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Short Communication

Fertilization rather than aggregate size fractions shape the nitrite-oxidizing microbial community in a Mollisol

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ABSTRACT

How nitrite-oxidizing bacteria (NOB) respond to long-term fertilization and variations in soil aggregate levels remains unclear. In this study, the potential nitrite oxidation activity (PNO), abundance, diversity, and community compositions of *Nitrobacter*- and *Nitrospira*-like NOB were examined in three aggregate fractions (2000–250, macroaggregate; 250–53, microaggregate; < 53 µm, silt + clay) of a Mollisol under four fertilization regimes. NOB abundances were higher in macro- and micro-aggregates, and best explained by aggregate size variation. The PNO, Shannon diversity index and community composition of NOB were more affected by the fertilization regimes. We found PNO significantly correlated with the structure of *Nitrospira*-like NOB, followed by the abundances and Shannon diversity indexes of NOB. Soil aggregate phosphorus level, total potassium and NH_4^+ were associated with the NOB community structure. Our results suggested that PNO directly link to the variations for the abundance, diversity and community structure of NOB, which are regulated by the nutrient level in the microhabitat.

Nitrite-oxidation is the second step in nitrification catalyzed by nitrite oxidizing bacteria (NOB) (Daims et al., 2016). Among NOB, *Nitrobacter*- and *Nitrospira*-like NOB were believed to play important functional roles in terrestrial ecosystems (Bartosch et al., 2002; Kim and Kim, 2006; Ke et al., 2013). Soil aggregates, differing in physico-chemical properties, provide spatially heterogeneous habitats for various microbial communities (Six et al., 2000; Sessitsch et al., 2001; Davinic et al., 2012). How the abundance, alpha diversity, and community structure of *Nitrobacter*- and *Nitrospira*-like NOB affect their potential functions of various aggregates under long-term fertilization remains poorly understood. We hypothesized that both fertilization and particle sizes could affect the abundance, diversity and, community structure of NOB, which ultimately lead to the change in their potential function, by regulating the soil properties. In addition, this also might prompt us to ask which or both of NOBs drive PNO in soils. Here we used a Mollisol (USDA taxonomy) for a case study.

The long-term fertilization experiment was conducted in the Mollisol Ecological Experimental Station (45°40′N, 126°37′E) in Harbin, Heilongjiang Province, China. Four fertilization treatments were employed in a completely randomized design with three replications: without fertilization (CK); horse manure (M); chemical fertilizers including nitrogen, phosphorus, and potassium fertilizers (NPK); and chemical fertilizers plus horse manure (MNPK). Details on the experimental site, soil sampling, aggregates fractionation and analysis were described in Wang et al. (2018). The chemical properties of soil aggregates are shown in Fig. S1, and the total phosphorus (TP), available phosphorus (AP), total potassium (TK) and NH_4^+ were significantly ($p < 0.01$) enhanced by fertilization. Two-way analysis of variance (ANOVA) indicates that the soil aggregate fractions were significantly related to those measured properties (Table S1). The potential nitrite oxidation (PNO) activity was determined using the method described by Wertz et al. (2007). The PNO ranged from 0.02 to 1.33 µg NO_2^- -N $g^{-1}h^{-1}$ (Fig. 1A) and was markedly ($p < 0.01$) affected by the fertilizer treatments (Table S2). The PNO displays significant and positive correlations with the C/N ratio ($r = 0.39$, $p < 0.01$), TP ($r = 0.57$, $p < 0.01$), TK ($r = 0.61$, $p < 0.01$), NH_4^+ ($r = 0.44$, $p < 0.01$) and AP ($r = 0.66$, $p < 0.01$) (Table S3). It is likely that the inorganic nutrient supply is the key determinant for soil aggregates PNO. The larger

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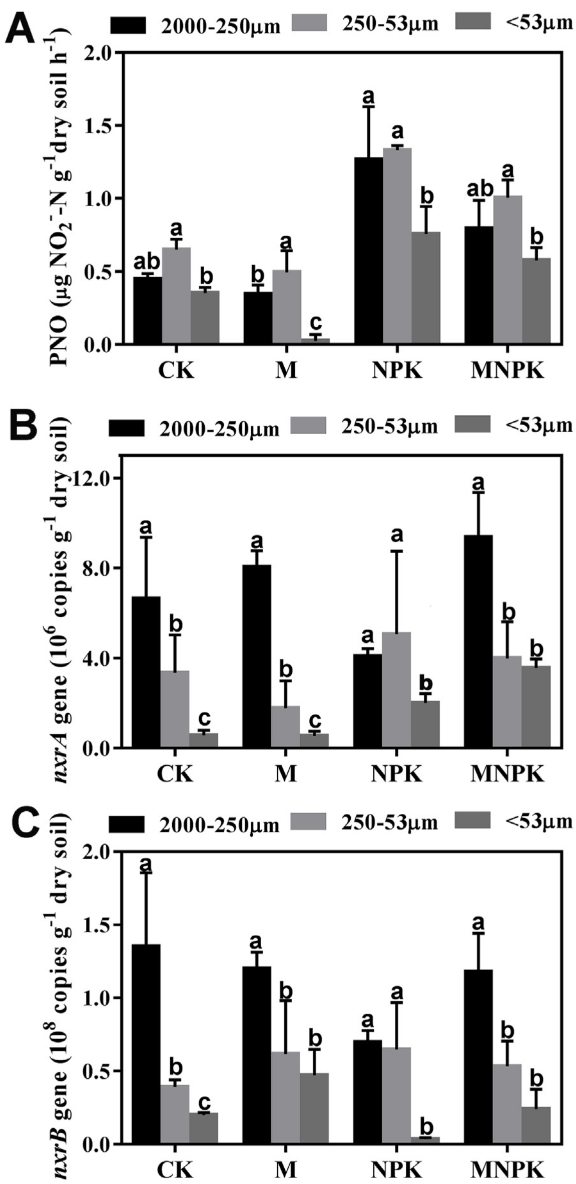

Fig. 1. Soil aggregate potential nitrite oxidation (A) activity among three fractions under different fertilization treatments. Abundances of *Nitrobacter*- (B) and *Nitrospira*-like NOB (C) of three fractions under different fertilizer treatments. Error bars represent the standard error (n = 3) and are followed by a lowercase letter indicating a significant difference among three factions within each fertilizer according to the Tukey test ($p < 0.05$).

Table 1
Multiple regression analysis between PNO and abundance, diversity, and community structure PCA1 values of *Nitrobacter*- and *Nitrospira*-like NOB, respectively.

PNO = 1.21 + 0.77 $\log_{10}$(*Nitrobacter* abundance)-0.72 $\log_{10}$(*Nitrospira* abundance)	equation (1)
$R^2 = 0.50$, $p < 0.01$	
PNO = 3.97–0.52(*Nitrobacter* Shannon index)-0.30(*Nitrospira* Shannon index)	equation (2)
$R^2 = 0.50$, $p < 0.01$	
PNO = 0.67–0.37(*Nitrobacter* PCA1 values) + 2.28(*Nitrospira* PCA1 values)	equation (3)
$R^2 = 0.64$, $p < 0.01$	

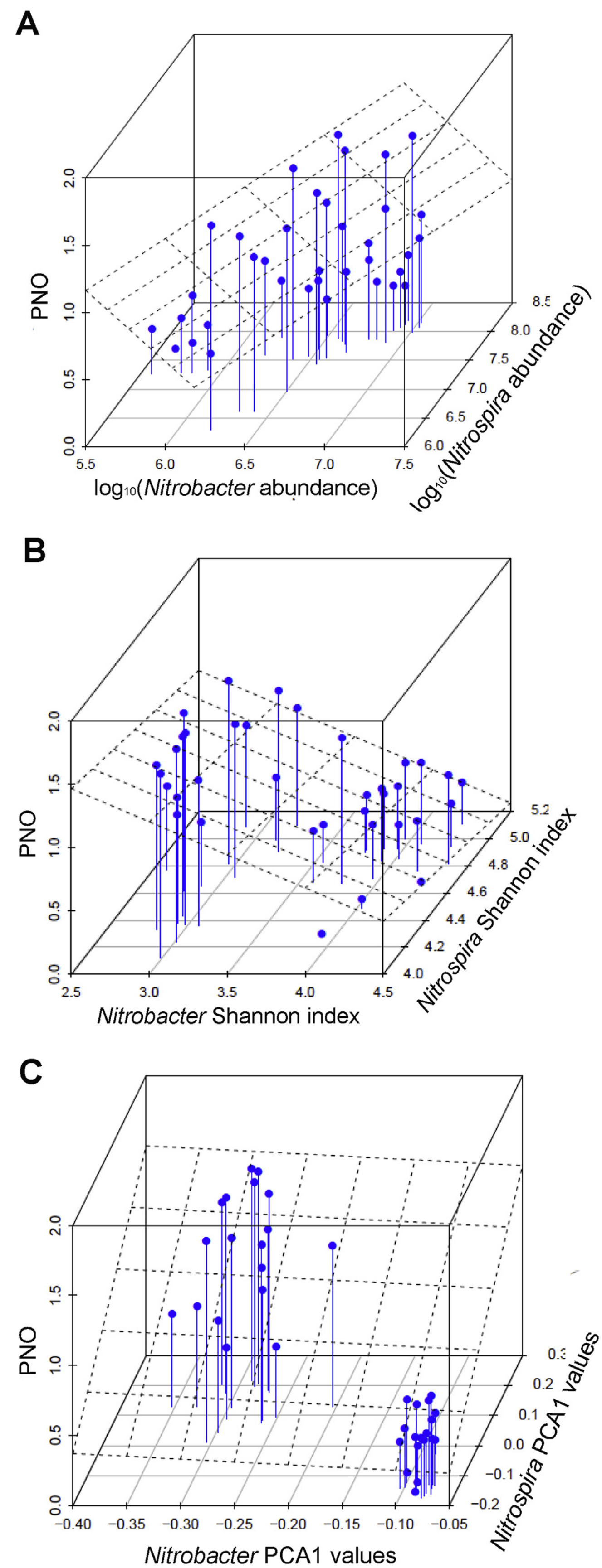

Fig. 2. PNO values vs. abundance (A), Shannon diversity index (B), and community structure PCA1 values (C) of *Nitrobacter*- and *Nitrospira*-like NOB, respectively. Plane represents a significant multiple linear regression with the equation in Table 1.