Journal of Integrative Agriculture 2020, 19(10): 2530–2548

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ScienceDirect

RESEARCH ARTICLE

Aggregate-associated changes in nutrient properties, microbial community and functions in a greenhouse vegetable field based on an eight-year fertilization experiment of China

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Abstract

Soil aggregation, microbial community, and functions (i.e., extracellular enzyme activities; EEAs) are critical factors affecting soil C dynamics and nutrient cycling. We assessed soil aggregate distribution, stability, nutrients, and microbial characteristics within >2, 0.25–2, 0.053–0.25, and <0.053 mm aggregates, based on an eight-year field experiment in a greenhouse vegetable field in China. The field experiment includes four treatments: 100% N fertilizer (CF), 50% substitution of N fertilizer with manure (M), straw (S), and manure plus straw (MS). The amounts of nutrient (N, P_2O_5, and K_2O) input were equal in each treatment. Results showed higher values of mean weight diameter in organic-amended soils (M, MS, and S, 2.43–2.97) *vs*. CF-amended soils (1.99). Relative to CF treatment, organic amendments had positive effects on nutrient (i.e., available N, P, and soil organic C (SOC)) conditions, microbial (e.g., bacterial and fungal) growth, and EEAs in the >0.053 mm aggregates, but not in the <0.053 mm aggregates. The 0.25–0.053 mm aggregates exhibited better nutrient conditions and hydrolytic activity, while the <0.053 mm aggregates had poor nutrient conditions and higher oxidative activity among aggregates, per SOC, available N, available P, and a series of enzyme activities. These results indicated that the 0.25–0.053 mm (<0.053 mm) aggregates provide suitable microhabitats for hydrolytic (oxidative) activity. Interestingly, we found that hydrolytic and oxidative activities were mainly impacted by fertilization (58.5%, P<0.01) and aggregate fractions (50.5%, P<0.01), respectively. The hydrolytic and oxidative activities were significantly (P<0.01) associated with nutrients

Received 10 February, 2020 Accepted 29 May, 2020
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doi: 10.1016/S2095-3119(20)63269-5

(SOC and available N) and pH, electrical conductivity, respectively. Furthermore, SOC, available N, and available P closely (P<0.05) affected microbial communities within >0.25, 0.25–0.053, and <0.053 mm aggregates, respectively. These findings provide several insights into microbial characteristics within aggregates under different fertilization modes in the greenhouse vegetable production system in China.

Keywords: fertilization, soil aggregate distribution, microbial characteristics

1. Introduction

Soil, as a highly complex system, provides a heterogeneous environment (e.g., non-uniform distribution of organic C and nutrients) for microbes (Liao *et al.* 2018). Soil aggregate is an important facet of soil structure, which can contribute to spatial heterogeneity by creating microhabitats that differ in C and nutrients status, soil aeration, and pore spaces (Regelink *et al.* 2015; Wang S *et al.* 2018b). Macro-aggregates (>0.25 mm) normally have more big pores (Regelink *et al.* 2015) and contain large amounts of labile substrates that originate principally from plant residues or organic resources (ORs) inputs (Six *et al.* 2004). In contrast, micro-aggregates (<0.25 mm) are composed of more recalcitrant C fractions formed by the high degree of humification of organic matter or microbial residues (Davinic *et al.* 2012) and provide a protective microhabitat for microbes and soil organic C (SOC) sequestration (Totsche *et al.* 2018). Moreover, the variations in soil structural characteristics induced by different fertilization modes influence soil aggregate distribution and stability (Chen *et al.* 2015), which impacts the spatial heterogeneity of soil physicochemical properties, and, consequently, the distribution of microbes and their activity among various aggregates (Jiang *et al.* 2013; Kim *et al.* 2015).

Soil microbes and extracellular enzyme activities (EEAs) together not only play important roles in maintaining soil fertility and productivity through controlling essential biochemical processes, such as SOC dynamics and nutrient recycling (Sinsabaugh 2010), but also provide more comprehensive information involved in changes in soil environment than physicochemical properties (Luo *et al.* 2017). Commonly, soil microbial communities and functions (i.e., a series of EEAs) are strongly affected by fertilization, and their responses to manure, straw, and/or chemical fertilizers in soils have been well investigated in recent years (Zhang *et al.* 2012; Li *et al.* 2016). The impacts of fertilization on microbial characteristics in soils were interpreted by direct effects of C and nutrient resource supply (Dong *et al.* 2014), or indirect variations in soil physical and other characteristics (Rousk *et al.* 2010). However, information on the microbial characteristics within soil aggregates is relatively scarce in agricultural soils under different fertilization patterns.

Recognizing the spatial distribution of microbial communities and functions at the aggregate scale is crucial for the exploration of microbial ecology and its influence on biogeochemical processes (e.g., the transformations of SOC and nutrients) in soils (Upton *et al.* 2019). The different aggregates supply spatially heterogeneous microhabitats for microbes, which are distinguished by discrepancies in organic C complexity, nutrient abundance, moisture status, and oxygen concentrations (Liao *et al.* 2018; Wang S *et al.* 2018b). Research on microbial communities and functions within soil aggregates has shown inconsistent results about the spatial distribution in microbial characteristics (Jiang *et al.* 2011; Wang *et al.* 2015). Greater microbial biomass or activities have been detected in the small aggregate-size fractions, i.e. <0.053 or 0.25–0.053 mm aggregates (Kong *et al.* 2011; Bach *et al.* 2018), or the large aggregate-size fractions, i.e. >0.25 mm aggregates (Kuzyakov and Blagodatskaya 2015; Li J *et al.* 2015). This inconsistency motivates the need for additional studies to investigate the heterogeneous distribution of microbial communities and functions (Wang S *et al.* 2017).

Recently, greenhouse vegetable production (GVP) systems have developed rapidly in China (Zhang *et al.* 2017). In 2010, the planting area of GVP systems reached 3.7 million ha in China, which accounts for approximately 85% of the worldwide greenhouse cultivation area (Yang *et al.* 2014). In intensive agricultural production systems (e.g., GVP systems), farmers adopted unreasonable agricultural management practices, such as overuse fertilizers, to obtain high yields and economic benefits (Huang *et al.* 2017). Moreover, GVP systems are characterized by a large cropping index, unique environment with high internal temperature and humidity, and the absence of rain leaching (Hu *et al.* 2017). These unique characteristics have resulted in a series of consequences, such as soil structure deterioration, soil acidification, and salination (Wang *et al.* 2014), which may influence soil microbial properties in GVP systems (Moeskops *et al.* 2010). To solve these problems, organic amendments are increasingly recommended as promising fertilization strategies to ameliorate soil physicochemical and microbial attributes (Chen *et al.* 2015). Examination of how ORs

(e.g., manure and/or straw) addition induces changes in microbial characteristics at the soil aggregate scale will deepen our understanding of the improvement of soil quality in response to ORs addition. However, studies that have explored the effects of ORs application on soil aggregates and its associated microbial communities and functions in GVP systems are scarce.

Consequently, an 8-year field experiment was established to systematically evaluate the effects of 50% substitution of N fertilizer with ORs (i.e., manure and/or straw) on soil aggregates (>2, 2–0.25, 0.25–0.053, and <0.053 mm aggregates) as well as aggregate-associated microbial communities and functions in a GVP system in Tianjin, China. The aims were: (i) to evaluate the effects of different fertilization patterns (organic *vs*. chemical fertilization) on soil aggregate distribution, aggregate-associated physicochemical and microbial properties and (ii) to assess the spatial distribution in physicochemical and microbial properties within different aggregates.

2. Materials and methods

2.1. Study sites description

The field experiment was conducted in October 2009 at a typically managed vegetable agroecosystem in a suburban area of Xiqing District (117°0′E, 39°13′N), Tianjin City, China, where cropping system is leafy vegetables (celery)–fruit vegetables (tomato) rotation. This area belongs to a typical warm sub-humid continental climate zone. The mean annual temperature inside and outside the shed is 21.4 and 11.6°C, respectively. Annually, there are 203 days free of frost and over 2 810 h of sunshine. The soils in this site are classified as medium-loam Chao (aquic cambisols) soil by FAO soil classification. The initial soil properties of plough horizon (0–20 cm) were: pH, 7.9; soil organic C, 15.3 g kg^{-1}; available N, 186.2 mg kg^{-1}; available P, 144.6 mg kg^{-1}; and available K, 404.0 mg kg^{-1}.

2.2. Experimental design

The trial was designed in a randomized block design with three replicates and four treatments. The area of one individual plot was 2.4 m wide and 6.0 m long (14.4 m^2). Each plot is separated with PVC plates within 105 cm deep to prevent nutrient and water cross-contamination between neighboring plots. Four fertilization treatments were: (1) 100% N fertilizer (CF), (2) 50% N fertilizer plus 50% manure (commercial pig manure; M), (3) 50% N fertilizer plus 25% manure and 25% straw (MS), and (4) 50% N fertilizer plus 50% straw (S). The detailed description of nutrients (N, P_2O_5, and K_2O) and C inputs, and information about the fertilizers used, was shown in Table 1 and Appendix A.

2.3. Soil sampling and aggregate size preparation

After the celery harvest in mid-January 2017, five undisturbed surface soil block (0–20 cm in depth) samples were collected randomly from each plot and then mixed for one representative soil sample. The combined soil samples were preserved in sterile containers (rigid plastic boxes) under 4°C conditions and rapidly transferred to the laboratory for sieving. All combined soil samples were passed through an 8-mm sieve by carefully breaking soil clods along the natural planes of fracture to eliminate macrofauna, stones, and large roots (Yu *et al*. 2015).

Table 1 The amounts of nitrogen and carbon application (kg ha^{-1}) in the present study

Treatment[1)]	N inputs				C inputs		
	Chemical fertilizer	Manure	Straw	Sum	Manure	Straw	Sum
Spring vegetable season (tomato)							
CF	450.0	0	0	450.0	0	0	0
M	225.0	225.0	0	450.0	2 260.0	0	2 260.0
MS	225.0	112.5	112.5	450.0	1 130.0	4 618.0	5 748.0
S	225.0	0	225.0	450.0	0	9 236.0	9 236.0
Autumn–winter vegetable season (celery)							
CF	450.0	0	0	450.0	0	0	0
M	225.0	225.0	0	450.0	2 260.0	0	2 260.0
MS	225.0	112.5	112.5	450.0	1 130.0	4 618.0	5 748.0
S	225.0	0	225.0	450.0.	0	9 236.0	9 236.0

[1)] CF, 100% N fertilizer; M, 50% N fertilizer plus 50% manure; MS, 50% N fertilizer plus 25% manure and 25% straw; S, 50% N fertilizer plus 50% straw.

The amounts of N, P_2O_5, and K_2O inputs (900.0, 525.0 and 1 200.0 kg ha^{-1} yr^{-1}, respectively) were equal in each treatment. All the organic manure and corn straw were applied into soils (15 cm in depth) as basal fertilization before vegetables (e.g., celery or potato) planting.

Soil aggregates were separated by the wet-sieving method described by Yu *et al.* (2012a) for microbial-associated analysis. Briefly, the sieved field-moist clods (<8 mm), the equivalent of 100 g dry weight equivalent, were placed on top of three stacked sieves (2, 0.25, and 0.053 mm in diameter), and gently immersed in deionized water, pre-wetting for 10 min prior to sieving. Then, the sieves were manually moved (amplitude 3 cm) vertically 50 times within 2 min using a digital metronome to separate the clods into four water-stable aggregates as follows: >2, 2–0.25, 0.25–0.053, and <0.053 mm aggregates. Afterwards, >2, 2–0.25, and 0.25–0.053 mm aggregates were then collected from the corresponding sieves, while the <0.053 mm aggregates passed through 0.053 mm sieve were centrifuged (5 000 r min^{-1}, 2 min) for collection. Each separated aggregate was divided into two portions. One portion was air-dried for dry-weight conversion and determination of nutrient-related properties (e.g., soil organic C (SOC) and available P (AP)), whereas the other portion was stored at 4°C (less than one week) until subsequent analysis of soil microbial characteristics (e.g., soil microbial community and EEAs) and available N (nitrate-N (NO_3^--N) and ammonium-N (NH_4^+-N)) contents. Notably, loss of microbial biomass and nutrients could happen through diffusion into the supernatant, but the loss could be negligible as shown in Appendix B and by Balser *et al.* (2002).

2.4. Physicochemical property analysis within aggregates

The pH in the four aggregates was gauged in soil/water mixture (1:2.5) through a compound electrode pH meter. The electrical conductivity (EC) was measured in soil/water mixture (1:5) using an electromagnetic device. SOC was analyzed following the heated dichromate/titration method (Mebius 1960). Soil NO_3^--N and NH_4^+-N were extracted by 2 mol L^{-1} KCl and determined through a flow injection auto-analyzer. AP was extracted with 0.5 mol L^{-1} $NaHCO_3$ and measured through the Olsen method (Olsen and Oisen 1982). Available K (AK) was extracted with 1 mol L^{-1} NH_4OAc and measured by atomic absorption spectrometry. Soil bulk density (BD) was evaluated through the core sampling method (Li *et al.* 2019).

2.5. Soil microbial community analyses

Soil microbial community composition within aggregates was evaluated using phospholipid fatty acids (PLFAs) analysis (Jiang *et al.* 2013). The nonadecanoic acid methyl ester (19:0) was added as the internal standard, and 24 individual PLFAs were applied to evaluate microbial community as follows: Gram-positive bacteria (G^+; i15:0, a15:0, i16:0, a17:0 and i17:0), Gram-negative bacteria (G^-; 16:1ω7c, cy17:0, 18:1ω7c and cy19:0), bacteria (B; 15:00, 17:00 and G^+ plus G^-), actinomycetes (Acti; 10Me-16:0, 10Me-17:0 and 10Me-18:0), arbuscular mycorrhizal fungi (AMF, 16:1ω5c), saprotrophic fungi (SF; 18:2ω6c and 18:1ω9c), fungi (F; AMF and SF) and general microorganisms (14:00, 16:00 and 18:00) (Wang C *et al.* 2018). The sum of bacteria, actinomycetes, fungi, and general microorganisms was expressed as the total biomass. Ratios of F/B and G^+/G^- were calculated to evaluate the changes of microbial community composition by fertilization (Frostegård *et al.* 2011).

2.6. Soil EEA analysis

Seven EEAs (α-glucosidase (αG), β-glucosidase (βG), β-cellobiosidase (CBH), β-xylosidase (BX), N-acetyl-glucosaminidase (NAG), phenol oxidase (PHOs), peroxidase (PerX)) were measured through a micro-plate enzyme assay (Bell *et al.* 2013). Three labelled fluorogenic substrates (4-methylumbelliferone-β-D-glucoside, 7-amino-4-methylocumarin, and 1-3,4-dihydroxyphenylalanine (L-DOPA)) were used for the determination of enzyme activities. Briefly, aggregate samples (1.0 g in dry weight) was dissolved in 50 mL Na-acetate (50 mmol L^{-1}) buffer to create soil suspension. For hydrolase (αG, βG, CBH, BX, and NAG) analysis, the buffer, sample suspension, 10 mmol L^{-1} references, and 200 μmol L^{-1} substrates were placed into the wells of a black 96-well microplate. The black 96-well microplates were incubated at 25°C for 4 h in the dark. Afterwards, to terminate the enzymatic reaction, 10 μL NaOH (1 mol L^{-1}) solution was added immediately to each well. Fluorescence was quantified by a microplate fluorometer with 365 nm excitation and 450 nm emission filters. For oxidase (PHOs and PerX) activities, the buffer, sample suspension, 25 mmol L^{-1} L-DOPA, and 0.3% (w/v) H_2O_2 were dispensed into the wells of a clear 96-well microplate. The microplates were incubated at 20°C for 20 h in the dark. Afterwards, oxidase activities were gauged by the microplate fluorometer with the absorbance at 450 nm. The units of EEAs were nmol g^{-1} soil h^{-1}.

2.7. Calculations

Soil aggregate stability Mean weight diameter (MWD; mm) was calculated to evaluate soil aggregate stability (Wang S *et al.* 2018b):

$$\mathrm{MWD}=\sum_{i=1}^{4}(Xi\times Wi)$$

where Xi is the mean diameter of aggregate fraction i (mm); and Wi is relative mass of aggregate fraction i (%).

The geometric mean of EEAs The geometric mean of

the assayed enzyme activities (Gmea), hydrolase (GH) and oxidase (GOR) were calculated as (Wang H *et al.* 2017):

$$Gmea=\sqrt[7]{\alpha G\times\beta G\times CBH\times BX\times NAG\times PHOs\times PeroX}$$

$$GH=\sqrt[5]{\alpha G\times\beta G\times CBH\times BX\times NAG}$$

$$GOR=\sqrt[2]{PHOs\times PeroX}$$

where αG, βG, CBH, BX, NAG, PHOs, and PeroX are α-glucosidase, β-glucosidase, β-cellobiosidase, β-xylosidase, N-acetyl-glucosaminidase, phenol oxidase, and peroxidase, respectively.

Calcuations of soil bacterial stress indices The ratio of saturated to monounsaturated PLFAs (sat/mono) and cyclopropyl to precursor PLFAs (cy/pre) were used as two indices to estimate the bacterial stress in soils, which were calculated as follows (Moeskops *et al.* 2012; Yu *et al.* 2018):

$$sat/mono=\frac{(14:00+15:00+16:00+17:00+18:00)}{(16:1\omega5c+16:1\omega7c+18:1\omega7c+18:1\omega9c+18:2\omega6c)}$$

$$cy/pre=\frac{cy19:0}{18:1\omega7c}$$

2.8. Statistical analyses

One-way analysis of variance (ANOVA) with Duncan tests was applied to evaluate the significance (P<0.05) of physicochemical and microbial attributes (e.g., microbial FAME and EEAs) using the SPSS 16.0 Software (SPSS Inc. Chicago, IL, USA). Permutational multivariate analysis of variance (PERMANOVA) and two-way ANOVA based on 48 soil samples (4 fertilizer treatments×4 aggregate fractions×3 replicates) were performed to evaluate the effects of fertilization, aggregate fractions and their interactions on the obtained parameters (e.g., microbial community, EEAs, and physicochemical properties) using PRIMER-E (PRIMER-E, Plymouth, UK) and SPSS 16.0 Software. Pearson's correlation and linear regression analysis were conducted to investigate the relationships between physicochemical and microbial characteristics using SPSS 16.0. By using CANOCO 4.5 Software, principal component analysis (PCA) and redundancy analysis (RDA) were used to investigate the shifts in microbial community among different fertilization patterns and explore the correlations between microbial community and physicochemical properties, respectively.

3. Results

3.1. Vegetable yields

The total yields of autumn-winter celery and spring tomato from 2009 to 2017 were shown in Fig. 1. The total yield of celery (2009–2017; 9 seasons) was the highest for the MS treatment (1 082.8 t ha^{-1}), decreasing in the order MS>S (1 071.7 t ha^{-1})>M (1 020.7 t ha^{-1})>CF (986.2 t ha^{-1}). Similarly, the total yield of tomato (2010–2016, 7 seasons) was the highest in straw-amended treatments (S and MS, 724.9 and 719.0 t ha^{-1}, respectively), followed by M treatment (671.7 t ha^{-1}) and the lowest in CF treatment (623.7 t ha^{-1}).

3.2. Distribution and stability of soil aggregates

The soil aggregates in the surface soil layer (0–20 cm) were found to be dominated by >0.25 mm aggregates (74.7–82.8%), followed by 0.25–0.053 and <0.053 mm aggregates (9.1–11.4 and 7.9–13.9%, respectively) (Table 2). Compared with CF treatment, organic amendments (M, MS, and S) significantly (P<0.05) increased the mass proportions of >2 mm aggregates by 34.1–78.4%, decreased the mass proportions of <0.053 mm aggregates by 21.2–43.3%, but had no significant effect on the mass proportions of 0.25–0.053 mm aggregates (Table 2). Moreover, the values of MWD, varied from 1.99 to 2.97 mm, were higher in ORs-amended soils by 22.1–48.9% than those in CF-treated soils. BD values were the highest in CF-treated soils (1.32 g cm^{-3}), intermediate in manure-amended soils (M and MS, 1.24 g cm^{-3}), and the lowest in straw-amended

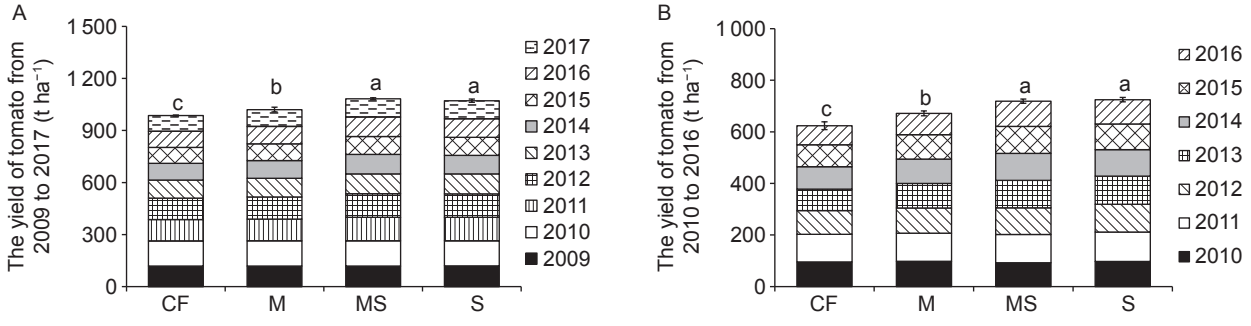

Fig. 1 Effects of different fertilization treatments on the total yields (t ha^{-1}) of autumn–winter celery (A) and spring tomato (B) from 2009 to 2017. CF, 100% N fertilizer; M, 50% N fertilizer plus 50% manure; MS, 50% N fertilizer plus 25% manure and 25% straw; S, 50% N fertilizer plus 50% straw. Different lowercase letters donated significant differences (P<0.05) among different fertilization treatments. Error bars indicate SE.

soils (S, 1.14 g cm^{-3}).

3.3. Carbon and nutrient concentrations within soil aggregates

In the >0.053 mm (i.e., >2, 2–0.25, and 0.25–0.053 mm) aggregates, the contents of SOC and available N (i.e., NO_3^--N and NH_4^+-N) increased with increasing C addition rate (CF<M<MS<S; Table 1), whereas AP contents were the highest for manure-amended soils (MS and S), and decreased in the order M≈MS>S≥CF (Table 3). However, in the <0.053 mm aggregates, the contents of NO_3^--N, NH_4^+-N, and AP were 6.0–8.9, 26.1–38.2, and 160.3–180.4 mg kg^{-1}, respectively, and did not differ significantly across different fertilization patterns. In contrast to these parameters (SOC and available N), the AK contents in all aggregates showed the following trend, with the order S<MS<M<CF.

In all fertilization treatments, 0.25–0.053 mm aggregates had higher contents of SOC, available N (NH_4^+-N and NO_3^--N), and AP, but lower AK contents than other aggregates (>2, 2–0.25, and <0.053 mm) (Table 3). Moreover, aggregate-associated pH declined with decrease in aggregate size, whereas aggregate-associated EC values were the highest for >2 mm aggregates and decreased in the order (>2 mm)>(<0.053 mm)>(0.25–0.053 mm)>(2–0.25 mm) (Appendix C).

3.4. Microbial community within soil aggregates

The microbial community composition at the aggregate scale was measured by PLFA analysis. In the >0.053 mm aggregates, the contents of total microbes (Fig. 2-A)

Table 2 Effects of different fertilization treatments on soil aggregate distribution, stability, and bulk density

Treatment[1)]	Distribution of aggregate fractions (%)				Mean weight diameter (mm)	Bulk density (g cm^{-3})
	>2 mm	2–0.25 mm	0.25–0.053 mm	<0.053 mm		
CF	29.2±0.9 d	45.4±1.4 a	11.4±0.2 a	13.9±0.3 a	1.99±0.03 d	1.32±0.04 a
M	39.2±1.6 c	40.7±2.8 b	9.1±1.2 a	11.0±0.4 b	2.43±0.05 c	1.24±0.04 b
MS	45.2±4.2 b	35.8±1.8 c	9.5±1.6 a	9.6±1.2 bc	2.68±0.19 b	1.14±0.06 c
S	52.1±2.6 a	30.7±0.4 d	9.3±1.5 a	7.9±1.4 c	2.97±0.13 a	1.14±0.03 c

[1)] CF, 100% N fertilizer; M, 50% N fertilizer plus 50% manure; MS, 50% N fertilizer plus 25% manure and 25% straw; S, 50% N fertilizer plus 50% straw.
Data are mean±SE. Different letters indicate significant differences (P<0.05) among different fertilization treatments.

Table 3 Effects of different fertilization treatments on soil aggregate-associated chemical characteristics

Soil variable	Treatment[1)]	Aggregate size			
		>2 mm	2–0.25 mm	0.25–0.053 mm	<0.053 mm
Soil organic C	CF	14.2±0.4 Bd	16.6±0.3 Ac	17.5±1.2 Ac	11.0±0.4 Cc
(g kg^{-1})	M	19.0±0.6 Cc	21.5±0.6 Bb	24.7±1.6 Ab	13.3±0.4 Db
	MS	21.9±0.5 Bb	24.8±0.9 Aa	27.0±2.3 Aab	15.0±0.3 Ca
	S	23.0±0.5 Ca	26.1±1.2 Ba	28.5±0.8 Aa	15.2±0.9 Da
NH_4^+-N	CF	4.5±0.2 Ac	5.5±0.6 Ab	6.3±1.4 Ab	6.0±1.3 Aa
(mg kg^{-1})	M	5.8±0.2 Bb	6.7±0.5 Ba	9.8±0.5 Aa	6.3±1.8 Ba
	MS	6.6±0.7 Cab	7.4±0.4 Ba	8.9±0.0 Aa	8.3±0.8 ABa
	S	8.0±1.4 ABa	7.4±0.4 Ba	10.3±1.0 Aa	8.9±1.9 ABa
NO_3^--N	CF	25.3±0.3 Ab	24.9±3.5 Ab	23.6±1.0 Ab	26.1±2.3 Aa
(mg kg^{-1})	M	29.2±4.2 Bb	42.1±7.3 Aa	49.3±6.7 Aa	29.3±2.9 Ba
	MS	39.6±5.4 Ab	49.0±10.1 ABa	54.2±1.7 Aa	35.1±10.9 Ba
	S	55.1±13.2 Aa	56.6±9.6 Aa	54.1±2.9 Aa	38.2±15.0 Aa
Available P	CF	159.5±11.8 Bb	172.8±14.4 ABb	185.3±9.2 Ab	160.3±8.5 Ba
(mg kg^{-1})	M	206.6±11.0 Ba	220.3±19.9 Ba	252.0±17.2 Aa	175.2±8.0 Ca
	MS	218.3±17.1 Ba	222.4±9.5 ABa	245.4±12.7 Aa	180.4±10.2 Ca
	S	201.6±8.2 Aa	195.6±13.3 Aab	187.6±17.6 ABb	161.4±16.6 Ba
Available K	CF	593.1±66.0 Aa	528.8±18.2 Aa	435.0±18.2 Ba	537.3±18.8 Aa
(mg kg^{-1})	M	540.2±46.9 Aab	496.9±14.6 Ab	415.4±8.0 Ba	513.6±1.8 Aab
	MS	485.9±28.0 Ab	491.1±2.2 Ab	418.1±7.0 Ba	495.8±11.8 Abc
	S	469.8±50.5 Ab	482.8±14.6 Ab	378.6±24.4 Bb	470.1±19.5 Ac

[1)] CF, 100% N fertilizer; M, 50% N fertilizer plus 50% manure; MS, 50% N fertilizer plus 25% manure and 25% straw; S, 50% N fertilizer plus 50% straw.
Data are mean±SE. Capital letters indicate significant differences (P<0.05) among different aggregate fractions within the same fertilization treatment, while lowercase letters indicate significant differences (P<0.05) among different fertilization treatments within the same aggregate fraction.

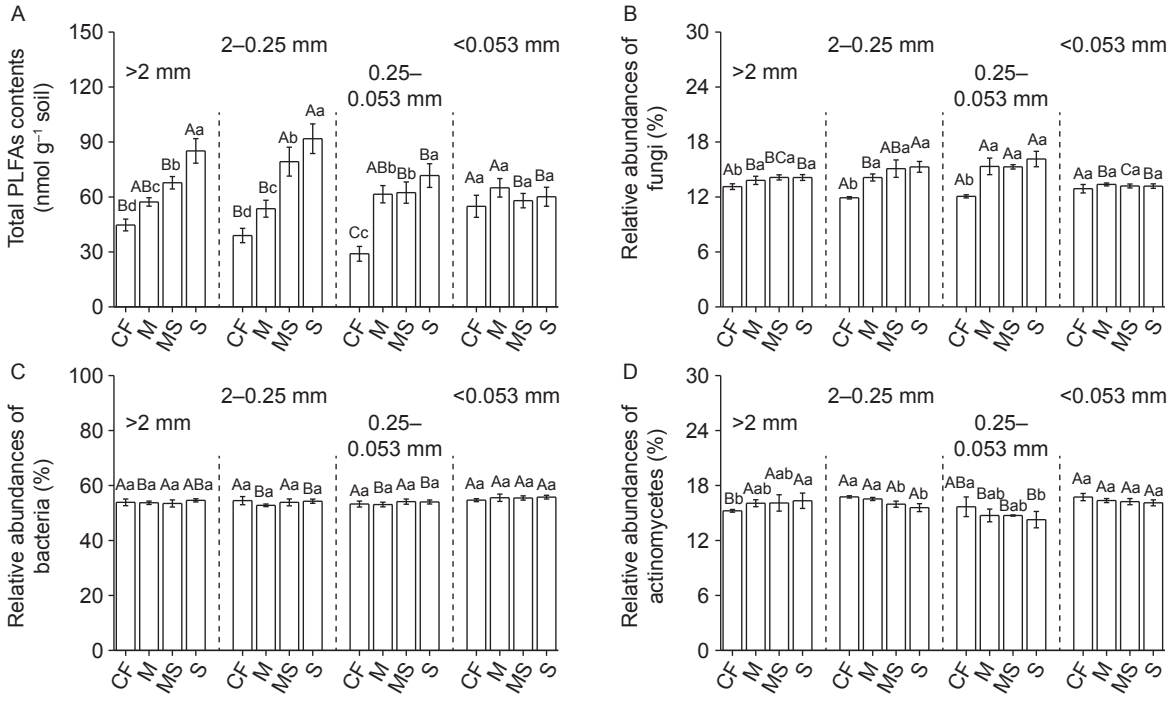

Fig. 2 Effects of different fertilization treatments on the contents of total PLFAs (A) and relative abundances of microbial subgroups of PLFAs (fungi (B); bacteria (C) and actinomycetes (D)) within soil aggregates. CF, 100% N fertilizer; M, 50% N fertilizer plus 50% manure; MS, 50% N fertilizer plus 25% manure and 25% straw; S, 50% N fertilizer plus 50% straw. Capital letters indicate significant differences among different aggregate fractions, while lowercase letters indicate significant differences among different treatments within the same aggregate fraction at the P<0.05 level. Error bars indicate SE.

and microbial subgroups (e.g., fungi, bacteria, and actinomycetes; Appendix D) showed similar trends among different fertilization patterns as follows: CF<M<MS<S. Additionally, no significant changes in these parameters were observed between organic amendments and CF treatment in the <0.053 mm aggregates (Appendix D). Compared with the CF treatment, organic amendments significantly increased the relative abundance of fungi in >0.053 mm (>2, 2–0.25, and 0.25–0.053 mm) aggregates by 5.2–7.7%, 18.6–28.4%, and 26.7–33.8%, respectively (Fig. 2-B), and had little effect on the relative abundance of bacteria among all aggregates (Fig. 2-C). The relative abundance of actinomycetes tended to decline with ORs application among all aggregates (except for >2 mm aggregates) (Fig. 2-D).

Total biomass of microbes was higher in the <0.053 mm aggregates under CF treatment, but higher in the >2 and 2–0.25 mm aggregates under straw-amended treatment (Fig. 2-A). Similarly, the changes in the contents of fungi, bacteria, and actinomycetes across different aggregates showed similar distribution patterns to those of total microbes (Appendix D). Regardless of fertilization treatments, the relative abundance of fungi was the highest and of actinomycetes was the lowest in the 0.25–0.053 mm aggregates, whereas the relative abundance of bacteria was the highest in the <0.053 mm aggregates (Fig. 2-B–D). Besides, the relative abundance of G^+ declined with decrease in aggregate size, whereas the relative abundance of G^- showed the opposite tendency with decreasing aggregate size (Appendix E). PERMANOVA results (Fig. 3) showed that fertilization (35.8%**; P<0.01) exhibited more pronounced effects on microbial community composition

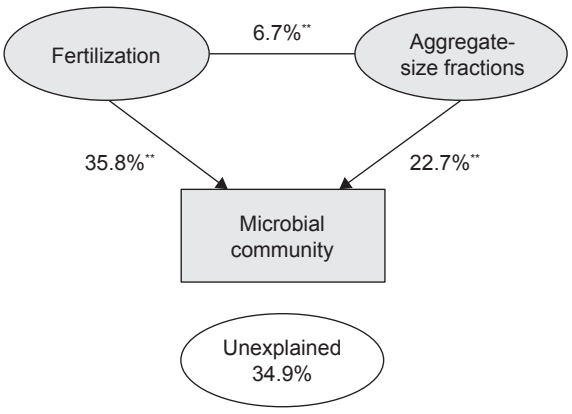

Fig. 3 The permutational multivariate analysis of variance values revealing the percent that fertilization and aggregate fractions contributed to the variation in microbial community. **, P<0.01.

than aggregate fractions (22.7%**; P<0.01).

Principal component analysis (PCA) revealed that organic amendments altered the profiles of microbial communities within the four aggregates (Fig. 4). The PLFA profiles of organic amendments had a distinct boundary with the CF treatment in the larger aggregates, but not in the <0.053 mm aggregates (Fig. 4). In addition, the PLFA profiles in the <0.053 mm aggregates were distinctly separated from the other aggregates within all fertilization treatments (Appendix F).

The changes in microbial community composition within the four aggregates were driven by organic amendment-induced changes in aggregate-associated basic properties: the eight selected soil factors (SOC, NO_3^--N, NH_4^+-N, AP, AK, pH, EC, and C/N) together accounted for >68.9, 68.6, 72.9, and 58.5%, of the total variation in PLFAs within >2, 2–0.25, 0.25–0.053, and <0.053 mm aggregates, respectively (Fig. 5). The results of redundancy analysis

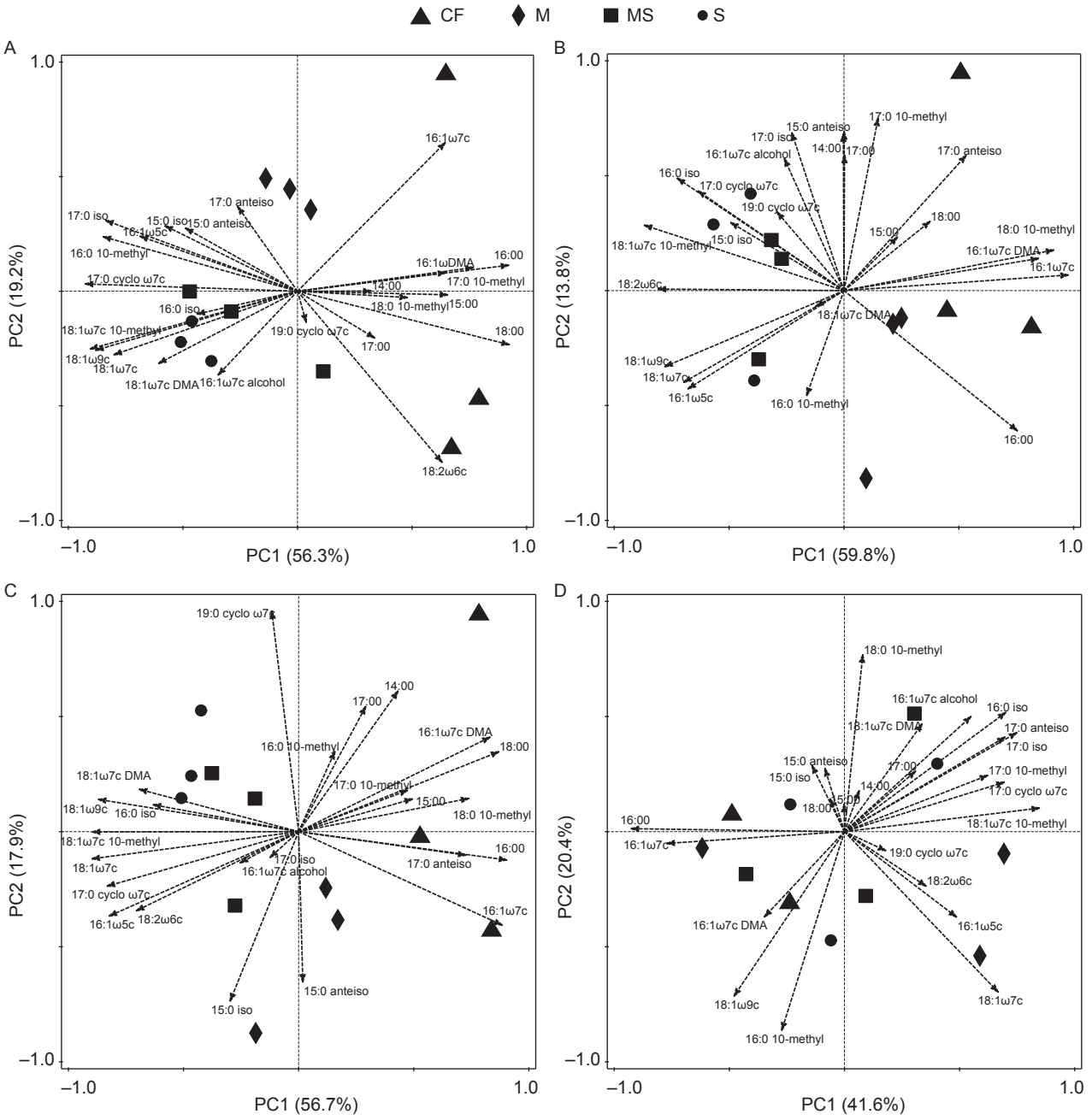

Fig. 4 Principal component analysis (PCA) of PLFAs across different fertilization treatments within aggregate fractions (>2 mm aggregates (A), 2–0.25 mm aggregates (B), 0.25–0.053 mm aggregates (D), <0.053 mm aggregates (D)).

(RDA; Fig. 5-A and B) showed that SOC content (50.8%, *P*=0.004 and 52.3%, *P*=0.002) was the most important contributor to variation in microbial community composition within >2 and 2–0.25 mm aggregates. In 0.25–0.053 mm aggregates, available N (NO_3^--N, 51.3%, *P*=0.002 and NH_4^+-N, 9.0%, *P*=0.016) and EC (14.8%, *P*=0.006) had significant influences on microbial community composition (Fig. 5-C). Meanwhile, AP content (22.1%, *P*=0.024) was the main driver for regulating microbial community composition within <0.053 mm aggregates (Fig. 5-D).

3.5. Microbial community structure within soil aggregates

The ratios of F/B and G^+/G^- as indices of microbial community structure were affected by fertilization and aggregate fractions (Fig. 6-A and 6-B). The F/B ratio was markedly higher under organic amendments compared with those under CF treatment in aggregates (except for <0.053 mm aggregates) (Fig. 6-A). No significant changes in the G^+/G^- ratio were observed among different fertilization

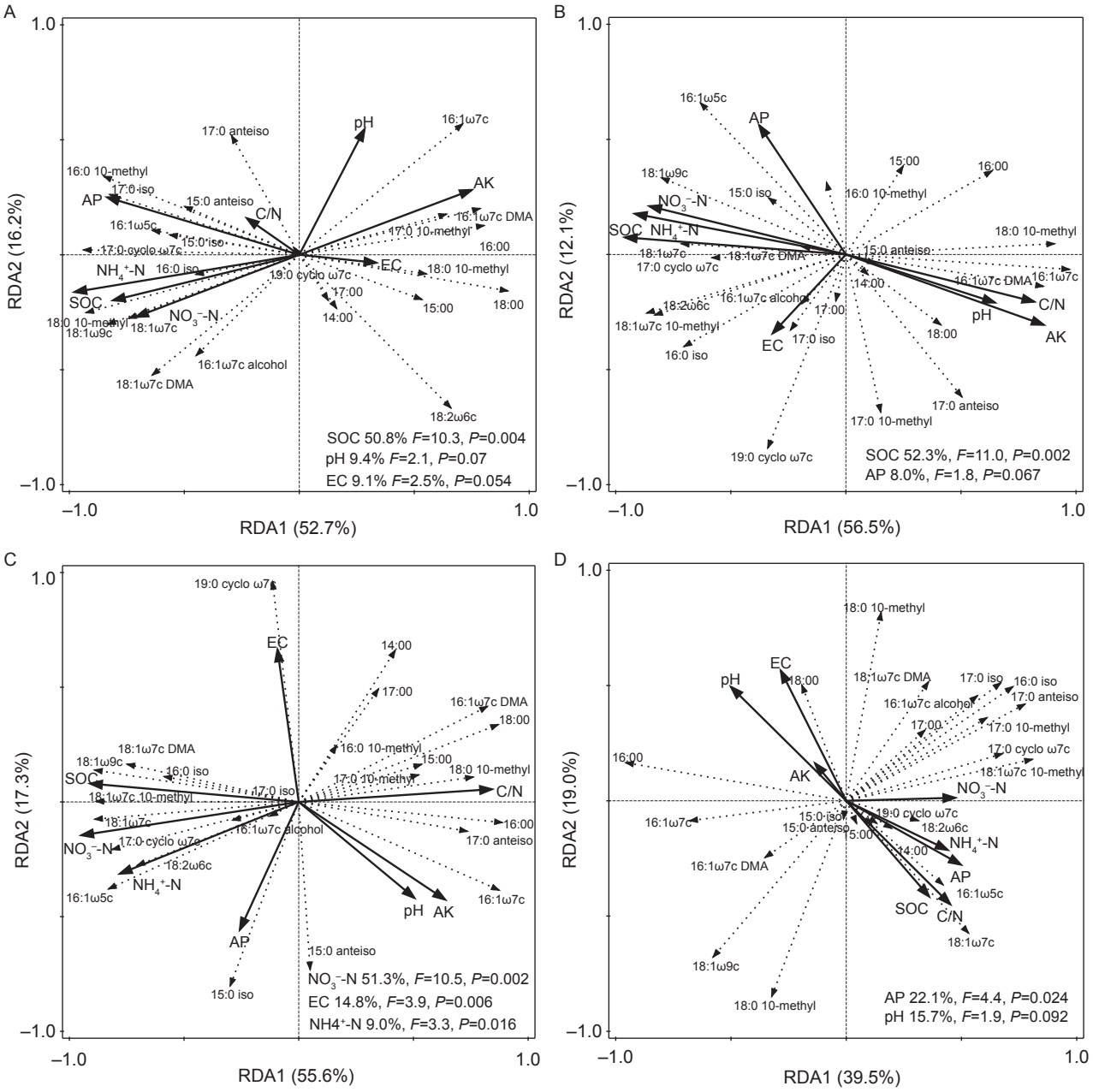

Fig. 5 Redundancy analysis (RDA) of PLFAs constrained by soil physicochemical properties within aggregate fractions (>2 mm aggregates (A), 2–0.25 mm aggregates (B), 0.25–0.053 mm aggregates (C) and <0.053 mm aggregates (D)) under different fertilization treatments.

patterns within all aggregates (Fig. 6-B). Regardless of fertilization treatments, the F/B ratio was the highest in the 0.25–0.053 mm aggregates and the lowest in the <0.053 mm aggregates, whereas the G^+/G^- ratio declined with decrease in aggregate size.

The values of cy/pre and sat/mono were lower in ORs-amended soils compared with those in CF-treated soils in the >0.053 mm aggregates, but not in the <0.053 mm aggregates (Fig. 7). Moreover, these stress indices increased with decrease in aggregate size, regardless of fertilization treatments (Fig. 7).

3.6. Microbial functions within soil aggregates

Hydrolase (i.e., αG, βG, CBH, BX, and NAG) activities (Table 4) and GH (Fig. 8-B) in all aggregates increased as follows: CF<M<MS<S. In all fertilization patterns, these hydrolase activities were the highest within the 0.25–0.053 mm aggregates among four aggregates (Table 4). However, the changes in oxidase activities (e.g., PHOs, PerX, and GOR) with increasing C supply were less pronounced than those in hydrolytic activity. Specifically, oxidase activities (Table 4) and GOR (Fig. 8-C) were the highest for MS treatment and decreased in the order MS>S>M>CF in all aggregates. Regardless of fertilization treatments, these oxidase activities increased basically with decrease in aggregate size (Table 4). In addition, the ratios of GH/GOR differed remarkably among the four aggregates and followed the rank order (0.25–0.053 mm)>(>2 mm)≈(2–0.25 mm)>(<0.053 mm) (Fig. 8-D). Two-way ANOVA

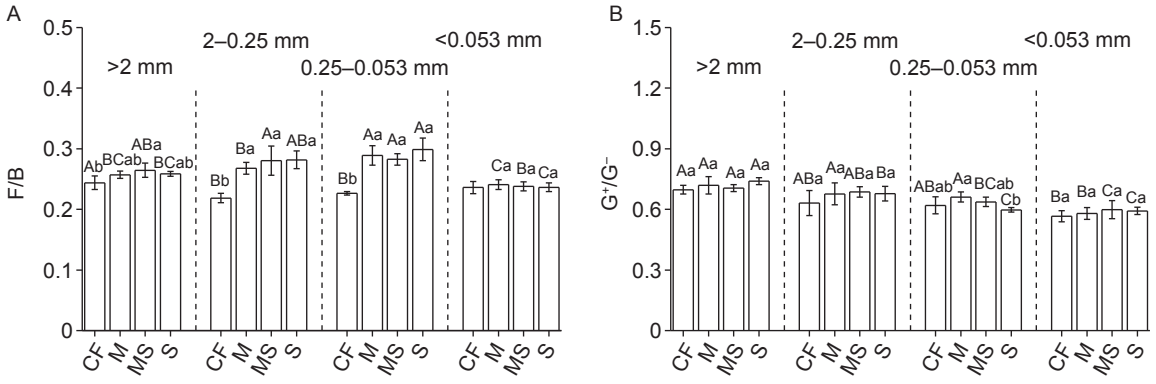

Fig. 6 Effects of different fertilization treatments on microbial associated ratios (F/B (A) and G^+/G^- (B)) within soil aggregates. CF, 100% N fertilizer; M, 50% N fertilizer plus 50% manure; MS, 50% N fertilizer plus 25% manure and 25% straw; S, 50% N fertilizer plus 50% straw; F, fungi; B, bacteria; G^+, gram-positive bacteria; G^-, gram-negative bacteria. Capital letters indicate significant differences among different aggregate fractions, while lowercase letters indicate significant differences among different treatments within the same aggregate fraction at the P<0.05 level. Error bars indicate SE.

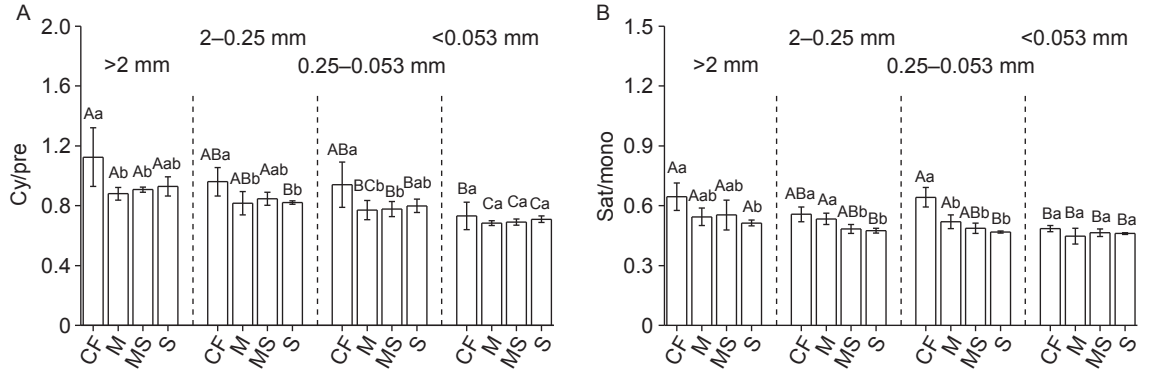

Fig. 7 Effects of different fertilization treatments on the bacterial physiological stress indices (cy/pre (A); sat/mono (B)) within soil aggregates. CF, 100% N fertilizer; M, 50% N fertilizer plus 50% manure; MS, 50% N fertilizer plus 25% manure and 25% straw; S, 50% N fertilizer plus 50% straw; sat/mono, (14:00+15:00+16:00+17:00+18:00)/(16:1ω5c+16:1ω7c+18:1ω7c+18:1ω9c+18:2ω6c); cy/pre, (cy19:0)/(18:1ω7c). Capital letters indicate significant differences among different aggregate fractions, while lowercase letters indicate significant differences among different treatments within the same aggregate fraction at the P<0.05 level. Error bars indicate SE.

Table 4 Effects of different fertilization treatments on seven extracellular enzyme activities (EEAs) within soil aggregates

EEAs (nmol g^{-1} soil h^{-1})[1)]	Treatment[2)]	Aggregate size			
		>2 mm	2–0.25 mm	0.25–0.053 mm	<0.053 mm
βG	CF	91.2±17.7 Cd	159.6±4.3 Bc	205.7±9.6 Ac	152.1±14.3 Bd
	M	178.4±21.9 Bc	195.6±13.6 Bc	298.6±16.1 Ac	207.3±19.0 Bc
	MS	289.3±27.7 Bb	365.7±27.3 Bb	631.1±134.0 Ab	251.3±14.1 Bb
	S	354.1±14.1 Ca	501.6±72.1 Ba	857.5±100.9 Aa	343.6±28.2 Ca
CBH	CF	12.3±1.9 Cd	24.8±2.0 Bd	34.9±4.9 Ad	22.0±2.4 Bc
	M	29.8±2.1 Cc	39.8±1.2 Bc	60.8±1.8 Ac	38.4±4.6 Bb
	MS	51.4±6.3 Cb	84.5±8.4 Bb	126.6±4.7 Ab	45.4±2.0 Cb
	S	72.4±6.7 Ca	119.8±11.1 Ba	197.0±13.9 Aa	80.4±8.5 Ca
NAG	CF	11.6±1.7 Cd	17.2±1.0 Bc	27.9±3.8 Ac	12.9±1.0 BCd
	M	29.7±4.1 Bc	28.6±3.2 Bc	39.4±2.2 Ac	20.6±1.8 Cc
	MS	61.2±6.9 Bb	70.2±12.5 Bb	124.9±16.1 Ab	32.9±5.4 Cb
	S	75.7±8.7 Ba	92.7±5.6 Ba	180.9±21.8 Aa	45.3±5.0 Ca
BX	CF	28.3±3.2 Cc	52.0±3.3 Bd	67.3±10.5 Ad	57.8±5.3 ABc
	M	58.7±4.7 Cb	67.0±4.5 Cc	101.8±3.9 Ac	80.9±7.4 Bb
	MS	81.5±11.6 Ca	114.7±2.5 Bb	162.3±9.5 Ab	91.8±5.9 Cb
	S	99.2±13.9 Ca	153.6±10.2 Ba	225.7±31.9 Aa	130.1±15.4 BCa
αG	CF	37.6±3.9 Cc	51.8±3.9 Bd	75.2±11.5 Ad	68.6±5.6 Ac
	M	64.9±12.2 Cb	82.4±3.8 Bc	106.3±1.7 Ac	95.3±6.1 ABb
	MS	90.6±9.6 Ca	109.9±10.0 Bb	148.5±8.7 Ab	104.9±10.9 BCb
	S	99.7±14.4 Ca	144.1±13.1 Ba	198.7±18.3 Aa	137.2±11.1 Ba
PHOs	CF	1.21±0.08 Cc	1.55±0.06 Bb	1.51±0.14 Bc	1.74±0.08 Ab
	M	1.30±0.08 Cbc	1.78±0.15 ABa	1.65±0.07 Bbc	1.85±0.09 Ab
	MS	1.78±0.16 Ba	1.87±0.09 Ba	1.99±0.09 ABa	2.12±0.11 Aa
	S	1.40±0.03 Bb	1.81±0.10 Aa	1.82±0.15 Aab	1.86±0.10 Ab
PerX	CF	1.07±0.04 Cc	1.45±0.10 Bc	1.48±0.02 ABa	1.56±0.04 Ac
	M	1.27±0.13 Bb	1.58±0.10 Abc	1.54±0.15 Aa	1.61±0.02 Abc
	MS	1.55±0.10 Ba	1.93±0.18 Ba	1.66±0.09 Ba	2.05±0.15 Aa
	S	1.29±0.07 Cb	1.71±0.09 ABab	1.53±0.13 Ba	1.80±0.15 Ab

[1)] βG, β-glucosidase; CBH, β-cellobiosidase; NAG, N-acetyl-glucosaminidase; BX, β-xylosidase; αG, α-glucosidase; PHOs, phenol oxidase; PeroX, peroxidase.
[2)] CF, 100% N fertilizer; M, 50% N fertilizer plus 50% manure; MS, 50% N fertilizer plus 25% manure and 25% straw; S, 50% N fertilizer plus 50% straw.
Data are mean±SE. Capital letters indicate significant differences (P<0.05) among different aggregate fractions within the same fertilization treatment, while lowercase letters indicate significant differences (P<0.05) among different fertilization treatments within the same aggregate fraction.

revealed that microbial functions (i.e., a series of EEAs) were strongly affected by fertilization and aggregate fractions. Hydrolase activity was mainly driven by fertilization (51.5−62.7%; P<0.01), whereas oxidase activity was preferentially impacted by aggregate fractions (45.1−47.1%; P<0.01; Appendix G).

Table 5 showed that significant positive correlations were found between hydrolase activities (βG, CBH, NAG, BX, and αG) and the contents of fungi (r=0.62**–0.66**), bacteria (r=0.50**–0.57**), and actinomycetes (r=0.40**–0.47**). Similarly, soil nutrient-related properties were closely associated with hydrolase activities (e.g., SOC, r=0.65**–0.79**; NO_3^--N, r=0.68**–0.72**) (Appendix H). Oxidase (i.e., PHOs and PerX) activities were correlated with pH (−0.62** and −0.50**) and EC (−0.43** and −0.47**), but not significantly correlated with microbial subgroups (e.g., fungi and bacteria) and nutrient-related properties (e.g., SOC and AP) (Appendix H and Table 5).

4. Discussion

4.1. Organic amendments promote soil aggregation and stability

As indicated by the MWD values, organic amendments had more positive effects on soil aggregation and aggregate stability than CF treatment (Table 2). These observations were in line with the findings of studies on the upland Ultisol of Jiangxi Province (Lin *et al.* 2019) and the brown soil of Northeast China (Xie *et al.* 2015), where manure addition promoted >0.25 mm aggregates formation and enhanced aggregate stability. The possible reason for these findings was that ORs application could increase organic binding agents of different origins (e.g., organic matter derived from ORs and microorganism exudates) (Guo *et al.* 2019), and thus promoted soil aggregation (Huang *et al.* 2010). Similarly, Yan *et al.* (2013) and Liu

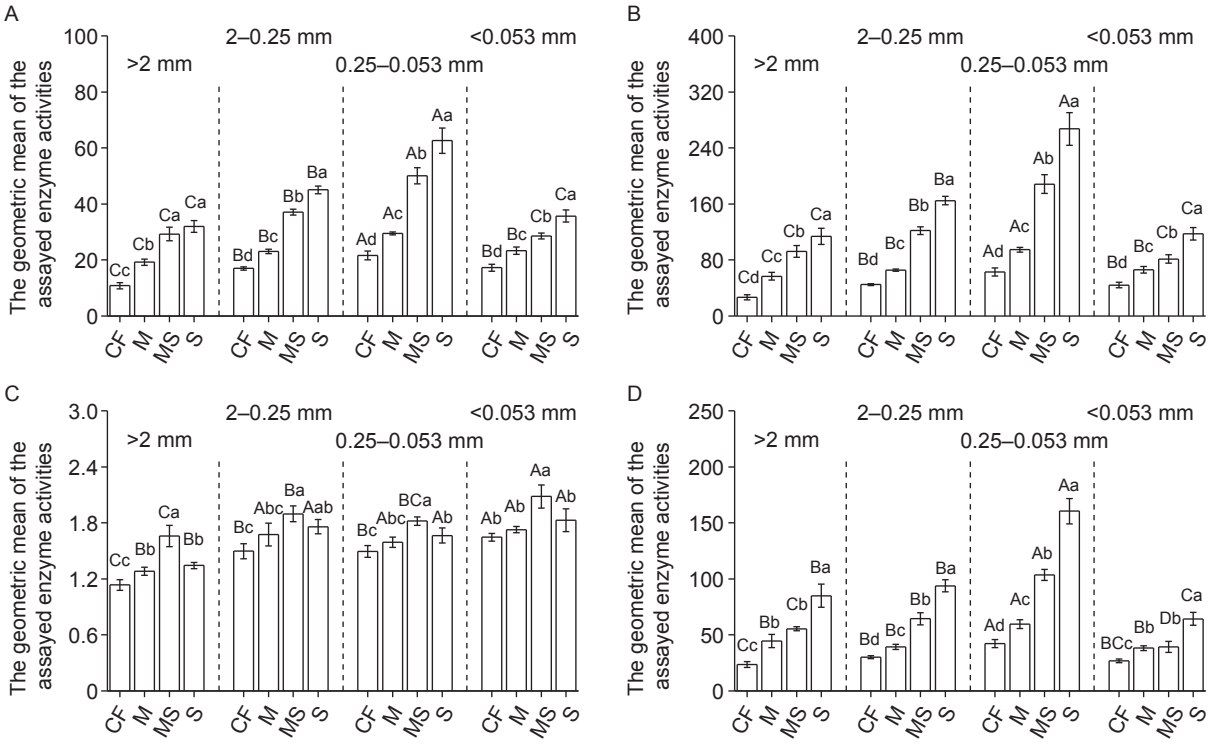

Fig. 8 Effects of different fertilization treatments on the geometric mean of the assayed enzyme activities (Gmea (A), GH (B), GOR (C) and GH/GOR (D)) within soil aggregates. CF, 100% N fertilizer; M, 50% N fertilizer plus 50% manure; MS, 50% N fertilizer plus 25% manure and 25% straw; S, 50% N fertilizer plus 50% straw. Capital letters in the top indicate significant differences among different aggregate fractions, while lowercase letters indicate significant differences among different treatments within the same aggregate fraction at the P<0.05 level. Error bars indicate SE.

Table 5 Pearson's correlation coefficients between the contents of microbial subgroups and extracellular enzyme activities (n=48)[1)]

	βG	CBH	NAG	BX	αG	PHOs	PerX
Fungi	0.62**	0.66**	0.63**	0.64**	0.64**	0.29	0.25
Bacteria	0.51**	0.55**	0.50**	0.54**	0.57**	0.30	0.25

[1)] βG, β-glucosidase; CBH, β-cellobiosidase; NAG, N-acetyl-glucosaminidase; BX, β-xylosidase; αG, α-glucosidase; PHOs, phenol oxidase; PeroX, peroxidase.
**, P<0.01.

et al. (2014) indicated that there was a positive relationship between the amounts of C inputs and soil aggregate stability in several agricultural soils. This was supported by our findings, i.e., the linear relationships between SOC contents and MWD (R^2=0.84**, Appendix I). Additionally, the intrinsic recalcitrance of ORs inputs has much effects on aggregate stability dynamics (Abiven *et al.* 2007). Abiven *et al.* (2009) reported that easily degradable ORs have a strong and transitory influence on aggregate stability, while recalcitrant ORs have a weak but long-term effect on aggregate stability. Thus, in our experimental sites, straw-amended treatments (recalcitrant ORs and high C inputs; Table 1) had more strong effects on aggregate stability than manure-amended treatment (easily degradable ORs and low C inputs).

4.2. Responses of nutrient-related properties to fertilization and aggregate fractions

Mounting evidence indicates that organic amendments promote SOC and nutrients accumulation in soil aggregates (Zhang *et al.* 2014). In the current study, organic amendments strongly increased SOC and nutrient (NO_3^--N, NH_4^+-N, and AP) contents in the >0.053 mm aggregates. This may be explained by Li *et al.* (2016), who indicated that ORs (e.g., manure) application could improve soil nutrient holding ability and incorporate large amounts of C resources into soils. Thus, although many nutrients are taken away during vegetable growth (Fig. 1), more nutrients are retained in ORs-amended soils than in CF-amended soils. Additionally, we found that organic amendments had little effect on

nutrient properties in the <0.053 mm aggregates (Table 3). This could be explained by the following two reasons. Firstly, several studies suggested that the addition of exogenous ORs are mainly preferentially preserved in >0.25 mm aggregates, and then transferred to <0.053 mm aggregates over long-term microbial metabolism (Grandy and Neff 2008; Guan *et al.* 2015). This was supported by Wang Y *et al.* (2017), who reported that ORs-derived nutrients are difficult to reach the <0.053 mm aggregates because of the priority of nutrient localization in >0.25 mm aggregates. Second, the <0.053 mm aggregates are physically protected by the mineral surface, as well as iron and aluminum oxides, which are the most stable fractions and their properties are difficult to be influenced by fertilization (Chung *et al.* 2008). Therefore, organic amendments had different effects on the nutrient-related properties within different aggregates (e.g., strong effects on >0.053 mm aggregates, and weak effects on <0.053 mm aggregates; see Table 3).

Many researchers have found that the contents of SOC and available nutrients increase with increase in aggregate size (Ayoubi *et al.* 2012; Yao *et al.* 2019). This may be explained by the findings of Six *et al.* (2004), who demonstrated that >0.25 mm aggregates are formed by 0.25–0.053 and <0.053 mm aggregates plus organic binding agents, which subsequently result in the accumulation of organic matter and nutrients in the >0.25 mm aggregates. However, in the present study, higher concentrations of SOC, available N (NO_3^--N and NH_4^+-N) and AP were observed in the 0.25–0.053 mm aggregates than that in the other aggregates (Table 3). Recent evidence suggests that 0.25–0.053 mm aggregates are physically more stable than >0.25 mm aggregates and preserve greater quantities of SOC and nutrients (Simonetti *et al.* 2017; Totsche *et al.* 2018). Similarly, Yu *et al.* (2012b) pointed out that although the microhabitats in 0.25–0.053 mm aggregates were suitable for enzymes, especially for hydrolase (Table 4), the enzymes were not physiologically capable of degrading organic C because of their micropore- to nanopore-dominated structure as well as the tortuosity of the pore network. These studies could explain the present results that the contents of SOC, available N, and P were higher in the 0.25–0.053 mm aggregates than in other aggregates.

Moreover, the AK contents were consistently lower in the 0.25–0.053 mm aggregates than in other aggregates (Table 3), which were different from the distribution patterns of SOC, available N (NO_3^--N and NH_4^+-N), and P among aggregates in our study. These discrepancies may be primarily explained by the following two possible reasons. Firstly, the properties of K are different from those of other nutrients, such as SOC (Arienzo *et al.* 2009). Liu *et al.* (2019) indicated that K mostly exists in soils as ion forms, which are hardly protected by soil aggregates; however, other nutrients, e.g., SOC, can exist in organic forms in soils and are protected at different levels within different size aggregates (Totsche *et al.* 2018). Secondly, Liao *et al.* (2013) reported that K absorption capacity in soils was largely controlled by SOC contents. Specifically, SOC had positive effects on K absorption at low K level (<120 mg kg^{-1}) in soils; inversely, SOC would prohibit K absorption when soils at high K level (>160 mg kg^{-1}). In the current study, under the premise of high AK content (469.8−593.1 mg kg^{-1}) in soils, the high SOC contents in 0.25–0.053 mm aggregates were not beneficial for K absorption and reduced AK contents. These studies may explain the present results (Table 3) and suggest that 0.25–0.053 mm aggregates can be regarded as a suitable microenvironment for the preservation of organic C and nutrients (except for AK).

4.3. Responses of microbial communities to fertilization and aggregate fractions

Results showed that organic amendments strongly increased total microbial biomass (total PLFAs) and most microbial subgroups within aggregates (except for <0.053 mm aggregates) (Fig. 2 and Appendix D). These findings were supported by Wang Y *et al.* (2017), who observed that manure application over 23 years mainly promoted microbial (e.g., fungal and bacterial) growth in large aggregate-size fractions (> 0.25 mm). These impacts were mainly attributed to the adequate supply of C and nutrient resources by ORs application (Appendix J; Ma *et al.* 2016). Similar results were also reported by Liu *et al.* (2009), who found that C and nutrients from ORs (e.g., manure or straw) application play a vital role in stimulating agricultural soil microbial growth. In addition, ORs addition was beneficial for vegetable growth (Fig. 1), thereby providing active C resources (e.g., roots and their secretions) that are easily utilized for microorganisms. Meanwhile, at the aggregate scale, straw-amended treatments have stronger positive effects on microbial growth than manure-amended treatment (Fig. 2). In the present study, the large amounts of C input from straw was more useful than manure in improving soil structure and nutrient conditions for microbial growth (Tables 3 and 4), which were consistent with several studies (Abiven *et al.* 2009; Bei *et al.* 2018). These findings could be the reason for the present results (see Fig. 2 and Appendix D) that different fertilization patterns had different effects on microbial (fungal and bacterial) biomass within aggregates.

Organic fertilizer application may also result in some microbial subgroups becoming competitively dominant while restraining other microbial subgroups (Wang Y *et al.* 2017). In this study, organic amendments increased the ratio of F/B in the >0.053 mm aggregates, but not in the

<0.053 mm aggregates (Fig. 6). These changes indicate that fungi adapt better to ORs application over 8 years than bacteria. Lazcano *et al.* (2013) observed that the utilization of exogenous ORs by microbes has a community succession effect: fast-growing microbes (e.g., bacteria) proliferate soon after ORs addition, and subsequently, their population size decreases, which promotes the growth of other microorganisms that grow more slowly, such as fungi. Zhou *et al.* (2016) indicated that long-term (21 years) organic fertilizer application could create more intra- and inter-aggregate pores, which are favorable for fungal growth (Simonetti *et al.* 2017). These results may explain the increase in the F/B ratio in ORs-amended soils in the present investigation.

The microbial community is unevenly distributed among different aggregates among various ecosystems (Wang S *et al.* 2018a). Murugan *et al.* (2019) indicated that microbial biomass declines with decrease in aggregate size, whereas Zhang *et al.* (2013) reported an inverse relationship between microbial biomass and aggregate size. These variations in microbial biomass among different aggregates are generally considered to be associated with aggregate-associated physicochemical properties (Zhang *et al.* 2016). In our study, different distribution patterns involved in microbial biomass were found in four fertilization treatments, where microbial (e.g., fungal and bacterial) biomass was enriched in the <0.053 mm aggregates under CF-amended soils, and enriched in the >0.25 mm aggregates under ORs-amended soils (Fig. 2 and Appendix D). The present observations are supported by Zhang *et al.* (2014), who indicated that microbial biomass C is enriched in the >0.25 mm aggregates under manure-amended soils, and that this difference may be ascribed to manure increasing the availability of C and nutrients (Li J *et al.* 2015) and creating suitable pore characteristics (Wang Y *et al.* 2017) for microbial growth within the >0.25 mm aggregates. Bach *et al.* (2010) indicated that <0.053 mm aggregates could protect soil microbes from predation, due to its low porosity. This may be responsible for high microbial biomass in the <0.053 mm aggregates on the premise of no ORs inputs (i.e., CF treatment).

Interestingly, we observed that the F/B ratio was higher in 0.25–0.053 mm aggregates and lower in <0.053 mm aggregates (except for the CF treatment) among soil aggregates (Fig. 6). This result may be explained by the results of Kong *et al.* (2011), which indicated that the 0.25–0.053 mm aggregates provide a relatively unique micro-environment (e.g., a suitable pore size, low pH values (Appendix C)) for fungal growth (Rousk *et al.* 2010; Simonetti *et al.* 2017), whereas the <0.053 mm aggregates are not suitable for fungal growth due to its low porosity (Chen *et al.* 2014).

Lower values for cy/pre and sat/mono ratios were observed in ORs-amended treatments than in CF treatment among different aggregates (Fig. 7). Lower values for these ratios were associated with increased nutrient turnover, increased bacterial growth rates, and reduced SOC limitation (Fierer *et al.* 2003; Yu *et al.* 2018). These studies indicate that organic amendments may increase nutrient turnover (i.e., increase nutrient availability; Table 3), reduce SOC limitation (i.e., increase SOC contents; Table 3), and then promote bacterial growth (Appendix D) within different aggregates. Moreover, the ratios of cy/pre and sat/mono decline with decrease in aggregate size, suggesting that nutrient availability and C resources were less limiting to the bacterial population in the <0.053 mm aggregates than in other aggregates.

In the present study, we found that SOC (P=0.002), available N (NO_3^--N and NH_4^+-N; P=0.002 and P=0.016), and AP (P=0.024) contents had the significant influence on microbial community composition within >0.25, 0.25–0.053, and <0.053 mm aggregates, respectively. The current results may be partially explained by the findings of Regelink *et al.* (2015), who demonstrated that soil aggregates can provide spatially heterogeneous microhabitats for microorganisms because the different aggregates create microhabitats that differ in resource availability, soil aeration, and pore spaces. Consequently, this specific and heterogeneous microclimate within aggregates may be that the main factors for regulating the distribution of microbes among different aggregate fractions in our study.

4.4. Responses of EEAs to fertilization and aggregate fractions

Extracellular enzyme production by microorganisms, which is closely related to SOC and nutrient cycling (Nie *et al.* 2014). Our findings confirmed that organic amendments strongly enhanced hydrolase activities among the four aggregates (Table 4 and Fig. 8). Similar to these findings, Wang Y *et al.* (2017) observed that manure addition over 23 years increased a series of hydrolase activities in soils under a rice–barley rotation. The changes in organic C and nutrient contents in soils induced by ORs addition may be partially responsible for this observation (Banerjee *et al.* 2016; Appendix H). Zhang *et al.* (2016) also confirmed that the variation in hydrolase activities could be explained by C and nutrient availability, which are recognized to be greatly affected by ORs application. Moreover, ORs inputs may trigger hydrolases production *via* microbial activation (Table 5; Schnecker *et al.* 2015), which may be another reason for the enhanced hydrolase activities observed in this study. Meanwhile, the positive effects on oxidase activities (Table 4) induced by organic amendments are

weaker than that of hydrolase activities among aggregates. We suggest one possible explanation for these results in this study. Oxidase activities were mainly affected by soil pH (Keeler *et al.* 2009; Sinsabaugh 2010), rather than nutrient properties, whereas the changes in soil pH induced by organic amendments are small in the present study. Furthermore, several studies reported that fungi are the major producer of oxidases (Burns *et al.* 2013), and its growth is inhibited in alkaline soils (Rousk *et al.* 2010), which suggested that fungal growth was inhibited in CF-treated soils (high pH level; Appendix C) and subsequently reduce oxidase expression.

Based on several studies, aggregate size has been found to affect EEAs; however, research results have been inconsistent in recent years, with positive (Kim *et al.* 2015), negative (Nie *et al.* 2014) and neutral effects (Awad *et al.* 2018) on EEAs being reported. However, in our study, hydrolase activities were generally higher in 0.25–0.053 mm aggregates than in other aggregates (Table 4). Liu *et al.* (2013) indicated that the distribution of hydrolase activities among aggregates was mainly regulated by C and nutrient resources. Banerjee *et al.* (2016) also observed that hydrolase activities were the greatest in C-rich aggregates. Thus, the 0.25–0.053 mm aggregates with high levels of C and nutrients (Table 3) exhibit high hydrolase activities among different aggregates, and suggest that the 0.25–0.053 mm aggregates provide a relatively unique microenvironment for hydrolase activity. Additionally, several researchers reported that the smaller aggregates, especially <0.053 mm aggregates, have the highest activities of oxidase among different aggregates (Allison and Jastrow 2006; Sinsabaugh 2010). These studies are consistent with our observation that oxidase activities decreased with increase in aggregate size (Table 4). Oxidases (e.g., PHOs) are produced primarily by fungi and their activities are sensitive to agricultural management practices as well as soil properties (Sinsabaugh 2010; Burns *et al.* 2013). For example, low organic C level in soils creates positive feedback on oxidase activity (Li N *et al.* 2015), whereas N addition or high N availability in soils inhibits oxidase activities or expression by fungi (Sinsabaugh 2010; Jian *et al.* 2016). Thus, in the present study, oxidase activities were higher in the <0.053 mm aggregates owing to the low SOC and available N level (Table 3).

4.5. Organic amendments increase vegetable yields over 8 years

Our results showed that organic amendments improved the 8-year total vegetable (celery and tomato) yields compared to CF treatment (Fig. 1), in agreement with several studies conducted in cropland (e.g., maize and wheat) and vegetable fields (Rong *et al.* 2018; Li *et al.* 2019). These results could be explained by the following two possible reasons. First, ORs inputs provide plenty of C and nutrient resources into soils and improve soil nutrient conditions, which is beneficial for improving crop (e.g., vegetable) yields (Table 3; Muhammad *et al.* 2020). Meanwhile, Agegnehu *et al.* (2016) indicated that organic amendments can improve soil physical quality (e.g., soil porosity and aggregate stability; Table 2), which provide a suitable microenvironment for root growth and development, finally in turn promote crop growth and increase crop yields. Second, as important ORs, manure and straw could provide C resource for microbial growth and activity in soils, which in turn improved soil nutrient status and consequently the vegetable yield (Zhang *et al.* 2019). Tautges *et al.* (2016) also found that microbial utilization of ORs may have promoted ORs-derived C and nutrient cycling, activating C and nutrient availability, then became available for vegetable growth. Taken together, these studies showed that organic amendments over 8 years could enhance the yields of vegetables (i.e., celery and tomato) through promoting microbial growth and activity, improving soil structure and nutrient conditions, which was supported by evidence from Appendix K. Meanwhile, several studies indicated that these features (e.g., crop growth, soil nutrient conditions, microbial growth and activity, etc.) influence each other, e.g., crop growth, especially root growth, promoted microbial metabolism and nutrient activation in soils (Tautges *et al.* 2016; Jing *et al.* 2019). Therefore, we should notice that the effects of fertilization on soil physical, chemical, and microbial characteristics, as well as crop yields in the soil–crop system are complicated, which were influenced by multi-factors. Moreover, based on several studies (Liu *et al.* 2018, 2019), together with our previous study (Luan *et al.* 2019), we speculated that soil physical, chemical and microbial characteristics (e.g., SOC, BD, etc.) improved gradually with an increase in the years of organic fertilization; and then these characteristics (e.g., SOC) would reach relative constant levels after several years.

5. Conclusion

After 8-year of ORs (organic manure and/or corn straw) application in a GVP system in Tianjin, China, we observed significant changes in soil aggregate distribution and aggregate-associated physicochemical and microbial properties. Firstly, we confirmed that ORs application over 8 years improved vegetable yields and aggregate stability; meanwhile, ORs application had stronger effects on the nutrient and microbial characteristics in the >0.053 mm aggregates than those in the <0.053 mm aggregates. Secondly, across aggregates, the 0.25–0.053 mm

aggregates provide suitable microsites for conserving nutrients and hydrolytic activity; meanwhile, the microsites of <0.053 mm aggregates was beneficial for oxidative activity. Thirdly, we found that SOC, available N, and available P contents had significant influences on microbial community composition within the >0.25, 0.25–0.053, and <0.053 mm aggregates, respectively. Finally, by combining aggregate-associated nutrient and microbial properties and vegetable yields, we recommend 2/4CN+1/4MN+1/4SN is continuous high-yield fertilization patterns in GVPs. These findings will help us enhance our understanding of the factors and mechanisms responsible for driving microbial characteristics at the aggregate scale under different fertilization patterns in GVP systems.

Acknowledgements

The authors sincerely acknowledge the financial support provided by the earmarked fund for China Agriculture Research System (CARS-23-B02), the National Key Research and Development Program of China (2016YFD0201001), and the Key Research and Development Program of Shandong Province, China (2017CXGC0206).

Appendices associated with this paper can be available on http://www.ChinaAgriSci.com/V2/En/appendix.htm

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