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Soil aggregation regulates distributions of carbon, microbial community and enzyme activities after 23-year manure amendment

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A B S T R A C T

Manure amendment affects soil organic carbon (SOC) sequestration, microbial biomass and activity, and aggregate formation. However, how soil aggregation regulates SOC sequestration and microbial activity after manuring has received less attention. We studied the distribution of SOC, microbial community composition and activity in four aggregate classes (>2, 1–2, 0.25–1, and <0.25 mm) using field moisture sieving of soil from a 23-year manure addition field experiment under a rice–barley rotation. Long-term manuring increased the portion of large macroaggregates (>2 mm) by 2.4% ($p < 0.05$), and reduced the portion of microaggregates (<0.25 mm, including sand and silt) by 5.9% ($p < 0.05$) compared with soil without manure (control). Manuring increased SOC and total nitrogen contents of the large macroaggregates by 9.1% and 7.1%, respectively, but not of the microaggregates. Also, manuring increased the phospholipid fatty acids (PLFAs) contents of bacteria, fungi, arbuscular mycorrhizal fungi, and total microbes of the macroaggregates (>2, 1–2, and 0.25–1 mm) but not of the microaggregates. The fungal/bacterial PLFA ratio remained unchanged in all aggregates. Manuring increased β-glucosidase and chitinase activities in two macroaggregate classes (>2, and 1–2 mm), but not in the microaggregates. In conclusion, SOC, microbial biomasses and enzyme activities in the macroaggregates are more sensitive to manuring than in the microaggregates. Soil aggregation regulates the distributions of SOC and microbial parameters after 23-year manure amendment.

1. Introduction

Agroecosystem productivity and sustainability are dependent on soil structure and fertility. Aggregation is the formation of soil structure, physically protecting organic matter (OM) from microbial decomposition, regulating water, gas and nutrient dynamics, and reducing erosion (Jastrow, 1996; Six et al., 2004; Jastrow et al., 2007). Increasing aggregate-protected soil organic carbon (SOC) has potential to mitigate climate change because SOC decomposition is governed by accessibility by decomposers (Dungait et al., 2012).

Soil aggregates, composed of primary particles and binding agents, are the basic units of soil structure (Bronick and Lal, 2005). Two functional aggregate classes are commonly differentiated. Microaggregates (<0.25 mm) comprise primary mineral granules and organic debris, and macroaggregates (>0.25 mm) comprise microaggregates and particulate OM (Tisdall and Oades, 1982; Gupta and Germida, 1988; Miller and Jastrow, 1990). SOC is embedded and bound within hierarchical aggregates, and SOC is likely inaccessible by decomposers within microaggregates rather than within macroaggregates (Bird et al., 2002; Tian et al., 2015). The formation and turnover of macroaggregates are critical processes influencing SOC dynamics (Six et al., 2004).

Manure amendment firstly promotes aggregates formation and its-associated carbon (C) incorporation due to direct and indirect microbial-derived binding agents (Aoyama et al., 1999a; Mikha and Rice, 2004; Six et al., 2004; Ding and Han, 2014). The

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microaggregates, as preliminary aggregates, generally respond little to manuring (Mikha and Rice, 2004). In contrast, the macroaggregates occlude more manure-derived SOC due to the physical entrapment of particulate OM (Aoyama et al., 1999b; Bhattacharyya et al., 2009; Yu et al., 2012). Furthermore, manuring affects crop growth and further influences aggregate-associated SOC. Finally, increase in crop-derived SOC resulted from manure amendment stimulates microbial activities (Fontaine et al., 2007; Kuzyakov, 2010). Therefore, it is important to differentiate how manuring affects SOC between micro- and macro-aggregates with different stability and turnover rates.

Microbial biomass and community are primarily influenced by soil structure and substrate availability (Elliott and Coleman, 1988; Garcia-Franco et al., 2015; Li et al., 2015). Microbial biomass generally increases with aggregate size (from micro- to macro-aggregates) (Kanazawa and Filip, 1986; Helgason et al., 2010) due to the increasing OM amount (Chen et al., 2015). Therefore, manuring was reported to increase microbial biomass and change microbial community composition through forming aggregates and bringing surplus of OM (Zhang et al., 2015). In turn, the shift in microbial community further affects the aggregation (Gupta and Germida, 1988) and the turnover of encapsulated SOC. In general, fungi and bacteria have partly different functions in SOC turnover and stabilization (Jastrow et al., 2007). For example, fungi are more important for macroaggregate formation (Ding and Han, 2014) because of their hyphae as compared with bacteria (De Gryze et al., 2005). Fungi predominantly proliferate in larger pores among macro- and micro-aggregates; whereas bacteria reside in smaller pores within microaggregates (Tisdall and Oades, 1982; Ding and Han, 2014). However, the redistribution of microbial groups within macro- vs. micro-aggregates after long-term manuring has received little attention in the literature.

Enzyme activity is highly sensitive and related to C turnover (Aon et al., 2001; Wang et al., 2015a). Even though the C turnover in aggregates has received much attention, there are only few studies investigating the impacts of soil structure (macro- vs. micro-aggregates) on enzyme activities (Dorodnikov et al., 2009). The relationships between aggregate size and enzyme activities were found to be complex, and both positive (Kanazawa and Filip, 1986; Gupta and Germida, 1988) and negative relationships (Allison and Jastrow, 2006; Dorodnikov et al., 2009; Wang et al., 2015a) were reported. Because of the higher C accumulation level of macroaggregates compared with microaggregates, we hypothesize higher increase of enzyme activities in macroaggregates than that in microaggregates in response to manuring.

The lower plain of the Yangtze River is one of the most important agricultural regions in China (Wang et al., 2015b). The croplands in this region have been under intensive management with the use of conventional chemical fertilizers or additional manure to ensure high grain yields for a long period. However, it is not clear how additional manure amendment affects the distributions of SOC, microbial community composition and enzyme activities within macro- vs. micro-aggregates.

The objectives of this study were to investigate changes of SOC and microbial characteristics within micro- vs. macro-aggregates in response to a 23-year manure amendment in a rice–barley rotation system. We hypothesize that: (1) manuring increases macroaggregate-associated SOC but has little effect on microaggregate-associated SOC; consequently, (2) manuring increases microbial biomass and enzyme activities in macroaggregates but has little effect on those in microaggregates; and (3) higher fungi/bacteria biomass increase in macroaggregates compared to in microaggregates when manured because fungi are more important than bacteria in macroaggregate formation.

2. Materials and methods

2.1. Study site

This study was carried out in a rice–barley rotation field (30°26′04″ N, 120°25′01″ E, elevation 3–4 m a.s.l.) at the National Monitoring Station for Soil Fertility and Fertilizer Efficiency in Hangzhou (Zhejiang Province, China). The study site is flat and well-drained. The region is characterized by a subtropical humid monsoon climate, with a mean air temperature of 16–17 °C, an annual rainfall of 1500–1600 mm, an annual evapotranspiration of 1000–1100 mm, an annual frost-free period of 240–250 d and an annual sunshine duration of 1900–2000 h (Wang et al., 2015b). The soil, classified as an Inceptisol (US Soil Taxonomy), has a loam texture with 42% sand, 38% silt, and 20% clay (Chen et al., 2010a, 2010b).

The long-term field experiment was launched in autumn 1990. Prior to the formal experiment, the field had been intensively cultivated for more than 30 years with conventional chemical fertilizers, and afterward, the soil was homogenized by growing barley, early rice and late rice in rotation for 2 years (from autumn 1988 to autumn 1990) without fertilization. The main characteristics of the initial soil (0–20 cm) in 1990 were as follows: bulk density, 1.24 g cm^{-3}; porosity, 53.2%; SOC, 16.6 g kg^{-1}; total N, 1.67 g kg^{-1}; available N, 94.1 mg kg^{-1}; total phosphorus (P), 2.53 g kg^{-1}; available P, 37.4 mg kg^{-1}; available potassium (K), 67.5 mg kg^{-1}; cation exchange capacity (CEC), 14.6 cmol kg^{-1}; and pH, 6.4.

2.2. Experimental design

Two fertilization regimes, conventional chemical NPK fertilization (CK), and the NPK fertilization combined with composted pig manure amendment (manuring) were established using a random design. The manuring treatment had same amounts of N, P, and K fertilization with same chemical fertilizers as the CK treatment. Each fertilization regime has three field replicates. The N, P and K fertilizations were applied as urea (46% N), calcium superphosphate ($CaP_2H_4O_8$, 7.0% P) and potassium chloride (KCl, 49.8% K), at rates of 315 kg N ha^{-1} year^{-1}, 68.7 kg P ha^{-1} year^{-1} and 130.7 kg K ha^{-1} year^{-1}, respectively. The fresh composted pig manure, with 68.9% gravimetric water content, was applied at a rate of 22.5 Mg ha^{-1} year^{-1}. The dry matter of pig manure had a C content of 197 g kg^{-1}, N content of 14.5 g kg^{-1}, P content of 14.2 g kg^{-1}, and K content of 13.1 g kg^{-1}. Thus, the application rates of C, N, P and K of pig manure are equivalent to 1.4 Mg C ha^{-1} year^{-1}, 101 kg N ha^{-1} year^{-1}, 99.5 kg P ha^{-1} year^{-1} and 91.8 kg K ha^{-1} year^{-1}, respectively.

Each replicated plot has an area of 100 m^2. During the first 10 years (1990 – 2000), a rotation of barley–early rice–late rice was arranged for annual cropping. The annual fertilization quotas were 20% for barley, 40% for early rice, and 40% for late rice. After 10 years, soils were converted to barley–rice rotation, and the annual fertilization quotas were 32% for barley and 68% for rice. In each single growing season, the pig manure, P, and K and 70% N were applied as base fertilizer; while the remaining 30% N was used as top dressing. All other management practices (e.g. tillage and planting) were the same for both treatments. The barley was seeded in late November and harvested in early May of the next year in both the two and three crops of annual rotations over the 23-years. In the three crops rotation, the early rice was seeded in mid-May and harvested in late July, and the late rice was seeded in late July and harvested in early November. In the two crops rotation, the rice was seeded in middle June and harvested in the early November. The barley and rice were both harvested by hand. The aboveground biomass was removed leaving less than 3-cm stubble (Wang et al., 2015b).