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Seasonal and clonal variations of microbial biomass and processes in the rhizosphere of poplar plantations

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ABSTRACT

Plant nutrient acquisition and dynamics are closely coupled with microbial activities and rhizosphere processes. This study was conducted using an *in situ* rhizobox approach and incubation experiments, with the primary goal to elucidate rhizosphere effects of different poplar clones at different sampling seasons on soil microbial processes and biomass content. Evaluations included pH, inorganic N (NO_3^--N and NH_4^+-N), microbial biomass carbon (MBC) and nitrogen (MBN), enzyme activities related to N cycling, as well as net N mineralization and nitrification rates in rhizosphere soils of three poplar clones (Nanlin-895, NL-80351 and 75) at three periods of the year. The magnitude of rhizosphere effects varied with time and poplar genotypes. Significant variations in microbial biomass content and enzyme activities were observed among the tested three poplar clones; and microbial biomass content was positively correlated with soil NO_3^--N concentration. The obtained results suggested that genotypes (clones or cultivars) had profound impacts on the rhizosphere microbial communities; and NO_3^--N concentration rather than total inorganic N was the primary determinant of seasonal dynamics of microbial biomass and processes in the rhizosphere soils of poplar trees in a seasonally flooded soil.

1. Introduction

The rhizosphere is a zone characterized by high microbial activity, and is clearly distinct from bulk soil with regard to pH, redox potential and availability of nutrients, water and oxygen (Hinsinger et al., 2009). It has long been recognized that tree species can exert a strong influence on rhizosphere nutrient cycling through root and rhizosphere processes and create feedback in the patterns of nutrient cycling in forest ecosystems (Lambers et al., 2009). Several studies investigated the influence of plant roots on specific biochemical and microbial processes, such as soil enzyme activities (Fang et al., 2010; Kaiser et al., 2010; Weintraub et al., 2007), nitrogen (N) mineralization (Norton and Firestone, 1996), nitrification and denitrification (Priha et al., 1999). However, limited efforts have been made to link rhizosphere processes with soil processes, with even less being done to address the importance of spatially and temporally dynamic processes in the rhizosphere of different plants or different genotypes of the same plant species. Results reported in the literature are inconsistent (Cheng, 2009), but demonstrate close relationships between soil processes and soil conditions, as well as tree species (Zhao et al., 2010). Available N and P levels have been found to accumulate (Turpault et al., 2005), diminish (Chen et al., 2002) or remain unchanged (Ehrenfeld et al., 1997) in the rhizosphere when compared to bulk soils. Rhizosphere processes have been recognized as the most important but least understood soil processes that impact nutrient cycling and plant growth (Cheng, 2009).

Microbial biomass, an important labile pool of nutrients in soil (Henrot and Robertson, 1994), plays a significant role in nutrient transformation and ecosystem conservation under tropical and temperate climates (Wardle, 1998; Smith and Paul, 1990). Turnover of microbial biomass influences the amount of N being stored or released from, and thus availability of N for plant uptake (Wardle, 1998; Vitousek and Matson, 1984). Seasonal variations in soil temperature and moisture directly control temporal fluctuations in microbial biomass and activity in ecosystems (Bell et al., 2008; Corre et al., 2002; Vangestel et al., 1992). However, studies of seasonal effects on microbial biomass in rhizosphere soils have often produced inconsistent results. Marschner et al. (2002) observed high microbial biomass in rhizosphere soils during the wet season (late fall and early winter) under polyculture agroforestry systems in Central Amazonia. Other studies showed that soil microbial biomass were high in spring and fall but low in summer and winter (Luizao et al., 1992; Sarathchandra et al., 1984).

Tree species vary with respect to root morphology and physiology, as well as nutrient requirements (Jones et al., 2004; Wang et al.,

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2001). Rhizosphere effects, therefore, vary with tree species (Garcia et al., 2005) and tree species composition (Fang et al., 2013). Knowledge of rhizosphere effects on nutrient cycling associated with different species is fundamental for characterizing nutrient acquisition of different tree species and for interpreting the influence of tree species on soil processes. Unfortunately, only a few attempts have been made to understand rhizosphere nutrient cycling under different tree species (Phillips and Fahey, 2006; Kuzyakov, 2002; Wang et al., 2001) or different genotypes of the same tree species. Therefore, field studies on rhizosphere nutrient cycling under different tree species and genotypes may provide a more realistic view of rhizosphere processes. Using an *in situ* rhizobox approach, this study was conducted with the primary objective to elucidate rhizosphere effects of different poplar clones on soil microbial processes and soil microbial biomass in seasonally flooded soils sampled at different periods of the year. We hypothesized that the magnitude of rhizosphere effects would vary with sampling periodtime and genotype due to differences in intrinsic biological characteristics and environmental conditions.

2. Materials and methods

2.1. Site description

The study site (31°41′N, 118°26′E) is located at the eastern lower reaches of the Yangtze River near Ma-an-shan City in Anhui Province, southeast China. Seasonal flooding at this site lasts for about one month in summer each year. The area has a humid subtropical climate with an average growing season of 234 frost-free days. Mean annual precipitation is about 1023 mm, which is mostly distributed in the period from July to September. Mean annual temperature is 15.8 °C, ranging from −8 °C to 37.2 °C.

Historically, the site was a floodplain by the Yangtze River and was covered with common reed (*Phragmites australis*). In 2007, the site was reconstructed to improve drainage and establish plantations (Liu et al., 2012). Currently, soil bulk density and organic matter content at 0–60 cm depth are 1.38 g cm^{-3} and 19.0 g kg^{-1}, respectively. Total N, phosphorus (P) and potassium (K) are 1.93, 0.40 and 6.96 g kg^{-1}, respectively.

2.2. Plantation establishment

Three poplar clones (Nanlin-895, NL-80351, and 75) were selected for use in this study. Nanlin-895 is a hybrid of I-69 (*Populus deltoides* Bartr. cv. 'Lux') × I-45 (*P. euramericana* (Dode) Guinier cv. 'I-45/51'); NL-80351 is a hybrid of I-69 × I-63 (*P. deltoides* Bartr. cv. 'Harvard'), and 75 is a hybrid of I-72 (*P* × *euramericana* (Dode) Guinier cv. 'San Matino' ex I-72/58) × I-63. One-year-old rooted seedlings were planted in March 2008 with spacing 3m × 5 m. The average survival rate one year after planting was about 97%.

2.3. Rhizosphere study technique

Rhizoboxes were made of two attached PVC cylindrical compartments that were separated by a 100 μm thick polyamide filter with a 30 μm pore diameter (PA6366, 150-30WPW, Tiantai, Zhejiang, China). The upper compartment volume was 265 mL, while the lower compartment was 408 mL (Liu et al., 2012). The two compartments were packed with alluvia soil at a bulk density of 1.19 g cm^{-3}. The soil was collected from about 10–15 cm depth near the respective trees to which the rhizoboxes were later installed. Prior to use, the soil was sieved through a 2.2-mm sieve and stored at 4 °C. After packing, the top opening of the upper compartment was covered with a 1 mm plastic mesh which allowed trees roots to grow. The bottom of the lower compartment was covered with a 30 μm polyamide filer to prevent other plant roots from penetrating the rhizobox (Liu et al., 2012).

Twenty trees for each poplar clone were selected for installing the rhizoboxes. To meet typical root growth habit, the rhizoboxes were placed at 40–60 cm from the tree trunk with a 33° angle (Liu et al., 2012). Top of the rhizoboxes was 20–30 cm below the surface. All rhizoboxes were installed in early April 2010, and retrieved in late October 2010, late March 2011, and mid July 2011, respectively. Six rhizoboxes were retrieved for each poplar clone at each sampling time.

2.4. Sampling of soils and root mats

After the rhizoboxes were retrieved from the field, the two compartments were separated and the mesh was carefully removed. The rhizosphere soils packed in the lower compartment were then slowly pushed out. Based on results from our previous study (Liu et al., 2012), soils 0–4 mm from the root mats (i.e. Polyamide filer) were sampled as rhizosphere soils. Each sample was divided into two parts: one part was air dried for analysis of soil chemical properties and for the incubation experiments; the other was stored at 4 °C for an analysis of biological properties.

All root mats in each rhizobox were carefully collected from the upper compartments, washed with water, and dried to a constant weight at 65 °C in a drying oven. Biomass of root mats in rhizobox was determined as oven-dry basis weighted mass.

2.5. Chemical analysis

Soil pH was measured in a soil–water suspension (1:5 w/v) using an automatic acid–base titrator (SH/T0983, Zhejiang, China). Soil inorganic N (NH_4^+-N and NO_3^--N was extracted with 2 M KCl (1 g of soil to 5 mL KCl solution ratio), and quantified colorimetrically with an auto analyzer (Auto Analyzer III, Bran + Luebbe GmbH, Germany) (Fang et al., 2007).

2.6. Nitrogen mineralization and nitrification

Fresh soils were pre-incubated at 60% of water holding capacity (WHC) prior to the evaluation of N mineralization based on the differences in KCl extractable N with or without 30 days incubation (Zhang et al., 2010). Briefly, 5 g (oven-dry equivalent of field-moist soil) of pre-incubated soil was placed in a 25 mL glass beaker, and six beakers were prepared for each sample. Three were extracted with 25 mL 2 M KCl immediately; and three were incubated in the dark for 30 days at 25 °C in an incubator chamber (DYX-DHS-40 × 50, Zhejiang, China). During incubation, soil moisture was checked every 2–3 days by weight and restored to 60% WHC by adding ultra-pure sterilized water. After incubation, soil samples were transferred to 50 mL PVC tubes, followed with the addition of 25 mL 2 M KCl solution. The suspensions were then shaken for 1 h at 25 °C and filtered through No. 203 quantitative filter paper (Jiangsu, China). Concentrations of NH_4^+-N and NO_3^--N in the filtrates were measured colorimetrically with an auto analyzer as described above.

The potential net N mineralization rate was calculated based on the difference in the sum of (NH_4^+-N + NO_3^--N) before and after incubation. The potential net nitrification rate was calculated based on the difference in NO_3^--N concentrations before and after incubation.

2.7. Microbial biomass and enzyme activities

Microbial biomass carbon (MBC) and microbial biomass nitrogen (MBN) were measured using a fumigation–extraction method (Vance et al., 1987). Briefly, 5 g of oven-dry equivalent of field-moist