European Journal of Soil Biology 80 (2017) 53–58

Contents lists available at ScienceDirect

European Journal of Soil Biology

journal homepage: http://www.elsevier.com/locate/ejsobi

The impact of modified nanoscale carbon black on soil nematode assemblages under turfgrass growth conditions

Lian Duo, Lu He, Shulan Zhao*

Tianjin Key Laboratory of Animal and Plant Resistance, College of Life Sciences, Tianjin Normal University, Tianjin 300387, China

ARTICLE INFO

Article history:
Received 16 January 2017
Received in revised form
11 April 2017
Accepted 21 April 2017
Available online 29 April 2017

Handling editor: Bryan Griffiths

Keywords:
Nano-carbon black
Soil nematode
Trophic group
Community index
Turfgrass

ABSTRACT

At present, nanoscale materials have been produced with unprecedented speed due to their widespread use and may inevitably be released into the environment. As an effective adsorbent for heavy metals, nanoscale carbon black (CB) can be used to immobilize metals in contaminated soil, but no information is available regarding its effect on soil nematodes. In the present study, laboratory bioassays were performed to investigate the impact of three rates (1%, 3%, and 5% mass ratios) of HNO_3 or H_2SO_4 modified nanoscale carbon black (NCB or SCB) on the abundance and composition of nematode communities during 120 d of turfgrass growth. The results showed that 5% NCB and SCB treatments significantly increased plant shoot biomass of two crops. The total number of nematode genera in the control was greater than in nano-CB treatments. Plant parasites dominated across all treatments, with dominant genus *Helicotylenchus*. The abundance of total nematodes and each of the four trophic groups responded negatively to nano-CB addition. Three percent and 5% NCB addition significantly decreased the abundance of total nematodes, plant parasitic and bacterivorous nematodes, compared to the control. The species diversity, richness, community evenness and maturity index of soil nematodes were significantly decreased by 3% and 5% nano-CB compared to the control. This study revealed that modified nano-CB, as an amendment with a rate of 3% or higher, could cause significant disturbance to the soil food web and thus affect soil nematodes in the short-term.

1. Introduction

Human activities, such as mining, manufacturing, energy production, the disposal of waste materials and agricultural activities, result in soils contaminated with heavy metals, such as Pb, Cd, Cu, Zn, Cr, and Ni [1,2]. Unlike many organic pollutants, metals do not undergo chemical or microbial degradation, and they are highly persistent in soil over many years. Various *in situ* and *ex situ* cleanup techniques have been developed to remove heavy metals from soils, but many of these techniques are labor intensive, cost prohibitive and environmentally disruptive [3]. However, *in situ* immobilization techniques, using inorganic and organic amendments, such as lime, calcium carbonate, red-mud, bonemeal, and organic matter, to reduce the mobility and availability of heavy metals in soil, have become more attractive because they are environmentally friendly, cost effective and more convenient than conventional *ex situ* cleanup techniques [4–6].

Carbon black (CB) is a group of intensely black, finely divided forms of amorphous carbon [7]. In China, more than 5 million tons of CB are produced in 2015, accounting for 41% of world's total production and ranking first in the world [8]. Besides using in automobile tires and other rubber products as reinforcing agents and in printing ink, paint, etc., CB is also found to use as adsorbents for a wide variety of solutes [9,10]. With small particle sizes in the nanometer range, nano-CB has a large specific surface area and a porous structure of aromatic compounds with many functional groups such as the carboxyl group, phenolic hydroxyl group and carbonyl group, which enable nano-carbon black to adsorb pollutants from the environment. However, the virgin CBs often have low adsorption capacity for metal ions [7]. Nano-CBs modified by the oxidation of activated carbon with mineral acids, such as HNO_3, H_2SO_4, have shown good adsorption abilities for metal ions in environmental applications [11–13]. As a soil amendment, HNO_3 modified nano-CB was used to immobilize heavy metals in soil and found to reduce the bioavailability of soil Cu and Zn significantly [14]. Borah et al. (2008) found that carbon black modified with an acid mixture comprising HNO_3 and H_2SO_4 can remove 93% As from

* Corresponding author.
E-mail address: zhaosl_tjnu@126.com (S. Zhao).

http://dx.doi.org/10.1016/j.ejsobi.2017.04.004
1164-5563/

a 50 mg L^{-1} solution at the time of equilibration [10]. Although modified nano-CB provides an effective method for the remediation of heavy metals in the environment, the risks associated with its use have not been extensively assessed in soil organisms.

As the most abundant microfauna, soil nematodes occupy key positions at most trophic levels in the detritus food web [15,16]. They are sensitive to perturbations in soil conditions and play an important role in the decomposition of organic matter, nitrogen mineralization and nutrient cycling [17–20]. Various soil changes may affect the trophic structure, species richness and structure of nematode communities [21,22]. Soil nematodes have a short generation time, generally several days or months, and thus respond rapidly to soil disturbances (e.g. pollution, agriculture management, land-use change). It is well documented that soil nematodes can be useful ecological indicators [23–25].

To our knowledge, there are no studies available directly concerning the impacts of nano-CB addition on nematode community structure. Exploring the effects of nano-CB on soil nematodes can improve our understanding of how nano-CB addition alters ecosystem processes. Therefore, our objective was to examine the effects of modified nano-CB on nematode trophic abundance and community structure under turfgrass growth conditions. We characterized the community structure using diversity indices, e.g., Shannon–Wiener H', Margalef's richness SR, Pielou's evenness J', maturity index MI. The investigations may provide a basis for nano-CB application in the remediation of heavy metal contaminated soil.

2. Materials and methods

2.1. Materials

The soil used in this study was collected from the depth of 0–20 cm of the Research Base of Tianjin Normal University, Tianjin, China. The structure of soil particle was determined using hydrometer method. It was classified as sandy clay with 34.3% clay, 9.5% silt, and 56.2% sand according to international standard for soil texture classification. The following soil properties were characterized: pH value 7.44; total organic C 46.8 g/kg; total nitrogen 2.1 g/kg; available P 55 mg/kg. Soil pH was determined in 1:2.5 (w/v) soil:water solutions. Soil organic C was measured by the loss-on-ignition technique by ashing in a muffle furnace, total N was analyzed with an ultraviolet spectrophotometer after Kjeldahl digestion. Available P content was quantified using $NaHCO_3$ extraction method. A commercial nano-CB with a particle size of 20–70 nm and a specific surface area of 1200 m^2/g was purchased from the Tianjin Qiushi carbon black factory, China. The virgin carbon black often show low adsorption capacity for metal ions. Chemical modification using mineral acids could result in the change of textural properties as well as morphology and the creation of surface acidic functional groups (-COOH, -SO_2OH) which are responsible for the sorption mechanisms. H_2SO_4 and HNO_3 were often used in the oxidation of carbon blacks [7]. A species of turfgrass, *Festuca arundinacea* Schreb. (tall fescue), was chosen as the experimental model plant.

2.2. Nano-CB modification

A modification was conducted by refluxing commercial nano-CB, either with 20% H_2SO_4 at 110 °C for 90 min or 65% HNO_3 at 110 °C for 2 h. After cooling, the product was filtered and washed with deionized water until the filtrate was free of sulfates or nitrates. Finally, the modified nano-CB was dried at 110 °C for 24 h to a constant weight. The signatures SCB and NCB were used to designate nano-CB modified with H_2SO_4 and HNO_3, respectively. Borah et al. (2008) characterized the structural properties of modified nano-CB and found that chemical modification resulted in the creation of surface acidic functional groups (−COOH). FTIR spectra verified the introduction of nitro groups (−NO_2) in NCB and sulphonic acid group (−SO_2OH) in SCB. The variation appears to depend upon the nature of H_2SO_4 and HNO_3 used for modification [7].

2.3. Experimental design

Fresh soil was passed through a 6 mm sieve to remove stones and coarse plant residues. Modified nano-CB was added to the potting soil at rates of 1%, 3% and 5% (w/w) and thoroughly mixed with the soil. A control treatment was also prepared without adding nano-CB. Plastic pots (25 cm in height and 12 cm in diameter) were filled with treated soils (2 kg/pot). Approximately 4.5 g of seeds of *F. arundinacea* were sown in each pot. The pots were arranged according to a completely randomized design, with three replications. The turfgrass culture was exposed to natural light, with a temperature range of 25–34 °C, and a relative humidity of 31–48%. All of the pots were adjusted daily by weight to 70% water holding capacity with distilled water to maintain vigorous plant growth. The plant shoots were harvested at 35 d and 120 d for the first and the second crops, respectively, washed with deionized water, oven-dried and weighed. Soil was sampled from each pot after four months of turfgrass growth.

2.4. Nematode extraction and identification

Nematodes were extracted from 100 g of fresh soil from each sample using the method of centrifugal flotation in sucrose solutions, fixed in a 5% formalin acetic acid [26,27]. Nematode abundance was measured and expressed as individuals per 100 g dry soil. All isolated nematodes from each sample were identified to genus using a light microscope and assigned to four trophic groups: bacterivores, fungivores, plant parasites, and omnivore-predators based on stoma and esophageal morphology and the known feeding habitats of easily recognizable genera [15,28]. We identified them on the bases described by Yardirn and Edwards [28].

2.5. Indices of nematode community

Nematode diversity, richness and evenness were calculated using the following formulae: the Shannon-Wiener Index $H' = -\Sigma p_i \ln p_i$, where p_i is the proportion of genus n_i in the nematode community n [29]; the Margalef's Richness Index $SR = (G-1)/\ln n$, where G is the total number of genera and n is the total number of individuals in a community; and the Pielou's Evenness Index $J' = H'/\ln G$, where H' is the diversity index and G is the total number of genera [30].

Nematode genera are classified on a colonizer–persister (c-p) scale of 1–5, with *r*-strategists or colonizers (short life cycle, high fecundity, tolerant to disturbance, regarded as “enrichment opportunists”) = 1 and *K*-strategists or persisters (long life cycle, low colonization ability, few offspring, sensitive to disturbance) = 5 [31,32]. The Maturity Index, considering free-living nematodes only, was calculated using the following formula: $MI = (\Sigma v_i f_i)/n$, where v_i is the c–p value assigned to nematode genus i, f_i is the frequency of nematode genus i, and n is the total number of individuals in the soil sample. The Plant Parasitic Index was calculated based only on plant parasitic nematodes as $PPI = (\Sigma v_i f_i)/n$, where v_i is the c–p value for the plant parasite nematode genus i, f_i is the frequency of the plant parasite nematode genus i, and n is the total number of individuals [32].