., 2012; Zhang et al., 2020a). Clonal fragmentation destroys the interconnection spacers and narrows the range of physiological integration, resulting in resource sharing within a limited zone. Clonal epiphytes, such as *Selliguea griffithiana*, rely on physiological integration for surviving and growing in both canopy and understory habitats, especially in the canopies (Lu et al., 2015), so do some invasive or wetland clonal plants (Back et al., 2013; Song et al., 2022). However, clonal fragmentation has been observed to significantly augment the growth potential of certain aquatic and invasive plants, as detailed in research by Zhang et al. (2019), and Rosef et al. (2020). These studies have collectively demonstrated the consequential enhancement of proliferation and regenerative capabilities arising from such fragmentation (to compete with local species and occupy the new habitats). Moreover, specific attention has been directed towards the advantageous effects of root fragmentation in fast-growing and ecologically adaptable species, as highlighted by Huber et al. (2014).

In this study, the epiphytic fern of *P. nuda* could provide more support for survival and growth from interconnected clonal ramets under natural and anthropogenic disturbance. The adverse effects on survival rate and frond length are likely due to the limited resources for growth and adaptation in the juvenile fiddlehead leaf stage of *P. nuda* ramets subjected to fragmentation, leading to worse self-adjustment and adaptability of clonal ramets after disturbance (Rudolphi and Gustafsson, 2011; Lu et al., 2015; Song et al., 2022; Dai et al., 2023), and such effects on biomass are likely to be the inability of ramets to share resources between them, as the reduced regeneration capacity and the increased risk of infection after ramet fragmentation can prevent the allocation and share of resources between ramets (Song et al., 2013b; Lu et al., 2020). As the level of

TABLE 1 Effects of fragmentation level (F), growth stage (S) and their interaction (F × S) on survival, growth, the maximum quantum yield of PS II (F_v/F_m), and frond length of *P. nuda* in (generalized) linear mixed models.

Fixed factors	Survival			Biomassζ			F_v/F_mζ			Frond lengthζ		
	df	χ^2	*p*	df	χ^2	*p*	df	χ^2	*p*	df	χ^2	*p*
Fragmentation (F)	3	50.6	<0.001	3	95.4	<0.001	3	9.2	0.026	3	184.3	<0.001
Stage (S)	2	0.9	0.651	2	62.6	<0.001	2	0.5	0.783	2	49.8	<0.001
F × S	5	15.9	0.007	4	62.0	<0.001	4	3.4	0.487	4	260.3	<0.001
Frond sizeζ	1	49.6	<0.001	1	9.5	0.002	1	0.3	0.600	1	13.3	<0.001

ζdata were log-transformed. Fragmentation level, and growth stage, were treated as fixed effects, host trees nested in study sites as random factors, and original plant height as covariate. "df" means the degrees of freedom, "χ^2" is the value of the chi-square test, "p" is the significance of the test.

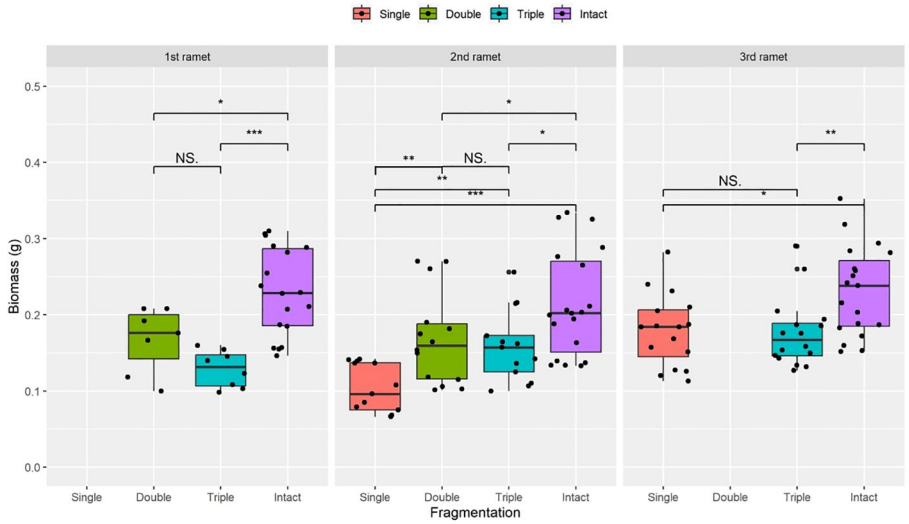

FIGURE 2
Biomass of surviving clonal ramet at different growth stages of each clonal fragment (single, double, triple, control/intact). Significant differences between each treatment is denoted by asterisks::*P < 0.05; **P <0.01; and ***P < 0.001; NS indicates non-significant differences between each treatment (P ≥ 0.05); Within each rhizome, the three adjacent ramets closest to the rhizome apex (labeled as 1st, 2nd, and 3rd).

clonal fragmentation decreases, there is a positive effect on ramet survival, biomass, and frond length, which could be because of the connection between ramets allowing epiphytes to obtain more shared resources and to better cope with stresses (Lu et al., 2015, Lu et al., 2020; Dai et al., 2023) and resulting in improved growth and reproductive regeneration capabilities. There is also a significant effect on the clonal ramet F_v/F_m, which is likely because the fragmentation process leads to water deficiency in fragmented ramets, resulting in an insufficient supply of essential raw materials for photosynthesis (Wang et al., 2020). Rubber plantations and epiphytes in the canopy are susceptible to weather and disturbances, which, together with water scarcity that reduces carbon dioxide movement into the fronds, can lead to a lack of carbon and energy demand for photochemistry and photosynthesis (Zheng et al., 2015; Wei et al., 2019; Li et al., 2020).

4.2 Response of *P. nuda* to different growth ages

There were significant effects of developmental age on biomass and frond growth; that is, the higher the ramet developmental age,

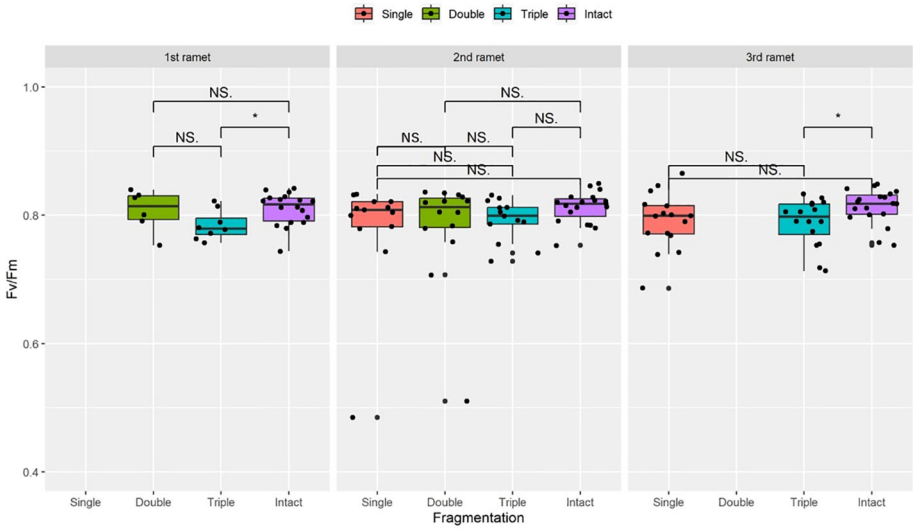

FIGURE 3
The maximum quantum yield of PS II (F_v/F_m) surviving clonal ramet at different growth stages of each clonal fragment (single, double, triple, control/intact). Significant differences between each treatment is denoted by asterisks:*P < 0.05; NS indicates non-significant differences between each treatment (P ≥ 0.05); Within each rhizome, the three adjacent ramets closest to the rhizome apex (labeled as 1st, 2nd, and 3rd).

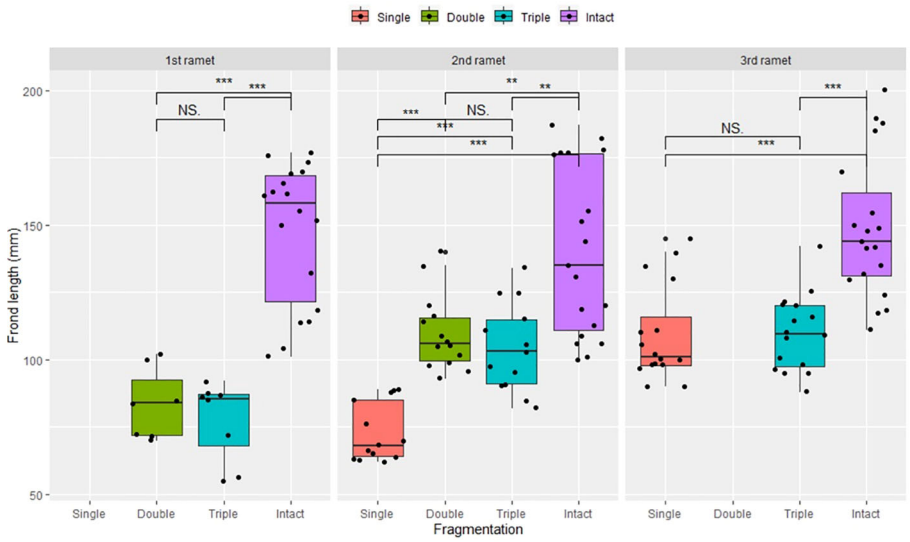

FIGURE 4
Frond length of surviving clonal ramet at different growth stages of each clonal fragment (single, double, triple, control/intact). Significant differences between each treatment is denoted by asterisks::*P < 0.05; NS indicates non-significant differences between each treatment (P ≥ 0.05); Within each rhizome, the three adjacent ramets closest to the rhizome apex (labeled as 1st, 2nd, and 3rd).

the larger the ramet biomass and frond length. Similar findings in previous studies suggest that this might be attributed to the insufficient self-regulation capacity of epiphytes, lack of nutrient reserves and rapid growth capability during the early stages of ramet formation when just-extended frond ramets are prevented from resource sharing (Lu et al., 2015; Lin et al., 2018). For example, the rooting position significantly influenced the growth of individual ramets, with the second and third most apical ramets exhibiting optimal growth when positioned as the most apical rooted ramet. This effect was more pronounced under higher nitrogen levels (Wang et al., 2017). In addition, the rapid development of clonal ramet root systems in the extended frond and mature stages potentially broadens their ecological niche, making them less susceptible to the affects of fragmentation (Truscott et al., 2006). As plant age increases, mature ramets share and transfer nutrients to young ramets, indicating that the growth of young ramets depends more on the closely related mature maternal ramets (Alpert, 1999; de Kroon et al., 2005; Song et al., 2013a). Therefore, severing the connection between the clonal offspring and the older parents' ramets at a younger age may result in inadequate nutrient supply, and hindered sharing of water resources and photosynthetic products, thereby reducing their survival and growth rate.

The adjustment and adaptive performance of the 1st age younger fragmented clonal ramets may diminish after disturbance, with their reproductive and resistance capabilities enhanced with growth stage and maturity (Rudolphi and Gustafsson, 2011). There is relatively little variation in the impact on F_v/F_m and ramet survival with increased ramet developmental age (Table 1, Figures 2-4), which is in contrast to previous studies which, indicated that clonal integration has varying effects on the maximum photosynthetic efficiency of ramets. Clonal integration significantly influences the photosynthetic rates of *Alternanthera philoxeroides* and *Fragaria vesca* but does not affect the photosynthetic rate of the epiphytic fern species *Diplopterygium glaucoma* (Luo et al., 2014; Reynolds et al., 2014). The yield effect of benefits and resource acquisition does not necessarily rely entirely on the frond photochemistry to obtain the energy required for growth. The lack of significance could be linked to the depletion of most plant resources and energy for recovering damage after fragmentation. The impact on leaf photosynthesis can translate into varying effects on ramet survival and growth. Therefore, an extended period of resource accumulation is required to enhance plant photosynthetic performance and stress resistance (Wei et al., 2019; Li et al., 2020; Lu et al., 2020). Furthermore, the presence of the forest canopy may block some light, resulting in insignificant effects on F_v/F_m at different growth stages for clonal ramets situated in the canopy, under normal circumstances, the F_v/F_m in plants typically ranges from 0.70 to 0.80 (Butler and Kitajima, 1975; Bolharnordenkampe et al., 1989). After disconnection, the F_v/F_m is significantly reduced. The changes in the F_v/F_m depend on the species and the light condition of the plants (Wei et al., 2019; Li et al., 2020). The negative impact of clonal fragmentation on epiphytes is age-dependent and influenced by clonal integration effects (Lu et al., 2016, Lu et al., 2020).

4.3 High dependence of clonal fragmentation on growth stage

The interaction between the level of clonal fragmentation and the developmental stage of the ramet showed that clonal fragmentation had entirely different effects on the survival, biomass, and frond length of *P. nuda* ramets at different growth ages. This is consistent with previous research which found *Pyrrosia nummulariifolia* and *Lemmaphyllum* microphyllum exhibit significant interaction effects of clonal fragmentation and ages on ramet survival and growth in natural forests (Dai et al., 2023). This

appears to stem from the transient reliance of relatively early-maturing clonal ramets on clonal integration (Saine et al., 2018). Slow-developing or mature epiphytes may exhibit a higher dependence on growth stages. The impact of clonal fragmentation on clonal ramet growth and dependence on clonal integration varied with growth stages for the same species, diminishing growth in younger ramets while enhancing growth in older ramets (Stuefer et al., 2001; Dong et al., 2012; Song et al., 2013b; Luo et al., 2014; Wang et al., 2014). Additionally, this age-dependent response to clonal fragmentation might be associated with the clonal division of labor in plants during ontogenesis. Clonal plants undergo morphological or functional specialization at different growth stages, enhancing their ability to acquire heterogeneous resources, thus elevating their position and role in competitive relationships (Fan et al., 2018). Consequently, the capacity of epiphytes to acquire resources varies at different growth stages, leading to distinct affects on clonal ramet survival and growth after clonal fragmentation.

The effect of clonal fragmentation on clonal ramets depended on growth stages. Clonal fragmentation affects early juvenile fiddlehead, extended frond, and mature *P. nuda* ramets differently, with a significantly negative impact on survival during the early growth stage. But there is no significant effect on survival during the extended frond and mature stages. Clonal fragmentation is likely to have a negative impact on the growth of young ramets, while positively influence the growth of older ramets (Ortego et al., 2002; Dong et al., 2012; Zhang et al., 2023). Stored carbohydrates in clonal ramets might be used to prevent the risk of future ramet death (Suzuki and Stuefer, 1999), which could be a result of a ramet response to the current growth potential and a trade-off between the current growth potential and future risk regulation that could explain the observed differences. This is further supported by the enhanced resistance of mature ramets to clonal fragmentation, especially in epiphytic ferns (Wei et al., 2019; Zhang et al., 2023). This highlights the high dependence of clonal integration on growth ages, and resource sharing facilitated by clonal integration contributes significantly to the survival and performance of epiphytic ferns.

5 Conclusion

In artificial rubber plantations, clonal fragmentation exerted negative age-dependent effects on survival and performance of the epiphytic fern *P. nuda*, especially at the juvenile fiddlehead leaf stage. When clonal ramets are fragmented by natural or anthropogenic disturbance, the plant's resource sharing will be limited to fragments of small numbers of interconnected ramets, leading to resource scarcity and declined performance. Such negative effects of clonal fragmentation were much more severe on juvenile individuals than aged ones, which may have resulted from the higher stress-resilience and resource-storage of adults. Based on the results of the survival, biomass, and frond length analysis of the *P. nuda* clonal ramets, it is imperative to minimize and avoid anthropogenic disturbance causing fragmentation in artificial forests, such as tropical rubber plantations, to mitigate its impact on plant biodiversity. This research indicates that clonal integration plays a crucial role in the growth, reproduction, and performance of clonal plants in tropical artificial forests under adverse stress conditions. It sheds light on the biodiversity conservation and maintenance during the establishment of environment-friendly rubber plantations establishment with increased human activities, land-use changes and extreme weather.

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.

Author contributions

XY: Data curation, Visualization, Writing – original draft, Writing – review & editing. NJ: Data curation, Supervision, Writing – review & editing, Writing – original draft. RB: Data curation, Writing – review & editing, Visualization, Writing – original draft. YM: Data curation, Writing – review & editing, Visualization, Investigation. XP: Data curation, Writing – review & editing, Investigation. JL: Writing – review & editing, Visualization. H-ZL: Supervision, Writing – review & editing, Visualization, Data curation, Investigation, Writing – original draft, Conceptualization, Funding acquisition, Methodology.

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 Yunnan Revitalization Talent Support Program (H-ZL), the National Natural Science Foundation of China (No.31872685), the 14th Five-Year Plan of the Xishuangbanna Tropical Botanical Garden Chinese Academy of Sciences (E3ZKFF7B, E3ZKFF2B, E3ZKFF9B), the CAS-UCLA Scholarship (H-ZL), and the Yunnan Natural Science Foundation (2019FB037).

Acknowledgments

We thank Mr. Yuan-Xiang Lu and Ms. Yu-Xian Hu for their fieldwork. We also appreciate Ms. Yaqiong Cui and Ms. Yang Chen for wording revision and the center laboratory of public technology for measuring devices.

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

Alpert, P. (1999). Clonal integration in Fragaria chiloensis differs between populations: ramets from grassland are selfish. *Oecologia*. 120, 69–76. doi: 10.1007/s004420050834

Back, C. L., Holomuzki, J. R., Klarer, D. M., and Whyte, R. S. (2013). Herbiciding invasive reed: indirect effects on habitat conditions and snail-algal assemblages one year post-application. *Wetl. Ecol. Manage.* 21, 155–156. doi: 10.1007/s11273-013-9296-4

Barlow, J., Gardner, T. A., Araujo, I. S., Avila-Pires, T. C., Bonaldo, A. B., Costa, J. E., et al. (2007). Quantifying the biodiversity value of tropical primary, secondary, and plantation forests. *Proc. Natl. Acad. Sci. U. S. A.* 104, 18555–18560. doi: 10.1073/pnas.0703333104

Bates, D., Mächler, M., Bolker, B. M., and Walker, S. (2015). Fitting linear mixed-effects models using lme4. *J. Stat. Software* 67, 1–48. doi: 10.18637/jss.v067.i01

Baumbach, L., Warren, D. L., Yousefpour, R., and Hanewinkel, M. (2021). Climate change may induce connectivity loss and mountaintop extinction in Central American forests. *Commun. Biol.* 4, 869. doi: 10.1038/s42003-021-02359-9

Bedogni, G. (2010). A beginner's guide to R. J. R. Stat. Soc. *Ser. A-Stat. Soc* 173, 697–698. doi: 10.1111/j.1467-985X.2010.00646_12.x

Bohnert, T., Wenzel, A., Altenhövel, C., Beeretz, L., Tjitrosoedirdjo, S. S., Meijide, A., et al. (2016). Effects of land-use change on vascular epiphyte diversity in Sumatra (Indonesia). *Biol. Conserv.* 202, 20–29. doi: 10.1016/j.biocon.2016.08.008

Bolharnordenkampe, H. R., Long, S. P., Baker, N. R., Oquist, G., Schreiber, U., and Lechner, E. G. (1989). Chlorophyll fluorescence as a probe of the photosynthetic competence of leaves in the field: a review of current instrumentation. *Funct. Ecol.* 3, 497–514. doi: 10.2307/2389624

Brezina, S., Koubek, T., Münzbergová, Z., and Herben, T. (2006). Ecological benefits of integration of *Calamagrostis epigejos* ramets under field conditions. *Flora.* 201, 461–467. doi: 10.1016/j.flora.2005.10.003

Butler, W. L., and Kitajima, M. (1975). Fluorescence quenching in photosystem II of chloroplasts. *Biochim. Biophys. Acta* 376, 116–125. doi: 10.1016/0005-2728(75)90210-8

Choat, B., Jansen, S., Brodribb, T. J., Cochard, H., Delzon, S., Bhaskar, R., et al. (2012). Global convergence in the vulnerability of forests to drought. *Nature.* 491, 752–75+. doi: 10.1038/nature11688

Dai, Q., Mo, Y. X., Chen, Q., Song, L., Zhang, L. M., Dossa, G. G. O., et al. (2023). Clonal fragmentation drives performance deterioration of epiphytic and lithophytic ferns in a karst forest. *Flora.* 302, 152258. doi: 10.1016/j.flora.2023.152258

De Frenne, P., Zellweger, F., Rodríguez-Sánchez, F., Scheffers, B. R., Hylander, K., Luoto, M., et al. (2019). Global buffering of temperatures under forest canopies. *Nat. Ecol. Evol.* 3, 744–749. doi: 10.1038/s41559-019-0842-1

de Kroon, H., Huber, H., Stuefer, J. F., and van Groenendael, J. M. (2005). A modular concept of phenotypic plasticity in plants. *New Phytol.* 166, 73–82. doi: 10.1111/j.1469-8137.2004.01310.x

Derzhavina, N. M. (2020). Ecological morphology of proto-epiphyte fern *lemmaphyllum microphyllum* C. Presl and its relation to the adaptogenesis. *Contemp. Probl. Ecol.* 13, 205–213. doi: 10.1134/S1995425520030051

Dong, B. C., Alpert, P., Guo, W., and Yu, F. H. (2012). Effects of fragmentation on the survival and growth of the invasive, clonal plant *Alternanthera philoxeroides*. *Biol. Invasions.* 14, 1101–1110. doi: 10.1007/s10530-011-0141-5

Ellis, C. (2012). Lichen epiphyte diversity: A species, community and trait-based review. *Perspect. Plant Ecol. Evol. Syst.* 14, 131–152. doi: 10.1016/j.ppees.2011.10.001

Fan, B. L., Zhao, C. M., Zhang, X. W., and Sun, K. (2018). Impacts of sand burial and wind erosion on regeneration and growth of a desert clonal shrub. *Front. Plant Sci.* 9. doi: 10.3389/fpls.2018.01696

Foster, P. (2001). The potential negative impacts of global climate change on tropical montane cloud forests. *Earth-Sci. Rev.* 55, 73–106. doi: 10.1016/S0012-8252(01)00056-3

Gargallo-Garriga, A., Sardans, J., Alrefaei, A. F., Klem, K., Fuchslueger, L., Ramírez-Rojas., I., et al. (2021). Tree species and epiphyte taxa determine the "*metabolomic niche*" of canopy suspended soils in a species-rich lowland tropical rainforest. *Metabolites.* 11, 718. doi: 10.3390/metabo11110718

Gauslaa, Y., Ohlson, M., Solhaug, K. A., Bilger, W., and Nybakken, L. (2001). Aspect-dependent high-irradiance damage in two transplanted foliose forest lichens, *Lobaria pulmonaria* and *Parmelia sulcata*. *Can. J. For. Res.* 31, 1639–1649. doi: 10.1139/x01-098

Grimshaw, H. J., Sharfstein, B., and East, T. (2005). The effects of shading on Chara zeylanica Klein ex Wild. and associated epiphytes. *Archiv für Hydrobiologie*. 162, 253–266. doi: 10.1127/0003-9136/2005/0162-0253

Heidbüchel, P., and Hussner, A. (2020). Fluorescence quenching in photosystem II of chloroplasts. *Aquat. Bot.* 160, 103164. doi: 10.1016/j.aquabot.2019.103164

Huber, H., Visser, E. J. W., Clements, G., and Peters, J. L. (2014). Flooding and fragment size interact to determine survival and regrowth after fragmentation in two stoloniferous *Trifolium* species. *Aob Plants.* 6, plu024. doi: 10.1093/aobpla/plu024

Jha, S., and Bawa, K. S. (2006). Population growth, human development, and deforestation in biodiversity hotspots. *S.Conserv. Biol.* 20, 906–912. doi: 10.1111/j.1523-1739.2006.00398.x

Kreft, H., Köster, N., Küper, W., Nieder, J., and Barthlott, W. (2004). Diversity and biogeography of vascular epiphytes in Western Amazonia, Yasuni, Ecuador. *J. Biogeogr.* 31, 1463–1476. doi: 10.1111/j.1365-2699.2004.01083.x

Kuznetsova, A., Brockhoff, P. B., and Christensen, R. H. B. (2017). lmerTest package: tests in linear mixed effects models. *J. Stat. Software* 82, 1–26. doi: 10.18637/jss.v082.i13

Laurance, W. F., Delamônica, P., Laurance, S. G., Vasconcelos, H. L., and Lovejoy, T. E. (2000). Conservation -: Rainforest fragmentation kills big trees. *Nature.* 404, 836–836. doi: 10.1038/35009032

Li, Q., Liu, X., Chai, Y. F., Yue, M., and Quan, J. X. (2020). Physiological integration ameliorates the effects of UV-B radiation in the clonal herb *Duchesnea indica*. *Folia Geobot.* 55, 141–150. doi: 10.1007/s12224-020-09369-1

Li, X. X., Shen, Y. D., Huang, Q. Q., Fan, Z. W., and Huang, D. D. (2013). Regeneration capacity of small clonal fragments of the invasive *Mikania micrantha* H.B.K.: effects of burial depth and stolon internode length. *PloS One* 8, e84657. doi: 10.1371/journal.pone.0084657

Lin, H. F., Alpert, P., Zhang, Q., and Yu, F. H. (2018). Facilitation of amphibious habit by physiological integration in the clonal, perennial, climbing herb *Ipomoea aquatica*. *Sci. Total Environ.* 618, 262–268. doi: 10.1016/j.scitotenv.2017.11.025

Liu, J. Q., Liu, W. J., Li, W. X., Jiang, X. J., and Wu, J. E. (2018). Effects of rainfall on the spatial distribution of the throughfall kinetic energy on a small scale in a rubber plantation. *Hydrol. Sci. J.* 63, 1078–1090. doi: 10.1080/02626667.2018.1473580

Liu, J. Q., Liu, W. J., and Zhu, K. (2020). Fluorescence quenching in photosystem II of chloroplasts. *Catena* 161, 113–121. doi: 10.1016/j.catena.2017.10.014

Liu, W. G., Zhang, J. Q., Yan, Y., Beckschäfer, P., Kleinn, C., Dossa, G. G. O., et al. (2023). Encouraging the reconversion of rubber plantations by developing a combined payment system. *Glob. Ecol. Conserv.* 43, e02415. doi: 10.1016/j.gecco.2023.e02415

Lu, H. Z., Brooker, R., Song, L., Liu, W. Y., Sack, L., Zhang, J. L., et al. (2020). When facilitation meets clonal integration in forest canopies. *New Phytol.* 225, 135–142. doi: 10.1111/nph.16228

Lu, H. Z., Liu, W. Y., Yu, F. H., Song, L., Xu, X. L., Wu, C. S., et al. (2015). Higher clonal integration in the facultative epiphytic fern *Selliguea griffithiana* growing in the forest canopy compared with the forest understorey. *Ann. Bot.* 116, 113–122. doi: 10.1093/aob/mcv059

Lu, H. Z., Song, L., Liu, W. Y., Xu, X. L., Hu, Y. H., Shi, X. M., et al. (2016). Survival and growth of epiphytic ferns depend on resource sharing. *Front. Plant Sci.* 7. doi: 10.3389/fpls.2016.00416

Luo, F. L., Chen, Y., Huang, L., Wang, A., and Zhang, M. X. (2014). Shifting effects of physiological integration on performance of a clonal plant during submergence and de-submergence. *H.Ann. Bot.* 113, 1265–1274. doi: 10.1093/aob/mcu057

Malta, E., Rijstenbil, J. W., Brouwer, P. E. M., and Kromkamp, J. C. (2003). Vertical heterogeneity in physiological characteristics of *Ulva* spp. mats. *Mar. Biol.* 143, 1029–1038. doi: 10.1007/s00227-003-1134-4

Morton, D. C., Le, Page., Y., DeFries, R., Collatz, G. J., and Hurtt, G. C. (2013). Understorey fire frequency and the fate of burned forests in southern Amazonia. Philos. *Trans. R. Soc B-Biol. Sci.* 368, 20120163. doi: 10.1098/rstb.2012.0163

Nadkarni, N. M., and Solano, R. (2002). Potential effects of climate change on canopy communities in a tropical cloud forest: an experimental approach. *Oecologia.* 131, 580–586. doi: 10.1007/s00442-002-0899-3

Ortego, J., Bonal, R., and Muñoz, A. (2002). Genetic consequences of habitat fragmentation in long-lived tree species: the case of the mediterranean holm oak (*Quercus ilex, L.*). *J. Hered.* 101, 717–726. doi: 10.1093/jhered/esq081

Platt, L., Hickman, M., Rhodes, T., Mikhailova, L., Karavashkin, V., Vlasov, A., et al. (2004). The prevalence of injecting drug use in a Russian city: implications for harm reduction and coverage. *Addiction.* 99, 1430–1438. doi: 10.1111/j.1360-0443.2004.00848.x

Polson, D., Hegerl, G. C., and Solomon, S. (2016). Precipitation sensitivity to warming estimated from long island records. *Environ. Res. Lett.* 11, 7. doi: 10.1088/1748-9326/11/7/074024

Qi, D. L., Yang, C., Yun, T., and Wu, Z. X. (2023). The main service functions and driving forces of rubber (*Hevea brasiliensis*) plantation ecosystem in China. *J. Rubber Res.* 26, 155–164. doi: 10.1007/s42464-023-00202-w

Reynolds, P. L., Richardson, J. P., and Duffy, J. E. (2014). Field experimental evidence that grazers mediate transition between microalgal and seagrass dominance. *Limnol. Oceanogr.* 59, 1053–1064. doi: 10.4319/lo.2014.59.3.1053

Roa-Fuentes, L., Villamizar-Peña, L. A., Mantilla-Carreño, J. A., and Jaramillo, M. A. (2022). Functional diversity and species diversity in flooded and unflooded tropical forests. *Acta Oecol.-Int. J. Ecol.* 114, 103814. doi: 10.1016/j.actao.2022.103814

Rosef, L., Ingebrigtsen, H. H., and Heegaard, E. (2020). Vegetative propagation of Solidago canadensis - do fragment size and burial depth matter? *Weed Res.* 60, 132–141. doi: 10.1111/wre.12395

Rudolphi, J., and Gustafsson, L. (2011). Forests regenerating after clear-cutting function as habitat for bryophyte and lichen species of conservation concern. *PloS One* 6, e18639. doi: 10.1371/journal.pone.0018639

Ruiz, J. M., Marín-Guirao, L., García-Muñoz, R., Ramos-Segura, A., Bernardeau-Esteller, J., Pérez, M., et al. (2018). Experimental evidence of warming-induced flowering in the *Mediterranean seagrass. Posidonia oceanica.* 134, 49–54. doi: 10.1016/j.marpolbul.2017.10.037

Saine, S., Aakala, T., Purhonen, J., Launis, A., Tuovila, H., Kosonen, T., et al. (2018). Effects of local forest continuity on the diversity of fungi on standing dead pines. *For. Ecol. Manage.* 409, 757–765. doi: 10.1016/j.foreco.2017.11.045

Song, Z. L., Huang, Y. Y., Liu, Q., and Hu, X. K. (2022). Discovering the characteristics of community structures and functional properties of epiphytic bacteria on *spartina alterniflora* in the coastal salt marsh area. *J. Mar. Sci. Eng.* 10, 1981. doi: 10.3390/jmse10121981

Song, Y. B., Yu, F. H., Keser, L. H., Dawson, W., Fischer, M., Dong, M., et al. (2013a). United we stand, divided we fall: a meta-analysis of experiments on clonal integration and its relationship to invasiveness. *Oecologia.* 171, 317–327. doi: 10.1007/s00442-012-2430-9

Song, Y. B., Yu, F. H., Li, J. M., Keser, L. H., Fischer, M., Dong, M., et al. (2013b). Plant invasiveness is not linked to the capacity of regeneration from small fragments: an experimental test with 39 stoloniferous species. *Biol. Invasions.* 15, 1367–1376. doi: 10.1007/s10530-012-0374-y

State Key Laboratory of Systematic and Evolutionary Botany. (2023). Institute of Botany, Chinese Academy of Sciences. Flora of China. http://www.iplant.cn/foc. [Accessed October 12, 2023].

Stuefer, J. F., Erschbamer, B., Huber, H., and Suzuki, J. I. (2001). The ecology and evolutionary biology of clonal plants: an introduction to the proceedings of Clone-2000. *Evol. Ecol.* 15, 223–230. doi: 10.1023/A:1016017822027

Suzuki, J. I., and Stuefer, J. F. (1999). On the ecological and evolutionary significance of resource storage in clonal plants. *Plant Species Biol.* 14, 11–17. doi: 10.1046/j.1442-1984.1999.00002.x

Truscott, A. M., Soulsby, C., Palmer, S. C. F., Newell, L., and Hulme, P. E. (2006). The dispersal characteristics of the invasive plant *Mimulus guttatus* and the ecological significance of increased occurrence of high-flow events. J. *Ecol.* 94, 1080–1091. doi: 10.1111/j.1365-2745.2006.01171.x

Wang, P., Alpert, P., and Yu, F. H. (2017). Clonal integration affects allocation in the perennial herb *Alternanthera philoxeroides* in N-limited homogeneous environments. *Folia Geobot.* 52, 303–315. doi: 10.1007/s12224-016-9273-9

Wang, M. Z., Bu, X. Q., Li, L., Dong, B. C., Li, H. L., and Yu, F. H. (2018). Constraints on the evolution of phenotypic plasticity in the clonal plant *hydrocotyle vulgaris. J. Evol. Biol.* 31, 1006–1017. doi: 10.1111/jeb.13281

Wang, P., Xu, Y. S., Dong, B. C., Xue, W., and Yu, F. H. (2014). Effects of clonal fragmentation on intraspecific competition of a stoloniferous floating plant. *Plant Biol.* 16, 1121–1126. doi: 10.1111/plb.12170

Wang, J. Y., Xu, T. T., Wang, Y., Li, G. Y., Abdullah, I., Zhong, Z. W., et al. (2020). A meta-analysis of effects of physiological integration in clonal plants under homogeneous vs. heterogeneous environments. *Funct. Ecol.* 35, 578–589. doi: 10.1111/1365-2435.13732

Wei, Q., Li, Q., Jin, Y., Wu, S. L., Fan, L. H., Lei, N. F., et al. (2019). Transportation or sharing of stress signals among interconnected ramets improves systemic resistance of clonal networks to water stress. *Funct. Plant Biol.* 46, 613–623. doi: 10.1071/FP18232

Wei, X. P., Qi, Y. D., Zhang, X. C., Luo, L., Shang, H., Wei, R., et al. (2017). Phylogeny, historical biogeography and characters evolution of the drought resistant fern *Pyrrosia* Mirbel (Polypodiaceae) inferred from plastid and nuclear markers. *Sci. Rep.* 7, 12757. doi: 10.1038/s41598-017-12839-w

Willmer, P. (2012). Ecology: pollinator-plant synchrony tested by climate change. *Curr. Biol.* 22, R131–R132. doi: 10.1016/j.cub.2012.01.009

Yang, B., Zhang, W. J., Meng, X. J., Singh, A. K., Zakari, S., Song, L., et al. (2020). Effects of a funnel-shaped canopy on rainfall redistribution and plant water acquisition in a banana (*Musa* spp.) plantation. *Soil Tillage Res.* 203, 104686. doi: 10.1016/j.still.2020.104686

Zambrano, J., and Salguero-Gomez, R. (2014). Forest fragmentation alters the population dynamics of a late-successional tropical tree. *Biotropica.* 46, 556–564. doi: 10.1111/btp.12144

Zellweger, F., De Frenne, P., Lenoir, J., Vangansbeke, P., Verheyen, K., and Bernhardt-Römermann, M. (2020). Forest microclimate dynamics drive plant responses to warming. *Science.* 368, 772–77+. doi: 10.1126/science.aba6880

Zeng, H. H., Wu, J. E., Singh, A. K., Zhu, X. A., Zhang, W. J., Hahn, P., et al. (2018). Effect of intercrops complexity on water uptake patterns in rubber plantations: Evidence from stable isotopes (C-H-O) analysis. *Agric. Ecosyst. Environ.* 338, 108086. doi: 10.1016/j.agee.2022.108086

Zhang, L. M., Alpert, P., Si, C., and Yu, F. H. (2019). Interactive effects of fragment size, nutrients, and interspecific competition on growth of the floating, clonal plant *Salvinia natans. Aquat. Bot.* 153, 81–87. doi: 10.1016/j.aquabot.2018.12.001

Zhang, Q., Chen, J. W., Li, B. G., and Cao, K. F. (2009). The effect of drought on photosynthesis in two epiphytic and two terrestrial tropical fern species. *Photosynthetica.* 47, 128–132. doi: 10.1007/s11099-009-0020-9

Zhang, R., Chen, Z. H., Lyu, W. Y., Hu, Y. F., Yang, J. X., Xin, J. C., et al. (2023). Effects of fragmentation, cadmium, and nutrients on the growth of a wetland invasive plant Alternanthera philoxeroides. *Plant Spec. Biol.* 38, 180–191. doi: 10.1111/1442-1984.12405

Zhang, L. M., Jin, Y., Yao, S. M., Lei, N. F., Chen, J. S., Zhang, Q., et al. (2020b). Growth and morphological responses of Duckweed to clonal fragmentation, nutrient availability, and population density. *Front. Plant Sci.* 11. doi: 10.3389/fpls.2020.00618

Zhang, J., Li, N. N., Song, A., You, W. H., and Du, D. L. (2023). Clonal integration can promote the growth and spread of *Alternanthera philoxeroides* in cadmium-contaminated environments. *Plant Physiol. Biochem.* 202, 107966. doi: 10.1016/j.plaphy.2023.107966

Zhang, L. M., Yao, S. M., Jin, Y., Yao, S. M., Lei, N. F., Chen, J. S., et al. (2020a). Effects of clonal fragmentation and nutrient availability on the competitive ability of the floating plant *Salvinia natans*. *Folia Geobot.* 55, 63–71. doi: 10.1007/s12224-020-09365-5

Zheng, G., Li, S. Q., and Yang, X. D. (2015). Spider diversity in canopies of Xishuangbanna rainforest (China) indicates an alarming juggernaut effect of rubber plantations. *For. Ecol. Manage.* 338, 200–207. doi: 10.1016/j.foreco.2014.11.031

Zotz, G., and Hietz, P. (2001). The physiological ecology of vascular epiphytes: current knowledge, open questions. *J. Exp. Bot.* 52, 2067–2078. doi: 10.1093/jexbot/52.364.2067

frontiers | Frontiers in Plant Science

TYPE Original Research
PUBLISHED 14 May 2024
DOI 10.3389/fpls.2024.1358924

OPEN ACCESS

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RECEIVED 20 December 2023
ACCEPTED 10 April 2024
PUBLISHED 14 May 2024

CITATION
Xing L, Quan J, Zhang S, Liu X, Bai H and Yue M (2024) Changes induced by parental neighboring touch in the clonal plant *Glechoma longituba* depend on the light environment.
Front. Plant Sci. 15:1358924.
doi: 10.3389/fpls.2024.1358924

Changes induced by parental neighboring touch in the clonal plant *Glechoma longituba* depend on the light environment

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Introduction: Touch by neighboring plants is a common but overlooked environmental variable for plants, especially in dense vegetation. In addition, shade is inevitable for understory plants. The growth performance of clonal plant to the interaction between thigmomorphogenesis and shade response, and their impact on light adaptability is still unknown.

Methods: At the present study, parental ramets of *Glechoma longituba* were exposed to two conditions (neighboring touch and shade), and their offspring ramets were in ambient or shaded environment. The phenotype and growth of parental and offspring ramets were analyzed.

Results: The results showed that neighboring touch of parental ramets regulated the performance of offspring ramets, while the effect depended on the light environment. The parental neighboring touch occurring in ambient environment suppressed the expansion of leaf organ, showed as a shorter petiole and smaller leaf area. Moreover, *G. longituba* exhibited both shade avoidance and shade tolerance characters to shaded environment, such as increased leaf area ratio and leaf mass ratio, longer specific petiole length and specific stolon length. It was notable that these characters of shade response could be promoted by parental neighboring touch to some extent. Additionally, parental light environment plays an important role in offspring growth, parent with ambient light always had well-grown offspring whatever the light condition of offspring, but the growth of offspring whose parent in shaded environment was inhibited. Finally, for the offspring with shaded environment, the touch between parental ramets in shade environment showed a disadvantage on their growth, but the influence of the touch between parental ramets in ambient environment was slight.

Discussion: Overall, the interaction of parental neighboring touch and shade environment complicate the growth of understory plants, the performance of plants is the integrated effect of both. These findings are conducive to an in-depth understanding of the environmental adaptation of plants.

KEYWORDS

clonal plant, parental neighboring touch, thigmomorphogenetic, shade response, maternal effect, light adaptability

Introduction

Plants in dense vegetation compete for resources and optimize their growth based on neighbor detection cues. Accordingly, neighbor detection and response strategies are important mediators of interactions among species, which plays significant roles in plant coexistence and community assembly (de Wit et al., 2012; Pierik et al., 2013). The underlying mechanism of interactions among plants is related to light quality variation (Crepy and Casal, 2015; Zhang et al., 2020), root chemicals (Semchenko et al., 2014; Kong et al., 2018), airborne volatile organic compounds (Baldwin et al., 2006; Ninkovic et al., 2019), and mechanical stimuli caused by neighboring plants (Elhakeem et al., 2018; Douma and Anten, 2019). Among these, mechanical stimuli exist commonly in nature but are often ignored (Li and Gong, 2011). Except for stimuli from neighboring touch, mechanical stimuli are also related to wind, insect herbivory, heavy rains, buzzing bees, tree strangling, acoustic vibration, and the navigation of roots around obstacles (Monshausen and Haswell, 2013; Brenya et al., 2022). As one of the environmental factors, mechanical stimuli have been shown to induce a range of anatomical, physiological, morphological, and developmental responses, termed thigmomorphogenesis (Jaffe, 1973). Thigmomorphogenetic plants typically have a reduced leaf area, petiole length, height, and stem length, and enhanced flexural rigidity in stem and roots; these make plants more resistant to mechanical stimuli and more likely to survive in stressful environments (Liu et al., 2007; Braam and Chehab, 2017). Significantly, the touch signal of neighboring leaf tips occurs before light signals and appears to be the earliest means of aboveground plant–plant signaling (de Wit et al., 2012). As a unique way for plants to rapidly detect future competitors, the touch by neighbors may play an important role in the environmental adaptability of plants.

Understory plants employ two different strategies to cope with shade conditions: shade avoidance syndrome (SAS) and shade tolerance syndrome (STS). Although both will optimize light capture and utilization by increased specific leaf area (SLA) and photosystem II and I ratio (PSII : PSI), and reduced chlorophyll a:b ratio, the plants with STS appear to have a slight elongation in low light (Valladares and Niinemets, 2008). Plants with SAS mainly manifested a suite of traits to grow towards the light including stem and petiole elongation reaction and leaves bending upward (Fraser et al., 2016; Fiorucci and Fankhauser, 2017; Xu et al., 2021; Martinez-Garcia and Rodriguez-Concepcion, 2023). The overlap between two strategies is due to the common signaling components associated with photoreceptors and phytohormone (Gommers et al., 2013). For example, phytochrome-mediated signaling pathways trigger the increase of gibberellins in shading plants, subsequently promoting stem and petiole elongation (Pierik et al., 2004; Ballare and Pierik, 2017). In contrast, the reduction of gibberellins is a key factor of phenotype change induced by thigmomorphogenesis (Boernke and Rocksch, 2018; Telewski, 2021; Wang et al., 2023). Therefore, the change in gibberellins level and the corresponding growth response of thigmomorphogenesis and SAS usually occur in opposite directions (Anten et al., 2005). However, for dense understory vegetation, the touch by neighbors and the shaded environment often coexist. Accordingly, plant performance is the result of the interaction of both. There have been several studies involving the interaction between mechanical stimuli and shade, but the results were different. Some studies showed that competition for light in dense vegetation suppressed thigmomorphogenesis because any reduction in height growth associated with thigmomorphogenesis resulted in reduced fitness (Sterck and Bongers, 1998; Henry and Thomas, 2002), while others did not find any thigmomorphogenesis inhibition for plants in dense stands (Liu et al., 2007). As such, the interaction of these two conditions is still unknown, and more research is required to thoroughly understand the growth of understory plants.

In addition, the performance of plants is also affected by the environment of their parents (Galloway and Etterson, 2007; Marshall and Uller, 2007; Dong et al., 2019), which regulates the phenotype, growth, and life history strategies of the offspring so that they may pre-adapt to the future environment (Galloway, 2005; Wang et al., 2022). This effect of parental environment on their offspring was termed "maternal effect". This impact is not transmitted through genetic inheritance but rather through the environment or other non-genetic factors provided by the parent during the developmental and reproductive processes (Galloway, 2005; Marshall and Uller, 2007). Many studies indicate that maternal effects could persist in the offspring. For instance, compared to the *Polygonum persicaria* offspring of sun-grown parents, the offspring of parents with a shaded environment produced greater leaves, larger SLA, higher total leaf area and biomass, and greater fitness (Galloway and Etterson, 2007; Baker et al., 2018). In addition, maternal effects induced light adaptability of *Campanula americana* offspring by directly influencing their germination rate and season (Galloway, 2005). In summary, the growth of offspring is often affected by the light environment of the parents. To our knowledge, there are very few reports on the maternal effects of the interaction between neighboring touch and shade.

In the present study, the interaction of parental neighboring touch and shade was explored with the clonal herb *Glechoma longituba* (Nakai) Kuprian, a "faithful" transmitter of the maternal effect (Guo et al., 2022; Tie et al., 2022). Two environmental factors (light and touch) were conducted on the parental ramets. By analyzing the growth parameters of parental ramets and their offspring, the following questions were addressed: (a) Does the effect of parental neighboring touch vary with a light environment? (b) If SAS occurs, what is the result of the interaction between thigmomorphogenesis and SAS? (c) Is the effect of parental light environment on clonal offspring affected by parental neighboring touch? We predicted that the impact of parental neighboring touch on clonal plants was linked to their light environment, which also influenced the maternal effect on environmental adaptability of clonal offspring.

Materials and methods

Plant material and propagation

Glechoma longituba (Nakai) Kuprian is a perennial herb widely distributed in the forest, on the roadside, and by creeks of tropical, subtropical, and temperate areas in China. The monopodial stolons

are able to creep on the ground, and ramets can develop on each stolon node (Quan et al., 2021). Each ramet produces two petioles and leaves. The blades are heart-shaped, with margins bearing rounded teeth (Figure 1). *G. longituba* has high phenotypic plasticity in response to light (Liu et al., 2015; Tie et al., 2022). In addition, for the rapid clonal propagation, *G. longituba* forms a complex ramet network; touch between ramets was inevitable.

The *G. longituba* in our experiment was collected from Jiwozi in the Qinling Mountains, Shaanxi, China. To ensure the uniformity of the genotypes and reduce the impact of the previous environment, the plant materials were collected from the same genet and then were vegetatively propagated for at least 4 months in a greenhouse at Northwest University in Xi'an (397 m a.s.l., 34.3°N, 108.9°E).

A total of 96 healthy and similarly sized (0.78 g ± 0.29 g) ramets were selected as parental ramets, and then were divided into 48 ramet pairs randomly. Each ramet pair was transplanted into a plastic pot (55 cm length × 28 cm width × 5.5 cm depth). The peat soil, perlite, and vermiculite (4:1:1, v/v/v) were mixed and utilized as the culture soil. The greenhouse conditions were a 24/20°C day/night temperature cycle, a 12/12-h light/dark cycle, and a 300 μmol m^{-2} s^{-1} photosynthetic photo flux density (PPFD) during the light cycle. PPFD levels were measured with a Quantum Metre (LQS-QM, Apogee Instruments Inc., USA). The relative humidity was maintained at 40%. Plants were watered into the soil directly every 3 days throughout the experiment and researchers avoided the droplets touching the leaves during watering. To minimize the effects of microenvironment variation in the greenhouse, the position of each pot was changed weekly.

Experimental design

After a week of transplanting, a 45-day experiment was conducted from 1 November 2022 to 15 December 2022. At first, 48 parental ramet pairs were divided into two groups randomly; half was placed under ambient light conditions, and the rest was placed in a shaded environment. Then, half of each group was subjected to touch treatment, and the other had no action. During the experiment, the newborn offspring was placed in an ambient light environment or under shade conditions (Figure 2). Accordingly, the experiment included a total of eight treatments (two parental light conditions × two parental neighboring touch treatments × two offspring light conditions). Each treatment was designed with six replicates.

Shading treatment

The shaded environment was achieved by hanging a shading net 20 cm above the top of ramets. The PPFD was about 80–100 μmol m^{-2} s^{-1} in the shaded environment. During the experiment, according to the experiment's design, if the parent and offspring were in different light environments, the shading treatment was only performed above the parent or offspring.

Parental neighboring touch treatment

The touch treatment was conducted between parental ramet pairs. Two petioles of the neighbor parental ramets were waved with a thin wooden stick gently to make the two leaf blades touch. During the treatment, we tried to minimize the bending angle of the petiole to avoid damage. The touch treatment was only performed on parental ramets and continued for 45 days. The touch was handled twice a day at 9:00 a.m. and 6:00 p.m., respectively; three contacts were made each time. To ensure the uniform touch intensity during repeated action, all the touch treatments were controlled by the same person. Furthermore, during the experiment, the distance between parental ramet pairs was maintained at 5 cm, a distance that is long enough for leaves of ramet pairs to not touch each other naturally during growth except for human-facilitated touch.

Measurement of growth parameters

At the end of the experiment, we performed growth architecture and biomass measurements. For parents, the blade area and petiole

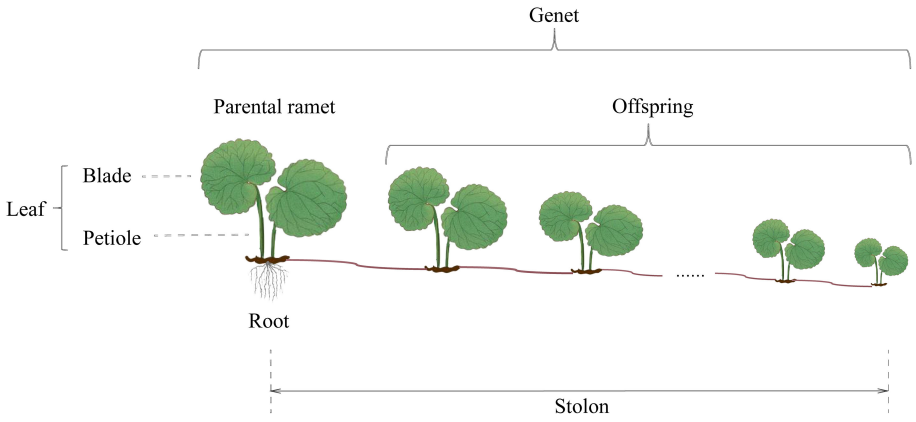

FIGURE 1
Clonal growth pattern of *Glechoma longituba* (Nakai) Kuprian.

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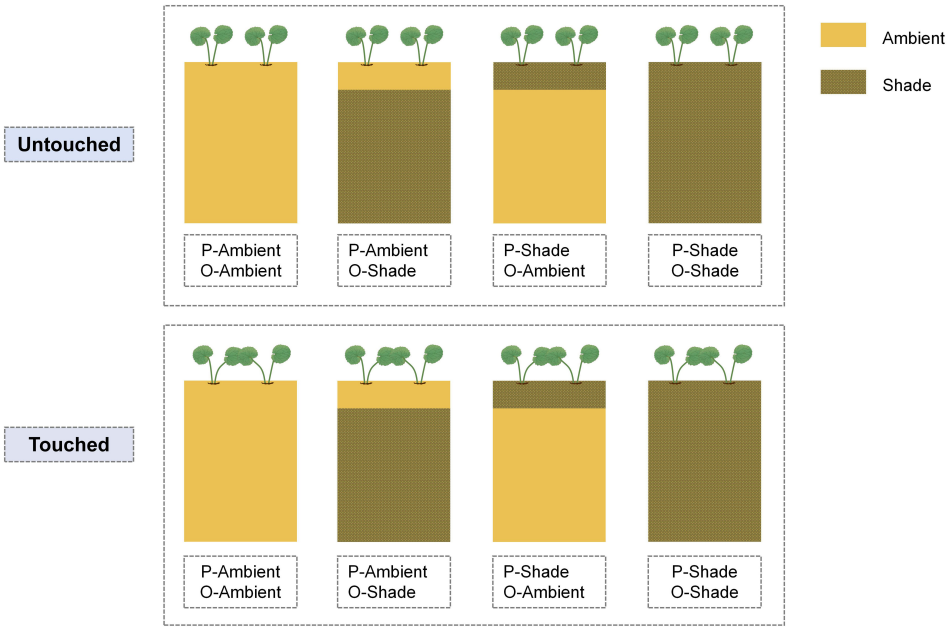

FIGURE 2
Schematic of the experiment. P-ambient: parents grew in an ambient environment; P-shade: parents grew in a shaded environment; O-ambient: offspring grew in an ambient environment; O-shade: offspring grew in a shaded environment. Touched: neighboring parental ramets were waved with a thin wooden stick gently to make two blade touch; Untouched: no touch happened between a pair of parental ramets.

length were first measured. Then, biomass indexes were measured, including total biomass, aboveground biomass (leaf biomass and node biomass), and root biomass. Finally, the following indicators of parental ramets were estimated: SLA (leaf area per leaf mass), specific petiole length (SPL; length of petiole per petiole biomass), leaf area ratio (LAR; blade area per total biomass), and leaf mass ratio (LMR; blade biomass per total biomass).

For offspring, the length of the longest stolon, total blade area, and the number of total ramets were recorded. After that, total biomass, leaf biomass, and stolon biomass were measured. The following indicators were estimated: specific stolon length (SSL; length of stolon per stolon biomass), stolon biomass/leaf biomass (S/L), LAR (blade area per total biomass), and LMR (blade biomass per total biomass). The comparison table of abbreviations is shown in Table 1.

Length of petiole and stolon was measured with a vernier caliper and tapeline, respectively. Blade area was calculated with Motic software (Motic Images Plus 2.0. Ink, Motic, China) after being scanned with a scanner (Perfection V19, EPSON, China). Biomass was measured after the samples were dried at 80°C for 48 h to a constant weight with an electronic balance (Sartorius BT25S, Beijing, China).

Statistical analysis

Before statistical analyses, to meet the assumptions of homoscedasticity and normality, the following data were subjected to logarithmic transformation: total biomass and root biomass of parent, and total biomass, stolon biomass, and S/L of offspring. We analyzed the integrated differences of phenotypic and growth among treatments of parental ramets and offspring, respectively, using a PERMANOVA (McArdle and Anderson, 2001), where parental neighboring touch treatment (NT; touch or not), parental light environment (PL; ambient or shade), offspring conditions (ambient or shade) and their interactions, and the covariates (initial fresh weight of parental ramet) were used as predictors, estimating the significance value by 999 permutations. Then, we conducted ANCOVAs to analyze the effects of PL, offspring light environment (OL), NT, and interaction regimes on all traits with parental fresh weight as a covariate. The LSD test was chosen as the method of multiple comparisons to test the significance among different treatments. Any effect of parental conditions, either direct (PL or NT) or in interaction with the offspring conditions (PL × OL, NT × OL, PL × NT × OL) that remained significant after removing the linear part of the maternal

TABLE 1 Abbreviation comparison.

Abbreviation	Complete spellings
SAS	Shade avoidance syndrome
STS	Shade tolerance syndrome
SLA	Specific leaf area
SPL	Specific petiole length
SSL	Specific stolon length
R/S	Root biomass/shoot biomass
S/L	Stolon biomass/leaf biomass
LAR	Leaf area ratio
LMR	Leaf mass ratio

investment (initial fresh weight of parent), was considered a maternal effect (Bolker et al., 2009; Galloway et al., 2009; Puy et al., 2022). All data analyses and diagrams were carried out using R (v.3.2.3, R Core Team, 2016) with $\alpha = 0.05$ as significance level. The images were further processed with Adobe Illustrator (v 28.1, Adobe Systems Incorporated, 2023).

Results

The PL, OL, NT, the interaction of PL and OL (PL × OL), and the interaction of PL and NT (PL × NT) significantly affected the growth of parental ramets and offspring. Nevertheless, the interaction of OL and NT (OL × NT) and three factors (PL × OL × NT) had no effect on them. Among three factors, PL displayed the most dissimilar traits of *G. longituba* among treatments ($R^2 = 0.297$ for parent and $R^2 = 0.519$ for offspring). For the parent, the next important factor was OL ($R^2 = 0.167$), followed by PL × NT ($R^2 = 0.075$), PL × OL ($R^2 = 0.071$), and NT ($R^2 = 0.068$). For the offspring, OL ($R^2 = 0.173$), NT ($R^2 = 0.048$), PL × OL ($R^2 = 0.036$), and PL × NT ($R^2 = 0.032$) played significant effects sequentially (Supplementary Table S1).

The influence of parental neighboring touch on parental ramets in different light environments

For parental ramets, there were significant effects of PL, OL, and NT on biomass (including total, aboveground, and root biomass). Additionally, PL affected SLA, SPL, LAR, and R/S; OL showed an effect on the blade area, SPL, LAR, LMR, and R/S, while NT influenced SLA, LAR, and petiole length; the interaction of PL and OL affected the blade area, petiole length, LMR, LAR, and R/S significantly; PL × NT influenced the blade area, SLA, and LAR. Moreover, OL × NT and PL × OL × NT did not show an effect on parents (Table 2).

When the genet (including parent and their offspring ramets) was in ambient light, neighboring touch (NT) mainly reduced the blade area and the petiole length of parental ramets but had no significant effect on other parental parameters. Additionally, compared with the ambient environment, the growth of genet in a shaded environment was depressed. Parental ramets displayed reduction in the blade area, biomass (aboveground, roots, and total biomass), and R/S, but increased SPL, LAR, and LMR. However, in the shaded environment, if touch between neighboring parental ramets happened, some traits induced by shade were changed. Upon recovery from the reduction in the parental blade area, SLA and LAR even became larger (Figure 3).

The influence of parental neighboring touch on offspring ramets in different light environments

For the offspring ramets, PL and OL displayed effects on almost all traits involved in our study, except for OL on stolon biomass. Neighboring touch treatment (NT) had effects on most traits except for stolon biomass/leaf biomass and LMR. Moreover, the PL × OL interaction affected the blade area, leaf biomass, and SSL of the longest stolon; PL × NT influenced total biomass, stolon biomass, blade area, and stolon/leaf biomass, but OL × NT only affected leaf biomass. Same as the parent, PL × OL × NT did not show an effect on the offspring (Table 3).

When the genet was in ambient light, in offspring, parental neighboring touch decreased total leaf biomass and blade area, but enhanced S/L. When the genet was in a shaded environment, as compared with the ambient environment, shade leads to a reduction in biomass (stolon, leaf, and total biomass), the total blade area, and the length of the longest stolon. In contrast, there is an increase in SSL and S/L. However, if touch between neighboring parental ramets happened, it caused a greater decrease in the total biomass, stolon biomass, and length of the longest stolon than in those without touch (Figure 4).

The influence of parental light environment on offspring light adaptability

We compared the offspring performance in the same light environment as their parents or in a different light environment (Figure 4). When the parental ramets were in ambient light, the offspring with a shaded environment did not show a significant difference from offspring with ambient light in the total biomass, stolon biomass, and length of longest stolon. However, they had a lower number of ramets, leaf biomass, blade area, LAR, and LMR, and higher SSL and stolon biomass/leaf biomass. Additionally, if the parental ramets are in a shaded environment, even if their offspring grew in an ambient environment, these offspring will still have a lower leaf, stolon, and total biomass, a smaller blade area, a shorter stolon length, but a larger SSL. In contrast to the offspring with ambient light, the offspring with a shaded environment will have the lowest total biomass, leaf biomass, blade area, LAR, LMR and stolon length, but the largest SSL.

The effect of parental neighboring touch on offspring light adaptability

When parents grew in ambient light, the following traits of offspring with ambient light were changed by parental neighboring touch: leaf biomass, leaf area, ramet number, LAR, and LMR were decreased, and stolon biomass/leaf biomass was increased. Total biomass, stolon biomass, SSL, and stolon length did not change significantly. For the offspring with a shaded environment, touch just reduced leaf area and LAR; others were unchanged (Figure 4).

When parents grew in a shaded environment and offspring grew in ambient light, except for ramet number, stolon biomass/leaf biomass, LAR, and LMR without any changes, SSL was increased and all other traits decreased due to parental neighboring touch. For offspring with a shaded environment, however, leaf biomass, leaf area, ramet number, stolon biomass/leaf biomass, SSL, LAR, and LMR were not influenced by parental neighboring touch, with only total biomass and the length and biomass of stolon decreased (Figure 4).

TABLE 2 Three-way ANCOVAs for effects of parental light environment (PL), offspring light environment (OL), parental neighboring touch (NT), and their interactions on growth indicators of parental ramets.

Source of variation	df	Total biomass (g) [a]		Aboveground biomass (g)		Root biomass (g) [a]		R/S		Blade area (cm^2)		SLA (cm^2/g)		Petiole length (cm)		SPL (cm/g)		LAR(cm^2/g) [a]		LMR (g/g)	
		F	*p*	*F*	*p*	*F*	*p*	*F*	*p*	*F*	*p*	*F*	*p*	*F*	*p*	*F*	*p*	*F*	*p*	*F*	*p*
Fresh weight	1	6.993	**0.014**	4.508	**0.045**	7.484	**0.012**	2.195	0.152	0.541	0.470	3.642	0.069	0.028	0.869	4.701	**0.041**	5.830	**0.024**	0.637	0.433
Parental light environment (PL)	1	48.609	**<0.001**	32.886	**<0.001**	46.368	**<0.001**	23.781	**<0.001**	0.246	0.625	58.640	**<0.001**	0.303	0.588	9.997	**0.004**	77.721	**<0.001**	1.888	0.183
Offspring light environment (OL)	1	24.918	**<0.001**	11.064	**0.003**	51.234	**<0.001**	44.822	**<0.001**	7.510	**0.012**	0.693	0.414	<0.001	0.995	7.422	**0.012**	9.724	**0.005**	21.517	**<0.001**
Neighbor touch (NT)	1	7.121	**0.014**	5.333	**0.03**	6.016	**0.022**	0.291	0.595	0.147	0.704	17.118	**<0.001**	16.543	**<0.001**	0.317	0.579	12.841	**0.002**	0.401	0.533
PL×OL	1	0.406	0.530	0.806	0.378	1.863	0.186	15.859	**0.001**	9.461	**0.005**	2.257	0.147	9.250	**0.006**	0.147	0.704	6.867	**0.015**	10.420	**0.004**
PL×NT	1	0.329	0.572	0.029	0.866	0.077	0.784	0.228	0.638	22.088	**<0.001**	30.731	**<0.001**	4.150	0.053	1.425	0.245	35.503	**<0.001**	0.133	0.719
OL×NT	1	1.226	0.280	1.537	0.228	0.606	0.444	<0.001	0.997	0.696	0.413	2.411	0.134	0.232	0.634	1.542	0.227	2.519	0.126	0.001	0.999
PL×OL×NT	1	0.007	0.934	0.035	0.853	0.639	0.432	2.183	0.153	1.197	0.285	0.262	0.614	2.162	0.155	<0.001	0.997	0.613	0.442	0.975	0.334

Values in bold indicate significant effects ($p < 0.05$) of factors and their interactions. The lowercase letter "a" indicates that the data are log-transformed.
The shading indicates that the p-value is significant.

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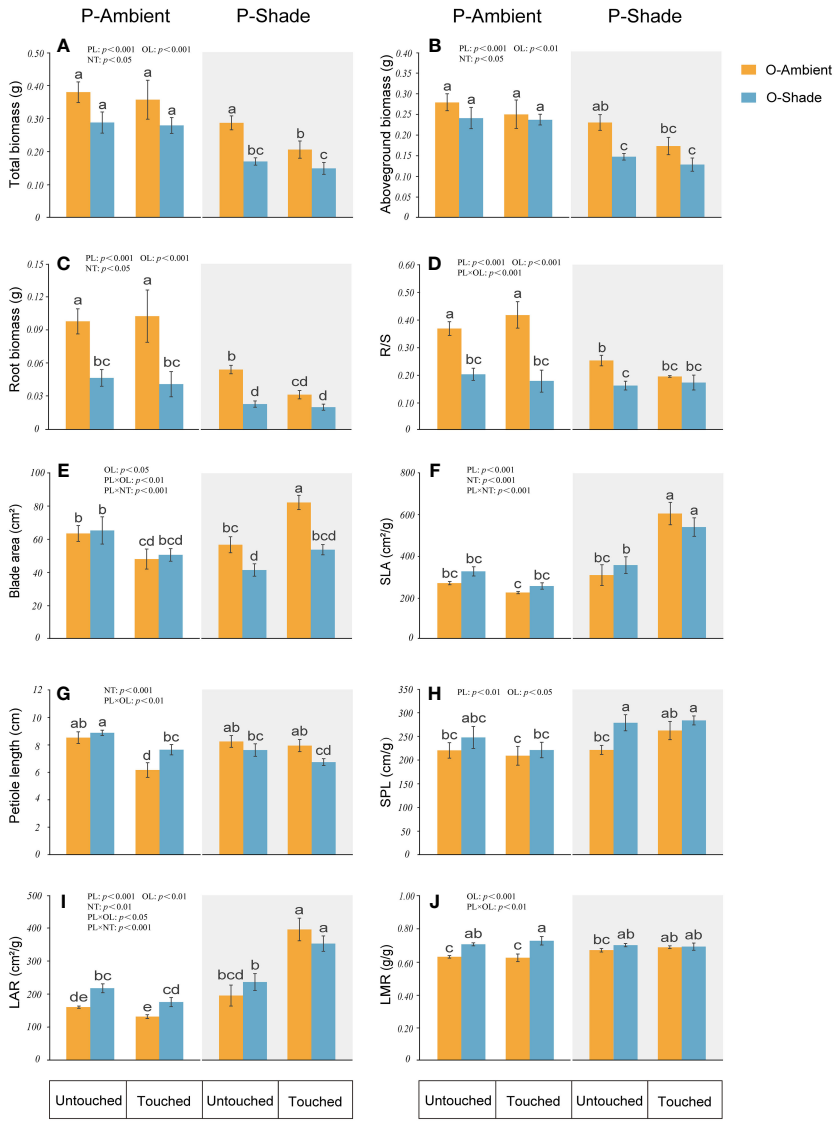

FIGURE 3
Growth of parental ramets in different treatments. **(A)** Total biomass; **(B)** aboveground biomass; **(C)** root biomass; **(D)** root biomass/shoot biomass (R/S); **(E)** blade area; **(F)** specific leaf area (SLA); **(G)** petiole length; **(H)** specific petiole length (SPL); **(I)** leaf area ratio (LAR); **(J)** leaf mass ratio (LMR). P-ambient: parents grew in an ambient environment; P-shade: parents grew in a shaded environment; O-ambient: offspring grew in an ambient environment; O-shade: offspring grew in a shaded environment. Different letters indicate significant difference among the treatments, and the same letter indicates no significant difference at the 0.05 level with the LSD test. Multiple comparisons of total biomass, root biomass, and LAR are based on log-converted data. Error bars show the SE.

Discussion

Our findings reveal complex interactions between parental neighboring touch and light environments on the clonal plant *G. longituba*. Namely, the effect of parental neighboring touch changed with light conditions. When plants grew in ambient light, the reduction in leaf investment induced by touch could help plants minimize damage caused by mechanical stimuli. When plants grew in a shaded environment, SAS or STS response of plants was promoted by parental neighboring touch to some extent, which was conducive to survival in the shade. Moreover, the maternal effect of PL on light adaptability of offspring ramets also relied on parental neighboring touch. If touch occurred between parental ramets in ambient light, the shaded offspring was slightly affected, while the effect was disadvantageous when touch happened on parental ramets in the shaded environment.

The effect of parental neighboring touch associated with light environment

In the present study, the growth of the clonal plant *G. longituba* was affected by the touch between neighbor parents and not only parental ramets but also their offspring ramets. However, the effect varied with a light environment. For example, in an ambient environment, it seems that the effect of parental neighboring

TABLE 3 Three-way ANCOVAs for effects of parental light environment (PL), offspring light environment (OL), parental neighboring touch (NT), and their interactions on growth indicators of offspring ramets.

Source of variation	df	Total biomass (g) [a]		Stolon biomass (g) [a]		Leaf biomass (g)		Stolon biomass/ Leaf biomass [a]		Total blade area (cm^2)		Number of ramets		Length of the longest stolon (cm)		SSL of the longest stolon (cm/g)		LAR (cm^2/g)		LMR (g/g)	
		F	*p*	*F*	*p*	*F*	*p*	*F*	*p*	*F*	*p*	*F*	*p*	*F*	*p*	*F*	*p*	*F*	*p*	*F*	*p*
Fresh weight	1	5.603	**0.027**	1.562	0.224	8.296	**0.008**	2.411	0.134	1.889	0.183	14.027	**0.001**	4.336	**0.049**	8.884	**0.007**	0.036	0.851	2.536	0.125
Parental light environment (PL)	1	210.375	**<0.001**	221.496	**<0.001**	92.739	**<0.001**	8.832	**0.007**	65.724	**<0.001**	82.482	**<0.001**	107.583	**<0.001**	338.294	**<0.001**	17.211	**<0.001**	10.370	**0.004**
Offspring light environment (OL)	1	31.672	**<0.001**	2.704	0.114	37.770	**<0.001**	27.823	**<0.001**	58.122	**<0.001**	23.117	**<0.001**	10.137	**0.004**	255.398	**<0.001**	44.476	**<0.001**	30.298	**<0.001**
Neighbor touch (NT)	1	21.647	**<0.001**	10.552	**0.004**	13.145	**0.001**	2.184	0.153	31.515	**<0.001**	9.292	**0.006**	21.820	**<0.001**	23.768	**<0.001**	12.487	**0.002**	2.155	0.156
PL×OL	1	4.134	0.054	3.903	0.06	8.334	**0.008**	0.131	0.720	9.886	**0.005**	17.531	**<0.001**	0.675	0.420	62.936	**<0.001**	0.102	0.752	0.116	0.737
PL×NT	1	6.757	**0.016**	15.056	**0.001**	1.412	0.247	4.472	**0.045**	5.444	**0.029**	2.388	0.136	3.863	0.062	0.486	0.493	4.056	0.056	4.193	0.052
OL×NT	1	0.596	0.448	0.622	0.439	9.095	**0.006**	3.517	0.073	3.557	0.072	5.795	**0.024**	1.783	0.195	1.756	0.198	0.002	0.969	3.410	0.078
PL×OL×NT	1	0.027	0.872	0.580	0.454	2.010	0.170	1.837	0.188	0.28	0.602	6.180	**0.021**	0.067	0.798	0.178	0.677	0.651	0.428	1.889	0.183

Values in bold indicate significant effects ($p < 0.05$) of factors and their interactions. The lowercase letter "a" indicates that the data are log-transformed.
The shading indicates that the p-value is significant.

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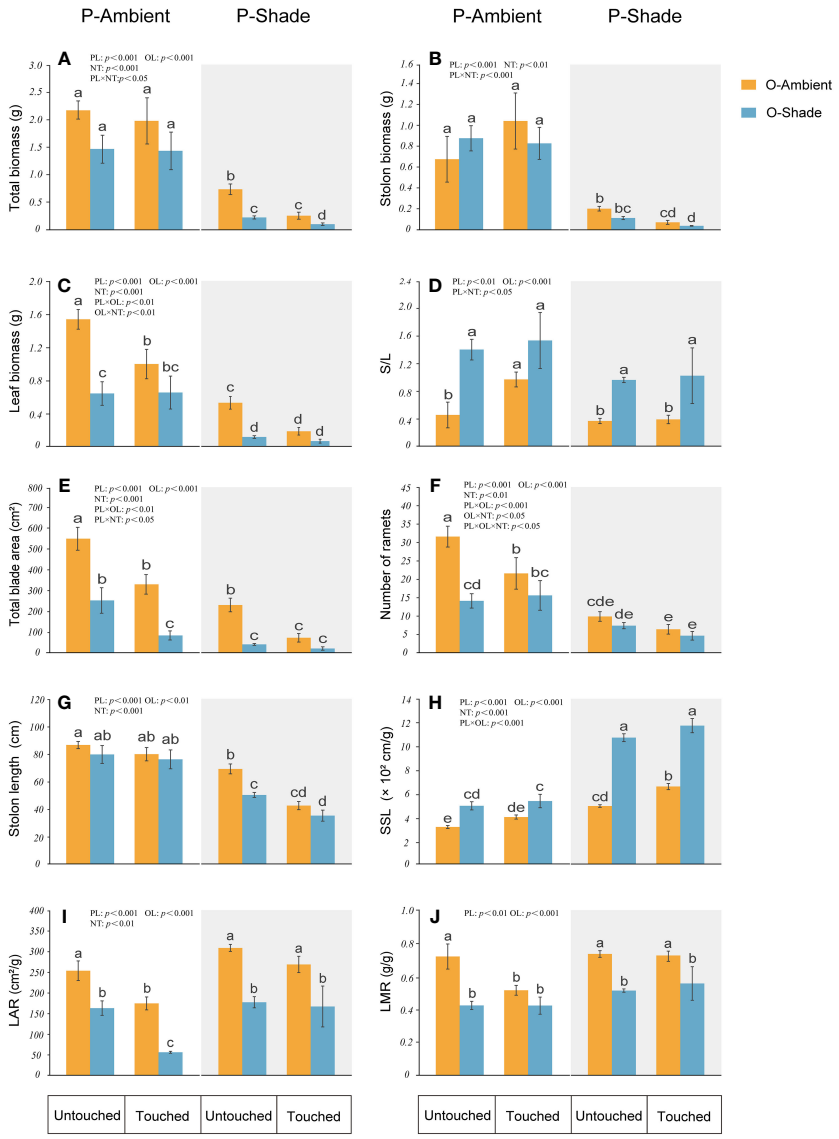

FIGURE 4
Growth of offspring ramets in different treatments. **(A)** Total biomass; **(B)** stolon biomass; **(C)** leaf biomass; **(D)** stolon biomass/leaf biomass (S/L); **(E)** total blade area; **(F)** number of ramets; **(G)** length of the longest stolon; **(H)** specific stolon length (SSL); **(I)** leaf area ratio (LAR); **(J)** leaf mass ratio (LMR). P-ambient: parents grew in an ambient environment; P-shade: parents grew in a shaded environment; O-ambient: offspring grew in an ambient environment; O-shade: offspring grew in a shaded environment. Different letters indicate significant differences among the treatments, and the same letter indicates no significant differences at the 0.05 level with the LSD test. Multiple comparisons of total biomass, stolon biomass, and stolon biomass/leaf biomass are based on log-converted data. Error bars show the SE.

touch was mainly on leaves; the reduction in the blade area and petiole length of the parental ramets was the most intense phenotypic change observed after direct touch by a neighbor, which is also regarded as the core morphological change of thigmomorphogenesis (Brenya et al., 2022). The compression of leaf and petiole was associated with the increases in cell wall stiffness and decreases in cell elongation, which is correlated with ethylene-regulated pectin degradation induced by touch (Wu et al., 2020). Being a clonal herb with a long stolon, the petiole is the vertical support of *G. longituba*; a shorter petiole limits the bending moment, and together with a smaller blade, they reduce the risk of various mechanical strains, such as plastic deformation, uprooting, and buckling, and are likely adaptive in crowded vegetation (Langer et al., 2021; Telewski, 2021; Langer et al., 2022).

Parental environmental signals that can be perceived by clonal offspring via a connected spacer (stolon or rhizome) have been proven (Liu et al., 2015; Guo et al., 2022; Tie et al., 2022; Xue et al., 2022). Moreover, in this study, although touch only took place between parents, its impact was transferred to their offspring, causing the decrease of ramet number, leaf area, leaf biomass and leaf investment (decreased LAR and LMR), but relatively less reduction in stolon biomass (increased S/L). The performance of offspring ramets induced by parental neighboring touch owing to clonal integration was also proved with *Leymus secalinus* (Sui et al.,

2011). The sharing of environmental signals among interconnected ramets was considered to favor young ramets (Wei et al., 2019). In this study, to some extent, the compression of phenotype and growth, and more allocation to the stolon, those induced by touch benefits plants in withstanding mechanical stress, which is also the form of plants in high mountain and grazed areas to protect plants from the stresses caused by high wind, rain, physical touch from other plants, or trampling (Braam, 2005; Sui et al., 2011).

When *G. longituba* was in a shaded environment, growth was obviously inhibited, as shown by the lower biomass accumulation and ramet production, which was due to the limitation of light resources. Shade induced a significant impact on both leaf and stolon. Despite the decreased biomass, some changes in parental ramets caused by shade were in favor of adapting to low-light conditions. For instance, notwithstanding the smaller leaf area, more investment to leaf in parental ramets showed increased LAR and LMR, which optimized light capture in the shade. Moreover, the longer SPL in parents and SSL in offspring represented expansion in the vertical and horizontal directions, which suggested an effort of clonal plants to escape from the shade (Figures 3, 4). Evidently, the clonal plant *G. longituba* exhibited both STA and SAS characters to tolerate the shaded environment.

Interestingly, if parental neighboring touch happened in the shade, it seems that some responses of *G. longituba* were more beneficial to survival in the shaded environments. For example, the reduction of parental leaf area in the shade was offset, and even more resources were allocated to the leaf area, causing larger SLA and more LAR (Figure 3). Additionally, although the length and biomass of stolon decreased, the long SSL and SPL was maintained the same as in the shade (Figure 4). In addition, the touch of the parent caused larger SLA in the shaded offspring, and these responses were not even represented in the shaded environment. Consequently, SAS or STS responses induced by shade were not restrained by parental neighboring touch and, instead, were promoted to some extent.

Parental neighboring touch and maternal effect on offspring light adaptability

The connected stolon provides physical support for communication among ramet nets and guarantees the parental and ongoing effects on offspring ramets. The maternal effect and the light adaptation of clonal offspring induced by it have been discussed in many studies. Some documented that the phenotype induced by the maternal effect may facilitate offspring to pre-adapt to a light environment (Guo et al., 2022; Tie et al., 2022; Xue et al., 2022). The alternative view, however, stated that parental shading effects contributed little to the tolerance of clonal offspring to shading (Dong et al., 2019). Our results displayed that a parental high-light environment was in favor of offspring performance. If the parental ramets were in ambient light, their offspring always maintained a higher total biomass and ramet number regardless of OL. The assurance in total biomass of the shaded offspring was mainly related to stolons. By contrast, the offspring with shaded parents usually had a lower biomass even if they were under ambient light conditions. In brief, it seemed from our results that the offspring with a shaded environment did not benefit from the shading experience of their parent despite the matching parent–offspring environment. However, plants will avoid excessive growth or defense through a negative feedback–regulatory loop and achieve balance in response to adverse environments (Li et al., 2019), More precisely, the decrease in growth of *G. longituba* does not necessarily mean that they are completely unadaptable; resources may be more devoted to other traits not involved in the present study, such as defense and life span.

If parental ramets are in an ambient environment, the offspring with a shaded environment showed similar growth whether touch occured or not, and they both exhibited the same biomass (leaf, stolon, and total biomass), ramet number, LMR, SSL, and stolon biomass/leaf biomass. Instead, if touch happened when parents grew in a shaded environment, the biomass of offspring with a shaded environment was decreased, accompanied by a reduction in length and biomass of stolons. Of course, the longest SSL and a high S/L were still kept (Figure 4). These traits responded in opposite directions, suggesting the potential for complex trade-offs among traits. Therefore, the effects of parental neighboring touch on offspring under shaded conditions depended on PL. If light resource in the parental environment was abundant, the impact of parental neighboring touch on the offspring with a shaded environment was not obvious, whereas when the parent was under limited light conditions, touch from a neighbor was more disadvantageous to the growth of the clonal plant *G. longituba*. The interaction of mechanical stimuli and shade has been reported by several studies; some documented that shading decreased or eliminated the thigmomorphogenesis (Henry and Thomas, 2002), while others showed opposite results (Feng et al., 2019). These results disclosed a complexity of interaction between shade and mechanical stimuli on plant morphology, growth, and allocation. Note that the mechanical stimuli in these studies were simulated with wind; the effect not only is a single mechanical stimulus, but also affects the exchange of heat, water vapor, and CO_2 around leaves (Feng et al., 2019), which made the findings more diverse. Our study focused on the effect of contact between neighboring leaves, which broadens our understanding of mechanical stimuli.

Conclusion

Our results illustrated that the impact of parental neighboring touch on the clonal plant *G. longituba* was dependent on the light environment. For instance, in an environment with sufficient light, depressed leaf investment induced by touch was regarded as a trade-off to resist potential mechanical damage. In a shaded environment, SAS or STS response induced by shade was promoted by parental neighboring touch to some extent, which was conducive to the survival of plants in a shaded environment. If touch occurred on the parental ramets in ambient light, the light adaptability of the shaded offspring was slightly affected, while the effect was disadvantageous when touch happened on the parental ramets in the shaded environment. In sum, the role of neighboring touch varied, relying on the light environment, which complicated the plant–

plant interactions under dense vegetation. The nuanced interactions between neighboring touch and light conditions highlight the complexity inherent in the dynamics of plant development within dense vegetation. Our research contributes to understanding the growth dynamics of understory plants. Given that this study was conducted in a controlled environment, there may be discrepancies between the experimental conditions and natural conditions. Therefore, future research should explore conducting field experiments to bridge this gap.

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.

Author contributions

LX: Formal analysis, Writing – review & editing, Data curation, Investigation, Writing – original draft. JQ: Data curation, Formal analysis, Writing – review & editing. SZ: Writing – review & editing. XL: Writing – review & editing, Formal analysis, Funding acquisition, Methodology, Project administration, Resources, Supervision. HB: Writing – review & editing. MY: Writing – review & editing.

Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article. This research was supported by the National Natural Science Foundation of China (no. 32201295).

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.2024.1358924/full#supplementary-material

References

Anten, N. P. R., Casado-Garcia, R., and Nagashima, H. (2005). Effects of mechanical stress and plant density on mechanical characteristics, growth, and lifetime reproduction of tobacco plants. *Am. Nat.* 166, 650–660. doi: 10.1086/497442

Baker, B. H., Berg, L. J., and Sultan, S. E. (2018). Context-dependent developmental effects of parental shade versus sun are mediated by DNA methylation. *Front. Plant Sci.* 9. doi: 10.3389/fpls.2018.01251

Baldwin, I. T., Halitschke, R., Paschold, A., von Dahl, C. C., and Preston, C. A. (2006). Volatile signaling in plant-plant interactions: "Talking trees" in the genomics era. *Science* 311, 812–815. doi: 10.1126/science.1118446

Ballare, C. L., and Pierik, R. (2017). The shade-avoidance syndrome: multiple signals and ecological consequences. *Plant Cell Environ.* 40, 2530–2543. doi: 10.1111/pce.12914

Boernke, F., and Rocksch, T. (2018). Thigmomorphogenesis - Control of plant growth by mechanical stimulation. *Sci. Horticamsterdam.* 234, 344–353. doi: 10.1016/j.scienta.2018.02.059

Bolker, B. M., Brooks, M. E., Clark, C. J., Geange, S. W., Poulsen, J. R., Stevens, M. H. H., et al. (2009). Generalized linear mixed models: a practical guide for ecology and evolution. *Trends Ecol. Evol.* 24, 127–135. doi: 10.1016/j.tree.2008.10.008

Braam, J. (2005). In touch: plant responses to mechanical stimuli. *New Phytol.* 165, 373–389. doi: 10.1111/j.1469-8137.2004.01263.x

Braam, J., and Chehab, E. W. (2017). Thigmomorphogenesis. *Curr. Biol.* 27, R863–R864. doi: 10.1016/j.cub.2017.07.008

Brenya, E., Pervin, M., Chen, Z.-H., Tissue, D. T., Johnson, S., Braam, J., et al. (2022). Mechanical stress acclimation in plants: Linking hormones and somatic memory to thigmomorphogenesis. *Plant Cell Environ.* 45, 989–1010. doi: 10.1111/pce.14252

Crepy, M. A., and Casal, J. J. (2015). Photoreceptor-mediated kin recognition in plants. *New Phytol.* 205, 329–338. doi: 10.1111/nph.13040

de Wit, M., Kegge, W., Evers, J. B., Vergeer-van Eijk, M. H., Gankema, P., Voesenek, L. A. C. J., et al. (2012). Plant neighbor detection through touching leaf tips precedes phytochrome signals. *P Natl. Acad. Sci. U.S.A.* 109, 14705–14710. doi: 10.1073/pnas.1205437109

Dong, B. C., Meng, J., and Yu, F. H. (2019). Effects of parental light environment on growth and morphological responses of clonal offspring. *Plant Biol.* 21, 1083–1089. doi: 10.1111/plb.13001

Douma, J. C., and Anten, N. P. R. (2019). Touch and plant defence: volatile communication with neighbours. *J. Exp. Bot.* 70, 371–374. doi: 10.1093/jxb/ery444

Elhakeem, A., Markovic, D., Broberg, A., Anten, N. P. R., and Ninkovic, V. (2018). Aboveground mechanical stimuli affect belowground plant-plant communication. *PloS One* 13(5): e0195646. doi: 10.1371/journal.pone.0195646

Feng, J., Huang, P., and Wan, X. (2019). Interactive effects of wind and light on growth and architecture of poplar saplings. *Ecol. Res.* 34, 94–105. doi: 10.1111/1440-1703.1013

Fiorucci, A. S., and Fankhauser, C. (2017). Plant strategies for enhancing access to sunlight. *Curr. Biol.* 27, R931–R940. doi: 10.1016/j.cub.2017.05.085

Fraser, D. P., Hayes, S., and Franklin, K. A. (2016). Photoreceptor crosstalk in shade avoidance. *Curr. Opin. Plant Biol.* 33, 1–7. doi: 10.1016/j.pbi.2016.03.008

Galloway, L. F. (2005). Maternal effects provide phenotypic adaptation to local environmental conditions. *New Phytol.* 166, 93–99. doi: 10.1111/j.1469-8137.2004.01314.x

Galloway, L. F., and Etterson, J. R. (2007). Transgenerational plasticity is adaptive in the wild. *Science* 318, 1134–1136. doi: 10.1126/science.1148766

Galloway, L. F., Etterson, J. R., and McGlothlin, J. W. (2009). Contribution of direct and maternal genetic effects to life-history evolution. *New Phytol.* 183, 826–838. doi: 10.1111/j.1469-8137.2009.02939.x

Gommers, C. M. M., Visser, E. J. W., St Onge, K. R., Voesenek, L., and Pierik, R. (2013). Shade tolerance: when growing tall is not an option. *Trends Plant Sci.* 18, 65–71. doi: 10.1016/j.tplants.2012.09.008

Guo, Y. H., Quan, J. X., Wang, X. B., Zhang, Z. X., Liu, X., Zhang, R. C., et al. (2022). Predictability of parental Ultraviolet-B environment shapes the growth strategies of clonal *Glechoma longituba*. *Front. Plant Sci.* 13. doi: 10.3389/fpls.2022.949752

Henry, H. A. L., and Thomas, S. C. (2002). Interactive effects of lateral shade and wind on stem allometry, biomass allocation, and mechanical stability in *Abutilon theophrasti* (Malvaceae). *Am. J. Bot.* 89, 1609–1615. doi: 10.3732/ajb.89.10.1609

Jaffe, M. J. (1973). Thigmomorphogenesis: The response of plant growth and development to mechanical stimulation: With special reference to *Bryonia dioica*. *Planta* 114, 143–157. doi: 10.1007/BF00387472

Kong, C.-H., Zhang, S.-Z., Li, Y.-H., Xia, Z.-C., Yang, X.-F., Meiners, S. J., et al. (2018). Plant neighbor detection and allelochemical response are driven by root-secreted signaling chemicals. *Nat. Commun.* 9:3867. doi: 10.1038/s41467-018-06429-1

Langer, M., Hegge, E., Speck, T., and Speck, O. (2022). Acclimation to wind loads and/or contact stimuli? A biomechanical study of peltate leaves of *Pilea peperomioides*. *J. Exp. Bot.* 73, 1236–1252. doi: 10.1093/jxb/erab541

Langer, M., Kelbel, M. C., Speck, T., Muller, C., and Speck, O. (2021). Twist-to-bend ratios and safety factors of petioles having various geometries, sizes and shapes. *Front. Plant Sci.* 12. doi: 10.3389/fpls.2021.765605

Li, Z.-G., and Gong, M. (2011). Mechanical stimulation-induced cross-adaptation in plants: an overview. *J. Plant Biol.* 54, 358–364. doi: 10.1007/s12374-011-9178-3

Li, Y., Yang, Y., Hu, Y., Liu, H., He, M., Yang, Z., et al. (2019). Della and EDS1 form a feedback regulatory module to fine-tune plant growth-defense tradeoff in *Arabidopsis*. *Mol. Plant* 12, 1485–1498. doi: 10.1016/j.molp.2019.07.006

Liu, X., Li, Q., Yue, M., Zhang, X. F., Zhang, R. C., Zhang, B., et al. (2015). Nitric oxide is involved in integration of UV-B absorbing compounds among parts of clonal plants under a heterogeneous UV-B environment. *Physiol. Plant.* 155, 180–191. doi: 10.1111/ppl.12313

Liu, Y., Schieving, F., Stuefer, J. F., and Anten, N. P. R. (2007). The effects of mechanical stress and spectral shading on the growth and allocation of ten genotypes of a stoloniferous plant. *Ann. Botlondon.* 99, 121–130. doi: 10.1093/aob/mcl230

Marshall, D. J., and Uller, T. (2007). When is a maternal effect adaptive? *Oikos* 116, 1957–1963. doi: 10.1111/j.2007.0030-1299.16203.x

Martinez-Garcia, J. F., and Rodriguez-Concepcion, M. (2023). Molecular mechanisms of shade tolerance in plants. *New Phytol.* 239, 1190–1202. doi: 10.1111/nph.19047

McArdle, B. H., and Anderson, M. J. (2001). Fitting multivariate models to community data: a comment on distance-based redundancy analysis. *Ecology* 82, 290–297. doi: 10.1890/0012-9658(2001)082[0290:FMMTCD]2.0.CO;2

Monshausen, G. B., and Haswell, E. S. (2013). A force of nature: molecular mechanisms of mechanoperception in plants. *J. Exp. Bot.* 64, 4663–4680. doi: 10.1093/jxb/ert204

Ninkovic, V., Rensing, M., Dahlin, I., and Markovic, D. (2019). Who is my neighbor? Volatile cues in plant interactions. *Plant Signal Behav.* 14. doi: 10.1080/15592324.2019.1634993

Pierik, R., Cuppens, M. L. C., Voesenek, L., and Visser, E. J. W. (2004). Interactions between ethylene and gibberellins in phytochrome-mediated shade avoidance responses in tobacco. *Plant Physiol.* 136, 2928–2936. doi: 10.1104/pp.104.045120

Pierik, R., Mommer, L., and Voesenek, L. A. C. J. (2013). Molecular mechanisms of plant competition: neighbour detection and response strategies. *Funct. Ecol.* 27, 841–853. doi: 10.1111/1365-2435.12010

Puy, J., Carmona, C. P., Hiiesalu, I., Opik, M., de Bello, F., and Moora, M. (2022). Mycorrhizal symbiosis alleviates plant water deficit within and across generations via phenotypic plasticity. *J. Ecol.* 110, 262–276. doi: 10.1111/1365-2745.13810

Quan, J. X., Latzel, V., Dan, T., Zhang, Y., Münzbergová, Z., Chai, Y., et al. (2021). Ultraviolet B radiation triggers DNA methylation change and affects foraging behavior of the clonal plant *Glechoma longituba*. *Front. Plant Sci.* 12. doi: 10.3389/fpls.2021.633982

Semchenko, M., Saar, S., and Lepik, A. (2014). Plant root exudates mediate neighbour recognition and trigger complex behavioural changes. *New Phytol.* 204, 631–637. doi: 10.1111/nph.12930

Sterck, F., and Bongers, F. (1998). Ontogenetic changes in size, allometry, and mechanical design of tropical rain forest trees. *Am. J. Bot.* 85, 266–266. doi: 10.2307/2446315

Sui, Y., He, W., Pan, X., and Dong, M. (2011). Partial mechanical stimulation facilitates the growth of the rhizomatous plant *Leymus secalinus*: modulation by clonal integration. *Ann. Bot-London* 107, 693–697. doi: 10.1093/aob/mcr012

Telewski, F. W. (2021). Mechanosensing and plant growth regulators elicited during the thigmomorphogenetic response. *Front. Forests Global Change.* 3. doi: 10.3389/ffgc.2020.574096

Tie, D., Guo, Y., Zhu, C., Quan, J., Liu, S., Zhou, Z., et al. (2022). Parental UV-B radiation regulates the habitat selection of clonal *Duchesnea indica* in heterogeneous light environments. *Functi. Plant Biol.* 49, 600–612. doi: 10.1071/FP21253

Valladares, F., and Niinemets, U. (2008). Shade tolerance, a key plant feature of complex nature and consequences. *Annu. Rev. Ecol. Evol. S* 39, 237–257. doi: 10.1146/annurev.ecolsys.39.110707.173506

Wang, L., Ma, C., Wang, S., Yang, F., Sun, Y., Tang, J., et al. (2023). Ethylene and jasmonate signaling converge on gibberellin catabolism during thigmomorphogenesis in Arabidopsis. *Plant Physiol.* 194:758–773. doi: 10.1093/plphys/kiad556

Wang, L.-H., Si, J., Luo, F.-L., Dong, B.-C., and Yu, F.-H. (2022). Parental effects driven by resource provisioning in alternanthera philoxeroides-a simulation case study. *Front. Plant Sci.* 13. doi: 10.3389/fpls.2022.872065

Wei, Q., Li, Q., Jin, Y., Wu, S., Fan, L., Lei, N., et al. (2019). Transportation or sharing of stress signals among interconnected ramets improves systemic resistance of clonal networks to water stress. *Funct. Plant Biol.* 46, 613–623. doi: 10.1071/FP18232

Wu, Q., Li, Y., Lyu, M., Luo, Y., Shi, H., and Zhong, S. (2020). Touch-induced seedling morphological changes are determined by ethylene-regulated pectin degradation. *Sci. Adv.* 6. doi: 10.1126/sciadv.abc9294

Xu, H. Y., Chen, P. R., and Tao, Y. (2021). Understanding the shade tolerance responses through hints from phytochrome A-mediated negative feedback regulation in shade avoiding plants. *Front. Plant Sci.* 12. doi: 10.3389/fpls.2021.813092

Xue, W., Huang, L., Yu, F.-H., and Bezemer, T. M. (2022). Light condition experienced by parent plants influences the response of offspring to light via both parental effects and soil legacy effects. *Funct. Ecol.* 36, 2434–2444. doi: 10.1111/1365-2435.14136

Zhang, N., van Westreenen, A., Anten, N. P. R., Evers, J. B., and Marcelis, L. F. M. (2020). Disentangling the effects of photosynthetically active radiation and red to far-red ratio on plant photosynthesis under canopy shading: a simulation study using a functional-structural plant model. *Ann. Botlondon.* 126, 635–646. doi: 10.1093/aob/mcz197

frontiers | Frontiers in Plant Science

TYPE Original Research
PUBLISHED 24 May 2024
DOI 10.3389/fpls.2024.1348080

OPEN ACCESS

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RECEIVED 01 December 2023
ACCEPTED 08 May 2024
PUBLISHED 24 May 2024

CITATION
Jing S, Ren X, Lin F, Niu H, Ayi Q, Wan B, Zeng B and Zhang X (2024) Stem elongation and gibberellin response to submergence depth in clonal plant *Alternanthera philoxeroides*. *Front. Plant Sci.* 15:1348080. doi: 10.3389/fpls.2024.1348080

Stem elongation and gibberellin response to submergence depth in clonal plant *Alternanthera philoxeroides*

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Clonal plants are widely distributed in the riparian zone and play a very important role in the maintenance of wetland ecosystem function. Flooding is an environmental stress for plants in the riparian zone, and the response of plants varies according to the depth and duration of flooding. However, there is a lack of research on the growth response of clonal plants during flooding, and the endogenous hormone response mechanism of clonal plants is still unclear. In the present study, *Alternanthera philoxeroides*, a clonal plant in the riparian zone, was used to investigate the time-dependent stem elongation, the elongation of different part of the immature internodes, and the relationship between growth elongation and the phytohormone gibberellin (GA) under a series of submergence depths (0 m, 2 m, 5 m, and 9 m). The results showed that stem elongation occurred under all treatments, however, compared to 0 m (control), plants grew more under 2 m and 5 m submergence depth, while grew less under 9 m water depth. Additionally, basal part elongation of the immature internode was the predominant factor contributing to the stem growth of *A. philoxeroides* under different submergence depths. The phytohormone contents in basal part of the mature and immature internodes showed that GA induced the differential elongation of internode. Plant submerged at depth of 2 m had the highest GA accumulation, but plant submerged at depth of 9 m had the lowest GA concentration. These data suggested that GA biosynthesis are essential for stem elongation in *A. philoxeroides, and* the basal part of the immature internode was the main position of the GA biosynthesis. This study provided new information about the rapid growth and invasion of the clonal plant *A. philoxeroides* around the world, further clarified the effects of submergence depth and duration on the elongation of the stem, and deepened our understanding of the growth response of terrestrial plants in deeply flooded environments.

KEYWORDS

clonal plant, alligator weed, gibberellin, hydrostatic pressure, submergence times, submergence depth

1 Introduction

Flooding is an environmental stress for plants in the riparian zone, and the response of plants varies according to the depth and duration of flooding (Müller et al., 2019). Flooding depth and duration concomitantly influence the growth traits and yield of plant (Meng et al., 2022). A longer waterlogging duration caused a greater reduction in the above parameters (Wang et al., 2017). Clonal plants are widely distributed in the riparian zone and play a very important role in the maintenance of wetland ecosystem function, and often adapt to environmental changes through phenotypic plasticity, especially invasive clonal plants (Dong et al., 2017; Guo et al., 2023). Previous studies have demonstrated that the clonal plant *Alternanthera philoxeroides* is an invasive amphibious weed that is native to South America but has now invaded into the temperate and tropical regions across the world (Wu et al., 2017). *A. philoxeroides* has rapid clonal reproduction and is phenotypically plastic (leaf area, internode length, shoot diameter, etc.) (Gao et al., 2015).

Compared to the terrestrial environmental factors, the environmental factors in the water body can change considerably, such as light, water temperature, dissolved O_2 and CO_2 concentrations, etc (Vervuren et al., 2003). Light quantity and quality in rivers vary with depth and turbidity, for example, in the River Rhine, light quantum flux decreases with increasing water depth both in freshwater lakes and in flooded environments (Vervuren et al., 1999; Li et al., 2024). Light is reduced to 90% of the total solar radiation entering the water column at a depth of 50 cm, and less than 1% of the light intensity is available underwater when the water depth reaches more than 1.5 m (Vervuren et al., 2003). In completely submerged environments, O_2 concentration in plants is reduced and low levels of O_2 stimulate ethylene biosynthesis, whose diffusion rate in water is very slow, leading to a rapid rise in ethylene levels in plants in a short period of time (Voesenek et al., 2016; Wang and Komatsu, 2022).

The clonal plant *A. philoxeroides* is a common plant in floodplains, riparian zones, and water-level drawdown zones of large reservoirs with inundation-disturbed habitats (Yang et al., 2019), and it is also distributed in areas with deeper inundation (Zheng et al., 2021). For example, the Three Gorges Reservoir (TGR) which is the largest hydroelectric power project in the world, the water level of the reservoir fluctuates regularly from 145 m to 175 m in elevation (Chen et al., 2021). Thus, the water-level drawdown zones with a maximum drop of 30 m are formed along the banks of the Yangtze River (Lei et al., 2017). We have been conducting long-term research on plant growth in the drawdown zone, and we have found that the clonal plant *A. philoxeroides* exhibits a fast-growing in shallow submerged environments and a slow-growing or even stop-growing in deep submerged conditions (Ayi et al., 2016; Jing et al., 2022), but there is little discussion on the growth response of *A. philoxeroides* at different water depths and its response mechanism. Our previous study has shown that the formation of pith cavity and adventitious roots, and non-structural carbohydrate metabolism play important roles in the changes of different growth strategies in *A. philoxeroides* (Jing et al., 2022). The stem growth of *A. philoxeroides* at any submergence depth was chiefly caused by the elongation of the basal parts of immature internodes, which was highly correlated to both cell proliferation and cell enlargement (Jing et al., 2024). However, the time depended stem elongation and the hormone regulatory mechanisms remain unclear, this characteristic of the plant species is crucial for explaining the successful invasion of clonal plants (You et al., 2016).

Phytohormone plays a very important role as a signalling substance in the response of plants to biotic and abiotic stresses (Taiz and Zeiger, 2010). The response and adaptation strategies of plants in flooded environments are very closely related to hormone concentrations, and the regulation of plant morphology, anatomy, physiology, ecology, molecules and signalling under low oxygen or low light stress conditions is largely influenced by hormones (Bailey-Serres and Voesenek, 2008; Lin et al., 2021). When plants were submerged, the content of ethylene increases due to the diffusion of ethylene is weakened, which inhibits abscisic acid (ABA) synthesis and promotes ABA decomposition, whereas ABA inhibits gibberellin (GA) synthesis (Fukao and Bailey-Serres, 2008), so an increase in ethylene promotes an increase in GA content, which in turn promotes stem growth (Sasidharan and Voesenek, 2015). Physiological and genetic analyses indicated that GA biosynthesis and signal transduction are essential for internode elongation in deep-water rice (Ayano et al., 2014). Its role in plant response to submergence has been widely reported (Sasidharan and Voesenek, 2015; Wang et al., 2021). However, changes in the concentration of the endogenous hormone GA in different water depth environments have not been reported for the clonal plant *A. philoxeroides*.

To explore the time-dependent stem elongation and the hormone regulatory mechanisms under different submergence depths, taking the clonal plant *Alternanthera philoxeroides* (Mart.) Griseb., a submergence-tolerant plant as a model, we hope to solve the following scientific questions in this study: (1) What are the trends in the stems of different maturity levels of *A. philoxeroides* as the duration of submergence changes? (2) Is there a difference in the content of endogenous hormone GA in the immature internodes of *A. philoxeroides* under different water-depths? In order to clarify the above questions, we measured the length of stem, immature stem of *A. philoxeroides* at different water-depths, and determined the endogenous hormone GA content of mature and immature stem by using high-performance liquid chromatography and quantified by tandem mass spectrometry (HPLC-MS/MS). The answers to these questions will help understanding the phytohormone regulatory mechanisms of clonal plant tolerance to extreme flooding and explain why *A. philoxeroides* remains highly invasive worldwide.

2 Materials and methods

2.1 Plant material and cultivation

Alternanthera philoxeroides (Mart.) Griseb. can spread quickly via clonal growth, in this study, plants were grown as described in Jing et al. (2022). *A. philoxeroides* plants were cultivated from

cuttings obtained from plants naturally growing on the banks of the Jialing River in Chongqing, Southwest China (29°49'42''N, 106°26'46''E). Each selected cutting was planted in a plastic pot (diameter and depth were both 13 cm) containing riparian soil from the Jialing River banks. All plants were cultivated under the same conditions. The temperature, relative humidity, daily maximum light (PAR) intensity, and water provision were maintained at 10~15 °C, 75~85%, 600~800 μmol m^{-2} s^{-1}, and approximately 80~90% of the soil water-holding capacity, respectively. After approximately one month of cultivation, plants with approx. 288 mm height and 12 internodes were selected for submergence treatments.

2.2 Experimental design

The plants subjected to complete submergence treatments were suspended at planned water depths, as described in Jing et al. (2022). The design of 2 m, 5 m, and 9 m deep submergence was based on our long-term field observation on the elevational distribution of *A. philoxeroides* in the water level fluctuation zone of the Three Gorges reservoir. Unsubmerged control plants were placed under dark conditions and watered regularly to ensure adequate water supply. Four submergence treatments were applied in a fully randomized design using selected plants. Control plants were placed under dark conditions and were not submerged, in this article it is called 0 m. Additionally, three groups of plants were submerged in a water-filled concrete reservoir, with the top of plants 2 m, 5 m, and 9 m beneath the water surface (Supplementary Figure S1A). The plants in pots were suspended at planned water depths as described in Jing et al. (2022). According to the previous observation and pre-experimental results, the submergence treatments lasted to 11 d in the growth measured experiments, but 4 d in the endogenous GA measurements.

To investigate the effects of submergence depth on plants, the physicochemical status of water body (light, dissolved oxygen (DO), pH, and temperature) in the concrete reservoir were kept constant at any depths as described in Jing et al. (2022). DO concentration, photosynthetically active radiation (PAR) intensity, temperature and pH of the water column at different depths in the reservoir were checked twice per day (morning and evening) using a multi-parameter water quality analyzer (Hydrolab DS5, Hach, United States). During the experiment, no significant differences in these factors were found between different water depths (Table 1).

2.3 Growth measurements

Each plant had approximately 12 internodes at the start of treatments. From the stem base upwards, the 1st to 6th internodes were relatively more mature and the 7th to 12th internodes were immature (Supplementary Figure S1B). We marked nondestructively immature stems so as to distinguish the mature, immature stems formed before treatment. The length of mature stems, immature stems were measured every day. As plants may produce gaseous substances such as ethylene during submergence, it was ensured that the measurements were taken underwater, at the same time, the plants are not exposed to the atmosphere. Once the daily measurement was completed, the plants were quickly submerged to the appropriate water-depth for continuation of the treatment. In order to investigate the submergence time-dependent growth pattern of different parts of immature internodes, we selected an immature internode (the length of the internode was usually between 2.5 and 3.2 cm, as shown in Supplementary Figure S1D) from each plant before treatments and divided the internode into three equilong parts (basal, middle, and upper part, as shown in Supplementary Figure S1E) by marking with red polyester threads (Jing et al., 2024), measured the lengths of all parts every day.

2.4 Phytohormone concentration analysis

The endogenous GA concentration in the basal part of the internode of *A. philoxeroides* plants were mainly composed of GA_1, GA_3, GA_4, and GA_7. According to the results of the pre-experiment and the research objectives of the present study, the growth of plants under different submergence depths had already shown significant differences at the 4th day, and the endogenous hormone content of the plants was relatively high, the sampling time was set as the 4th day. The basal parts of each internode were harvested after submergence or control treatments, frozen in liquid nitrogen immediately, and kept at -80 °C before freeze drying. Ten basal parts of the mature and immature internodes were pooled to obtain enough material per sample. There were three replicates for GAs (GA_1, GA_3, GA_4 and GA_7) analyses. Measurement of endogenous GAs concentration was performed by HPLC-MS/MS. The HPLC-MS/MS system was composed of a high-performance liquid chromatography (HPLC, Agilent Technologies 1200 series, USA) connected to AB Sciex API 6500 Qtrap mass spectrometer

TABLE 1 Physico-chemical properties of water body in submergence reservoir during the experiment.

Submergence Depth (m)	Dissolved oxygen concentration (mg L^{-1})	Temperature (°C)	pH	PAR (μmol m^{-2} s^{-1})
0	n.a.	25.46 ± 0.03 a	n.a.	0 a
2	8.25 ± 0.08 a	25.48 ± 0.06 a	7.02 ± 0.02 a	0 a
5	8.16 ± 0.07 a	25.45 ± 0.07 a	7.01 ± 0.02 a	0 a
9	8.11 ± 0.06 a	25.46 ± 0.04 a	7.03 ± 0.01 a	0 a

The dissolved oxygen, temperature, photosynthetically active radiation (PAR), and pH of the water body in the concrete reservoir for submergence treatments were checked at different depths twice per day (in the morning and evening) using a multi-parameter water quality analyzer (Hydrolab DS5, Hach, United States) during the experiments (mean ± s. e.; n = 20); n.a. indicates no data. Same lower-case letter indicates no significant difference ($p > 0.05$) between submergence depths.

(Concord, ON, Canada). The Analyst 1.6.3 software (Concord, ON, Canada) controlled the HPLC-MS/MS system and Multiquant 3.2 to process the data (Pan et al., 2010).

2.5 Statistical analysis

Elongation difference of stem (or immature stem) between submergence depths were checked by one-way ANOVA. Separate ANOVA with Repeated Measures was used to detect the difference in stem length, basal, middle, and upper parts within internodes, One-way ANOVA was used to examine the difference in contents of gibberellin in mature and young stems respectively between different treatments. Logarithm data transformation was performed to equalize variance if necessary. Differences between treatments were detected using the Tukey HSD test, and the significance level was set at $p = 0.05$. All analyses were conducted using SPSS 22 (SPSS Inc., Chicago).

3 Results

3.1 Elongation of stem and immature stem parts

A. philoxeroides plants subjected to four treatments all elongated their stems during the experiment day by day (Figure 1A). At the end of treatments, the stem elongation were 198.00 mm, 245.67 mm, 223.17 mm, and 87.50 mm averagely in plants submerged at water depths of 0 m (control), 2 m, 5 m, and 9 m, respectively (Figure 1A). Compared to the 0 m, the stem presented apparent elongation when submerged at water depth of 2 m and 5 m, but the stem only had very slight elongation when submerged at water depth of 9 m. Stem elongation decreased with increasing submergence depth after 6 days treatment (Figure 1A). From the 7th day, the stem elongation of *A. philoxeroides* was significantly inhibited in the 9 m submergence depth, no elongation was observed. At this time, the stem elongation of plants submerged at water depth of 2 m and 5 m were still faster, indicating that the growth response of the stem of *A. philoxeroides* varied greatly at different water depths (Figure 1A). It was shown that water depth of 2 m and 5 m promoted elongation of the stems, while water depth of 9 m inhibited elongation of the stems, and this inhibition was more severe from the 7th day after the onset of submergence.

The immature stem elongation trend (Figure 1B, the 7th to 12th internodes) of *A. philoxeroides* under different water depths was consistent with that of the stems (Figure 1A). At the end of treatments, the elongation of immature stems in plants submerged at water depth of 0 m (control), 2 m and 5 m were 192.17 mm, 231.67 mm, and 206.83 mm, respectively (Figure 1B), and the immature stems were rapidly elongated throughout the treatment period. However, the water depth of 9 m promoted plant growth during the first 6 days of the experiment and then turned to inhibit plant growth as the duration of submergence increased (Figure 1B, especially on 7th day after the start of submergence), during the whole treatment period, the immature stem only grew 76.33 mm averagely.

3.2 Elongation of the different parts of immature internodes

The elongation of the basal (Figure 2A), middle (Figure 2B) and upper (Figure 2C) of immature internodes of *A. philoxeroides* showed different growth trends, which grew from about 8 mm at the start of the treatments to about 19 mm, 11 mm and 6 mm at 7 day under 2 m water depth, respectively. The growth rate of the basal parts was significantly faster than that of the middle parts, which in turn was faster than that of the upper parts (Figures 2A–C). However, when submergence treatment was carried out for 8 days, there was no significant growth in all parts of the marked immature internode, except for the internodes of the 0 m (unsubmerged group). In the same period of time, the elongation of basal, middle, and upper parts gradually decreased with increasing water depth (Figures 2A–C). Overall, 2 m water depth promoted the basal parts elongation of immature internodes at the early stage of treatments, while 9 m water depth had a certain inhibitory effect on the elongation of the immature internodes, and 5 m water depth had faster growth than 0 m at the beginning of submergence for 1~4 days, but the final length of all parts of the immature internodes was lower than that of the 0 m (Figure 2C).

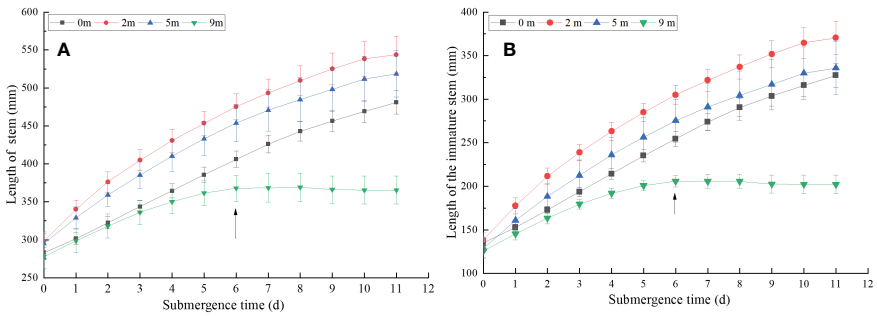

FIGURE 1
Length of stem **(A)** and immature stem **(B)** of *Alternanthera philoxeroides* after the start of different submergence treatments (Mean ± s. e.; n = 6). The arrows indicate *Aphiloxeroides* stops growing at 9 m water depth.

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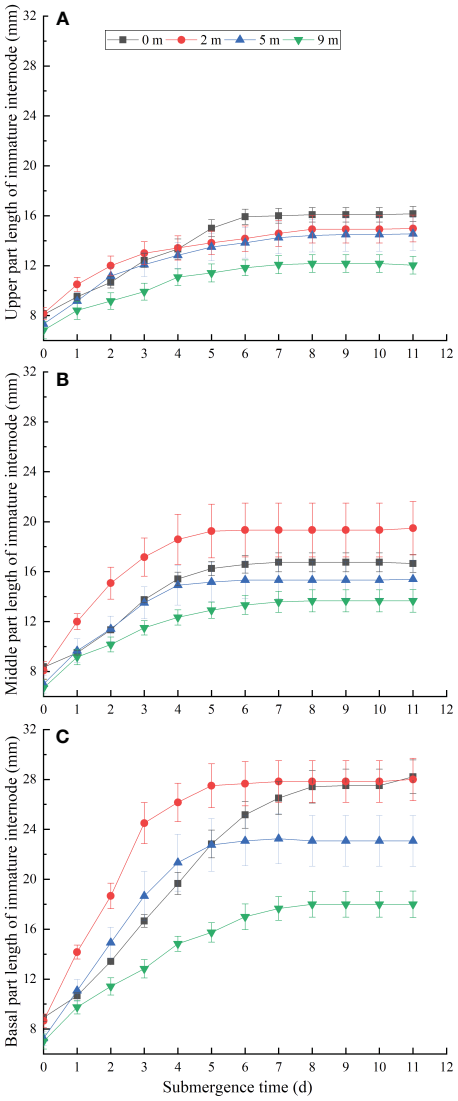

FIGURE 2
Upper **(A)**, middle **(B)** and basal **(C)** length of the immature internode for *Alternanthera philoxeroides* after the start of different submergence treatments (Mean ± s. e.; n = 6).

3.3 Endogenous GA concentration in the basal part of the internode

The concentration of GAs in immature internodes was higher than that in the mature internodes of *A. philoxeroides* (Figure 3). The concentration of GAs in the basal parts of immature internodes decreased with increasing submergence depth at the fourth day of experiment ($p < 0.05$, Figure 3). Plants submerged at 2 m water depth had the highest GAs concentration but plants submerged at 9 m depth had the lowest GAs concentration ($p < 0.05$, Figure 3). However, no significant difference was found between 0 m and 5 m submergence depths in GAs concentration in immature stems of *A. philoxeroides* ($p > 0.05$, Figure 3). The concentration of GAs in mature internodes of unsubmerged plants was significantly higher than that of submerged plants, but no difference was found among the three submerged treatments (i.e., 2 m, 5 m and 9 m). This

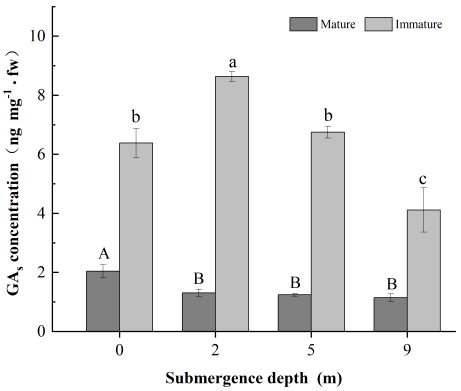

FIGURE 3
Concentration of GA_S in the basal of mature and immature internodes of *Alternanthera philoxeroides* at the fourth day of the submergence treatments (Mean ± s. e.; n =3). Different upper-case letters indicate statistically significant differences ($p < 0.05$) between mature internode for treatments. Different lower-case letters indicate statistically significant differences ($p < 0.05$) between mature stems for treatments. fw indicates fresh weight.

indicates that 2 m water depth promoted GAs biosynthesis in immature internodes, whereas 9 m water depth inhibited GAs biosynthesis.

4 Discussion

The influences of submergence on plant growth are different according to different flooding depths and durations. It has been reported that the yield of rice is almost non-existent when the flooding time is 5~6 days (Meng et al., 2022), longer flooding durations limited the basal area growth of larger trees and reduced sexual reproduction (Greet et al., 2020). In the present study, the stems of *A. philoxeroides* were elongating with the durations of submergence in all treatments (Figures 1A, B), this is mainly due to the growth of immature internodes. But the elongation of immature stems decreased when water depth increased (Figures 1A, B). This is consistent with the results of our previous studies, which found that immature internodes comparatively made the largest contribution to plant stem elongation (Jing et al., 2022). Moreover, as previous studies have found that the immature internodes showed intra-internodal variation in elongation among their basal, middle, and upper parts, and the variation was affected by submergence depth (Jing et al., 2024). The basal parts achieved much longer elongation than the middle and upper parts at 2 m water depth, but this elongation difference faded away when the water depth increased gradually to 9 m (Figures 2A–C).

In fact, after 7 days of submergence, the stems of *A. philoxeroides* were no longer growing under 9 m water depth (Figure 1A), the same to the immature stems (Figure 1B). Plants submerged at depth of 0 m, 2 m, 5 m continued to grow until the experiment was terminated after 11 days of submergence (Figures 1A, B). However, elongation of immature internodes was over by day 5 (Figure 2A–C), suggesting that *A. philoxeroides* produces new internodes to increase its total stem length during

submergence. This is consistent with our previous findings that *A. philoxeroides* can produce new internodes during submergence, but very few new internodes were produced during submergence at water depth of 9 m, and 1.85, 2.40, 1.85 new internodes on average were produced at water depth of 0 m, 2 m, and 5 m, respectively (Jing et al., 2022). This is an important feature for clonal plants to be able to reproduce in stressful environments (Jing et al., 2022).

It has been shown that ethylene content in plants rises rapidly within a short period of time in a completely submerged environment (Raskin and Kende, 1984; Voesenek et al., 2013, Voesenek et al., 2016), and that ethylene markedly increases the activity of the endogenous hormone GA, which in turn promotes cell division and cell elongation, and ultimately plant growth (Sasidharan and Voesenek, 2015). Therefore, in this study, when *A. philoxeroides* was completely submerged in a 2 m water depth environment, as in the completely submerged environment, the stem length was increased due to the rapid increase of ethylene concentration thereby inducing an increase in the biosynthesis of GAs, which promotes elongation and growth of the plant's immature parts (Figure 3). GA promotes cell division and cell elongation (Sauter and Kende, 1992), this is consistent with our previous study, the difference in the internode elongation is mainly due to the difference in cell growth and development. Cells in the basal parts of immature internodes were shorter and numerous, whereas those in the middle and upper parts were relatively longer and smaller in number (Jing et al., 2024). Plants possess higher concentrations of GA under 2 m water depth, but have lower concentrations of growth-promoting GA and less plant growth under 9 m water depth (Figure 3). In our experimental system, all factors except water depth were kept constant (Table 1). The main difference between 2 m and 9 m of complete submergence is the difference in hydrostatic pressure. For every 1 m increase in water depth, the pressure of water acting on an object increases by about 9.8×10^3 Pa, and the pressure under 9 m water depth is about 0.088 MPa. How do deep submergence environments affect the biosynthesis of the endogenous hormone GAs and thus inhibit plant growth?

For living cells, GAs promotes hydrolysis of hemicellulose by Xyloglucan Endotransfer glycosidase thereby softening and relaxing the cell wall and promoting cell elongation and cell division (Ayano et al., 2014; Wang and Komatsu, 2022). The percentage of S-phase cells significantly increased within 4~7 h of treating rice with GA_3 through [^{3}H] thymidine and DNA admixture experiments, suggesting that GA promotes cell division in meristematic tissues of internode and shortens the cell cycle, and that internode elongation in deep-water rice is ultimately regulated by GA (Fukao et al., 2019). Our findings are also consistent with previous studies that the response of plants in submerged environments is very closely linked to the GAs. Consequently, GAs content at the base of immature internodes of *A. philoxeroides* decreased with increasing water depth, and as we expected.

It was demonstrated in previous studies that mechanical stress has important effects on the biosynthesis of endogenous plant hormones (Chehab et al., 2012; Lange and Lange, 2015), such as the effect of soil stress on ethylene synthesis (Potocka and Szymanowska-Pułka, 2018). From a mechanical perspective, when the force environment is altered, the distribution of mechanical stresses within plant tissues also undergoes localized changes, followed by cascading effects at the cellular (Richter et al., 2009) and molecular (Laskowski et al., 2008) levels. Therefore, it is possible that the higher hydrostatic pressure under 9 m water depth resulted in changes in the distribution of mechanical stress in the immature internodes of *A. philoxeroides*, which in turn affected the biosynthesis of GAs. Hydrostatic pressure is a specific type of mechanical stress, and its effects on plant growth include the effects of submergence and the effects of force. The stress response produced by plants varies depending on the severity and duration of the stress occurrence (Shi et al., 2016). The growth response of plants should be different for different intensities of hydrostatic pressure and different times of action on the plants.

5 Conclusion

Entirely consistent with the conjecture, our results suggested that the stem elongation of *A. philoxeroides* responded significantly to submergence depth and duration, especially the response of immature stems. Elongation of immature stems decreased when water depth increased, which was associated with elongation at the basal of immature internodes, and the basal parts made the biggest contribution to the elongation of internodes. Moreover, the elongation of the basal part of the immature stems was related to the concentration of endogenous hormone GAs. Therefore, in flood-prone environments, *A. philoxeroides* was able to grow rapidly through clonal integration, and its growth response differences to water depth were regulated by endogenous hormones. The results of this study provide strong evidence to demonstrate the important role of the hydrostatic pressure induced by flooding. Investigating the growth response of plants in different water depth can deepen our understanding of clonal plant submergence tolerance mechanisms, and help to explore the future management of water level regulation in the drawdown zone of large reservoirs (e.g., Three Gorges Reservoir).

Data availability statement

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

Author contributions

SJ: Data curation, Investigation, Writing – original draft. XR: Data curation, Investigation, Writing – original draft. FL: Writing – review & editing. HN: Data curation, Writing – original draft. QA: Formal analysis, Methodology, Writing – review & editing. BW:

Data curation, Writing – original draft. BZ: Conceptualization, Funding acquisition, Methodology, Writing – original draft. XZ: Conceptualization, Funding acquisition, Methodology, Writing – original draft, 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 supported by National Natural Science Foundation of China (grant numbers U22A20448, 31400480, 31800331), National Key R&D Program of China (2023YFF1305204), Chongqing Talents Program (grant number (cstc2021ycjh-bgzxm0316), the Science and Technology Major Projects of Henan Province of China (Grant number 232102321047) and Key Scientific Research Project of Colleges and Universities in Henan Province (Grant number 23B180010).

Acknowledgments

We thank Xingrong Wu, Senrui Zhang, Dongdong Ji, Meng Wang, Jine Huang, Zhao Zhang and all the people who helped with the fieldwork over the course of the 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.

The author(s) declared that they were an editorial board member of Frontiers, at the time of submission. This had no impact on the peer review process and the final decision.

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The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpls.2024.1348080/full#supplementary-material

References

Ayano, M., Takahiro, K., Mikiko, K., Hitoshi, S., Takuya, K., Takeshi, K., et al. (2014). Gibberellin biosynthesis and signal transduction is essential for internode elongation in deepwater rice. *Plant Cell Environ.* 37, 2313–2324. doi: 10.1111/pce.12377

Ayi, Q., Zeng, B., Liu, J., Li, S., van Bodegom, P. M., and Cornelissen, J. H. C. (2016). Oxygen absorption by adventitious roots promotes the survival of completely submerged terrestrial plants. *Ann. Bot.* 118, 675–683. doi: 10.1093/aob/mcw051

Bailey-Serres, J., and Voesenek, L. A. C. J. (2008). Flooding stress: acclimations and genetic diversity. *Annu. Rev. Plant Biol.* 59, 313–339. doi: 10.1146/annurev.arplant.59.032607.092752

Chehab, E. W., Yao, C., Henderson, Z., Kim, S., and Braam, J. (2012). *Arabidopsis* touch-induced morphogenesis is jasmonate mediated and protects against pests. *Curr. Biol.* 22, 701–706. doi: 10.1016/j.cub.2012.02.061

Chen, Z., Arif, M., Wang, C., Chen, X., and Li, C. (2021). Effects of hydrological regime on foliar decomposition and nutrient release in the riparian zone of the Three Gorges Reservoir, China. *Front. Plant Sci.* 12. doi: 10.3389/fpls.2021.661865

Dong, B., Fu, T., Luo, F., and Yu, F. (2017). Herbivory-induced maternal effects on growth and defense traits in the clonal species *Alternanthera Philoxeroides*. *Sci. Total Environ.* 605–606, 114–123. doi: 10.1016/j.scitotenv.2017.06.141

Fukao, T., and Bailey-Serres, J. (2008). Submergence tolerance conferred by sub1a is mediated by SLR1 and SLRl1 restriction of gibberellin responses in rice. *Proc. Natl. Acad. Sci. U. S. A.* 105, 16814–16819. doi: 10.1073/pnas.0807821105

Fukao, T., Barrera-Figueroa, B. E., Juntawong, P., and Peña-Castro, J. M. (2019). Submergence and waterlogging stress in plants: a review highlighting research opportunities and understudied aspects. *Front. Plant Sci.* 10. doi: 10.3389/fpls.2019.00340

Gao, L., Geng, Y., Yang, H., Hu, Y., and Yang, J. (2015). Gene expression reaction norms unravel the molecular and cellular processes underpinning the plastic phenotypes of *Alternanthera Philoxeroides* in contrasting hydrological conditions. *Front. Plant Sci.* 6. doi: 10.3389/fpls.2015.00991

Greet, J., Fischer, S., and Russell, K. (2020). Longer duration flooding reduces the growth and sexual reproductive efforts of a keystone wetland tree species. *Wetl. Ecol. Manage.* 28, 655–666. doi: 10.1007/s11273-020-09738-9

Guo, X., Hu, Y., Ma, J., Wang, H., Wang, K., Wang, T., et al. (2023). Nitrogen deposition effects on invasive and native plant competition: implications for future invasions. *Ecotox. Environ. Safe.* 259, 115029. doi: 10.1016/j.ecoenv.2023.115029

Jing, S., Ren, X., Lin, F., Niu, H., Ayi, Q., Wan, B., et al. (2024). Water depth-dependent stem elongation of completely submerged *Alternanthera Philoxeroides* is mediated by intra-internodal growth variations. *Front. Plant Sci.* 15. doi: 10.3389/fpls.2024.1323547

Jing, S., Zhang, X., Niu, H., Lin, F., Ayi, Q., Wan, B., et al. (2022). Differential growth responses of *Alternanthera Philoxeroides* as affected by submergence depths. *Front. Plant Sci.* 13. doi: 10.3389/fpls.2022.883800

Lange, M. J. P., and Lange, T. (2015). Touch-induced changes in *Arabidopsis* morphology dependent on gibberellin breakdown. *Nat. Plants* 1, 14025. doi: 10.1038/nplants.2014.25

Laskowski, M., Grieneisen, V. A., Hofhuis, H., Hove, C. A., Hogeweg, P., Maree, A. F., et al. (2008). Root system architecture from coupling cell shape to auxin transport. *PLoS. Biol.* 6, e307. doi: 10.1371/journal.pbio.0060307

Lei, S., Zeng, B., Xu, S., and Zhang, X. (2017). Response of basal metabolic rate to complete submergence of riparian species *Salix Variegata* in the three gorges reservoir region. *Sci. Rep.* 7, 1–11. doi: 10.1038/s41598–017-13467–0

Li, H., Lv, J., He, X., Bao, Y., and Nsabimana, G. (2024). Precipitation-dependent sensitivity of suspended sediment concentration to turbidity in a mountainous river in Southwestern China. *Ecol. Indic.* 159, 111644. doi: 10.1016/j.ecolind.2024.111644

Lin, C., Ogorek, L. L. P., Pedersen, O., and Sauter, M. (2021). Oxygen in the air and oxygen dissolved in the floodwater both sustain growth of aquatic adventitious roots in rice. *J. Exp. Bot.* 72, 1879–1890. doi: 10.1093/jxb/eraa542

Meng, Y., Yu, S., Yu, Y., and Jiang, L. (2022). Flooding depth and duration concomitantly influence the growth traits and yield of rice. *Irrig. Drain.* 71, 94–107. doi: 10.1002/ird.2632

Müller, J. T., van Veen, H., Bartylla, M. M., Akman, M., Pedersen, O., Sun, P., et al. (2019). Keeping the shoot above water - submergence triggers antithetical growth responses in stems and petioles of watercress (nasturtium officinale). *New Phytol.* 229, 140–155. doi: 10.1111/nph.16350

Pan, X., Welti, R., and Wang, X. (2010). Quantitative analysis of major plant hormones in crude plant extracts by high-performance liquid chromatography-mass spectrometry. *Nat. Protoc.* 5, 986–992. doi: 10.1038/nprot.2010.37

Potocka, I., and Szymanowska-Pułka, J. (2018). Morphological responses of plant roots to mechanical stress. *Ann. Bot.* 122, 711–723. doi: 10.1093/aob/mcy010

Raskin, I., and Kende, H. (1984). Role of gibberellin in the growth response of submerged deep water rice. *Plant Physiol.* 76, 947–950. doi: 10.1104/pp.76.4.947

Richter, G. L., Monshausen, G. B., Krol, A., and Gilroy, S. (2009). Mechanical stimuli modulate lateral root organogenesis. *Plant Physiol.* 151, 1855–1866. doi: 10.1104/pp.109.142448

Sasidharan, R., and Voesenek, L. A. C. J. (2015). Ethylene-mediated acclimations to flooding stress. *Plant Physiol.* 169, 3–12. doi: 10.1104/pp.15.00387

Sauter, M., and Kende, H. (1992). Gibberellin-induced growth and regulation of the cell division cycle in deepwater rice. *Planta* 188, 362–368. doi: 10.1007/BF00192803

Shi, H., Liu, R., Xue, C., Shen, X., Wei, N., Deng, X. W., et al. (2016). Seedlings transduce the depth and mechanical pressure of covering soil using cop1 and ethylene to regulate ebf1/ebf2 for soil emergence. *Curr. Biol.* 26, 139–149. doi: 10.1016/j.cub.2015.11.053

Taiz, L., and Zeiger, E. (2010). *Plant physiology.* (Sunderland: Sinauer Associates).

Vervuren, P. J., Beurskens, S. M., and Blom, C. W. (1999). Light acclimation, CO_2 response and long-term capacity of underwater photosynthesis in three terrestrial plant species. *Plant Cell Environ.* 22, 959–968. doi: 10.1046/j.1365-3040.1999.00461.x

Vervuren, P. J., Blom, C. W., and De Kroon, H. (2003). Extreme flooding events on the Rhine and the survival and distribution of riparian plant species. *J. Ecol.* 91, 135–146. doi: 10.1046/j.1365-2745.2003.00749.x

Voesenek, L. A. C. J., Sasidharan, R., Visser, E. J. W., and Bailey-Serres, J. (2016). Flooding stress signaling through perturbations in oxygen, ethylene, nitric oxide and light. *New Phytol.* 209, 39–43. doi: 10.1111/nph.13775

Voesenek, L. A. C. J., Sasidharan, R., and Weber, A. (2013). Ethylene-and oxygen signalling-drive plant survival during flooding. *Plant Biol.* 15, 426–435. doi: 10.1111/plb.12014

Wang, X., and Komatsu, S. (2022). The role of phytohormones in plant response to flooding. *Int. J. Mol. Sci.* 23, 6383. doi: 10.3390/ijms23126383

Wang, X., Deng, Z., Zhang, W., Meng, Z., Chang, X., and Lv, M. (2017). Effect of waterlogging duration at different growth stages on the growth, yield and quality of Cotton. *PLoS One* 12, e169029. doi: 10.1371/journal.pone.0169029

Wang, Q., Wang, L., Chandrasekaran, U., Luo, X., Zheng, C., and Shu, K. (2021). ABA biosynthesis and signaling cascades under hypoxia stress. *Front. Plant Sci.* 12. doi: 10.3389/fpls.2021.661228

Wu, H., Carrillo, J., and Ding, J. (2017). Species diversity and environmental determinants of aquatic and terrestrial communities invaded by *Alternanthera Philoxeroides*. *Sci. Total Environ.* 581–582, 666–675. doi: 10.1016/j.scitotenv.2016.12.177

Yang, C., Yang, X., Zhang, X., Zhang, F., Zhou, C., Wang, Q., et al. (2019). Anatomical structures of alligator weed (*Alternanthera Philoxeroides*) suggest it is well adapted to the aquatic-terrestrial transition zone. *Flora* 253, 27–34. doi: 10.1016/j.flora.2019.02.013

You, W. H., Han, C. M., Fang, L. X., and Du, D. L. (2016). Propagule pressure, habitat conditions and clonal integration influence the establishment and growth of an invasive clonal plant, *Alternanthera Philoxeroides*. *Front. Plant Sci.* 7. doi: 10.3389/fpls.2016.00568

Zheng, J., Arif, M., Zhang, S., Yuan, Z., Zhang, L., Dong, Z., et al. (2021). The convergence of species composition along the drawdown zone of the Three Gorges Dam Reservoir, China: implications for restoration. *Environ. Sci. pollut. Res.* 28, 42609–42621. doi: 10.1007/s11356-021-13774-0

frontiers | Frontiers in Plant Science

TYPE Original Research
PUBLISHED 26 June 2024
DOI 10.3389/fpls.2024.1325052

OPEN ACCESS

EDITED BY
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RECEIVED 20 October 2023
ACCEPTED 29 April 2024
PUBLISHED 26 June 2024

CITATION
Watson MA and Vuorisalo T (2024) Interactions between developmental phenology, carbon movement, and storage constrain demography in the understory clonal herb *Podophyllum peltatum* L. *Front. Plant Sci.* 15:1325052. doi: 10.3389/fpls.2024.1325052

Interactions between developmental phenology, carbon movement, and storage constrain demography in the understory clonal herb *Podophyllum peltatum* L.

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Little is known about how carbon integration and storage dynamics affect and are affected by demography in field populations. We sought to elucidate this link by examining dynamic patterns of carbon integration relative to the timing of demographically significant developmental decisions regarding shoot type determination in mayapple, *Podophyllum peltatum*, a clonal plant with large and persistent rhizomes. Individual rhizome systems growing in natural populations were fed $^{14}CO2$ either in late-April, early-May, or mid-June, then harvested at intervals throughout the current season and into the next. When distribution of label was examined we found that carbon fixed at different times in the growing season is used differently: April-fixed assimilate remained in the labeled shoot or was moved into the old rhizome, May-fixed assimilate was found predominantly in the old rhizome, while early-June fixed assimilate moved into the old rhizome and the extending new ramet. Movement of assimilate into the old rhizome appeared to have precedence over formation of additional new ramets. Despite significant within season changes in location of dominant sinks within rhizome systems, there was little redistribution of labeled assimilate: early fixed assimilate was not used to fuel later within season growth, however, assimilate was redistributed between seasons. Vegetative and sexual systems differed in the distribution only of April-fixed assimilate. This was observed even though early labeling occurred prior to anthesis. Sexual systems retained a greater proportion of assimilate in the stem than did vegetative ones, which exported more to the old rhizome. ^{14}C-distribution patterns did not vary between systems differing in future demographic status suggesting that the developmental decision regarding shoot type is based on resources acquired in prior years. We explore the hypothesis that preformation and storage are functionally linked traits that permit plants to coordinate the developmental determination of structures differing in cost and demographic function with known resource status. We conclude that demography influences and is

influenced by integrative physiology and that physiological restrictions on within season redistribution of assimilates constrain plants' capacities to respond to short-term environmental variation. Such constraints may affect plants' abilities to respond to rapid environmental change in the Anthropocene.

KEYWORDS

developmental phenology, seasonal carbon integration, demography, development and demography, ^{14}C-translocation, storage and integration, phenology

1 Introduction

The Anthropocene marks a period of man-made and rapid environmental change (Corlett, 2015). Limits on plants' capacities to respond to such rapid change may be due either to constraints on plasticity and/or to the lack of appropriate genetic variation on which selection may act (e.g., Griffith and Watson, 2006; Dodd and Douhovnikoff, 2016; Kudoh et al., 2023; Williamson et al., 2023). Clonal plants may be particularly susceptible because clonality is at its heart a conservative growth strategy. It is of greatest advantage when environmental conditions are predictable. While observational data allow us to document changes in growth and demographic expression induced by environmental change, to move from documentation to prediction requires more nuanced understanding of how particular plants work. We need to know how plants' developmental programs and their integrative physiologies interact to create a particular pattern of demographic expression (Diggle, 1994; Watson et al., 1995; Geber et al., 1997a, b; Meloche and Diggle, 2003; Goldberg et al., 2020) and how these interactions facilitate or constrain their capacities to persist in a rapidly changing world.

Plant growth is modular and earlier developmental events influence later ones through their effects on resource status and patterns of resource distribution (Watson, 1984; Diggle, 1994; Meloche and Diggle, 2003). In turn, temporal patterns of resource distribution influence subsequent developmental decisions (Diggle, 1994; Watson et al., 1995, 1997; Sachs and Novoplansky, 1997; Geber et al., 1997a; Worley and Harder, 1999; Lapointe, 2001; Weinig and Delph, 2001; Meloche and Diggle, 2003; Huber et al., 2004). Storage is another important component of resource status (Lubbe et al., 2021), yet little is known about how storage and storage dynamics affect and are affected by demography (Goldberg et al., 2020). To examine the interactions between resource dynamics, storage and demography requires information about temporal changes in demographic status, developmental phenology (i.e., the timing of meristem commitment to alternate demographic functions) and the integrative physiology of the plant; an approach we termed developmental ecology (Watson, 1984; Watson et al., 1997).

Work on crop species and a few native species indicates that pathways of resource flow within plants change with temporal changes in sink strength, type of sink and relative positions of organs – all of which result from developmental decisions regarding patterns of meristem determination made throughout the life of the plant (Marshall, 1990; Watson et al., 1995, 1997; Meloche and Diggle, 2003). Relatively little data of this kind exist for native species in their natural habitats and most address changes in sink strength due to shading or herbivory rather than developmental phenology (Flanagan and Moser, 1985; Jónsdóttir and Callaghan 1989; Tissue and Nobel, 1990a, b; Zimmerman and Whigham, 1992; Tissue et al., 1995; Babst et al., 2008; MaChado et al., 2013). However, phenological data about resource use and integration should provide insight into several key issues, including: (1) the differential use of resources gained at different times in the growing season; (2) the degree to which plants can reallocate resources gained at one time in the growing season to support later growth and sexual reproduction; (3) the effect of demographic status on these patterns; (4) the effects of variation in patterns of resource integration on developmental events that affect future growth form and demography and (5) plants capacities to respond to rapid changes in their environment.

Clonal plants provide convenient systems for examining these relationships. Not only are their behaviors governed by physiological and developmental rules that generally apply to all plants (Watson et al., 1995, 1997; Huber et al., 1999, 2004; Rashid et al., 2023) but they also maintain unique organs – stolons and rhizomes – that have important integrative functions (Jónsdóttir and Watson, 1997), provide storage capacity (Suzuki and Hutchings, 1997; Suzuki and Stuefer, 1999), and retain meristem banks that can be used in damage recovery (e.g., Eisen et al., 2021). The role of storage generally is evaluated indirectly or through examination of changes in total nonstructural carbohydrate (TNC) pools. Little is known, for example, about the developmental equivalency of carbon fixed and stored at different times in seasonal phenology or constraints on the remobilization of stored resources. Chapin et al. (1990) suggest that all stored carbon is not equally available at any given time in plants' developmental phenology and data from other studies are consistent with this hypothesis (Jónsdóttir and Callaghan, 1989; Suzuki and Hutchings, 1997; Stuefer and Huber, 1999; Suzuki and Stuefer, 1999).

Approaches that take a dynamic perspective on patterns of resource use have proved valuable in other systems (e.g., Ashmun, 1994),

and we take such an approach here. We address the link between the timing of demographically significant developmental events, current and future demographic status, and temporal patterns of resource movement by feeding the radioactive isotope $^{14}CO_2$ to plants in natural field populations. Using a matrix of label and harvest dates, we test the following hypotheses: (1) Assimilate fixed at different times in the growing season is transported to different structures. (2) There is significant remobilization of assimilate fixed early in the season, when light levels are high, to support the growth of later-developing structures, when light levels are low. (3) Current and future demographic status of the aerial shoot influences seasonal patterns of assimilate movement within rhizome systems (physically and physiologically interconnected sets of ramets within a clone).

1.1 Study system

We work with mayapple, *Podophyllum peltatum* L. (Berberidaceae), a long-lived rhizomatous clonal plant that is widely distributed in deciduous forests east of the Rockies in North America (Gleason and Cronquist, 1963). They grow in discrete patches of a few to many thousand aerial shoots belonging to one or more clones (Watson et al., unpublished). Mayapple rhizome systems consist of annually produced rhizome segments or ramets that remain morphologically and physiologically inter-connected generally for 6-7 years (Sohn and Policansky, 1977; Landa et al., 1992) (Figure 1). Most rhizome systems produce only one new ramet each year, rarely two or three (Geber et al., 1997a); but have the developmental capacity to produce up to five (Jones and Watson, 2001). In our experimental populations individual systems produced only a single terminal rhizome segment (ramet) per year. These newly forming ramets give rise to only one of two types of aerial structure; either a single vegetative leaf or a two-leaved sexual shoot that bears a terminal flower; the larger the terminal rhizome segment, the more likely it is to produce a sexual shoot, suggesting that the larger sexual shoot is more costly for the plant (Sohn and Policansky, 1977; Benner and Watson, 1989; de Kroon et al., 1991; Geber et al., 1997a, b).

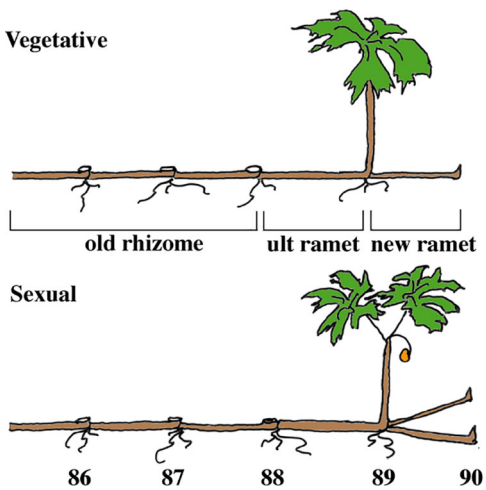

FIGURE 1
The architecture of vegetative and sexual rhizome systems of mayapple, *Podophyllum peltatum*. Plants are typical of those found in late June in Indiana. The four compartments used in the analyses are: new ramet, ultimate (ult) ramet, old rhizome, and shoot. In some analyses, the ultimate ramet is included in the old rhizome compartment. See text for explanation. The numbers beneath the ramets indicate the year in which each bore an aerial shoot.

Mayapples are obligately outcrossing but nectarless, and pollination rates of the most important pollinators, i.e., bumblebee queens (*Bombus* spp.) and honeybees (*Apis mellifera*) are usually low (Sohn and Policansky, 1977; Laverty and Plowright, 1988; Crants, 2008). The large fleshy fruits of mayapple are known to be dispersed by eastern box turtles (*Terrapene carolina*) (Rust and Roth, 1981). In our study area, another likely seed disperser is the raccoon (*Procyon lotor*). Raccoons feed on fruits of native vegetation in northern Indiana (Lehman, 1977) and are known to defecate germinable seeds (Willson, 1993; Niederhauser, 2015). Neither of these likely frugivores would aid in long distance dispersal. Insect herbivory in our study area was modest, but a few mayapple stems hosted stem boring larvae probably belonging to *Papaipema rutila* (cf. Bess, 2005).

Mayapple development is characterized by preformation, a common feature of plants of the deciduous forest understory (Foerste, 1891; Randall, 1952; Ott et al., 2019; Schnablova et al., 2020; Rünk et al., 2021; Rashid et al., 2023). New mayapple ramets are initiated almost two full years before their maturation aboveground (Watson et al., 1997; Geber et al., 1997a, b; Jones and Watson, 2001); they become morphologically determined as vegetative or sexual almost one full year before they emerge as aerial shoots and before the terminal bud reaches its final location on the forest floor and forms roots (Foerste, 1884; Geber et al., 1997a, b; Jones and Watson, 2001). The early determination of the demographic fate of aerial shoots almost one full year before their emergence aboveground suggests that the developmental decision to produce one or the other shoot type is contingent upon more than just the net amount of carbon accumulated by the developing terminal ramet within the season of shoot expansion. Further it suggests that assessments regarding the amount of assimilate fixed and stored in prior years also are important (Watson et al., 1997; Rünk et al., 2021; Rashid et al., 2023).

Because mayapple rhizome systems are highly integrated for carbon (Landa et al., 1992; Jónsdóttir and Watson, 1997) they, like other such plant species, should be able to move carbon stored in older ramets to developing axes, serving as a link between non-overlapping periods of high assimilate production and high carbon demand (Pitelka and Ashmun, 1985; Zimmerman and Whigham, 1992; Tissue et al., 1995; Wijesinghe and Whigham, 1997). In mayapple, integration for nitrogen-based resources is less extensive and more unidirectional with nitrogen-based resources generally moving from roots on intermediate-aged and new ramets to the developing terminal ramet (Jónsdóttir and Watson, 1997).

Mayapple rhizome systems exhibit extreme developmental division of labor among ramets, a phenomenon reported in an array of clonal plants (Alpert and Stuefer, 1997; Jónsdóttir et al., 1996). The current year's ramet maintains both above and belowground function through the presence of aerial leaves and subterranean roots (Figure 1). All older ramets function only below ground and in the absence of injury to the rhizome system, these

older ramets do not produce an aerial shoot again, but their roots do continue to function in mineral nutrient acquisition (Jónsdóttir and Watson, 1997) and the rhizome in storage. The older portions of rhizome systems also maintain a bank of dormant buds that is important in damage recovery (Watson et al., 1997; Ott et al., 2019; Eisen et al., 2021). Not surprisingly, in this understory herb carbon appears to be the critical limiting resource (Sohn and Policansky, 1977; Benner and Watson, 1989; de Kroon et al., 1991; Watson and Lu, 1999, 2004). We focus on carbon here.

These combined traits make mayapple a good subject for the study of the interaction between physiology, developmental phenology, and demographic determination. Mayapples grow slowly on relatively long-lived and highly integrated rhizome systems, and developmental determination of aerial shoot type takes place over an extended period of almost two years. This permits us to see interactions that would be difficult to detect but, no doubt, occur in faster growing taxa.

2 Materials and methods

Our study was conducted in an upland beech-maple-hickory temperate deciduous forest, on a private farm in Greene County, south-central Indiana (39°10'11"N, 86°42'58"W), the site of related work (Landa et al., 1992; Geber et al., 1997a; Watson and Lu, 1999, 2004). The study was initiated at the beginning of the 1989 growing season in several large colonies on a south-facing slope. Upon harvest we verified that there was little branching in our experimental rhizome systems, and most systems were made up of six to seven ramets, making these integrated subunits fairly uniform in age.

2.1 ^{14}C-labeling and harvest protocols

Rhizome systems were labeled with $^{14}CO_2$ either in late April, early May or early June and harvested either in mid-May, mid-June or September of 1989, or in mid-March of 1990, creating a matrix of seven label by harvest combinations that differed in the length of the chase period (Table 1). In late April (first label), mayapple shoots were almost fully expanded and the forest canopy was open. Two weeks later (May label/harvest), the forest canopy was closing and mayapple flowers were in anthesis. By June the forest canopy had been closed for several weeks and in this study all flowers and young fruits aborted.

June is an important period in mayapple development. The new rhizome segment that will give rise to next year's ramet experiences rapid extension growth (Geber et al., 1997a), the critical stages of morphological determination of the demographic status of next year's aerial shoot occur (Jones and Watson, 2001), endogenous leaf senescence was first observed (Watson and Lu, 1999, 2004) and fruits when present enlarge. In late summer, the determination of next year's shoot type, senescence of the current year's shoot and expansion of the new rhizome segment are complete and the new ramet is forming roots. By March of the following year new aerial shoots are about to emerge above ground, with emergence regularly occurring in the first week in April at our location (Watson, pers. obs.).

For each label by harvest treatment combination, 10 rhizome systems bearing a vegetative and 10 bearing a sexual aerial shoot were labeled, for a total of 140 rhizome systems (Table 1). Selected shoots were sufficiently far apart that no two of them were interconnected within branching rhizome systems. This was confirmed when systems were harvested. Rhizome systems differed in the number of ramets making up the old rhizome, though virtually all systems had at least five old ramets. Label was introduced by exposing the single vegetative leaf, or the larger of the two leaves of sexual shoots to $^{14}CO_2$ (100 μCi ^{14}C per rhizome system (Amersham CFA.3, 50-60 mCi/mmole $NaHCO_3$)) (Landa et al., 1992). Shoots that senesced prior to the scheduled harvest of their rhizome system were collected when they turned completely brown. At harvest, rhizome systems were washed, separated into individual ramets and their associated roots, and dried to constant weight at 65°C. For plants harvested in September 1989 and March 1990, the demographic status of the 1990 shoot (vegetative or sexual) was determined by dissection and recorded. The few

TABLE 1 Matrix of label dates and length of the chase period, in weeks, used to examine the dynamic pattern of carbon movement in mayapple, *Podophyllum peltatum* L.

Harvest Dates					
	FL	FR	PS	ES	Total #
	May[a]	June	August	March	RhizomeSystems
Labeling Dates					
April (LE[a])	2[b]	7	16		60
May (FL[a])		5	14		40
June (FR[a])			10	50	40
Total # Rhizome Systems					
Systems Harvested	20	40	60	20	140

[a]Labeling and harvest dates corresponded to the following periods in mayapple seasonal phenology: LE, leaf expansion; FL, flower anthesis; FR, fruit development; PS, post senescence; ES, early spring. Labeling dates were as follows: LE, April 25-28, 1989; Fl, May 5-8, 1989; FR, June 6-8, 1989. Harvest dates were as follows: FL, May 8-10; FR, June 8-15; PS, August 28-September 5; ES, March 22, 1990.
[b]Length of the chase period = number of weeks between labeling and harvest.

plants that failed to incorporate $^{14}CO_2$ were excluded from the translocation analyses but were used in the biomass analyses.

Following biomass determination, structures were ground in a Wiley mill using a 20 mesh, and two replicate subsamples of known weight oxidized in a Harvey Biological Oxidizer (model OX-400). Released $^{14}CO_2$ was trapped in scintillation fluid (Harvey Biological ^{14}C Cocktail) and the amount of label present in each subsample determined by scintillation counting (Landa et al., 1992). If the two subsamples gave similar values, they were averaged together, and that average value used to calculate ^{14}C activity in each structure. If the two values differed, additional subsamples were run and included in calculations, but this was rarely necessary because subsamples showed a high degree of homogeneity. The resulting measure of activity per milligram dry weight specifies the specific activity. The total activity of a structure or compartment was calculated by multiplying its specific activity by its dry mass.

Labeled assimilate was used as a marker of the movement of total assimilate fixed at a given time. Data were aggregated into four compartments (Figure 1). (1) The ultimate old rhizome segment or ramet (Ult), which gives rise to the current year's shoot. (2) The old rhizome compartment (Old), composed of all ramets proximal to (older than) the ultimate ramet. (3) The new ramet (New), is the ramet that morphologically differentiates next year's aerial shoot during the current growing season, and (4) the current year's aerial shoot (Shoot), which includes the leaves, petioles, stem and flower, if present. While the Ultimate, New and Shoot compartments were about the same age, the Old compartment, because it consisted of varying numbers of annual rhizome segments was not. Despite this variability in the size and age of the OLD compartment between rhizome systems, statistically significant differences were found. Root data were aggregated as described above. This differs from Landa et al. (1992) in which roots that were associated with the current year's ramet were analyzed with those of the new ramet. Values of percent distribution were obtained by dividing the total amount of ^{14}C in each compartment at the time of harvest by the total amount of label in the rhizome system at that time.

Specific activity was used as an indicator of differences among structures in sink strength. Because only the larger of the two leaves of sexual shoots was labeled, we normalized specific activity between vegetative and sexual rhizome systems by multiplying the specific activity of each structure in sexual systems by 1.67, the average ratio of total leaf area of sexual systems to the leaf area labeled. We assumed that seasonal photosynthetic profiles of sexual and vegetative systems were similar, which field gas exchange studies suggest is reasonable (Basha, Griffith, Carlson and Watson, unpubl.).

2.2 Data analysis

All analyses used the Fit Model module of JMP v 3.0 (SAS, 2000).

2.2.1 Biomass

The labeling treatments did not affect overall rhizome system size or the distribution of biomass among compartments (analysis not shown) allowing data from the three labeling treatments to be combined within harvests and demographic categories for biomass analysis.

We performed two separate ANOVAs on the biomass of new rhizome and old rhizome compartments of the rhizome system (including the 1989 (Ult) ramet) to test for changes in biomass over time. We also performed planned comparisons between subsequent harvests and between vegetative and sexual systems within harvest. For statistical analysis, root and rhizome biomass was pooled within compartments as the analyses of the pooled data gave results similar to those for the un-pooled data with respect to harvest and shoot type effects.

2.2.2 ^{14}C distribution

The structure of the data sets on total ^{14}C activity and specific activity are similar and, thus, the two analyses have many elements in common. MANOVA designs were used because multiple measurements of total and specific activity were made within rhizome systems. Repeated measures designs (Potvin et al., 1990) were used both because the within-rhizome ramet data is serially correlated due to the shared history of ramets within rhizome systems (Geber et al., 1997a, b) and ^{14}C levels of ramets belonging to the same rhizome system are not independent because they are a function of the total amount of ^{14}C taken up by each rhizome system. We used Pillai's Trace because of its relative robustness and power (JMP v 4.0, SAS, 2000; Scheiner and Gurevitch, 2000); the more commonly used Wilke's Lambda gave similar results.

Because not all combinations of label and harvest dates are represented in the experiment (Table 1), we performed five separate MANOVAs each for total and specific activity. Three of the five MANOVAs examined changes in labeled-assimilate distribution across the season for each label date separately, while the other two MANOVAS analyzed differences between label dates at two different harvests. However, because total activity and specific activity distribution were analyzed at different resolutions (total activity was examined across compartments, specific activity on a ramet-by-ramet basis), analyses of total activity and specific activity required different MANOVA designs.

Total activity. A sum design was used to examine between subject effects and a contrast design to examine within subject effects. Because all MANOVAs showed significant departures from sphericity, the degrees of freedom of all within subject tests were reduced using the Greenhouse-Geisser correction (JMP v 4.0, SAS, 2000; Scheiner and Gurevitch, 2000). Total activity data were grouped into the four compartments described above, with roots and rhizome pooled within each compartment. Overall differences among plants in the summed activity were reflected in the between-subjects effects: harvest dates (for MANOVAs by label date), label date (for MANOVAs by harvest date), and shoot type. All between-subject effects were treated as fixed factors. Differences in the distribution of activity within the plants were tested as within-subject effects. These included differences among compartments within individuals (compartment) and differences among individuals in the distribution of label among harvests, label dates

or shoot types (Compartment * harvest, Compartment * label, Compartment * type effects respectively).

Specific activity. Analyses of specific activity differed from those of total activity in three ways: they involved only the below-ground organs, each ramet was analyzed separately, and the roots and rhizomes were analyzed separately, resulting in 14 dependent variables rather than just four as in the total activity analyses. These differences primarily affected the design of the within-subject portion of the MANOVAS, requiring a compound MANOVA design. There were two levels of belowground organ (root or rhizome) and seven levels of ramet (i.e., seven years of ramet growth) (Figure 1). Specific activities of missing organs, all of which were older ramets, were taken to be zero. We included root versus rhizome effects, ramet (i.e., positional) effects and their interaction as terms in the analysis. Between-subject effects were assessed with a sum design as described above for total activity.

3 Results

3.1 Biomass distribution

3.1.1 Temporal changes in rhizome biomass as a function of current (1989) demographic status

Old rhizome systems that were sexual in 1989 were heavier than those that were vegetative at each of the 1989 harvests (Figure 2A; Table 2, 1989 Type in May, June and September). This difference vanished by March 1990 when, instead, the demographic status of the 1990 shoot (1990 Type) drove the relationship (Figure 2A; Table 3).

Old rhizome biomass increased by ca. 2.0g from May to June in both 1989 vegetative and sexual systems, but vegetative old rhizome biomass increased by 73% compared to 33% in the already larger sexual systems (Figure 2A; Table 2, 1989 Type: May contrast). The period of old rhizome biomass increase was followed by a period of progressive decline in biomass that extended over the remainder of the current growing season and into the next, for both vegetative and sexual rhizome systems. Systems that were sexual in 1989 showed a greater drop in average old rhizome biomass (3.4 g, 46%) than those that were vegetative (1.2 g, 26%), even though no fruiting occurred, resulting in a progressive convergence in old rhizome biomass between the two. By the following spring there was no difference in old rhizome biomass between systems that had been sexual versus vegetative in 1989 (Figure 2A; Table 2, 1989 Type: March contrast).

Increase in new ramet biomass was similar in 1989 vegetative and sexual systems (Figure 2B; Table 2, Shoot * Harvest). However, at every harvest, 1989 sexual systems produced new ramets that were larger than those produced by 1989 vegetative systems, although the differences were statistically significant only in September (Figure 2B; Table 2, 1989 Type: September contrast).

3.1.2 Temporal changes in rhizome biomass as a function of future (1990) demographic status

When rhizome systems that were harvested in September 1989 or March 1990 were categorized by 1990 rather than 1989 shoot type different patterns of biomass distribution were found (Figure 2A; Table 3). Systems that gave rise in 1990 to sexual

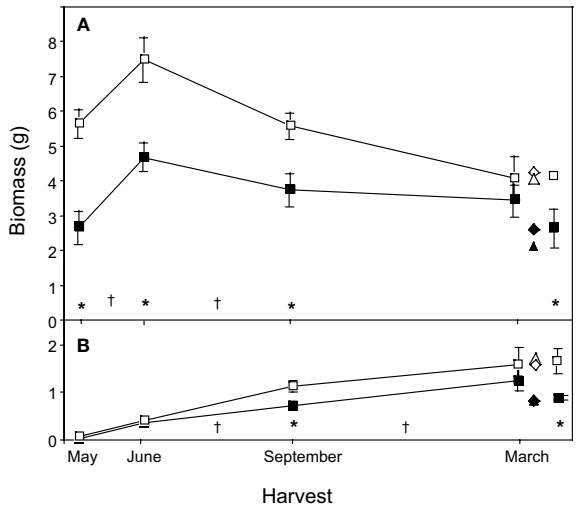

FIGURE 2
Changes in total rhizome biomass through time for vegetative and sexual rhizome systems of mayapple, *Podophyllum peltatum*. Biomass data for roots and rhizomes are combined, and data from the Ult ramet is included in the Old rhizome compartment. Data are represented as the mean ± 1 s.e. **(A)** Temporal changes in Old rhizome biomass for rhizome systems terminated in 1989 by either a sexual (□) or a vegetative (■) shoot. Data from the March harvest is represented in three columns. The left-hand column represents data from rhizome systems that were terminated by either a sexual (□) or a vegetative (■) shoot in 1989, as in earlier harvests. In contrast, the right-hand column represents data from rhizome systems that were terminated by either a sexual (□) or a vegetative (■) shoot in 1990. The center column represents the life history status of each rhizome system in both 1989 and 1990: (open symbols represent rhizome systems that were sexual in 1990; closed symbols represent systems that were vegetative in 1990; (△) represent systems that were sexual in 1989; (◆), those that were vegetative in 1989. **(B)** Changes in biomass for the new ramet. Symbols as **(A)**. Asterisks designate statistically significant pairwise planned comparisons within harvests between systems differing in life history status (vegetative or sexual shoot in 1989); daggers (†) designate significant planned comparisons between harvest dates (see Table 2).

TABLE 2 ANOVA of effects of Harvest Date and 1989 Life History Status (1989 Type, vegetative or sexual) on Old Rhizome Biomass (left) and New Rhizome Biomass (right)[1].

Source	Old Rhizome Biomass				New Rhizome Biomass			
	DF	Sum Squares	F Ratio	Prob>F	DF	Sum Squares	F Ratio	Prob>F
1989 Type	1	178113361	23.3938	< **0.0001**	1	1339823	4.1705	**0.0435**
Harvest	3	143115664	6.2657	**0.0006**	3	32128158	33.3355	**<0.0001**
1989 Type * Harvest	3	35238645	1.5428	0.2075	3	1228609	1.2748	0.2867
Planned Comparisons	**Estimate**	**t-Ratio**	**Prob. > |t|**		**Estimate**	**t-Ratio**	**Prob. > |t|**	
Harvest: May v June	-2700	-3.159	**0.0020**		-0.337	-1.952	(0.0535)	
Harvest: June v Sept	1671.9	2.7218	**0.0076**		-0.6888	-5.459	**<0.0001**	
Harvest: Sept v March	1504.5	1.9285	(0.0564)		-0.6351	-3.963	**0.0001**	
1989 Type: May	3983.4	2.8872	**0.0047**		0.0523	0.1844	0.8541	
1989 Type: June	3868.3	4.0248	**0.0001**		0.0531	0.2688	0.7886	
1989 Type: Sept.	2657.6	3.4726	**0.0007**		0.4890	3.1107	**0.0024**	
1989 Type: March	585.57	0.4306	0.6676		0.3679	1.3173	0.1905	

[1]See Figure 1 – Vegetative for a description of the compartments.
Planned comparisons between subsequent harvests and between shoot types are shown in the bottom section of the table. Values in bold face are significant at $p < 0.05$; those in parentheses are of borderline significance.

shoots had significantly heavier old rhizomes in both September and March (Table 3, Type contrast, Figure 2A for March harvest, right column), but the difference between the two was not as large as in 1989 (Figure 2A). Similarly, new ramets that would be sexual in 1990 were significantly larger than those that would be vegetative, and the difference was statistically significant at both harvests (1990 Type: September and March contrasts; Figure 2B for March harvest).

3.1.3 Distribution of biomass among ramets within rhizome systems

Virtually all ramets of 1989 sexual systems were larger than vegetative systems at the May through September 1989 harvests, but they converged in size by March 1990 (Figure 3). Biomass shifted to the younger ramets between May and June and the shift was more striking in current (1989) sexual systems. From June through March, biomass was lost throughout the rhizome system, but

TABLE 3 ANOVA of effects of 1990 Life History Status (1990 Type, vegetative or sexual) and Harvest Date (March v September) on Old Rhizome Biomass (left) and New Rhizome Biomass (right) [1].

Source	Old Rhizome Biomass				New Rhizome Biomass			
	DF	Sum Squares	F Ratio	Prob>F	DF	Sum Squares	F Ratio	Prob>F
Harvest (Mar v Sept)	1	44411160	6.7015	**0.0119**	1	2687927.2	6.2844	**0.0148**
1990 Type	1	142231739	21.4622	**<.0.0001**	1	9200633.3	21.5112	**<.0.0001**
Harvest (Mar v Sept) *1990 Type	1	78308	0.0118	0.9138	1	200039.9	0.4677	0.4966
Planned Comparisons	**Estimate**	**t - ratio**	**Prob > |t|**		**Estimate**	**t-ratio**	**Prob. > |t|**	
Harvest, Sexual: Sept v March	-2000	-2.179	**0.0331**		606.33	2.5776	**0.0123**	
Harvest,Vegetative: Sept v March	-1855	-1.579	0.1194		346.42	1.1605	0.2502	
1990 Type: September	3546.6	4.8669	**<0.0001**		751.39	4.0588	**<0.0001**	
1990 Type: March	3384	2.5901	**0.0119**		1011.3	3.0469	**0.0034**	

[1]See Figure 1 – Vegetative for a description of the compartments.
Planned comparisons between subsequent harvests and between shoot types are shown in the bottom of the table. Values in bold face are significant at $p < 0.05$.

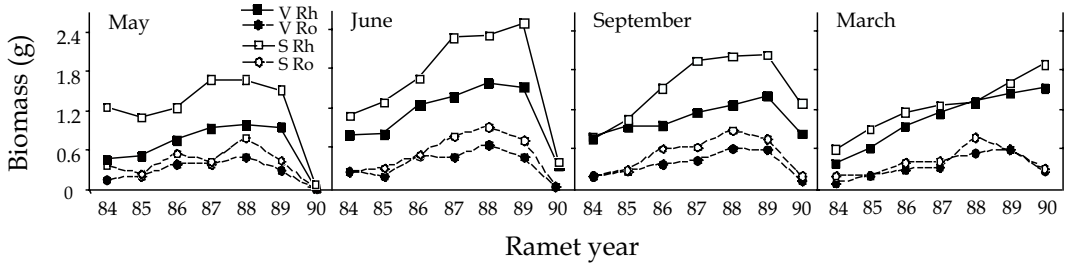

FIGURE 3
The distribution of root and rhizome biomass by ramet, at each harvest, for rhizome systems that were sexual or vegetative in 1989. The numbers on the X-axis represent the locations of ramets within rhizome systems, the number indicates the year that the ramet bore an aerial shoot.

particularly from the younger ramets of the old rhizome (Figure 3: 87, 88, 89 ramets). Although root and rhizome biomass were correlated (r = 0.4591), roots exhibited much less variation in biomass distribution among ramets from one harvest to the next. Considering our findings from the ^{14}C data presented below, it is of particular interest that root biomass of the 1989 ramet did not increase significantly between June and September (F = 0.6435, p = 0.427).

3.2 Assimilate distribution

3.2.1 The relationship between time of labeling and ^{14}C distribution patterns

Plants labeled at different times in the growing season showed different patterns of label distribution (Figure 4, vertical comparisons, June and September harvests, Table 4, Compartment * Label Date): April label was found predominantly in the shoot (63% (V) to 80% (S)), May label in the old rhizome and ultimate rhizome segment (83%) and June label in the new ramet (45%) and old rhizome.

The demographic status of the current year's shoot (1989, V or S) was a significant factor in how assimilate was distributed among compartments only for April-fixed assimilate; it was of marginal significance for May-fixed and non-significant for June-fixed assimilate. In systems labeled in April, sexual systems retained a greater proportion of label in their shoot (80% vs. 63% in vegetative systems) (Figure 4; Table 5, Compartment * 1989 Type). The effect of the 1989 shoot type diminished as the season progressed.

3.2.2 Within and between season changes in patterns of assimilate distribution

There was little redistribution of label among compartments within the growing season irrespective of label date (Figure 4, horizontal comparisons). Statistically significant redistribution of label was noted for April-fixed assimilate, from the shoot into the old rhizome and new ramet compartments, and marginally significant redistribution for May-fixed assimilate (Table 5, Compartment * Harvest), but the magnitude of redistribution was small compared to the pronounced seasonal changes in dominant sink location (Figure 4, horizontal vs. vertical comparisons). The pattern of redistribution did not differ between 1989 sexual *vs* vegetative systems (Table 5, Compartment * Harvest * 1989 Type interaction).

While we saw little evidence of within season redistribution of label there was significant redistribution of label between the September 1989 and March 1990 harvests, from the 1989 old rhizome and ultimate rhizome segment into the 1990 ramet (Figure 4; Table 5).

Despite the small amount of within season remobilization of assimilate, rhizome systems did lose significant amounts of label through time: 10% between May and June, 50% between June and September, and 30% between September 1989 and March 1990 (Figure 4 horizontal comparisons, Table 5, Harvest, April and May Label Dates), indicating that significant amounts of labeled carbon remain in metabolizable forms.

3.2.3 Changing temporal patterns of specific activity among compartments

We examined changes in the specific activity of underground organs to detect whether ramets at different locations in the rhizome system, and their root and rhizome components, differed significantly in sink strength.

Seasonal changes in overall specific activity levels within rhizome systems mirrored the patterns observed for total activity. Rhizome systems labeled later in the season had lower overall specific activities than those labeled earlier (Figure 5; Table 6, Label Date) and overall specific activity levels generally decreased through time (Table 6, May and June Label, Harvest). The old rhizomes of 1989 vegetative systems were a stronger sink for April-fixed assimilate than those of 1989 sexual systems; while the reverse pattern was observed for May-fixed assimilate (Figure 5; Table 6, Label Date * Type; Table 6, Type).

Significant differences in the specific activities of roots and rhizomes of individual ramets were detected in systems that were labeled in April or in May (rt/rhz: Table 7) but, for the most part, these differences were small (Figure 5) and were unaffected by 1989 shoot type (Table 7). However, for plants labeled in June, striking differences in the specific activity of roots and rhizome segments were found between the 1989 and 1990 ramets in both the September and March Harvests. The roots of the 1989 ramet labeled more heavily than the associated rhizome segment, while the opposite was true for the 1990 ramet (Figure 5, bottom panel).

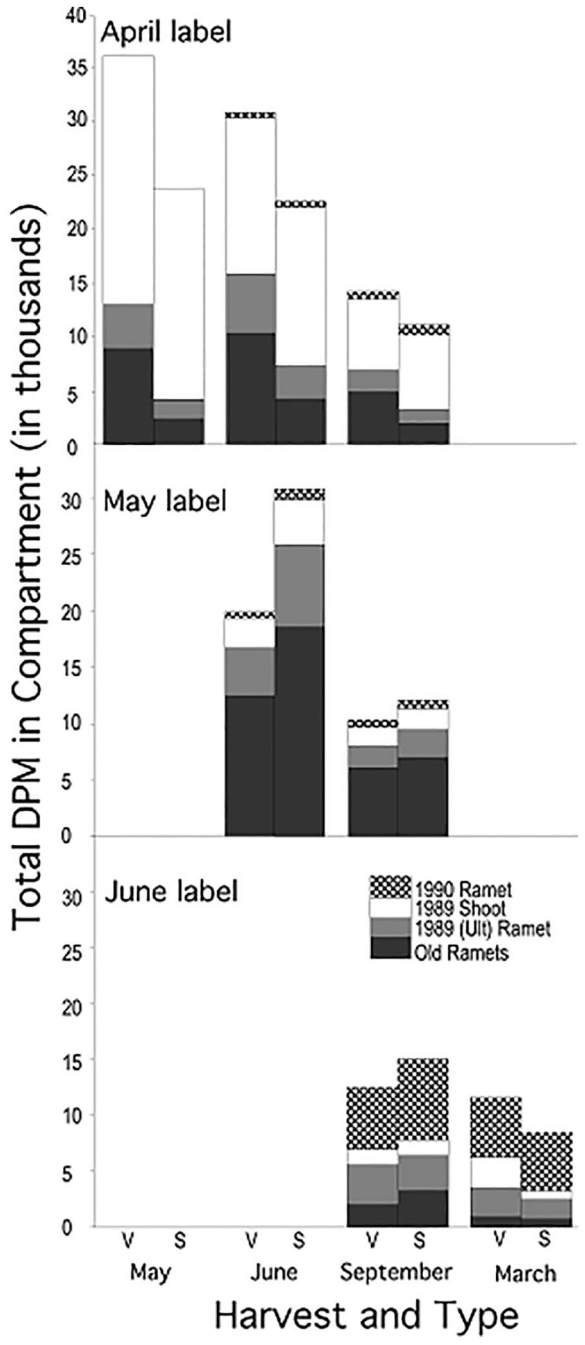

FIGURE 4
Distribution of mean total ^{14}C activity among the four compartments of mayapple: New (1990) ramet, 1989 shoot, Ult ramet and old rhizome compartment (see Figure 1). Data for each label date are presented in separate horizontal panels: top (April), middle (May), bottom (June). Within each label date each harvest is represented as paired bars representing plants that were vegetative (left bar) or sexual (right bar) in 1989.

3.2.4 Relationship between patterns of assimilate movement and biomass changes within rhizome systems

The ^{14}C data explain some aspects of biomass change better than others. The gain in biomass by the old rhizome compartment observed early in the season (Figure 2A) must result from the extensive movement of April- and May-fixed assimilate into the old rhizome (Figure 4). It is harder to explain the loss of biomass from the old rhizome later in the season (from June through September) and continuing at a reduced rate into the following year, in part because it is unclear what assimilate is being lost and how. Some of the carbon found in the old rhizome must be in a metabolizable form. Comparison of data from the short and long chase periods following the April and May labeling periods indicate that only a small, though statistically significant, amount of recently fixed assimilate could have been moved from the old rhizome into the new ramet over the course of the summer (Figure 4). We find that 79% of the May-fixed label remaining in the rhizome system in September is still found in the old rhizome (versus 84% in June), while only 6% of it is found in the new ramet (versus 3% in June), indicating that little remobilization from the old rhizome has occurred to build the new ramet. Further studies are needed before we can exclude the role of respiratory costs or mycorrhizae, as opposed to retranslocation, in the loss of old rhizome weight. These data suggest that the new ramet is constructed not only from current assimilate, as shown here, but by assimilate fixed in prior years. Longer-term studies are needed to address this question.

4 Discussion

By studying the dynamic patterns of carbon uptake and use within mayapple rhizome systems we have found apparent explanations for several demographic responses observed both by us in mayapple and in an array of other species. Our work demonstrates how the interaction between seasonal developmental phenology (i.e., the seasonal timing of meristem commitment), resource integration and storage both support and constrain plants' capacities to respond to environmental variation. Further, we reveal constraints on the seasonal use of stored assimilate that can affect the timing and pathway of developmental decisions and, hence, demographic expression.

4.1 Storage and development

Mayapple behaves like a classic stress tolerator, foregoing growth for storage (Grime, 2001; Chapin et al., 1993). Up to 70% of recently fixed assimilate is allocated to the old rhizome rather than the production of additional new ramets, a developmental alternative for which meristems are available (Jones and Watson, 2001). Storage of large quantities of recently fixed assimilate is a common feature of perennial plants of seasonal environments (Chapin et al., 1990; Suzuki and Hutchings, 1997; Lapointe, 1998, 2001; Suzuki and Stuefer, 1999; Hart et al., 2024), with storage rates of 34-67% (Danckwerts and Gordon, 1987; Jónsdóttir and Callaghan, 1989; Wyka, 1999; Price et al., 2002; Sanz-Pérez et al., 2009). Yet, given the severely light-limited environment of the deciduous forest where mayapple grows, the amount of assimilate

TABLE 4 Contrast MANOVA of the distribution of total activity by label date and 1989 Life history status (1989 Type, vegetative or sexual) for the June (left) and September (right) harvests.

	June Harvest				September Harvest			
Between Subjects [Sum]	**DF**	**Pillai's Trace**	**Exact F**	**Prob>F**	**DF**	**Pillai's Trace**	**Exact F**	**Prob>F**
Label Date	1, 29	0.0013	0.038	0.8471	2, 46	0.0161	0.377	0.688
1989 Type	1, 29	0.1452	4.926	**0.0344**	1, 46	0.1301	6.880	**0.0118**
Label Date * 1989 Type	1, 29	0.0681	2.119	0.1562	2, 46	0.1790	0.413	0.6636
Within Subjects [Contrast]	**DF**	**Pillai's Trace**	**Exact F**	**G-G P**	**DF**	**Pillai's Trace**	**Exact F**	**G-G P**
Compartment[1]	3, 27	0.7011	21.114	**<.0.0001**	3, 44	0.3660	8.464	**0.0011**
Compartment * Label Date	3, 27	0.5996	13.478	**0.001**	6, 90	0.9383	13.258	**<0.0001**
Compartment * 1989 Type	3, 27	0.1654	1.784	0.1928	3, 44	0.0845	1.354	0.2731
Compartment * Label Date * 1989 Type	3, 27	0.2925	3.720	(0.0643)	6, 86	0.2812	2.454	(0.0549)

[1]See Figure 1 – Vegetative for a description of the compartments.
Between-subject effects reflect differences in total activity among rhizome systems. Within-subjects effects reflect differences in the distribution of label among the four compartments (New (1990 Root/Rhizome segment), Ult, 1989 ramet), old rhizome) within rhizome systems[1]. Values in bold face are significant at $p < 0.05$; those in parentheses are of borderline significance. Within subject tests have been corrected for departures from sphereicity using the Greenhouse-Geisser correction.

stored is striking. Our study finds that in mayapple, storage is an active process, in that it occurs simultaneously with growth and, hence, does not represent the passive accumulation of luxury resources (*sensu* Chapin et al., 1990).

Chapin et al. (1990) suggest that storage is selected under conditions where there is strong asynchrony between supply and demand, where risk of damage is high and/or when rapid shifts in the amount or nature of productivity occur, such as in the transition from vegetative to reproductive growth. Based on these assertions we predicted that in mayapple early fixed assimilate, which comprises the largest assimilate pool (Basha, Griffith, Carlson and Watson, unpubl.; this study, Figure 4), would be used to fuel late season growth, particularly the differentiation and extension of the terminal rhizome segment that forms the new ramet. However, we found that in mayapple there is little within season reallocation of recently fixed assimilate. Most of the assimilate that is moved into storage remains there at least until the end of the current growing season (Figure 4), but is remobilized to support growth of the new ramet in the following year (Landa et al., 1992); a pattern consistent with the findings of Eisen et al. (2021) on the response of mayapple rhizome systems to rhizome severing. Thus, our findings of temporal constraints on the use of stored assimilate more accurately support another thesis of Chapin et al. (1990) that stored carbohydrate is not necessarily available for use at any given time.

Such physiological constraints on within season remobilization of recently fixed assimilate appear to be widespread. Several studies in which leaf area, fruit set, or leaf shading were manipulated infer that recently fixed assimilate is unavailable to support short-term compensatory responses but is available in the long term (Zimmerman and Whigham, 1992; Cunningham, 1997; Wyka, 1999; Ehrlén and van Groenendael, 2001; Tixier et al., 2017; Eisen et al., 2021). In related work of ours Landa et al. (1992) also found significant reallocation of stored assimilate among years. This pattern appears to act as buffer that holds rates of fruit maturation constant when leaf area is manipulated (Sohn and Policansky, 1977). Further, we find that storage reserves are rapidly replenished by assimilate fixed in the current year (Figures 4, 5 and Landa et al., 1992), requiring bi-directional transport, a phenomenon observed in a variety of taxa from an array of habitats (Tissue and Nobel, 1990a, b; Jónsdóttir et al., 1996; Alpert and Stuefer, 1997; Jónsdóttir and Watson, 1997; Tixier et al., 2017).

Time lags of a year or more are commonly observed in responses of plants with extensive storage to short-term environmental variation (Zimmerman and Whigham, 1992; Cunningham, 1997; Geber et al., 1997a, b; Watson and Lu, 1999; Worley and Harder, 1999; Ehrlén and van Groenendael, 2001; Rünk et al., 2021). The failure to remobilize recently fixed assimilate within a growing season provides one explanation for the presence of such lags.

In mayapple, developmental phenology – when meristems are committed to alternate demographic functions - also appears to play a crucial role (Watson et al., 1995, 1997; Geber et al., 1997b) such that development phenology and integrative physiology work in concert to influence plants' capacities to respond quickly to buffer environmental variation.

4.2 Preformation and storage

Plants that store significant amounts of assimilate also frequently exhibit preformation (Foerste, 1891; Randall, 1952; Lapointe, 2001; Schnablova et al., 2020; Rünk et al., 2021), a pattern of development in which organs are partially or completely determined one or more years before their expansion into mature structures (Watson et al., 1995, 1997; Diggle, 1997; Jones and Watson, 2001). The frequent co-occurrence of preformation with extensive storage suggests that the development and commitment of meristems to one or another structure is based upon the amount of assimilate stored, or being stored, at the time the developmental commitment is made. In this

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TABLE 5 Contrast MANOVAs of differences in the distribution of total activity by Harvest Date and 1989 Life history status (1989 type, vegetative or sexual).

	April Label				May Label				June Label			
Between-Subjects [Sum]	DF	Pillai's Trace	F	Prob>F	DF	Pillai's Trace	F	Prob>F	DF	Pillai's Trace	F	Prob>F
Harvest	2, 44	0.2495	7.314	**0.0018**	1, 30	0.2411	9.530	**0.0043**	1, 24	0.0403	1.010	0.325
1989 Type	1, 44	0.0232	1.043	0.312	1, 30	0.2410	9.530	**0.0043**	1, 24	0.1152	3.119	0.0901
Harvest * 1989 Type	2, 44	0.0009	0.021	0.9795	1, 30	0.0801	2.618	0.1161	1, 24	0.0113	0.275	0.605
Within-Subjects [Contrast]												
Compartment[1]	3, 42	0.768	46.356	**<0.0001**	3, 28	0.6466	17.080	**0.0020**	3, 22	0.4474	5.938	**0.0375**
Compartment * Harvest	6, 86	0.5357	5.244	**0.0102**	3, 28	0.3377	4.760	(0.0540)	3, 22	0.2838	2.907	0.1224
Compartment * 1989 Type	3, 42	0.3126	6.376	**0.0043**	3, 28	0.2986	3.974	(0.0742)	4, 19	0.2399	0.240	0.2666
Compartment * Harvest * 1989 Type	6, 86	0.0419	0.307	0.738	3, 28	0.1373	1.486	0.2507	4, 19	0.1914	0.191	0.3297

Analyses were performed separately for the April, May and June label dates. Each analysis is based on all available harvests of that label date. Between-subject effects reflect differences in total activity among rhizome systems. Within-subject effects test for differences in the distribution of activity among the four compartments within rhizome systems[1]. Values in bold face are significant at $p < 0.05$; those in parentheses are of borderline significance. Within subject tests have been corrected for departures from sphereicity using the Greenhouse-Geisser correction.

[1]See Figure 1A for a description of the compartments.

way, the plant could be assured of having sufficient resources to mature a particular structure, irrespective of subsequent environmental conditions. We see evidence of such a mechanism in mayapple, in which the new ramet is initiated two years (year X-2) before it emerges above ground (in year X) and one year before its rhizome elongates and the aerial shoot becomes irreversibly determined (in year X-1) (Watson et al., 1997). The elongation rate of the developing ramet in year X-1 is strongly correlated with the likelihood of that ramet differentiating a sexual shoot; ramets that elongate faster tend to be larger and are more likely to be sexual (Geber et al., 1997a). Interestingly, expanding ramets have a proclivity to grow faster or slower from the very onset of their growth in year X-1, suggesting that conditions, perhaps resource conditions at the time the bud is initiated (in year X-2), establish its growth trajectory in year X-1. Our data are consistent with the hypothesis that new ramet growth is supported by assimilate fixed in prior years (Eisen et al., 2021), given that assimilate fixed early in year X-1 (i.e., 1989) is not extensively used in the early stages of new ramet construction (Landa et al., 1992). Such a developmental program would match the pattern of meristem commitment with known levels of carbon resource availability, but at a cost, the loss of the capacity to respond quickly to changed environmental conditions (Watson et al., 1997; Worley and Harder, 1999; Werger and Huber, 2006). It is a pessimistic rather than an optimistic strategy (*sensu* Jones, 1992).

4.3 The role of current and future demographic status on carbon distribution and growth

Sexual and vegetative rhizome systems of mayapple differ in how they distribute assimilate, even in the absence of fruit initiation and maturation (Figure 4, April label). These differences could simply be caused by differences in the seasonal timing of developmental phenology, as reported between male and female plants of other species (e.g., Putwain and Harper, 1972; Watson, 1995; Laporte and Delph, 1996). But, in mayapple, differences in seasonal phenology between sexual and vegetative plants early in the season tend to be small (ca. 1-2 days) and are probably of insufficient magnitude to explain the differences between them in ^{14}C-transport pattern. Phenological differences do increase later in the season - sexual shoots with fruit senesce on average one month later than vegetative ones (Watson and Lu, 1999, 2004), but because we lacked fruit-bearing sexuals in this study we could not assess how this difference affected carbon transport.

It also is possible that sexual shoots retain more assimilate in the leaf and stem in preparation for the possibility that a resource demanding fruit will be set; a form of short-term storage that has been observed by others (Chapin et al., 1990; Lapointe, 1998). If a sexual shoot fails to set fruit the unused assimilate could be re-allocated to other plant functions before the leaves senesce. In mayapple, sexual shoots that fail to mature fruit effectively become two-leafed vegetative shoots and they do give rise to disproportionately long new rhizomes (Sohn and Policansky, 1977), an observation consistent with this hypothesis. However,

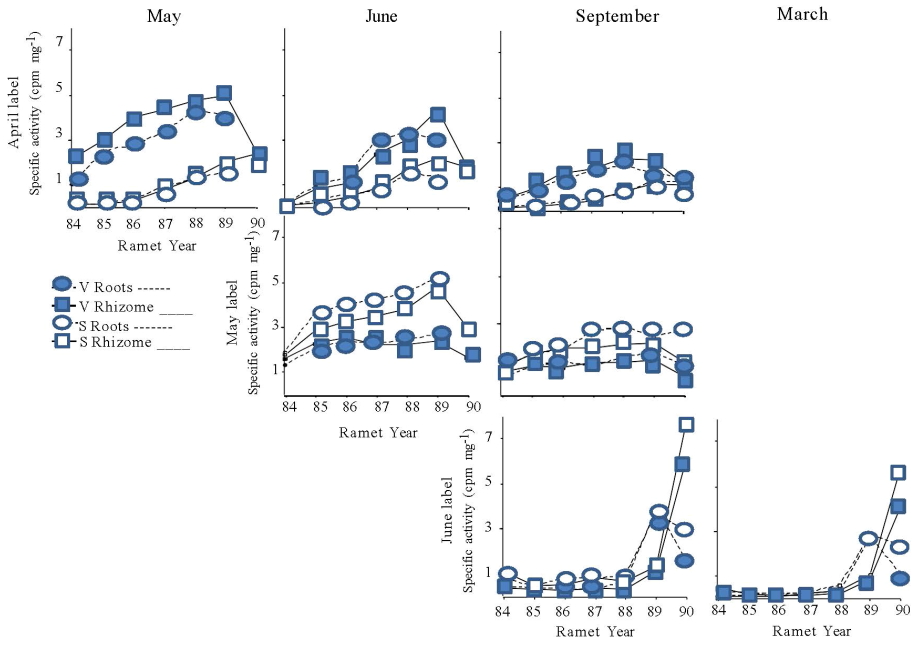

FIGURE 5
Specific activity of the below ground structures (roots = ○; rhizomes = □) of the ramets of mayapple in systems terminated by either a sexual (open symbols) or a vegetative shoot (closed symbols) in 1989. Rhizome systems were labeled at one of three times during the 1989 growing season: April (top panel), May (middle panel), or June (bottom panel), and then harvested either in May (far left), June (second from left) or September 1989 (second from right), or March 1990 (far right). The numbers on the X-axis identify the individual ramets within the rhizome system and correspond to the year in which each ramet bore an aerial shoot (Figure 1).

in mayapple, we do not see significant withdrawal of labeled assimilate from the senescing leaves of failed sexual shoots. Moreover, these failed sexual shoots senesce their leaves sooner than those that mature fruit (Watson and Lu, 1999, 2004), shortening their period of net positive photosynthesis and, resulting in the permanent loss of this locally stored assimilate when the leaf is shed, a potentially substantial and rarely considered cost to even unsuccessful reproduction. Despite loss of assimilate via senescence, mayapple sexual systems that fail to form fruit tend to be more robust in subsequent years than vegetative or successful sexual systems, perhaps owing to their greater leaf area alone. Not only do they produce larger new rhizome segments, they branch more frequently, and give rise to additional new sexual *vs* vegetative ramets (Sohn and Policansky, 1977; Geber et al., 1997a). Seemingly, the larger leaf area of the sexual shoot and its greater longevity (Watson, 1990; Watson and Lu, 1999, 2004) permits more assimilate to be fixed, thereby compensating for the higher loss of carbon by the senescing sexual leaf.

Interestingly, we found no significant differences in the patterns of distribution (Figure 4) and sink strength (Figure 5) of recently fixed assimilate between those systems that will become vegetative or sexual, even though larger new ramets tend to experience very different demographic fates (Sohn and Policansky, 1977; Geber et al., 1997a, b). These observations further support the idea that the performance of the new expanding ramet is based on the resources acquired in prior years and is consistent with the hypothesis that the preformation of structures influences the pathway of development based on the quantity of resources accumulated at the time of meristem determination rather than that of outgrowth.

4.4 Changing seasonal environments in the Anthropocene

Several issues remain unclear about controls on development and physiological integration in long-lived plants, of resource-poor environments, that may significantly affect their ability to rapidly respond to changing environmental cues in a warming world. In mayapple, for instance, we have a poor understanding of what triggers seasonal aerial growth. Observational data over many years indicates a one-week interval of emergence that is insensitive to temperatures experienced by plants during the preceding month (Watson, unpubl.), suggesting to us photoperiodic control. In contrast, the expansion rate of new rhizome segments *is* sensitive to environmental temperatures and thus can result in effects on flowering time (Watson, pers. obs.). Mayapple fertilization is dependent on outcrossing by insects, and rainy or cold conditions during flowering leads to reduced fruit set; typically less than 10% of sexual shoots mature fruit (Geber et al., 1997a). Thus, temperature and rainfall effects on phenology of either mayapple or its pollinators could have serious demographic consequences involving fruit set and, hence, the genetic diversity of local populations.

TABLE 6 Compound MANOVA of specific activity distribution within rhizome systems in the June and September harvests as a function of Label Date and 1989 Life history status (1989 Type, vegetative or sexual).

Between Subject [Sum]	June Harvest			September Harvest		
	DF	Exact F	Prob>F	DF	Exact F	Prob>F
Label Date	1, 29	8.4647	**0.0069**	2, 46	2.592	(0.0858)
1989 Type	1, 29	0.1994	0.6585	1, 46	0.071	0.7908
Label Date * 1989 Type	1, 29	6.0451	**0.0202**	2, 46	2.783	(0.0723)
Within subject [Compound]	**DF**	**Pillai's trace**	**F**	**G-G P**	**DF**	**Pillai's trace**	**F**	**G-G P**
Rt/Rhz	1,29	0.146	4.968	**0.0337**	1,46	0.018	0.855	0.3600
Rt/Rhz * Label Date	1,29	0.053	1.614	0.2141	2,46	0.188	5.313	**0.0084**
Rt/Rhz * 1989 Type	1,29	0.009	0.269	0.6081	1,46	0.004	0.162	0.6895
Rt/Rhz * Label Date * 1989 Type	1,29	0.071	2.213	0.1476	2,46	0.016	0.369	0.6937
Ramet	6,24	0.738	11.29	**0.0027**	6,41	0.618	11.063	**0.0013**
Ramet * Label Date	6,24	0.575	5.422	**0.0254**	12,84	0.758	4.276	**0.0080**
Ramet * 1989 Type	6,24	0.124	0.568	0.5842	6,41	0.082	0.610	0.5571
Ramet * Label Date* 1989 Type	6,24	0.330	1.969	0.1902	12,84	0.284	1.159	0.3501
Rt/Rhz * Ramet	6,24	0.684	8.655	**0.0055**	6,41	0.340	3.514	(0.0876)
Rt/Rhz * Ramet * Label Date	6,24	0.317	1.860	0.2015	12,84	0.809	4.759	**0.0096**
Rt/Rhz * Ramet * 1989 Type	6,24	0.119	0.540	0.5975	6,41	0.087	0.651	0.4368
Rt/Rhz * Ramet * Label Date* 1989 Type	6,24	0.143	0.665	0.5336	12,84	0.329	1.378	0.2734

[1]See Figure 1 for description of compartments.

The between-subject analysis examines differences among rhizome systems based on a sum MANOVA design. Within-subject tests are a compound MANOVA with two levels of dependent variables (Root and Rhizome) for each of seven ramets.[1] Within-subject effects test differences between roots and rhizomes (Rt/Rhz) within ramets, or differences between roots and rhizomes in the distribution among ramets (Rt/Rhz * Ramet). Values in bold face are significant at $p < 0.05$; those in parentheses are of borderline significance. Within subject tests have been corrected for departures from sphereicity using the Greenhouse-Geisser correction.

More closely aligned with the study reported here, we do not know what physiological mechanisms lead to movement of large amounts of assimilate into the old rhizome, nor do we understand what regulates the timing of this movement. Movement of resources into storage organs is generally thought to be controlled by sink-driven processes (Geiger, 1987) but, in mayapple, we have found that the largest concentration of newly fixed assimilate is in the oldest rhizome segment (Landa et al., 1992), (although this was less evident in the study reported here), and that segment is excised during the growing season and the assimilate it contains presumably lost. Arbuscular mycorrhizae also may play a role in creating sinks in mayapple rhizomes, but do not explain the oft-seen accumulation of label in the oldest rhizome segment, because mycorrhizae are found at highest concentrations at nodes closer to the younger end of the rhizome system (nodes X-4 to X-5) (Watson et al., 2001).

5 Concluding comments

Mayapple growth and development is characterized both by extensive storage and complete preformation of new structures. The co-occurrence of these two syndromes, one developmental and the other physiological, in stress tolerant organisms (Grime, 2001; Chapin et al., 1993) of strongly seasonal environments (Diggle, 1997; Watson et al., 1997) suggest that they act in concert to regulate the relative production of structures differing in demographic function and in long and short-term costs, based

TABLE 7 Compound MANOVA of specific activity distribution within the rhizome system for April, May and June label dates as a function of harvest date and 1989 Life history status (1989 Type, vegetative or sexual).

	April Label				May Label				June Label			
Between-Subject [Sum]	DF	Exact F	Prob>F		DF	Exact F	Prob>F		DF	Exact F	Prob>F	
Harvest	2, 44	2.612	0.0847		1, 30	6.221	**0.0184**		1, 30	5.982	**0.0205**	
1989 Type	1, 44	12.393	**0.0010**		1, 30	4.375	**0.0450**		1, 30	1.344	0.2555	
Harvest * 1989 Type	2, 44	1.687	0.1969		1, 30	1.021	0.3203		1, 30	0.309	0.5824	
Within Subject [Compound]	DF	Pillai's tr.	F	G-G P	DF	Pillai's tr.	F	G-G P	DF	Pillai's tr.	F	G-G P
Rt/Rhz	1,44	0.327	21.37	**<0.0001**	1,30	0.020	0.60	0.4439	1,30	0.201	7.54	**0.0101**
Rt/Rhz * Harvest	2,44	0.221	6.23	**0.0041**	1,30	0.131	4.51	**0.0421**	1,30	0.028	0.85	0.3642
Rt/Rhz * 1989 Type	1,44	0.044	2.01	0.1624	1,30	0.083	2.70	0.1107	1,30	0.003	0.09	0.7699
Rt/Rhz * Harvest * 1989 Type	2,44	0.098	2.40	0.1025	1,30	0.044	1.38	0.2487	1,30	0.002	0.06	0.8017
Ramet	6,39	0.699	15.05	**0.0003**	6,25	0.688	9.19	**0.0020**	6,25	0.729	11.20	**0.0155**
Ramet * Harvest	12,80	0.400	1.66	0.1838	6,25	0.486	3.93	**0.0363**	6,25	0.166	0.83	0.3976
Ramet * 1989 Type	6,39	0.371	3.83	**0.0452**	6,25	0.263	1.49	0.2676	6,25	0.212	1.12	0.3311
Ramet * Harvest * 1989 Type	12,80	0.373	1.53	0.2186	6,25	0.367	2.41	0.1175	6,25	0.163	0.81	0.4016
Rt/Rhz * Ramet	6,39	0.584	9.11	**0.0026**	6,25	0.439	3.27	0.0532	6,25	0.673	8.56	**0.0328**
Rt/Rhz * Ramet * Harvest	12,80	0.534	2.42	(0.0686)	6,25	0.620	6.80	**0.0047**	6,25	0.190	0.98	0.3689
Rt/Rhz * Ramet * 1989 Type	6,39	0.206	1.68	0.2192	6,25	0.161	0.80	0.5135	6,25	0.075	0.34	0.5860
Rt/Rhz * Ramet * Harv. * 1989 Type	12,80	0.197	0.72	0.5791	6,25	0.216	1.15	0.3635	6,25	0.153	0.75	0.4259

Between-Subject terms test overall differences among plants. See the caption of Table 6 for explanation of abbreviations and terms. Values in bold face are significant at $p < 0.05$; those in parentheses are of borderline significance. Within subject tests have been corrected for departures from sphericity using the Greenhouse-Geisser correction.

on estimates of resources on hand at the time the developmental commitment is made which, as in mayapple, is years earlier. These syndromes can be thought of as evolved solutions to problems posed by the physical and biological environments in which they are found (Watson et al., 1997; Watson, 2008). In mayapple, the interactions between developmental program and resource integration reflect a conservative growth strategy that is consistent with the constraints imposed by the environment in which they evolved; this strategy conserves resources over time but precludes rapid response to short term environment variation. Without knowing more about what factors govern the initiation of various developmental and physiological process – daylength, temperature, rainfall, symbionts – and how these may be affected by a warming habitat (e.g., changes in the timing of anthesis *vis-à-vis* the availability of pollinators; changes in the mycorrhizal symbiosis with changes in the intensities and patterns of rainfall), it remains difficult to assess the capacity of these tightly bound processes to continue to successfully interact and remain functional. For plants like mayapple, whose migration capacity is limited, the answers will be crucial to their persistence (Whigham, 2004).

Data availability statement

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

Author contributions

MW: Conceptualization, Funding acquisition, Methodology, Project administration, Writing – original draft, Writing – review & editing. TV: Investigation, Writing – review & editing.

Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article. The work was supported by a grant to MW by the National Science Foundation, funding to TV by the Academy of Finland, and support from the Department of Biology, the College of Arts and Sciences and the University Graduate School of Indiana University to MW. Support for open access publication charges provided by IU libraries.

Acknowledgments

We thank Barbara Benner who was involved in the initial planning of this experiment, Janna Gartner who helped work out the protocol, and Polley McClure and John Smith who gave us the unrestricted use of their property. This study would not have been possible without Henry Briggs, Indiana University Radiation Control Officer, and David Parkhurst, both of whom helped in important ways during the NRC permitting process. Keith Landa managed the lab and he and Doug Karpa-Wilson ran the statistical analyses. Thanks also are due to the many work-study students who ground and oxidized voluminous quantities of material. The manuscript benefited greatly from comments by Monica Geber, Cynthia Jones, Hans de Kroon, Dennis Whigham, Ying Lu and reviewers. Omer Falik assisted in preparing the figures.

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.

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References

Alpert, P., and Stuefer, J. F. (1997). ""Division of labor in clonal plants,"," in *The Ecology and Evolution of Clonal Plants*. Eds. H. de Kroon and J. M. van Groenendael (Backhuys Academic Publishers, Leiden, Netherlands), 137–154.

Ashmun, T.-L. (1994). A dynamic perspective on physiological cost of reproduction in plants. *Am. Nat.* 144, 300–316. doi: 10.1086/285676

Babst, B. A., Ferrieri, R. A., Thorpe, M. R., and Orians, C. M. (2008). *Lymantria dispar* herbivory induces rapid changes in carbon transport and partitioning in *Populus nigra*. *Entomol. Exp. Appl.* 128, 117–125. doi: 10.1111/j.1570-7458.2008.00698.x

Benner, B. L., and Watson, M. A. (1989). Developmental ecology of mayapple: seasonal patterns of resource distribution in sexual and vegetative rhizome systems. *Funct. Ecol.* 3, 539–547. doi: 10.2307/2389568

Bess, J. (2005). *Conservation Assessment for the mayapple borer moth (*Papaipema rutila *(Guenee))* (Eastern Region: USDA Forest Service).

Chapin, F. S. III, Autumn, K., and Pugnaire, F. (1993). Evolution of suites of traits in response to environmental stress. *Am. Nat.* 142, S78–S92. doi: 10.1086/285524

Chapin, F. S. III, Schulze, E.-D., and Mooney, H. A. (1990). The ecology and economics of storage in plants. *Annu. Rev. Ecol. Syst.* 21, 423–447. doi: 10.1146/annurev.es.21.110190.002231

Corlett, R. T. (2015). The Anthropocene concept in ecology and conservation. *Trends Ecol. Evol.* 30, 36–41. doi: 10.1016/j.tree.2014.10.007

Crants, J. E. (2008). Pollination and pollen limitation in mayapple (*Podophyllum peltatum* L.), a nectarless spring ephemeral. Ann Arbor: University of Michigan. Available at: https://deepblue.lib.umich.edu/bitstream/handle/2027.42/60727/jcrants_1.pdf?sequence=1.

Cunningham, S. A. (1997). The effect of light environment, leaf area, and stored carbohydrates on inflorescence production by a rainforest understory palm. *Oecologia* 111, 36–44. doi: 10.1007/s004420050205

Danckwerts, J. E., and Gordon, A. J. (1987). Long-term partitioning, storage and re-mobilization of ^{14}C assimilated by *Lolium perenne* (cv. Melle). *Ann. Bot.* 59, 55–66. doi: 10.1093/oxfordjournals.aob.a087285

de Kroon, H., Whigham, D. F., and Watson, M. A. (1991). Effects of rhizome severing, fertilization and timing of shoot senescence on clonal growth in mayapple. *Funct. Ecol.* 5, 360–368. doi: 10.2307/2389807

Diggle, P. K. (1994). The expression of andromonoecy in *Solanum hirtum (Solanaceae)*: phenotypic plasticity and ontogenetic contingency. *Am. J. Bot.* 81, 1354–1365. doi: 10.1002/j.1537-2197.1994.tb11457.x

Diggle, P. K. (1997). Extreme preformation in alpine *Polygonum viviparum*, an architectural and developmental analysis. *Am. J. Bot.* 84, 154–169. doi: 10.2307/2446077

Dodd, R. S., and Douhovnikoff, V. (2016). Adjusting to global change through clonal growth and epigenetic variation. *Front. Ecol. Evol.* 26. doi: 10.3389/fevo.2016.00086

Ehrlén, J., and van Groenendael, J. M. (2001). Storage and delayed costs of reproduction in the understory perennial *Lathyrus vernus*. *J. Ecol.* 8, 237–246. doi: 10.1046/j.1365-2745.2001.00546x

Eisen, K. E., Siegmund, G.-F., Watson, M. A., and Geber, M. A. (2021). Variation in the location and timing of experimental severing demonstrates that the persistent rhizome serves multiple functions in a clonal forest understory herb. *Funct. Ecol.* 36, 356–367. doi: 10.1111/1365-2435.13970

Flanagan, L. B., and Moser, W. (1985). Pattern of ^{14}C assimilate distribution in a clonal herb, *Aralia nudicaulis*. *Can. J. Bot.* 63, 2111–2114. doi: 10.1139/b85-297

Foerste, A. F. (1884). The may apple. *Bull. Torrey Bot. Club* 11, 62–64. doi: 10.2307/2477021

Foerste, A. F. (1891). On the formation of flower buds in spring-blossoming plants during the preceding summer. *Bull. Torrey Bot. Club* 18, 101–106. doi: 10.2307/2476568

Geber, M. A., de Kroon, H., and Watson, M. A. (1997a). Organ preformation in Mayapple as a mechanism for historical effects on demography. *J. Ecol.* 85, 211–223. doi: 10.2307/2960652

Geber, M. A., Watson, M. A., and de Kroon, H. (1997b). ""Development and resource allocation in perennial plants: The significance of organ preformation"," in *Plant Resource Allocation*. Eds. F. A. Bazzaz and J. Grace (San Diego: Academic Press), 115–141. doi: 10.1016/B978-012083490-7/50006-2

Geiger, D. R. (1987). Understanding source and sink regions of plants. *Plant Physiol. Biochem.* 25, 659–666.

Gleason, H. A., and Cronquist, A. (1963). *Manual of the vascular plants of Northeastern United States and adjacent Canada* (New York: D. Van Norstrand Co), 810.

Goldberg, D. E., Batzer, E. E., Elgersma, K. J., Martina, J., and Klimešová, J. (2020). Allocation to clonal growth: critical questions and protocols to answer them. *Perspect. Plant Ecol. Ecol. Syst.* 43, 125511. doi: 10.1016/j.ppees.2020.125511

Griffith, T. M., and Watson, M. A. (2006). Is evolution necessary for range expansion? Manipulating reproductive timing of a weedy annual transplanted beyond its range. *Am. Nat.* 167, 153–164. doi: 10.1086/498945

Grime, J. P. (2001). *Plant Strategies, Vegetation Processes, and Ecosystem Properties* (Chichester: Wiley). pp. 417.

Hart, A. T., Landhäusser, S. M., and Wiley, E. (2024). Tracing carbon and nitrogen reserve mobilization during spring leaf flush and growth following defoliation. *Tree Physiol.* 2024, tpae015. doi: 10.1093/treephys/tpae015

Huber, H., Lukács, S., and Watson, M. A. (1999). Spatial structure of stoloniferous plants: an interplay between structural blue-print, ontogeny and plasticity. *Plant Ecol.* 141, 107–115. doi: 10.1023/A:1009861521047

Huber, H., Whigham, D. F., and O'Neill, J. (2004). Timing of disturbance changes the balance between growth and survival of parent and offspring ramets in the clonal forest understory herb *Uvularia perfoliata*. *Evol. Ecol.* 18, 521–539. doi: 10.1007/s10682-004-5142-8

Jones, H. G. (1992). *Plants and Microclimate* (Cambridge, UK: Cambridge University Press).

Jones, C. S., and Watson, M. A. (2001). Heteroblastic shoot development in mayapple (*Podophyllum peltatum*, Berberidaceae), a rhizomatous forest herb with limited options. *Am. J. Bot.* 88, 1340–1358. doi: 10.2307/3558442

Jónsdóttir, I. S., and Callaghan, T. V. (1989). Localized defoliation stress and movement of ^{14}C-photoassimilates between tillers of *Carex bigellowii*. *Oikos* 54, 211–219. doi: 10.2307/3565269

Jónsdóttir, I. S., Callaghan, T. V., and Headley, A. D. (1996). Resource dynamics within arctic clonal plants. *Ecol. Bull.* 45, 53–64.

Jónsdóttir, I. S., and Watson, M. A. (1997). ""Ecological significance of physiological integration in plants,"," in *The Ecology and Evolution of Clonal Plants*. Eds. H. de Kroon and J. M. van Groenendael (Backhuys Academic Publishers, Leiden), 109–136.

Kudoh, A., Megonigel, P., Langley, J. A., and Whigham, D. (2023). Increased shoot density and global change drivers in a widespread clonal wetland species, Schoenoplectus americanus. *Estuaries Coasts* 47, 176–188. doi: 10.1007/s12237-023-01249-z

Landa, K., Benner, B., Watson, M. A., and Gartner, J. (1992). Physiological integration for carbon in mayapple (*Podophyllum peltatum)*, a clonal perennial herb. *Oikos* 63, 348–356. doi: 10.2307/3544960

Lapointe, L. (1998). Fruit development in *Trillium*. Dependence on stem carbohydrate reserves. *Plant Physiol.* 117, 183–188. doi: 10.1104/pp.117.1.183

Lapointe, L. (2001). How phenology influences physiology in deciduous forest spring ephemerals. *Physiologia Plantarum* 113, 151–157. doi: 10.1034/j.1399-3054.2001.1130201.x

Laporte, M. M., and Delph, L. F. (1996). Sex-specific physiology and source-sink relations in the dioecious plant *Silene latifolia*. *Oecologia* 106, 63–72. doi: 10.1007/BF00334408

Laverty, T. M., and Plowright, R. C. (1988). Fruit and seed set in Mayapple (*Podophyllum peltatum*): influence of intraspecific factors and local enhancement near *Pedicularis canadensis*. *Can. J. Bot.* 66, 173–178. doi: 10.1139/b88-027

Lehman, L. E. (1977). *Population ecology of the Raccoon on the Jasper-Pulaski wildlife study area. Pittman-Robertson Bull. No. 9* (Indianapolis: Division of Fish and Wildlife, Indiana Dept. of Nat. Res.), 97.

Lubbe, F., Klimeš, A., Dolezal, J., Jandová, V., Mudrak, O., Janecek, S., et al. (2021). Carbohydrate storage in herbs: The forgotten functional dimension of the plant economic spectrum. *Ann. Bot.* 127, 813–825. doi: 10.1093/aob/mcab014

MaChado, R. A. R., Ferrieri, A. P., Robert, C. A. M., Glauser, G., Kallenbach, M., Baldwin, I. T., et al. (2013). Leaf-herbivore attack reduces carbon reserves and regrowth from the roots via jasmonate and auxin signaling. *New Phytol.* 200, 1234–1246. doi: 10.1111/nph.12438

Marshall, C. (1990). "Source-sink relations of interconnected ramets," in *Clonal Growth in Plants: Regulation and Function*. Eds. J. M. van Groenendael and H. de Kroon (SPB Academic Publishing, The Hague), 23–42.

Meloche, C. G., and Diggle, P. K. (2003). The pattern of carbon allocation supporting growth of preformed shoot primordia in *Acomastylis rossii* (Rosaceae). *Am. J. Bot.* 90, 1313–1320. doi: 10.3732/ajb.90.9.1313

Niederhauser, E. C. (2015). Seed Dispersal of the Forest Herb Podophyllum peltatum by *Multiple Vectors*. Athens: Ohio University. Available at: http://rave.ohiolink.edu/etdc/view?acc_num=ohiou1429701798.

Ott, J., Klimešová, J., and Hartnett, D. (2019). The ecology and significance of below-ground bud banks. *Ann. Bot.* 123, 1099–1118. doi: 10.1093/aob/mcz051

Pitelka, L. F., and Ashmun, J. W. (1985). ""Physiology and integration of ramets in clonal plants,"," in *Population Biology and Evolution of Clonal Organisms*. Eds. J. B. C. Jackson, L. W. Buss and R. E. Cook (Yale University Press, New Haven,CT), 399–435.

Potvin, C., Lechowicz, M. J., and Tardif., S. (1990). The statistical analysis of ecophysiological response curves obtained from experiments involving repeated measures. *Ecol.* 71, 1389–1400. doi: 10.2307/1938276

Price, E. A. C., Gamble, R., Williams, G. G., and Marshall, C. (2002). ""Seasonal patterns of partitioning and remobilization of 14C in the invasive rhizomatous perennial Japanese knotweed (Fallopia japonica (Houtt.) Ronse Decraene)"," in *Ecology and Evolutionary Biology of Clonal Plants*. Eds. J.F. Stuefer, B. Erschbamer, H. Huber and J. Suzuki (Dordrecht: Springer). 347–362. doi: 10.1023/A:1016036916017

Putwain, P. D., and Harper, J. L. (1972). Studies in the dynamics of populations. V. Mechanisms governing the sex ratio in *Rumex acetosa* and *R. acetosella*. *J. Ecol.* 60, 113–129. doi: 10.2307/2258045

Randall, W. J. (1952). Interrelations of autecological characteristics of forest herbs. University of Wisconsin, Madison (WI).

Rashid, K., Rashid, S., Islam, T., Ganie, A. H., Nawchoo, I. A., and Khuroo, A. A. (2023). Hidden from the harsh: Belowground preformation prior to winter determines life history strategy of a temperate perennial herb. *Flora* 308, 152387. doi: 10.1016/j.flora.2023.152387

Rünk, K., Zobel, K., and Liira, J. (2021). Long-term growth of three sympatric *Dryopteris* fern speccies shows the accumulation of climatic effects over 2 years because of organ preformation. *Climatic Change* 164, 16. doi: 10.1007/s10584-021-02967-8

Rust, R. W., and Roth, R. R. (1981). Seed production and seedling establishment in the mayapple, *Posophyllum peltatum* L. *Am. Midl. Natur.* 105, 51–60. doi: 10.2307/2425009

Sachs, T., and Novoplansky, N. (1997). "What does aclonal organization suggest concerning clonal plants)?," in *The Ecology and Evolution of Clonal Plants*. Eds. H. de Kroon and J. M. van Groenendael (Backhuys Academic Publishers, Leiden, The Netherlands), 55–77.

Sanz-Pérez, V., Castro-Díez, P., and Joffre, R. (2009). Seasonal carbon storage and growth in Mediterranean tree seedlings under different water conditions. *Tree Physiol.* 29, 1105–1116. doi: 10.1093/treephys/tpp045

SAS (2000). *SAS/STAT Users Guide* (Cary NC: SAS Insitute Inc).

Scheiner, S. M., and Gurevitch, J. (2000). *Design and Analysis of Ecological Experiments* (New York: Oxford University Press). doi: 10.1093.oso/9780195131871.001.0001

Schnablova, R., Huang, L., Klimešová, J., Smarda, P., and Herben, T. (2020). Inflorescence preformation prior to winter: a surprisingly widespread strategy that drives phenology of temperate perennial plants. *New Phytol.* 229, 620–630. doi: 10.1111/nph.16880

Sohn, J. J., and Policansky, D. (1977). The costs of reproduction in the mayapple *Podophyllum peltatum* (Berberidaceae). *Ecol.* 58, 1366–1374. doi: 10.2307/1935088

Stuefer, J. F., and Huber, H. (1999). The role of stolon internodes for ramet survival after clone fragmentation in *Potentilla anserina*. *Ecol. Let.* 2, 135–139. doi: 10.1046/j.1461-0248.1999.00066.x

Suzuki, J., and Hutchings, M. J. (1997). ""Interactions between shoots in clonal plants and the effects of stored resources on the structure of shoot populations."," in *The Ecology and Evolution of Clonal Plants*. Eds. H. de Kroon and J. M. van Groenendael (Backhuys Academic Publishers, Leiden, The Netherlands), 311–329.

Suzuki, J., and Stuefer, J. F. (1999). On the ecological and evolutionary significance of storage in clonal plants. *Plant Species Biol.* 14, 11–17. doi: 10.1046/j.1442-1984.1999.00002.x

Tissue, D. T., and Nobel, P. S. (1990a). Carbon relations of flowering in a semelparous clonal desert annual. *Ecol.* 71, 272–281. doi: 10.2307/1940266

Tissue, D. T., and Nobel, P. S. (1990b). Carbon translocation between parents and ramets of a desert perennial. *Ann. Bot.* 66, 551–557. doi: 10.1093/oxfordjournals.aob.a088064

Tissue, D. T., Skillman, J. B., McDonald, E. P., and Strain, B. R. (1995). Photosynthesis and carbon allocation in *Tipularia discolor* (Orchidaceae), a winter evergreen understory herb. *Am. J. Bot.* 82, 1249–1256. doi: 10.1002/j.1537-2197.1995.tb12658.x

Tixier, A., Sperling, O., Orozco, J., Lampinen, B., Roxas, A. A., Saa, S., et al. (2017). Spring bud growth depends on sugar delivery by xylem and water recirculation by phloem Münch flow in *Juglans regia*. *Planta* 245, 495–508. doi: 10.1007/s00425-017-2707-7

Watson, M. A. (1984). Developmental constraints: Effect on population growth and patterns of resource allocation in a clonal plant. *Am. Nat.* 123, 411–426. doi: 10.1086/284212

Watson, M. A. (1990). "Phenological effects on clone development and demography," in *Clonal Growth in Plants: Regulation and Function*. Eds. J. M. van Groenendael and H. de Kroon (SPB Academic Publishing, The Hague), 43–55.

Watson, M. A. (1995). Sexual differences in plant developmental phenology affect plant-herbivore interactions. *Trends Ecol. Evol.* 10, 180–182. doi: 10.1016/S0169-5347(00)89046-1

Watson, M. A. (2008). Resource storage and the expression of clonal plant life histories. *Evol. Ecol.* 22, 471–475. doi: 10.1007/s10682-007-9226-0

Watson, M. A., Geber, M. A., and Jones, C. S. (1995). Ontogenetic contingency and the expression of plant plasticity. *Trends Ecol. Evol.* 10, 474–475. doi: 10.1016/S0169-5347(00)89191-0

Watson, M. A., Hay, M. J. M., and Newton, P. C. D. (1997). ""Developmental phenology and the timing of determination of shoot bud fates: ways in which the developmental program modulates fitness in clonal plants."," in *The Ecology and Evolution of Clonal Plants*. Eds. H. de Kroon and J. M. van Groenendael (Backhuys Academic Publishers, Leiden: The Netherlands), 31–53.

Watson, M. A., and Lu, Y. (1999). Timing of shoot senescence and demographic expression in the clonal perennial *Podophyllum peltatum* (Berberidaceae). *Oikos* 86, 67–78. doi: 10.2307/3546570

Watson, M. A., and Lu, Y. (2004). "Factors regulating senescence in the annual shoots of perennial plants." in *Cell Death in Plants*. Ed. L. D. Nooden (San Diego: Academic Press), 259–269. doi: 10.2307/3546570

Watson, M. A., Scott, K., Griffith, J., Dieter, S., Jones, C. S., and Nanda, S. (2001). The developmental ecology of mycorrhizal associations in mayapple, *Podophyllum peltatum*, Berberidaceae. *Evol. Ecol.* 15, 425–442. doi: 10.1023/A:1016097217834

Weinig, C., and Delph, L. F. (2001). Phenotypic plasticity early in life constrains developmental responses later. *Evol.* 55, 930–936. doi: 10.1554/0014-3820(2001)055[0930:PPEILC]2.0.CO;2

Werger, M. J. A., and Huber, H. (2006). Tuber size variation and organ preformation constrain growth responses of a spring geophyte. *Oecologia* 147, 396–405. doi: 10.1007/s00442-005-0280-4

Whigham, D. (2004). Ecology of woodland herbs in temperate deciduous forests. *Annu. Rev. Ecol. Evol. Syst.* 35, 583–621. doi: 10.1146/annurev.ecolsys.35.021103.105708

Wijesinghe, D., and Whigham, D. F. (1997). Costs of producing clonal offspring and the effects of plant size on population dynamics of the woodland herb *Uvularia perfoliata* (Liliaceae). *J. Ecol.* 85, 907–919. doi: 10.2307/2960611

Williamson, M., Gerhard, D., Hulme, P. E., Millar, A., and Chapman, H. (2023). High performance plastic clones best explain the spread of yellow monkey flower from lowland to higher elevation areas in New Zealand. *J. Evol. Biol.* 36, 1455–1470. doi: 10.1111/jeb.14218

Willson, M. F. (1993). Mammals as seed-dispersal mutualists in North America. *Oikos* 67, 159–176. doi: 10.2307/3545106

Worley, A. C., and Harder, L. D. (1999). Consequences of preformation for dynamic resource allocation by a carnivorous herb, *Pinguicula vulgaris* (Lentibulaceae). *Am. J. Bot.* 86, 1136–1145. doi: 10.2307/2656977

Wyka, T. (1999). Carbohydrate storage and use in an alpine population of the perennial herb, *Oxytropis sericea*. *Oecologia* 120, 198–208. doi: 10.1007/s004420050849

Zimmerman, J. K., and Whigham, D. F. (1992). Ecological functions of carbohydrates stored in corms of *Tipularia discolor* (Orchidaceae). *Funct. Ecol.* 6, 575–581. doi: 10.2307/2390055

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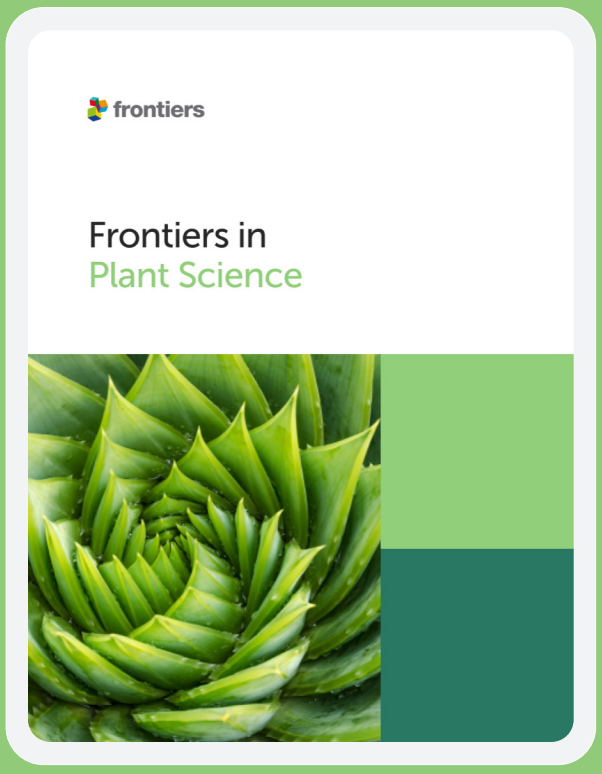

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