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Soil aggregate size-dependent relationships between microbial functional diversity and multifunctionality

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ABSTRACT

Soil stability and aggregates are important drivers of soil fertility and microbial diversity and are highly vulnerable to land degradation. However, the role of soil aggregates in driving the responses of microbial functional diversity and multiple ecosystem services and functions (multifunctionality) to further degradation (e.g., fertilization) remains largely unexplored and poorly understood. In this study, we used soils from long-term experiments involving inorganic and organic fertilization treatments to investigate the role soil aggregates (microscale) play in driving microbial functional gene diversity (via GeoChip) and the activity of multiple extracellular enzymes in an agricultural ecosystem. We found that microbial functional gene diversity has a significant and positive relationship with soil multifunctionality, which is enhanced in soil aggregates by organic fertilizer but is reduced by inorganic fertilizer. We also found that soil aggregate fractions indirectly controlled multiple ecosystem functions via changes in functional diversity. Smaller soil aggregates with higher resource availability (carbon and nitrogen) supported more ecological functions than larger aggregates under contrasting fertilizer management regimes. Soil multifunctionality is regulated by the differences in resource availability and not by microbial functional gene composition, which suggests that microbial functional diversity contributed more to multifunctionality than gene composition. Random forest analysis and structural equation modeling indicated that soil carbon and nitrogen and microbial functional diversity together determined the multifunctionality, whereas soil traits have more standardized total effects than functional diversity. Our study highlights that soil aggregation stratifies soil nutrition and microbial functional diversity, which leads to the differentiation of aggregate ecosystem multifunctionality.

1. Introduction

Soil aggregates are the basic components of soil structure and differences in pore size, oxygen potential, moisture content, organic matter, and predation pressure provides microscale heterogeneous habitats for distinct microorganisms (Ranjard et al., 2001; Davinic et al., 2012). Soil aggregates are thus considered to be the major factor governing microbial biodiversity, abundance, and community composition in soils at the microscale organization level (Vos et al., 2013; Rillig et al., 2017; Liao et al., 2018; Han et al., 2020a). These soil microbial characteristics could also further impact a number of microbial functions, e.g., soil carbon and nitrogen cycling (Garcia-Franco et al., 2015; Trivedi et al., 2017; Wang et al., 2018; Lin et al., 2019). Additionally, soil aggregate turnover caused by multiple factors, e.g., fertilizer management regimes, across different sizes is fundamental to understanding microbial composition and potential metabolism in soil ecosystems. Therefore, determining the microbial community and functional diversity within soil aggregate fractions is crucial when attempting to assess the effects of degradation (e.g., that caused by different fertilizer management regimes) on the maintenance of soil health and fertility.

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Soil carbon plays an essential role in forming and stabilizing soil aggregate, which contains different resource availability (e.g., carbon and nitrogen) with vary sizes (e.g., Jiang et al., 2014; Liao et al., 2018). The spatial heterogeneity of soil aggregates could maintain bacterial diversity (Vos et al., 2013), which in turn affects soil enzyme activity and special functional processes (Nannipieri et al., 2012). Attention has been paid to the heterogeneous distribution of microbial functional guilds (e.g., ammonia- and nitrite-oxidizing microbes) that possess specific functional gene at the soil aggregate level (Jiang et al., 2014; Han et al., 2018a; Han et al., 2018b; Wang et al., 2018). For example, soil aggregates with different resource availabilities (carbon and nitrogen) control the abundance/composition of many nitrifying microorganisms and their potential activity in a Vertisol under different fertilization regimes (Han et al., 2020b). Recent investigations have also revealed that soil aggregate fractions affected the bacterial diversity and multiple enzyme activities (Ling et al., 2014), while the carbon and nitrogen shaped the bacterial community structure under the soil aggregate level when different fertilizations regimes were applied to a Mollisol (Liao et al., 2018).These results suggest the pivotal role of soil aggregate nutrient heterogeneity (carbon and nitrogen) in regulating the soil microbial community and biodiversity, which further impacts microbial functional processes. However, the role played by soil aggregates when regulating the relationship between microbial functional diversity and multiple ecosystem functions (i.e., multifunctionality) remains poorly understood. In addition, whether soil aggregate with nutrient heterogeneity control the multiple ecosystem functions directly or indirectly also need to be explored.

Soils have the ability to simultaneously maintain multiple ecosystem functions and services (multifunctionality) (Jing et al., 2015; Lefcheck et al., 2015; Delgado-Baquerizo et al., 2016a), and soil biodiversity plays an important role in supporting the sustainable productivity of ecosystems (Cardinale et al., 2006; Balvanera et al., 2006; Hooper et al., 2012; Singh et al., 2014). Ecosystem multifunctionality indices have been widely used as an effective tool to assess complex and interactive functional processes (Allan et al., 2015; Jing et al., 2015; Bender et al., 2016; Mori et al., 2016; Delgado-Baquerizo et al., 2020). Over the past two decades, numerous studies have been conducted on the relationships between soil biodiversity and ecosystem functioning. Furthermore, biodiversity has been shown to enhance ecosystem multifunctionality (Lefcheck et al., 2015; Delgado-Baquerizo et al., 2016a), and this relationship is more linear than saturating (e.g., Peter et al., 2011; Reich et al., 2012; Mora et al., 2014). However, most of these previous studies focused on larger scales (Jing et al., 2015; Delgado-Baquerizo et al., 2016a; Gross et al., 2017; Bagousse-Pingue et al., 2019) and there is limited information on the variations in soil multifunctionality at the micrometer-scale (e.g., soil aggregates). And whether soil aggregate sizes shaping multifunctionality is still an open question. Therefore, it is very important to fill this knowledge gap and provide a more comprehensive understanding of the relationship between biodiversity and soil multifunctionality at the microscale.

Functional gene arrays (GeoChip) detect up to thousands of functional gene simultaneously and have become an important molecular tool for assessing the functional gene composition, biodiversity, abundance, and dynamics of microbial communities from diverse ecosystems (Rhee et al., 2004; Zhou et al., 2012; Xu et al., 2014). GeoChip 5.0 (Shi et al., 2019), the most advanced version, contains 161 961 distinct probes covering 385 417 gene sequences that target genes involved in microbial functional groups that play important roles in element cycling, including C (e.g., *amyA*, *xylA*, *glx*, *mcrA* and *pmoA*), N (e.g., *amoA*, *hao*, *narG*, *nirS/K* and *nifH*), P (e.g., *ppK* and *ppX*), and S (e.g., *sir*, *dsrA* and *dsrB*). The diverse functional guilds in soil ecosystems mean that GeoChip 5.0 could be used evaluate the relationship between microbial functional diversity and gene composition and multifunctionality.

In this study, we focused on how microbial functional diversity (GeoChip-based microbial functional gene diversity) and soil carbon and nitrogen influenced multifunctionality at the aggregate level in agricultural soils that have been subjected to different fertilizer treatment regimes. We hypothesized that (i) soil aggregate with nutrient heterogeneity (carbon and nitrogen) may play key roles in regulating the microbial functional diversity, which is positively linked to ecosystem multifunctionality; (ii) both soil carbon and nitrogen availability and microbial functional diversity may be strong predictors of soil ecosystem multifunctionality; and (iii) soil aggregate sizes mediate the differentiation of the multifunctionality.

2. Material and methods

2.1. Experimental site and soil sampling

The experimental site was located in Laiyang (36.9° N, 120.7° E), Shandong Province, northern China, which has a warm-temperate, semi-cloudy monsoon, climate. The annual average precipitation and temperature are 779 mm and 11.2 °C, respectively. The soil is a non-calcareous fluro-aquic and contains 19.2% clay, 28.7% silt and 52.1% sand. It has a pH of 6.8, total nitrogen of 0.5 g/kg, and organic carbon of 4.1 g/kg (Tian et al., 2017). A maize (*Triticum aestivum* L.) - wheat (*Zea mays* L.) crop rotation system commenced in 1978 and there were three randomly replicated plots with three fertilization treatments: (i) no fertilizer (control, CK); (ii) inorganic fertilizer (nitrogen, potassium, and phosphate, NPK); and (iii) organic manure fertilizer (M). The inorganic fertilizer treatments were supplied with 276 kg N ha^{-1} as urea, 135 kg K ha^{-1} as potassium chloride (KCl) and 90 kg P ha^{-1} as superphosphate [$Ca(H_2PO_4)_2$]. The manure was applied at a rate of 60 000 kg N ha^{-1}, which contained 18.4 g/kg P, 19.7 g kg^{-1} N and 331 g/kg carbon.

Soil samples were obtained in June 2017 after maize had been harvested. Six soil subsamples (approximately 5 cm in diameter) were removed from each experimental plot at a depth of 0–20 cm using a small trowel and then combined to make one soil sample per plot. All soil samples were immediately delivered to the laboratory and manually divided into two parts. One was stored at 4 °C for further experiments, such as soil aggregate fractionation (within one week), and the other was stored at −80 °C for storage.

2.2. Soil aggregate fractionation and soil properties

Wet-sieving method is a way to obtain water stable soil aggregate for microbiology study (e.g., Elliott, 1986; Ling et al., 2014; Liao et al., 2018). Therefore, three size of soil aggregates were manually fractionated by wet sieving into the following size fractions: (i) 2000-250 μm (macroaggregates), (ii) 250-53 μm (microaggregates) and (iii) < 53 μm fractions (silt and clay) as previously described (Elliott, 1986; Han et al., 2018a). The separated soil aggregates were then freeze-dried and stored at −80 °C for GeoChip analysis. The soil properties, including total nitrogen (TN), total carbon (TC) and SOC (soil organic carbon) content, were collected from Wan et al. (2020). And soil aggregate exchangeable NH_4^+-N (ammonium) was extracted with 2 M KCl (1:5, w/v) and measured on an FIAstar 5000 analyzer (Foss Tecator, Hillerød, Denmark) (Han et al., 2017). Soil nitrate levels were well below the detection limit because it is easily leached when sieving soil aggregates. Specifically, only soil aggregate carbon and nitrogen were measured due to that soil carbon content plays key roles in forming and stabilizing soil aggregate, promoting soil physical properties and nutrient recycling, and those variables could also predict soil aggregate multifunctionality.

2.3. Quantification of multiple soil ecosystem functions

We detected 14 extracellular soil enzymes involved in elemental cycling and used these to represent ecosystem functions (i.e., as indicators). These indicators were C-cycling enzymes (β-D-glucopyranoside, β-D-xylanase, α-D-glucopyranoside, β-D-cellobioside, invertase, phenoloxidase, peroxidase, and cellulase), N-cycling enzymes (β-N-acetyl-glucopyranoside and urease), P-cycling enzymes (acid, neutral

and alkaline phosphatase) and S-cycling enzyme (arylsulphatase). β-D-glucopyranoside, β-D-xylanase, α-D-glucopyranoside and β-D-cellobioside activities were assessed using the MUB-linked model substrates method (Saiyacork et al., 2002; Deforest, 2009); peroxidase and phenol oxidase activities were detected spectrophotometrically using pyrogallol as a substrate (Allison et al., 2008); phosphatase, invertase, urease and arylsulphatase activity were measured using the method described by Luo et al. (2016); and cellulase activity was determined according to method reported by Pancholy et al. (1973). In addition, the activities of soil enzymes attached to the same functional group were normalized. For instance, the P-cycling enzyme activity was assessed according to the following equation:

$$\text{Pase} = \sqrt[3]{(\text{neutral} \times \text{acid} \times \text{alkaline phosphatase}}$$

2.4. Soil DNA extraction and GeoChip 5.0 analysis

DNA was extracted from composite soil samples using a PowerSoil® DNA isolation kit (Mo Bio Laboratories, Carlsbad, CA, USA) following the manufacturer's protocol. The extracted DNA was then purified using DNA-EZ Reagent M Humic acid-Be-Gone B (Sangon Biotech, Shanghai, China). The quality of the extracted DNA was checked spectrophotometrically at 260 nm and 280 nm (NanoDrop, ND-2000, Thermo-Scientific, Wilmington, DE, USA). The absorbance ratios at 260 nm/280 nm were larger than 1.80.

The GeoChip 5.0 (60K) analysis was carried out to assess microbial functional gene composition, functional gene abundances, and diversity as described previously (Shi et al., 2019); Han et al., 2020a). Briefly, the DNA was labeled with fluorescent dye Cy3 using a random priming approach and purified by a QIA quick purification kit (Qiagen, Valencia, CA, USA) following the manufacturer's instructions. After measuring dye incorporation on a NanoDrop ND-1000 spectrophotometer (Nano-Drop Technologies), the DNA was then dried in a SpeedVac (ThermoSavant, Milford, MA, USA) at 45 °C for 45 min. The labeled DNA was suspended in hybridization buffer and hybridized at 67 °C in the presence of 10% formamide for approximately 16 h, and then scanned by a NimbleGen MS200 scanner (Roche, Madison, WI, USA) using a laser power and photomultiplier tube gain of 100%. The signal intensities were detected using Agilent Feature Extraction software (v. 12.1.1.1; Agilent, Santa Clara, CA, USA). Spots with signal-to-noise ratios lower than 2.0 were removed before statistical analysis, as described previously (He et al., 2008).

2.5. Statistical analysis

Soil enzyme activities were Z-score transformed, and a heat-map was generated by GraphPad Prism 7 (GraphPad Software, Inc., La Jolla, CA, USA). The coefficients of variation (CV) of the individual soil enzyme activities were then calculated. Different algorithms were used to assess the relationship between functional diversity and soil multifunctionality. First, Z-score standardized rates of the soil enzyme functions were averaged to obtain a multifunctionality index (average approach). An ordinary least squares (OLS) linear regression model was then constructed to test the relationships between GeoChip microbial functional gene diversity and soil multifunctionality (lowest Akaike formation criterion (AIC) = 17.65)). Second, the thresholds approach was also implemented to decode the correlation between GeoChip microbial functional gene diversity and multifunctionality using the "multifunc" package in R (Byrnes et al., 2014). A nonmetric multidimensional scaling (NMDS) analysis based on the Bray-Curtis dissimilarity was performed to explore any differences in the soil enzyme activity patterns among soil aggregates under the diverse fertilization regimes using the "vegan" package in R. A two-way PERMANOVA was employed to separate and quantitatively evaluate the impacts of the soil aggregate fractions and fertilization regimes on multiple enzyme activity patterns. The pivotal and credible predictors of soil multifunctionality among different factors were evaluated using a random forest analysis, which was performed using the "randomForest" package in R (Breiman, 2001). In addition, a structural equation model (SEM) was constructed to assess the direct and indirect effects of soil intrinsic variables on multifunctionality using the AMOS software (IBM SPSS AMOS, Chicago, IL, USA 21.0.0). All the data were normalized prior to modeling. The requirements of the parameters to fit the model included a root mean squared error of approximation of (RMSEA) $<$ 0.05, a low chi-square value (χ^2), a Fisher's P value of $0.05 < p \leq 1.00$ and a low Akaike formation criterion.

3. Results

3.1. Aggregate distribution and soil attributes under contrasting fertilization types

Inorganic/organic fertilizers did not significantly alter the mass distribution of the soil aggregates compared to the no fertilizer soil (Fig. S1). The mass proportion of the aggregates was highest in the [2000-250 μm] fraction, followed by the [<53 μm], [250-53 μm], and [> 2000 μm] fractions. The soil total carbon (TC), total nitrogen (TN), soil organic carbon (SOC), and NH_4^+ contents were higher in the [<53 μm] and [250-53 μm] fractions, and lower in the [2000-250 μm] fraction across the three fertilizer treatments (Table S1, Wan et al., 2020). Long-term fertilization increased the soil carbon and nitrogen contents in the fertilized treatments compared to the control across all aggregate sizes.

3.2. Soil aggregate enzyme activity, microbial functional diversity, and multifunctionality

The different soil aggregate sizes had clearly distinct functional traits when subjected to the different fertilizer management treatments (Fig. 1A). Among the enzyme activities assessed, C-, N-, P- and S-cycling were stimulated by fertilization following the aggregate size order [<53 μm] > [250-53 μm] > [2000-250 μm] for each fertilization treatment. Furthermore, the organic fertilization treatment enhanced soil functional traits more than inorganic fertilization for all soil aggregate sizes. The coefficient of variation for the C-, N-, P- and S-cycling enzyme activities in the soil aggregates showed different sensitivities, with coefficients of variation (CV) of 0.47, 0.65, 0.47 and 0.87, respectively. Nonmetric multidimensional scaling (NMDS), based on all soil enzyme activities, was performed to visualize the differences in soil functional traits among the various soil aggregate sizes and fertilizer management treatments (Fig. S2). Notable multi-functional enzyme activity differences were found among the three soil aggregate sizes (Fig. S2). In addition, the multi-functional enzyme activities of the inorganically and organically fertilized soil aggregates were clearly distinguishable from the control along the horizontal axis of the NMDS analysis (Fig. S3). Two-way permutational multivariate analysis of variance (PERMANOVA) demonstrated that soil aggregate size, rather than fertilization management, was the major driver that shifted the soil aggregate multifunction traits. Additionally, most of the soil C-, N-, P- and S-cycling related enzyme activities were positively correlated with each other (Fig. S4). The measured soil properties, such as TC, SOC, TN, and NH_4^+ also showed positive correlations with multiple enzyme activities, except for phenoloxidase and peroxidase (Fig. S5).

GeoChip data demonstrated that the microbial functional Shannon diversity was the highest in the [<53 μm] fractions, followed by that in the [250-53 μm] and the [2000-250 μm] fractions in the no fertilizer and inorganic fertilizer soils (Fig. 1B). There were no significant differences among the three soil aggregates following organic fertilizer treatment. Furthermore, the abundances of multiple functional genes involved in C- and N-cycling were also the greatest in the [<53 μm] size fractions, followed those in the [250-53 μm] and [250–2000 μm] fractions in the no fertilizer and inorganic fertilizer soils, but this was not the case for

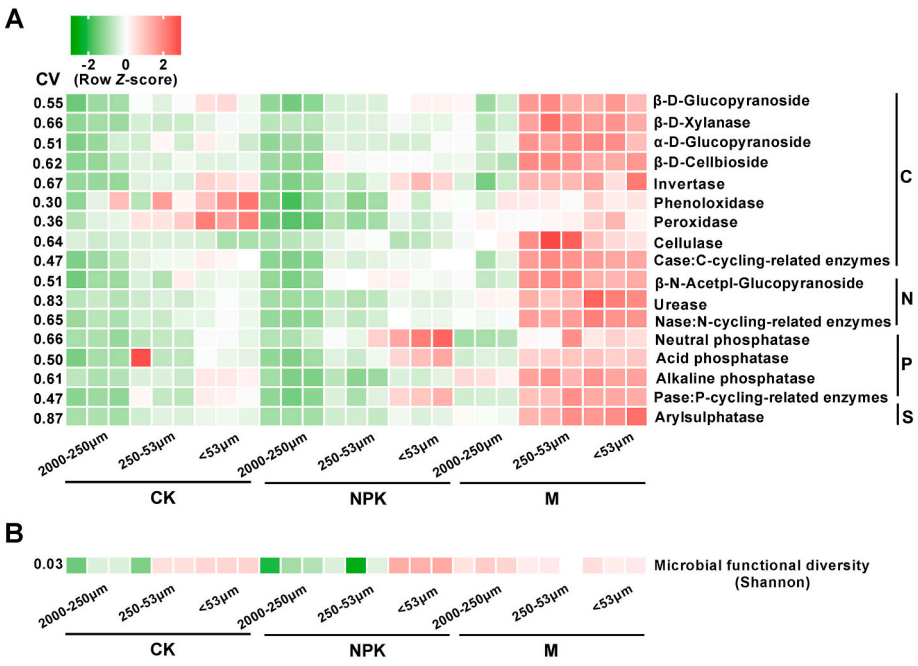

Fig. 1. Heat map showing the change in multiple enzyme activities (A) and microbial functional diversity (B) in the soil aggregates under three fertilization treatments. The coefficient of variation (CV) indicated the sensitivity for enzyme activity variation. The capital letters C, N, P and S show the respective C, N, P and S-cycle enzymes categories.

organic fertilizer soils (Figs. S6 and S7). In addition, there was significant soil multifunctionality stratification among the soil aggregates following both the no fertilizer and inorganic fertilizer treatments in the following order [<53 μm] > [250-53 μm] > [2000-250 μm] (Fig. 2A). The exception was the [250-53 μm] fraction following the organic fertilizer treatment.

3.3. Relationships between microbial functional diversity and multifunctionality

The average approach showed that the microbial functional diversity had a significant and positive linear relationship with soil multifunctionality ($R^2 = 0.24$, $p < 0.01$) (Fig. 2B). Simultaneously, there were also positive relationships between functional diversity and the C-, N- and P-cycling related enzyme activities, but not S-cycling (Fig. S8).

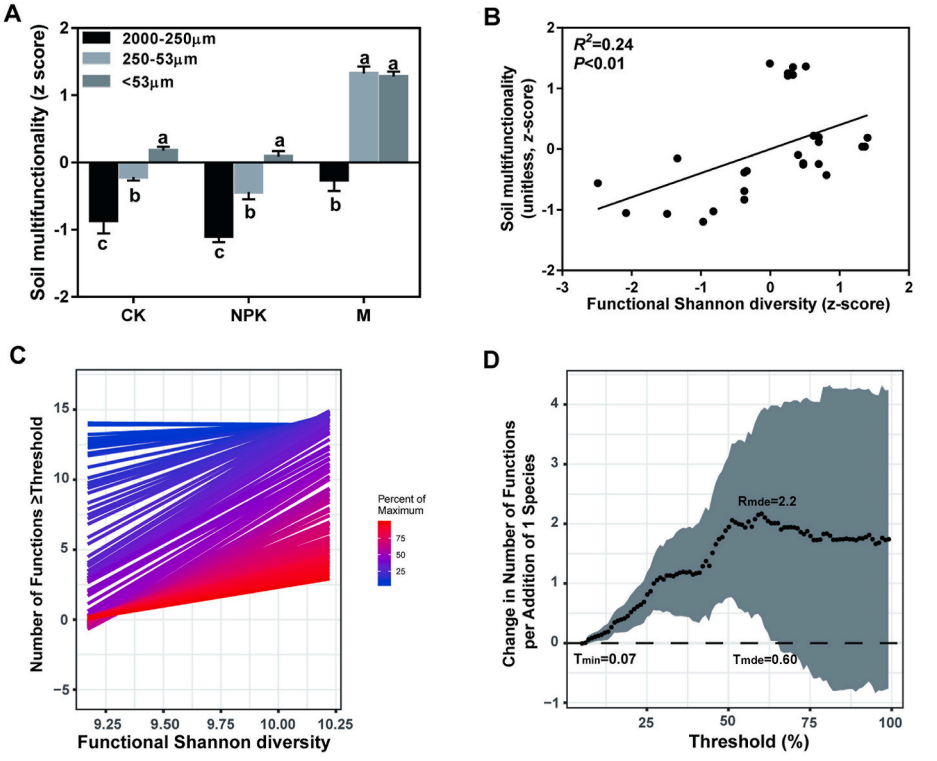

Fig. 2. Average multifunctionality index in response to soil aggregate stratification (A), and relationships between microbial functional Shannon diversity and ecosystem multifunctionality (B). Results of regression are as follows: $R^2 = 0.24$, $P < 0.01$, AIC = 17.65. Diversity effects for a range of ecosystem multifunctionality thresholds. Effect of the functional Shannon diversity on the number of functions above thresholds (C). Lines represent the slope between diversity and the number of functions greater than or equal to a threshold value ranging from 5 to 99% of maximum for each function. The dotted curves indicate the changes in number of functions per unit increment of diversity of bacteria (D). Tmin, is the minimum threshold that multifunctionality becomes influenced by changes in diversity, and Rmed, is the realized maximum effect of diversity on multifunctionality.

When reanalyzed by using the multiple threshold approach, a positive correlation was also observed between microbial functional diversity and soil multifunctionality (Fig. 2C). The minimum threshold (T_{min}) was 7%. At this point, functional diversity starts to have a positive impact on soil multifunctionality (Fig. 2D). The realized maximum effect of diversity (R_{mde}) was 2.2. Furthermore, functional diversity had the strongest positive influences at the 60% threshold, which suggests that increases in the diversity of one specific functional gene diversity could increase function by 2.2.

SOC and NH_4^+ had a significant ($p < 0.05$) positive correlation with microbial functional gene diversity, whereas soil TC and TN had little effect on microbial functional diversity ($p > 0.05$) (Fig. S9). The dissimilarity of soil multifunctionality was positively correlated ($r = 0.84$, $p < 0.01$) with soil microenvironmental factors (Fig. 3A), but not with soil microbial functional gene composition (Fig. 3B); although the microbial functional gene composition of the various soil aggregates was quite different (Fig. S10).

3.4. Direct and indirect effect s of environmental factors on soil multifunctionality

The random forest model suggested that soil TC, TN and organic C were reliable predictors ($p < 0.05$) of individual C-, N-, P- and S-cycling related enzyme activities (Fig. S11). Soil aggregate size plays vital roles in predicting C- and P-cycling enzyme activities, whereas fertilization treatments can be used to predict C-, N- and S-cycling enzyme activities. The random forest model explained 89.5% of the overall soil multifunctionality variance and soil organic carbon was the most prominent predictor (Fig. 4). Soil total carbon and nitrogen and microbial functional diversity were also important indicators ($p < 0.05$). Furthermore, the soil abiotic factors may be more important predictors of soil multifunctionality than microbial functional diversity (biotic factors). Interestingly, the results also showed that soil aggregate stratification ($p < 0.05$) was more important than fertilization management regimes ($p > 0.05$) when predicting soil multifunctionality.

The structural equation model (SEM) showed that soil variables, such as soil TC, TN, SOC, and NH_4^+, and microbial functional diversity could explain 98% of the variance in soil ecosystem multifunctionality (Fig. 5A). The SOC (path coefficient = 0.78; $p < 0.01$) had direct and positive effects on soil e cosystem multifunctionality, followed by TN (path coefficient = 0.20) and NH_4^+ (path coefficient = 0.11). The microbial functional diversity (path coefficient = 0.07) also had a direct and positive impact on soil ecosystem multifunctionality, whereas NH_4^+ had an indirect effect by regulating functional diversity. We analyzed the standardized total effects of a number of different individual parameters to further assess the comprehensive regulatory effect of the driving factors on soil multifunctionality (Fig. 5B). Our data confirmed that soil carbon had the greatest positive and integrated effect on soil multifunctionality, followed by soil TN and fertilization type. In particular, SOC had completely standardized direct effects, whereas TC had completely standardized indirect effects on soil multifunctionality. In contrast to fertilization, soil aggregate size had a high negative total effect on soil multifunctionality, which indicated that smaller soil aggregates have greater impacts on soil multifunctionality than larger ones.

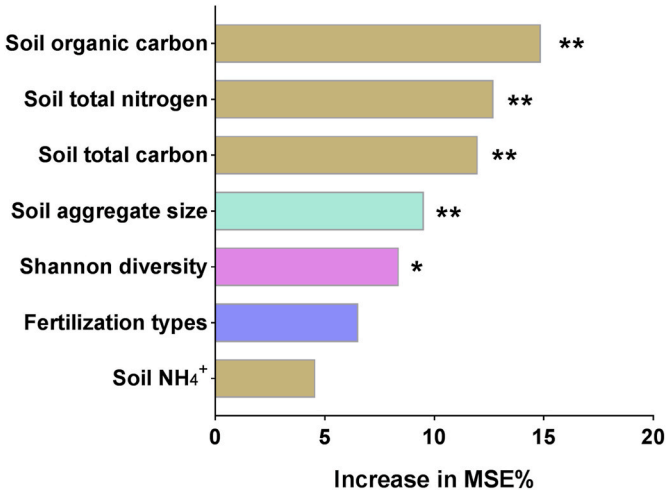

Fig. 4. Main predictors of ecosystem multifunctionality. The figure shows the Random Forest mean predictor importance (% of increase of MSE) of soil variables drivers and functional microbial diversity (Shannon index, bits) on ecosystem multifunctionality. Significance levels of each predictor are as follows: *$p < 0.05$ and **$p < 0.01$.

4. Discussion

The microscale spatial heterogeneity and complexity of soil aggregates impact the distribution, abundance, activity, and taxonomic composition of microorganisms, and determines how microbial diversity and ecosystem functions are sustained and evolve. In our study, multiple enzyme activities in the soil aggregate fractions followed the order of [<53 μm] > [250-53 μm] > [2000-250 μm] regardless of fertilization treatment. This agreed with some previous results that enzyme activity/specific processes (e.g., β-xylosidase, N-acetyl-β-glucosaminidase and potential nitrification activity) and microbial abundance were higher in smaller soil aggregates with a greater soil carbon and nitrogen content than larger aggregates (Jiang et al., 2014; Nie et al., 2014; Trivedi et al., 2015). The distribution pattern for microbial functional abundance (e.g., C- and N-cycling related microbial functional abundances) and multiple enzyme activities appeared to be in line with resource

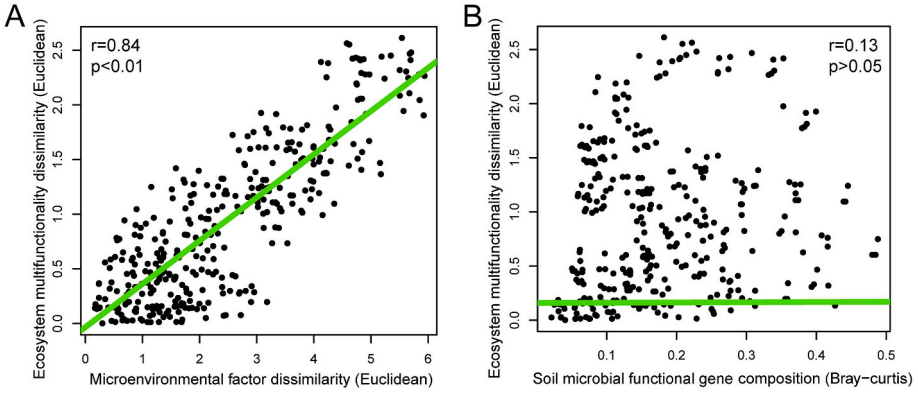

Fig. 3. The relationship between the dissimilarity matrices of ecosystem multifunctionality dissimilarity and microenvironmental factors dissimilarity (including soil total carbon and nitrogen, organic carbon and NH_4^+) (A) and the microbial functional gene composition dissimilarity using linear regression (B).

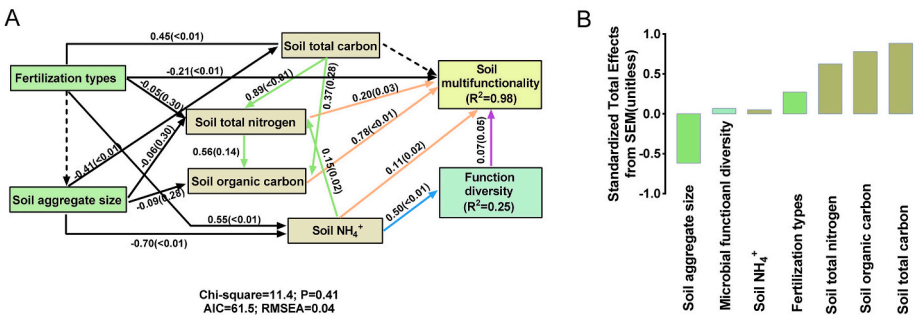

Fig. 5. Direct and indirect effects of soil variables and microbial functional diversity on ecosystem multifunctionality (A). Standardized total effects (direct plus indirect effects) derived from the structural equation models depicted above (B). Numbers adjacent to arrows are indicative of the effect-size of the relationship. Significance p-value is in the brackets. Numbers following the included variables show the explained percentage of their variance by their predictors.

availability within the soil aggregates, which suggests that greater resource availability in smaller soil aggregates could support larger microbe abundance levels and multiple enzyme activities. Indeed, most of the enzyme activities were positively related to soil nutrition (Fig. S5).

In this study, the variation in soil aggregate size with different microbial functional diversities was found to be a major factor influencing the variability in multiple soil enzyme activities (Fig. 1 and Fig. S2), which suggests that soil aggregate stratification could indirectly result in soil multifunctionality differentiation. These results support the hypothesis that smaller soil aggregates, rather than larger ones, have a greater positive effect on soil multifunctionality (Fig. 2A). Furthermore, the smaller soil aggregates had a greater microbial diversity than the larger ones when they were subjected to different fertilizer management regimes (Fig. 1B). The results also show that microbial functional diversity positively and directly regulates soil multifunctionality (Fig. 2C). These observations are in agreement with the hypothesis that a strong positive relationship exists between microbial functional diversity and multifunctionality (Wagg et al., 2014; Delgado-Baquerizo et al., 2016a; Mori et al., 2016). This relationship also imply that there is a lack of functional redundancy in soil aggregates, since a loss in microbial functional diversity could result in a reduction in some functions (Griffiths et al., 2012; Pasari et al., 2013; Delgado-Baquerizo et al., 2016b). Our findings suggest that microbial functional diversity, stratified by soil aggregate size, is an important factor that affects the simultaneous maintenance of soil ecosystem multifunctionality by supporting different soil processes, such as organic matter decomposition and nitrogen cycling, at the microscale (Fig. 1). One possible explanation for this is that soil ecosystem function could be coupled with biodiversity due to niche complementarity and/or microbial interactions, so that related species having dissimilar functional traits coexist in order to enhance the utilization of resources. For example, the enzyme-mediated degradation of organic matter from complex polymers into simpler and more labile monomers requires the cooperation of a large and distinct group of microorganisms (Hooper et al., 2000; Wardle et al., 2004; van der Heijden et al., 2008). Carbon-cycling enzyme activity is thought to have a positive relationship with SOC and microbial functional diversity, and this hypothesis was supported by our experimental data (Fig. S5, Fig. S9). Additionally, our random forest model clearly demonstrated that soil TC, TN, SOC, and NH_4^+ were pivotal determinants of C-, N-, P- and S-cycling related enzyme activities and soil multifunctionality (Fig. S11). Therefore, soil aggregates with relatively higher carbon and nitrogen availabilities could support more diverse microbes, which would further accelerate biogeochemical cycles (Fig. S5) and enhance ecological functional processes. To our knowledge, this is the first study to link microbial functional diversity and soil properties to multifunctionality at the soil microscale.

Microbial functional genes encoding multiple enzymes involved in primary biogeochemical processes can connect microbial taxonomic composition to its potential metabolic capacity and ecological functions (Torsvik and Øvreås, 2002; Xie et al., 2011; Bai et al., 2013). Our GeoChip data revealed that soil aggregates could significantly shape functional gene composition regardless of fertilization treatment (Fig. S10). However, no obvious relationship was detected between the dissimilarity of soil multifunctionality and functional gene composition (Fig. 3), which suggests that ecosystem multifunctionality is not determined by microbial functional gene composition at the soil aggregate scale under contrasting fertilizer regimes. This contradicts several previous findings (Wagg et al., 2014; Zheng et al., 2019), which suggested that both soil biodiversity and community composition govern multifunctionality in soil ecosystems. Unlike the methods used in the studies by Wagg et al. (2014) and Zheng et al. (2019), the GeoChip was used in our study to evaluate functional community composition. This is one possible reason for the different results. More importantly, the differences may also result from the variation in the spatial heterogeneity of microbial community composition and soil multifunctionality at the soil microscale. In addition, our study indicates that microbial diversity and functional gene composition affected multiple ecosystem functions in a non-cooperative way (Fig. 3) and that microbial diversity contributed more to multifunctionality than functional gene composition. Furthermore, our study highlights that the variation in soil microbial functional gene diversity was more important than variation in the microbial functional gene composition when attempting to predict multifunctionality at the microscale level.

This study focused on the effect of soil resource availability (carbon and nitrogen, abiotic factors) and microbial functional diversity (biotic factors) on multifunctionality at the soil aggregate level. Furthermore, the high variability (98%) could account for the multifunctionality identified by structural equation modeling (Fig. 5). It is intuitive to speculate that other biotic and abiotic factors may not be able to further improve the prediction of soil multifunctionality despite of their contributing. Soil organic carbon exerted strong impacts on multifunctionality dynamics. The direct effects of carbon and nitrogen could be partly due to the supply of available nutrients and energy that can be used by diverse heterotrophic microorganisms to produce enzymes, which means that higher nutrition levels could indirectly improve the multifunctionality. On the other hand, the soil organic carbon could improve the microenvironment for distinct microbes by promoting soil aggregate physical stability, which is of importance for the function of microorganisms.

5. Conclusions

In this study, our results showed that soil aggregate size could indirectly and negatively determine soil multifunctionality, and that smaller soil aggregates have greater influence on soil multifunctionality than larger ones. Soil multifunctionality was enhanced in soil aggregates by organic fertilizer, but was reduced by inorganic fertilizer. A combination of these biotic (microbial functional diversity) and abiotic characteristics (soil carbon and nitrogen) could improve our assessment of soil multifunctionality in soil aggregates subjected to different fertilizer

management regimes. Overall, our study provides new insights into the importance of soil attributes and microbial functional diversity during the regulation of ecosystem functions at the microscale.

Authors' contributions

SH, WLC, QYH planned the research and collected soil samples; SH conducted the laboratory analyses and collected the raw data; SH, MDB, XSL, QYH wrote the draft of the manuscript, and MDB, YRL, JoyDVN, WLC, JZZ, QYH contributed to revisions; All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and material

Not applicable.

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.2021.108143.

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