



Bacterial diversity in soils subjected to long-term chemical fertilization can be more stably maintained with the addition of livestock manure than wheat straw



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ABSTRACT

Addition of organic matter such as livestock manures and plant residues is a feasible practice to mitigate soil degradation caused by long-term application of chemical fertilizers, and the mitigation is largely mediated through activities of the soil-dwelling microorganisms. However, the roles of different kinds of organic matter in maintaining bacterial community structure have not been assessed in a comparative manner. In this study, 454 pyrosequencing of 16S rRNA gene was employed to compare the bacterial community structure among soils that had been subjected to 30 years of NPK fertilization under six treatment regimes: non-fertilization control, fertilization only, and fertilization combined with the use of pig manure, cow manure or low- and high-level of wheat straws. Consistent with expectation, long-term application of NPK chemical fertilizers caused a significant decrease of bacterial diversity in terms of species richness (i.e. number of unique operational taxonomic units (OTU)), Faith's index of phylogenetic diversity and Chao 1 index. Incorporation of wheat straw into soil produced little effects on bacterial community, whereas addition of either pig manure or cow manure restored bacterial diversity to levels that are comparable to that of the non-fertilization control. Moreover, bacterial abundance determined by quantitative PCR was positively correlated with the nutritional status of the soil (e.g., nitrate, total nitrogen, total carbon, available phosphorus); however, bacterial diversity was predominantly determined by soil pH. Together, our data implicate the role of livestock manures in preventing the loss of bacterial diversity during long-term chemical fertilization, and highlight pH as the major deterministic factor for soil bacterial community structure.

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1. Introduction

Large amounts of chemical fertilizers have been applied into arable fields over the past few decades in order to maximize the crop yields and prevent food shortage worldwide (Savci, 2012). However, excessive use of chemical fertilizers can cause serious soil degradation such as nitrogen leaching, soil compaction and reduction in soil organic matter; and consequently, the efficacy of chemical fertilizers on crop yields decreases over time (Horrigan et al., 2002). Therefore, new strategies are urgently required to

mitigate the deleterious effects of long-term application of chemical fertilizers. Addition of organic matter is a traditional method of increasing soil quality (Reeves, 1997). Previous studies revealed that a combined use of chemical fertilizers with organic matter such as straw and manure is an efficient practice of maintaining soil productivity (Steiner et al., 2007; Bhattacharyya et al., 2008; Verma and Sharma, 2008). However, the underlying mechanistic details remains largely elusive, particularly the roles of microorganisms in maintaining soil productivity. More specifically, empirical evidence on the specific responses of soil microbial community composition and structure to different treatment regimes is seldom available (Enwall et al., 2007).

Microorganisms are abundant and diverse in soils (Nannipieri et al., 2003). They play vital roles in maintaining soil

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functionalities, including nutrients cycling and bioremediation (Thomas, 2001). They also make significant contributions to the plant health (Berg, 2009). Apart from the soil-borne plant pathogens, the microbial communities in the soil also contain a wide range of species that benefit plant growth. Of particular note are certain bacterial species, known as plant growth-promoting rhizobacteria (PGPR), which can aggressively colonize rhizosphere and produce various disease-suppression activities (Lugtenberg and Kamilova, 2009). Therefore, maintaining high levels of microbial diversity in soil is of crucial importance for sustainable agriculture (Kennedy and Smith, 1995; Bhat, 2013). However, it is also a challenging task, as microorganisms (bacteria in particular) are sensitive to environmental changes caused by natural disturbance and human activities (Yin et al., 2010). It has recently been proposed that bacterial community can be used as an indicative marker for the assessment of environmental impacts of agricultural practices on soil quality (Sharma et al., 2011). Alteration of soil quality is normally coupled with a shift of bacterial community structure (Kennedy and Smith, 1995). Indeed, loss of microbial diversity is the general consequence of long-term chemical fertilization (Ramirez et al., 2010; Coolon et al., 2013). Therefore, any effective mitigation technology should ideally be able to restore the levels of soil microbial diversity, not just the soil abiotic properties.

Species composition and diversity index are two important measures of a microbial community, and they are determined by a combination of both historical and contemporary effects (Martiny et al., 2006; Sun et al., 2014). Previous studies revealed that chemical fertilization causes significant changes of the structural composition of bacterial community, but the levels of bacterial diversity remain unaltered in short-term treatments of less than a few years (Wakelin et al., 2007; Zhao et al., 2014). There was a significant correlation between alteration of soil nutrient contents (e.g., available K, total nitrogen, total carbon) and shift of bacterial community structure (Wakelin et al., 2007; Zhao et al., 2014). Interestingly, long-term fertilization results in significant changes of both structure composition and diversity of bacterial communities in the soil, and a decrease of bacterial diversity has been reported in a number of recent studies (Ramirez et al., 2010; Wu et al., 2011; Coolon et al., 2013; Yuan et al., 2013).

Livestock manure and crop residue are two major types of organic matter that have the potential to mitigate soil degradation caused by long-term chemical fertilization. Several studies have addressed the impacts of livestock manure and plant residues on soil-dwelling bacterial communities (Chu et al., 2007; Enwall et al., 2007; Wakelin et al., 2007; Wessén et al., 2010; Wu et al., 2011; Poulsen et al., 2013; Yuan et al., 2013). For example, addition of rice residues into paddy field eliminated the effects of chemical fertilization on bacterial “species” composition, but it produced no effects on levels of bacterial diversity (Ahn et al., 2012; Yuan et al., 2013). Amendment of livestock manures raised the levels of bacterial diversity no matter they were used alone or used in combination with chemical fertilizers (Sun et al., 2004; Enwall et al., 2007; Guanghua et al., 2008; Chaudhry et al., 2012); and notably, the effects can be detected within a short period of application (Lazcano et al., 2012). Together, these separate lines of investigation implicate stronger effects of livestock manure than crop residues on bacterial communities in the soil. However, the relative influences of livestock manure versus crop residue have so far not been assessed under the same experimental setup, particularly in the context of agricultural practice involving long-term chemical fertilization.

Immigration and diversification are the ultimate sources of biological diversity, and the agricultural soil systems should not be an exception (Fukami et al., 2007; Prosser et al., 2007). Microorganisms inhabiting plant residues and livestock manures represent

newly arrived immigrants that will likely affect the indigenous soil microbial communities. Livestock manures contain much more abundant bacteria than crop residues, but most bacterial species in the livestock manures originate from the guts of animals, which are less competitive when compared with members of the established soil bacterial communities (Unc and Goss, 2004). In the contrast, bacteria from crop residues are mostly of soil origin and thus have a much higher chance to invade the indigenous bacterial communities in the soil (Hallmann et al., 1997; Ryan et al., 2008). Therefore, it would be interesting to know the ecological significance of microorganisms that are introduced into the soil during the application of organic matter, specifically, to which extent they determine the microbial community structure. In term of diversification, environmental factors such as soil nutritional status and soil pH play important roles in bacterial community assembly (Prosser et al., 2007). Soil acidification is one of the major deleterious effects caused by chemical fertilization, especially the use of nitrogen fertilizers (Schroder et al., 2011); importantly, many studies have revealed a deterministic role of soil pH for bacterial communities in the soil (Fierer and Jackson, 2006; Lauber et al., 2009; Griffiths et al., 2011).

Here we describe a comparative analysis of bacterial communities in soils that have been subjected to 30 years of chemical fertilization (NPK) with the addition of low- or high-levels of wheat straw residues, cow manure (CM), or pig manure (PM). Also included in our analysis were a non-fertilization control and another control treatment of NPK fertilization only. The experiment was designed to assess the relative importance of livestock manures and crop straws in mitigating the long-term effects of chemical fertilization on soil bacterial communities. Bacterial abundance was estimated using quantitative PCR and structure and composition of the bacterial communities were analyzed using the high-throughput method of 454 pyrosequencing. Both techniques were based on the analysis of 16S rRNA genes (Chaudhry et al., 2012; Cummings et al., 2013). Having found greater effects of livestock manures on bacterial abundance and diversity than wheat residues, we proceeded to test the hypothesis that the observed effects were mediated by bacteria inhabiting in livestock manures. The bacterial communities in fresh pig and cow manures were compared with those in the soil. Our data implicate little role of the introduced bacteria in shifting soil bacterial community structure. Instead, we show that restoration of the bacterial communities was predominantly caused by alterations of the soil pH.

2. Materials and methods

2.1. Experiment design and soil sampling

The experimental was located in Mengcheng county, Anhui province, China (33°13'N, 116°35'E, with an elevation of 42 m). The mean annual temperature of this region is 14.8 °C, and the mean annual precipitation is 872 mm. The soils involved in this work belong to a typical lime concretion black soil. The experimental fields were set up in 1982 and had since been subjected to wheat-soybean crop rotation (of note, maize instead of soybean was grown during 1993–1998). This work has involved six treatments with four replicates (plots) for each: (1) control, no chemical fertilizers were added; (2) NPK control, mineral NPK fertilizer only; (3) mineral NPK fertilizers plus 3750 kg wheat straw ha⁻¹ y⁻¹ (NPK + LS); (4) mineral NPK fertilizers plus 7500 kg wheat straw ha⁻¹ y⁻¹ (NPK + HS); (5) mineral NPK fertilizers plus 15,000 kg fresh pig manure ha⁻¹ y⁻¹ (NPK + PM); (6) mineral NPK fertilizers plus 30,000 kg fresh cow manure ha⁻¹ y⁻¹ (NPK + CM). The size of each plot is 70 m² (14.9 m × 64.7 m). The NPK fertilizer comprised urea (180 kg N ha⁻¹ y⁻¹), superphosphate (90 kg P₂O₅ ha⁻¹ y⁻¹)

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