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Different responses of soil microbial metabolic activity to silver and iron oxide nanoparticles

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HIGHLIGHTS

- AgNPs decrease soil microbial metabolic activity and metabolic efficiency.
- FeONPs increase soil microbial metabolic activity and metabolic efficiency.
- AgNPs and FeONPs have the contrast effects on soil nitrification process.
- Nanoparticles invasion would influence the health and fertility of arable soil.

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ABSTRACT

The knowledge regarding the effects of metal or metal oxide nanoparticles on soil microbial metabolic activity and key ecological functions is limited, relative to the information about their species diversity. For this reason, the responses of soil microbial metabolic activity to silver (AgNPs) and iron oxide (FeONPs) nanoparticles, along concentration gradients of each, were evaluated by microcalorimetry and soil nitrification potential. The changes in abundances of bacteria, eukaryotes and ammonia-oxidizing bacteria were measured by real time quantitative PCR. It was found that AgNP (at 0.1, 1 and 10 mg kg^{-1} soil) amendments decreased soil microbial metabolic activity, nitrification potential and the abundances of bacteria and ammonia-oxidizing bacteria; on the contrary, FeONPs had the positive effects on soil microbial metabolic activity (at 1 and 10 mg kg^{-1} soil) and soil nitrification potential (at 0.1 and 1 mg kg^{-1} soil). Specific microbial metabolic activity and specific nitrification potential further revealed that metal or metal oxide nanoparticles could change the C and N cycles of the agricultural soil through influencing soil microbial metabolism. These findings could deepen the understanding of the influence of NPs on soil microorganisms and their driven soil ecology process.

1. Introduction

Soil microorganisms, the indispensable agents of soil ecosystem, are responsible for decomposition of organic residues and are the key players in driving nutrient cycling in arable soils, such as cycling of nitrogen, phosphorus and potassium (Kaye et al., 2005). Thereby soil microorganisms affect C and nutrient cycling of arable soil. Meantime, soil microorganisms sensitively respond to environmental changes and/or exotic stress. Furthermore, in some instances, the changes in microbial community structure or activity can even mirror the variations in soil physical and chemical properties in response to different exotic disturbances (Lin et al., 2012). For such reason, the influences of exotic stresses on the community composition and functional aspects of soil microorganisms are frequently reported. For example, the environmental stresses caused by heavy metals generally decrease the species diversity of the soil microbial community (Chen et al., 2014b; Wang et al., 2010).

The extensive use of manufactured nanoparticles (NPs) for a variety of industrial, commercial, medical and agricultural products leads to their inevitable release into the environment, where they

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may potentially become environmental contaminants (Nowack, 2009; Paschoalino et al., 2010). Concomitantly, the impact of NP release on crop and agroecosystem has been focused and reported. Silver nanoparticles (AgNPs) and multi-walled carbon nanotubes (MWCNTs) are documented to decrease plant biomasses by 60% and 75%, respectively (Stampoulis et al., 2009). Our previous investigation found that iron oxide nanoparticles (FeONPs) can stimulate the growth of the mung bean (*Vigna radiata*) by enhancing its physiological activities (Ren et al., 2011). Soil microorganisms play important roles in plant growth and agroecosystem. Therefore, in order to comprehensively unravel the ecotoxicity of NPs, the soil microbial responses to NP pollution have also been extensively and intensively recorded. Some metal or metal oxide NPs have been found to be toxic to soil microorganisms and influence soil microbial species diversity. Kumar et al. (2012) reported that AgNPs are highly toxic to the soil microbial community, especially to the plant-associated bacteria *Bradyrhizobium canariense*. Our previous study indicates that FeONPs can potentially stimulate some bacterial growth and change the soil bacterial community structure (He et al., 2011b).

NPs bring changes not only in soil microbial species diversity but also in their ecological functions. AgNPs have been shown to inhibit the activities of soil dehydrogenase activity (Murata et al., 2005), phosphomonoesterase, arylsulfatase, β-D-glucosidase and leucine-aminopeptidase (Peyrot et al., 2014). FeONPs can stimulate soil enzyme activities (He et al., 2011b). Soil enzyme activities and/or ecological functions are concomitant with soil microbial metabolism. Thus, the abovementioned phenomena imply the possible influences of nanomaterials on soil microbial metabolism. Soil respiration has been conducted to evaluate the responses of soil microbial metabolism to NPs (Ge et al., 2013; Kumar et al., 2014; Yang et al., 2014a). In contrast, microcalorimetric technique can provide more detailed information on soil microbial metabolic property than soil respiration does. Indeed, microcalorimetry has been used to evaluate the effects of different pollutants on soil microbial metabolism (Herrmann et al., 2014). However, to the best of our knowledge, there is almost no microcalorimetric report regarding the effect of nanoparticles on soil microbes to date. This type of information would be of great help towards a comprehensive understanding of the effect of NPs on soil microbial ecological functions and their ecological processes. For example, soil microbial metabolic efficiency is closely connected to soil carbon conversion efficiency and soil C cycling (Barros et al., 2003).

Soil microorganisms assimilate or dissimilate soil C to drive nutrient cycles. The changes in soil microbial metabolic activity, therefore, can also imply the influence of NPs on, for instance, the soil N cycle. The rate-limiting step in microbial nitrification is the oxidation of ammonia to nitrite. Ammonia oxidation is mainly carried out by autotrophic ammonia-oxidizing microbes. However, the investigation on the influence of NPs on ammonia oxidation and its drivers in arable soil is at the infant stage. At strain level, Anjum et al. (2013) summarized the inhibitive effects of AgNPs on nitrifying bacterial growth and their activity. The condition of pure culture is quite different from that of soil matrix; only one strain rather than AOB (ammonia-oxidizing bacteria) community is focused. The information on the responses of soil ammonia-oxidizing microbial community to NPs was limited.

In view of the abovementioned information gaps, in this study, AgNPs and FeONPs (iron oxide NPs) are focused, because they are the widely used NPs in a wide range of technical applications and consumer products (Ramimoghadam et al., 2014; Yang et al., 2014b); as a consequence, they are also the NP pollutants (Nair et al., 2010). The changes of soil microbial metabolic activity in a microcosm amended with FeONPs or AgNPs were monitored by microcalorimetry. The responses of the soil nitrification process to two metal (oxide) NPs were also analyzed to evaluate the potential effect of NPs on the N cycle of arable soil. In addition, the shifts in population size of soil bacteria, eukaryotes and AOB were determined by real-time quantitative PCR (qPCR). Collectively, these findings will greatly contribute to the information regarding the assessment of NP ecotoxicity in agroecosystems.

2. Materials and methods

2.1. Preparation and characterization of nanoparticles

The standard procedure (Feng et al., 2013) was followed to prepare two types of NPs solutions. Briefly, FeONPs were synthesized by chemical co-precipitation of Molday. Colloidal AgNPs were synthesized by reducing $AgNO_3$ in a polyvinylpyrrolidone (PVP) solution using glucose as reducer and NaOH to accelerate the reaction.

For TEM analysis, 5 μl of each sample solution was placed onto a carbon coated copper grid. Once the solvent evaporated, TEM images were collected with JEM-2000EX (accelerating voltage of 120 KV), and used for particle morphology characterization. For each sample, a minimum of 400 NPs were randomly selected for the analysis of diameter distribution. The zeta potential (ζ) of the NPs was measured with a zeta potential analyzer (BECKMAN, Delsa 440SX). To determine their dispersibility in soil matrix, the NPs in soils were extracted following the protocol of Wang et al. (2014) and were examined by TEM analysis.

Bulk silver and iron oxide particles (about 5 μm) were purchased from Shanghai Demo Chemical Technology Co., China.

2.2. Soil description and sampling

Soils were collected from Fengqiu County, Henan Province, China (35°00′N, 114°24′E). The soil, a typical soil in the North China region with a profile of sandy loam (approximately 9% clay, 21.8% silt) in the plough layer and loam in the subsoil, was derived from alluvial sediments of the Yellow River and classified as Aquic Inceptisol (a calcareous fluvo-aquic soil). The soil contained 5.83 g kg^{-1} of organic matter, 0.45 g total N kg^{-1}, 0.50 g total P kg^{-1}, and 18.6 g total K kg^{-1} and had a pH of 8.65. The crop succession was winter wheat (*Triticum aestivum* L.) and summer maize (*Zea mays* L.).

After wheat harvest, soil samples were collected from the surface (0–15 cm depth) of arable soils. Soil samples were packed on-site into sealed polythene bags and then transported to the laboratory. Large roots, macro-fauna and stones were removed from the samples, and then the residual soil was crushed and passed through a sieve (2 mm) for the nanoparticle amendment microcosm.

2.3. The nanoparticle amendment microcosm

The 20-g soil was adjust to 60% of maximum water holding capacity (the maximum ability of a soil to contain and to retain water, 28% in the current investigation) by additions of 1.76 ml distilled water based on the 8% soil water content. Then, different concentrations of silver nanoparticles (AgNPs) (0.1 (L), 1 (M), 10 (H) mg kg^{-1} soil) (Arnaout and Gunsch, 2012) and iron oxide nanoparticles (FeONPs) (0.1 (L), 1 (M), 10 (H) mg kg^{-1} soil) (Zhang et al., 2011) were added to the soil and mixed thoroughly. To demonstrate the nanoparticle effect, Fe_2O_3 (5 μm diameter) and Ag (5 μm diameter) bulk materials were established for comparison, as well as a control without nanoparticle amendment. Specifically, NPs and bulk materials were sonicated for 10 min in deionized water and then transferred into corresponding soil samples. To ensure thorough mixing, each soil sample was first divided into