Effects of Clonal Integration on Biological Characteristics of Rhizosphere Soil Bacteria in Shaded Ramets of Phyllostachys nidularia Postprint

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Abstract

Clonal integration is considered an important mechanism for clonal plants to maintain ecological advantages, as it alleviates stress imposed by resource heterogeneity through physiological integration among ramets. This study used the rhizomatous clonal plant Phyllostachys bissetii as the research subject to investigate the effects of clonal integration on rhizosphere soil bacterial biological characteristics of P. bissetii ramets under heterogeneous light conditions. Clonal fragments of P. bissetii consisted of one proximal ramet and one distal ramet, with either the proximal or distal ramet placed in an 80% shaded environment while the other ramet was placed in a full-light environment; simultaneously, rhizomes between ramets were either kept connected or severed. The results showed that regardless of whether the proximal or distal ramet of P. bissetii clonal fragments was shaded, clonal integration significantly promoted dissolved organic carbon (DOC) content and microbial biomass carbon and nitrogen contents (MBC, MBN) in the rhizosphere soil of shaded ramets; the activities of extracellular N-acetyl- -glucosaminidase (NAGase) and urease in the rhizosphere soil of shaded ramets under rhizome-connected conditions were significantly higher than those of shaded ramets under rhizome-severed treatment; sequencing results of the 16S rDNA V3-V4 hypervariable regions of genomic DNA from the rhizosphere soil of shaded ramets showed that operational taxonomic units (OTUs) were assigned to 13 phyla and 415 genera. The relative abundances of bacterial groups associated with rhizosphere soil nitrogen transformation, including Nitrosomonadaceae (uncultured), Nitrospira, Nitrospinaceae (uncultured), Xanthobacteraceae (uncultured), and Bradyrhizobium, were relatively low (with a maximum value of 11.8%). Principal component analysis (PCA) based on OTUs indicated that rhizome severing treatment did not significantly affect the bacterial community structure in the rhizosphere soil of shaded ramets. Clonal integration significantly promoted C availability in the rhizosphere soil of shaded ramets, thereby stimulating the microbially regulated soil organic matter (SOM) turnover process. The effects of clonal integration on soil bacterial biological characteristics of clonal plants in heterogeneous habitats may partially explain the ecological advantages of clonal plants.

Full Text

Preamble

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Effect of Clonal Integration on Soil Microbial Properties in the Rhizosphere of *Phyllostachys bissetii* Subjected to Heterogeneous Light

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Abstract

Clonal integration is considered a crucial mechanism enabling clonal plants to maintain ecological dominance, as it alleviates resource heterogeneity stress through physiological integration among ramets. This study investigated the effects of clonal integration on rhizosphere soil bacterial biological characteristics of *Phyllostachys bissetii* under heterogeneous light conditions. Clonal fragments of *P. bissetii* containing two successive ramets—a proximal ramet and a distal ramet—were used as experimental subjects. Shading (80% light reduction) was applied to either the distal or proximal ramet, while the counterpart ramet was placed in full sunlight. The rhizome connecting the two ramets was either severed or left intact.

Our results demonstrate that regardless of whether the proximal or distal ramet was shaded, clonal integration significantly promoted dissolved organic carbon (DOC) content and microbial biomass carbon (MBC) and nitrogen (MBN) in the rhizosphere soil of shaded ramets. The activities of extracellular enzymes N-acetyl- -D-glucosaminidase (NAGase) and urease in the rhizosphere soil of shaded ramets were also significantly enhanced under rhizome-connected conditions compared to severed conditions.

High-throughput sequencing of the 16S rDNA V3–V4 variable region yielded a total of 556,519 reads and 18,898 operational taxonomic units (OTUs) from 12 soil samples. Bacterial OTUs were assigned to 13 phyla and 415 genera. Key bacterial groups associated with nitrogen cycling, including *Xanthobacteraceae*, *Bradyrhizobium* (uncultured), *Nitrosomonadaceae* (uncultured), *Nitrospira*, and *Nitrospinaceae* (uncultured), were detected but at relatively low abundances.

Principal component analysis (PCA) revealed that rhizome severance did not significantly affect the bacterial community structure in the rhizosphere of shaded ramets, which maintained similar microbial diversity at the OTU level.

In summary, clonal integration significantly increased carbon availability and soil microbial biomass in the rhizosphere of shaded ramets, thereby stimulating microbially mediated soil organic matter (SOM) turnover. We tentatively conclude that the effects of clonal integration on rhizosphere soil microbial properties may partially explain the ecological advantages of clonal plants, particularly those inhabiting heterogeneous light environments.

Keywords: clonal integration; rhizosphere soil; Illumina MiSeq sequencing; microbial community composition; functional redundancy

Introduction

Clonal growth connects ramets within a clone through horizontal structures such as stolons and rhizomes, enabling the sharing of resources like photosynthates among connected ramets—a process termed clonal integration. Studies using isotopic tracers and dye labeling have demonstrated that assimilates can be transported both acropetally (toward distal ramets) and basipetally (toward proximal ramets) within clonal fragments. Clonal integration may thus represent a key mechanism by which clonal plants maintain ecological advantages, mitigating stress from resource heterogeneity through physiological integration among ramets.

Root exudates released into the rhizosphere are considered the primary source of readily decomposable carbon inputs to soil. These labile organic compounds serve as important energy sources for heterotrophic rhizosphere microorganisms, promoting their rapid growth and reproduction. Soil organic matter mineralization is a complex biochemical process catalyzed by soil enzymes, which originate primarily from soil microbes. While some enzymes participate in microbial metabolic processes, others are excreted or secreted into the soil matrix. Soil enzyme activities are considered sensitive indicators of microbial activity, and rhizosphere ecological processes such as organic matter degradation and nutrient availability may be influenced by both microbial activity and changes in community structure.

Soil represents a complex and dynamic system. Traditional cultivation-based methods can only isolate a tiny fraction of soil microorganisms, as the vast majority cannot be cultured or identified under laboratory conditions. With the rapid development of sequencing technologies, 16S rDNA sequencing has been increasingly applied to analyze bacterial community structure in environmental samples. In this study, we collected rhizosphere soil from shaded ramets of *P. bissetii* to investigate the effects of clonal integration on rhizosphere soil biological processes under heterogeneous light conditions. We measured dissolved organic carbon, microbial biomass, and enzyme activities, and extracted rhizosphere soil genomic DNA for 16S rDNA V3–V4 region sequencing to examine how resource sharing between ramets affects bacterial community structure.

2. Experimental Design

Phyllostachys bissetii, also known as Rongcheng bamboo (Gramineae), is a typical clonal plant that expands horizontally through underground rhizomes. Culms reach 3–6 m in height and are used as timber or for weaving, and the species serves as a primary food source for giant pandas.

The experimental site was located in Nanbaoshan Town, Qionglai City, Sichuan Province (103°14 14 E, 30°14 31 N) at an elevation of 1,217 m, with a mean annual temperature of 16.3°C and annual precipitation of 1,117.3 mm. A relatively flat pure stand of *P. bissetii* was selected as the experimental area. Clonal fragments with uniform ramet size and consistent rhizome spacing were chosen, each containing two connected ramets that were designated as proximal and distal ramets. The experiment comprised two treatment groups: (1) an acropetal transport group where the distal ramet was shaded and the proximal ramet received normal light, and (2) a basipetal transport group where the proximal ramet was shaded and the distal ramet received normal light. Rhizome connections were either severed or left intact.

To exclude external soil from influencing rhizosphere ecological processes in shaded ramets, the following procedures were implemented: polyethylene film and boards (50 cm × 50 cm) were placed around the soil block at the base of each ramet to prevent water and nutrient infiltration from the external soil environment. The soil surface was covered with shade netting to prevent litter contamination. All experimental treatments were completed by mid-May 2015.

The experimental design is illustrated in [Figure 1: see original paper]. Each treatment was replicated 6 times. Soil within approximately 2 mm of the root surface was collected as rhizosphere soil from shaded ramets within clonal fragments. Plant residues were manually removed, and samples were stored in sterile self-sealing bags at ultra-low temperature for later analysis.

3. Soil Physicochemical Index Measurement

Rhizosphere soil microbial biomass carbon (MBC) and nitrogen (MBN) were measured using the chloroform-fumigation extraction method (CFAP). Soils were extracted with 0.5 mol/L K SO solution after chloroform fumigation or without fumigation. Filtrates were analyzed for dissolved organic carbon (DOC) and dissolved organic nitrogen (DON) using a TOC/TN analyzer (TOC-L, SHIMADZU, Japan). Microbial biomass was calculated using established formulas, where EB represents the difference in extractable carbon/nitrogen between fumigated and non-fumigated samples.

N-acetyl- -D-glucosaminidase (NAGase) activity was determined using the method of Parham and Deng, expressed as micrograms of *p*-nitrophenol hydrolyzed per gram fresh soil during incubation. Urease activity was measured using the method of Kandeler and Gerber with urea as substrate, expressed as micrograms of ammonium nitrogen hydrolyzed per gram dry soil.

4. Soil Sample High-Throughput Sequencing

Soil microbial genomic DNA was extracted using a commercial kit and verified by "GENEOUT gel electrophoresis" (LabGene). The V3-V4 variable region of bacterial 16S rRNA was amplified using primers 338F (5 -ACTCCTACGGGAGGCAGCAG-3) and 806R (5 -GGACTACHVGGGTWTCTAAT-3). PCR reactions contained 4 L 5× Fast Pfu buffer, 2 L 2.5 mmol/L dNTPs, 0.4 L each primer (5 mol/L), 0.4 L Fast Pfu polymerase, and 20 L ddH O. Annealing temperature was 55°C.

To ensure analytical accuracy and reliability, PCR used minimal cycle numbers while maintaining consistency across samples. Amplicons from the same sample were pooled, purified using AxyPrep DNA Gel Extraction Kit (AxyGen Biosciences, Union City, CA, USA), and quantified with the Quant-iT PicoGreen dsDNA Assay Kit (Promega, USA). Purified amplicons were mixed in equimolar amounts and sequenced on the Illumina MiSeq platform (Majorbio, Shanghai, China) using paired-end sequencing (2×300 bp) with a PhiX control library.

5. Data and Bioinformatics Analysis

One-way ANOVA was performed using SPSS 22 (SPSS, Chicago, IL, USA) to analyze the effects of rhizome connection status on soil enzyme activities and microbial biomass in shaded ramets. Data analysis and figure preparation were conducted using OriginPro 9 (OriginLab Corp., Massachusetts, USA).

Raw sequencing data were processed as follows: (1) Paired-end reads were extracted based on barcode sequences; (2) Low-quality bases (quality score <20) were truncated from the 3 end using a 50-bp sliding window; (3) Reads with primer mismatches, ambiguous bases, or reads shorter than 10 bp were removed; (4) Overlapping reads were merged into contigs with a minimum overlap of 10 bp and maximum mismatch ratio of 0.2 in the overlap region.

Quality-filtered contigs were analyzed using QIIME (version 1.17). To reduce computational redundancy, non-repetitive sequences were extracted and singletons removed. Sequences were clustered into operational taxonomic units (OTUs) at 97% similarity using uclust. Chimeras were identified and removed using UCHIME. Representative sequences were taxonomically classified against the Silva Release 119 database (https://www.arb-silva.de/documentation/release-119/). Rarefaction curves were generated using Mothur (v1.12.1) to assess sequencing depth, and Shannon diversity indices were calculated. Principal component analysis (PCA) was performed using the Vegan 2.0 package.

1. Soil Properties

In the acropetal transport group, shaded distal ramets with intact rhizomes exhibited significantly higher rhizosphere soil DOC, MBC, and MBN compared to severed controls. In the basipetal transport group, shaded proximal ramets with connected rhizomes showed significantly higher MBC and MBN, while DOC and MBC differences reached significant levels [Figure 2: see original paper].

Both acropetal and basipetal transport groups showed higher NAGase and urease activities in rhizosphere soil of shaded ramets with connected rhizomes compared to severed treatments. In the acropetal group, NAGase and urease activities in connected shaded distal ramets were significantly higher than in severed controls, though this significant difference was not observed in the basipetal group [Figure 3: see original paper].

Two-way ANOVA revealed that rhizome connection status, assimilate transport direction, and their interaction significantly affected microbial biomass and extracellular enzyme activities in shaded ramet rhizosphere soil. Specifically, DOC, MBC, MBN, NAGase, and urease activities were all significantly influenced by rhizome connection status, transport direction, and their interaction .

2. Soil Sample High-Throughput Sequencing Results

Illumina MiSeq sequencing of 12 soil samples yielded a total of 556,519 reads and 18,898 OTUs. Each sample library contained varying numbers of sequences. Rarefaction curves plateaued, indicating adequate sequencing depth [Figure 4: see original paper]. The curve shows that sample 2 (from basipetal treatment with connected shaded proximal ramet) differed substantially from other samples in OTU numbers, while samples 1, 12, 5, 6, 7, and 10 (from acropetal treatment with severed shaded distal ramets) showed similar OTU counts. Overall, differences in OTU numbers between rhizome connection treatments were minor and did not significantly contribute to variation among samples.

Shannon diversity index analysis revealed no significant effect of clonal integration on bacterial community diversity in the rhizosphere of shaded ramets .

Taxonomic assignment showed that bacterial OTUs across all samples belonged to 13 phyla and 415 genera, with relative abundances below 0.1% grouped as "others." *Proteobacteria*, *Chloroflexi*, and *Acidobacteria* dominated all samples. *Xanthobacteraceae*, which has nitrogen-fixing capabilities and includes the genus *Azorhizobium* (uncultured), was detected only in acropetal treatment samples (maximum relative abundance 2.99% in connected treatment, 2.05% in severed treatment). Another nitrogen-fixing genus, *Bradyrhizobium*, showed maximum relative abundances of 3.07% in acropetal and 1.17% in basipetal treatments.

Nitrosomonadaceae (uncultured), containing the ammonia-oxidizing genus *Ni-*

trosomonas, was present in all samples at low relative abundance (0.77-2.71%). *Nitrospira*, an important nitrite-oxidizing bacterium (NOB) in nitrogen cycling, was detected in all samples at 1.76–7.83% relative abundance. *Nitrospinaceae* (uncultured) showed 1.41-5.63% relative abundance in acropetal treatments and 1.77-11.80% in basipetal treatments [Figure 5: see original paper] and [Figure 6: see original paper].

Principal component analysis based on genus-level composition showed that regardless of transport direction, rhizome-connected and severed shaded ramets had similar bacterial community structures, with no significant separation between treatments [Figure 7: see original paper].

3. Discussion

The transport of photosynthates among clonal ramets has important ecological significance for clonal plant growth, particularly for plants in resource-heterogeneous patches. Previous studies have shown that ramets growing in favorable habitat patches translocate newly formed assimilates to ramets in less favorable patches, alleviating limitations in photosynthetic capacity or nutrient uptake. The higher DOC, MBC, and MBN observed in rhizosphere soil of connected shaded ramets in this study confirm the existence of clonal integration in *P. bissetii*.

Tree girdling experiments have shown that interrupting the transport of newly formed photosynthates to roots reduces rhizodeposition. The effect of clonal integration on labile carbon appears similar to girdling effects, as severed shaded ramets showed reduced DOC. A previous study on a stoloniferous herbaceous clonal plant reported similar results.

Soil extracellular enzymes produced by microbes regulate soil organic matter degradation, participating in processes such as hydrolysis, transfer, and mineralization. NAGase is involved in chitin hydrolysis and is an important source of organic carbon in soil, produced primarily by bacteria, fungi, and actinomycetes. Urease, produced by bacteria, filamentous fungi, and yeasts, plays a significant role in nitrogen mineralization. The increased microbial biomass (MBC, MBN) resulting from clonal integration likely contributed to enhanced activities of these two hydrolytic enzymes. The significant effects of rhizome connection status and transport direction on soil extracellular enzymes suggest that clonal integration directionality may differentially affect soil organic matter degradation, though underlying mechanisms require further investigation.

Principal component analysis is an important technique for analyzing environmental microbial community structure changes. Our PCA results showed that whether in acropetal or basipetal treatments, rhizome-connected and severed shaded ramets had similar bacterial community compositions at the genus level. This contrasts with previous research on the stoloniferous clonal plant *Glechoma longituba*, which found that clonal integration altered microbial community composition and promoted rhizosphere processes.

Increased carbon input can cause short-term changes in microbial activity and soil organic matter turnover. However, soil microbial communities appear to exhibit functional redundancy, where the reduction of specific microbial populations has minimal impact on particular soil processes because other microbes can perform the same functions. This functional redundancy may serve as an insurance strategy, maintaining system stability in fluctuating environments and conferring resilience to soil microbial communities in response to rhizosphere changes caused by clonal integration.

High-throughput sequencing offers greater breadth and resolution for diversity analysis compared to traditional fingerprinting techniques (DGGE, PLFA), enabling comprehensive analysis of microbial genetic diversity at the community level. However, the massive sequence datasets may obscure clear interpretation of community structure differences under different ecological processes, which may explain why clonal integration did not significantly affect rhizosphere bacterial community structure in this study. While high-throughput sequencing can comprehensively reflect overall bacterial diversity, the vast data may hinder clear elucidation of community structural differences.

The relationship between microbial diversity and soil ecosystem function depends on exploring connections between diversity and community structure, as well as between community structure and function. Targeting specific functional groups and combining quantification of low-abundance but functionally critical microbial populations with high-throughput sequencing may provide new avenues for microbial ecology research.

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