ARTICLE IN PRESS

Environmental Pollution xxx (2017) 1–14

Contents lists available at ScienceDirect

Environmental Pollution

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

Carbon nanomaterials alter plant physiology and soil bacterial community composition in a rice-soil-bacterial ecosystem☆

Yi Hao [a, 1], Chuanxin Ma [b, c, 1], Zetian Zhang [a], Youhong Song [d], Weidong Cao [e], Jing Guo [f], Guopeng Zhou [e], Yukui Rui [a, b, *], Liming Liu [a], Baoshan Xing [b]

[a] *Beijing Key Laboratory of Farmland Soil Pollution Prevention and Remediation, College of Resources and Environmental Sciences, China Agricultural University, Beijing 100193, China*

[b] *Stockbridge School of Agriculture, University of Massachusetts Amherst, MA 01003, United States*

[c] *Department of Analytical Chemistry, The Connecticut Agricultural Experiment Station, New Haven, CT 06504, United States*

[d] *Queensland Alliance for Agriculture and Food Innovation, The University of Queensland, Brisbane, Queensland 4072, Australia*

[e] *Institute of Agricultural Resources and Regional Planning, Chinese Academy of Agricultural Sciences, Ministry of Agriculture Key Laboratory of Crop Nutrition and Fertilization, Beijing 100081, China*

[f] *Dow Pharma and Food Solution, The Dow Chemical Company, 1801 Larkin Center Dr. Midland, MI 48642, United States*

ARTICLE INFO

Article history:
Received 12 June 2017
Received in revised form
14 August 2017
Accepted 9 September 2017
Available online xxx

Keywords:
Carbon nanomaterials
Rice
Phytohormones
Antioxidant enzymes
Soil bacterial community

ABSTRACT

The aim of this study was to compare the toxicity effects of carbon nanomaterials (CNMs), namely fullerene (C_{60}), reduced graphene oxide (rGO) and multi-walled carbon nanotubes (MWCNTs), on a mini-ecosystem of rice grown in a loamy potted soil. We measured plant physiological and biochemical parameters and examined bacterial community composition in the CNMs-treated plant–soil system. After 30 days of exposure, all the three CNMs negatively affected the shoot height and root length of rice, significantly decreased root cortical cells diameter and resulted in shrinkage and deformation of cells, regardless of exposure doses (50 or 500 mg/kg). Additionally, at the high exposure dose of CNM, the concentrations of four phytohormones, including auxin, indoleacetic acid, brassinosteroid and gibberellin acid 4 in rice roots significantly increased as compared to the control. At the high exposure dose of MWCNTs and C_{60}, activities of the antioxidant enzymes superoxide dismutase (SOD) and peroxidase (POD) in roots increased significantly. High-throughput sequencing showed that three typical CNMs had little effect on shifting the predominant soil bacterial species, but the presence of CNMs significantly altered the composition of the bacterial community. Our results indicate that different CNMs indeed resulted in environmental toxicity to rice and soil bacterial community in the rhizosphere and suggest that CNMs themselves and their incorporated products should be reasonably used to control their release/discharge into the environment to prevent their toxic effects on living organisms and the potential risks to food safety.

1. Introduction

With the rapid development of nanotechnology, the use of a variety of carbon nanomaterials (CNMs) is increasing because of their unique properties. For example, fullerene (C_{60}) is used in electronics, solar cells and biomedicine (Cho et al., 2010; Ngan et al., 2015; Liu et al., 2015c), and carbon nanotubes (CNTs) are broadly applied in computers, aircraft airframes, and as drug delivery carriers (De Volder et al., 2013; Lanone et al., 2013). Furthermore, reduced graphene oxide (rGO) is widely used in lithium ion batteries and for pollutant removal (Liu et al., 2015a; Zhang et al., 2016a). With the growth of these applications, CNMs will

☆ This paper has been recommended for acceptance by Dr. Yong Sik Ok.

* Corresponding author. Beijing Key Laboratory of Farmland Soil Pollution Prevention and Remediation, College of Resources and Environmental Sciences, China Agricultural University, Beijing 100193, China.

E-mail address: ruiyukui@163.com (Y. Rui).

[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.envpol.2017.09.024

inevitably be released into the environment during their life cycles (Bindraban et al., 2015; Liu and Lal, 2015; Oyelami and Semple, 2015; Pezzuto et al., 2015). Therefore, a thorough understanding of the ecological effects of CNMs is urgently needed.

The interactions between CNMs and plants have been widely investigated. For example, concentrations of graphene ranging from 250 to 1500 mg/L inhibited wheat growth in 30-day hydroponic experiments, primarily because of oxidative stress (Zhang et al., 2016b). ^{13}C-labeled fullerenol (2.5, 5, and 10 mg/L) had no influence on wheat stem and leaf development but enhanced root elongation during hydroponic cultivation for 7 days (Wang et al., 2016a). Hydroponic exposure to 1000 and 2000 mg/L water-soluble carbon nanodots for 4 weeks inhibited maize growth and increased hydrogen peroxide (H_2O_2) concentration and antioxidant enzyme activities (Chen et al., 2015). Multi-walled carbon nanotubes (MWCNTs) decreased the total number of flowers of red clover when applied to the soil at the concentration of 3 mg/kg and increased biological nitrogen fixation by 8% at the concentration 3000 mg/kg, but had no impact on plant biomass or root colonization by arbuscular mycorrhizal fungi (AMF) (Moll et al., 2016). At the concentration of 10 mg/L, MWCNTs promoted growth of salt-stressed broccoli (treated with 100 mM NaCl) as indicated by increased net CO_2 assimilation and water uptake (Carmen Martinez-Ballesta et al., 2016). Thus, CNMs could exhibit both positive and negative effects on plant growth. However, due to the limitations of the status of the previous studies, it is difficult to conclude whether such variance depends on plant species, CNM types, or other factors such as substrates, pH, and exposure time.

In a plant-soil system, soils play an essential role in maintaining plant growth. Majority of previous studies focused on CNM phytotoxicity to the terrestrial plants. Few of studies took the roles of microorganisms in rhizosphere into account under the background of CNM contaminations, although the effects of CNMs on alteration of soil bacteria were investigated (Jin et al., 2014). Therefore, the interaction between CNMs and the rhizosphere bacterial community is a focus of increasing research attention. As the major CNM, the effects of CNTs on soil microorganisms have been widely investigated. Shrestha et al. (2013) used a pyrosequencing method to analyze the bacterial community in sandy loam soil and found that MWCNTs at 1000 mg/kg altered the abundance of bacterial genera over a 90-day incubation period. By contrast, Khodakovskaya et al. (2013) used denaturing gradient gel electrophoresis (DGGE) and pyrosequencing techniques in a tomato plant–soil system and found that MWCNTs had no apparent effect on soil bacterial diversity after 9 weeks of exposure. The composition of the soil microbial community is also altered by single-walled carbon nanotubes (SWCNTs), with bacterial abundance positively correlated with the exposure dose ($R^2 = 0.27$) (Jin et al., 2014). Functionalized MWCNTs (fMWCNTs) are widely used in industrial products. Kerfahi et al. (2015) assembled carboxyl groups on the surface of MWCNTs in acidic solutions and observed significant changes in the relative abundance of the dominant bacterial phyla after 8 weeks exposure to the fMWCNTs, primarily reflecting their acidic nature, whereas raw multi-walled carbon nanotubes (rMWCNTs) did not affect composition of the soil microbial community. In addition to CNTs, researchers have studied the microbial toxicity of other important CNMs. For example, Ren et al. (2015) showed that 60-day exposure to a graphene concentration of 1000 mg/kg soil did not affect the abundance of most bacterial phylotypes but altered bacterial populations at the genus level, including *Nitrospira* and *Planctomyces*, which are involved in nitrogen biogeochemical cycles. Du et al. (2015a) reported that graphene oxide induced a targeted increase in some nitrogen-fixing and dissimilatory iron-reducing bacteria, with subsequent compositional changes in the entire soil bacterial community. Ge et al. (2016) observed that MWCNTs and graphene cause moderate changes to arid soil communities after 1 year of cultivation, although the effects were similar to those caused by biochar. Tong et al. (2016) observed that small-sized fullerene aggregates tended to negatively affect the soil biota and that soil organic matter regulated the effects of C_{60} on soil bacterial communities. Given the evidence that CNMs altered the diversity and composition of soil bacterial community, questions on how such alteration directly or indirectly influence on plants remain unsolved.

Besides analysis of the diversity of soil bacterial community as affected by CNMs, soil microbial biomass carbon (SMBC) is another crucial parameter, which indicates the balance in soil organic matter between soil carbon release and sequestration in the plant-soil-microbial ecosystem (Thakur et al., 2015). Although SMBC contributes only 1–4% of the total soil carbon, it is the most active portion of the soil carbon pool (Balota et al., 2003). Many recent studies demonstrate a close relationship between nanoparticles and SMBC. For example, silver, nickel or cobalt nanoparticles significantly decrease SMBC concentration after 28-day exposure (Antisari et al., 2015). Under flooding, copper oxide nanoparticles strongly decreased the SMBC concentration in paddy soil (Xu et al., 2015). However, exposure to TiO_2 nanoparticles has no effect on SMBC concentration. Exposure to SWCNTs at the concentration of 1000 mg/kg decreased the SMBC concentration in urban soils by 27% relative to the control group after 3-week incubation (Jin et al., 2013). Similarly, exposure to MWCNTs at the concentration 5000 μg/g strongly decreases SMBC concentration, in addition to depressing activities of soil phosphatase, 1,4-β-*N*-acetylglucosaminidase, 1,4-β-glucosidase, cellobiohydrolase, and xylosidase (Chung et al., 2011). The evidence that the presence of NMs altered the SMBC suggested that it is of importance to understand the consequence of such alteration resulted by different CNMs to plants in an identical plant-soil-bacterial system.

Furthermore, few studies have focused on the effects of CNM on plant–soil–microbe ecosystems as an entire system, even though plants and microorganisms both perform important functions in maintaining soil quality and nutrient structure in the rhizosphere of agricultural soils. Therefore, in the present study, we exposed a hybrid rice line to three types of CNMs, comprising MWCNTs, rGO, and C_{60}, applied at two concentrations (50 and 500 mg/kg) for 30 days. The main objective of the study was to investigate the effects of different types of CNMs on crop growth and concurrent impacts on the composition of the soil bacterial community. At harvest, the concentrations of four phytohormones, comprising indoleacetic acid (IAA), indolepropionic acid (IPA), brassinosteroid (BR), and gibberellin gibberellic acid4 (GA4), were determined to assess whether the presence of CNMs may affect plant growth by regulating phytohormone levels. The antioxidant defense system is an additional indicator of the plant's resistance to abiotic stresses induced by CNMs. In addition to categorization of antioxidant isozymes and determination of the predominant isoform, we measured the activities of superoxide dismutase (SOD) and peroxidase (POD) in the roots of rice plants treated with different concentrations of CNMs. Paired-end sequencing was used to analyze whether exposure to different types of CNMs may significantly alter the composition of the soil bacterial community. The findings provide important information on the effects of CNMs in a plant–soil–microbe system and of the environmental risks of specific CNM.

2. Materials and methods

2.1. Sample preparation and CNM characterization

The C_{60} was purchased from Puyang Yongxin Fullerene