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Original article

Fungi contribute more than bacteria to soil organic matter through necromass accumulation under different agricultural practices during the early pedogenesis of a Mollisol

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ABSTRACT

Living and dead microbial organisms contribute to the sequestration of soil organic carbon (SOC). The contributions of different community compositions are not well understood, particularly at the initial stage of soil development. Using an eight-year field experiment established on exposed parent material (PM) of a Mollisol, our objectives were (1) to differentiate microbial biomass and necromass of different microbial communities, and (2) to elucidate their contributions to SOC under different agricultural practices compared to PM and an arable Mollisol without C amendment (MO). The field treatments included two no-tilled soils supporting perennial plants (Alfalfa, and natural fallow), and four tilled soils under rotation between maize and soybean in alternate years, with or without chemical fertilization and crop residue amendment. Bacterial and fungal derived necromass were estimated by comparing amino sugars (ASs) contained in living and dead cell walls and phospholipid fatty acids (PLFAs) contained in living cell membranes, assuming that the conversion factor between cell membrane and wall for all microbes was one. The ratio of living microbial biomass estimated in ASs to that as indicated by total PLFAs was 0.76–0.87, indicating a high reliability of the estimation. Microbial biomass parameters in the field treatments were lower than those in MO and higher than those in PM. Both PLFAs and ASs demonstrated that bacteria dominated over fungi (70.2% v.s. 12.6%), but the fungal derived necromass were larger than bacterial derived necromass (70.7% v.s. 25.9%) in the studied soils. The microbial contribution to SOC was larger in necromass than in living biomass. The contribution of fungal derived necromass to SOC was dominant in soils and showed the same order among the soils as the fungal biomass, i.e. being larger under alfalfa than under natural fallow and in the tilled soils with organic C amendment than those without organic C input. These results suggested that only shift in fungal community due to land use change and organic C input could influence microbial contribution to soil organic carbon stabilization.

1. Introduction

Soil organic carbon (SOC) has beneficial effects on soil biological, chemical and physical properties, which in turn improves soil fertility and reduces soil erosion. Sequestration of SOC is also one of the important measures to mitigate climate change [1]. Soil organic carbon contains plant residues, microbial cell fragments and animal detritus as well as decomposed, humified refractory organic materials [2]. The content of SOC reflect the long-term balance between inputs of organic materials, primarily as plant residues, roots and root exudates, and losses of organic carbon due to microbial decomposition, erosion, and leaching [3]. Soil microbes directly contribute to SOC through protection of microbial biomass, metabolite excretion and dead cells in soil [4]. Therefore, the dynamics and compositions of microbial communities, and the degradation rate of their byproducts determine their contributions

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to SOC accumulation [4,5]. Soil microbial biomass carbon contributes to 1%–5% of SOC [6]. However, the standing biomass of microbes may not necessarily reflect microbial function in controlling SOC storage [7]. Soil microbes can reserve the senesced biomass and produce recalcitrant compounds, which will accumulate in soils due to their very slow turnover [4,8]. Incorporation of microbial necromass into stable SOC may play a greater role than previously believed [9–15]. However, there are no reliable approaches to differentiate between living and non-living microbial residues and between microbial C and native SOC.

Different soil microbial compositions have different functions in SOC decomposition [16,17]. Compared to bacteria, fungi and actinomycetes can degrade more recalcitrant organic compounds and those with high C:N [18], and survive under anaerobic and dry conditions [19,20]. Gram-positive (G(+)) bacteria (i.e. i15:0) can degrade complex substrates more easily than gram-negative (G(−)) bacteria, but have slower growth rates than G(−) bacteria [21,22]. In addition, soil microbial necromass may be selectively incorporated into native SOC, depending on the recalcitrance of their specific chemical structure and then influence the organo-mineral complexes [2,12,23], and the formation of soil structure [4]. Therefore, any change in soil microbial compositions may influence SOC content through their effects on SOC decomposition and microbial products [16,24,25]. Experimental data are needed to support these speculations.

Soil microbial biomass is often measured by using chemical extraction methods, such as the chloroform fumigation extraction method, phospholipid fatty acids (PLFAs) and amino sugars (ASs) [6]. The fumigation extraction estimates the total living microbial biomass [26], but cannot separate different microbial compositions, such as bacteria and fungi. Phospholipid fatty acids are essential membrane components of living microbes and measured to characterize the living microbial community compositions in soil [27–29]. Amino sugars are components of microbial cell walls of both living and dead microbes and measured to characterize both the living and dead community compositions in soil [25,30]. Due to the different origins and implication of microbial communities, there are few studies to measure PLFAs and ASs simultaneously [25,31]. Liang et al. suggested that the difference between Ass and PLFAs may provide information on microbial necromass [25], which is important for understanding microbial contribution to SOC accumulation.

Loss of surface soil is accelerated by soil water erosion and at mining and construction sites, resulting in soil parent materials becoming closer to the ground surface, or even exposed to the air. Restoration of such degraded soils is a great challenge, particularly in terms of restoration speed [32]. Accordingly, a field experiment was established in 2004 to examine soil development from the parent material (PM) of a Mollisol under different agricultural practices. It was demonstrated that soil microbial community compositions shifted among the field treatments [33] and different aggregate sizes in all treatments [34]. The objectives of this study were (1) to differentiate microbial biomass and necromass of different microbial communities, and (2) to elucidate their contributions to SOC under different agricultural practices compared to PM and an arable Mollisol without C amendment (MO). It was hypothesized that the shift of microbial community compositions could influence their microbial contributions to SOC through living microbial biomass and necromass differently.

2. Materials and methods

2.1. Study site and experimental design

The field experiment was established in 2004, at the National Observation Station of Hailun Agro-ecology System, Chinese Academy of Sciences, Heilongjiang province (47°26′N, 126°38′E). The experimental site is located in the central region of the Mollisols in Northeast China. The region has a typical temperate continental monsoon climate with a hot summer and a cold winter. The mean annual temperature is 1.5 °C, with the highest monthly temperature (35 °C) in July and lowest in January (−38 °C). The mean annual rainfall is 550 mm, with about 65% occurring from June to August. The soil was classified as Pachic Haploboroll according to the USDA Taxonomy [35]. The soil profile consists of following horizons: Ap1 (0–0.15 m) - Ap2 (0.15–0.25 m) - Ah1 (0.25–0.40 m) - Ah2 (0.40–0.70 m) - AB (0.70–1.00 m) - BC (1.00–1.70 m) and C (>1.70 m). The parent materials were clay loess-like materials [36], containing 42% clay and 35.6% silt and with dominant clay minerals of vermiculate, chlorite and illite.

The field experiment was established in June 2004 and the detailed information was reported elsewhere [33] and some selected soil characters are given in Table 1. Briefly, the parent materials were excavated from > 2-m C horizon, broken down to <0.5 m in size, and refilled to the plots, resulting in soil depth of 0.8 m. Randomized block design with four replicates was used to lay out six treatments in the plots. The treatments included natural fallow (NatF), with *Poa annua* L., *Equisetum arvense* L. and *Spodiopogon sibiricus* Trin, leguminous *Medicago sativa* L. (Alfa) and four tilled soil. The tilled soils were applied without chemical fertilization (F0) or with chemical fertilization (F1), without the above-ground biomass incorporated (C0) or with the above-ground biomass partially incorporated (C1) or totally incorporated (C2) into soil (Table 1). The cropped soils were tilled to 0.20 m depth using a spade before seeding. The cropping system was soybean (*Glycine max* (Merrill.) L.) and maize (*Zea mays* L.) rotation in alternative year. The first crop was soybean in 2004 and the crop was maize before the sampling in 2012. Following the practices of local farmers, the crops were generally sown in May and harvested in October at a density of 70,000 and 270,000 ha^{-1} for maize and soybean, respectively. Chemical N and P were applied as $(NH_4)_2HPO_4$ at a rate of 300 kg ha^{-1} yr^{-1}; K_2SO_4 was applied at a rate of 120 kg ha^{-1} yr^{-1} only in sowing. In F1C1, 2250 kg ha^{-1} ground soybean seeds that had been baked at around 200 °C, and 4500 kg ha^{-1} chopped maize straw were incorporated into the soil at the same time as in other treatments. The above-ground biomass was harvested, weighed and then chopped into 0.20–0.50 m long pieces. The chopped crop residues were incorporated into 0–0.20 m surface soil with tillage to make ridges (0.75 m wide and 0.25 m high) and the chopped plant residues in Alfa and NatF were mulched on the ground at the same time.

2.2. Soil sampling and analysis

Soil samples were collected from 0 to 0.20 m depth from three replicated plots of each treatment before sowing in May, 2012. The fourth replicate remained intact for other observation. The refilled soils at the 0.40–0.50 m depth under the three plots of NatF treatment were sampled and used as parent materials (PM). The archive air-dried parent material could not be used to measure soil microbial properties and fresh parent materials could not be sampled from >2 m depth during the sampling time when the deep soil was still frozen. The reference Mollisol (MO) was sampled from the A horizon (0–0.20 m) from an adjacent experimental field, which was cropped with maize and soybean rotation. Chemical N and P were applied as $CO(NH_2)_2$ and $(NH_4)_2HPO_4$ for maize at a rate of 210 kg ha^{-1} yr^{-1}, and 129 kg ha^{-1} yr^{-1}, respectively; and $(NH_4)_2HPO_4$ at a rate of 129 kg ha^{-1} yr^{-1} for soybean since 1993. All above-ground biomass was manually removed from the field of the reference Mollisol and soil tillage was also performed to 0.20 m depth using a spade.