



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

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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 (*cbhl* 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 ($>63\ \mu\text{m}$) 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 ($<63\ \mu\text{m}$), especially the clay fraction, showed lower *cbhl* 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.

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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 (Jolivet 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 ($\sim 1500\ \text{Pg C}$ 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).