Science of the Total Environment 514 (2015) 307–313

Contents lists available at ScienceDirect

Science of the Total Environment

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

Effects of graphene oxides on soil enzyme activity and microbial biomass

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HIGHLIGHTS

- The effects of graphene oxide (GO) on soil microbial activity was studied via a 59-day incubation experiment.
- Up to 1 mg GO g^{-1} soil was applied and the soil enzyme activities and microbial biomass were measured.
- Soil enzyme activity was lowered by 15–50% under 0.5–1 mg GO g^{-1} soil, but the effect subsided afterwards.
- Soil microbial biomass showed little change in response to GO treatment.
- GO can negatively affect soil enzyme activity in short term upon entrance to soils.

ARTICLE INFO

Article history:
Received 22 October 2014
Received in revised form 24 January 2015
Accepted 24 January 2015
Available online xxxx

Editor: D. Barcelo

Keywords:
Nanomaterial
Microbial biomass
Soil incubation
Extracellular enzymes
Soil microorganisms

ABSTRACT

Due to recent developments in nanotechnology, nanomaterials (NMs) such as graphene oxide (GO) may enter the soil environment with mostly unknown consequences. We investigated the effects of GO on soil microbial activity in a 59-day soil incubation study. For this, high-purity GO was prepared and characterized. Soils were treated with up to 1 mg GO g^{-1} soil, and the changes in the activities of 1,4-β-glucosidase, cellobiohydrolase, xylosidase, 1,4-β-N-acetyl glucosaminidase, and phosphatase and microbial biomass were determined. 0.5–1 mg GO g^{-1} soil lowered the activity of xylosidase, 1,4-β-N-acetyl glucosaminidase, and phosphatase by up to 50% when compared to that in the control soils up to 21 days of incubation. Microbial biomass in soils treated with GO was not significantly different from that in control soils throughout the incubation period, and the soil enzyme activity and microbial biomass were not significantly correlated in this study. Our results indicate that soil enzyme activity can be lowered by the entry of GO into soils in short term but it can be recovered afterwards.

1. Introduction

Graphene oxides (GOs) are layered graphene sheets that have oxygen-containing functional groups including epoxide, carboxyl, carbonyl, and hydroxyl group (Lerf et al., 1998). Among a wide range of nanomaterials (NMs) that are being manufactured, GO or chemically modified GO are considered to be highly promising novel material due to their superior electrical characteristics, colloidal property, and large surface area (Geim and Novoselov, 2007). These excellent material properties allow GO to be used in various applications such as energy-storage materials, paper-like materials, and bioenvironmental materials (Park and Ruoff, 2009; Wang et al., 2011; Zhao et al., 2012). For example, adsorbents and photocatalysts that are based on GO are employed in removing pollutants from the environment (Zhao et al., 2012). For GO to be successfully applied in such diverse fields, it is important to determine its fate, distribution, and potential environmental impacts (Anjum et al., 2013). Previous studies have determined the fate and toxicity of other carbon-based NMs including carbon nanotubes (CNTs) and fullerenes in the soil environment (Avanasi et al., 2014; Li et al., 2013a, 2013b; Navarro et al., 2013), but few studies have investigated the impacts of GO on the soil environment. Compared with other carbon-based NMs, GO showed higher mobility in sand, which suggests that highly mobile GO may increase the environmental risks once they enter the soil environment (Qi et al., 2014). However, how GO may impact soil microbial activity that is important for nutrient cycling in soil ecosystems remains to be investigated.

It has been shown via culture studies using model microorganisms such as *Escherichia coli* (*E. coli*) that GO has strong antimicrobial effect (Akhavan and Ghaderi, 2010; Hu et al., 2010; Liu et al., 2011). For example, a study using GO nanowalls showed that direct contact of bacteria with GO nanowalls can lead to cell damage; after 1 h of bacteria-GO

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http://dx.doi.org/10.1016/j.scitotenv.2015.01.077
0048-9697/

nanowall contact, only 41% survived. This cytotoxicity was attributed to the sharp edges of the nanowalls (Akhavan and Ghaderi, 2010). In another study, it was shown that after 2-h contact of bacteria with GO nanosheets, the cell metabolic activity was decreased to approximately 70% and 13% at 0.02 mg ml^{-1} and 0.085 mg ml^{-1}, respectively. The bacterial cell membranes were shown to have been severely damaged and the cytoplasm was flowing out (Hu et al., 2010). Additionally, GO treated on bacteria at 0.005–0.08 mg ml^{-1} for up to 4 h showed time- and concentration-dependent antibacterial activity (Liu et al., 2011).

Antimicrobial effects of GO on microorganisms other than model microorganisms, such as some plant pathogens, have also been shown (Chen et al., 2013; Wang et al., 2014). GO showed 94% cytotoxicity against phytopathogenic bacteria causing infections in rice even at a low concentration. This high antimicrobial efficiency was ascribed to the extremely sharp edges of GO and generation of reactive oxygen species (ROS) (Chen et al., 2013). The antifungal activity of GO was also shown for two important plant pathogenic fungi (Wang et al., 2014).

While the antimicrobial effects of GO have been demonstrated in culture studies, only a few studies determined the effects of GO on microbial communities inhabiting environmental samples (Ahmed and Rodrigues, 2013; Wang et al., 2013). GO exhibited a toxic effect on the wastewater microbial communities at concentrations from 0.05 to 0.3 mg ml^{-1}, and reduced their metabolic activity by 20–70% in a concentration-dependent manner. The potential mechanism for this toxicity was ascribed to the ROS generated by GO because GO at high concentrations produced higher levels of ROS compared to control samples (Ahmed and Rodrigues, 2013). On the other hand, one study reported enhanced bacterial activity by GO (Wang et al., 2013). The activity of anaerobic ammonium-oxidizing (anammox) bacteria that removes nitrogen from wastewater increased up to 10% and the production of carbohydrate, protein, and total extracellular polymeric substances increased in a dose-dependent manner when GO was treated within the concentration of 0.05–0.1 mg ml^{-1}. However, at 0.15 mg GO ml^{-1}, anammox bacterial activity and extracellular polymeric substance production decreased (Wang et al., 2013). Because both toxic and nontoxic effects of GO were observed as such, generalized conclusions on safety risks associated with GO are yet to be made (Seabra et al., 2014).

In this study, we determined the effects of GO exposure on soil microbial activity. Soil enzyme activity and microbial biomass are sensitive indicators of changes in soil ecosystem function under soil disturbance caused by nanomaterials, heavy metal, and organic pollutants (Chung et al., 2011; Kuperman and Carreiro, 1997; Liu et al., 2009; Shrestha et al., 2013). Therefore, the alterations in extracellular enzyme activity and microbial biomass were determined in soils that were treated with thoroughly characterized GO at 0.1–1 mg GO g^{-1} soil and incubated for 59 days, a point at which the effects of GO on microbial parameters could no longer be detected. We report for the first time in our knowledge the effects of GO on soil microbial activity. When soils were exposed to 0.5–1 mg GO g^{-1} soil, significant decrease was observed in the activities of xylosidase, 1,4-β-N-acetyl glucosaminidase, and phosphatase which are soil enzymes that mediate C, N, and P cycling, respectively. These effects subsided afterwards, however. Our results suggest that GO may have negative effects on soil enzyme activity in short term.

2. Materials and methods

2.1. Soil sampling

Soil samples were collected in October 2013 from top 15 cm of a site dominated by deciduous trees in Konkuk University campus. This site was chosen for our study because it can represent the urban ecosystem and NMs are most likely to enter soils in an urban environment (Chung et al., 2011; Jin et al., 2013). Upon collection, soil samples were sieved with an 8-mm sieve and kept frozen. Subsequently, 60-g soil subsamples to be incubated were placed in glass jars. The soil was a sandy loam, and the weight proportion of sand, silt, and clay was 52.77 (±2.12) (average (± one standard error), $n = 3$) %, 39.23 (±2.67) %, and 8.00 (±1.00) %, respectively. The pH of the soil was 4.62 (±0.05). The organic C and N concentrations in soil were 12.16 (±0.49) g C kg^{-1} soil and 0.80 (±0.03) g N kg^{-1} soil, respectively. The ratio of C:N in soil was 15.22 (±0.14).

2.2. Characteristics of GO

GO was prepared by modified Hummers method (Hummers and Offeman, 1958). Graphite flake (1 g, 99.8%, Alfa Aesar) and $NaNO_3$ (1 g, ≥99.0%, Sigma-Aldrich) were dissolved in H_2SO_4 (50 ml, 95–97%, Sigma-Aldrich), and $KMnO_4$ (6 g, ≥99.0%, Sigma-Aldrich) was slowly added. The solution mixture was magnetically stirred for 1 h at 35 °C on a hot plate. Deionized (DI) water (80 ml) was added to the solution and heated for 30 min at 90 °C. Subsequently, DI water (200 ml) and H_2O_2 (6 ml, 30 wt.%, Sigma-Aldrich) were successively added to the solution. Brownish GO powder was obtained by filtration with a glass microfiber filter (CHMLAB, GF4), and stored in vacuum at room temperature. The filtered GO cake was dispersed in 5 wt.% HCl aqueous solution with mild stirring for 12 h in order to remove the metal ions. Subsequently, the GO solution was allowed to settle for 1 day, and the supernatant was decanted away. The completely precipitated GO was purified via dialysis for 3 days through which the residual metal ions and acid were removed. After dialysis, the gel-type GO was redispersed in ethanol and then the mixture was centrifuged at 13,000 rpm for 60 min to separate the solid GO. Finally, the solid product obtained was dried in air.

The morphology of GO was investigated by scanning electron microscopy (SEM; Hitachi S-4800, HITACHI, Japan) and high-resolution transmission electron microscopy (HRTEM; Tecnai 20, FEI, USA). The HRTEM image of GO was obtained by drying drops of GO solution on holey carbon grid. The structure of GO was characterized by X-ray diffraction (XRD:D/MAX-2500V/PC, Rigaku INC., Japan) with Cu Kα radiation ($\lambda = 1.5406$ Å) at the scanning rate of 4° min^{-1}. Fourier transform infrared spectroscopy (FTIR; Varian 640-IR, Agilent Technologies, USA) was performed to identify chemical functional groups over the wave number range of 800–3800 cm^{-1} using KBr pellets. X-ray photoelectron spectroscopy (XPS) spectra were obtained with an Automated XPS Microprobe PHI X-tool (ULVAC-PHI, Japan). Raman spectroscopy (Labram ARAMIS IR2, HORIBA, USA) was used to analyze the crystallinity (e.g., crystal structure, disorder, and defects) of GO (Kudin et al., 2008). Thermogravimetric analysis (TGA) was performed (DSC2010, TA Instrument, USA) to confirm the content of metal catalysts and purity of GO by heating it up to 1000 °C at the rate of 10 °C min^{-1} under air atmosphere. The specific surface area of GOs was determined by the Brunauer, Emmet, and Teller (BET) method (Brunauer et al., 1938) using BEL SORP-mini II (BEL Japan, Japan) at 350 °C. The suspension behavior of GO at 7.14 mg ml^{-1}, which was the concentration used for 0.5 mg GO g^{-1} soil treatment, was analyzed by taking images of GO suspension at various time points after it was sonicated for 1.5 h (Fig. SI-1). In addition, to compare the suspension behavior of GO with that of single-walled CNTs (SWCNTs), SWCNT suspension at 7.14 mg ml^{-1} was prepared and analyzed in the same way as the GO suspension (Fig. SI-2).

2.3. Soil incubation

The soil incubation experiment was conducted using suspended form of GO which was prepared by bath-sonicating (Branson, USA) the mixture of GO and DI water at room temperature for 1.5 h. GO suspension was added to 60 g of field-moist soil subsamples placed in glass jars and mixed. The concentrations of GO applied to soils were 0 (DI water only), 0.1, 0.5, and 1.0 mg g^{-1} soil, and there were 4 replicates for each concentration. In preparing the GO suspension of all