Geoderma 292 (2017) 17–24

Contents lists available at ScienceDirect

Geoderma

journal homepage: www.elsevier.com/locate/geoderma

Fertilization alters microbial community composition and functional patterns by changing the chemical nature of soil organic carbon: A field study in a Halosol

Hongyan Wang [a], Ying Nie [a,b], Clayton R. Butterly [c], Lei Wang [b,d,*], Qiuhui Chen [b], Wei Tian [b], Bingbing Song [b], Yunguan Xi [b], Yu Wang [d,*]

[a] College of Resources and Environmental Sciences, Northeast Agricultural University, Harbin 150030, China
[b] Nanjing Institute of Environmental Sciences, Ministry of Environmental Protection, Nanjing 210042, China
[c] Department of Animal, Plant and Soil Science, AgriBio, Centre for AgriBioscience, La Trobe University, Melbourne Campus, Victoria 3086, Australia
[d] State Key Laboratory of Soil and Sustainable Agriculture, Institute of Soil Science, Chinese Academy of Sciences, Nanjing 210008, China

ARTICLE INFO

Article history:
Received 25 May 2016
Received in revised form 6 December 2016
Accepted 4 January 2017
Available online 15 January 2017

Keywords:
Carbon cycling
^{13}C NMR
Microbial enzyme
Linkage
Fertilization

ABSTRACT

Insight into the effects of fertilization on soil organic carbon (C) content and composition, and on microbial community and functions related to C decomposition are being gained, although the linkage between them remains elusive. To address this knowledge gap, a field experiment was conducted under different fertilization regimes, no fertilization (CL), chemical fertilization for 11 years (CF), organic fertilization for 6 years (OF6) and organic fertilization for 11 years (OF11) in an alkaline sandy loam soil. Changes in soil C chemistry using ^{13}C nuclear magnetic resonance (NMR) spectroscopy, microbial community using phospholipid fatty acid (PLFA) analysis and microbial functions using six hydrolase and three oxidoreductase enzyme activities were assessed. Fertilization significantly increased soil carbon contents, microbial biomass, and the abundance of fungi and bacteria. Nevertheless, CF induced a higher fungal-to-bacterial ratio due to a lower response in bacterial than fungal growth. In contrast, OF11 increased bacterial abundance, hydrolase activity, and consequently depleted labile C, resulting in higher alkyl-C contents and alkyl C/O-alkyl C ratios compared to other fertilizer treatments. Changes in microbial community composition and enzyme activity were tightly linked to soil C forms. Alkyl-C, carbonyl-C and ketone-C explained most of the variation (>89%) in soil microbial community, while alkyl-C and ketone-C explained most of the variation (>91%) in enzyme activity. Our results indicate that C composition rather than C quantity shaped soil microbial community composition and enzyme activity, restricting soil C decomposition. The practice of long-term organic fertilizer use is suggested to increase the quantity and recalcitrance of soil organic C.

1. Introduction

Soil organic carbon (SOC) represents a significant C reservoir (Schmidt et al., 2011) and can act as sink or source of CO_2 depending on the balance of organic matter accumulation and decomposition processes. The input of SOC such as crop residues has been suggested as an option for increasing the SOC level, while supplying nutrients to support agricultural production (Quilty and Cattle, 2011). Organic and inorganic fertilizer inputs are used primarily to increase crop yields, and meanwhile elevate litter-fall and root-derived C inputs into soil. Nevertheless, organic fertilization may also accelerate SOC decomposition (Fontaine et al., 2004; Luo et al., 2015). Consequently, mineral N fertilizer application may also enhance the CO_2 efflux (Sainju et al., 2008). The disparate effects on C inputs from roots and litter-fall and SOC decomposition result in inconsistent reports on the impact of fertilization on SOC pools (Alvarez, 2005; Li et al., 2013), and cause uncertainties in predicting the potential of fertilization on C balance (Cusack et al., 2011; Schmidt et al., 2011).

Soil microorganisms are the primary decomposers of SOC and drivers of soil nutrient cycling in agricultural systems (Lützow et al., 2006). Fertilization, the non-equilibrium flow of both nutrients [e.g. nitrogen (N) and phosphorus (P)] and energy (C) into soils can affect the population, composition, and functions of soil microorganisms (Marschner et al., 2003). Soil enzyme activities mediate microbial nutrient and energy acquisition from organic matter, these activities vary independently of microbial community structure (Bowles et al., 2014). Environmental factors such as pH (Sinsabaugh et al., 2008), moisture (Kotroczó et al., 2014), temperature (Creamer et al., 2015; Marschner and Bredow, 2002) and soil texture (Grandy et al., 2009) affect enzyme activities as well. Soil enzymes can be divided into two broad groups,

* Corresponding authors.
E-mail addresses: pingfanshangren@126.com (L. Wang), wangyu@issas.ac.cn (Y. Wang).

http://dx.doi.org/10.1016/j.geoderma.2017.01.006
0016-7061/

hydrolytic (hydrolase) and oxidation-reduction (oxidoreductase) enzymes. Generally, oxidoreductase enzymes are produced to scavenge N and P occluded in organic matter when nutrient availability is low relative to C acquisition (Cusack et al., 2011). This nutrient demand is determined by the elemental stoichiometry of microbial biomass in relation to environmental nutrient availability (Sinsabaugh et al., 2008). Thus, SOC content and quality are considered as key factors affecting soil microbial community structure and function.

Nevertheless, the lack of correlation between microbial community structure, enzyme activities and SOC content (Schnecker et al., 2015) brought out the hypothesis that C chemistry rather than content determines substrate availability for the microbial community. The addition of different organic materials are reported to result in various changes in microbial community composition (Elfstrand et al., 2007), since organic amendment addition could alter the chemical composition of SOC (Xu et al., 2006). Further, Kätterer et al. (2014) also found that repeated addition of organic amendments altered soil C-to-N ratio, which was associated with the changes in the contents of gram positive (G^+) and gram negative (G^-) bacteria. However, Ushio et al. (2008) observed that the contents of specific microbial biomarker lipids correlated with total C and N of soil but not the C-to-N ratios in a tropical montane forest ecosystem. Interestingly, Baumann et al. (2009) observed that changes in microbial community structure were associated with changes in soil C-chemistry, mainly the relative content of aryl-C and O-alkyl-C using ^{13}C NMR. Ng et al. (2014) also reported strong linkage between soil microbial community composition and enzyme activities to soil C chemistry induced by different organic amendments. Nevertheless, information on the effect of different fertilization practices on soil C chemistry quantified by C species remains sparse, and its' linkage with microbial community composition and functional capacity needs further investigation. Importantly, some labile C compounds (e.g. sugars) can persist in soil for decades, and this has been suggested to occur due to these compounds requiring co-metabolism with another (missing) compound (Schmidt et al., 2011). It is possible that interactions between C compounds and/or stages of decomposition exist which restrict the ability of C decomposers to utilize C compounds effectively interrupting C cycling.

A field experiment was conducted to assess whether changes in the amounts and composition of soil C, via different fertilizer treatments, alters microbial community composition and functional capacity (activity of C cycle enzymes). Four fertilizer treatments including; no fertilizer, chemical fertilizer for 11 years, organic fertilizer for 6 years, and organic fertilizer for 11 years were imposed in an alkaline loam soil. This type of soil, characterized with fast C decomposition, is widely distributed in the world (Singh, 2015). We hypothesized that (i) chemical fertilizer would decrease fungal-to-bacterial (F/B) ratio, and increase hydrolytic enzyme abundance toward increasing C acquisition due to the relatively high available N contents, (ii) organic fertilizer would increase F/B ratio, and oxidation-reduction enzyme abundance to facilitate the acquisition of N due to low N availability, (iii) chemical fertilizer would consume more labile C and result in greater recalcitrant C compared with organic fertilizer and (iv) C chemistry would reflect the changes in microbial community composition and enzyme activity.

2. Materials and methods

2.1. The field experiment and soil sampling

The field experiment was established in May 2004 at Daqing city (46° 42′ 20″ N, 124° 46′ 25″ E). This region has a warm temperate climate with average annual rainfall of 427.5 mm and mean annual temperature of 4.2 °C. The soil was classified as a Halosol according to the Chinese Soil Taxonomy (Gong et al., 2003) and an Inceptisol according to the USA Soil Taxonomy (Soil Survey Staff, 2014), and had a sandy loam texture (812 g kg^{-1} sand, 108 g kg^{-1} silt and 80 g kg^{-1} clay), with a pH 9.2 (H_2O), total C 15.1 g kg^{-1} and total N 1.6 g kg^{-1}.

An experimental field including 36 plots (60 m^2 in area per plot) in a randomized block design was subjected to a maize-soybean crop rotation. Both maize and soybean were planted in May and harvested in October. Four fertilizer practices were selected and applied with three replicates two weeks before sowing crops. Each plot (10 × 6 m, 12 in total) received either no fertilizer (Control, CL) established at 2004, inorganic nitrogen (N), phosphorus (P) and potassium (K) fertilizer (CF) established at 2004, organic fertilizer (OF) based on the same N dose as the NPK treatment for 6 consecutive years (OF 6) established at 2009, or organic fertilizer (OF) based on the same N dose as the NPK treatment for 11 consecutive years (OF 11) established at 2004. The CF treatment comprised 60 kg N ha^{-1} $year^{-1}$, 8 kg P ha^{-1} $year^{-1}$ and 14 kg K ha^{-1} $year^{-1}$, added as urea, calcium superphosphate and potassium sulfate, respectively. The OF compost was applied at 30 t ha^{-1} $year^{-1}$ equivalent to 60 kg N ha^{-1} $year^{-1}$, 14 kg P ha^{-1} $year^{-1}$ and 14 kg K ha^{-1} $year^{-1}$. The soils were sampled in October 2014 after soybean harvest. Five sampling points within each plot were randomly selected and 6 cores (10 cm diameter × 20 cm depth) were taken randomly at each point. All soil cores from each plot were bulked, crumbled, and thoroughly mixed within a plastic bag. The composite samples were passed through a 2-mm sieve and plant material, stones and other visible debris were removed manually. Thereafter, the sieved samples were kept at 4 °C before further analyses.

2.2. ^{13}C nuclear magnetic resonance (NMR) spectroscopy

The chemical composition of soil C was characterization using solid state magic-angle spinning (MAS) nuclear magnetic resonance (NMR). Soil samples were treated five times with 50 mL HCl (10%), 50 HF (10%) and distilled water, then dried and pressed into 7-mm zirconia rotors and spun at 6 kHz using a Bruker Avance III 400 spectrometer (Bruker BioSpin, Rheinstetten, Germany) with a resonance frequency of 100.62 MHz for ^{13}C. The signal-to-noise ratio was enhanced by applying cross polarization with total suppression of spinning sidebands (TOSS) pulse program and used a contact time of 1000 μs, a relaxation delay of 0.5 s, a ramp-contact and small phase incremental alternation with 64 steps (SPINAL64) decoupling pulse program. The spectra were recorded as the sum of 15,000 scans and calibrated using the methane C atoms of adamantine as an external standard ($\delta = 29.47$ ppm). The spectra across the following chemical shift limits were used to provide estimates of broad C types: 0–45 ppm (alkyl C), 45–60 ppm (N-alkyl C), 60–90 ppm (O-alkyl C), 90–110 ppm (di-O-alkyl-C), 110–145 ppm (aryl C), 145–165 ppm (O-aryl-C), 165–190 ppm (Carbonyl C), 190–220 ppm (ketone C). The ratios between the relative intensities of the 0–45 and 45–110 ppm regions (alky C/O-alkyl C) and of the 110–165 and 45–110 ppm regions (aryl C/O-alkyl C) were used as indicators of decomposition (Shrestha et al., 2015).

2.3. Phospholipid fatty acid (PLFA) analysis

Microbial community composition was assessed using phospholipid fatty acid (PLFA) analysis. PLFA are a vital component of the membrane of all microbes and are rapidly degraded as cells die, making them good indicators of living organisms (White et al., 1979). Thus, analysis of microbial populations using PLFA provides direct identification, classification and quantification of microbial community composition (Kätterer et al., 2014; Schnecker et al., 2015; Zhong et al., 2015). Freeze-dried soil samples were extracted with a single-phase mixture of chloroform-methanol-citrate buffer (1:2:0.8, v v v^{-1}; 0.15 M, pH 4.0) (Frostegård et al., 1993). The resulting fatty acid methyl esters were then separated and identified using a gas chromatograph (Agilent 7890 N, Wilmington, DE) fitted with a flame-ionization detector (FID) and using the MIDI Sherlock microbial identification system (Version 4.5, MIDI, Newark, NJ). Separation was accomplished with an Agilent 19091B-102E Ultra 25% phenyl methyl siloxane column (25.0 m × 200 μm × 0.33 μm). The oven temperature was raised to