Soil Biology & Biochemistry 86 (2015) 24–33

Contents lists available at ScienceDirect

Soil Biology & Biochemistry

journal homepage: www.elsevier.com/locate/soilbio

Distinctive effects of TiO_2 and CuO nanoparticles on soil microbes and their community structures in flooded paddy soil

Chen Xu, Cheng Peng, Lijuan Sun, Shu Zhang, Haomin Huang, Yingxu Chen, Jiyan Shi*

Department of Environmental Engineering, Zijingang Campus, Zhejiang University, 388 Yuhangtang Road, Hangzhou, Zhejiang, 310058, People's Republic of China

ARTICLE INFO

Article history:
Received 4 September 2014
Received in revised form
3 March 2015
Accepted 12 March 2015
Available online 30 March 2015

Keywords:
CuO nanoparticles
TiO_2 nanoparticles
Soil microbes
Enzyme activity
Phospholipid fatty acids (PLFAs)
Paddy soil

ABSTRACT

The wide use of metal oxide nanoparticles (MNPs) will inevitably increase their environmental release into soil, which consequently raises concerns about their environmental impacts and ecological risks. In this study, two typical MNPs (TiO_2 and CuO NPs) in different doses (0, 100, 500 and 1000 mg kg^{-1} soil) were applied to evaluate their effects on microbes in flooded paddy soil. The negative effects of CuO NPs were stronger than that of TiO_2 NPs on soil microbes, as reflected by the significant decline in soil microbial biomass (as indicated by the reduced microbial biomass carbon [MBC] and the total phospholipid fatty acids [PLFAs]) and enzyme activities including urease, phosphatase and dehydrogenase. The principle component analysis (PCA) of the PLFAs and the diversity indices reveal that not TiO_2 NPs but CuO NPs reduced the composition and diversity of the paddy soil microbial community. The reduced impact of TiO_2 NPs may be due to their particle characteristics. The bioavailability of CuO NPs is thought to induce the major toxicity to microbes in the flooded paddy soil, as determined by the increased Cu contents in the soil extractions and the microbial cells. The elevated stress ratio values demonstrate that CuO NPs may also indirectly affect soil microbes by changing nutrient bioavailability. Over all, both TiO_2 NPs and CuO NPs may induce perturbations on the microbes in flooded paddy soil and showed potential risks to the paddy soil ecosystem. Therefore, attentions toward the effects of MNPs to the ecological environment should be paid from now on.

1. Introduction

Rapid development of nanotechnology has enabled the production of metal oxide nanoparticles (MNPs) for industrial, agricultural, medical and consumer applications that take advantage of their unique electrical, magnetic and catalytic properties (Godwin et al., 2009; Kumar et al., 2011a). For example, TiO_2 nanoparticles (TiO_2 NPs) are widely used in sunscreens, cosmetics, catalysts and bottle coatings, while CuO nanoparticles (CuO NPs) are commonly used in semiconductor devices, catalysts, and photovoltaic cells (Jiang et al., 2002; Godwin et al., 2009). The handling of MNPs among their production, application and disposition is leading to their inevitable release to the environment, which consequently raises a great deal of concerns regarding the potential environmental risks of MNPs (Gottschalk and Nowack, 2011; Pan and Xing, 2012). While the concentrations of most MNPs in the environments still remain unknown, the exposure modeling suggests that soil could be a major sink of MNPs, with higher accumulation than that in water or air (Gottschalk et al., 2009).

Since soil acts as an important interface to sustain plant and animal productivity, maintain or enhance water and air quality, and support human health and habitation (Doran and Parkin 1996), the assessment and protection of soil quality and health is critically important and urgently needed. Among the various factors that have been proposed to influence soil health, biological indicators (including soil organism and biotic parameters) are reported to be vitally important (Doran and Zeiss 2000) because ecosystem functioning is governed largely by soil microbial dynamics. So far,

---

Abbreviations: MNPs, metal oxide nanoparticles; NPs, nanoparticles; CuO NPs, CuO nanoparticles; TiO_2 NPs, TiO_2 nanoparticles; MBC, microbial biomass carbon; PLFAs, phospholipid fatty acids; EDTA, ethylene diamine tetraacetic acid; DTPA, diethylene triamine pentacetate acid; FAAS, flame atomic absorption spectrometry.

* Corresponding author. Department of Environmental Engineering, College of Environmental and Resource Sciences, Zhejiang University, Hangzhou 310058, People's Republic of China. Tel./fax: +86 571 88982019.

E-mail addresses: smilechenbanban@126.com (C. Xu), xhpmc@163.com (C. Peng), sunliuliu2012@126.com (L. Sun), shuzhangspower@gmail.com (S. Zhang), skeeter19881030@163.com (H. Huang), yingxuc@gmail.com (Y. Chen), shijiyan@zju.edu.cn (J. Shi).

http://dx.doi.org/10.1016/j.soilbio.2015.03.011
0038-0717/

many nanoparticles (NPs) have been reported to be microbial toxic and thus directly affect microorganisms. For example, TiO_2 NPs damaged the cell wall/membrane of *Nitrosomonas europaea*, leading to increased cell permeability and ultimately cell death (Fang et al., 2010). CuO NPs displayed notable toxicity towards bacteria by generating free radicals (Gajjar et al., 2009). However, most of the investigations were conducted on a pure microbial culture or cell culture under constant physicochemical conditions, which are far from the complex natural environment. Debates regarding how significantly that NPs are released into soil will affect soil microorganisms still remain. Rousk et al. (2012) found that the application of CuO NPs caused a sigmoidal decay in bacterial growth in mineral soil, while no statistical significant relationship was observed in organic soil. The bacterial community composition and size were also affected differently by CuO NPs in two different soil types (Frenk et al., 2013). In the case of TiO_2 NPs, rather confusing effects on soil microorganisms were demonstrated by different studies. Ge et al. (2012) reported that the addition of TiO_2 NPs altered the bacterial communities significantly, whereas Burke et al. (2014) observed a variation in arbuscular mycorrhizal fungal community in the TiO_2-NP amended soil. Moreover, TiO_2 NPs reduced the diversity of microbial community in a grassland soil (Ge et al., 2011), and in contrast, a striking increase in soil richness was observed by Shah et al. (2014). This discrepancy of soil microbial response to MNPs could be attributed to the coactions of many factors, including the inherent toxicity differences among MNPs, exposure dose and time, the treated microbiological species, soil property (pH, Eh, soil organic matter, water content, iron strength etc.), or other experimental conditions. So far, there is no standard to assess the potential toxicity of MNPs to soil microorganisms due to the complicated environmental factors and the lack of traceability of MNPs in soil. Understandably, gaps in the understanding of some crucial ecosystems that may be vulnerable to MNPs must be addressed.

Agricultural soil is more likely to be exposed to MNPs than wild land soil due to the use of sewage sludge as fertilizer and the application of MNPs in plant protection products (Suppan, 2013; Larue et al., 2014). Thus, MNPs have potentially profound impacts on terrestrial ecosystems and the safety of food chain. Paddy soil is the most typical and widespread agricultural soil in both China and Western Asia, and the quality of paddy soil is critical to the national economy and people's livelihood. While the effects of MNPs on the microbial communities of dryland soil (grassland soil, arctic soil, forest soil, etc.) have been discussed recently (Kumar et al., 2011b; Ge et al., 2013; Vittori Antisari et al., 2013), wetland soil with specific properties, such as flooded paddy soil, has yet to receive sufficient attention. In this study, the soil samples collected from a typical paddy field in Hangzhou, China were exposed to different doses of TiO_2 NPs and CuO NPs in rhizo-boxes for 110 days. The soil microbial biomass carbon (MBC) and the total phospholipid fatty acids (PLFAs) were used to assess the effects on the soil microbial biomass. Urease, phosphatase and dehydrogenase activities were measured as the indication of soil microbial activities. The effects on the microbial community composition and diversity were evaluated using PLFAs analysis. The main purpose of this paper is to provide a relatively full view of the impact of MNPs on a paddy soil micro-ecosystem.

2. Material and methods

2.1. Nanoparticles

Both TiO_2 NPs and CuO NPs were procured from Nachen Sci. & Tech Ltd., Beijing, China. Semispherical TiO_2 NPs (white, bare coated) have a reported average particles size of 20 nm, a specific surface of 57.7 cm^2 g^{-1}, and a purity > 99.9% with the anatase crystalline phase. The hydrodynamic diameter of TiO_2 NPs in Mill-Q water is 252.8 nm, and the zeta potential is −14.17 mV. Spherical CuO NPs (black, bare coated) have a reported average particles size of 40 nm, a specific surface of 131 cm^2/g with a purity > 99.9%. Details of the characterization of CuO NPs are presented in prior studies (Shi et al., 2014).

2.2. Soil microcosm and experimental design

The soils were sampled from a typical paddy field at the Huajiachi Campus of Zhejiang University in March 2013. To minimize spatial heterogeneity, soils were collected from ten random sites in the field, and mixed homogeneously to form a composite sample. The top layer of the sampled soil was removed by a shovel, and then the upper layer (0–20 cm) of the soil was collected and placed in a sealed sterile plastic bag, and transported to the laboratory in an ice box. The soil is mildly alkaline (pH 7.17), containing 6.4% total carbon. The total concentrations of major metals listed as follows: Cu 151.05 mg kg^{-1}, Ti 2793.20 mg kg^{-1}, Pb 252.22 mg kg^{-1}, Zn 429.23 mg kg^{-1} and Fe 39303.07 mg kg^{-1}. After removal of visible rocks, roots and fresh litters, the soil was air dried, sieved to 2 mm, and stored in the dark at room temperature (25 ± 2 °C) under aerobic condition.

Each microcosm consisted of 2.5 kg soil (dry weight) in a three chambered plexiglass rhizo-box (Fig. 1). Rice (*Oryza sativa* L.) was cultivated in the glass beads filled rhizo-zone to maximally simulate the flooding-drying process during the rice cropping cycle. Three doses of TiO_2 NPs and CuO NPs (100, 500 and 1000 mg kg^{-1} soil) with three replicates were set in this study according to their toxicity to the pure-cultivating bacteria (Heinlaan et al., 2008), with NP-free samples as controls. The MNPs were introduced into the soil by mixing the final doses of the powdered test material with 25 g air-dried soil thoroughly so that the settling and aggregation of MNPs will be alleviated (Manzo et al., 2010). The spiked carrier soil was then mixed with the untreated air-dried soil by step-by-step amplify through to homogeneity, and followed by adjusting to the upper 5 cm of the soil surface using deionized water. Microcosms with crops were incubated in a greenhouse for 3 months. During the incubation, the water content was maintained at the

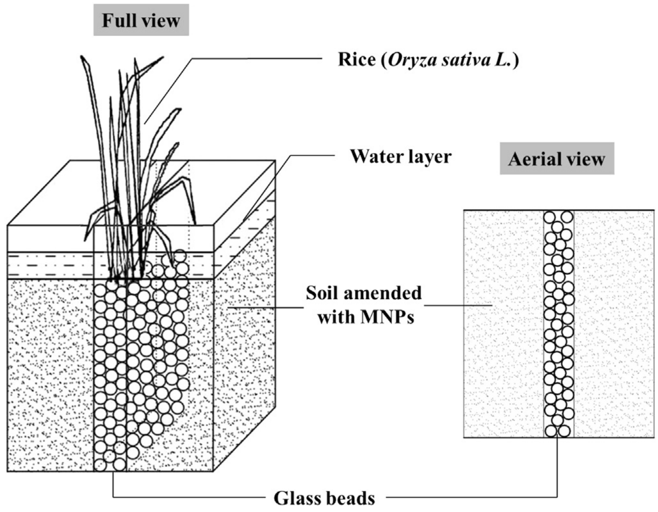

Fig. 1. Schematic representation of the microcosm: Rice (*Oryza sativa* L.) was cultivated in the microcosm to maximally simulate the actual flooding-drying process during the rice cropping cycle; the glass beads filled in the rhizo-zone were used to differentiate the non-rhizosphere from the rhizosphere.