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RESEARCH ARTICLE

Clonal integration in *Vallisneria natans* alters growth and the rhizosphere microbial community of neighboring plants under heterogeneous conditions

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Abstract

Background Resource translocation among interconnected ramets can improve the growth performance of the recipient ramets and influence soil properties and microbial communities in the rooting zone. However, scanty attention has been paid to the effect of this clonal integration on soil biotic and abiotic characteristics of neighboring species around the recipient ramets.

Methods We conducted a soil heterogeneous experiment in which the mother ramet of the ramet pair for *Vallisneria natans* was planted in a high-nutrient patch, and the daughter ramet was planted in a low-nutrient patch with conspecific neighbors *V. natans* or heterospecific neighbors *Myriophyllum spicatum*. The stolons between ramet pairs were severed or left intact.

Results Our results showed that effects of clonal integration on growth of the daughter ramet depend on the identity of neighboring species. Overall growth of neighbors *V. natans* was not affected by clonal integration, while growth of neighbors *M. spicatum* was greatly reduced. Soil properties and microbial community composition (especially bacteria) in the rhizosphere of neighboring plants were significantly influenced by clonal integration, and these effects were more obvious in the rhizosphere of neighbor *V. natans* than those in the rhizosphere of neighbors *M. spicatum*.

Conclusion Our study suggests that clonal integration may play a vital role in facilitating nutrient cycling, modifying habitat heterogeneity and affecting interspecific interactions and even the community structure.

Keywords Clonal integration · *V. natans* · Neighboring plants · Heterogeneity · Soil properties · Microbial community

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Introduction

In natural habitats, crucial resources required for plant growth are usually heterogeneously distributed on a spatial scale, which creates certain obstacles for plants to fully absorb and utilize resources (Janeček et al. 2008; Zhang et al. 2016). Compared with nonclonal plants, clonal plants in heterogeneous habitats have a significant advantage: clonal integration (Herben 2004; Xu et al. 2010; Lin et al. 2018). Ramets growing in resource-rich patches can transfer resources (e.g., water, carbohydrates, nutrients) through stolons, rhizomes or horizontally growing roots to connected ramets growing in resource-poor patches (Hutchings and Wijesinghe 1997; You et al. 2013; Li et al. 2017; Duchoslavová and Jansa 2018). Therefore, clonal integration generally improves the growth performance of the recipient ramets in resource-poor patches and the whole clone (Xiao et al. 2011; Yu et al. 2019; Wang et al. 2020). Although this benefit of clonal integration has been extensively studied, most research has focused on the morphological, physiological and biomass characteristics (Evans 1992; He et al. 2011; Zhang et al. 2016; Xue et al. 2022), while few studies have investigated how clonal integration affects the underground dynamic characteristics of clonal ramets.

Soil nutrient availability plays an important role in the growth of individual plants and the productivity and structure of plant communities (Hartnett and Bazzaz 1985). Aquatic clonal plants are sensitive to the heterogeneous distribution of soil nutrients during growth (Bonanno et al. 2018). Previous studies have shown that the growth performance of ramets in low-nutrient patches can be significantly improved based on their connection to ramets in high-nutrient patches (You et al. 2014; Zhang et al. 2016). A few studies explored the process underlying this phenomenon. In clonal fragments of *Sasa palmata*, translocation of nitrogen increased from individual ramets in high-N patches to ramets in low-N patches (Saitoh et al. 2006). The study of Roiloa et al. (2014) showed that preferential transport of ammonium from parents to connected water-stressed offspring ramets, improving survival of offspring ramets growing in water-stressed conditions. This translocation of resources either has no impact on the recipient or benefits the recipient. In a previous study based on ^{15}N and deuterium labeling, Ye et al. (2016) showed that water and nitrogen are translocated from the donor ramet of *Potentilla anserina* to the recipient ramet through clonal integration and then released by the roots of the recipient ramet into the soil, benefiting neighboring plants. Studies have shown that the survival, growth and reproduction of individual plants are largely determined by the identity, number, location and growth of neighboring plants (Mack and Harper 1977; Hartnett and Bazzaz 1985). Genets, or fragments of clonal plants, usually encounter a variety of neighboring species while maintaining and expanding their natural populations. Therefore, we wondered what effects clonal integration in aquatic clonal plants would have on ramets and neighboring species growing in nutrient-poor habitats when different neighboring species are present.

Soil properties and microbial community composition in the root zone of the recipient ramet can be influenced by clonal integration among connected ramets. For example, in an oil-contaminated wetland, clonal integration in *Phragmites australis* can alter soil microbial communities in the root zone of ramets subjected to crude oil contamination (Xue et al. 2020). In stoloniferous herb *Glechoma longituba*, clonal integration could facilitate N assimilation and have a significant influence on microbial community composition in the rhizosphere of shaded, connected offspring ramets by translocation of photosynthates from exposed mother ramets (Chen et al. 2015). Similarly, the results of Li et al. (2018) showed that clonal integration significantly increased rhizospheric C availability and microbial biomass of shaded ramets of *Phyllostachys bissetii*. Previous studies show that resources released by the target plant through hydraulic lifting can be used by neighboring plants and microbes (Sekiya et al. 2011; Pang et al. 2013). However, no study has been conducted to test whether soil properties and microbial community composition in the rhizosphere of neighboring species are also affected by clonal integration among connected ramets when different neighboring species are present around the recipient ramet.

To address these unknowns, we grew connected and disconnected ramet pairs of the submerged clonal plant *Vallisneria natans* in heterogeneous soil environments. The mother ramet of the ramet pair was planted in a high-nutrient patch, and the daughter ramet was planted in a low-nutrient patch with the conspecific or heterospecific neighbors. We predicted that 1) clonal integration would improve the growth of daughter ramets and

their neighboring plants in low-nutrient patches; 2) soil properties and microbial community composition in the rhizosphere of neighboring plants would be affected by clonal integration among connected ramets; 3) These effects of clonal integration would depend on the identity of neighboring species.

Materials and methods

Species

V. natans is a submerged macrophyte with a wide geographical range that represents a dominant native species in many freshwater habitats in China (Zhou et al. 2019). It usually expands its population by producing stolons that spread horizontally above ground and form many clonal ramets. Studies have shown that clonal integration can benefit *V. natans* in heterogeneous environments (Xiao et al. 2007; Xiao et al. 2011). Early in August 2020, we collected seedlings from the precultured populations of *V. natans* and cultivated in large containers filled with lake clay at the National Field Station of Liangzi Lake. At the same time, approximately 50 shoots of *Myriophyllum spicatum*, each 10 cm high, were collected from the National Field Station of Liangzi Lake and cultivated until rooting. *M. spicatum* is a common submerged macrophyte that has a high rate of survival, and strong resistance to pollution (Sun et al. 2021).

Experimental design

On 19 August 2020, twenty ramet pairs of *V. natans* with uniform size were selected as testing plants. Thirty plants of *M. spicatum* and thirty mature individual plants of *V. natans* (15 cm in plant height each, which is consistent with the daughter ramets of ramet pairs) were selected as neighboring plants. Twenty small containers (24 cm in diameter and 11 cm in height) were filled with lake mud (TOC 3.18 $mg{\cdot}g^{-1}$, TN 0.41 $mg{\cdot}g^{-1}$) to create high nutrient patches, and the other 20 were filled with yellow mud (TOC 0.15 $mg{\cdot}g^{-1}$, TN 0.02 $mg{\cdot}g^{-1}$) to create low nutrient patches. A high-nutrient patch and a low-nutrient patch were placed into every large aquarium (length: 100 cm, width: 50 cm, height: 60 cm) filled with 200 L of lake water (TN 1 $mg{\cdot}L^{-1}$ and TP 0.03 $mg{\cdot}L^{-1}$). Three plants of *M. spicatum* or 3 individual plants of *V. natans* were planted evenly in low nutrient patches. The mother ramets for ramet pairs of *V. natans* were planted in high nutrient patches, and the daughter ramets for ramet pairs of *V. natans* were planted in low nutrient patches with neighbors *V. natans* or *M. spicatum*. The experimental containers were placed at random on an outdoor cement platform. After four days of acclimatization, the stolons between ramet pairs of *V. natans* were severed or left intact, with five replicates for each treatment (Fig. 1). The day temperatures were 25–34 °C during the experimental period.

Growth parameters of daughter ramets and neighboring plants

The experiment was harvested on 1 October. Several fully developed and healthy leaves of the daughter ramets were selected to measure the chlorophyll content with the dimethylsulfoxide (DMSO) chlorophyll extraction method (Richardson et al. 2002). Then these leaves were oven-dried and fully ground to determine the content of leaf N with an organic elemental analyzer (FLASH 2000, Thermo Fisher Scientific Inc. USA). Furthermore, the daughter ramets were separated into shoots and roots, dried in an oven at 70 °C for 48 h, and weighed to obtain the shoot biomass, root biomass, total biomass and root/shoot ratio. Meanwhile, we counted the number of offspring ramets that the daughter ramets produced. In the apical fragment, offspring ramets and stolons were dried in an oven at 70 °C for 48 h and weighed to obtain the biomass of the apical offspring, stolons and apical fragment.

The chlorophyll content and leaf N contents of neighboring plants *V. natans* and *M. spicatum* were measured using similar methods. For *V. natans*, the plants were separated into shoots and roots, dried in an oven at 70 °C for 48 h, and weighed to obtain the shoot, root, and total biomasses and root/shoot ratio. We also counted the number of offspring ramets produced by the neighboring plant *V. natans*. Similarly, the biomass of offspring, stolons and clonal fragments were obtained. For *M. spicatum*, we counted the shoot, root, and total biomasses and root/shoot ratio.

Soil properties

At the end of the experiment, rhizosphere soil of neighboring plants *V. natans* and *M. spicatum* was sampled and sieved. Half of the soil samples used for chemical analysis were sealed in sterile plastic bags

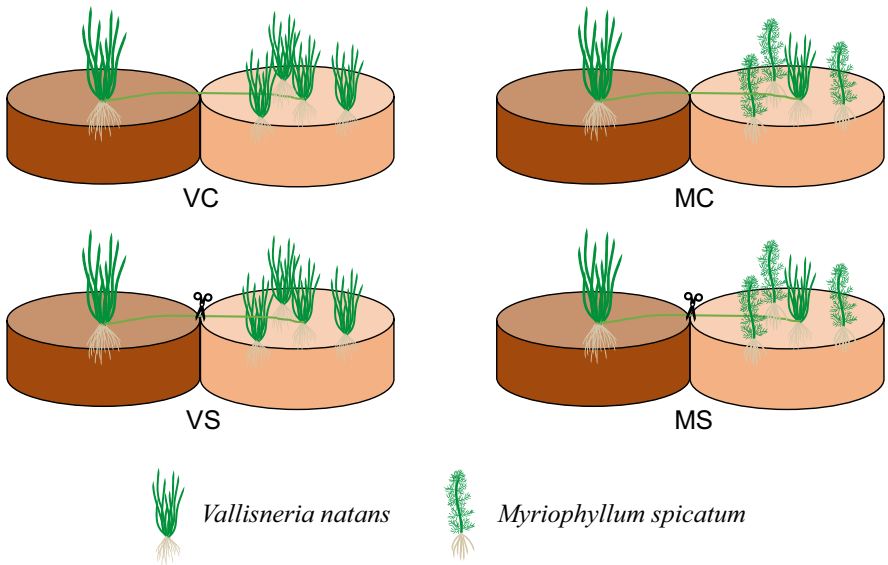

Fig. 1 Schematic representation of the experimental design. The mother ramet of the ramet pair for *Vallisneria natans* was planted in a high-nutrient patch, and the daughter ramet was planted in a low-nutrient patch that included neighbors *V. natans* or *Myriophyllum spicatum*. Stolon connections between the mother and daughter ramets were either connected or severed. VC means that neighbors were *V. natans* and stolons between ramet pair were connected; VS means that neighbors were *V. natans* and stolons between ramet pair were severed; MC means that neighbors were *M. spicatum* and stolons between ramet pair were connected; MS means that neighbors were *M. spicatum* and stolons between ramet pair were severed

and stored at 4 °C and the other half of the samples used for DNA extraction and sequencing were sealed in 15 mL sterilized polypropylene tubes and stored at −80 °C. All samples were analyzed as soon as possible. The soil pH was determined with a pH meter in a 1:2.5 soil-water suspension (Hong et al. 2015). Urease activity was measured by colorimetric determination of ammonium with short-term incubation (Kandeler and Gerber 1988). The activity of β-1, 4-*N*-acetylglucosaminidase (NAGase) was assayed by an improved colorimetric determination of the intensity of the yellow color produced by *p*-nitrophenyl (*p*NP) release (Ekenler and Tabatabai 2002). Spectrophotometric determination of the red compound developed from the reaction of POXase activity and proline was used to test for POXase activity (Perucci et al. 2000). A newly developed chloroform-fumigation extraction method was used to determine the microbial biomass C and N (Witt et al. 2000). Ten grams of fumigated or unfumigated (without adding chloroform) soil samples were mixed with 40 ml of 0.5 M K_2SO_4 at a ratio of 1:4 (w/v), shaken for 60 min on a shaker and filtered through Whatman No. 42 filter paper. Then, the extracts were used to analyze the dissolved organic carbon (DOC) content with a TOC analyzer (TOC-L CPN CN200/Lachat Instruments) and dissolved organic nitrogen (DON) content with a flow injection analyzer (QC8500, LACHAT, United States). Microbial biomass carbon (MBC) and nitrogen (MBN) were calculated according to the equation MBC (N) = $2.22*E_B$, where E_B is the difference between DOC/DON from fumigated and nonfumigated soil (Wu et al. 1990). Spectrophotometry using the ammonium indophenol blue method and the correction factor method were applied to determine the NH_4^+-N and NO_3^--N concentrations, respectively (Ivančič and Degobbis 1984; Song et al. 2007).

DNA extraction and PCR amplification

Three soil samples from each treatment were randomly selected for DNA extraction and sequencing. Microbial DNA was extracted from the soil samples using the FastDNA spin kit (MP Biomedicals, USA) according to the manufacturer's protocols. The quality and concentration of the extracted DNA were checked with a NanoDropTM 2000 Spectrophotometer (Nanodrop, Wilmington, DE, USA).

For the bacterial community, the V3-V4 regions of the microbial 16S rRNA gene were amplified using the primers 338F (5′-barcode-ACTCCTACGGGA GGCAGCAG-3′) and 806R (5′-GGACTACHVGGG TWTCTAAT-3′). For the fungal community, ITS sequences were amplified using the primers ITS1F (5′-CTTGGTCATTTAGAGGAAGTAA-3′) and ITS2R (5′-GCTGCGTTCTTCATCGATGC-3′). PCR amplification was performed in a 20 μL reaction volume containing 4 μL of 5×FastPfu Buffer, 0.8 μL of each primer (5 μM), 0.4 μL of FastPfu polymerase, 2 μL of 2.5 mM dNTPs, and 10 ng of template DNA. The PCR procedure parameters were as follows: 95 °C for 3 min, followed by 30 cycles at 95 °C for 30 s, 55 °C for 30 s and 72 °C for 45 s; and a final extension at 72 °C for 10 min. Amplicons were extracted from 2% agarose gels and quantified using QuantiFluor™-ST (Promega, USA). Purified amplicons were collected in equimolar amounts and paired-end sequenced (250×2) on an Illumina MiSeq platform (Majorbio, Shanghai, China). Bacterial 16S rDNA sequences were aligned with the reference sequences in the SILVA database (http://www.arb-silva.de). Fungal ITS sequences were aligned with the reference sequences in the UNITE database (https://unite.ut.ee/). Operational taxonomic units (OTUs) with a 97% similarity cutoff were clustered, and chimeric sequences were identified and removed.

Statistical analyses

The effects of connection, species identity and their interactions on growth parameters (physiological indicators, biomass indicators and clonal propagation) of the daughter ramets were analyzed by two-way ANOVA. The effects of connection on the growth parameters of neighboring plants *V. natans* and *M. spicatum* were tested by independent-samples *T* Test. Two-way ANOVA was also applied to test the effects of connection, species identity and their interaction on soil properties, microbial alpha diversity indices (number of observed OTUs and Shannon diversity indices) and community composition (relative abundances of dominant genera) in the rhizosphere of neighboring species. Nonmetric multidimensional scaling analysis (NMDS) and Adonis tests were performed to examine differences in the microbial community composition (at the OTU level) in the rhizosphere of neighboring species among different treatments using the '*vegan*' package. Before the analysis, the data were log-transformed to meet the assumptions of normality and homogeneity if necessary. All the above analyses were performed using R version 3.5.2.

Results

Growth characteristics of the daughter ramets

Leaf physiological indices of the daughter ramets were significantly affected by the interaction between connection and species (Table S1). Clonal integration significantly increased the leaf N of the daughter ramets adjacent to *V. natans* and strongly reduced the chlorophyll content of the daughter ramets adjacent to *M. spicatum* (Figs. 2a and S1a). The effects of clonal integration on individual growth of the daughter ramets and growth of the apical fragment were reversed between the two neighboring plants. Clonal integration significantly decreased the total biomass of the daughter ramets and biomass of the apical fragment adjacent to *V. natans* but greatly increased those adjacent to *M. spicatum* (Table S1; Figs. 2b and d). Only when the neighboring plant was *V. natans*, connection had significant negative effects on the shoot biomass and root biomass of the daughter ramets but had significant positive effects on the root/shoot ratio (Fig. S1b, c and d). Clonal integration strongly increased the number of ramets and biomass of stolons of the daughter ramets adjacent to *V. natans* but had no significant effect on those adjacent to *M. spicatum* (Table S1; Figs. 2c and S1f).

Growth characteristics of neighboring plants

Connection had no significant effect on the leaf physiological indices, root biomass, root/shoot ratio and biomass of clonal fragments of neighboring plant *V. natans* (Fig. 3a and d; Fig. S2a, c, d). Significantly lower shoot biomass and total biomass and higher number of ramets, biomass of offspring and biomass of stolons of neighboring plant *V. natans* were found in the connected treatments than in the severed treatments (Fig. 3b and c; Fig. S2b, e, f). Connection had no significant effect on the chlorophyll content, shoot biomass, root/shoot ratio of neighboring plant *M. spicatum* (Fig. S2g, h and j). Clonal integration

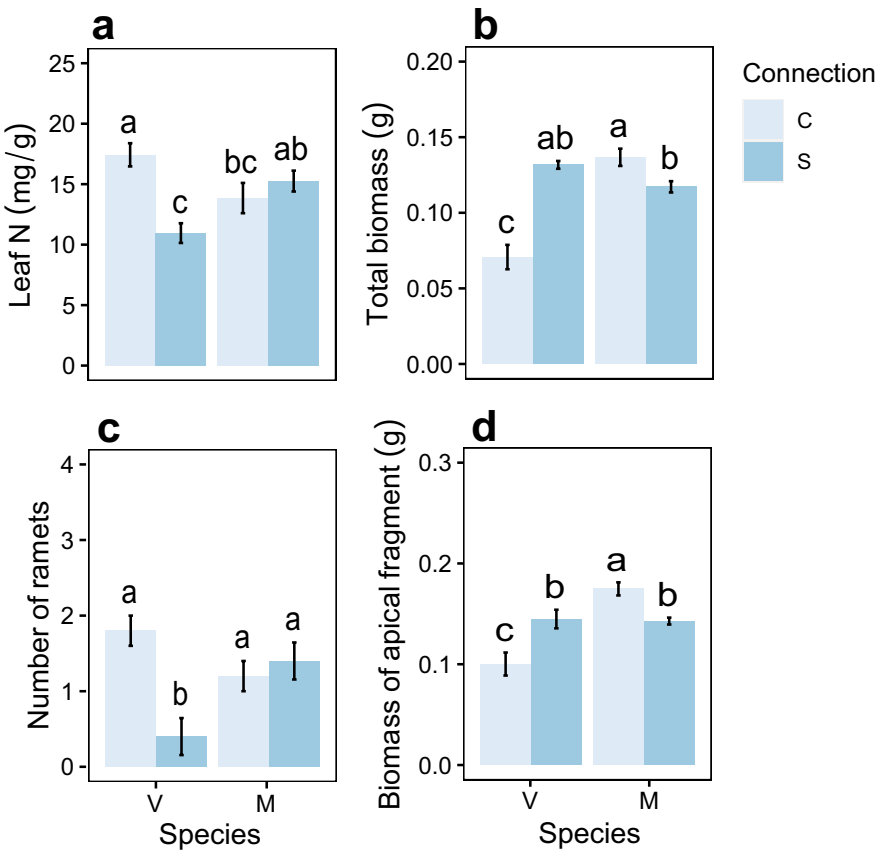

Fig. 2 Growth characteristics of the daughter ramets with two neighboring plants under the connected and severed treatments: (**a**) leaf N, (**b**) total biomass, (**c**) number of ramets, (**d**) biomass of apical fragment. V means that neighbors were *V. natans*; M means that neighbors were *M. spicatum*. C means that stolons between ramet pair were connected; S means that stolons between ramet pair were severed. The data indicate the mean ± SE ($n = 5$). Different lowercase letters indicate significant differences ($P < 0.05$)

significantly decreased leaf N, the root biomass and total biomass of neighboring plants *M. spicatum* (Fig. 3e and f; Fig. S2i).

Soil properties in the rhizosphere of neighboring plants

Connection and the interaction between connection and species did not have a significant effect on the pH in the rhizosphere (Fig. 4a). No significant effect of connection, species, or their interaction was detected for the activities of urease and phenol oxidase (POXase) (Table S2; Fig. 4b and c). The activity of NAGase in the rhizosphere of neighboring plant *V. natans* was significantly higher in the connected treatments than in the severed treatments, while clonal integration had no significant effect on that in the rhizosphere of neighboring plant *M. spicatum* (Table S2; Fig. 4d). Across both neighboring species, the MBC and MBN in the rhizosphere were higher in the connected treatments (Fig. 4e and f). The positive effects of the connection on DOC and NO_3^--N were greater in the rhizosphere of the neighboring plant species *V. natans* than in the rhizosphere of the neighboring plant species *M. spicatum* (Fig. 4h and l). In the connected treatments, the NAGase, MBN, TOC, DOC, TN, DON and NO_3^--N in the rhizosphere of the neighboring plant species *V. natans* were higher than those in the rhizosphere of the neighboring plant species *M. spicatum* (Fig. 4d–l). In the severed treatments, the MBN, TN and DON in the rhizosphere of neighboring plant *V. natans* were higher than those in the rhizosphere of neighboring plant *M. spicatum* (Fig. 4f–j).

Microbial community compositions in the rhizosphere of neighboring plants

After filtering, a total of 6744 OTUs for bacteria and 1027 OTUs for fungi were obtained from 12 samples. The number of observed OTUs and Shannon diversity indices of bacterial communities and Shannon diversity indices of fungal communities were

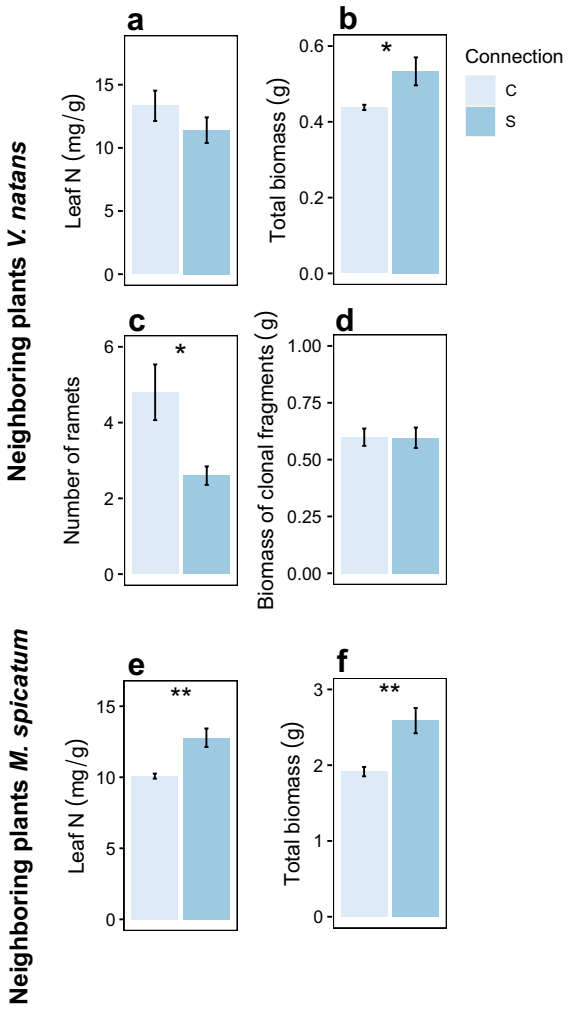

Fig. 3 Growth characteristics of two neighboring plants under the connected and severed treatments: (**a**) leaf N, (**b**) total biomass, (**c**) number of ramets and (**d**) biomass of clonal fragments of neighboring plants *V. natans*, (**e**) leaf N and (**f**) total biomass of neighboring plants *M. spicatum*. The data indicate the mean ± SE ($n = 5$). Significant differences at $P < 0.05$, indicated with asterisk (*): one asterisk means $P < 0.05$, two asterisks mean $P < 0.01$, and three asterisks mean $P < 0.001$

significantly affected by the interaction between connection and species (Table S3). The effects of clonal integration on the Shannon diversity indices of the bacterial communities were reversed in the rhizosphere of two neighboring plants. Clonal integration greatly reduced the Shannon diversity indices of the bacterial communities in the rhizospheres of the neighboring plant *V. natans* but greatly increased that in the rhizospheres of the neighboring plant *M. spicatum* (Fig. 5b). In the fungal communities, only the Shannon diversity indices in the rhizosphere of neighboring plant *V. natans* were greatly reduced by connection (Fig. 5d).

The first axis of the NMDS plots showed that the bacterial communities were roughly separated at the integration level and species level. Furthermore, the Adonis analysis implied that the differences in bacterial communities could be explained by the species and the interaction between species and connection (Fig. 6a). The fungal community composition did not show significant differences among the different treatments (Fig. 6b). Among the dominant bacterial genera, clonal integration greatly increased the relative abundance of *Lysobacter*, *norank_f__norank_o__Subgroup_7* and *Sphingomonas* in the rhizosphere of neighboring *V. natans* (Figs. 7a and 8b–e). Except for the lower abundance of the genus *norank_f__norank_o__norank_c__KD4.96* under the connected treatments, connection had no significant effect on the other dominant bacterial genera in the rhizosphere of the neighboring plant *M. spicatum* (Figs. 7a and 8a-f). Except for the relative abundance of *Talaromyces* in the rhizosphere of the neighboring plant *M. spicatum*, connection had no significant effects on the dominant fungal genera in the rhizosphere of the two neighboring plants (Figs. 7b and 8g-l).

Discussion

Clonal integration significantly decreased individual growth of the daughter ramets adjacent to *V. natans* but greatly increased that adjacent to *M. spicatum*, which supported our third hypothesis and partially supported our first hypothesis. Clonal integration did not affect overall growth of neighbors *V. natans* but reduced growth of neighbors *M. spicatum*, which did not support our first hypothesis but supported our third hypothesis. Clonal integration altered soil properties and microbial community composition in the rhizosphere of two neighboring plants to varying degrees, which supported our second and third hypothesis.

Growth of daughter ramets in response to clonal integration and neighboring species identity

When the neighboring plant was *V. natans*, daughter ramets had higher leaf N content in the connected

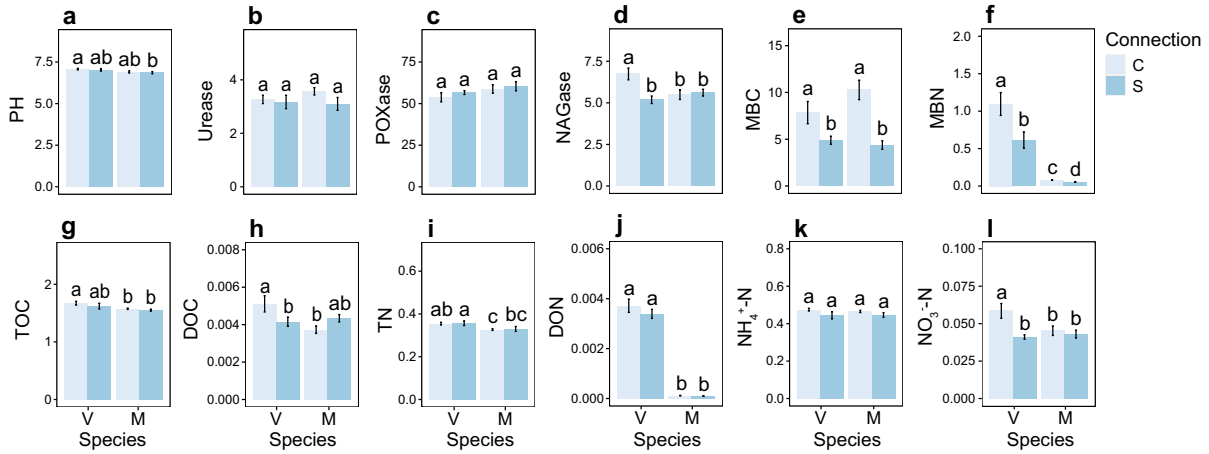

Fig. 4 Soil properties in the rhizosphere of two neighboring plants under the connected and severed treatments. The data indicate the mean ± SE ($n = 5$). Different lowercase letters indicate significant differences ($P < 0.05$)

treatments, which may be because translocation patterns of nitrogen were especially distributed to the leaves (Saitoh et al. 2006). Individual growth of daughter ramets were strongly reduced by connection, as well as growth of apical fragment, while the clonal growth of daughter ramets was enhanced by connection. A possible reason is that mother plants may not choose to invest in daughter plants to resist competition with congeners but invest in clonal growth to escape the canopy of neighboring plants. This result

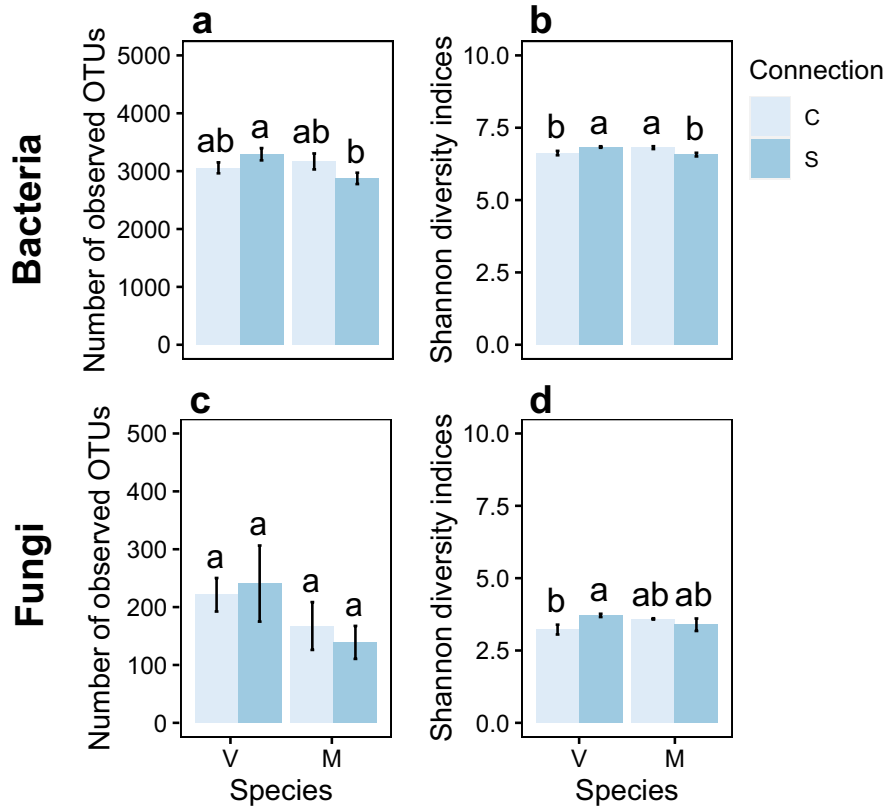

Fig. 5 Alpha diversity estimates of the bacterial and fungal communities in the rhizosphere of two neighboring plants under the connected and severed treatments. The data indicate the mean ± SE ($n = 3$). Different lowercase letters indicate significant differences ($P < 0.05$)

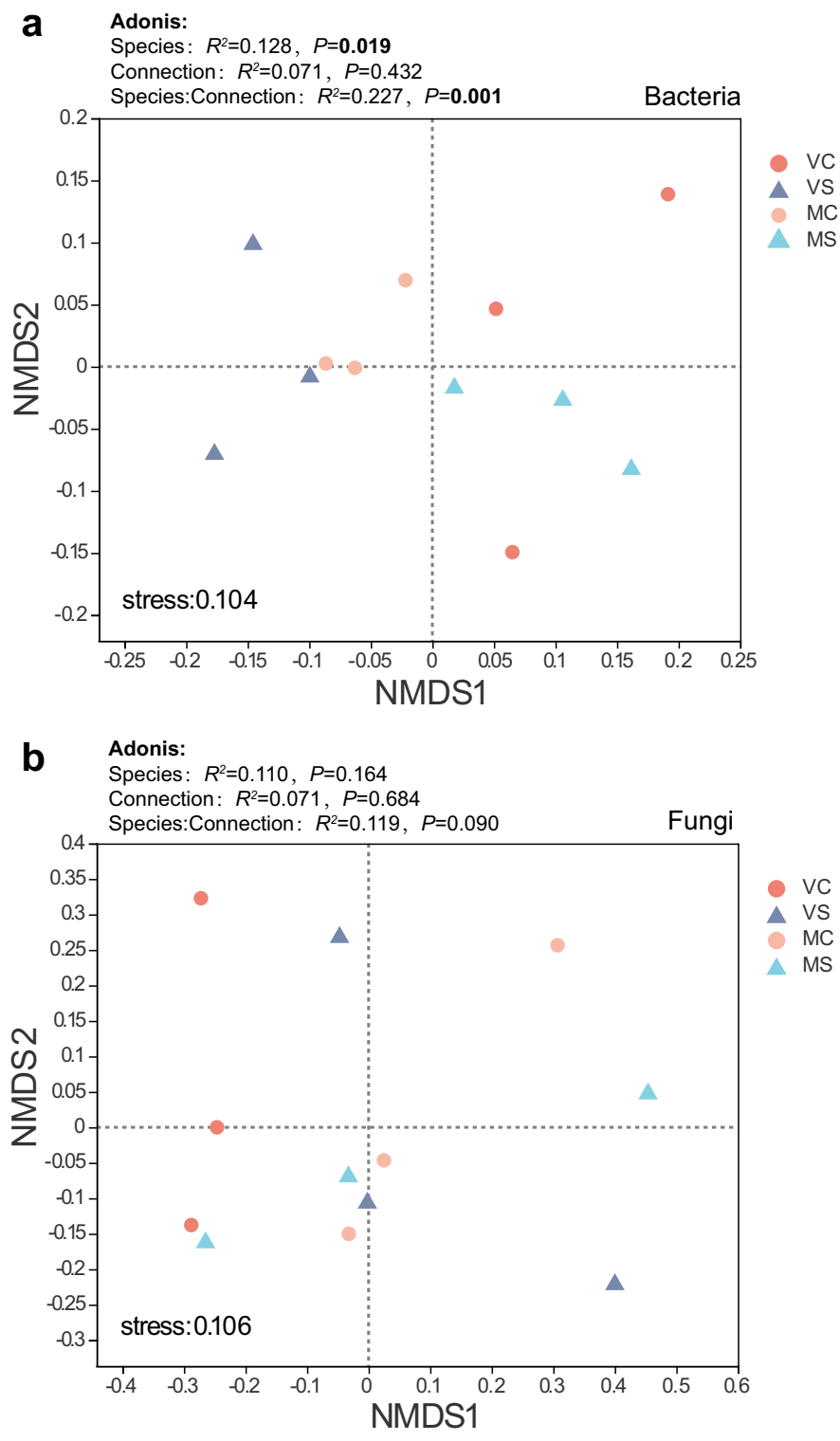

Fig. 6 Nonmetric multidimensional scaling diagram showing differences in the bacterial and fungal community compositions among the different treatments. The results of the Adonis tests are presented

is consistent with a previous study reporting that the beneficial effect of clonal integration is much smaller with competition (Wang et al. 2008). Root/shoot ratio was strongly increased by connection, which may be

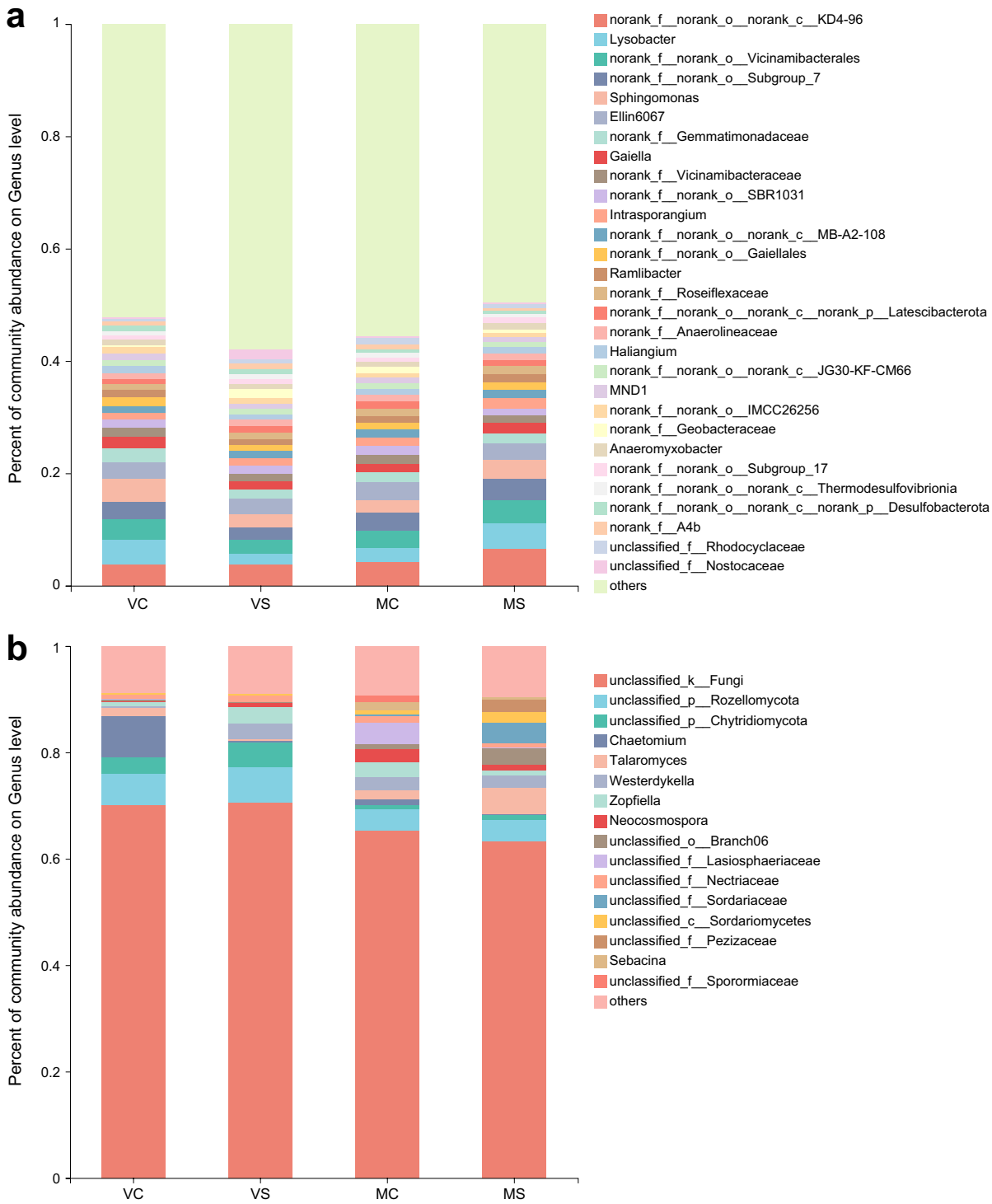

Fig. 7 Community compositions of bacteria (**a**) and fungi (**b**) at the genus level in the rhizosphere of two neighboring plants under the connected and severed treatments

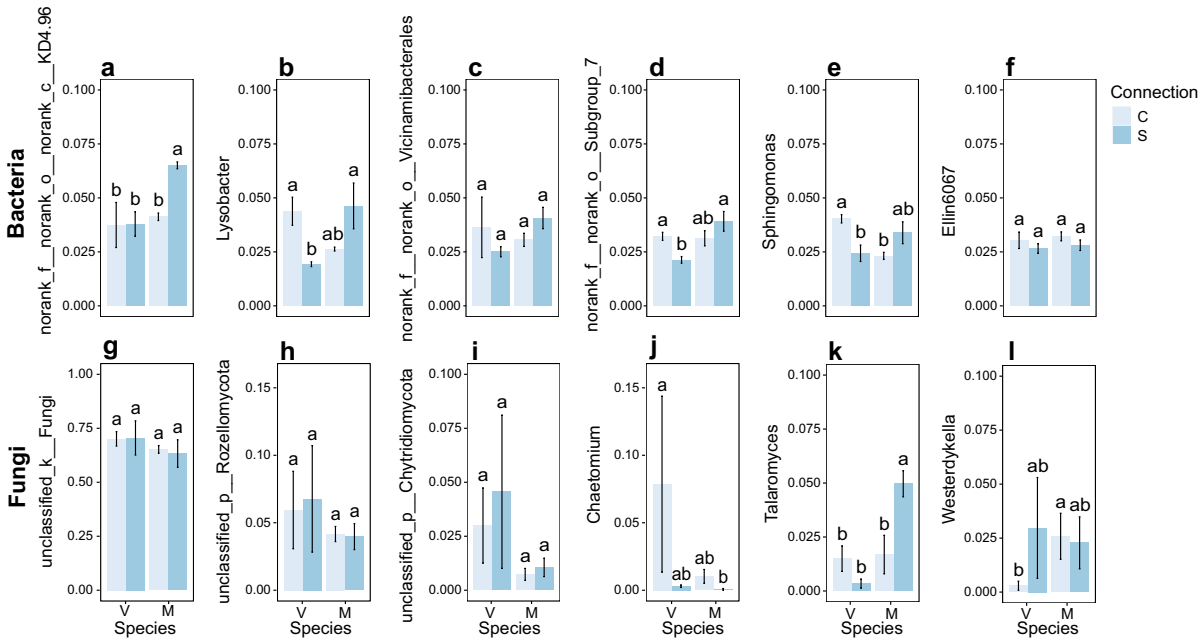

Fig. 8 Relative abundance of six dominant bacterial and fungal genera ranked in decreasing order in the rhizosphere of two neighboring plants under the connected and severed treatments. The data indicate the mean ± SE ($n = 3$). Different lowercase letters indicate significant differences ($P < 0.05$)

because improved underground resource conditions alleviate the reduction of root growth to some extent. These phenomenons only occurred when the neighboring plant was *V. natans*, indicating the different responses of the clonal plant *V. natans* to conspecific neighbors compared with heterospecific neighbors. When the neighboring plant was *M. spicatum*, the growth of daughter ramets and apical fragments were significantly increased by clonal integration, which demonstrates that clonal integration may directly or indirectly contribute to the greater competitive ability of *V. natans* under nutritionally heterogeneous conditions when facing other species. This appears consistent with the conclusion that clonal integration can increase the competitive ability of clonal plants when clonal fragments grow from open areas into stands of other plants (Wang et al. 2021). Xiao et al. (2011) also found that clonal integration helped *Vallisneria spiralis* invade vegetated habitats occupied by *M. spicatum*. In contrast, some studies showed that the competitive ability of several clonal plants was not influenced by clonal integration (Price and Hutchings 1996; Pennings and Callaway 2000; Březina et al. 2006). The effects of clonal integration on the competitive ability of clonal plants may be species-specific and depend on environmental conditions and competitor identity.

Growth of neighboring plants in response to clonal integration

Individual growth of neighboring plants *V. natans* was reduced by connection, which could be because the increased number of apical ramets intensified competition between the same species. In addition, the benefits of neighboring plants *V. natans* from resource translocation between ramets may do not compensate for the reduction of their growth but contribute to their clonal growth. Enhanced clonal growth may counteract the negative effects of competition, which is responsible for the unaffected growth of clonal fragments of neighboring plants *V. natans*. These results suggest that the clonal plant *V. natans* tends to enhance clonal growth under stressful environments, facilitating foraging for resources over large areas (Yu et al. 2001). An opposing view is that the most important factor for a clone established under a canopy should be to improve individual performance, rather than producing new ramets (Xiao

et al. 2007). In our study, clonal growth might be a critical survival tactic of *V. natans* in the competitive environment. The growth and leaf performance of neighboring plant *M. spicatum* were decreased by clonal integration; thus, *M. spicatum* may benefit less from resource translocation by *V. natans*. Moreover, the increased growth of daughter ramets of *V. natans* due to clonal integration may have exposed *M. spicatum* to greater competition.

Soil properties in response to clonal integration and species identity

Overall, the stolon connection led to higher extracellular enzyme activities, microbial biomass and C availabilities in the rhizosphere of neighboring plant *V. natans*.

The strong carbon sinks may be created by connected ramets in vegetated habitats (Xiao et al. 2011). Consequently, in addition to mineral nutrient, more carbohydrates could be transported to connected apical ramets and released to soil, affecting soil properties in the rhizosphere of neighboring plant *V. natans*. Increased C availability resulting from clonal integration stimulated the reinforcement of microbial activity (Butler et al. 2004), which further enhanced the activity of hydrolytic enzymes (Li et al. 2018), especially NAGase. NAGase activity is often used as an indicator of N mineralization in soil (Ekenler and Tabatabai 2004). A previous study demonstrated that nitrogen mineralization and nitrification in the rhizosphere of stressed ramets can be significantly increased by clonal integration (Chen et al. 2015), which is consistent with the increased NO_3^--N in our study. DON and NH_4^+ -N did not present significant changes, which was probably because we could not distinguish between enhanced production and decreased uptake (Koranda et al. 2011). However, positive effects of connection on soil properties occur mainly in the rhizosphere of neighboring plant *V. natans*, although the microbial biomass (MBC and MBN) in the rhizosphere of *M. spicatum* was significantly increased by connection. This result is likely due to more adequate resources flow and utilization among conspecifics.

Microbial community composition in response to clonal integration and species identity

Our sequencing results showed that clonal integration reduced the bacterial and fungal alpha diversity (Shannon diversity indices) in the rhizosphere of the neighboring plant species *V. natans*, which suggested that the dominant species might have been more abundant. Correspondingly, the relative abundances of the dominant bacterial genera *Lysobacter*, *norank_f__norank_o__Subgroup_7* and *Sphingomonas* in the rhizosphere of neighboring plant *V. natans* were greatly increased by clonal integration. *Lysobacter* has a high-level production of extracellular enzymes and bioactive metabolites (Xie et al. 2012). *Sphingomonas* could significantly promote biodegradation and nutrient release and represents a good microbial resource for soil remediation (Gou et al. 2008). Recruitment of these beneficial microorganisms might be associated with variations in soil properties. Nevertheless, the relative abundance of dominant fungal genera in the rhizosphere of neighboring plant *V. natans* was little affected by connection, which indicates that fungi may be insensitive to variations in soil properties. Only bacterial alpha diversity in the rhizosphere of neighboring plant *M. spicatum* was greatly increased by clonal integration, indicating that different plant species may shape different microbial communities (Shi et al. 2021). This result was in accordance with the lower abundance of *norank_f__norank_o__norank_c__KD4.96* in the connected treatments, implying that rare species might have been more abundant (Dang et al. 2020). Furthermore, the composition of soil bacterial communities (beta diversity) also showed significant differences in the different treatments. The variation may be due to the changes in the soil microenvironment by interactions between species and clonal integration, which determines the differential bacterial community assembly (Dai et al. 2019). Contrary to previous studies, the composition of fungal communities may be slightly influenced by the variation in the soil microenvironment (de Menezes et al. 2015).

Conclusions

Our results showed that, under heterogeneous soil environments, both daughter ramets of *V. natans* and neighboring plants *V. natans* benefited less from resource translocation between ramets when neighboring plants were *V. natans*. When the neighboring plant was *M. spicatum*, clonal integration provided

daughter ramets of *V. natans* greater competitive advantage, reducing growth of *M. spicatum*. Though neighboring plants benefited less from clonal integration, soil properties and microbial community composition in the rhizosphere of neighboring plants were significantly influenced by clonal integration, especially when neighboring plants were *V. natans*. But one caveat is that we cannot distinguish whether the performance of neighboring plants is the result of direct effects of clonal integration on plant interactions, or the result of indirect effects of clonal integration on neighboring plants via changing soil nutrient availability, or both. Furthermore, another caveat is that we cannot distinguish whether the variations of soil nutrient conditions are primarily affected by resource translocation from donor ramets or the recipient ramets' own root exudates. Untangling these complex relationships would be challenging in future research. Even so, our study provides insights into the implications of clonal integration in heterogeneous environments: facilitating nutrient cycling, modifying habitat heterogeneity and affecting interspecific interactions and even the community structure.

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Author contributions Xiaowen Ma, Chunhua Liu and Dan Yu conceived the study. Xiaowen Ma conducted the experiments. Xiaowen Ma, Weicheng Yu, Min Tao, Chang Zhang, and Zhiqiang Zhang collected the data. Xiaowen Ma and Weicheng Yu analyzed the data; Xiaowen Ma and Chunhua Liu led the manuscript writing. All authors read and approved the contents of this paper.

Data availability The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Competing interests The authors have no competing interests to declare that are relevant to the content of this article.

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