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Research article

Implication of graphene oxide in Cd-contaminated soil: A case study of bacterial communities

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ABSTRACT

The application of graphene oxide (GO) has attracted increasing concerns in the past decade regarding its environmental impacts, except for the impact of GO on a metal-contaminated soil system, due to its special properties. In the present work, the effects of GO on the migration and transformation of heavy metals and soil bacterial communities in Cd-contaminant soil were systematically evaluated. Soil samples were exposed to different doses of GO (0, 1, and 2 g kg^{-1}) over 60 days. The Community Bureau of Reference (BCR) sequential extraction procedure was used to reflect the interaction between GO and Cd. Several microbial parameters, including enzyme activities and bacterial community structure, were measured to determine the impacts of GO on polluted soil microbial communities. It was shown that Cd was immobilized by GO throughout the entire exposure period. Interestingly, the structure of the bacterial community changed. The relative abundance of the major bacterial phyla (e.g., *Acidobacteria* and *Actinobacteria*) increased, which was possibly attributed to the reduced toxicity of Cd in the presence of GO. However, GO exerted an adverse influence on the relative abundance of some phyla (e.g., *WD272* and *TM6*). The diversity of bacterial communities was slightly restricted. The functional bacteria related to carbon and the nitrogen cycling were also affected, which, consequently, may influence the nutrient cycling in soil.

1. Introduction

Graphene oxide (GO), which is considered a precursor for the preparation of graphene and its derivatives, is a one-atom thick, two-dimensional, nanomaterial made of sp^2-hybridized carbon (Wu et al., 2016; Zhu et al., 2010). It is considered the oxidized form of graphene and consists of various reactive oxygen functional groups (epoxides, hydroxyl, ketones, and carboxyl groups) (Dreyer et al., 2010). GO has excellent physiochemical, chemical, mechanical and electrical properties, and it has been widely applied in electronics, super-conductor materials, optical devices, water treatment, biomedicine, and even agricultural fertilizers in both pure and nanocomposite forms (Liu et al., 2013; Wang et al., 2013a, 2015b; Wu et al., 2017; Yuan et al., 2015b, 2016a, 2016b; Zhu et al., 2010; Jiang et al., 2017; Guan et al., 2017).

Given the increasing application of GO, it is inevitable that GO will be released into terrestrial and aquatic environments in increasing amounts, potentially resulting in eco-toxicological effects (Akhavan and Ghaderi, 2010). Graphene-based materials have been found to be toxic toward organisms, with GO being the foremost toxic (Liu et al., 2011). GO may enter soil through many paths, such as the application of biosolids originating from waste treatment or nano-containing fertilizers and release during their production and manufacturing. Therefore, GO potentially has profound impacts on terrestrial ecosystems since soil is one of the ultimate recipients for GO (Ge et al., 2011; Zhou et al., 2017). Bacteria are the most diverse and abundant organisms on the earth. They usually react quickly to changes in the living environment due to low homeostasis and a high surface-to-volume ratio (Boivin et al., 2002). In addition, microorganisms play an important role in the biogeochemical cycling of carbon and nitrogen (Falkowski et al., 2008). For these reasons, changes in bacterial activity,

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diversity, richness and community structure could be early signals of alterations in the whole ecosystem. Recent studies demonstrated that GO possesses antibacterial activity (Ahmed and Rodrigues, 2013; Liu et al., 2011; Chung et al., 2015). GO has the potential to disturb the functions of essential microorganisms in activated sludge processes (Ahmed and Rodrigues, 2013). Bacterial metabolic activity significantly decreased after GO exposure, which was caused by membrane disruption and oxidation stress (Liu et al., 2011). Moreover, a study focusing on the impact of GO on the soil microbial community found that the enzyme activity was reduced by the entry of GO into soils in the short-term, which could be recovered afterward (59 days), and the biomass was mainly unaffected (Chung et al., 2015). After 90 days of GO interaction, bacterial communities became richer and more diverse, and some functional bacterial genera were selectively enriched (Du et al., 2015).

Most studies have focused on the environmental behaviors and the effects of GO on pure cultures or simple terrestrial environments. However, many parts of the world face grave soil pollution, especially in China. Approximately 3.33 million hectares of cropland are too contaminated with both organic and inorganic pollutants to grow food (Larson, 2014). Cd is a common heavy metal in soil. It has been reported that Cd can inhibit soil enzyme activities due to its toxicity (Wang et al., 2010). Likewise, Cd exerted adverse effects on the bacterial richness and diversity (Wang et al., 2010). However, the toxicity of heavy metals can be reduced by soil amendments, such as zero valent iron and limestone (Lee et al., 2011). With abundant oxygen-containing functional groups and a large specific surface area, GO was found to adsorb and eliminate heavy metals from a contaminated soil via surface complexation and electrostatic interaction (Wang et al., 2013c; Khalil et al., 2016). Thus, it can be inferred that the interaction between heavy metal and GO may also affect bacterial communities after GO enters the soil. However, little is known about the effects of GO on the bacterial communities in heavy metal-contaminated soil (Shao et al., 2016).

The present study underlines the impact of GO on bacterial communities in heavy metal-contaminated soil by culture-independent methods. Soils were exposed to different doses of GO (0–2 g kg^{-1}). The interaction between GO and Cd was analyzed by BCR technology. The changes in soil enzyme activities and microbial composition, as well as the diversity of the whole community, were systematically investigated. This study will help to define the soil microbial impact portion of the discussion framework concerning the environmental impacts of GO.

2. Materials and methods

2.1. Graphene oxide

GO was successfully synthesized by the modified Hummers' method (Hummers and Offeman, 1958). The detailed processes were presented in the authors' previous research studies (Wang et al., 2013b, 2015a), and the characterizations of GO are presented in the Supporting Information (Figs. S1 and S2). Furthermore, all chemicals and reagents used in this experiment were of analytical grade. All aqueous solutions were prepared using deionized (DI) water.

2.2. Collection and preparation of soil sample

Uncontaminated soils were collected from the Yuelu Mountain (Changsha, China). The air-dried soil was sieved to 2 mm, and the main physico-chemical properties of soil are presented in Table S1. This soil was homogenized and mixed with $CdCl_2$ solution (10 mg kg^{-1} dry weight soil) to obtain Cd-contaminated soil. The added concentration was based on the background value and the situation of present heavy metal contamination in Hunan Province (Wang et al., 2008). Then, the soil was preincubated at 25 °C for 8 weeks to ensure that the stimulated Cd-contaminated soil became relatively stable, as described in a previous study (Huang et al., 2014).

2.3. Experimental design

The pre-contaminated soil samples were divided into 9 experimental pots (2.5 L), with 1000 g each, to obtain triplicate samples to expose to three different concentrations of GO (0, 1 or 2 g kg^{-1} d.w.s). A specific amount of GO suspension was added to the soil in order to reach the desired concentrations, followed by intensive mixing with the soil. DI water was added to maintain the soil moisture at 60% gravimetric water holding capacity every week. There is no report regarding the specific concentration of GO in the environment. Therefore, this research chose the high concentration of GO (1 or 2 g kg^{-1}) to represent worst-case scenarios of GO discharge (e.g., industrial spill) based on published studies (1–5 g GO was added into 1 kg soil samples) (Chung et al., 2015; Du et al., 2015). The pre-contaminated soil without GO was designed as a control in this experiment.

2.4. Extraction and quantitation of Cd

The Community Bureau of Reference (BCR) sequential extraction procedure was used to analyze the effect of GO on Cd in soil, and the detailed extraction procedure is shown in Table S2 (Davidson et al., 1998; Shao et al., 2015). In brief, a 0.5 g soil sample was extracted by 0.11 M acetic acid, 0.1 M hydroxylamine hydrochloride, 30% m/v H_2O_2 and 1 M NH_4OAc, successively. The residual fraction was determined after a digestion process with mixed acid (HNO_3, $HClO_4$ and H_2O_2) (Yuan et al., 2015a). All solutions were pre-filtrated through 0.45 μm filter paper, and the filtrate was analyzed by an atomic absorption spectrometer (AAS, Perkin-Elmer, USA).

2.5. Soil enzyme activity assays

The enzyme activities of dehydrogenase, urease and catalase were analyzed in triplicate air-dried pooled samples that were collected on days 0, 3, 7, 15, 30 and 60. The methods were described by Li et al. (2008). The specific methods are provided in the Supporting Information. The urease activity was measured by the colorimetric method using toluene and 10% urea. Dehydrogenase activity was assayed by the reduction of 2,3,5-triphenyltetrazolium chloride to triphenyl formazan. Catalase activity was measured by the titrimetric method using potassium permanganate titration. The effects of GO on enzyme activities were determined using a one-way analysis of variance. If the p value was less than 0.05, it indicated a significant difference. All statistical analyses were performed with SPSS version 19 (Chicago, IL).

2.6. Soil DNA extraction

Total genomic DNA was extracted from an approximately 0.25 g soil sample using the TIANamp Soil DNA kit (DP336, TIANGEN BIOTECH, China), according to the manufacturer's instructions. Agarose gel electrophoresis and an ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE) were implemented to determine the concentration and quality of the genomic DNA. The detailed process is provided in the Supporting Information. DNA was stored at −20 °C before the PCR amplification occurred.