European Journal of Soil Biology 74 (2016) 16–22

Contents lists available at ScienceDirect

European Journal of Soil Biology

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

Original article

Temporal responses of microorganisms and native organic carbon mineralization to ^{13}C-glucose addition in a sandy loam soil with long-term fertilization

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ARTICLE INFO

Article history:
Received 14 May 2015
Received in revised form
23 February 2016
Accepted 25 February 2016
Available online 11 March 2016

Handling Editor: C.C. Tebbe

Keywords:
Inorganic fertilizer and compost
^{13}C-glucose
PLFA
Microbial abundance
Priming effect

ABSTRACT

The altered mineralization rate of soil organic carbon (SOC) in the presence of exogenous organic substrates occurs by stimulating microbial activity. In this study, ^{13}C-glucose was applied at a rate of 1000 μg ^{13}C g^{-1} soil to arable soils following a 20-year application of compost (CM), inorganic NPK fertilizer (NPK) and a no-fertilizer Control. It was incubated for 30 days to evaluate how the labile substrate affected the microbial abundance and native SOC decomposition. Phospholipid fatty acids (PLFAs) were used as biomarkers for bacteria (Gram-positive bacteria, Gram-negative bacteria and actinobacteria) and fungi. ^{13}C-glucose application resulted in a significant increase in microbial abundance and positive priming effect for all treatments. The primed CO_2 flux derived from native SOC peaked on day 11, then increased gradually again from day 15 onwards in all treatments. The increase of abundance peaked on days 7 and 15 for Gram-negative (G^-) bacteria and Gram-positive (G^+) bacteria, however, fungal and actinobacterial PLFAs increased steadily from day 3 onwards under all three fertilization regimes. The results suggest that G^+ and G^- bacteria make a greater contribution to priming effects during the first 15 days of incubation, while fungi and actinobacteria are more important at the latter stages. The difference between glucose-derived ^{13}C remaining in soils and primed CO_2 from native SOC was 480, 381 and 263 mg C kg^{-1} in CM, NPK and Control treatments, respectively. Our study demonstrates that the exogenous labile organic substrate addition can more effectively promote C sequestration in organic C-rich soil (CM) than in organic C-poor soil (NPK or Control).

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1. Introduction

Preserving and increasing organic carbon (C) content in agricultural soils is of paramount importance, because of its central role in determining soil properties that strongly affect both crop production and the quality of the environment [1]. Soil has the largest stock of organic C in the terrestrial ecosystem [2], with the process of mineralization representing a gross flux to the atmosphere ten times greater than that from burning fossil fuels [3]. The mineralization of soil organic C (SOC) is therefore an important biochemical process related to greenhouse gas emissions and organic C loss [4].

Altered rates of native SOC mineralization in the presence of labile C substrates ('priming') is increasingly recognized as being central to the coupling of plant and soil-biological productivity, and also potentially as a key process mediating the C-balance of soils. The structure and activity of the microbial community both mediate the magnitude of the change in the SOC mineralization rate, and also determine the direction of that change [5]. Recent studies have shown that the increase in SOC mineralization following the labile substrate addition involves a succession of different microbial groups, and is related to the dynamics of

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http://dx.doi.org/10.1016/j.ejsobi.2016.02.007
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microbial community [6–8]. As shown by ^{13}C incorporation into PLFA, bacteria are the first group to trap and metabolize most of the easily-available organics inputted into the soil [9,10]. Other groups of microorganisms, such as fungi, preferentially utilize poorly-available substrates, benefit from bacterial necromass remaining after the easily-available organics was exhausted [7]. In contrast to bacteria, fungi can grow through low nutrient zones to the distantly-located substrates using their hyphae, as a result increases SOM decomposition and thus real priming effect (PE) [11]. However, some studies reported that Gram-negative bacteria may also contribute to real PE [12]. By now, there are little data available on changes in microbial community composition during PE [12], the responsibility of microbial groups during the PE process remains an open question, and we don't know which microbial groups are responsible for PE at different stages [7].

In addition to the microorganisms in soil, the nutrient status has also been found to affect the magnitude of the PE [13]. Applying of organic or inorganic fertilizers can increase the SOC and soil nutrient contents and affect the abundance, composition, diversity and functioning of microorganisms in soils [14,15]. In the present study, ^{13}C-glucose was added to soils following 20 years (1989–2009) of treatment with different fertilizers (compost, NPK or a no-fertilizer Control). The dynamic variation in soil respiration and SOC-priming were monitored during the incubation using the ^{13}C stable isotopic technique. The dynamic changes in soil microbial abundance and composition affected by the labile C addition for the different fertilization regimes over the course of the incubation were also measured. The experiments were designed to test the following hypotheses: (1) that microbial groups drive the PE dynamics, and bacteria, fungi and actinobacteria contribute differently to PE in the process of incubation, and (2) that increased availability of nutrients reduces the extent to which labile C stimulates the PE.

2. Material and methods

2.1. Site description and experimental design

The field experiment was located at the Fengqiu Agro-ecological Experimental Station, Chinese Academy of Sciences, in Henan, China (35°00′N, 114°24′E). The soil developed from alluvial sediments of the Yellow River, and was classified as aquic inceptisol under the U.S. soil taxonomy. The crop succession was winter wheat (*Triticum aestivum*) and summer maize (*Zea mays*); and there has been no substantial change in agronomic practice for more than 50 years. The soil had a pH of 8.65, and contained 4.48 g kg^{-1} organic C, 0.430 g kg^{-1} total N, 0.495 g kg^{-1} total P and 18.6 g kg^{-1} total K at the beginning of the experiment in 1989.

The detailed experimental design and fertilization regimes have been documented by Zhang et al. [16]. Briefly, three treatments with four replicates in completely randomized blocks were established. The treatments were (1) no added fertilizer (Control), (2) inorganic fertilizer NPK (NPK) and (3) compost (CM). A total of 150 kg ha^{-1} N for each crop was applied in the NPK and CM treatments. In the NPK treatment for wheat or maize, 60 or 90 kg N (urea) ha^{-1} was used as the basal fertilizer and 90 or 60 kg N (urea) ha^{-1} was used in the supplemental fertilizer, respectively. For the NPK treatment, 75 kg ha^{-1} P_2O_5 (superphosphate) and 150 kg ha^{-1} K_2O (potassium sulfate) were also added to each crop. In the CM treatment, a total of 2.76 tons ha^{-1} of compost (made up of wheat straw, oil cake and cotton cake; see Meng et al. [17]), which contained 1164 kg C, 150 kg N, 51 kg P_2O_5 and 65 kg K_2O, was applied as the basal fertilizer for maize and wheat. To match the amounts of P and K with the NPK treatment, 24 kg ha^{-1} P_2O_5 and 85 kg ha^{-1} K_2O were added to the CM plot prior to applying compost.

2.2. Soil sampling and preparation

Ten soil cores (0–20 cm) from each replicate plot of the CM, NPK and Control treatments were collected on 7 June 2009 with a 2.5 cm diameter auger, and mixed carefully to form one composite sample. Fresh samples were stored at 4 °C in the field, then transported to the laboratory for subsequent analysis. Fresh soils were passed through a 6-mm sieve for the incubation experiments, and an air-dried and sieved (<2 mm) subsample was used to analyze soil properties.

2.3. Incubation experiment and microbial abundance measurements

Fresh soil samples (110.0 g, oven-dried basis) were packed into individual 500-mL incubation bottle with the same bulk density (~1.20 g cm^{-3}) as in the field. Using a mini-pipette, 13.44 mL of a solution of ^{13}C-labeled glucose (99 atom % ^{13}C, Sigma–Aldrich, St. Louis, MO, USA) was added uniformly to the soil in each bottle at a rate of 1000 μg ^{13}C g^{-1} soil (bottles were labeled 'CCM' for CM soil, 'CNPK' for NPK soil, and 'CControl' for Control soil). Only deionized water was added to the corresponding control soils (labeled 'CM', 'NPK' and 'Control', respectively). Soil moisture in the bottles was maintained at 60% water-holding capacity by adding deionized water every two days. All bottles were covered with plastic wrap that had needle-punctured holes to maintain aerobic conditions, then incubated at 20 °C in the dark. Four replicate samples from the incubation bottles were removed from the incubator at 0, 3, 7, 15 and 30 days of incubation to measure changes in soil microbial abundance and composition.

The extraction of PLFAs was carried out using a modified Bligh-Dyer technique [18]. Briefly, fresh soil samples (3.00 g oven-dried) were extracted with a 2:1:0.8 solution of methanol:-chloroform:phosphate buffer. The soil extracts were centrifuged and the chloroform phases were collected. Phospholipids were separated from glycolipids and neutral lipids using silicic acid bonded solid-phase-extraction columns by sequential leaching with chloroform, acetone, and methanol, respectively. Phospholipids were saponified and methylated to fatty acid methyl esters (FAMEs) under N_2 at 37 °C and then dissolved in hexane, which contained a 19:0 (methyl nonadecanoate fatty acid) FAME standard. The resulting FAMEs were analyzed with a gas chromatograph mass spectrometer (GC–MS) QP 2010 PLUS (Shimadzu, Kyoto, Japan) equipped with a Varian VF23-MS column (30 m × 0.25 mm i.d. × 0.25 m film thickness) (Varian Associates Inc., Walnut Creek, CA, USA). Conditions for GC with helium as the carrier gas were an oven temperature programmed at 50 °C for 2 min, increased at a rate of 3 °C min^{-1} to 200 °C for 4 min, then at a rate of 5 °C min^{-1} to 240 °C for 10 min. The interface of MS was set to the maximum 240 °C oven temperature, the ion source was set to 210 °C and the MS scanned the range of 50–650 m/z at 1.7 scans s^{-1}. Peaks were identified by comparing retention times with known standards. Concentrations of PLFAs were obtained by comparing peak areas with the 19:0 FAME standard peaks.

The PLFAs 16:3ω3, 18:1ω9, 18:2ω6,9, 18:2ω9,12 and 20:1ω9 were used as biomarkers for fungi [19,20]; 10Me17:0, 10Me18:0 and 10Me20:0 were biomarkers for actinobacteria [21,22]; a15:0, i15:0, a16:0, i16:0, a17:0, i17:0 and i19:0 were specific for G^+ bacteria [21]; and cy17:0, cy19:0, 16:1ω7c, 16:1ω7t, 18:1ω7c and 18:1ω7t were specific for G^- bacteria [21,22].

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