



Alteration of the soil bacterial community during parent material maturation driven by different fertilization treatments



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ABSTRACT

Soil parent materials are potential arable land resources that have great value for utilization. Soil bacteria play vital roles in soil formation, and soil parent material provides the basic nutritional environment for the development of the microbial community. Due to the extremely limited available nutrients in most parent materials, fertilization management is important for providing necessary available nutrients and for enhancing the maturation process of the parent materials. After 30 years of artificial maturation driven by different fertilization treatments, the soil development of three different parental materials was evaluated, and the bacterial community compositions were investigated using a high-throughput nucleic acid sequencing approach. The results showed that fertilization management increased the soil fertility and microbial biomass and enhanced soil parent material maturation compared with cultivation alone. Supplying available nutrients via chemical fertilization was more effective than cultivation alone for soil nutrient accumulation, microbial biomass promotion, and copiotrophic bacterial enrichment during soil parent material development. The soil bacterial community structure was determined by both parent material and fertilization strategies. Compared to straw returning, chemical fertilization-driven parent material maturation decreased soil bacterial diversity and significantly changed the soil bacterial community structure. However, compared to chemical fertilization, straw returning had a less negative effect on soil bacterial diversity, but was not as efficient in resolving the nutrient limitation during soil parent material maturation. This study provided insight into the maturation of soil parent materials for agriculture production to support the ever constant need for food by an increasing human population.

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1. Introduction

There is a variety of parent materials distributed in the subtropical region of China and reclaim this kind of land is in urgent need to meet an increasing demand for agricultural products (Xu and Cai, 2007). However, soil parent material is inherently less

fertile than fully formed soil and cannot support high amounts of food production (Li et al., 2014). Exploiting and utilizing soil parent material is an effective measure to alleviate the shortage of arable land resources and develop agricultural production. Nutrient accumulation processes are affected by parent materials (Anderson, 1988) and nutrient concentrations in parent materials, that is often limited, is needed to be increased to increase crop yields (Robertson and Vitousek, 2009). Fertilizer application and crop straw return are two effective methods to improve soil nutrient concentrations and availability (Marschner et al., 2003). Straw incorporation is an important strategy to improve soil quality and has a long-term positive effect on crop yield (Tejada et al., 2008; Han and He,

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2010; Ortiz Escobar and Hue, 2008). Nitrogen is often limiting in soil parent materials (Anderson, 1988), and its input can lead to higher net primary production (LeBauer and Treseder, 2008). Fertilizer and straw application provide nutrition (especially nitrogen) for plants growing in soil parent materials.

Microorganisms (especially bacteria) are the foundation of the earth's biosphere and play unique roles in ecosystem functions. In agricultural soil, bacteria are indispensable maintainers of soil productivity (Smith and Paul, 1990). Bacteria contribute to soil productivity through their involvement in organic matter decomposition, humus formation, nutrient transformation, and element cycling. The latest studies have demonstrated that the microbial necromass (e.g. the remains of dead bacterial cells) could be a significant source of soil organic matter (Langerhuus et al., 2012). Moreover, microbial products initiate and enhance the formation of long-term stabilized soil organic matter (Ludwig et al., 2015). Soil microorganisms are actively involved in soil aggregation dynamics and play a key role in soil structure formation processes. Thus, microorganisms are considered architects that can have an effect on the surrounding soil environment (Vogel et al., 2014). Moreover, during the early period of soil formation, microorganisms enhance weathering through the production of organic acids, promotion of hydrolysis reactions, and the release of nutrients such as phosphorus and potassium from soil minerals (Lian et al., 2008; Uroz et al., 2009).

Soil parent material provides the foundation elements that comprise the basic nutritional environment for microbial community development (Anderson, 1988; Ulrich and Becker, 2006). During soil formation, soil physical–chemical properties (i.e., soil pH and organic matter concentration) change with time and can influence soil microbial communities (Lombard et al., 2011). Moreover, soil microbial communities mediate many soil biogeochemical processes (Balser and Firestone, 2005). Therefore, there are complex interactions between soil microbial communities and the soil physical–chemical environment during the different stages of soil development (Tarlara et al., 2008). Consequently, measuring soil microbial communities can indicate the status of soil development and the effectiveness of management interventions (Harris, 2009; Li et al., 2014).

Soil parent material is a key factor that determines the bacterial community of the mature soil (Ulrich and Becker, 2006). Sun et al. (2015a) reported that the use of cultivation as a management strategy to accelerate parent material maturation had an important effect on the bacterial community of the soil. Fertilization and crop straw returning are major management strategies to accelerate soil parent material maturation, but their effects on bacterial community development during the maturation of soil parent materials have generally been less studied, especially in long-term field experiments.

Fertilization supplies available nutrients for both soil microbes and crop plants, which in turn enhance soil nutrient input through rhizosphere deposition. Soil parent material and fertilization (the main factors involved in soil maturation) should also be the main driving forces for soil microbial community formation during the maturation process. To investigate the bacterial community alteration during soil maturation under different fertilization regimens, we measured the soil properties, soil enzymatic activity and bacterial community composition.

2. Materials and methods

2.1. Site description and soil sampling

The long-term experimental research was conducted in Qiyang (111°52'32"E, 45°26'42"N, 150–170 m a.s.l), Hunan province, South

China, and was established in 1982 by the Chinese Academy of Agricultural Sciences (CAAS) to study how to accelerate the artificial maturation process for the rapid fertilization of parent materials. This region has a subtropical monsoon climate with an average annual temperature of 17.8 °C and mean annual rainfall of 1255 mm, of which 70%–80% occurs from April to October. There are three soil parent material types in this long-term soil maturation experiment: quaternary red clay soil (Q), granite soil (G) and purple sandy shale (P). These three types represent the three major soil parent materials of the agricultural soils in Hunan province in South China (soil material information is shown in Table_S1_1). To assess the effect of chemical fertilization and crop straw returning, four treatments were selected for each parent material: control without fertilizer (CK), straw returning (SR), chemical fertilizer without straw returning (NPK) and chemical fertilizer with straw returning (NPK + SR) (the treatments are described in Table_S1_2). The experimental plots were consisted of cement pools that were 4 m long × 2 m wide × 1 m deep, with open bottoms, the cement walls were about 10 cm above the soil surface to avoid the cross contamination of soils from different plots. All of the plots were cultivated in the same way using a Poaceae, Leguminosae, Cruciferae and Tuber crop rotation pattern. The soils were sampled in May, 2012, as described previously (Sun et al., 2015a). All collected samples were sieved (2 mm) and divided equally into two parts: one was frozen at –80 °C for DNA extraction and the second was stored at 4 °C prior to analysis.

2.2. Soil chemical analysis, soil microbial biomass and soil enzyme analysis

All chemical properties were determined by routine methods (Bao, 2010). Soil pH was measured with a glass electrode (soil/water = 1:5). Soil microbial biomass C (MBC) was determined by the chloroform fumigation extraction method (Vance et al., 1987). After 24 h of fumigation, 12.5 g of soils were extracted using 0.5 M K₂SO₄ with a 1:5 ratio for 60 min on a rotary shaker. The amount of organic C in the extract was measured by a LiquiTOC II total organic C analyzer (Elementar, Shanghai, China). The MBC was calculated using the following equation: $MBC = EC \times kEC$, where EC was the difference between the amount of C extracted from the fumigated and non-fumigated soils and kEC was 2.64 (Vance et al., 1987; Zhong and Cai, 2007).

Catalase activity was measured by back-titrating residual H₂O₂ with KMnO₄ (Edwards et al., 1992). Two grams (<2 mm) of air-dried soils were added to a 40 mL aliquot of distilled water and 5 mL of 30% H₂O₂. The mixture was shaken for 10 min, and then 5 mL of 1.5 N H₂SO₄ was added. A 25 mL aliquot of the filtered solution was titrated with 0.02 N KMnO₄. Controls were generated in the same way without the addition of H₂O₂.

Invertase activity was determined using sucrose as the substrate (Hu et al., 2006). Five grams (<2 mm) of air-dried soil, 15 mL of sucrose solution (8%) and 5 mL of phosphate buffer (pH5.5) were added to a 50 mL triangular flask and incubated for 24 h at 37 °C. After filtration, 1 mL of filtrate was added to a test tube and heated for 5 min with 3 mL of 5-dinitrosalicylic acid (DNS). The colour was measured using a colourimetric assay at 508 nm.

Acid and alkaline phosphatase were assayed using disodium phenyl phosphate as the substrate (Dick et al., 2000). Briefly, 1 g of soil (<2 mm) and 5 drops of toluene were added to a 250 mL triangular flask and shaken for 15 min; then, 20 mL of 0.5% disodium phenyl phosphate was added, and the mixtures were incubated at 37 °C for 24 h. Next, 40 mL of 0.3% aluminium sulphate solution was added to the mix and filtered. After filtration, 3 mL of filtrate was added to a 50 mL volumetric flask for the colour reaction measured by a colourimetric assay at 660 nm. We used acetate

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