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Time-dependent effect of graphene on the structure, abundance, and function of the soil bacterial community

Wenjie Ren [1], Gaidi Ren [1], Ying Teng *, Zhengao Li, Lina Li

Key Laboratory of Soil Environment and Pollution Remediation, Institute of Soil Science, Chinese Academy of Sciences, Nanjing 210008, China

HIGHLIGHTS

- Graphene transiently promoted the enzyme activities and soil bacterial biomass.
- A transiently significant shift in soil bacterial community structure was caused.
- The effect of graphene on soil bacterial community was time dependent.
- Graphene significantly suppressed *Nitrospira* and *Planctomyces*.
- Graphene promoted some bacteria populations degrading organic pollutants.

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ABSTRACT

The increased application of graphene raises concerns about its environmental impact, but little information is available on the effect of graphene on the soil microbial community. This study evaluated the impact of graphene on the structure, abundance and function of the soil bacterial community based on quantitative real-time polymerase chain reaction (qPCR), pyrosequencing and soil enzyme activities. The results show that the enzyme activities of dehydrogenase and fluorescein diacetate (FDA) esterase and the biomass of the bacterial populations were transiently promoted by the presence of graphene after 4 days of exposure, but these parameters recovered completely after 21 days. Pyrosequencing analysis suggested a significant shift in some bacterial populations after 4 days, and the shift became weaker or disappeared as the exposure time increased to 60 days. During the entire exposure process, the majority of bacterial phylotypes remained unaffected. Some bacterial populations involved in nitrogen biogeochemical cycles and the degradation of organic compounds can be affected by the presence of graphene.

1. Introduction

Graphene is an atomically thin, two-dimensional, carbon-based nanomaterial consisting of sp^2 hybridized carbon atoms [1]. Because of its unique and versatile physiochemical, mechanical and electrical properties, graphene exhibits wide applications in superconductor materials, electronics, optical devices, biomedicine, water treatment, and even agricultural fertilizers [2–4]. The global market for graphene-based materials is expected to grow steadily from $81 M in 2012 to $122.9 M in 2017 and to reach up to $986.7 M in 2022 [5]. During the course of the production, application and disposal of graphene-containing materials, graphene will inevitably be released into the environment. Therefore, concerns over its potential environmental and health risks have been raised [6]. Nevertheless, knowledge of the environmental impacts of graphene is currently very limited.

To date, the majority of studies on the toxicological effects of graphene has focused mainly on pure cultures [7,8]. Graphene has been observed to possess antibacterial effects. Graphene can bind the cell surface and cause physical and chemical damage to the cell membrane [7]. Additionally, graphene may interact with proteins and nuclei acids, altering their structure and function [9]. On the other hand, graphene may generate ROS, which can also cause disruption of membrane lipids, proteins and nuclei acids [10]. Few studies are available on the impact of graphene on the microbial community. Ahmed and Rodrigues [11] investigated the acute effect of graphene oxide on the wastewater microbial community and found that graphene led to a significant decrease in microbial metabolic activity and reduced the degradation of organic matter and nutrients.

* Corresponding author. Tel.: +86 25 85881531; fax: +86 25 86881126.
E-mail address: yteng@issas.ac.cn (Y. Teng).
[1] These authors contributed equally to the work.

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The soil system is predicted to act as the ultimate sink for nanomaterials, including graphene [12]. Soil microbial communities play an important role in nutrient cycling, sustaining soil quality and the remediation of contaminated soil. However, few studies addressing the effect of graphene on soil microbial communities have been published to date. Compared with pure cultures and wastewater systems, the soil environment is much more complex and uncontrolled. When graphene enters the soil, it may interact with organic matter or clay minerals present in the soil [13], stabilizing graphene and making it less bioavailable, which would mitigate the effect of graphene on microbial communities. On the contrary, dissolved organic matter (DOM) might promote the mobility and bioavailability of graphene by acting as natural surfactants [14]. Therefore, the impact of graphene on the soil microbial community must be considered in light of recent results indicating its adverse effects on microorganisms. However, it is very difficult to predict the effect of graphene on soil microbial communities based only on its toxicity in pure cultures.

Several studies have investigated the impacts of other carbon-based nanomaterials on soil microbial communities. Intriguingly, even the same type of material may display different impacts. Tong et al. [15] observed a negligible impact of fullerene (C_{60}) (1000 mg/kg soil) on the structure and function of the soil microbial community, while Johansen et al. [16] reported that the counts of fast-growing bacteria in the soil were reduced by 3–4-fold after exposure to C_{60} at a concentration of 50 mg/kg soil. The discrepancy between these studies might result from variations in the shape and size of the nanomaterial due to different preparation methods. Both single-walled (SWCNTs) and multi-walled (MWCNTs) carbon nanotubes can significantly lower enzyme activities and microbial biomass [17–19], and SWCNTs exhibit a more severe impact than MWCNTs [18]. Compared with these nanomaterials, graphene has a smaller diameter and, thus, a larger surface area, indicating higher antimicrobial activity. However, these characteristics might also influence the interaction between graphene and soil components, possibly resulting in a different effect compared with CNTs and C_{60}.

The objective of the present study was to investigate the impact of graphene on the soil bacterial community. The changes in the biomass and structure of the soil bacterial community were determined via real-time PCR and pyrosequencing. Pyrosequencing has been demonstrated to provide detailed information about the microbial composition of the whole community following exposure to nanomaterials [20–22]. The effects of graphene on microbial function were also evaluated based on soil enzyme activities, which are considered sensitive indicators of changes in the microbial community under nanomaterial stress [17,20,23]. Fluorescein diacetate (FDA) esterase and soil dehydrogenase activity were selected because they were usually considered to represent overall microbiological activity of soil. Dehydrogenase occurs in all intact and viable microbial cells, so its activity is usually related to the presence of viable microorganisms and their oxidative capability [24]. As the fluorogenic substrate is taken up by active cells and then transformed by FDA esterase, the enzyme has been considered a measure of soil microbial activity [25]. Besides, FDA esterase activity has often been used as a sensor and functional indicator of soil health [26]. This study is expected to expand the current understanding of the potential risks of graphene to the soil environment.

2. Materials and methods

2.1. Graphene and soil

Graphene (0.5–2 μm diameter, 0.8 nm thickness, purity >99% wt.) was purchased from XFNANO Materials Tech Co. Ltd., Nanjing, China. The BET surface area of the dry powder was 500–1000 m^2/g.

Surface soil samples were collected from a paddy field at the Changshu Agroecological Experimental Station, Chinese Academy of Sciences (31°32′56″N, 120°41′53″E), Jiangsu Province, China. The soil is classified as a Gleyi-Stagnic Anthrosol. Visible rocks, roots, and fresh litter were removed, and the soil was homogenized via passage through a 2.0 mm sieve and thorough mixing and subsequently stored in plastic bags at 4 °C until the incubation experiment to minimize microbial activity. The properties of the soil were as follows: organic matter content, 18.95 g/kg; pH, 7.31; total nitrogen, 0.92 g/kg; available phosphorus, 9.71 mg/kg; available potassium, 64 mg/kg; cationic exchange capacity, 19.53 cmol/kg.

2.2. Microcosm experiment

Prior to the incubation experiment, the soil samples were pre-incubated in the dark at 25 °C for 3 days. A specific amount of graphene was added as dry powder to the soils to reach a concentration of 10, 100 or 1000 mg/kg d.w.s., followed by intensively mixing with the soil. Soil samples (10 g dry weight equivalent) were weighed into 120 ml serum bottles for each treatment, and sterile distilled water was added to adjust the soil moisture to a 60% water holding capacity (WHC). Soil without graphene was used as the control. All treatments were performed in triplicate. All of the bottles were capped with black butyl stoppers, and the microcosms were then incubated at 28 °C for 60 days. Throughout the experiments, soil moisture was maintained at 60% WHC through the gravimetric addition of sterile distilled water every week, after which the microcosms were aerated.

2.3. Soil enzyme activities

The enzyme activities of dehydrogenase and FDA esterase in the soil were evaluated in the four treatments described above, and three replicate subsamples for each treatment were collected on days 4, 8, 15, 21, 28 and 60. The assay methods for dehydrogenase and FDA esterase were modified from Li et al. [27]. The detailed methods are provided in the Supplementary material.

2.4. DNA extraction

On days 4, 21 and 60, 0.5 g of soil was collected from each microcosm in triplicate for all four treatments. Total genomic DNA was extracted with the FastDNA™ SPIN kit for soil (MP Biomedicals LLC, Ohio, USA) according to the manufacturer's instructions. The quality of the DNA extracts was checked via electrophoresis on a 0.8% agarose gel. The quantity and purity of DNA in the extracts were determined using a Nanodrop® ND-1000 UV–vis spectrophotometer (NanoDrop Technologies, Wilmington, DE). The soil DNA was stored at −20 °C until use.

2.5. Real-time PCR

For relative quantification of the bacterial communities in the soil, qPCR was performed with the CFX96 optical real-time detection system (Bio-Rad Laboratories Inc., Hercules, CA, USA) using the universal bacterial primers 515F (5′-GTGCCAGCMGCCGCGG-3′) and 907R (5′-CCGTCAATTCMTTTRAGTTT-3′), which amplify the V4 region of the bacterial 16S rRNA gene. The final volume of the reaction mixtures was 20 μl, containing 10 μl of SYBR® *Premix Ex Taq*™ (TaKaRa Biotech, Dalian, China), 0.5 μM each primer, and 1 μl of template DNA (ranging from 1 to 10 ng). Standard curves were obtained by performing serial dilutions of linearized plasmids containing the evaluated genes. Amplification of the 16S rRNA gene was initiated through denaturation at 95 °C for 30 s, followed by 40 cycles of 30 s at each of 95 °C, 55 °C and 72 °C and, finally, 30 s for