



A long-term hybrid poplar plantation on cropland reduces soil organic carbon mineralization and shifts microbial community abundance and composition



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ABSTRACT

Poplar plantations have been established around the world to provide timber or fuelwood and to control erosion in degraded areas. The objective of this study was to investigate the effects of converting croplands to long-term hybrid poplar (*Populus × euramericana* cv. I-72) plantations for 10, 15 and 20 years on soil bacterial and fungal communities and on their relationships with soil organic carbon (SOC) mineralization. Overall, the results indicated that the long-term hybrid poplar plantations increased soil pH, SOC, total N and moisture contents and decreased dissolved organic carbon (DOC), NH_4^+ and NO_3^- contents compared to that on the cropland. There were lower cumulative amounts of CO_2 respired and SOC mineralization rates in the hybrid poplar plantation soils compared to that of the cropland. The hybrid poplar plantation with 20 years caused a greater fungal internal transcribed spacer (ITS) gene copy number and a lower bacterial 16S rRNA gene copy number and dehydrogenase and β -glucosidase activities compared to that in the cropland soils. As indicated by Illumina MiSeq sequencing, the establishment of hybrid poplar plantations harbored distinct soil bacterial and fungal communities, which were strongly correlated with specific soil properties. We further found that the SOC mineralization rate was positively correlated to the bacterial abundance and the relative abundances of Actinobacteria and Bacteroidetes, whereas negatively correlated to the fungal abundance. This study suggested that the long-term hybrid poplar plantation reduced SOC mineralization, causing changes in habitats that favor fungal community growth and shifts in bacterial community composition to more facultative and/or obligate anaerobes and less microbes that are capable of decomposing recalcitrant carbon, which may help enhance SOC accumulation in soil and mitigate climate change.

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1. Introduction

Because poplar (*Populus spp.*) grows quickly and has a short rotation cycle (usually less than 20 years) and high yield, it is an important bioenergy crop (Calfapietra et al., 2010). Poplar plantations have been established around the world, especially

in mid-latitude plains, to provide timber or fuelwood and to control erosion in degraded areas. With its fast response to environmental changes and its growing importance among managed forests for its impacts on the global carbon balance, poplar has been demonstrated to have an important impact on environment protection and sustainability (Gielen and Ceulemans 2001; Dhondt et al., 2004). In China, more than 7 million ha of poplar plantations were established by 2007, widely distributed in temperate regions, such as the Sanjiang Plain, Yellow River, Huai River region, Jianghuai Plain and Inner Mongolia Plateau (Fang, 2008). Croplands have frequently been converted to poplar plantations for obtaining greater economic benefits.

Converting cropland to tree plantations is known to affect soil organic carbon (SOC) sequestration (Guo and Gifford, 2002). Zhang

Abbreviations: SOC, soil organic carbon; MBC, microbial biomass carbon; MBN, microbial biomass nitrogen; DOC, dissolved organic carbon; ITS, internal transcribed spacer.

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et al. (2007) observed that conversion of cropland to spruce plantations for more than 40 years could enhance SOC storage. While a few studies reported that conversion of cropland to pine forest or tree plantations could decrease SOC stocks, particular for the first few years (Parfitt et al., 1997; Ross et al., 1999). As a typical afforestation practice, few literatures reported the changes of SOC under poplar plantations converted from cropland soils (Hu et al., 2013). In a recent study, lower soil respiration was observed in 15-year-old poplar forests than in 5- or 10-year-old plantations from a forest soil in northwestern China, which indicated that poplar plantations resulted in lower soil carbon loss (Gong et al., 2012). In an investigation, Hu et al. (2013) suggested that converting croplands into poplar stands increased C sequestration in surface nutrient-poor mineral soil by comparing a number of adjacent croplands in semiarid region in northeast China. So far, however, the effect of long-term poplar plantations on dynamics of SOC and its potential mechanism is still poorly understood, particularly in warm temperate regions where poplars are widely planted.

Soil microorganisms regulate the multiple input and loss pathways of soil carbon (Kennedy, 1999). The abundance, structure and activity of soil microbial communities play important roles in SOC mineralization and sequestration (Six et al., 2006). Tree plantations on cropland or afforestation were reported to influence soil microbial community and subsequent ecosystem sustainability (Ding et al., 1992; Lauber et al., 2008). For instance, Zhang et al. (2007) found that conversion of cropland to long-term spruce plantations could increase the abundance and diversity of microbial communities with increasing amounts of SOC. Nevertheless, little is known about the effect of long-term poplar plantations on soil microbial communities. Therefore, this study aims to investigate the impacts of converting cropland to long-term poplar (*Populus × euramericana* cv. I-72) plantations on soil physico-chemical properties, abundance, composition and activity of bacterial and fungal community and their relationships with SOC accumulation.

2. Materials and methods

2.1. Site and soil sampling

This study was conducted at the Chenwei forest farm (39°15'N, 118°18'E) located in Sihong County, Jiangsu Province. The local climate conditions were governed by a warm temperate continental monsoon climate with a mean annual temperature of 14.1 °C and an annual precipitation of 897 mm. The total number of sunlight hours per year averages 2327, with 197 frost-free days per year. The soil is a silty clay loam (33% sand, 41% silt and 26% clay) derived from lacustrine deposits from Hongze Lake and is classified as an Alfisol. Previously, the land was mainly used for agricultural crops (i.e., wheat, peanut and soybean). A portion of the land (30 ha) was planted with poplar (*Populus spp.*) in 1970. The poplar plantations were manually planted with 1-year-old seedlings in 1994, 1999 and 2004. The soil was tilled before planting by using a disk pulled by a tractor. Plants were spaced at 4 m × 7 m, and the planted poplar clone was Nanlin-95 (*Populus × euramericana* cv. I-72). Fertilizer, mainly nitrogen-based fertilizer applied at a rate of 250 g N/plant/year, was applied during the first 2–4 years of the growth stage.

Following a space-for-time substitution procedure, four different hybrid poplar stand age-classes were categorized: (1) ten-year-old stand (10YR), (2) fifteen-year-old stand (15YR) and (3) twenty-year-old stand (20YR), and (4) cropland soil (Control). The experimental design was a randomized complete block design with three replications laid out on the similar soil type and with consistent management. During the spring and summer, the soil surface between the rows was mainly occupied by annual

vegetation. In the autumn, poplar leaves were found on the soil surface. At the end of March 2014, the average heights of the 10-, 15- and 20-year-old poplars were 18 m, 20 m and 24 m, while the diameters at breast height were 93 cm, 98 cm and 102 cm, respectively. Local cultivars of wheat and corn were used in rotation in the cropland treatment since 1994 using a conventional tillage and inorganic fertilization (250 kg N ha⁻¹, 60 kg P₂O₅ ha⁻¹ and 80 kg K₂O ha⁻¹) practices.

A composite soil sample at depths of 0–15 cm was collected in March from each poplar stand after removing the litter layer and cropland before tillage and application of fertilizers. The composite samples were transferred to sterile plastic bags, sealed and placed on ice for transport to the laboratory for preparations using standard procedures. One sub-sample (50 g) was frozen at –70 °C for molecular analysis, one was further air-dried at room temperature and passed through a 0.25-mm sieve before soil chemical analysis, and another one was stored at –4 °C for later soil biological analysis.

2.2. Analysis of soil properties, microbial biomass and enzyme activity

All the analysis of soil properties was conducted according to the protocol described by Lu (2000). The soil pH was determined using a soil-to-water ratio of 1:2.5. The soil organic carbon (SOC) and total nitrogen (TN) contents were determined via wet digestion using K₂Cr₂O₇ oxidation and the Kjeldahl method, respectively. The bulk density of the topsoil was measured at each site using 100-cm³ cylinders. Ammonium (NH₄⁺) and nitrate (NO₃⁻) contents were assayed using Nessler's reagent and the phenol disulfonic acid colorimetric method, respectively (Hart et al., 1994). Soil dissolved organic carbon (DOC) was measured according to Chen et al. (2013). The chloroform fumigation-extraction protocol with K₂SO₄ extraction was employed to determine the soil microbial biomass C (MBC) and N (MBN) using 15-g oven-dry equivalent field-moist soil (Vance et al., 1987). The dehydrogenase β-glucosidase activities were assayed as described by Chen et al. (2013). The enzyme activities were expressed as products per unit dry weight of soil and incubation time.

2.3. Analysis of SOC mineralization

SOC mineralization was determined according to the method described by Zheng et al. (2007) with minor modifications. Briefly, 20-g (fresh weight) samples were moistened to 60% of their water-holding capacity and incubated under aerobic conditions at 25 °C in the dark for 28 days in a 125-mL jar. A 2-mL gas sample was collected from the headspace of the jar using a gas-tight syringe daily during the first 10 days and every other day following the eleventh day of incubation. The CO₂ concentration was analyzed using a gas chromatograph (Agilent 7890A) equipped with a flame ionization detector.

2.4. DNA extraction and real-time PCR assay

DNA extractions from 0.5 g of soil from each sample were performed using the PowerSoilR DNA Isolation Kit (Mo Bio Laboratories Inc., CA) according to the manufacturer's instructions.

The abundances of total bacteria and fungi were quantified using real-time PCR based on the 16S rRNA gene (primer set 338F/518R) and the internal transcribed spacer (ITS) gene (primer set 5.8s/ITS1F; Fierer et al., 2005), respectively. All samples were evaluated in triplicate following the protocol described by Chen et al. (2013). The reaction was performed in an iCycler IQ5 thermal cycler (Bio-Rad, Hercules, CA) by fluorometric monitoring with SYBR Green 1 dye. No-template controls were included in the assays. A triplicate 10-fold series containing known amounts of

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