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Long-term fertilization regimes drive the abundance and composition of N-cycling-related prokaryotic groups via soil particle-size differentiation

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ABSTRACT

Even though organic fertilization is known to promote soil nitrogen cycling (*N*-cycling), it is unclear how this effect is mediated. Here we studied the potential roles of soil physiochemical properties and soil particle-size fraction (PSF) in relation to the diversity and abundance of *N*-cycling-related prokaryotes by sampling field sites that had been subjected to either mineral or organic fertilization for over 30 years (two independent fields subjected to both fertilization treatments). We found that organic fertilization increased the 63–200 μm sized soil fraction and the abundance of various *N*-cycling-related prokaryotes including diazotrophs, ammonia-oxidizers and denitrifiers. Moreover, increase in the abundance of different *N*-cycling-related prokaryotes varied between different size fractions: the abundance of ammonia-oxidizers and nitrate-reducers was the highest in the 63–200 μm size fraction, while the abundance of diazotrophs, ammonifiers and denitrifiers was the highest in the 200–2000 μm, 2–63 μm and 0.1–2 μm size fractions, respectively. According to structural equation modelling (SEM), soil PSF, physiochemical properties and the community structure of *N*-cycling-related prokaryotes within different PSFs were associated with the abundance of *N*-cycling-related prokaryotes in non-fractionated bulk soils. In conclusion, these results suggest that organic, but not mineral, fertilization can support the abundance of *N*-cycling-related prokaryotes and that the strength of the effects depends on their association with specific particle-size fractions.

1. Introduction

Soils are under increasing pressure to provide a range of agroecological services for meeting the growing demand for food and bioenergy production (Hayden et al., 2010). Nitrogen (N) is a critical nutrient in agroecosystems, and crop production largely depends on the extent to which agricultural *N*-requirements can be achieved (Greenwood, 1982). The transfer of nitrogen into, within, and out of the soils requires interaction between various microorganisms that carry out the ecological functioning of N_2-fixation, ammonia-oxidation, denitrification and ammonification (Hayden et al., 2010; Vitousek et al., 2002). Soil *N*-cycling reflects therefore a key linkage between above- and below-ground ecosystems and is one of the most important biogeochemical processes on the Earth (Sun et al., 2015).

The soil microorganisms responsible for *N*-cycling are often very sensitive to external disturbance (Bandyopadhyay et al., 2010; Melero et al., 2007). For example, long-term agricultural fertilization has been shown to significantly affect the soil *N*-cycling processes by shaping the structure of corresponding prokaryotic communities (Sun et al., 2015). In general, it has been found that mineral fertilization can have dramatic effects on the abundance of different *N*-cycling-related prokaryotic groups (Fan et al., 2011; Shen et al., 2008). In contrast, organic fertilization has generally been shown to have a positive but group-specific effect on the abundance of diazotrophs (Hai et al., 2009), nitrifiers (Wang et al., 2014) and denitrifiers (Chen et al., 2010). The effects of fertilization on *N*-cycling-related prokaryotic groups can be further modified by various soil physiochemical properties such as soil structure, pH and resource availability (Hallin et al., 2009; Bru et al., 2011; Levy-Booth et al., 2014). For example, fertilization can change the soil particle size fraction (PSF), which could potentially affect the composition and functioning of PSF-associated prokaryotic communities (Neumann et al., 2013; Blaud et al., 2016; Jiang et al., 2013). However, it is still unclear how the type of long-term fertilization (e.g. organic vs. mineral) affects the abundance, diversity and composition

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of different *N*-cycling-related prokaryotic groups in agroecosystems.

Soils are composed of a wide range of different sized soil fractions that act as microhabitats for diverse microbial communities (Miller and Dick, 1995). Such microenvironments are important for determining mineral-organic interactions of soil biochemical processes scale on a micro-scale (Zhang et al., 2007). Soil PSF have been associated with variation in microbial community structure (Poll et al., 2003; Hemkemeyer et al., 2014, 2015) having effects on of *N*-cycling (potentially via enzymatic activity (Marx et al., 2005)) and other *N*-cycling-related microbiological processes such as ammonification (Nacro et al., 1996), denitrification (Lensi et al., 1995) and ammonia-oxidation (Jiang et al., 2014). Even though fertilization can affect the distribution of microbial groups connected to organic carbon turnover and accumulation in the soil PSF (Sessitsch et al., 2001; Zhang et al., 2007, 2014a, 2015), the effects on *N*-cycling-related prokaryotes are still unclear. Specifically, the interactive effects between organic fertilization, soil physiochemical properties and soil PSF for the abundance and activity of *N*-cycling-related microorganisms, and the soil biological potential for N transformation, remain elusive (Hayden et al., 2010).

The objective of this study was to characterize the effects between long-term mineral and organic fertilization on the soil PSF differentiation and the abundance and composition of *N*-cycling-related prokaryotic communities. Quantitative PCR (q-PCR) approach was used to measure the abundances of different *N*-cycling-related prokaryotic groups across various soil fractions by using samples collected from two independent fields that had been subjected to organic or mineral fertilization over a 30-year period. We chose the *nifH* gene as a bio-marker for the molecular analysis of diazotrophs (Levy-Booth et al., 2014) that include microorganisms with nitrogenase enzymes used for fixing atmospheric N_2 into a biologically usable form either as free-living or in symbiosis with their host plants. Nitrification is the biological oxidation of NH_3 to NO_3^- and involves two distinct groups of microorganisms, chemolithotrophic ammonia-oxidizers and nitrite-oxidizers (Chain et al., 2003). To study this group, we used the *amoA* marker gene (*AOA* and *AOB*), which encodes the subunit of the AMO enzyme. Denitrification is the full or partial dissimilative reduction of NO_3^- to N_2 by microorganisms and it is the primary pathway of N_2O emissions from the soils (Kowalchuk and Stephen, 2001; Shaw et al., 2006). Denitrifiers belong to a variety of physiological and taxonomic groups (Zumft, 1997) and the process of denitrification is carried out by many enzymes. As a result, several genes were used as bio-markers for denitrifiers including nitrate reductase (encoded by *napA* or *narG* genes), nitrite reductase (encoded by *nirS* or *nirK* genes), nitric oxide reductase (encoded by *norB* gene) and nitrous oxide reductase (encoded by *nosZ* genes). Ammonification, which is involved in the decomposition of organic N to ammonia is carried out by NAD-dependent glutamate dehydrogenase encoding *gdh* gene. Thus, the *gdh* gene was used as a bio-marker for ammonification (Govindarajulu et al., 2005). We used permutational multivariate analysis of variance (PERMANOVA), multivariate regression tree (MRT) and structural equation modelling (SEM) to analyse and test two following main hypotheses. First, we hypothesized that long-term fertilization would have an impact on the soil prokaryotes involved in the *N*-cycling via the soil PSF differentiation and soil physiochemical properties. Second, if these effects are very general, we might expect that this impact would depend on the association of the organisms with specific soil PSF, but not vary between soils from two different geographical regions.

2. Materials and methods

2.1. Description of the field sites and soil sampling

To explore general effects of fertilization, we chose two independent fields for sampling that had different rotation system histories, soil types, physiochemical properties and annual climatic conditions (Fig. S1). One of the field sites was located at Suixi County, Anhui Province, China (116° 45′ E, 33° 37′ N), and had been used for corn–wheat rotation since it was established in 1981. The annual mean temperature and precipitation for this region are approximately 15.0 °C and 870 mm, respectively. The other field site located in Jiangxi Province, China (116°20′ E, 28°15′ N) and had been used for corn-corn-fallow rotation since it was established in 1986. The mean annual temperature and precipitation for this region are approximately 17.3 °C and 1549 mm, respectively. The soil properties at the beginning of the field experiments for both sites are shown in Table S1. The exact location and cropping regimes are shown in Fig. S1 (including also the major soil groups based on the China Soil Classification System (Zhang et al., 2014b)). All plots were arranged in a randomized block design and the fertilizer application rates, fertilizer type and the plot sizes are summarized in Table 1.

Table 1
Application rates, fertilizer types and plot sizes for fertilization treatments in the two long-term field sites.

	Treatment [a]		Amount of fertilizer ($kg{\cdot}ha^{-1}{\cdot}y^{-1}$)	Replicate plot sizes (m^2)
I	A-NPK	Application of mineral fertilizer only [b]	N 525, P_2O_5 210, K_2O 210	30
II	A-OF	Application of organic fertilizer only [c]	10, 000	30
III	J-NPK	Application of mineral fertilizer only [d]	N 240, P_2O_5 120, K_2O 240	22
IV	J-OF	Application of organic fertilizer only [e]	30, 000	22

[a] A, Anhui experimental site; J, Jiangxi experimental site; NPK: application of mineral NPK fertilizer; OF: application of organic fertilizer.
[b] The mineral NPK fertilizer composed of urea, superphosphate and potassium chloride.
[c] The organic fertilizer was prepared from bean cake compost and contained 30–40% organic matter, 6–7% total N, 1–3% P_2O_5, 2–3% K_2O, and 10–15% water.
[d] The mineral NPK fertilizer composed of urea, calcium magnesium phosphate and potassium chloride.
[e] The organic fertilizer was composed of pig manure.

Within both fields, soils were treated with organic fertilizer (OF) or mineral NPK fertilizer (NPK) and both fertilization treatments were replicated three times (three plots) within each field (two different experimental sites with two fertilization treatments each replicated for three times: N = 12). We collected soil samples from all the plots in 2016 before the harvest as follows. Four soil cores (5 cm diameter covering 0–20 cm depth) were collected from each plot and carefully mixed to provide a single sample per replicate plot. The soil cores were gently broken apart along the natural break points and sieved (< 5 mm) to remove visible plant matter and organic debris. One part of each sample was stored at 4 °C for analysing the soil physiochemical properties, while the other part was stored at −20 °C for DNA extraction.

2.2. Determination of soil physiochemical properties and DNA extraction

The soil physicochemical properties were determined by following previously described methods (Ling et al., 2014). Briefly, soil total carbon (TC) and total nitrogen (TN) were measured with an elemental analyser (Vario MAX; Elementar, Germany), while the total soil phosphorus (TP) was determined by digestion with HF–$HClO_4$ followed by molybdenum-blue colorimetry (Xun et al., 2016).

The total genomic soil DNA was extracted from 0.25 g of soil per each sample (dry weight equivalent) by using a Power Soil® DNA Isolation Kit (MoBio, Carlsbad, CA, USA), which efficiently eliminates humic substances and other potential quantitative PCR (qPCR) inhibitors. Additionally, DNA extracts were purified with a Wizard DNA Clean-Up System (Axygen Bio, USA) as recommended by the manufacturer. Even though it is possible that some qPCR inhibitors were still left to our samples, their effect was likely small, which should not cause