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Effect of clonal integration on nitrogen cycling in rhizosphere of rhizomatous clonal plant, *Phyllostachys bissetii*, under heterogeneous light

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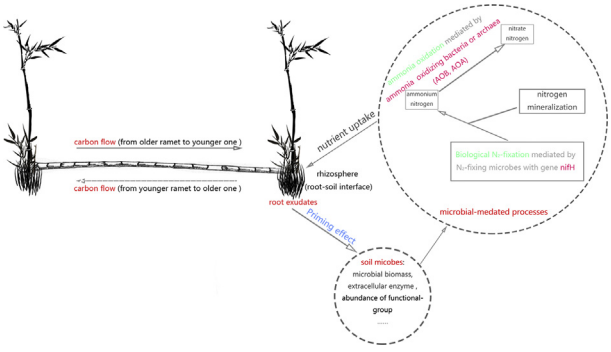
HIGHLIGHTS

- Resource sharing increased rhizospheric C availability and N turnover of offspring ramet.
- Negative effect of clonal integration on rhizospheric N availability was observed.
- Direction of assimilates transport exerted different influences on N turnover.
- Gene abundance correlated with soil enzyme was markedly affected by resource sharing.

GRAPHICAL ABSTRACT



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ABSTRACT

Clonal integration plays an important role in clonal plant adapting to heterogeneous habitats. It was postulated that clonal integration could exhibit positive effects on nitrogen cycling in the rhizosphere of clonal plant subjected to heterogeneous light conditions. An in-situ experiment was conducted using clonal fragments of *Phyllostachys bissetii* with two successive ramets. Shading treatments were applied to offspring or mother ramets, respectively, whereas counterparts were treated to full sunlight. Rhizomes between two successive ramets were either severed or connected. Extracellular enzyme activities and nitrogen turnover were measured, as well as soil properties. Abundance of functional genes (archaeal or bacterial *amoA*, *nifH*) in the rhizosphere of shaded, offspring or mother ramets were determined using quantitative polymerase chain reaction. Carbon or nitrogen availabilities were significantly influenced by clonal integration in the rhizosphere of shaded ramets. Clonal integration significantly increased extracellular enzyme activities and abundance of functional genes in the rhizosphere of shaded ramets. When rhizomes were connected, higher nitrogen turnover (nitrogen mineralization or nitrification rates) was exhibited in the rhizosphere of shaded offspring ramets. However, nitrogen turnover was significantly decreased by clonal integration in the rhizosphere of shaded mother ramets. Path analysis indicated that nitrogen turnover in the rhizosphere of shaded, offspring or mother ramets were primarily driven by the response of soil microorganisms to dissolved organic carbon or nitrogen. This unique in-situ experiment

Abbreviations: SOM, soil organic matter; TOC, total organic carbon; TN, total nitrogen; DOC, dissolved organic carbon; DON, dissolved organic nitrogen; MBC, microbial biomass carbon; MBN, microbial biomass nitrogen; N_{min}, nitrogen mineralization rate; N_{nitri}, nitrogen nitrification rate; NAGase, *N*-acetyl-β-D-glucosaminidase; POXase, phenol oxidase; PODase, peroxidase.

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provided insights into the mechanism of nutrient recycling mediated by clonal integration. It was suggested that effects of clonal integration on the rhizosphere microbial processes were dependent on direction of photosynthates transport in clonal plant subjected to heterogeneous light conditions.

1. Introduction

Resources for plant growth, including light, water and nutrients, are heterogeneously distributed within various habitats, which increases the difficulty for plants to capture essential nutrients (Hutchings and John, 2004; Jackson and Caldwell, 1996; Stark et al., 2017). Clonal plant rapidly expands into open habitat by forming inter-connected ramets, thus placing ramets in different environments (Janeček et al., 2008). Resource sharing among inter-connected ramets, referred to as clonal integration, plays an important role in clonal plant adapting to heterogenous habitats. (Saitoh et al., 2006; Zhang and He, 2009; Cornelissen et al., 2014).

Rhizosphere, affected by C and N availabilities from plant root exudates, is a zone of high microbial activity and turnover (Koranda et al., 2011; Lambers et al., 2009). Release of root exudates, such as sugars, organic acids, amino-acids, lipids and phenols, are a primary source of labile carbon inputs to rhizosphere (Jones and Darrah, 1994; Kuzyakov, 2002). As a major source of growth substrates and structural materials, labile carbon is metabolized by root-associated microorganisms (Kuzyakov and Cheng, 2001; Rajaniemi and Allison, 2009). Tree girdling, interrupting photosynthates flow to roots, significantly reduced plant C exudation and negatively influenced N turnover (N mineralization or nitrification rates) and extracellular enzyme activities in the rhizosphere of *Fagus sylvatica* and *Pinus contorta* (Chen et al., 2012; Dannenmann et al., 2009; Weintraub et al., 2007). Similarly, severing rhizome or stolon of clonal plant interrupted photosynthates flow from illuminated ramets to shaded ones. Based on phospholipid fatty acids method (PLFA), rhizosphere microbial community composition of stoloniferous herb *Glechoma longituba* was significantly altered by clonal integration under heterogeneous light conditions (Lei et al., 2014). As biomarkers for main groups of soil microorganisms, PLFA analyses provided limited taxonomic resolution, which did not allow us to identify the specific functional groups that shift in abundance (Chen et al., 2015; Rousk et al., 2010).

Generally, microbial functional groups play a crucial role in soil N cycling processes such as N_2 fixation and nitrification (Ma et al., 2016). Quantification of functional genes can serve as a good predictor of microbial functional group, since the calculation of gene abundance is based on a theoretical single copies number per microorganism (Petersen et al., 2012). Alterations of microbial functional group would provide important and direct information about biogeochemical cycling in the rhizosphere (Malchair and Carnol, 2013; Hawkes et al., 2005). In addition, degree of clonal integration varies among different clonal species and life phases (Cornelissen et al., 2014). So, further studies are needed to extend our understanding on effects of clonal integration on soil C and N cycling in the rhizosphere of clonal plant.

Phyllostachys bissetii is a perennial woody clonal plant with ramets connected through belowground rhizomes. An in-situ experiment was conducted using clonal fragments of *Phyllostachys bissetii* with two successive ramets. Shading treatments were applied to offspring or mother ramets, respectively, whereas counterparts were subjected to full sunlight. Rhizomes between two successive ramets were either severed or connected. Compared to severing rhizomes, we predicted that 1) rhizospheric C and N availabilities of shaded ramets were significantly increased when rhizomes were connected; 2) rhizospheric microbial biomass of shaded ramets was significantly increased when rhizomes were connected; 3) higher extracellular enzyme activities and greater N mineralization or nitrification rates were observed in the rhizosphere of shaded ramets when rhizomes were connected. Finally, compared to severing rhizomes, we also expected higher *nifH* and *amoA* abundance in the rhizosphere of shaded ramets when rhizomes were connected.

2. Materials and methods

2.1. Study species

Phyllostachys bissetii is a perennial and monocarpic woody clonal plant which can form linearly and monopodially growing rhizomes. *P. bissetii* stands consist of a large number of ramets produced from active nodal buds on the rhizomes. Rhizome and root of *P. bissetii* can last for many years, forming dense and multilayered underground networks. As staple food of panda, *P. bissetii* is mainly distributed in Sichuan and Zhejiang Province, China.

2.2. Experimental design and soil sampling

The experiment was carried out in a bamboo stand, where *P. bissetii* was a dominant species. The study site is located in Qionglai City, Sichuan Province, China (longitude 103°14′14″E, latitude 30°14′31″N), with the altitude of 1217 m above the sea level. Soil texture (clay loam) is uniform across the site. The mean annual air temperature is approximately 16.3 °C and the average annual precipitation amounts to 1117.3 mm.

The experiment was initiated in January 2015, setting up as a randomized block design with rhizome connection status (connected vs. severed) and photosynthates transport direction (acropetal vs. basipetal). Ten blocks (16 m^2) were established. Each block consisted of four clonal fragments of *P. bissetii*. Each clonal fragment included two successive ramets. The height and diameter of ramet was about 3 m and 2 cm, respectively and the length of rhizome between inter-connected ramets was about 50 cm. Each block was spaced out at least 100 m (Wang et al., 2017). Older ramets in each clonal fragment were referred to as mother ramets and the younger ones as offspring ramets. Previous studies indicated that assimilates were predominantly moved acropetally (towards developmentally younger ramets) in several clonal species, and to a much lesser extent in a basipetal direction (towards relatively older ramets) (Marshall, 1990; Pitelka and Ashmun, 1985; Stuefer, 1996). The experiment included two groups: an acropetal group (photosynthates transporting to younger ramets) and a basipetal group (photosynthates transporting to older ramets).

Shade cages (3.5 m in height) were covered with shade cloth, which transmitted approximately 20% of ambient photosynthetically active photon flux density (PPFD). In acropetal group, offspring ramet in each clonal fragment was in 80% shading and mother ramet was in full sunlight. In basipetal group, mother ramet in each clonal fragment was in 80% shading and offspring ramet was in full sunlight. Rhizomes between two successive ramets were either severed or connected. Soil block (50 cm × 50 cm) was established around each shaded offspring or mother ramet, respectively. At a depth of 50 cm, the soil blocks were respectively isolated using excavation and surrounded by polythene and PVC to prevent water and nutrient diffusion from ambient soil (Fig. 1). The top of each soil block was covered with mesh to avoid potential effects of litter decomposition.

Our experiment aimed to explore effects of photosynthates from illuminated ramets on microbial-mediated processes in the rhizosphere of shaded ones. So, rhizosphere processes of shaded ramets only were taken into consideration. Bulk soil, defined as soil not directly attached