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Distribution of soil nutrients, extracellular enzyme activities and microbial communities across particle-size fractions in a long-term fertilizer experiment

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ABSTRACT

Soils were particle-size fractionated to evaluate changes in carbon and nitrogen contents, enzyme activities and microbial community composition in response to 33 years of fertilization. This study focused on yellow–brown paddy soil and the particle-size fractions of >2000, 2000–200, 200–63, 63–2 and 2–0.1 μm. Microplate fluorometric assays and phospholipid fatty acid analysis (PLFA) were used to determine soil biological characteristics under no fertilizer (control, CK), fertilizer N (N), fertilizer N and P (NP), fertilizer N, P and K (NPK), organic manure plus fertilizer N, P and K (NPKM) and organic manure (M) treatments. The results showed that fertilizer and soil fraction individually and interactively ($P < 0.05$) affected soil C, N contents, enzyme activities and microbial communities except for α-glucosidase activity, bacterial relative abundance and the G+:G− ratio. Particularly, organic treatments significantly increased soil organic carbon (SOC) and total nitrogen (N) contents of all five fractions. The highest C and N contents and enzyme activities were observed in the 200–63 μm fraction, except for phosphatase and sulfatase, which showed the highest activities in the 2–0.1 μm fraction. The highest activities of β-glucosidase, β-cellobiosidase, α-glucosidase, aminopeptidase, phenol oxidase and peroxidase in each fraction were obtained in the organic treatments (NPKM and M). Activities of phosphatase, sulfatase, *N*-acetyl-glucosaminidase and β-xylosidase in the 2000–200 μm fraction were highest under NPK treatment. PLFA analysis showed that the >63 μm fraction contained higher abundance of total PLFAs than that in the 63–0.1 μm fraction. Organic treatments significantly enhanced total PLFAs abundance in >2000 μm fraction, but decreased PLFAs abundance in the 2000–200 μm fraction compared with the NPK treatment. Larger fractions (>2000 μm and 2000–200 μm) held relatively lower G+:G− ratios and higher fungi:bacteria ratios, which indicated better soil conditions in these fractions. Principal component analysis showed a smaller variability of microbial community composition among treatments than particle-size fractions. Most treatments of larger fractions (>2000 μm and 2000–2 μm) were well separated from the other fractions. Redundancy analysis showed total N, C:N ratio, phosphatase, sulfatase, *N*-acetyl-glucosaminidase and β-cellobiosidase activities significantly affected the composition of the microbial community. Significant correlations were also obtained between enzyme activities with SOC, total N and C:N ratio. We concluded that the long-term application of organic fertilizers contributed to improvements in the soil organic carbon and total nitrogen and most of the enzyme activities, especially for the 200–63 μm fraction, along with abundant and diverse microbial community composition in larger particles.

1. Introduction

Soils consist of particles of sand, silt and clay which bound into aggregates of various sizes by organic and inorganic agents. The distribution and stability of aggregates, and of the pores within and between them, affect soil properties and the composition and activity of soil biotic communities (Tisdall, 1994; Mikha and Rice, 2004). At the same time, aggregate formation and stabilization are

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affected by various factors, including climate conditions, mineral composition and types and amount of soil organic carbon (SOC) (Degens and Harris, 1997; Denef and Six, 2005). The main agents of aggregate formation and stabilization are organic materials, including persistent cementing agents, such as humic matter involved in stabilizing microaggregates, and transient bonding agents (e.g., polysaccharides derived from plants and micro-organisms) as well as temporary binding agents (e.g., fungal hyphae, fine roots, bacterial cells) related to formation and stabilization of macroaggregates (Tang et al., 2011). Therefore, the determination of SOC and the soil nitrogen content (total N) within different particle-size fractions is important for evaluating the effects of different fertilizer managements on soil quality.

Continuous use of imbalanced fertilizers under intensive rice–wheat cultivation in the Yangtze Plain has adverse impacts on the soil. Integrated nutrient management practice is seen as a viable option in restoring the soil physical structure and chemical fertility (Gattinger et al., 2012), improving soil organic C and therefore, sustaining the system productivity (Seufert et al., 2012) and reducing global warming potential (Cavigelli et al., 2013). The scientific literature is replete with studies on the effects of organic matter on soil structure and other properties, but frequently at the bulk soil scale. Little attention has been given to understanding the soil mechanism properties at the particle-size scale, which might manifest quite differently, but largely influence the bulk soil properties (Das et al., 2014). Therefore, the specific locations of enzyme activities within the soil matrix have attracted attention, especially as the area of these enzyme activities is affected by SOM quality (Kandeler et al., 1999) and turnover (Stemmer et al., 1998). Additionally, extracellular enzyme activities in soils play an important role in the degradation of polymeric material and the supply of low molecular weight substrates to microorganisms (Nannipieri et al., 2012). To date, it is unknown how soil nutrients and extracellular enzyme activities are distributed across particle-size fractions after different fertilizer treatments in a long-term experiment.

Definitely, balanced fertilizer application can influence both soil organic matter content and quality, substantially increasing microbial diversity in soils, thus improving soil functions and increasing the resistance of soil to environmental stress (Wardle and Ghani, 1995; Pankhurst et al., 1996; Kennedy and Gewin, 1997; Degens et al., 2000; Nsabimana et al., 2004). Microbial communities in soils produce extracellular enzymes to acquire energy and resources from complex biomolecules in the soil environment (Burns, 1982). These enzymes are of interest on an ecosystem scale because they catalyze important transformations in the carbon (C), nitrogen (N) and phosphorus (P) cycles (Wallenstein and Burns, 2011). It is well known that the addition of easily decomposable substrates to soil rapidly stimulates the soil microflora, resulting in a significant increase in aggregate stability (De Gryze et al., 2005; Abiven et al., 2007). Denef et al. (2001) and De Gryze et al. (2005) showed that fungi significantly affected macroaggregates formation. Besides the physical effects of enmeshment of macroaggregates by hyphae, extracellular polysaccharides can be produced by hyphae, attaching micro-aggregates and binding them together into stable macroaggregates (Neufeldt et al., 1999). Additionally, the hydrophobicity of microbial extracellular polysaccharides contributes to the stabilization of macroaggregates by decreasing their wettability (Liu et al., 2010). Besides fungi, bacteria also exude extracellular polysaccharides to bind soil particles and increase inter-particle cohesion (Degens and Harris, 1997). Increases in the fungi: bacteria ratio have been linked to increases in soil C and the C:N ratio across landscapes (Fierer et al., 2009 De Vries et al., 2012) and in response to organic amendments (Bernard et al., 2012). Other studies have shown increases in phospholipid fatty acid biomarkers for arbuscular mycorrhizal fungi (AMF) in response to long-term organic management (Bossio et al., 1998; Moeskops et al., 2010, 2012). Studies also supports that fungal communities has been suggested as a means of increasing agroecosystem N retention and other functions (De Vries and Bardgett, 2012; Jackson et al., 2012). However, the relationships among soil C and N pools, extracellular enzyme activities and microbial community composition and structure in soils amended with inorganic and organic fertilizers are still poorly understood.

As stated previously, aggregate formation could be influenced by many different factors, so it is still necessary and meaningful to conduct experiments in yellow–brown paddy soil, which is a typical paddy soil in the Yangtze Plain of China. Our objective was to elucidate the distribution of organic C and total N, enzyme activities and microbial community composition within different particle-size fractions to determine the significant positive effects of adequate fertilization. It is thus reasonable to predict that the application of balanced fertilization in combination with organic amendments to a long-term rice–wheat rotation would significantly impact aggregation, increase extracellular enzyme activities and enrich the microbial community, thereby resulting in a better soil structure and a higher soil quality for crops.

2. Materials and methods

2.1. Field design and sampling

The long-term field 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 total accumulated temperature of 5189.4 °C (>10 °C/day) and precipitation of 1300 mm. The tested yellow–brown paddy soil belongs to Udalfs with clay loam texture (USDA soil classification). At the beginning of the experiment in 1981, the soil had a pH (H_2O) of 6.3, organic matter of 27.43 g kg^{-1}, total N, P, K of 1.801 g kg^{-1}, 1.004 g kg^{-1} and 30.22 g kg^{-1}, respectively. The concentrations of available P and K were 5.0 mg kg^{-1} and 98.5 mg kg^{-1}. Six treatments (three replicates each) were randomly implemented in 18 plots (40 m^2 each) under a rotation of winter wheat and rice. Treatments consisted of soil without fertilizer (control, CK), fertilizer N (N), fertilizer N and P (NP), fertilizer N, P and K (NPK), organic manure plus fertilizer N, P and K (NPKM) and organic manure (M). For the NPKM treatment, fertilizer N, P and K were applied in the form of urea (300 kg N ha^{-1} per year), superphosphate (150 kg P_2O_5 ha^{-1} per year) and potassium chloride (150 kg K_2O ha^{-1} per year), respectively, while no PK or K was applied for the N and NP treatments, respectively. Organic manure was applied as pig manure (H_2O 69%) with properties of 15.1 g kg^{-1} total N, 20.8 g kg^{-1} P_2O_5 and 13.6 g kg^{-1} K_2O (22,500 kg ha^{-1} per year).

Sixty percent of chemical fertilizers were applied to rice and the other 40% were applied during the wheat season, while organic manure was applied equally (50:50) to the two crops. All fertilizer P and K and manure during the wheat season and the rice season were applied once as basal dressing. Meanwhile 40% of fertilizer N was applied as a basal fertilizer, 40% during tillering stage and 20% during booting stage in rice season. The amounts of N fertilizer applied to wheat were 50% as basal fertilizer, 25% for overwintering period and 25% during the jointing stage. Manure and mineral fertilizers were evenly broadcasted onto the soil surface and immediately incorporated into the plowed soil (0–20 cm depth) by tillage before sowing. According to the experimental design, the nutrient application rates of the other treatments were equal to the nutrients applied in the NPKM treatment.