



Single-walled carbon nanotubes alter soil microbial community composition

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HIGHLIGHTS

- The effects of SWCNTs on soil microbial community composition were investigated.
- SWCNTs significantly altered soil microbial community composition.
- Gram-positive and Gram-negative bacterial, and fungal biomass decreased with higher SWCNT concentrations.
- Soil microorganisms may be adversely affected when exposed to high concentrations of SWCNTs.

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ABSTRACT

Recent developments in nanotechnology may lead to the release of nanomaterials into the natural environment, such as soils, with largely unknown consequences. We investigated the effects of single-walled carbon nanotubes (SWCNTs), one of the most widely used nanomaterials, on soil microbial communities by incubation of soils to which powder or suspended forms of SWCNTs were added (0.03 to 1 mg g⁻¹ soil). To determine changes in soil microbial community composition, phospholipid fatty acid (PLFA) profiles were analyzed at 25th day of the incubation experiment. The biomass of major microbial groups including Gram-positive and Gram-negative bacteria, and fungi showed a significant negative relationship with SWCNT concentration, while the relative abundance of bacteria showed a positive relationship with SWCNT concentration. Furthermore, soils under distinct concentrations of SWCNT treatments had PLFA profiles that were significantly different from one another. Our results indicate that the biomass of a broad range of soil microbial groups is negatively related with SWCNT concentration and upon entry into soils, SWCNTs may alter microbial community composition. Our results may serve as foundation for scientific guideline on regulating the discharge of nanomaterials such as SWCNTs to the soil ecosystem.

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1. Introduction

Diverse types of nanomaterials (NMs) are being manufactured because of their versatile use (Chen et al., 2011; De Volder et al., 2013; Wallentin et al., 2013). Among these NMs, carbon nanotubes (CNTs) have great potential to be applied to multiple fields in large scale because they exhibit superior physical and chemical characteristics (De Volder et al., 2013; Lau and Hui, 2002; Upadhyayula et al., 2009; Welscher et al., 2009). As a result of such applications, CNTs may enter the environment and accumulate in the soils (Gottschalk et al., 2009; Klaine et al., 2008; Silva et al., 2012a,b). However, how CNTs may impact soil biota including microorganisms that are highly important for soil

ecosystem health and function are only beginning to be understood (Dinesh et al., 2012; Li et al., 2013; Zhao and Liu, 2012).

A few studies on the effects of CNTs on environmental microorganisms have shown that CNTs can repress microbial activity (Chung et al., 2011; Jin et al., 2013; Kang et al., 2009; Luongo and Zhang, 2010). In a short time, both multi-walled carbon nanotubes (MWCNTs) and single-walled carbon nanotubes (SWCNTs) can reduce the activity of soil enzymes and microbial biomass (Chung et al., 2011; Jin et al., 2013). In addition, the application of CNTs inhibited microbial respiration in soils and activated sludge (Luongo and Zhang, 2010; Tong et al., 2012). Very recently, a DNA-based study on soil microorganisms suggested adverse effects of SWCNTs on bacteria and fungi involved in soil nutrient cycling (Rodrigues et al., 2013). As inhibitory effects of CNTs on the activity of environmental microorganisms have been reported, detailed

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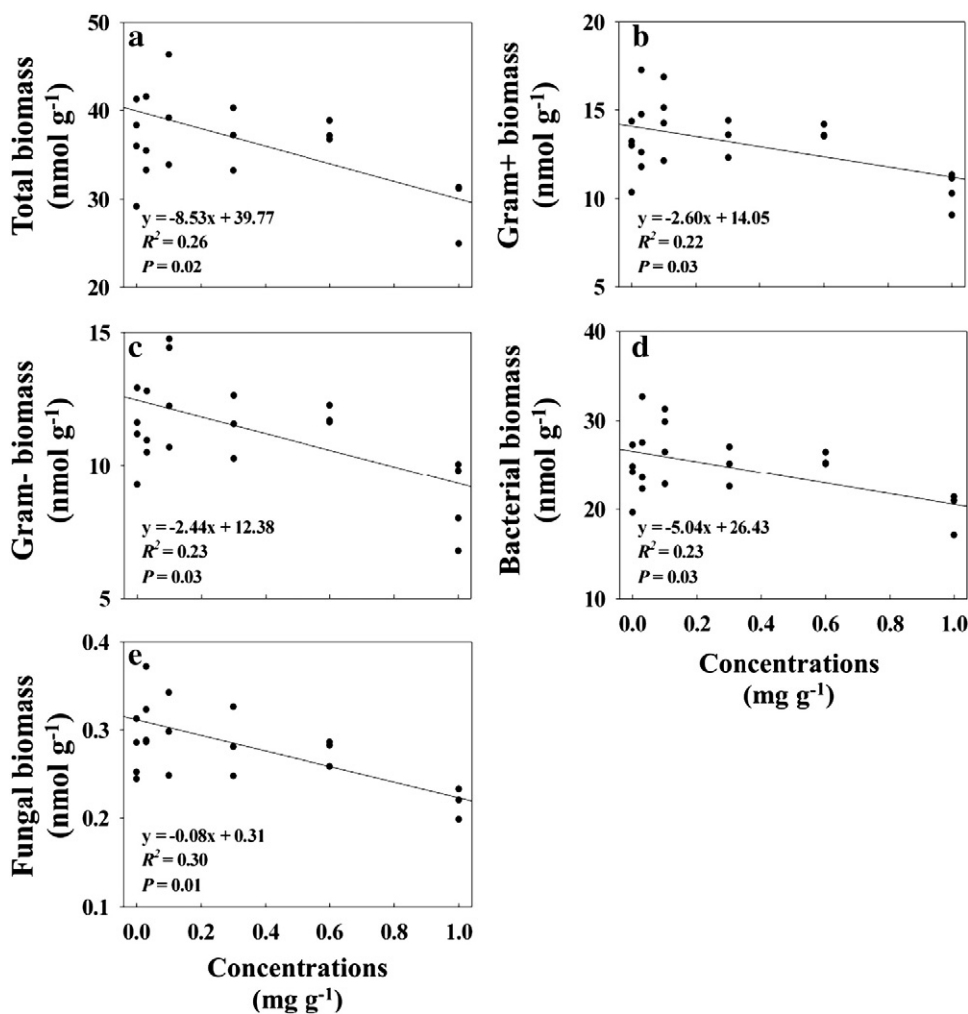


Fig. 1. Linear relationships between single-walled carbon nanotube (SWCNT) concentrations and biomass of major microbial groups in soils treated with powder form of SWCNTs.

investigations on the impacts of CNTs on specific microbial groups and microbial community composition are needed to better understand how CNTs repress microbial activity.

The changes in microbial community composition by SWCNTs were determined in this study by phospholipid fatty acid (PLFA) analysis of soils treated with SWCNTs. PLFAs were analyzed because they are excellent indices of living microbial cells and sensitive indicators of changes in soil microbial community composition in response to environmental stress such as contamination by nanomaterials and heavy metal (Kumar et al., 2012; Shah and Belozero, 2009; Witter et al., 2000). For SWCNT treatment, various concentrations (0.03 to 1 mg g⁻¹ soil) of SWCNTs in two different forms (powder- and suspended-form SWCNTs) that represent exposure to soil microorganisms were applied to soils. Previously, we have shown that soil microbial enzyme activities and biomass were reduced by such SWCNT treatment (Jin et al., 2013). In this study, the hypothesis that repression of soil enzyme activities by SWCNTs is due to a shift in microbial community composition was tested. We also determined whether these responses are due to SWCNTs influencing the community as a whole, or if certain microbial guilds (i.e. bacteria or fungi) are more sensitive to SWCNTs. We show for the first time that SWCNTs can significantly change microbial community composition and that the biomass of diverse soil microbial groups including Gram-positive and Gram-negative bacteria, and fungi are negatively correlated with SWCNT concentration.

2. Materials and methods

2.1. Soil sampling

Soil samples were collected in July 2011 from top 15 cm of a grass-dominated landscaped site in Korea 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; Silva and da Boit, 2011). Upon collection, soil samples were sieved with an 8-mm sieve. Subsequently, soil subsamples to be incubated were placed in glass jars and the remaining soils were air-dried. The soil is a sandy loam (sand 64.75 ± 0.57 (Mean ± 1 standard error) %, silt 27.75 ± 0.57%, and clay 7.50 ± 0.00%), and its pH is 6.98 ± 0.20. The soil has C and N concentrations of 17.69 ± 0.22 g C kg⁻¹ soil and 1.14 ± 0.03 g N kg⁻¹ soil, respectively. The cation exchange capacity (CEC) is 13.51 ± 0.78 cmol CEC kg⁻¹ soil (Chung et al., 2011).

2.2. Characteristics of single-walled carbon nanotubes

SWCNTs that are commercially available were used for this study (Southwest Nanotechnologies, Inc., USA) because they are widely distributed and have standard characteristics with small batch-to-batch variability. Detailed characteristics of the SWCNTs were described earlier (Jin et al., 2013). Briefly, the SWCNTs were produced with cobalt-

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