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Soil aggregate stratification of nematodes and microbial communities affects the metabolic quotient in an acid soil

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ABSTRACT

The addition of fresh organic matter is known to modify both soil aggregation and soil biotic community composition. We hypothesized that fertilization alters the composition of soil nematode and microbial communities in soil aggregates, and the interaction between nematodes and microbes can stimulate or inhibit microbial activity. We used a field experiment with 9 years of manure application to investigate changes in nematodes and microbial communities among aggregate size fractions in an acid soil planted with maize in subtropical China. Nematodes, microbial communities, and metabolic quotient (qCO_2) were examined within three aggregate size fractions from soils under four fertilization regimes. Three aggregate fractions include large macroaggregates (>2000 μm; LA), small macroaggregates (250–2000 μm; SA), and inter-aggregate soil and space (<250 μm; IA). Four manure treatments include no manure (M_0), low-rate manure with 150 kg N ha^{-1} y^{-1} (M_1), high-rate manure with 600 kg N ha^{-1} y^{-1} (M_2), and high-rate manure with 600 kg N ha^{-1} y^{-1} and lime at 3000 kg $Ca(OH)_2$ ha^{-1} 3 y^{-1} (M_3). Fertilization influenced the proportion of the aggregate size fractions. The proportion of the LA fraction significantly increased under M_2 and M_3 treatments compared to M_0 and M_1 treatments, while the SA fraction significantly decreased. Aggregate fractions significantly affected the total number of nematodes and the abundance of bacterivorous nematodes (dominant genus *Protorhabditis*) and plant parasitic nematodes (dominant genus *Pratylenchus*), with values following the trend of LA > SA > IA. A high value for the nematode structure index (SI) in the LA fraction suggested a complex community structure with many linkages in the food web. Aggregate fractions also influenced microbial biomass and diversity. PLFA signature analysis revealed that microbial biomass and diversity (Shannon index) increased with decreasing aggregate size. However, the SA fraction had a significantly higher soil metabolic quotient (qCO_2) than the IA fraction. Only fertilization had a significant effect on the compositions of nematode groups, while both fertilization and aggregate fractions significantly affected microbial community composition. The variations in the composition of nematode and microbial communities could be explained independently by fertilization treatments (44% and 48%, respectively) and aggregate size (6.0% and 21%, respectively). Aggregated boosted trees (ABT) analysis indicated that total C exerted the strongest influence on microbial biomass, while pH influenced the total number of nematodes. The abundance of bacterivores showed a significant positive association with bacterial biomass across fertilization treatments and aggregate fractions ($r^2 = 0.17$, $P = 0.026$), which could partly explain the significant negative correlation between the total number of nematodes and qCO_2 ($r^2 = 0.25$, $P = 0.002$). The grazing on microbes by microbivores may decrease microbial activity.

1. Introduction

Soil organic matter (SOM) and soil aggregation are important determinants of soil fertility and productivity. Soil structure depends on the association between mineral soil particles (sand, silt, and clay) and organic matter, which forms aggregates of different size and stability (Tisdall and Oades, 1982). Manure application is an important land management practice to improve the nutrient status of soil and increase the content of SOM (Yanget et al., 2004). Aoyama and Kumakura (2001) further pointed out that manure application increases the accumulation of macroaggregate-protected C and thus sequesters more organic C in soils.

Microorganisms are a fundamental component of soils, playing a key role in essential processes such as organic matter dynamics and development of soil aggregation. The structural organization of

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soil particles provides a spatially heterogeneous habitat for microorganisms characterized by differences in organic C content among aggregate size classes (Young et al., 2008), or by predation pressure, water potential, and oxygen concentrations (Balesdent et al., 2000; Ranjard and Richaume, 2001). Application of organic fertilizer improves soil aggregation and alters the chemical properties of aggregates, such as organic C content (Lehmann et al., 2007; Virto et al., 2010), which in turn affects the soil microbial community composition and activity simultaneously (Zhang et al., 2007; Davinic et al., 2012).

Nematodes are one of the most abundant groups of soil invertebrates, occupying central positions in the soil food web (Neher, 2001). Given that soil pore spaces depend to a great extent on the size and arrangement of soil aggregates (Lebron et al., 2002), the distribution of soil nematodes with particular feeding habits and body sizes likely depends on soil aggregation and the availability of resources associated with different soil structures (Quénéhervé and Chotte, 1996). The abundance of fungal- and bacterial-feeding nematodes tends to increase in soils under organic management (Briar et al., 2007), presumably because the increase of microbial biomass expands the food base for free-living nematodes (Nahar et al., 2006). Furthermore, grazing by microbivores typically stimulates microbial activity in microcosms (Ingham et al., 1985; Traunspurger et al., 1997) and field soils (Anderson et al., 1981; Bardgett et al., 1999). Recently, Briar et al. (2011) has revealed that the co-occurrence patterns for soil nematodes and microorganisms among aggregate fractions. It is still unclear, however, how the interaction between soil nematodes and microbes within different aggregate fractions affect microbial activity. To date, there have been few studies to quantitatively evaluate the influence of fertilization and aggregate fractions on soil biotic community composition.

In this study, we examined the role of soil aggregation in determining nematode and microbial community composition and function using a 9-year manure experiment on an acid soil with low organic matter and pH. Acidic soils with poor fertility are widely distributed in tropical and subtropical area, and accounts for 30% of the total farmland area of China. The application of livestock manure can improve soil productivity and resolve environmental contamination due to direct discharge of livestock excrement into water (Long and Sun, 2012). We hypothesized that the application of organic manure increased total carbon and nitrogen among aggregate fractions and improved soil properties affecting nutrient availability, which in turn enhanced microbial biomass and the total number of nematodes. Furthermore, we expected that the grazing by microbivores could stimulate or inhibit microbial activity, measured as soil metabolic quotient, across fertilization regimes and aggregate fractions. The stimulation impact of nematodes to qCO_2 could be contributed to the increase of microbial biomass gazing by microbivores, while the inhibition impact to the microbivores selectively feeding on actively growing bacteria. We tested the first hypothesis by analyzing the relationship among soil chemical properties (mainly include soil total carbon and nitrogen, soil pH), microbial biomass and nematode abundance in aggregate fractions. Then we quantitatively analyzed the relative contribution of different soil chemical properties to the change of soil nematodes and microbial communities. Finally, we tested the second hypothesis by assessing the interaction between nematodes and microbes and its relationship to the soil metabolic quotient.

2. Materials and methods

2.1. Experimental site and design

The long-term fertilization experiment was conducted at the Yingtan National Agroecosystem Field Experiment Station (28°15′20″N, 116°55′30″E) of the Chinese Academy of Sciences in Yujiang Country, Jiangxi Province, China. The mean annual temperature and precipitation were 17.6 °C and 1795 mm, respectively, and most precipitation occurred from March to June. The soil is an acid loamy clay (clay 36.3%, silt 42.5% and sand 21.2%) derived from Quaternary red clay (Udic Ferralsols in Chinese Soil Taxonomy and Ferric Acrisols in the FAO classification system).

Twelve concrete plots, 2 m wide × 2 m long × 1.5 m deep, were constructed in the field. Four pig manure treatments were compared in a completely randomized design with three replications: (1) no manure (M_0); (2) low-rate manure with 150 kg N ha^{-1} y^{-1} (M_1); (3) high-rate manure with 600 kg N ha^{-1} y^{-1} (M_2); (4) high-rate manure with 600 kg N ha^{-1} y^{-1} and lime applied once every 3 years at 3000 kg $Ca(OH)_2$ ha^{-1} (M_3). The pig manure had average total carbon of 386.5 g kg^{-1}, total nitrogen of 32.2 g kg^{-1} on a dry matter basis, and water content of 75%. The monoculture corn, cultivar No.11 from Denghai, was planted annually in April and harvested in July from 2002 to 2011. There were no tillage and management measures with the exception of weeding by hand.

2.2. Soil sampling and aggregate fractionation

Soil samples from each plot were collected from a depth of 0–15 cm in late July of 2011. Each soil sample pooled from five soil cores (5-cm diameter) was stored in an individual insulated bag, and immediately transported to the laboratory in a cooler. All field fresh soils were passed through an 8-mm sieve by gently breaking soil clods along natural planes of fracture, and then for aggregate fractionation. The soil samples in each aggregate fraction were divided into three subsamples which were used for nematode faunal, microbial community and soil chemical analyses, respectively.

Three aggregate size classes were fractionated through dry sieving of fresh soil as follows: large macroaggregates (>2000 μm; LA), small macroaggregates (250–2000 μm; SA) and fine material (<250 μm). Small aggregates passing through a 250 mm sieve are considered too small for nematodes to enter and this fraction will thus be referred to as inter-aggregate soil and space (IA) (Briar et al., 2011). Fresh soil was dry-sieved because both wet-sieving and air drying compromised the *in situ* link between the aggregates obtained and their indigenous biota. Moreover, nematodes have positive hydrotropism and once exposed to water, they will be attracted and move toward it (Stock and Goodrich-Blair, 2012). A substantial portion of the soil microbial biomass is thought to live on or near the aggregate surface (Oades, 1984). Therefore, there were substantial losses in the abundance of nematodes and microbes using the wet-sieving method, which could lead to erroneous interpretations about soil nematodes and microbial community composition. Naturally formed field aggregates (Pikul et al., 2009) were isolated manually by dry sieving 300 g of fresh soil (moisture content ~10%) on a series of 2 sieves (2 mm and 250 μm). Soil was placed on a 2 mm sieve, and then gently moved up and down by hand at oscillation 60 min^{-1} for 10 min. The material passing through the 2 mm sieve mesh was transferred to a 250 mm sieve for further fractionation, ultimately generating three aggregate fractions. The three aggregate fractions were placed in an oven at 60 °C until dry to determine the proportion of whole soil weight in each fraction. This gentle sieving procedure aimed to minimize excessive disturbance of larger aggregates and was tested to ensure consistent and repeatable aggregate distributions were obtained in soils of each treatment.

2.3. Soil chemical properties and metabolic quotient analyses

Soil pH was determined using a glass-calomel electrode in a suspension with soil:water 1:2.5 (w/v). Soil total C (TC) was analyzed by dry combustion, using a Shimadzu TOC 5000 Total C