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Organic fertilization promotes crop productivity through changes in soil aggregation

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ABSTRACT

Soil aggregates are the key functional units of soil ecosystems which are essential to biogeochemical cycling and plant growth. However, it remains unclear how fertilization regimes influence soil aggregation, associated resources, and microbial distribution among aggregates, as well as the potential subsequent impacts for other abiotic and biotic processes. For this, a long-term maize cropping field experiment was conducted in the subtropical region of China under four fertilization treatments: no fertilizer, chemical fertilization, organic fertilization, and chemical plus organic fertilization. Additionally, we classified soil aggregates into large macro-aggregates (>2 mm), small macro-aggregates (0.25–2 mm), and micro-aggregates (<0.25 mm) and compared soil nutrients, enzyme activities, and microbial communities of each aggregate fraction and bulk soil as well as crop productivity and plant carbon (C), nitrogen (N), and phosphorus (P). Results showed that long-term organic fertilization (1) increased soil C, N, and P contents within macro-aggregates, while increased bacterial and fungal biomass within all three aggregate size fractions, (2) increased N-acquiring enzyme activity, C:P, and N:P enzyme ratios but decreased phosphatase activity and C:N enzyme ratio regardless of aggregate fractions, and (3) promoted crop productivity but decreased plant C:N, C:P, and N:P ratios compared with chemical fertilization. Additionally, the fungi:bacteria ratio and phosphatase activity decreased, but the gram-positive to gram-negative bacterial ratio, C-acquiring enzyme activity, C:N and C:P enzyme ratios increased with decreasing aggregate size. The partial least squares models confirmed that macro-aggregates had strong effects on crop performance while micro-aggregates was the main determinant of microbial community. Taken together, long-term organic fertilization promotes soil functioning and crop productivity via increasing the proportion of soil macro-aggregates.

1. Introduction

Soil aggregates as functional units are important determinants of biotic and abiotic interactions in soil ecosystems (Amézketa, 1999; Rabot et al., 2018). Different sized aggregates show distinct properties due to specific aeration, moisture retention, organic matter content, and niche availability. For example, stabilized organic matter (e.g., mineral-associated organic carbon, recalcitrant plant or microbial by-products) is generally less accessible for biological degradation (Lützow et al., 2006) and is more stable in soil particles <0.25 mm (e.g., micro-aggregates, silt, and clay fraction) (Six and Paustian, 2014). By contrast, labile organic materials such as fresh litter, fine roots and fungal hyphae are more abundant in macro-aggregates (>0.25 mm diameter) (Plante et al., 2002), providing physical protection to organic matter (Six et al., 2000a,b). Agricultural fertilization is one of the most well-known drivers for increasing soil aggregation (Abiven et al., 2009; Bronick and Lal, 2005). The input of organic fertilizers such as compost derived from dung increases the physical protection of organic matter

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through enhancing macro-aggregate formation (Jiang et al., 2013; Singh, 2018).

Soil aggregates provide spatial heterogeneity for soil biota (Gupta and Germida, 2015; Smith et al., 2014). A growing body of work has found the different distribution patterns of soil microbes, extracellular enzyme activities and microfauna (e.g., nematodes) within soil aggregates (Jiang et al., 2018; Upton et al., 2019). The differences in microbial activity and community composition across aggregate size fractions are expected to be associated with nutrient cycling and other soil ecological processes that deliver multiple functions (Hallett et al., 2009; Trivedi et al., 2017). Many studies have suggested that soil aggregate distribution is critical for bulk soil nutrient retention and availability (Garland et al., 2018; Zhang et al., 2016). So far, most relevant studies separate bulk soils into aggregate size fractions and then center on the functional potentials of each fraction individually, at the cost of linking the specific contribution of different-sized aggregates to overall soil functioning (e.g., crop productivity).

Soil aggregate sizes can interact with microbial communities to influence element cycling (Gupta and Germida, 2015). Consequently, nutrient and organic carbon (C) turnover efficiency among different-sized aggregates can further affect plant growth. Soil microbes participate in the soil aggregation, and microbial community composition and function within aggregate fractions are therefore a proxy to explore the inherent mechanism in the bulk soil ecological processes (Rillig et al., 2017). In addition, soil microbe-derived enzymes are highly sensitive to resources variation within aggregates (Wang et al., 2015) and the enzymatic stoichiometry has been interpreted as an indicator of substrate availability (Sinsabaugh et al., 2009). Further, plant growth can in turn influence soil aggregation processes (e.g., increases soil aggregate stability) by releasing root exudates (De Gryze et al., 2005; Jin et al., 2017), enmeshing and realigning soil particles (Bronick and Lal, 2005). However, few studies have focused on the effects of fertilization on the complex interactions among plants, microbial communities, and nutrient cycling functions at the aggregate level.

In the present study, we therefore focused on the influences of fertilization regimes (chemical vs. organic) on microbial community composition and soil functions (e.g., nutrient retention and availability) at the aggregate scale, as well as the consequences for the overall functioning (e.g., supporting plant growth) at bulk soil scale, as well as the relationships with plant performance properties (i.e., element concentration and productivity). A 28-year long-term field experiment was employed in the subtropical region of China subjected to organic and/or inorganic fertilization management. Targeting to understand the above-mentioned complex relationships, the following hypotheses were raised (1) soil resource properties and microbial community vary with aggregate size fractions, playing distinct roles in soil functioning indicated by soil enzyme activities and stoichiometry characteristics; (2) soil functioning would be mediated by fertilization regime through restructuring the distribution pattern of soil aggregates; and (3) organic fertilization would boost the bulk soil C and nutrient pools by increasing the proportion of macro-aggregate, thereby plant productivity.

2. Materials and methods

2.1. Study site

This study was conducted at Jiangxi Institute of Red Soil in Jiangxi Province, China (116°20′24″E, 28°15′30″N). The area has a typical subtropical climate with a distinct wet season (from March to June) and a distinct dry season (from July to September) and a mean annual precipitation of 1537 mm. The minimum monthly mean temperature is 4.6 °C in January, and the maximum monthly mean temperature is 29.8 °C in July. The uneven distribution of rainfall causes strong seasonal drought in summer and/or early autumn. The soil in this study area is derived from quaternary red clay classified as Ultisols and Oxisols according to U.S. Soil Taxonomy (Soil Survey Staff, 1999), or Acrisols and Ferralsols according to World Reference Base (IUSS Working Group WRB, 2014). This soil has a clay-loam texture with 17% sand, 57% silt, and 26% clay.

The experiment was set up in 1986 with double cropping of Spring and Summer maize (*Zea mays* L.) with a density of 40, 000 plants ha^{-1} from early April to the end of November and pesticide-free during the whole growth period of maize, all plots were left fallow during the winter (Fig. S1). At the initiation of the experiment, the soil had a pH (H_2O) of 6.0, contained 9.39 g kg^{-1} of organic C, 0.98 g kg^{-1} of total nitrogen (N), 1.42 g kg^{-1} of total phosphorus (P), 15.83 g kg^{-1} of total potassium (K), and 60.3, 12.9 and 102 mg kg^{-1} of available N, P, and K, respectively. For this study, four treatments with three replicates each were selected: 1) no fertilization (Control), 2) chemical fertilization (application of mineral NPK fertilizer alone), 3) organic fertilization (application of pig manure alone), and 4) chemical plus organic fertilization (application of mineral NPK fertilizer plus pig manure). Pig manure and mineral NPK fertilizer were incorporated via tillage every growing season before planting maize. Pig manure was applied as composted manure (15, 000 kg ha^{-1}, fresh weight basis with about 75% gravimetric moisture content), and the nutrients of pig manure (dry weight basis) were on average 340 g kg^{-1} of organic C, 28.3 g kg^{-1} of total N, 10.3 g kg^{-1} of total P and 9.8 g kg^{-1} of total K. The nutrient inputs of pig manure each year were on average 106.13 kg N ha^{-1}, 38.63 kg P ha^{-1}, 36.75 kg K ha^{-1}, respectively. Mineral fertilizer was applied as urea (60 kg N ha^{-1}), calcium superphosphate (13.11 kg P ha^{-1}), and potassium chloride (49.80 kg K ha^{-1}), respectively. Plots were 5.5 m × 4 m and were separated by concrete borders (Fig. S1).

2.2. Soil and plant sampling

Soil samples were taken from the plow layer (0–20 cm depth) using a 3-cm diameter auger at eight randomly selected points on crop row or between rows which were about 10 cm away from maize stem during the maize tasseling stage in October 2013 and mixed to get one composite sample per plot. To minimize the destruction of soil structure, fresh soil samples were stored in hard plastic containers during transportation to the laboratory. Then, five randomly selected maize plants were collected from each treatment plot and separated into shoots (sum of leaves, stems, and ears) and roots (collected from 20 cm depth soil). Both fresh shoot and root samples were washed and then cut into small pieces and mixed thoroughly to get a composite sample and fresh weight was recorded. Approximately 100 g of fresh shoot or root materials were oven-dried at 65 °C to calculate water content and then ground to fine powders for C, N, and P analysis (see below). Crop productivity was represented by the sum of plant shoot and root dry biomass which was calculated from fresh weight and water content data.

2.3. Soil aggregate fractionation

To minimize the disturbance of soil microbes, aggregates were isolated by the modified dry-sieving method (Dorodnikov et al., 2009). Fresh soil samples were dried to 'optimal' moisture (about 15% gravimetric soil water content) in a dark, ventilated place, which allows limited mechanical stress to induce maximum, brittle failure along natural planes of weakness (Dorodnikov et al., 2009). Then, individual soil clods were gently broken apart along the natural breakpoints and all visible stones as well as other debris were removed by sieving through 8 mm mesh. After this, soils were divided into two parts, one part to be analyzed as bulk soil and another part for aggregate fractionation by a sieving procedure. Briefly, for each plot, about 500 g of 'optimal moisture' soil was transferred to a set of two sieves (2- and 0.25-mm mesh), which was softly shaken three times for 2 min each time. Soil retained on the 2-mm and 0.25-mm sieve was gently collected as large macro-aggregates (>2 mm) and small macro-aggregates (0.25–2 mm), respectively. The remaining soil sifted through the 0.25-mm sieve was gently collected as micro-aggregates + non-aggregated particles (e.g.,

silt and clay particles) (hereinafter termed as "micro-aggregates") (<0.25 mm). Then, each soil aggregate fraction was weighed and divided into two parts. One part was used for analysis of soil moisture content, phospholipid fatty acids (PLFA), and soil enzyme activities assays (see below). The other part was air-dried for analyses of chemical soil properties (see below).

The proportion of each soil aggregate fraction (based on dry weight) was calculated to obtain the soil aggregate distribution and the mean weight diameter (MWD) was calculated as an index of aggregate stability using the equation:

$$MWD = \sum_{i}^{3} x_i w_i \quad (1)$$

where, i is the number of aggregate size fractions; x_i is the mean diameter (mm) of the aggregates in any aggregate size fraction, and w_i is the weight of the aggregates in that aggregate size fraction as a proportion of the total dry weight of soil (van Bavel, 1950).

2.4. Chemical and biological analyses

2.4.1. Soil and plant chemical analyses

Soil moisture was determined from mass loss after drying at 105 °C for 24 h. Soil pH was measured using a soil:water ratio of 1:5 (*w/v*). Soil organic C (SOC) was determined by Walkley-Black's wet digestion method, soil total N was measured using the micro-Kjeldahl method (Lu, 2000). Soil total P was measured photometrically after soils were digested with $HClO_4$–H_2SO_4. Soil dissolved organic C (DOC) was extracted with ultrapure water (soil:solution = 1:5 (*w/v*)) and the extracts were analyzed with a total C analyzer (Elementar, Germany). Nitrate and ammonium were extracted with 2 M KCl (soil:solution = 1:5 (*w/v*)), and the extracts were analyzed by a continuous flow analyzer (Auto-Analyzer AA3, Germany). Soil available P was extracted with 0.03 M NH_4F-0.025 M HCl (soil:solution = 1:4 (*w/v*)) and determined by the molybdenum-blue method.

Nutrient content of each aggregate size fraction per kg bulk soil was calculated as:

$$Content = \frac{p_a \times c_a}{100} \quad (2)$$

where, p_a is the proportion of each aggregate size fraction (%), and c_a is the nutrient concentration of the aggregate size fraction (mg or g kg^{-1}).

Plant shoot and root C were measured by dichromate oxidation, while N and P were determined by digestion with H_2SO_4 and H_2O_2, after which P concentration was measured by the molybdenum-blue method and N concentration was measured by the Kjeldahl method.

2.4.2. Soil enzyme assays

Activities of seven hydrolytic soil enzymes were measured, including four enzymes involved in C-acquisition: α-glucosidase, β-glucosidase, cellobiohydrolase, β-xylanase; two enzymes involved in N-acquisition: leucine aminopeptidase, β-N-acetylglucosaminidase, and one enzyme involved in P-acquisition: phosphatase (Sinsabaugh et al., 2009). In addition, we measured activities of two oxidases involved in the degradation of recalcitrant organic C: phenoloxidase and peroxidase (Sinsabaugh, 2010). Hydrolytic enzymes were assayed using standard fluorometric techniques and the activities were represented by nmol g^{-1} dry soil h^{-1} (Bell et al., 2013). Oxidases were measured spectrophotometrically in a clear 96-well microplate using the substrate L-3, 4-dihydroxyphenylalanine (L-DOPA) and the activities were represented by μmol g^{-1} dry soil h^{-1} (Pind et al., 1994). Details of the measuring procedure are explained in supplementary materials. To represent the general potential C, N, and P acquisition activity, the C, N, and P acquiring enzymes were grouped and normalized as ln(α-glucosidase + β-glucosidase + cellobiohydrolase + β-xylanase + phenoloxidase + peroxidase), ln(leucine aminopeptidase + β-N-acetylglucosaminidase), and ln(phosphatase), respectively (Sinsabaugh et al., 2009).

2.4.3. Phospholipid fatty acid profiles

The PLFAs of bulk soil and each aggregate fraction were extracted according to the procedure described by Frostegård et al. (1991). The detailed protocol is provided in supplementary materials. We used the PLFAs a-13:0, i-14:0, a-14:0, i-15:0, i-16:0, a-16:0, i-17:0, a-17:0, i-18:0 as indicators for Gram-positive bacteria and the PLFAs 16:1ω9c, cy-17:0, 17:1ω8c, 18:1ω7c, 18:1ω5c, 19:1ω9c, cy-19:0 as indicators for Gram-negative bacteria. The sum of Gram-positive and Gram-negative bacteria represents total bacterial biomass (Zelles, 1999). The PLFAs 16:1ω5c, 18:1ω9c, 18:2ω6,9c, 18:3ω6c were used as indicators for fungi (Frostegård and Bååth, 1996; Olsson, 1999). The ratios of fungi to bacteria and Gram-positive to Gram-negative bacteria were commonly used to explain the response of microbial groups to fertilization (Jiang et al., 2018).

2.5. Statistical analysis

All statistical analyses were performed using R version 4.0.3 (R Core Team, 2020) with the R studio interface (version 1.2.1335, RStudio Team, 2018). Prior to analysis, all data were checked for normality using Shapiro-Wilk tests and for homogeneity of variance using Levene tests. The single and interactive effects of chemical fertilizer, organic fertilizer, and soil aggregate size fraction on soil physicochemical properties, enzyme activities, enzymatic stoichiometry, and phospholipid fatty acid profiles (PLFAs) were tested using three-way analysis of variance (ANOVA). Further, the single effects of fertilization regime on aggregate stability (MWD), plant shoot and root biomass, shoot and root C, N, and P, shoot and root element stoichiometry, soil physicochemical properties, enzyme activities, enzymatic stoichiometry, and PLFAs of bulk soil were tested using one-way ANOVA. In addition, the single effects of soil aggregate size fraction under each fertilization treatment on soil physicochemical properties, enzyme activities, enzymatic stoichiometry, and PLFAs were tested using one-way ANOVA. Where significant effects were found, post-hoc tests were run using Fisher's least significant difference (LSD) with a significance level of $\alpha = 0.05$.

Permutational Multivariate Analysis of Variance (PERMANOVA with 999 permutations) was performed by the 'adonis' function in the 'vegan' R package (Oksanen et al., 2020) to quantitatively evaluate the effects of chemical versus organic fertilizer on plant properties (i.e., shoot and root biomass, and shoot and root C, N, and P concentration) and to quantitatively evaluate the effects of fertilization regime and aggregate size fraction on soil nutrients (i.e., total N, total P, mineral N and available P) and microbial community composition (i.e., PLFA profiles). Statistical significance was tested using permutation tests (pseudo-*F* ratio).

Finally, partial least squares path model (PLS-PM) was performed using the 'plspm' R package (Sanchez et al., 2015) to further infer potential direct and indirect effects of chemical and organic fertilizer, large macro-aggregate, small macro-aggregate as well as micro-aggregate nutrient pools on maize root and shoot properties. Another PLS-PM was performed to further infer potential direct and indirect effects of chemical and organic fertilizer, large macro-aggregate, small macro-aggregate as well as micro-aggregate microbial community on bulk soil microbial community. Large macro-aggregate, small macro-aggregate, and micro-aggregate nutrient pools are latent variables, which are indicated by total N, total P, mineral N, and available P content of each aggregate, respectively. Maize root and shoot properties are latent variables measured by biomass, N, and P concentration of root and shoot, respectively. Furthermore, large macro-aggregate, small macro-aggregate, micro-aggregate, and bulk soil microbial communities are latent variables reflected by PLFA profiles of each aggregate and bulk soil, respectively. The quality of the PLS-PM was evaluated by examining the goodness of fit (GoF) index which >0.7 mean a good overall prediction performance of the model, and by examining the coefficients

of determination (R^2) of the latent variables which indicate the amount of variance of the dependent variables explained by their independent latent variables (Sanchez et al., 2015).

3. Results

3.1. Soil aggregate distribution and nutrients within aggregates

The soil aggregate distribution was significantly influenced by fertilization regime (Fig. 1A). Compared to chemical fertilizer, organic fertilizer increased macro-aggregate fractions. Compared to the Control, chemical fertilization decreased the proportion of macro-aggregate fractions (Fig. 1A). Compared to the Control, mean weight diameter was significantly decreased under chemical fertilization and significantly increased under organic fertilization (with or without chemical fertilizer) (Fig. 1B).

Both chemical and organic fertilization significantly increased bulk soil SOC, total N, and total P, with the highest values of SOC and total N under organic fertilization while highest value of total P when chemical and organic fertilizers were combined (Fig. 2A–C). Organic fertilization significantly increased bulk soil DOC and available P with the highest values when organic fertilizer was applied alone (Fig. 2D, F), while mineral N increased under organic fertilization regardless of whether chemical fertilizer was applied or not (Fig. 2E). Except for available P under Control and NPK treatments, all other resources showed a similar trend towards lower concentration of the bulk soil as aggregate size decreased (Fig. 2). For treatments without organic fertilizer, the available P content increased as aggregate size decreased. Furthermore, micro-aggregates in treatments without organic fertilizer contributed the most available P (>50%) to the bulk soil (Table S4); however, available P content of the bulk soil in treatments without organic fertilizer was very low (Fig. 2F). All other bulk soil nutrients were mainly contributed by macro-aggregates in both chemical and organic fertilizer treatments (Table S4).

3.2. Microbial community structure

The concentrations of soil microbial groups (based on PLFAs) varied with aggregate fractions and fertilization regime (Fig. 3, Table S2). Organic fertilization significantly increased bacterial and fungal biomass in bulk soils (Fig. 3A and B). Across all fertilization treatments, the concentrations of bacterial and fungal PLFAs were highest in small macro-aggregates followed by micro-aggregates, and both bacterial and fungal PLFAs in small macro-aggregate and micro-aggregates were significantly higher than in large macro-aggregates in organic fertilizer treatments (Fig. 3A and B). Organic fertilization significantly increased the Gram-positive to Gram-negative bacterial ratio (G^+:G^-) and the fungi to bacteria ratio (F:B) of bulk soil (Fig. 3C and D). Across all fertilizer treatments, the G^+:G^- ratio showed an increasing trend with decreasing aggregate size (except for large macro-aggregates in NPK + OM treatments).

3.3. Enzyme activities and enzymatic stoichiometry

Fertilization increased the activities of microbial enzymes involved in C and N-acquisition in bulk soil, with the highest values when chemical and organic fertilizers were applied in combination (Fig. 4A and B, Figs. S3A–F, Table S3). Chemical fertilization significantly increased P- acquiring enzyme activity while organic fertilizer applied alone significantly decreased P-acquiring enzyme activity in bulk soil (Fig. 4C, Fig. S3G, Table S3). However, when chemical and organic fertilizers were applied together, no significant effect on P-acquiring enzyme activity was detected in bulk soil (Fig. 4C, Fig. S3G, Table S3). Aggregate size fraction had significant effects on the distribution of C-acquiring enzymes, which increased with decreasing aggregate size (Fig. 4A). In contrast, N-acquiring enzyme activities did not differ among aggregate size fractions (Fig. 4B). The activities of P-acquiring enzymes tended to decrease with decreasing aggregate size in Control, NPK, and OM treatments but not in the NPK + OM treatment (Fig. 4C).

For bulk soil, organic fertilization significantly decreased C:N enzyme ratio while chemical fertilization had no significant effect on C:N enzyme ratio (Fig. 4D, Table S3). Fertilization significantly increased C:P and N:P enzyme ratios of bulk soil compared to the Control (Fig. 4E and F, Table S3). Across all fertilization treatments, the C:N and C:P enzyme ratios increased with the decreasing aggregate size (Fig. 4D and E). However, no significant differences among aggregate size fractions of N:P enzyme ratio were found across all fertilization treatments (Fig. 4F).

3.4. Crop productivity and stoichiometry

Fertilization significantly influenced crop productivity indicated by maize shoot and root biomass as well as shoot and root element stoichiometry (Fig. 5). Compared to the Control, fertilization (NPK, OM, and NPK + OM) significantly increased the shoot and root biomass (Fig. 5A) but significantly decreased the maize shoot and root C:N ratio (Fig. 5B). Root C:P and N:P ratios were significantly decreased under organic fertilization, while they were homeostatic under chemical fertilization (Fig. 5C and D). Furthermore, both shoot C:P and N:P ratios did not differ across fertilization treatments (Fig. 5C and D).

3.5. Quantitative examination of the effects of fertilization and aggregates

PERMANOVA showed that variation in plant properties, bulk soil nutrients and microbial community were all best explained by organic fertilizer (83.07%, 87.40% and 87.06%, respectively), and to a lesser extent by chemical fertilizer (8.17%, 5.27% and 2.47%, respectively) and the interaction between chemical and organic fertilizer (7.39%, 5.44% and 6.37%, respectively) (Table 1). Variation in aggregated soil nutrients was best explained by fertilization regime (73.31%), and to a lesser extent by aggregate size fractions (15.17%) and the interactions between fertilization regime and aggregate size fractions (9.48%) (Table 1). In contrast, variation in aggregated soil microbial community composition was best explained by soil aggregate size fractions

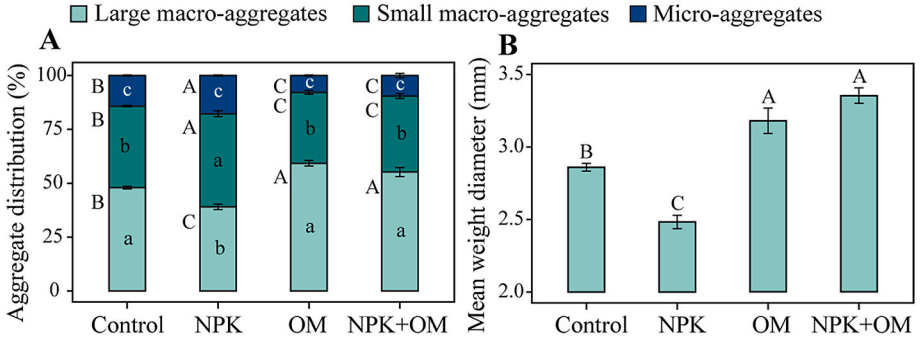

Fig. 1. Soil aggregate distribution (A), mean weight diameter of aggregates (B) under different fertilization treatments. Different lowercase letters indicate significant differences among aggregate size fractions in the same fertilization treatment ($P < 0.05$), while different uppercase letters indicate significant differences among fertilization treatments for each of the aggregate size classes ($P < 0.05$). Values are means ± SE (n = 3). Control = no fertilizer; NPK = chemical fertilizer; OM = organic fertilizer; NPK + OM = chemical fertilizer + organic fertilizer.

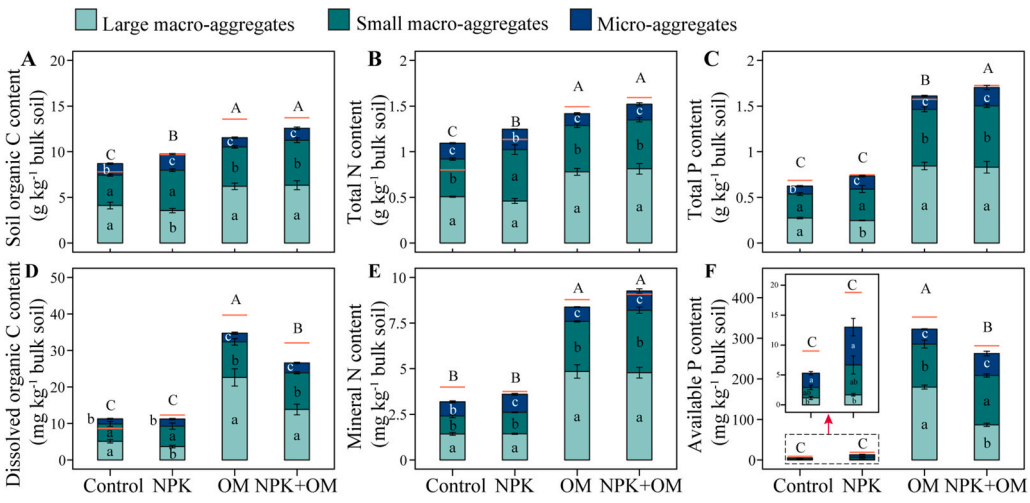

Fig. 2. Soil organic carbon (A), total nitrogen (B), total phosphorus (C), dissolved organic carbon (D), mineral nitrogen (E), and available phosphorus (F) content within aggregates under different fertilization treatments. The red lines are the means of bulk soils under the different fertilization treatments. Different lowercase letters indicate significant differences among aggregate size fractions in the same fertilization treatment ($P < 0.05$), while different uppercase letters indicate significant differences among fertilization treatments ($P < 0.05$). Values are means ± SE (n = 3). Control = no fertilizer; NPK = chemical fertilizer; OM = organic fertilizer; NPK + OM = chemical fertilizer + organic fertilizer. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

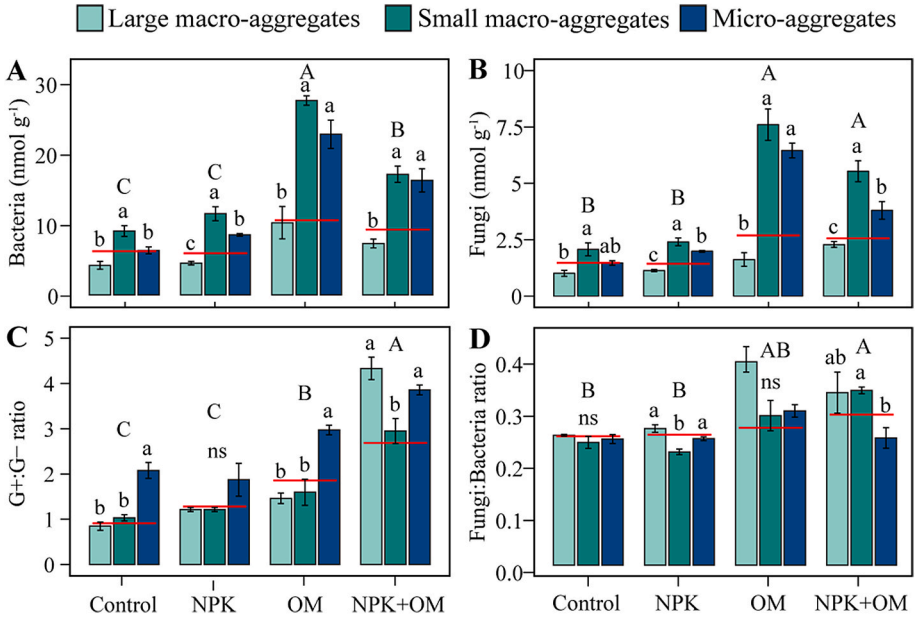

Fig. 3. Concentrations of bacterial (A) and fungal (B) PLFA (phospholipid fatty acids) markers, and Gram-positive to Gram-negative (C) and fungi to bacteria (D) PLFA ratios within soil aggregates under different fertilization treatments. The red lines are the means of bulk soils under the different fertilization treatments. Different lowercase letters indicate significant differences and ns indicate no significant differences among aggregate size fractions in the same fertilization treatment ($P < 0.05$), while different uppercase letters indicate significant differences among different fertilization treatments ($P < 0.05$). Values are means ± SE (n = 3). Control = no fertilizer; NPK = chemical fertilizer; OM = organic fertilizer; NPK + OM = chemical fertilizer + organic fertilizer. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

(34.90%) and fertilization regimes (43.75%) and to a lesser extent by the interaction between aggregate size fraction and fertilization regime (12.77%) (Table 1).

The partial least squares path model indicated that all predictor variables explained 97%, 99%, and 98% of variations in root, shoot properties, and in bulk soil microbial community, respectively (Fig. 6). Organic fertilizer showed significantly positive effects on nutrient pools (Fig. 6A) and microbial community (Fig. 6C) of all aggregate size fractions, while chemical fertilizer positively regulated the nutrient pools of small macro-aggregates and micro-aggregates (Fig. 6A). However, chemical fertilizer suppressed the nutrient pool of large macro-aggregate and the microbial community of small macro- and micro-aggregates (Fig. 6A, C). In general, both chemical and organic fertilizer and all sizes of aggregates promoted root and shoot properties, and the promoting effects decreased with decreasing aggregate size (Fig. 6B). Organic fertilizer and large macro-, micro- aggregates had positive total effects while chemical fertilizer and small macro-aggregate had negative total effects on bulk soil microbial community, and micro-aggregate exerted the highest positive effect among all aggregates (Fig. 6D).

4. Discussion

4.1. Nutrient pool within aggregate fractions depends on fertilization regimes

As expected, we found an increase of C, N, and P content with increasing mean aggregate size, because large and small macro-aggregates accounted for the largest proportion of aggregates while C and nutrient concentrations across aggregates were similar (Fig. S2). The nutrients in aggregates can reflect the contribution of individual soil aggregate fraction to the overall bulk soil nutrient status (Zhang et al., 2016). In our study, long-term organic fertilization resulted in the accumulation of total and available nutrients (especially the available P) in macro-aggregates. In treatments without organic fertilizer, although macro-aggregates contained more than 80% of the total P of bulk soil, only around half of the bulk soil available P was contributed by

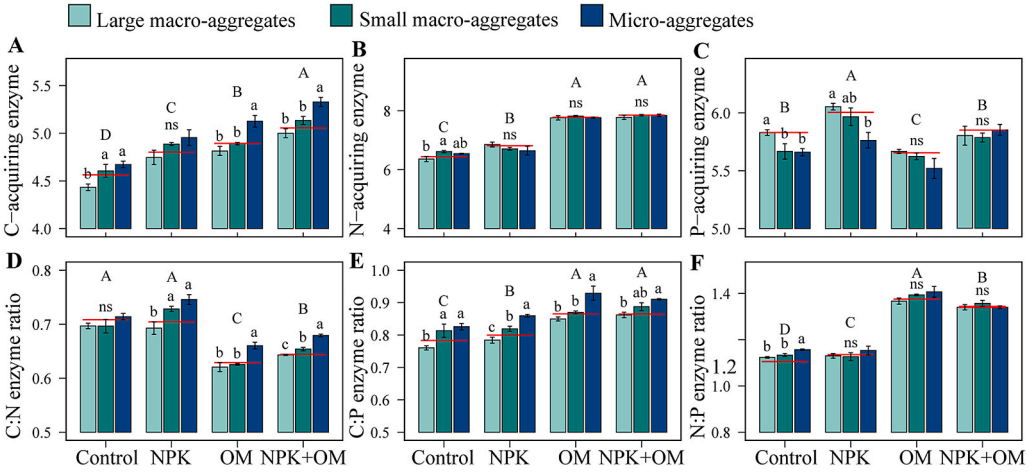

Fig. 4. Microbial enzyme activities and enzymatic stoichiometry within aggregates under different fertilization treatments. Shown are activities of normalized C-acquiring enzyme (A), N-acquiring enzyme (B), P-acquiring enzyme (C), C:N enzyme ratio (D), C:P enzyme ratio (E), and N:P enzyme ratio (F). The red lines are the means of bulk soils under different fertilization treatments. Different lowercase letters indicate significant differences and ns indicate no significant differences among aggregate size fractions in the same fertilization treatment ($P < 0.05$), while different uppercase letters indicate significant differences among different fertilization treatments ($P < 0.05$). Values are means ± SE (n = 3). Control = no fertilizer; NPK = chemical fertilizer; OM = organic fertilizer; NPK + OM = chemical fertilizer + organic fertilizer. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

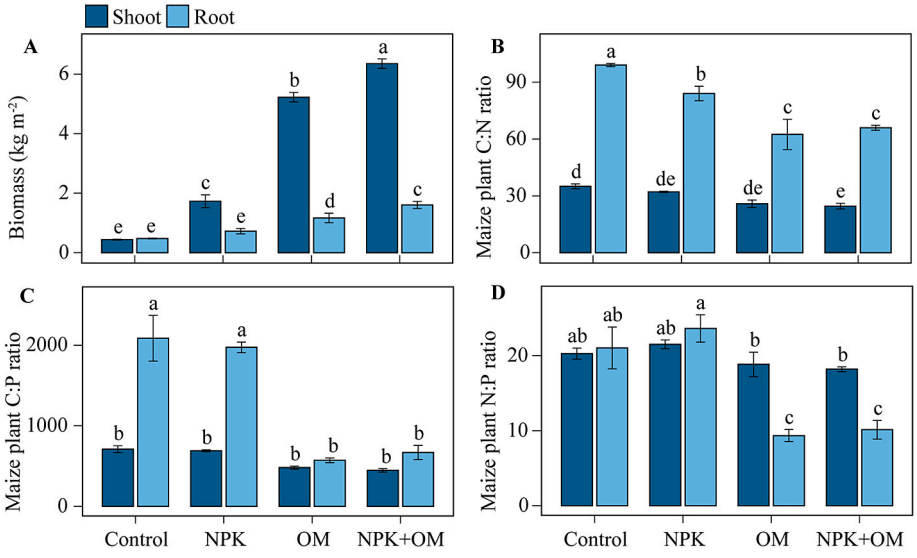

Fig. 5. Maize shoot and root biomass (A), plant C:N (B), C:P (C) and N:P (D) ratio of maize shoot and root under different fertilization treatments; values are means ± SE (n = 3). Different lowercase letters indicate significant differences among different fertilization treatments ($P < 0.05$). Control = no fertilizer; NPK = chemical fertilizer; OM = organic fertilizer; NPK + OM = chemical fertilizer + organic fertilizer.

Table 1
Results from PERMANOVA testing the effects of chemical versus organic fertilizer on plant properties (i.e., biomass, and root and shoot C, N, and P concentration), bulk soil nutrients (i.e., total N, total P, mineral N and available P), and bulk soil microbial community (i.e., PLFA profiles) as well as effects of fertilization regime and aggregate size on aggregated soil nutrients and microbial community.

	Df	Pseudo-F	*P*-value	R^2 (%)	Pseudo-F	*P*-value	R^2 (%)	Pseudo-F	*P*-value	R^2 (%)
		Plant properties			Bulk soil nutrients			Bulk soil microbial community		
Chemical fertilization	1	47.89	0.001	8.17	22.16	0.006	5.27	4.82	0.064	2.47
Organic fertilization	1	487.06	0.001	83.07	367.8	0.002	87.40	169.3	0.001	87.06
Chemical fertilization × Organic fertilization	1	43.33	0.003	7.39	22.87	0.005	5.44	12.43	0.003	6.37
Residuals	8			1.37			1.90			4.10
					Aggregated soil nutrients			**Aggregated soil microbial community**		
Fertilization	3				286.26	0.001	73.31	40.82	0.001	43.75
Aggregate size	2				88.87	0.001	15.17	48.85	0.001	34.90
Fertilization × Aggregate size	6				18.50	0.001	9.48	5.96	0.002	12.77
Residuals	24						2.05			8.57

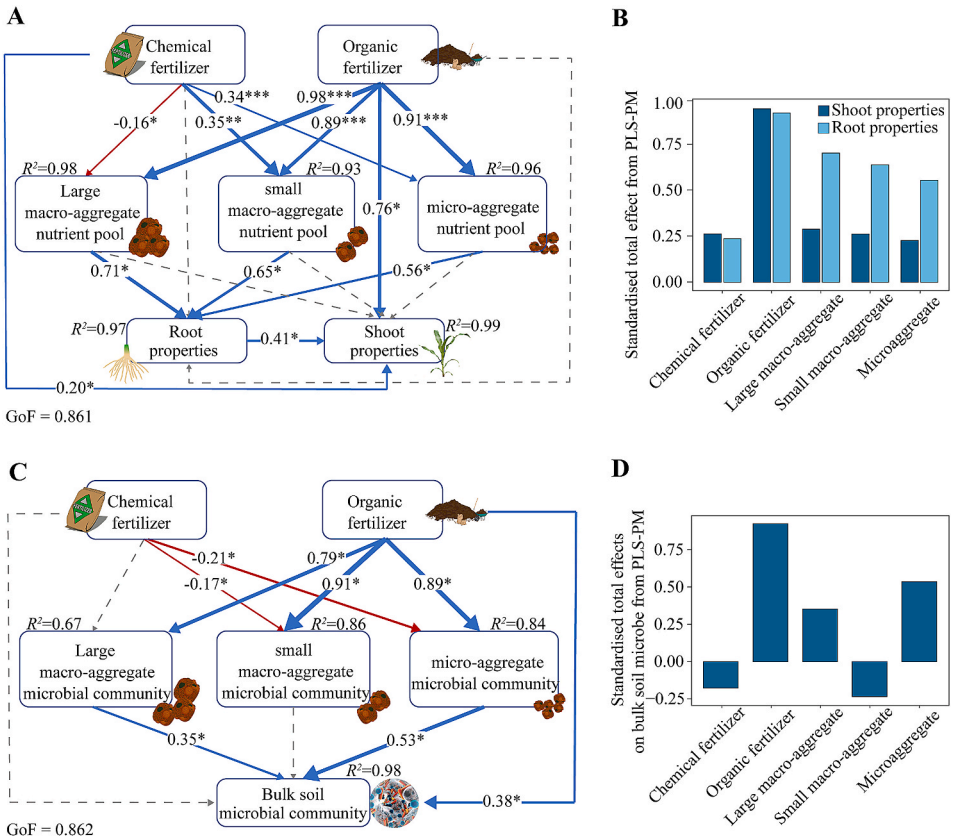

Fig. 6. The partial least squares path models (PLS-PM) illustrating the direct and indirect effects of chemical and organic fertilizer, large macro-aggregate, small macro-aggregate, and micro-aggregate nutrient pools on maize root and shoot properties (A) and standardized total effects on root and shoot properties from PLS-PM (B) as well as the direct and indirect effects of chemical and organic fertilizer, large macro-aggregate, small macro-aggregate, and micro-aggregate microbial community on bulk soil microbial community (C) and the standardized total effects on bulk soil microbial community from PLS-PM (D). Large macro-aggregate, small macro-aggregate, and micro-aggregate nutrient pools are latent variables, which are indicated by total N, total P, mineral N, and available P content of each aggregate, respectively. Maize root and shoot properties are latent variables measured by biomass, N, and P concentration of root and shoot, respectively. Large macro-aggregate, small macro-aggregate, micro-aggregate, and bulk soil microbial communities are latent variables reflected by PLFA profiles of each aggregate and bulk soil, respectively. The red and blue arrows indicate negative and positive flows of causality, respectively. Numbers on the arrowed lines and thickness of arrows indicate normalized path coefficient. The dotted gray arrows represent non-significant path relationships. R^2 beside the latent variables are the coefficients of determination. The GoF index represents the goodness of fit. Asterisks represent significant effects: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

macro-aggregates. This indicates that a large part of the P contained in macro-aggregates under treatments without organic fertilizer was difficult to acquire by soil microbes and/or plants. Such a phenomenon could be explained by the 'phosphorus paradox' theory in highly weathered (sub)tropical soils that large amount of secondary minerals (i.e., kaolinite and oxides of aluminum, iron, and manganese) strongly adsorb P to the soil and reduce P availability to plants and microbes (Garland et al., 2018; Hunt et al., 2007). These results partly support our first hypothesis and indicate that organic fertilization can increase nutrient availability of the bulk soil by increasing the nutrient content of macro-aggregate due to the high amounts of extractable and organic nutrients in the applied pig manure.

4.2. *Microbial community composition within aggregate fractions depends on fertilization regimes*

Organic fertilization enhanced concentrations of total bacterial and fungal PLFAs, probably because higher nutrient levels under organic fertilization could support more microbial biomass (Börjesson et al., 2012; Zhang et al., 2014), and manure-derived exogenous microbes may also play critical roles in regulating indigenous soil microbial community (Yang et al., 2022). Further, the present result revealed that the microbial community composition was not only explained by fertilization, but also by aggregate size and, importantly, by interactions of aggregates size and fertilization regime.

In line with previous studies such as Jiang et al. (2013) and Zhang et al. (2016), microbial biomass was higher in smaller aggregates than in larger aggregates. The increase in bacterial and fungal PLFA concentrations as soil aggregate size decreased may be due to small pore sizes in micro-aggregates (Chenu et al., 2001) and higher specific surface area of clay and silt for microbial cells to adhere to (van Gestel et al., 1996). We found that the ratio of fungi to bacteria declined with decreasing aggregate size which was in agreement with prior works (Jiang et al., 2018; Smith et al., 2014). Changes in the soil microbial communities may reflect differences in the chemical composition of aggregates due to differences in microbial preference for the substrates they use (Fanin et al., 2014). The higher relative abundance of fungi in macro-aggregates and organically-fertilized bulk soils could be explained by more favorable substrate properties (Huygens et al., 2008), in particular higher C:N ratio, which is known to favor fungi over bacteria (Waring et al., 2013). Furthermore, fungi may preferentially colonize larger soil aggregates with high porosity (Harris et al., 2003), and fungal hyphae physically bind smaller aggregates into larger aggregates (Duchicela et al., 2013; Rillig and Mummey, 2006).

We further found that distinct groups of bacteria dominated differently-sized aggregates, with gram-positive bacteria (G^+) being more dominant than gram-negative bacteria (G^-) in micro-aggregates compared to macro-aggregate in most treatments (except for NPK + OM). In general, G^+ bacteria are thought to favor older and more stabilized organic matter, whereas G^- bacteria preferentially use plant-derived labile organic matter as a C source (Kramer and Gleixner, 2008). Hence, the G^+:G^- ratio can be used as an indicator of the relative C availability for soil bacteria (Fanin et al., 2019; Kramer and Gleixner, 2006). The high proportions of G^- bacteria in macro-aggregates indicate that the macro-aggregates contain much more labile organic matter with relatively high turnover rates (Kramer and Gleixner, 2006). On the other hand, the higher G^+:G^- ratios in micro-aggregate suggests that micro-aggregates contain proportionally more stabilized organic matter (Kramer and Gleixner, 2008; Fanin et al., 2019). Furthermore, all fertilization treatments increased the G^+:G^- ratio in bulk soil, indicating that both chemical and organic fertilization increased the relative abundance of stabilized organic C, especially in micro-aggregates.

Finally, our results further revealed that organic fertilizer was more beneficial to bulk soil microbial community compared to chemical fertilizer, and micro-aggregates was the main contributor to bulk soil microbial community among all size of aggregates. These results also partly support our first hypothesis and indicated that fertilization could restructure microbial community within aggregates and potentially influence soil functioning (e.g., nutrient retention).

4.3. Enzyme distribution pattern within aggregate fractions depends on fertilization regimes

In this study, the C-acquiring enzymes showed relatively higher activities in micro-aggregate reflecting urgent microbial C demand, and activities of N-acquiring enzymes showed no differences among aggregates indicating similar N limitation for microbes among aggregates (Rosinger et al., 2019). The distribution of C- and N- acquiring enzymes among aggregates was similar under all fertilization treatments, indicating no interactions between aggregate size and fertilization regime. However, the activity of P-acquiring enzymes decreased as aggregate size decreased in all treatments except for NPK + OM, indicating that interactions among chemical and organic fertilization could change how aggregate size influences P-acquiring enzymes. The relatively high P-acquiring enzyme activity in macro-aggregates indicates higher microbial P demand (Luo et al., 2017), and this is consistent with the lower available P concentration in macro-aggregates which makes it hard to meet the microbial demand. Furthermore, the higher phosphatase activity in macro-aggregates could indicate the presence of plant debris and less-humified organic matter contained in larger aggregates (Rojo et al., 1990).

Organic fertilization decreased the C:N enzyme ratio but increased the N:P enzyme ratio, indicating a relatively high activity of the N-acquiring enzymes. This also suggests that long-term organic fertilizer could influence microbial nutrient demand by altering the soil resource stoichiometry (i.e., high C:N ratio). More specifically, the relatively high soil C content would stimulate microorganisms to produce more N-acquiring enzymes to meet their N demand and maintain the microbial stoichiometry homeostasis (Cleveland and Liptzin, 2007; Tian et al., 2017). Further, the increasing C:N and C:P enzyme ratios with decreasing aggregate size suggest a relatively high N and P mineralization rate within macro-aggregates and relatively high demand for organic C by microorganisms within micro-aggregates. These results support our second hypothesis that organic fertilization could change the distribution patterns of microbial enzyme activities among soil aggregates and thereby the role of aggregates in soil functioning (e.g., nutrient supply). More specifically, macro-aggregates have more labile organic C and higher N and P supply capacity while micro-aggregates contain relatively more stabilized organic C, and microorganisms might be C-limited in micro-aggregates.

4.4. Macro-aggregates promote crop growth

We found that the dynamics of maize plant C, N, and P concentrations and their ratios differed in their responses to fertilization treatments and that the shoot element ratios were more homeostatic than the root element ratios. The element stoichiometry of shoots, and particularly of roots, is a useful tool to investigate plant nutrient limitation (Schreeg et al., 2014). Hence, the increased root N:P ratio in treatments without organic fertilizer indicates that maize was more limited by P than by N.

In this study, we demonstrated that macro-aggregates contained the majority of soil nutrients (except available P under Control and NPK), contributing the most available nutrients to bulk soil and mostly affecting plant properties. Likewise, previous studies have suggested that macro-aggregates contain much more labile organic matter (Plante et al., 2002), the immobilized nutrients may become available to plants following subsequent microbial turnover (Zhang et al., 2015). Alternatively, the high proportion of fungi in macro-aggregates could also increase nutrient acquisition by the plant. For example, mycorrhizal hyphae could fulfill similar functions as root hairs, absorbing soil N and P and transferring them to the host plant (Frey and Schüepp, 1993; Richardson et al., 2011).

Overall, long-term chemical fertilization may reduce soil aggregate stability with inhibition effects on large macro-aggregate nutrient pool and negative effects on microbial communities within all aggregate sizes. In contrast, organic fertilization promoted soil macro-aggregate formation and the nutrient retention as well as microbial communities within all sizes of aggregates to further positively control plant growth. Our results suggested the performance of maize was mostly affected by organic fertilizer and macro-aggregates. In general, the nutrients in organic forms are critical to the long-term functioning and productivity of soils (Egan et al., 2018). Hence, the current study supports the third hypothesis that increasing the proportion of macro-aggregates as well as the nutrient pool is one important pathway of how organic fertilization enhances sustainable soil functioning. However, due to the method limitation, we didn't collect data of non-aggregated particles and solutions inter soil aggregates which also play vital roles in determining nutrient flux in soil ecosystems. Future research should give specific attention to the combination of aggregated and non-aggregated soil nutrient pools.

5. Conclusions

Long-term fertilization regimes could shift the microbial community compositions and enzyme activities, and chemical properties of soil aggregates and thus influence soil functioning (e.g., nutrient cycling and crop productivity). Irrespective of chemical or organic fertilizaitons, soil macro-aggregates contributed most of the available nutrients to the bulk soil and therefore an important resource of nutrients to crop growth. Soil microorganisms are particularly abundant in smaller aggregates, and resources contained in micro-aggregates are more likely utilized by microbes than by plants while microorganisms in micro-aggregates were C-limited. Furthermore, organic fertilization could enhance soil nutrient capacity by promoting the macro-aggregation, but there is a risk of nutrient imbalance (e.g., C:N ratio imbalance) that warrants further investigation. Together, the current findings advance the knowledge of fertilization effects on soil aggregation and its role in shaping soil multiple functions. Further, we suggest ecological intensification of agriculture by integrating organic amendments could enhance soil macro-aggregation and biological regulation of soil ecosystem processes, sustaining the synergies between nutrient cycling and crop productivity.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.soilbio.2021.108533.

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