



Effects of short-term fallow managements on soil microbial properties: A case study in China

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ABSTRACT

Allowing farmland to fallow can effectively improve the self-rehabilitation of arable soil and solve agroecological problems such as biodiversity loss, soil fertility loss and over-cultivation. However, long-term fallows cannot satisfy the provisionment requirements of China; therefore, the use of short-term fallows to restore cultivated soil has become a better option. Nevertheless, few studies have compared short-term fallow managements and the influence of fallows on the soil microbial community, especially vegetation plus fertilizer management. Therefore, we periodically analyzed the soil properties, microbial biomass nitrogen and bacterial community structure of farmland soil under different treatments during one year on Yellow River Delta of China. The experiment included the following five treatments: (1) control with traditional farming and chemical fertilization (CK), (2) natural fallow without any fertilizer input (NN), (3) pasture fallow without any fertilizer (NP), (4) natural fallow with cattle manure (MN), and (5) pasture fallow with cattle manure (MP).

The results made it apparent that the differences of soil physicochemical characteristics became more significant in the autumn than in the spring. In contrast to the CK treatment, the MN exerted an obvious improvement on the microbial biomass nitrogen ($P < 0.05$). According to the alteration in observed OTUs, the Shannon-Wiener Index and the Phylogenetic Diversity, the CK presented a significant decrease in the summer ($P < 0.05$). At the beginning, the microbial community showed noteworthy differences between the CK and fallow managements ($P < 0.05$), except for the MP. The gaps diminished in the autumn, whereas the differences between finer taxonomic groups became clearer. Compared to CK, *Nitrospira* and *Steroidobacter* were observably lower in NN and NP, whereas *Chthonomonas* was significantly higher ($P < 0.05$). *Lysobacter* and *Sphingobacterium* were notably higher in MN than CK while *Geobacter* and *Sphingobacterium* were remarkably higher in MP than CK ($P < 0.05$).

In summary, no fertilizer input could be favorable for carbon and nitrogen retention, and the NP improved some functional microorganisms, but it may lead to soil compaction. Cattle fertilizer could promote organic matter degradation, and MN enhanced the soil microbial biomass nitrogen and soil-borne disease resistance on crops, whereas MP could reduce the nitrogen and clay content loss and improve heavy metal bioremediation. During short fallowing periods, pasture plus farmyard fertilizer could markedly promote soil micro ecosystemic function. If there was no fertilizer input, natural fallow could be a considerable choice.

1. Introduction

Fallow is seen as an effective method to maintain biodiversity, control weeds and restore soil fertility in low fertility farmlands (Van Buskirk and Willi, 2004). This approach has been adopted as a sustainable agricultural method in the Common Agricultural Policy (CAP), Conservation Reserve Program (CRP), Canada's Permanent Cover Program (PCP) and Australian Summer Fallow (Henle et al., 2008; Zeleke, 2017). However, long-term fallow cannot satisfy the provisionment

requirement at the local level in China, and agrochemical inputs have been remarkably increased to guarantee food security and increase the income of farmers in the areas where long-term fallow was implemented (Feng et al., 2005; Wang et al., 2014). To make up for the deficiency, increasing studies have tried to improve the fallowing potential to shorten the set-aside time (under three years), especially with green manure and crop rotation (Jensen et al., 2010; Zhang et al., 2016).

The plants chosen for fallow gradually become mature, and they

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include various species and combinations such as native tree species, green manure crops and legume pasture (Biederbeck et al., 2005; Joslin et al., 2016; Nezomba et al., 2010). On the other hand, some studies have stated that farmyard manure could favor the restoration of vegetative diversity, and fertilizer could assist soil heavy metal pollution remediation (Sabir et al., 2015; Saviozzi et al., 2002; Smith et al., 2003). Currently, organic materials are used as frequently soil amendments, and these materials include farmyard manure, leaf litter and green manure (Neina et al., 2016; Sradnick et al., 2014). A great deal of evidence indicates that fertilizer inputs could also enhance the fertility and structure of the soil, especially soil humification processes (Kirkby et al., 2016). However, few researchers have explained the benefits of using fertilizer during fallowing (Ahmad et al., 2014; Ghosh, 2007; Li and Wu, 2017). Therefore, a comparison among different fallow fertilizer management has not been clearly performed, especially under different vegetation types.

The soil microbiota is a vital part of soil biogeochemical cycles, and it closely connects the functions of the agro-ecosystem (Arshad and Martin, 2002; Ma et al., 2016; van der Heijden and Wagg, 2013). Increasing numbers of studies have shown that microorganisms could affect the soil quality and plant productivity directly (Bender et al., 2016; Brookes et al., 2013; Epelde et al., 2009). It could also be helpful for understanding the functions of soil microbiota via a combination of soil physiochemical and microbial properties (Fierer et al., 2012; Nannipieri et al., 2017). The decreasing costs of performing a molecular characterization of microbiota mean that this characterization has been applied widely in agro-ecosystems (Kaschuk et al., 2010; Reeve et al., 2010; Shen et al., 2016). As a consequence, using a molecular characterization to describe the soil microbiota has become an important method in agroecological studies. Although fallow could optimize soil the physicochemical and biochemical quality as widely accepted by academics (Oliver et al., 2010; Wick et al., 1998), relatively few studies have addressed soil microbiota changes in improved fallow farmlands. Although some studies about fallow have mentioned soil microbial properties, such as microbial diversity, soil respiration and catabolic diversity, they are still insufficient for explaining the taxonomy of microbiota, especially comparing among different improved fallow management types (Badiane Ndour et al., 2008; Castro et al., 2016; Drijber et al., 2000).

The microbial biomass and diversity respond quickly to changes in soil conditions, and they could be valuable indicators for determining the effects of agricultural management approaches on the soil environment (Shen et al., 2016). Additionally, sequence assays of different species could provide predictive profiles of the microbial community functions (Langille et al., 2013). Specifically, we addressed the following questions: (i) how does the addition of farm manure affect microbial communities during short fallowing? (ii) What are the effect of adding pasture under different fertilization management approaches? (iii) We explored the soil microbial taxa responses to the application of short fallows. To answer the above questions, we conducted the field experiment in a major agricultural region, and performed by sequencing the 16S fraction of DNA directly extracted from soil under various fallow farmlands. To study the modifications in the microbiota more accurately, we have taken three seasonal dates (spring, summer and autumn) into account.

2. Materials and methods

2.1. Site description and experimental design

The study sites were located at Shandong Wudi Field Scientific Observation & Research Base for Land Use, in Binzhou, China (E 117°43', N 37°48', altitude of 5 m.a.s.l). The rainfall ranged from 0 mm to 421.8 mm, with a 10-year average of 55.3 mm. The average temperature was 13.9 °C, varying between -6.1 °C and 28.4 °C. With a silty loam texture (approximately 3% clay and 78% silt), the soil at all the

Table 1

The background soil property of the study area.

Property	Range
pH	8.43
Electrical conductivity ($\mu\text{S cm}^{-1}$)	0.18×10^3
Soil total nitrogen (g kg^{-1})	0.62
Soil organic carbon (g kg^{-1})	8.39
Available nitrogen (mg kg^{-1})	24.59
Available potassium (mg kg^{-1})	0.11
Available phosphorus (mg kg^{-1})	8.51

† The data was provided by Agriculture Testing Center of Wudi.

sites is derived from the diluvial sediments of the Yellow River and it is classified as a typical saline alluvial soil (Fluvisols, FAO). According to the local agriculture bureau, sorghum (*Sorghum bicolor* (L.) Moench) has been planted there for five years, and the sorghum yield has notably decreased in the study area. According to investigations by the local agricultural authorities, partial farmlands (45%) near the study sites were already abandoned, planted with other crops or transformed into an artificial lake. The experimental fields were set up in May 2016, and the fields are flat and relatively homogeneous farmlands. The soil background is shown in Table 1.

Five treatments were performed as follows: (i) CK, control with traditional agricultural tillage, which was planted with sorghum and treated with compound fertilizer and urea, in all about 330 kg ha^{-1} ; (ii) NN, no fertilizer input, no tillage and native grass fallow, which was also called natural fallow; (iii) NP, no fertilizer input, no tillage and a mixture of alfalfa (*Medicago sativa* L.) and dahurian wild rye (*Elymus dahuricus* Turcz.); (iv) MN, no tillage, natural fallow with cattle manure, 1500 kg ha^{-1} ; and (v) MP, no tillage, pasture fallow (Alfalfa and Dahurian wild rye) with cattle manure, 1500 kg ha^{-1} .

2.2. Soil sampling and preparation

In June 2016, we began sampling the soil. The second and third samplings were performed in August and October of 2016, respectively. According to the "S" sampling principle, soil samples were collected from 6 points at a depth of 0–20 cm from each plot by foil sampler and then mixed and homogenized, and half was discarded. The rest of the soil was passed through a $< 2 \text{ mm}$ sieve to remove aboveground plant materials, roots, and stones. Then each sample was divided into three parts. One was stored at -20 °C prior to DNA analysis. The next one was stored at 4 °C prior to microbial biomass nitrogen analysis. The rest was air-dried to assess the physical and chemical properties.

2.3. Soil physicochemical assay

The soil pH was measured with a glass electrode (soil: water = 1:2.5). The soil texture was determined by laser particle size analyzer (LS-POP, OMEC, China). The soil samples were suspended in deionized water, and ultrasonicated for five minutes. The clay content (Clay) was measured at under $2 \mu\text{m}$ in soil particle diameter. The soil particle density (D_p) was determined by pycnometer method (Grossman and Reinsch, 2002).

The soil inorganic carbon (SIC) was determined in air-dried, finely ground soil aliquots. Subsamples of 10 mg each were weighed into tin caps with phosphoric acid and analyzed with a CN analyzer (TOC-L Analyzer and SSM-5000A Unit, SHIMADZU, Japan).

2.4. Biological analysis

2.4.1. DNA extraction and MiSeq illumina sequencing

The total soil genomic DNA was extracted from 0.5 g of each soil sample using a PowerSoil Total DNA Isolation Kit (MoBio Labs, Solana Beach, CA, USA) according to the manufacturer's instructions. The DNA

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