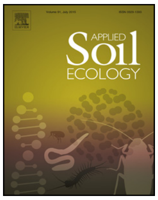
Applied Soil Ecology 105 (2016) 187–195

Contents lists available at ScienceDirect

Applied Soil Ecology

journal homepage: www.elsevier.com/locate/apsoil



Effect of 35 years inorganic fertilizer and manure amendment on structure of bacterial and archaeal communities in black soil of northeast China

Jianli Ding[a], Xin Jiang[a,b], Mingchao Ma[a,b], Baoku Zhou[c], Dawei Guan[a], Baisuo Zhao[c], Jing Zhou[a], Fengming Cao[a,b], Li Li[a], Jun Li[a,b,*]

[a] *Institute of Agricultural Resources and Regional Planning, Chinese Academy of Agricultural Sciences, Beijing 100081, China*
[b] *Laboratory of Quality & Safety Risk Assessment for Microbial Products (Beijing), Ministry of Agriculture, Beijing 100081, China*
[c] *The Institute of Soil Fertility and Environmental Sources, Heilongjiang Academy of Agricultural Sciences, Harbin 150086, China*

ARTICLE INFO

Article history:
Received 1 December 2015
Received in revised form 6 April 2016
Accepted 13 April 2016
Available online xxx

Keywords:
Black soil
Inorganic fertilizer
Manure amendment
Microbial community structure

ABSTRACT

Black soil is common in northeast China and plays an important role in Chinese crop production. However, in the past three decades, inappropriate use of fertilizer has caused a sequence of agroecological issues. The objective of this research was to evaluate the effect of long-term fertilizer on the microbial communities in black soil. The soil was subjected to four fertilization regimes: without fertilizer (CK); manure (M); nitrogen, phosphorus and potassium inorganic fertilizer (NPK); and inorganic fertilizers with manure (MNPK). The soil pH was decreased by inorganic fertilizers and increased by manure. Quantitative PCR analysis of microbial community size and Illumina platform-based analysis of the V4 16S rRNA gene region were performed to characterize soil microbial abundance and to compare community structure and diversity. Microbial community size was enhanced by the incorporation of inorganic fertilizer and manure. Microbial diversity was decreased by inorganic fertilizer and increased by the incorporation of inorganic fertilizer and manure. The predominate phyla in all samples were Proteobacteria (29.39–33.48%), Acidobacteria (13.14–16.25%) and Actinobacteria (9.32–10.77%). The relative abundance of different classes significantly differed among the different treatments, especially MNPK and NPK. Acidobacteria and Deltaproteobacteria were relatively stable in organic fertilizer treated soil. Gammaproteobacteria, Alphaproteobacteria and Betaproteobacteria were sensitive to all the fertilization regimes. Comparatively, Spartobacteria was stable in response to fertilization practices. Principal coordinate analysis indicated that microbial communities were primarily clustered into three groups: CK and M were clustered together; MNPK was improved by manure and separated from NPK. Shannon and Simpson indexes were significantly correlated with soil pH and the concentrations of available phosphorus and total phosphorus. Redundancy analysis indicates that microbial communities were closely positively correlated with soil nitrate nitrogen concentration ($P = 0.002$) and pH ($P = 0.002$). These results indicate that inorganic fertilizer plus manure increased microbial size and diversity and changed microbial composition.

1. Introduction

Soil ecosystems are highly complex and include a large number of microorganisms. It has been estimated that 1 g of soil contains up to 1 billion bacterial cells comprising tens of thousands of microbial taxa. Soil microorganisms play a vital role in ecosystems such as decomposing organic matter (Zhang et al., 2012), acting on soil development (Daynes et al., 2013), and microbial community structure influences carbon and nitrogen cycling (Mooshammer et al., 2014; Schimel and Schaeffer, 2012). The sustainability of terrestrial agroecosystems is indicated by the microbial diversity and activity (Su et al., 2015). Biological diversity and abundance are the foundations for ecosystem maintenance (Li et al., 2014b). In recent years, anthropogenic activities such as agricultural intensification and long-term fertilization can change the microbial abundance and overall diversity of soil organisms (Potthast et al., 2012; Fichtner et al., 2014). Fertilization is a significant agricultural

---

[*] Corresponding author at: Institute of Agricultural Resources and Regional Planning, Chinese Academy of Agricultural Sciences, Beijing 100081, China.
E-mail address: jli@caas.ac.cn (J. Li).

http://dx.doi.org/10.1016/j.apsoil.2016.04.010
0929-1393/

practice for improving plant nutrition and achieving high yield, and also changing the soil environment, such as soil carbon chemistry (Nemergut et al., 2008), and can change the activity and diversity of soil microorganisms (Jangid et al., 2008). Previous reports have shown the influence of fertilization on microbial community shifts in nitrogen-fixing bacteria (Simonsen et al., 2015), methanotrophs (Hu and Lu, 2015) and cellulolytic communities (Fan et al., 2012). Microorganisms can respond to changing environmental conditions by modifying community composition and microbial biomass (Tian et al., 2015), and microbial diversity is also a potentially valuable indicator of soil health and quality (Romaniuk et al., 2011). It is essential to manipulate the shifts in microbial community structure following long-term fertilization in order to develop better fertilizer regimes (Pan et al., 2014).

Chinese black soils would be better described as Phaeozems according to the World Reference Base (FAO), and belong to the pachic Haploborolls subtype of Haploborolls type in Borolls suborders (Yuri et al., 2011). Black soil is widely distributed in northeast China, which is one of the most vital food production areas in China. In the next 10–15 years, production from the northeast grain area should increase by 20 million tonnes and account for 50% of the increased production of the country (Song et al., 21 2014). Thus, it is important for China's food security. These untilled virgin black soils were fertile, but in recent decades, the land was excessively plowed and excessively treated with pesticides and chemical fertilizers, which caused a sequence of agroecological issues. Zhang et al. (2013) showed that arable Mollisols in northeast China were seriously degraded after reclamation and soil organic matter content decreased by 60%. In particular, the long-term use of inorganic fertilizers caused a series of environmental problems in black soil. Over-use of nitrogen (N) fertilizer led to soil acidification (Guo et al., 2010), chemical fertilizer decreased the soil microbial biomass (Hou et al., 2014) and reduced the catabolic activity of fast-growing or eutrophic bacteria (Wang et al., 2008). However, organic amendment can improve the soil, and manure increases soil organic matter, microbial biomass, and metabolic ability of the bacteria (Wei et al., 2008). Organic amendments from a variety of sources including agriculture, forestry and urban areas, added into inorganic fertilizer is an effective way to solve problems caused by inorganic fertilizer. Hence, it is crucial to know how the structure of microbial communities changes in black soil under long-term application of different fertilizers, especially incorporation of inorganic fertilizer with amendment.

We manipulated soil microbial biodiversity and community composition by long-term fertilizer applications through using next generation sequencing technologies (NGST), and tested how the incorporation of inorganic fertilizer with amendment changed the soil microbial biodiversity and community composition. Earlier studies on this site found that chemical fertilizers led to a decline in the catabolic activity of fast-growing or eutrophic bacteria as indicated by PCR-Denaturing Gradient Gel Electrophoresis (PCR-DGGE) methods (Wei et al., 2008). PCR-DGGE only revealed the dominant bacteria in the soil and there is limited information on the relationship between bacterial community changes and size shifts; comparatively, NGST can provide significant insights into species and functional diversity of microbial populations (Zhao et al., 2014). In addition, soil quality changes are slow and after a change of management it takes a reasonable period to achieve a long-term steady state, thus long-term agricultural field experiments are invaluable to detect changes that are absent in the short term (Chakraborty et al., 2011).

Our objective was to examine the changes of long-term inorganic and manure amendments on agricultural soil bacterial and archaeal communities, and analyze the relationships between microbial diversity, dominant groups and soil parameters associated with these changes. This work strengthens our current understanding about the effect of long-term organic and inorganic amendment applications on the structure of microbial communities, and provides a theoretical basis for rational fertilizer regimes that promote the sustainable development of black soils. Four different fertilizer treatments were selected to evaluate soil properties and structure and abundance of microbial communities in soil.

2. Material and methods

2.1. Site description, experimental design, and sampling

Field soil samples were collected from a long-term rotational field trial founded in 1979 and located in Harbin City, Heilongjiang Province, China (45°40′N, 126°35′E). This region has a temperate continental monsoon climate, with a frost-free period of 135 days, an annual average temperature of 3.5 °C, annual precipitation of 533 mm and an altitude of 151 m (Zhou et al., 2015). The experimental site was flat, with homogenous soil properties. The basic soil physicochemical properties (in 1980) were as follows: organic matter (OM), 26.7 g kg^{-1}; total nitrogen (TN), 1.47 g kg^{-1}; total phosphorus (TP), 1.07 g kg^{-1}; available nitrogen, 151.1 mg kg^{-1}; available phosphorus (AP), 51.0 mg kg^{-1}; available potassium (AK), 200 mg kg^{-1}; and pH 7.22. The total area of the site is 7000 m^2 and it has 96 plots (32 treatments and three replicates per treatment). Plots were randomly arranged and cement plates were inserted between plots. Tillage methods were shallow plowing combined with subsoiling, and rotary tillage combined with subsoiling. The site has been in wheat–maize–soybean crop rotation since 1980, with soybean planted in 2014.

In the present study, four fertilization regimes were selected: without fertilizer (CK); farmyard horse manure fertilizer (M); nitrogen, phosphorus and potassium inorganic fertilizer (NPK); and inorganic fertilizers with manure (MNPK). Each treatment was conducted with three replicates of 36 m^2 (9 m × 4 m). The fertilizers were applied in every growth season. The rates of inorganic fertilizers were 150 kg N ha^{-1}, 75 kg P_2O_5 ha^{-1} and 75 kg K_2O ha^{-1} for wheat and maize treatments; and 75 kg N ha^{-1}, 150 kg P_2O_5 ha^{-1} and 75 kg K_2O ha^{-1} for soybean. Horse manure was provided as organic amendment at the rate of 18,600 kg manure ha^{-1} for all crops in M and MNPK. The soybean growing period started in late April and ended in late September, and soil samples were collected on 19 September 2014. In the period of sampling, this region had the highest temperature of 21 °C and the lowest temperature of 8 °C, with a minimum relative humidity of 37%. From each replicate plot, ten soil cores (5–25 cm depth, 10 cm diameter) were collected and pooled to minimize within-plot variation. Soil was pooled in a sterile plastic bag, and taken to the laboratory on ice. Twelve soil samples (4 treatments × 3 replicates) were sieved at 2.0 mm, and divided into two parts: one stored at −80 °C for further molecular analysis, and the other was for soil property analysis.

2.2. Selected soil properties

Soil pH was measured with air-dried samples (sieved to 2.0 mm) using a glass combination electrode with a soil: water ratio of 1:1 (Sonmez et al., 2008). AP was determined by resin extraction following a protocol modified from Hedley and Stewart (1982). TP was measured using the colorimetric method with molybdenum in sulfuric acid (Garg and Kaushik, 2005). AK was extracted using acetic acid and ammonium leaching following the Mehlich III extraction method (Mehlich, 1984). Total potassium (TK) was estimated by digestion with concentrated hydrofluoric acid (Habib et al., 2014). Soil KCl-extractable nitrate nitrogen (NN)