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Soil bacterial community responses to long-term fertilizer treatments in *Paulownia* plantations in subtropical China

Jia Tu[a,b,c,d], Jie Qiao[e], Zhiwen Zhu[f], Peng Li[a,c,d], Lichao Wu[a,c,d,*]

[a] Central South University of Forestry and Technology, Key Laboratory of Cultivation and Protection for Non-Wood Forest Trees, Ministry of Education, Shaoshan South Road, No. 498, Changsha 410004, Hunan, China
[b] Hunan Academy of Forestry, Shaoshan South Road, No. 658, Changsha 410004, Hunan, China
[c] Central South University of Forestry and Technology, Key Laboratory of Non-wood Forest Products of State Forestry Administration, Shaoshan South Road, No. 498, Changsha 410004, Hunan, China
[d] Central South University of Forestry and Technology, Cooperative Innovation Center of Cultivation and Utilization for Non-Wood Forest Trees of Hunan Province, Shaoshan South Road, No. 498, Changsha 410004, Hunan, China
[e] Paulownia Research and Development Center of State Forestry Administration, Zhengzhou 450003, Henan, China
[f] Xiangyin Forestry Agency, Fourteen East Lake Community Groups, Wenxing Town, Xiangyin 410051, Hunan, China

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ABSTRACT

The use of fertilizers to overcome nutrient constraints is standard practice in *Paulownia fortunei* plantations to increase stem wood production and tree yields. However, little is known about the responses and important role of the soil bacteria involved in the increases of soil fertility and standing volume with different fertilization. The 16S rRNA was used to compare four different fertilizer treatments for soil bacterial communities in three *P. fortunei* plantations, in Hunan Province, China. The results showed that the soil bacteria richness was significantly higher in plantations fertilized for ten years than for shorter times. The phyla *Acidobacteria* and *Gemmatimonadetes* were more prevalent for ten-year old plantations, and *Firmicutes* significantly increased with organic manure treatment (denoted as OM; $p < 0.05$). OM significantly increased the ratio of *Bacilli*-like sequences in *Firmicutes* to 87%, 97% and 78% in seven-, nine- and ten-year-old plantations, respectively ($p < 0.05$), while control and inorganic fertilizer had no effect. Principal coordinate analysis revealed that the soil bacterial communities in OM were different from the control and inorganic fertilizer in the three different plantations. Canonical correspondence analyses showed that available phosphorus and available magnesium were the most strongly related to bacterial community composition. The addition of OM led to soil being more fertile and an increased standing volume compared with the other fertilizer treatments. *Bacilli*, the most abundant class of *Firmicutes* may represent the bacterial type that responded most distinctly to organic manure fertilization and could be highly effective bacteria to increase soil fertility and achieve sustainable development of *P. fortunei* plantations.

1. Introduction

Broad-leaved *Paulownia fortunei* (*Seem.*) *Hemsl.* is one of the three fastest growing timber tree species in the world and its wood is excellent material for manufacturing plywood, furniture and paper. There are large areas of commercial *P. fortunei* forest in subtropical and warm temperate regions, and there is great potential in mid-subtropical areas, but this requires adequate nutrients to support rapid growth (Wu et al., 2014). The use of fertilizers to overcome nutrient constraints to increase stem wood production is standard practice for increasing tree yields in many forest ecosystems (Albaugh et al., 2014). However, the type and quantity of fertilizer depends on the volume of timber growth required. Long-term application of mineral fertilizer may decrease microbial biomass and enzyme activities (Zhong et al., 2010), modify the composition of microorganism communities (Chu et al., 2007), lead to soil acidification (Steiner et al., 2007) and decrease soil organic matter (SOM) content (Zhong et al., 2010). The long-term application of organic fertilizer can improve soil quality (Reeves, 1997), and manure increases SOM (Li et al., 2007), microbial biomass and metabolic ability of microorganisms (Chang et al., 2007). Microorganisms can respond to changing environmental conditions by modifying community composition, and microbial diversity is a potentially valuable indicator of soil health and quality (Singh et al., 2011). Bacteria are the most abundant and diverse group of soil microorganisms (Fierer and Jackson, 2006)

* Corresponding author at: College of Forestry, Central South University of Forestry and Technology, Changsha, China.
E-mail address: wulichao@sina.com (L. Wu).

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and are the foundation of terrestrial ecosystems, regulating biogeochemical nutrient processes and playing vital roles in forest systems by decomposition of plant and animal organic matter for plant growth – they have essential roles in forest productivity by changing soil structure and fertility (Barton and Hamilton, 2007). It has long been recognized that study of the bacteria community structure in forest systems could effectively guide fertilization (Bååth et al., 1995). Hence, it is necessary to study the diversity and structure of the bacterial community with long-term application of different fertilizers in *P. fortunei* plantations. This research could guide the development a kind of highly effective fertilizer perhaps including inorganic, organic and microbial components to decrease consumption of chemical fertilizers and achieve sustainable development of *Paulownia* plantations.

Our previous study on soil microbial numbers in *P. fortunei* plantations relied on cultivation of bacteria on artificial media (Tu et al., 2017), and resulted in the formation of colonies by only 1.5–10% of the bacterial species in soil, which are not sufficient to describe information on soil microorganisms, because only a few microorganisms can be analyzed (Janssen, 2006). Additionally, gradient gel electrophoresis and terminal restriction fragment length polymorphism are also usually unable to detect rare species in complex environmental samples (Young et al., 2017). In contrast, high-throughput sequencing, as a next-generation sequencing technology, can vastly expand our understanding of the microbial world (Lee et al., 2011). The recently developed Illumina MiSeq platform can detect changes in low-abundance species with greater throughput but lower cost (Gołębiewski et al., 2014). However, information on Illumina MiSeq sequencing of soil bacterial communities of *Paulownia* plantations is still very limited.

Our objective was to determine the long-term effects of four different fertilizer treatments on soil bacterial diversity of *P. fortunei* plantations, and analyze the dominant groups and the relationships between soil parameters and diversity, to guide fertilizer use for *P. fortunei* plantations. Specifically, we addressed the following questions. (1) Do the bacterial communities in *P. fortunei* plantations differ due to long-term different fertilizer use? (2) Which bacterial species respond most distinctly to the organic manure (OM) fertilization that increases soil fertility and the standing volume compared to other fertilizer treatments?

2. Material and methods

2.1. Study area

The sampling sites were located in Hunan Province, China (28°32′40″–28°41′40″N,112°56′21″–112°58′44″E), in a basin surrounded by the Lohsiao, Nanling and Xuefeng Mountains from east to west (Fig. 1), in areas characterized by a humid, continental and subtropical monsoon climate. The soil texture, pH, bulk density and other key characteristics were similar before silviculture (Table 1). There are distinct seasonal climate changes, with a cold winter and a hot summer. The three study areas were waste land and originally covered with weeds and shrubs before use as *Paulownia* plantations. The *Paulownia* plantation rotations are 10–15 years.

2.2. Plot design and fertilization treatments

Geographical positions of the three *P. fortunei* plantations in Hunan Province selected as sampling sites were recorded using a handheld GPS. The plantations had three different ages: Yan Jiapu (Y), Zhang Jiacun (Z) and Huai Xicun (H) of seven, nine and ten years old, respectively. The experiment consisted of 12 plots (20 m × 20 m) of four fertilization treatments for the three plantations in a randomized complete block design (Table 1). The four treatments were: (1) soil without fertilizer in Y1, Z1 and H1 (control, CK); (2) OM treatment in Y2, Z2 and H2 contained 27.5% OM, 1.77% N, 5.13% P_2O_5 and 1.89% K_2O, applied at 1.25 t ha^{-1}; (3) inorganic fertilizer treatment (NPK) of (all kg ha^{-1}) 187.5 N, 187.5 P_2O_5 and 187.5 K_2O in Y3, Z3 and H3; and (4) inorganic fertilizer added with micronutrients (NPKM) of (all kg ha^{-1}) 187.5 N, 187.5 P_2O_5, 187.5 K_2O, 12.5 agricultural lime, 12.5 magnesium sulfate in Y4, Z4 and H4. The OM was chicken and cattle manure, composted by regular turning over a four-month period before application. Fertilizers were added into a ditch (25 cm depth) and 80–100 cm from the trunks. The field experiment treatments originated from the silviculture that was applied in each plantation. The fertilizers were applied during March of each year.

2.3. Soil sampling and processing

Soil sampling for this study was performed in September 2014 after the rainy season. The uniformity of the soil and land cover was based on visual examination of the plantation. In each plot, 10 sub-plots were selected and unusually dry or wet areas and highly compacted areas were avoided. Composite surface soil samples (0–15 cm depth) with use of a soil core 45 mm in diameter were collected from each plot where tillage depth was > 50 cm, which is the standard silvicultural practice for *P. fortunei* plantations. Each sample was air-dried in the shade, then ground to pass through a 2-mm sieve and used for determination of chemical properties. Samples for DNA extraction were stored on ice in the field and then immediately transported to and stored at −80 °C in a laboratory.

2.4. Soil chemical properties and standing volume analyses

The chemical components of soil quality were assessed: pH in water (Kader et al., 2015), nitrate-N according to Liu et al. (2005). Available potassium (AK) by the Mehlich 3 method (Bond et al., 2006), and flame photometry detection. SOM was assessed using the dichromate wet combustion method and a visible spectrophotometer (Van Gaans et al., 1995). Available calcium (ACa) and magnesium (AMg) were measured using the Mehlich 3 method and a Smartchem 200 (Bond et al., 2006). Available phosphorus (AP) using the hot water extraction method, the calcium phosphate solution method and the Mehlich 3 method and a Smartchem 200 (Daniels et al., 2001).

Bergmann (1998) studied *P. fortunei* plantations in Heze and measured diameter at breast height, tree height and the standing volume of 442 trees. The best regression equation Eq. (1) with a correlation coefficient (R = 0.973) was determined using regression analysis. After comparing the theoretical result for standing volume with Eq. (1), the relative error for the observed standing volume was less than 0.5%. Therefore, we calculated standing volume using Eq. (1):

$$V = 1.367857 \times 10^{-4} \times D^{1.666776} \times H^{0.893626} \quad (1)$$

where V is standing volume (m^3) of one tree, D is diameter at breast height (cm), and H is tree height (m).

2.5. Soil bacterial DNA extraction, amplicon library preparation and sequencing

Thirty-six samples were randomly chosen from the fertilizer treatments and used for DNA extraction. DNA was extracted from 0.5 g of each soil sample using a Fast DNA SPIN Kit for soil (MP Biomedicals, Santa Ana, CA, USA), according to the manufacturer's protocol. The extracted soil DNA was quantified using a NanoDrop ND-2000c UV–vis spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA).

To determine the diversity and composition of the bacterial communities in each sample, we used the PCR protocol of Caporaso et al. (2011). The bacterial 16S rDNA V4 region was amplified using custom 515F-806R primer. The PCR were carried out in 30 μL reactions with 15 μL of Phusion® High-Fidelity PCR Master Mix, 0.2 μM of forward and reverse primers, and about 10 ng of template DNA. Thermal cycling consisted of initial denaturation at 98 °C for 1 min; followed by 30