Soil Biology & Biochemistry 113 (2017) 1–13

Contents lists available at ScienceDirect

Soil Biology & Biochemistry

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

Evident variations of fungal and actinobacterial cellulolytic communities associated with different humified particle-size fractions in a long-term fertilizer experiment

Qian Zhang, Guoqing Liang, Tengfei Guo, Ping He, Xiubin Wang, Wei Zhou*

Ministry of Agriculture Key Laboratory of Plant Nutrition and Fertilizer, Institute of Agricultural Resources and Regional Planning, Chinese Academy of Agricultural Sciences, Zhongguancun No.12, Beijing 100081, PR China

ARTICLE INFO

Article history:
Received 8 September 2016
Received in revised form
24 May 2017
Accepted 26 May 2017
Available online 3 June 2017

Keywords:
Particle-size fraction
Fertilization
Humus
Cellulolytic fungi
Cellulolytic actinobacteria

ABSTRACT

Cellulose is the dominant form of carbon (C) existing in arable soils, however the ecology of its degradation in soil is still relatively poorly understood. Here, community abundance and composition of fungal and actinobacterial cellulolytic genes (*cbhI* and *GH48*) from glycoside hydrolase family 7 and 48 together with characterization of fulvic acid (FA) and humic acid (HA) determined by cross polarization magic angle spinning (CPMAS) ^{13}C nuclear magnetic resonance (NMR) spectroscopy were explored in five soil particle-size fractions (large macroaggregate, coarse sand, fine sand, silt and clay), collected from a 33-yr mineral and organic fertilizer experiment. The results revealed the significant effects of particle-size fraction and fertilization on the distribution of soil humus and cellulolytic microbial community abundance. Strong correlations were detected between C content and structure of soil humus with cellulolytic microbial abundance. Generally, larger fractions (>63 μm) especially fine sand, which showed a lower degree of humification with higher aromaticity, lower HA/FA ratio, aliphaticity and alkyl/O-alkyl ratio of HA, were associated with greater abundance of cellulolytic microbes. However, smaller fractions (<63 μm), especially the clay fraction, showed lower *cbhI* and *GH48* gene abundances with a greater degree of humification indicated by ^{13}C NMR spectra. Phylogenetic analysis of the obtained nucleotide sequences revealed undiscovered sequences of both fungal and actinobacterial cellulolytic microbes. However, no clear clustering of sequences from particular particle-size fraction or fertilizer treatment was observed, even though combined application of chemical fertilizer and manure significantly increased cellulolytic gene abundances.

1. Introduction

Soils, with non-uniform distribution of nutrients (Balser et al., 2006) across different particle-size fractions, create heterogeneous environments and diverse habitats for microorganisms (Stemmer et al., 1998). Particle-size fractionation, which allows for the separation of soil organic matter pools with varying degrees of microbial alteration and mineral association, might help elucidate microbially-mediated soil carbon (C) cycling characteristics (Joliveta et al., 2006; Ling et al., 2014). Studies that focused on bulk soil might ignore these variations and lead to ambiguous conclusions around the mechanisms of soil C transformation. Soil contains the largest portion of organic C (~1500 Pg C in the first meter of soil) in global terrestrial ecosystem, which exceeds the cumulative pool of atmospheric C (760 Pg) and biotic C (560 Pg) (Post et al., 1982; Batjes, 1996; Jobbágy and Jackson, 2000; Janzen, 2004). Humus, mainly composed of fulvic acid (FA), humic acid (HA) and humin (HM), are the most ubiquitous non-living natural organic compounds in the environment (Stevenson, 1994). As an essential part of soil organic matter that is mostly derived from the decomposition of animal and plant litter, soil humus components might be altered by ambient shifts like fertilization regarding their chemical structure and properties (Jindo et al., 2011). Although the literature is replete with studies about variations in humus under different land uses (Reddy et al., 2012), soil types (Schöning and Kögel-Knabner, 2006) and organic amendments (Jindo et al., 2011), limited reports are available in terms of detailed chemical

* Corresponding author. Institute of Agricultural Resources and Regional Planning, Chinese Academy of Agricultural Sciences, No.12, Zhong guan cun South Street, Beijing 100081, PR China.
E-mail address: wzhou@caas.ac.cn (W. Zhou).

http://dx.doi.org/10.1016/j.soilbio.2017.05.022
0038-0717/

and spectroscopic characteristics of soil humus after long-term fertilizations, especially at soil particle-size fraction level.

Soil humification is primarily a microbially-mediated process. The influence of exogenous nutrients addition such as nitrogen deposition and fertilization on quantity and quality of humus formed in soil, might sensitively reflect on biogeochemical C transformation characteristics (Haase et al. 2007; Edwards et al., 2011; Weber et al., 2012). Cellulose decomposition is a critical process of soil C transformation as cellulose is the most abundant polysaccharide in plant litter that enters soil (Šturšová et al., 2012). It is well known that decomposition of organic matter is carried out by a group of enzymes having the capability to catalyze the hydrolysis of the substrate (Ryckeboer et al., 2003; Tuomela et al., 2000). Likewise, the decomposition of cellulose is mainly accomplished through the synergistic activities of three major groups of enzymes: (i) endoglucanases that cleave cellulose into smaller oligosaccharides, (ii) cellobiohydrolases that cleave cellobiose from the reducing and non-reducing ends of cellulose oligosaccharides, and (iii) β-glucosidases that cleave cellobiose into its glucose constituents (Kubicek et al., 2010). Among them, cellulolytic enzymes encoded by the fungal glycoside hydrolase family 7 cellobiohydrolase I gene (*cbhI*) and bacterial glycoside hydrolase family 48 (*GH48*) were generally considered to catalyze the rate-limiting step of cellulose decomposition (Baldrian and Valášková, 2008). Amplification with PCR primers that target the catalytic region of the fungal *cbhI* in *Ascomycota* and *Basidiomycota* allows a representative group of cellulolytic fungi to be detected and monitored in soil ecosystems (Edwards et al., 2008; Weber et al., 2011). Recently developed primers for the determination of the abundance and diversity of *GH48* gene from actinobacteria showed great promise in elucidating the ecological role of these organisms in terrestrial C cycling (de Menezes et al., 2015). As a widely accepted strategy to sustain or even improve crop yield and soil fertility, reasonable fertilizer management generally emphasizes significant effects on soil biological characteristics (Ahn et al., 2012). Altered C input such as manure application influences competitive interactions among soil microorganisms, thereby changing community composition and/or richness and potentially impacting soil C cycling. Therefore, systematic investigation of the key functional genes corresponding to specific microbes involved in cellulose decomposition after long-term fertilizations would not only provide essential information about targeted microbial communities capable of cellulolytic activity, but also facilitate the potential explanation of C transformation mechanisms after fertilization (Yeh et al., 2013).

In this study, we focused on the abundance and phylogenetic analysis of *cbhI* and *GH48* genes across bulk soil and five particle-size fractions under 33-yr fertilizer treatments through quantitative PCR, cloning and sequencing. Solid-state cross-polarization and magic angle spinning ^{13}C nuclear magnetic resonance (CPMAS ^{13}C NMR) spectra was also applied to illustrate the chemical and structural characteristics of soil humus (FA, HA and HM). Since soil particle-size fractions provide spatially heterogeneous microclimatic conditions for microorganisms, cellulolytic microbial communities were expected to respond differently to them. Specifically, we hypothesized that, after 33-yr of fertilization, (i) chemical and structural characteristics of soil humus would show characteristic variability among different fertilizer treatments and soil particle-size fractions, (ii) fungal and actinobacterial cellulolytic community abundance and phylogenetic affiliation would be influenced by fertilization, particle-size fraction and their interaction effects, and (iii) correlations would be observed between cellulolytic microbial abundance with soil humus chemical and structural characteristics.

2. Experimental procedures

2.1. Site description and experimental setup

The long-term fertilizer experiment was initiated in 1981 at South Lake station (30°37′N, 114°20′1″E), Hubei Province, China, where rice–wheat rotation is the common cropping system. The site is located in the northern subtropical to middle subtropical transitional geographic climate zone with an annual average temperature and precipitation of 16.4 °C and 1300 mm, respectively. The tested yellow-brown paddy soil with a clay loam texture belongs to Udalfs (USDA soil classification). At the beginning of the experiment, the soil had a pH (H_2O) of 6.3, 27.4 g kg^{-1} organic matter, 1.8 g kg^{-1} total N, 1.0 g kg^{-1} total P, 30.2 g kg^{-1} total K and 5.0 and 98.5 mg kg^{-1} of available P and K, respectively. Six treatments (three replicates each) were randomly implemented in 18 plots (8 m × 5 m) under a rotation of winter wheat and middle-season rice. Each plot was separated with cement block (40 cm) to avoid the interference between different fertilizer treatments. Treatments consisted of no fertilizer application (control, CK), fertilizer N (N), fertilizer N and P (NP), fertilizer N, P and K (NPK), manure plus fertilizer N, P and K (NPKM) and manure (M). Mineral fertilizers were applied as annual rate of 150 kg N ha^{-1}, 75 kg P_2O_5 ha^{-1}, 150 kg K_2O ha^{-1}. The N, P and K fertilizers were applied as urea, superphosphate and potassium chloride, respectively. For the NPKM treatment, the same rate of chemical fertilizers as NPK treatment were used plus 22,500 kg ha^{-1} organic fertilizer per year. Organic fertilizer was applied as pig manure (H_2O 69%) with properties of 15.1 g kg^{-1} N, 20.8 g kg^{-1} P_2O_5 and 13.6 g kg^{-1} K_2O.

Sixty percent of mineral fertilizers were applied to rice and the other 40% were applied during wheat season, while manure was applied equally (50:50) to the two crops. All fertilizer P and K and manure were applied once as basal dressing during wheat season and middle-rice season, while 40% of fertilizer N was applied as a basal fertilizer, 40% during tillering stage and 20% during booting stage in the middle–rice season. The amounts of N fertilizer applied to winter wheat were 50% as basal fertilizer, 25% for overwintering period and 25% during the jointing stage. Manure and mineral fertilizers were evenly applied onto the soil surface and immediately incorporated into soil (0–20 cm depth) before sowing.

2.2. Soil collection and particle-size fraction procedure

Undisturbed soil samples from the three replicates of each treatment were collected in May 17th and September 20th, 2014, one week before wheat and rice harvesting. Three soil cores (5 × 10 × 18 cm) at a depth of 0–20 cm from each plot were collected and equally merged as representative soil sample for one replicate of each fertilizer treatment. Moist soils were gently broken apart along the natural breakpoints and passed through a 5-mm sieve to remove visible organic debris. The 5-mm sieve was used rather than a 2-mm sieve because of the unique viscid characteristic of the paddy soil; were forced through a 2-mm sieve, the natural structure of the soil would be destroyed. After thorough mixing, different particle-size fractions were separated according to Stemmer et al. (1998), as the procedure described below. Briefly, the soil–water suspension was dispersed by low-energy sonication (output energy of 0.2 kJ/g) and subsequently fractionated by a combination of wet sieving and repeated centrifugation to avoid disruption of microaggregates. Finally, five fractions were obtained for each sample: large macroaggregate (>2000 μm), coarse sand-sized fraction (2000–200 μm), fine sand-sized fraction (200–63 μm), silt-sized fraction (63–2 μm), and clay-sized fraction (2–0.1 μm). Field-moist soils (35 g equivalent dry weight for each sample) were suspended in 100 mL of distilled water and then