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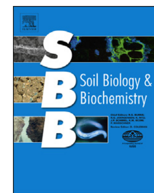

Impact of carbon nanomaterials on microbial activity in soil

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ABSTRACT

In this study, effects of an increase in concentration of fullerene-$C_{60,}$ single-walled carbon nanotubes (SWCNTs), multi-walled carbon nanotubes (MWCNTs) or fullerene soot (FS) on overall microbial activity was investigated over a 21 d incubation period. Microbial utilisation of ^{14}C-glucose and uptake of ^{14}C-glucose into the microbial biomass was investigated. For CNM-amended soils, greater extents of ^{14}C-glucose mineralisation were found in the C_{60}-amended soils compared to MWCNT-, SWCNT- or FS-amended soils. In addition, the 100 and 1000 mg kg^{-1} were consistently found to have higher extents of mineralisation in C_{60}, MWCNT, SWCNT or FS-amended soils, respectively. Further, the incorporation of ^{14}C-glucose into the microbial biomass declined slightly with an increase in concentration in the amended soils, but no consistent pattern was observed. As a result, the biophysical quotient (BQ) increased significantly ($P < 0.05$), as concentrations increased from 1 mg kg^{-1} to 1000 mg kg^{-1} in all C_{60}-, MWCNT-, SWCNT- and FS-amended soils. The results obtained from this study showed that the addition to carbon nanomaterials had no profound impacts on the overall microbial activity, and the overall influence of CNMs on soil microbial activity does not reveal a specific pattern in the short term.
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1. Introduction

The discovery and continued use of carbon nanomaterials (CNMs) in industrial applications has been one of the significant developments in recent times (Biercuk et al., 2002). Carbon nanotubes (CNTs) are a group of nanomaterials that have the potential to be used in a wide range of materials due to their enhanced physico-chemical, excellent thermal, electronic and mechanical properties as a result of their unique structure and size (Klaine et al., 2008). They have been shown to have potential applications in several areas, particularly in hydrogen storage, as semi-conductors, in biomedical applications and environmental remediation (Klaine et al., 2008; Mauter and Elimelech, 2008). Examples of these carbon nanomaterials are fullerene soot, Buckminster fullerene (C_{60}) and multi-walled carbon nanotubes (MWCNTs). Fullerenes are arranged in a spherical configuration forming a closed graphite ball with only an external surface, while several rolled-up graphite sheets form MWCNT structure, creating interstitial wall spaces inside the inner cavity (Yang and Xing, 2007). The growing usage of CNMs has led to concern over their potential release into and effects on the environment. This widespread use will result in their release to the environment through point sources (e.g., production facilities, landfills, or wastewater treatment plants), accidental release during transport or intentional release for groundwater remediation (Jaisi and Elimelech, 2009; Petersen et al., 2011).

Soil is one of the major sinks of nanomaterials in the environment (Klaine et al., 2008), and as a result, microflora may interact with these materials leading to impact on populations and/or function (Nowack and Bucheli, 2007; Johansen et al., 2008). 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., 2014). For example, Jin et al. (2014) reported that SWCNTs significantly altered microbial community composition, and microbial biomass was reduced when 0.03–1 mg g^{-1} SWCNT was added to soil. In addition, previous studies have reported that both MWCNTs and SWCNTs can reduce the activity of soil enzymes and microbial biomass (Chung et al., 2011; Jin et al., 2013, 2014). Furthermore, the application of CNMs inhibited microbial respiration in soils and activated sludge (Goyal et al., 2010; Tong et al., 2012). For example, Goyal et al. (2010) observed that SWCNT exposure was found to impact upon microbial community structure negatively, when it was added to activated sludge.

The patterns of ^{14}C-glucose utilisation by soil microflora have been used as indicators of differences in carbon availability between soils and the changes in physiological status and/or

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structure of the soil microbial community as a result of added substrate (Nguyen and Guckert, 2001; Boucard et al., 2008). It is generally accepted that in the short term, 50% of the carbon content of most organic substrates is converted to CO_2, with the remaining carbon taken up as part of the biomass or incorporated into humus (Chiellini et al., 2007). It is therefore imperative to understand how the different types of CNMs could affect microbial communities in case there should be an accidental discharge during transport, improper disposal and from manufacturing processes. Currently, there is little knowledge on how CNMs impact on soil microflora, despite concerns of how CNMs would affect the complex soil microbial communities and their associated activity in the environment.

In this study, the impact of buckminster fullerene (C_{60}), multi-walled carbon nanotubes (MWCNTs), single-walled carbon nanotubes (SWCNTs) or fullerene soot (FS) on the activity of soil microorganisms over a short period of time was assessed using ^{14}C-glucose and substrate induced respiration (SIR), and measuring the uptake of ^{14}C-carbon into the microbial biomass. To the authors' knowledge, no study has been carried out to compare the effects of the different CNMs.

2. Materials and methods

2.1. Chemicals

CNMs (C_{60}, MWCNTs, SWCNTs, and FS) were purchased from Sigma–Aldrich, UK. C_{60} had a purity of >99.5% and a diameter of 1 nm, SWCNTs had a purity of >90%, average length of 1.02 μm and a diameter of 1 nm, while MWCNTs had a purity >90%, with a length of 5–9 μm, diameter of 10–15 nm. Fullerene soot was used “as produced”. D-Glucose was purchased from BDH laboratory supplies, UK, while its corresponding ^{14}C-radiolabelled analogue was purchased from Sigma–Aldrich, UK. Goldstar liquid scintillation (LSC) and sample oxidiser cocktails (Carbosorb and Carbon count) were obtained from Meridian, UK, and Combustaid was obtained from Perkin–Elmer Life and Analytical Sciences, USA. Chloroform was obtained from Fisher-Scientific, UK. Sodium hydroxide (NaOH), potassium sulphate (K_2SO_4) and potassium carbonate (K_2CO_3) were purchased from Fisher-Scientific, UK.

2.2. Soil preparation and spiking

A pristine agricultural soil (Dystric Cambisol) was collected from a depth of 5–20 cm from Myerscough College, Preston, UK. Soil physico-chemical properties are as follows: pH 6.5, organic matter 2.7%, sand 60.4%, silt 20%, and clay 19.5%. The soil air-dried, sieved with a 2 mm sieve to remove roots and stones, and then stored at 4 °C until ready for use. Soil was rehydrated back to the original moisture content of 21% and aliquots of soil were then spiked with $C_{60,}$ SWCNTs, MWCNTs, or FS to give soil-CNM concentrations of 0, 1, 10, 100 and 1000 mg kg^{-1} (dry weight soil). Soil-CNM aliquots were blended following the method of Doick et al. (2003). Soil-CNM aliquots were then sealed in amber glass jars (in triplicate per treatment) and left to age in the dark at 20 ± 2 °C and analysed at 1, 7, 14 and 21 d, respectively.

2.3. Utilisation of ^{14}C-glucose by soil indigenous microorganism

At each time point, soil samples were removed from the amber jars so as to determine soil microbial activity. Soil microbial activity can be defined as the ability of soil microbes to utilise ^{14}C-glucose as a carbon source for respiration and ^{14}C-carbon incorporation into microbial biomass (Boucard et al., 2008).

2.3.1. Determination of microbial decomposition of ^{14}C-glucose in soil

Substrate induced respiration (SIR) was used assess the ability of the soil microbial community to mineralise ^{14}C-glucose to $^{14}CO_2$. The evolution of $^{14}CO_2$ was determined by respirometry following the method of Reid et al. (2001). A CO_2 trap, consisting of a 7 ml scintillation vial containing 1 ml NaOH (1 M) solution, was suspended from the lid of each flask to trap $^{14}CO_2$ evolved as a result of ^{14}C-glucose decomposition. To assess the catabolic potential of the soil microbial community, the following was added to each respirometer: soil (10 g wet weight) was added to each flask with 5 ml of 3 mM glucose solution and ^{14}C-glucose (50 Bq g^{-1}), following a modified method of Doick and Semple (2003). The respirometers were placed on an orbital shaker (IKA Labortechnik KS501 digital) and shaken at 100 rpm. The production of $^{14}CO_2$ was assessed daily by the addition of 6 ml LSC to the CO_2 traps. After storage in darkness overnight, trapped ^{14}C-activity was quantified using a Canberra Packard Tricarb 2250CA liquid scintillation analyser. Sampling was carried out at 1 h, 2 h, 4 h, 8 h, 12 h, 18 h, 24 h, 36 h, 48 h, 72 h, 4 d and 5 d. The maximum rates and overall extents of ^{14}C-glucose mineralisation were determined.

2.3.2. Uptake of ^{14}C-glucose into microbial biomass

To determine the biomass, soil was taken from the respirometers immediately after mineralisation, and was divided into three portions. The first portion was oven-dried at 30 °C and combusted in a Packard 307 sample oxidiser to determine the level of ^{14}C-activity remaining in soil after the end of the respirometric assay (residual ^{14}C-activity). The second and third portions were analysed using fumigation-extraction method (Vance et al., 1987). The second portion was immediately extracted with 0.5 M K_2SO_4 in a ratio of 1:5 (w/v) by shaking on an orbital shaker (IKA Labortechnik KS501 digital) at 30 min at 100 rpm. The soil solution was then filtered (Whatman No. 1 grade), 3 ml of the resultant supernatant was added to 17 ml of LSC and the amount of ^{14}C-activity was measured by liquid scintillation counting. The third portion was placed in a desiccator and fumigated with ethanol-free chloroform for 24 h so as to measure the amount of ^{14}C-activity within the microbial biomass. The samples in the desiccator were vented to remove residual chloroform in the soil samples, and the soil were then extracted with 0.5 M K_2SO_4 in a ratio of 1:5 (w/v) and analysed, as previously described.

Total initial ^{14}C-activity ($^{14}C_{init}$) was calculated as the addition of cumulative respiration and the residual ^{14}C-activity remaining in the soil after the respirometric assays. The extractable flush was ^{14}C-flush = ^{14}C-activity in fumigated soil − ^{14}C-activity in unfumigated soil (Boucard et al., 2008). The proportion of ^{14}C-glucose incorporated into the microbial biomass was calculated using the following equation based on previous experiments (Sparling and West, 1988; Sparling et al., 1990): ^{14}C-microbial biomass = ^{14}C-flush/$k_{EC.}$

Firstly, a fixed k_{EC} coefficient (0.35) was used to convert C-flush into ^{14}C-microbial biomass (Sparling et al., 1990). Secondly, k_{EC} coefficients were calculated for each treatment at each time point, and the ^{14}C-microbial biomass recalculated with this new coefficient.

This procedure is based on the assumptions that the ^{14}C-labelled microbial C is representative of the total microbial biomass, and that all the ^{14}C-activity not accounted for by mineralisation and extraction of unfumigated soil has been incorporated into the microbial biomass with negligible amounts of extracellular microbial metabolite (Sparling et al., 1990): k_{EC} = (^{14}C-flush)/($^{14}C_{init}$ − ^{14}C-respired − ^{14}C-activity in unfumigated soil). The ^{14}C-flush and ^{14}C-microbial biomass were all subsequently expressed as percentages of the initial ^{14}C-activity ($^{14}C_{init}$). Finally, biophysical quotients (BQ) were calculated as BQ = $^{14}CO_2$ respired/^{14}C-microbial biomass (fixed or variable k_{EC}).

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