



Soil microbial community structure and function are significantly affected by long-term organic and mineral fertilization regimes in the North China Plain



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ABSTRACT

An improved understanding of the complex interactions and relationships in the soil ecosystem is essential to predict the impact of farming practices on soil quality and its capacity for agricultural production. This study aims to improve our understanding of the impacts of fertilization strategy on key indicators of soil biological and chemical quality. We studied soils from a winter wheat–summer maize rotational experiment in the North China Plain with six different fertility treatments: no amendments (CK); standard mineral fertilizer treatment (SMF) or standard organic manure treatment (SMA) reflecting local farmer practice; mixed treatment with fertilizer and manure at half the rates for the SMF and SMA treatments (1/2 SMF + 1/2 SMA); double mineral fertilizer treatment (DMF); and double organic manure treatment (DMA). Soil organic C (SOC), total N (TN), total P (TP), pH, and dissolved organic C (DOC) and N (DON) and microbial biomass C (Cmic) and N (Nmic) were determined using standard methods. Soil bacterial community structure was assessed by denaturing gradient gel electrophoresis (DGGE), and activities for 10 extracellular enzymes (EEAs) were measured as indicators of soil function. Repeated application of either organic manure or mineral fertilizer increased SOC, TN, TP, DOC, DON, Cmic and Nmic, and decreased soil pH. Higher rates of organic manure fertilization significantly affected soil chemical properties compared to the lower rate. Soil bacterial community structure was significantly altered by the long-term fertilization regimes and diversity was significantly higher in the double manure rate treatment relative to mineral fertilizer. The higher urease, α -glycosidase, *N*-acetyl- β -glucosaminidase, *L*-leucine aminopeptidase (involved in N cycling), β -glucosidase, β -xylosidase and β -cellobiosidase (involved in C cycling), and alkaline phosphatase (involved in P cycling) activities for organic manure fertilized soils reflected a higher nutrient cycling capacity compared to mineral fertilized and control plots. Soil bacterial community diversities increased with Cmic and variations in EEAs were strongly correlated with soil DOC availability. Our study has demonstrated that a long-term fertilization strategy can be used to improve soil quality. Clearly, the use of organic fertilizers where available, is a win–win strategy for maintaining soil quality and crop productivity, while ensuring the delivery of soil ecosystem services into the future.

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1. Introduction

Chinese agriculture has intensified greatly since the early 1980s on a limited land area with large inputs of mineral fertilizers and other resources to meet the food demand of its increasing population (Guo et al., 2010). Cereal grain yields have increased by 65% between 1980 and 2010 (Zhang et al., 2012), but this success has come at a cost: overuse of mineral fertilizer not only induces low fertilizer use efficiency and the rapid depletion of known

P-deposits, but also has led to the degradation of the environment, through increased greenhouse gas emissions, nutrient run-off and biodiversity loss (Zhu and Chen, 2002; Kahrl et al., 2010; Miao et al., 2011; Zhang et al., 2013). All these phenomena have led Chinese people to become increasingly concerned about the sustainability of current intensive agricultural management practices.

In order to ensure the future sustainability of agricultural production, maintenance of soil quality is essential. Soil quality is “the capacity of a specific kind of soil to function, within natural or managed ecosystem boundaries, to sustain plant and animal productivity, maintain or enhance water and air quality, and support human health and habitation” (Doran and Parkin, 1994). Soil quality can be assessed by measuring a range of biological, physical and chemical indicators which are sensitive to changes in management (Fließbach et al., 2007), and linked to key soil functions. Measurement of soil quality indicators is therefore a useful approach for assessing and comparing the sustainability of different crop production strategies.

The replacement of mineral fertilizer with organic manure could be one way to solve the problems of excessive mineral fertilizer use while at the same time improving soil quality such as physical and chemical properties, carbon stocks, and biological properties including soil biodiversity (Mäeder et al., 2002; Edmeades, 2003; Hole et al., 2005; Fließbach et al., 2007; Gattinger et al., 2012; Sradnick et al., 2013). Use of organic manures as fertilizers can also reduce nutrient losses (Zhao et al., 2011) and climate change impacts (Kustermann et al., 2008), and support similar or higher crop yields than mineral fertilization in certain contexts (Melerio et al., 2006; Lin et al., 2009; Seufert et al., 2012). Mäeder et al. (2002) reported that while yields were 20% lower in organic systems relying on manure fertilization, they have higher nutrient use efficiencies than conventional systems. Lin et al. (2009) in our research group reported similar yields in manure fertilized treatments compared to mineral fertilized treatments after fifteen years in the same experiment.

Soil microbiota regulate biogeochemical nutrient processes and their activities play a critical role in terrestrial ecosystems. Meanwhile, microbial properties such as microbial biomass C, N and P, respiration, and metabolic quotient are often used as indicators of soil quality because of their sensitivity to environmental changes, land use (Ndiaye et al., 2000; Acosta-Martinez et al., 2008; Xu et al., 2009) and agricultural management (Li et al., 2008). Numerous reports indicated that fertilization management significantly affected soil microbial properties and community composition (Mäder et al., 2002; Enwall et al., 2005; Gu et al., 2009; Feng et al., 2015). The addition of different amounts of nitrogen fertilizer induced a shift in microbial community structure resulting in an increase in fungal biomarkers but a significant decrease in the soil microbial community's growth-response in treatments with higher rates of N addition (e.g. 2,000 $\mu\text{g N g}^{-1}$ soil (Yevdokimov et al., 2008)). Following addition of organic manure, soil microbial communities usually experience increases in biomass and activity that release nutrients in plant-available forms promoting plant vegetative growth and contributing to crop productivity (Kallenbach and Grandy, 2011; Jackson et al., 2012). Feng et al. (2015) revealed that the size of soil bacterial community increased in a long-term organic manure fertilized alkaline soil in the North China Plain (NCP).

Soil microorganisms depolymerize and mineralize organic matter by producing extracellular enzymes (Allison et al., 2007). These enzymes have been recognized by soil scientists as one of the more sensitive components of the soil ecosystem and their activities (EEA) provide an early indication of the soil's functional status and microbial nutrient demand (Allison et al., 2007; Giacometti et al., 2014). Previous research has shown that soil enzyme activities were influenced by tillage, land use and farming

practices (Li et al., 2008; Fan et al., 2012). For example, soil hydrolytic enzyme activities became higher after regular organic manure application (García-Ruiz et al., 2008; Li et al., 2008). Fan et al. (2012) demonstrated that long-term mineral fertilizer input decreased cellobiohydrolases (CBH) activity, while long-term manure input increased CBH activity.

This evidence demonstrates that different fertilization strategies can alter soil physical, chemical and biological quality, and have an impact on crop yields. It is also clear that there is a link between soil chemical/biological properties and soil biodiversity and function. Assessing the impact that organic manure and mineral fertilizers and their combined application could have on soil quality within a Chinese production system will therefore help to contribute to the development of more sustainable food production systems that meet the demands of a growing population while preventing soil degradation.

The objective of this study is to determine how long-term contrasting organic and mineral fertilization regimes have impacted on indicators of soil quality including: soil chemical/biological properties, bacterial community structure and extracellular enzyme activities (EEAs). We hypothesize that long-term organic manure fertilization will enhance soil chemical and biological quality indicators, and alter soil microbial community structure by increasing diversity. We also hypothesize that the changes in soil chemical and biological properties will drive changes in the soil microbial community function (EEAs). This research should result in an improved understanding of the relationship between soil fertilization strategy and soil quality which will contribute to the development of guidelines for more effective use of organic and mineral fertilizers in Chinese agricultural production systems.

2. Material and methods

2.1. Site descriptions and experimental design

This experiment was started in 1986 at Dezhou Experimental Station (116°34' E, 36°50' N, altitude: 20 m), Chinese Academy of Agricultural Sciences (CAAS), which is located in Yucheng City, Shandong Province, in North China Plain (NCP), China. The site's mean annual precipitation and temperature are 569 mm and 13.4 °C, respectively. The experimental soil is a Fluvo-aquic type formed from the sediments of the Yellow River with light loam texture (clay 21.4%; silt 65.6%; sand 13.0%). Baseline soil chemical properties at the start of the experiment in 1985 were 3.93 g total organic carbon kg^{-1} , 0.51 g total nitrogen kg^{-1} , 7.50 mg Olsen P kg^{-1} , 1,73.00 mg ammonium acetate-extractable K kg^{-1} , 0.96 g soluble salt kg^{-1} . The experiment mimicked the standard winter wheat-summer maize double cropping system which is widely used in the NCP and produces on average a total grain yield (wheat yield plus maize yield) of 15 $\text{t ha}^{-1} \text{annum}^{-1}$ (Lin et al., 2009). Standard commercial tillage and irrigation regimes are used.

2.1. Fertilization treatments

The experiment consists of 24 plots of six fertilization treatments with four replicates arranged in a randomized complete block design. Each plot is 28 m^2 (4 m \times 7 m), and is separated by a 0.8 m concrete slab to prevent the flow of water and fertilizer between the plots. The fertilization treatments include a control (CK) with no amendments added, a standard mineral fertilizer (SMF) treatment and a standard organic manure treatment (SMA) that both reflect local farmer practice, a mixed treatment (1/2SMF + 1/2SMA) with fertilizer and manure both applied at half the rates for the SMF and SMA treatments, a double mineral fertilizer treatment (DMF) and a double organic manure treatment

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