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Effects of long-term application of chemical and organic fertilizers on the abundance of microbial communities involved in the nitrogen cycle

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ABSTRACT

Long-term agricultural fertilization significantly affects nitrogen cycling in soils. However, there is little information about the response of different functional genes involved in the nitrogen cycle to chemical and organic fertilization, and the key factor influencing the community abundance. We investigated the influence of long-term application of chemical fertilizer (NPK) and combined with wheat straw or livestock manures on the abundance of six nitrogen cycle genes (*nifH*, archaeal and bacterial *amoA*, *nirS*, *nirK* and *nosZ*) by quantitative PCR. Compared with non-fertilization treatment, long-term application of NPK fertilizers significantly increased the abundance of *nirK*, *nosZ* and bacterial *amoA* genes but decreased archaeal *amoA* gene abundance, with no significant effect on the abundance of *nifH* and *nirS* genes. Compared with NPK treatment, the application of NPK + organic manure increased the abundance of all the nitrogen-cycling genes while the application of NPK + wheat straw had little effect. The abundance of *amoA* genes contributed the most to the variations in the abundance of the nitrogen cycling community between different fertilization strategies. Soil available P and total N were the most important factors influencing the abundance of microbial communities involved in the nitrogen cycle. These results indicated that, under the application of chemical fertilizers, the addition of livestock manures had much stronger effects on the abundance of nitrogen cycle genes than the addition of wheat straw, and bacterial and archaeal *amoA* genes were more sensitive to fertilization than other functional genes.

1. Introduction

Fertilization has been widely used as a common management practice to maintain soil fertility and crop productivity (Shen et al., 2010). Chemical fertilizers are generally high in nutrient content and are used rapidly and effectively by crops. To meet the needs of intensive agriculture, increasing amounts of chemical fertilizers have been applied to soil all over the world (Savci, 2012). However, risks to biodiversity, soil degeneration and environmental pollution are becoming increasingly serious with increasing chemical fertilization (Blanco-Canqui and Schlegel, 2013; Byrnes, 1990; Mozumder and Berrens, 2007). Organic fertilizers, which derive from animal matter, human excreta or vegetable matter, provide a more balanced nutrient supply, and the release of nutrients is more sustainable, when compared with chemical fertilizers (Dittmar et al., 2000; Chen, 2006). Additionally, organic fertilizers can modify soil physical conditions by improving soil aggregation, increasing soil hydraulic conductivity and by reducing mechanical resistance and bulk density (Bhattacharyya et al., 2007; Hati et al., 2006; Shirani et al., 2002). However, organic fertilizers are comparatively low in nutrient content and the nutrient release rate is too slow to meet crop requirements in a short time, the application of organic fertilizers alone could not meet the need of intensive agricultural production in usual. The combined application of organic and chemical fertilizers has proven to be a better approach to increase and sustain soil fertility and crop yields than the application of chemical or organic fertilizers alone (Aguilera et al., 2012; Bandyopadhyay et al., 2010; Bhattacharyya et al., 2008; Bokhtiar and Sakurai, 2005).

The cycling of nitrogen is a key component of agro-ecosystems, where nitrogen is a common limiting factor to the growth of crops. Crop yield depends largely on the extent to which the plants requirements for nitrogen can be met (Greenwood, 1982). However, nitrogen loss is a widespread problem in agricultural system

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(Harrison, 1989; Weier, 1994), which not only increases agricultural costs, but also causes many environmental problems, such as eutrophication, contamination of drinking water, global warming and atmospheric pollution (Choudhury and Kennedy, 2005; Stark and Richards, 2008). Therefore, it is urgent to find strategies to control and mitigate nitrogen loss from agricultural sources (Stark and Richards, 2008). Understanding the impact of different agricultural practices on the cycling of nitrogen will help us to solve the problems.

Nitrogen cycle is based on the redox transformation of nitrogen compounds and most of the processes are driven by a diverse range of microorganisms, especially bacteria and archaea (Simon and Klotz, 2013). Microorganisms are sensitive to habitat intervention (Jangid et al., 2008), and the application of fertilizers affects not only soil physical and chemical conditions, but also the biological environment (Bandyopadhyay et al., 2010; Liebig et al., 2002; Melero et al., 2007). Chemical fertilization dramatically but differentially affects the abundance of microbes involved in nitrogen cycling. Some studies found NPK fertilization increased the abundance of ammonia-oxidizing bacteria (AOB) (Fan et al., 2011; Wakelin et al., 2007) and denitrifiers (Chen et al., 2012a,b) while other studies found NPK fertilization had little effect on the abundance of *nifH*-containing microbes (Mårtensson et al., 2009) and AOB (Shen et al., 2008). Negative impacts of chemical fertilization on the abundance of ammonia-oxidizing archaea (AOA) (Fan et al., 2011) and AOB (He et al., 2007) have also been observed. Although chemical fertilization had different effects on the abundance of microbes involved in the nitrogen cycle, organic fertilization generally had positive effects on those functional microbes. Stubble retention or return of rice straw increased the abundance of the *nifH* gene (Wakelin et al., 2007; Wang et al., 2013). The addition of organic matter also resulted in higher abundance of AOB (Fan et al., 2011; He et al., 2007; Wakelin et al., 2007) and denitrifier communities (Chen et al., 2012a,b; Miller et al., 2008). The addition of organic matter and the associated increase in carbon content was an important factor in the positive effects on microbial habitats in soil. The addition of organic matters could provide abundant and balanced nutrients (such as high organic carbon and some inorganic salt) that are beneficial for the growth of microorganisms involved in nitrogen cycle (Chen et al., 2012a), as many microbial groups involved in the nitrogen cycle are heterotrophic or mixotrophic. Active nitrification could provide abundant substrate for denitrification, and the increase of oxygen consumption could lead to anoxic conditions in some soil area, which are favorable for denitrifiers (Miller et al., 2008). In addition, organic carbon compounds are also suitable electron donors for biological metabolism. The increase in organic carbon from the application of organic fertilizers could supply readily available organic carbon, thus stimulating denitrification (Philippot et al., 2007).

Although the effects of chemical and organic fertilization on the nitrogen cycling community have been widely investigated, most studies have focused on a single component of the nitrogen cycle, with a lack of research on the entire process especially the different response of nitrogen cycling community to fertilization (Bru et al., 2011; Hai et al., 2009). In addition, there is little information about the different effects of straw and livestock manures on the abundance of the nitrogen cycling community. In this study, we investigated the abundance of genes involved in different nitrogen cycle processes in a 30-year fertilization experimental field, which subjected to chemical fertilization with/without the addition of either wheat straw residues, cow manure, or pig manure. Our aim was to investigate the response of different functional genes involved in the nitrogen cycle to different fertilization strategies, and the key factors influencing nitrogen cycle gene abundance.

2. Materials and methods

2.1. Experimental field site

The long-term field experiment was initiated in 1982, located in Mengcheng, Anhui Province, China (33°13′ N, 116°35′ E). The average elevation of this region is 42 m, the mean annual temperature is 14.8 °C and mean annual precipitation is 872 mm. The soil is classified as Calcic Kastanozems according to the soil classification system of FAO (Food and Agriculture Organization) (Hua et al., 2014). Since 1982, the field has been under a winter wheat and summer soybean rotation, except for 1993–1998, when a winter wheat and summer maize rotation was in place.

Six treatments were included in this experiment: Control (without fertilization); chemical NPK fertilizers (NPK); chemical NPK fertilizers plus low amounts of wheat straw (NPK + LS); chemical NPK fertilizers plus high amounts of wheat straw (NPK + HS); chemical NPK fertilizers plus pig manure (NPK + PM); chemical NPK fertilizers plus cow manure (NPK + CM). Each treatment had four replicates (plots) and each plot was 60 m^2 (14.9 m × 4.7 m). N, P and K fertilizers were applied as urea (180 kg N ha^{-1} y^{-1}), superphosphate (90 kg P_2O_5 ha^{-1} y^{-1}) and potassium chloride (135 kg K_2O ha^{-1} y^{-1}), respectively. The application rates of low amounts of straw, high amounts of straw, pig manure and cow manure were 3750, 7500, 15,000 (fresh weight) and 30,000 (fresh weight) kg ha^{-1} y^{-1}, respectively. The C, N and P contents were 482.0 g kg^{-1}, 5.5 g kg^{-1} and 1.2 g kg^{-1} for wheat straw (Guo et al., 2014), 360 g kg^{-1}, 17 g kg^{-1} and 8.9 g kg^{-1} for pig manure and 370 g kg^{-1}, 7.9 g kg^{-1} and 4.2 g kg^{-1} for cow manure (Hua et al., 2014). Chemical fertilizers, wheat straw and manures were spread over the field and then the soil was ploughed for wheat seeding. All the NPK fertilizers, straw and manures were applied once a year in October.

2.2. Soil sampling and chemical analysis

Soil samples were collected on 11 June and 22 October 2012, just after wheat and soybean harvest, respectively. In each plot, the samples were collected from 12 points using a sterile blade at the depth of 0–10 cm and composited together as a single sample (replicate). After that, the samples were sieved through 2 mm mesh and impurities, such as fronds, roots and stones, were removed. Each sample was then divided into two portions: one was stored at 4 °C for biogeochemical analysis, and the other was stored at −40 °C for DNA analysis.

Soil pH was determined using a pH meter (FE20-FiveEasy™ pH, Mettler Toledo, Germany) at a soil:distilled water ratio of 1:5 (weight/volume). Soil total carbon (TC) and total nitrogen (TN) were measured using an elemental analyzer (Vario MAX, Elementar, Germany) after grinding and drying at 65 °C for 48 h. Nitrate (NO_3^--N), ammonium (NH_4^+-N), dissolved organic carbon (DOC) and dissolved total N (DTN) were extracted by placing 10 g fresh soil with 100 ml 2 M KCl, shaking for 1 h and then percolating through filters (Whatman, grade 2). The contents of nitrate, ammonium and DTN were determined using a continuous flow analytical system (San++System, Skalar, Holland) and DOC was determined using a total organic carbon analyzer (Multi N/C 3000, Analytik Jena, Germany). Dissolved organic nitrogen (DON) was calculated according to the following formula: DON = DTN − NH_4^+-N − NO_3^--N. Available K (AK) was extracted with 1 M ammonium acetate and determined using flame photometry (FP640, INASA, China). Soil available P (AP) was extracted with 0.5 M $NaHCO_3$ and determined using the molybdenum blue method. Soil nitrification potential was determined using the "shaken soil-slurry method" (Hart et al., 1994) with slight modifications. Briefly, 10 g of 1-mm sieved, field-moist soil was placed into a 250 ml flask.