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Biochar

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The application of biochar and organic fertilizer substitution regulates the diversities of habitat specialist bacterial communities within soil aggregates in proso millet farmland

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Abstract

Biochar and organic fertilizer substitution have been utilized to improve crop yields and promote sustainable agricultural development. However, few studies have focused on how biochar and organic fertilizer substitution regulate the diversities of bacterial meta-communities and sub-communities (i.e., habitat specialists and generalists) within soil aggregates on the Loess Plateau. A field experiment, containing six treatments, namely no fertilizer (CK), no fertilizer plus 10^4 kg ha^{-1} biochar (CK + B), traditional N fertilizer (TF, 150 kg N ha^{-1}), traditional N fertilizer plus 10^4 kg ha^{-1} biochar (TF + B), organic fertilizer replacing (OF), and organic fertilizer replacing plus 10^4 kg ha^{-1} biochar (OF + B), was adopted to explore the influences of biochar and organic fertilizer substitution on the size distribution, nutrient levels, extracellular enzymatic stoichiometry, bacterial meta-community and sub-community diversities, structures, ecological networks, and assembly processes within bulk soil, large macro-aggregates (> 2 mm), small macro-aggregates (0.25–2 mm), and micro-aggregates (< 0.25 mm) in proso millet farmland. Results showed that compared to CK + B treatment, OF + B treatment enhanced the distribution of large macro-aggregates while decreasing the distribution of micro-aggregates. OF + B treatment also markedly elevated the contents of total nitrogen (TN), soil organic carbon (SOC), nitrate nitrogen (NO_3^--N), ammonium nitrogen (NH_4^+-N), as well as activities of C-, N-, and P-acquiring enzymes, enhanced the carbon limitation, and reduced the nitrogen limitation in the bulk soil and macro-aggregates. Compared to CK treatment, OF and OF + B treatments significantly increased the observed OTUs of specialist bacterial communities in large and small macroaggregates, while having no effect on generalist bacterial communities. The impacts of biochar and fertilizer additions, as well as soil aggregates, on the community structure and composition of specialists were greater than those of generalists. RDA analysis showed that NH_4^+-N content had a significant influence on the meta-community and sub-community structure within three aggregates. Ecological network analysis indicated that OF + B treatment increased the whole bacterial network complexity and stability compared to CK + B treatment. Meanwhile, the bacterial network of specialists with biochar amendment was more complex and tighter than that without biochar. Null-model analysis showed that biochar and fertilizer regimes rarely affected the assembly processes of the bacterial meta-community and sub-community within bulk soil and three aggregates, mainly determined by stochastic process. These observations offer a theoretical basis for understanding the intrinsic relationships between bacterial microbial communities and soil aggregates regulated by biochar and fertilization regimes in the semi-arid region.

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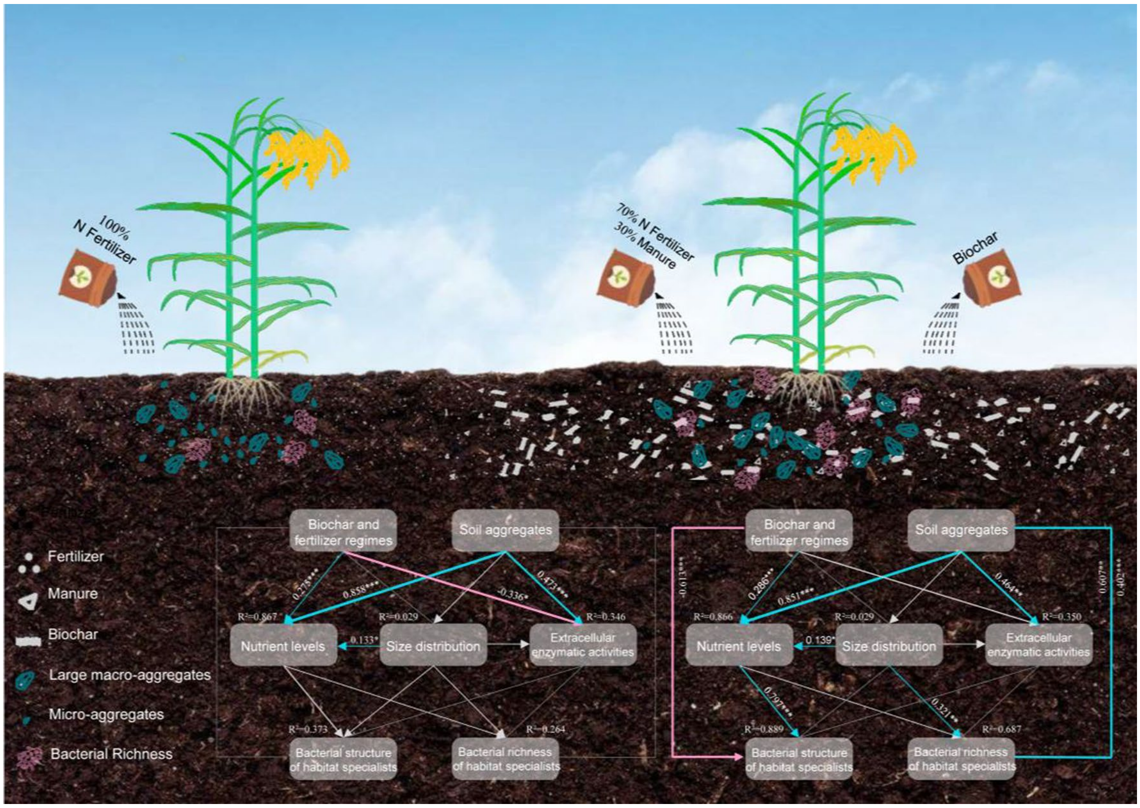
Highlights

- Biochar and fertilizer regimes had a profound influence on the richness and structure of bacterial communities in specialized habitats.
- Organic fertilizer substitution markedly enhanced the bacterial richness of habitat specialists in macro-aggregates.
- NH_4^+-N was the important variable regulating the bacterial community and sub-community structure within soil aggregates.
- Bacterial network of habitat specialists with biochar addition was more complex and tighter than that without biochar

Keywords Broomcorn millet, Biochar and organic fertilizer, Habitat generalists and specialists, Soil aggregates, Network analysis

Graphical Abstract



1 Introduction

Proso millet (*Panicum miliaceum* L.) is recognized as a promising "smart crop" for the future, known for its drought resistance and tolerance to barren conditions (Tian et al. 2023). It has become a predominant crop on the Loess Plateau, the largest dryland agricultural region characterized by low soil fertility and high erosion susceptibility, leading to decreased agricultural productivity (Lu et al. 2012; Qin et al. 2018; Zhang et al. 2023a). Therefore, it is imperative to develop efficient agricultural management strategies that focus on improving soil fertility, substantively boosting crop yield, and establishing environmentally sustainable agricultural regimes in the specified region.

Biochar, derived from the pyrolysis of plant biomass under anaerobic conditions, serves as a valuable organic carbon source material (Zhang et al. 2024). It offers unique advantages including strong adsorption capacity, substantial surface area, and nutrient-rich content (Wu et al. 2023), which could amend soil properties, improve the original soil structure, promote nutrient absorption, and facilitate aggregate formation (Zhang et al. 2020a, b; Zhong et al. 2024; Zhou et al. 2019). Although biochar boosts crop productivity in the short term by enhancing nutrient cycling, it does not yield the same effect as fertilizer (Zhang et al. 2023b). Hence, the integrated use of biochar and various types of fertilizers represents a sustainable approach deserving further exploration, especially concerning the reduction of fertilizer use while maintaining soil health and crop yields. However, excessive addition of nitrogen fertilizer not only wastes resources, raises production costs, thereby reducing farmers' income, but also leads to adverse effects such as soil acidification, compaction, and loss of fertility (Hammad et al. 2018; Park et al. 2023; Sun et al. 2020), whereas organic manure substitution is a preferable option to mitigate the adverse impacts of nitrogen fertilizer by increasing soil quality (Zhao et al. 2024). Several studies demonstrated that compared to solely adding biochar, application of biochar in conjunction with organic or nitrogen fertilizer can enhance crop yields, enhance soil nutrient levels, and shape microbial diversities (Dong et al. 2022a; Liu et al. 2022a; Wu et al. 2023). At present, the variations of soil properties, soil enzyme activities, and bacterial communities among various soil aggregates under biochar in conjunction with fertilizer regimes have not been thoroughly explored.

Microorganisms are prevalent and play vital roles on global biogeochemical processes (Li et al. 2024a, b). Microbial ecological groups are categorized into habitat specialists or generalists depending on the unique abilities to adapt to a broad spectrum of environmental filtering (Hu et al. 2019; Muller 2019). The specialists, with a narrower niche breadth, facilitate rapid responses to diverse environmental conditions, whereas the generalists demonstrate a wider niche breadth, which can contribute to the stability of the ecosystem (Gad et al. 2020; Jian et al. 2016; Székely and Langenheder 2014). Due to these variances in environmental adaptability, specialists and generalists are expected to undergo distinct assembly processes (e.g. deterministic process, stochastic process) (Pandit et al. 2009), and the influence of both assembly processes varies among the specialists and generalists. For example, previous studies confirmed that the deterministic process regulated the ecological assembly of specialists, whereas the colonization of generalists was influenced by stochastic process (Hou et al. 2019; Liao et al. 2016; Pandit et al. 2009). On the contrary, some argued that the generalists and specialists were mainly governed by deterministic process (Mo et al. 2021; Székely and Langenheder 2014). Therefore, it is imperative to determine whether the specialists and generalists of bacterial communities demonstrate similar or contrasting assembly patterns. Furthermore, in recent years, ecological network analysis has emerged as a potent tool for unraveling the stability and complexity of networks by computing node-level topological properties, namely nodes, edges, degree, and average pathway length (Faust and Raes 2012; Jiao et al. 2017), and the approach offers meaningful insights beyond microbial composition and diversities. Previous studies have indicated that compared with the absence of biochar, the application of an appropriate amount of biochar increased the network complexity, and the co-application of fertilizer and biochar also enhanced the complexity of the co-occurrence network (Duan et al. 2023; Mukhamed et al. 2023; Yan et al. 2021). However, the responses of network patterns and assembly processes of specialists and generalists within bacterial communities, especially in terms of soil aggregates in proso millet farmland, to biochar application and fertilization strategy on the Loess Plateau have not been fully elucidated.

In this study, a field experiment was carried out to explore the influences of the addition of biochar and fertilizer regimes (i.e., traditional nitrogen and substituting nitrogen with organic fertilizer) on the soil aggregate nutrient levels, eco-enzymatic stoichiometry, bacterial community and sub-community (habitat generalists and specialists) diversities, composition, ecological networks, and assembly processes in proso millet farmland. Specifically, the aims were to (i) explore the influences of combined application of biochar and fertilizer regimes on the nutrient properties and extracellular enzyme activities of soil aggregates; (ii) evaluate the effects of biochar and fertilizer application on bacterial diversity and composition within soil aggregates, and identify the key environmental variables that influence both the bacterial community and sub-community structures; (iii) elucidate how the addition of biochar and various fertilizers regulate the assembly processes and symbiotic networks of bacterial meta-communities and sub-communities within soil aggregates.

2 Materials and methods

2.1 Experimental design

A field trial was established at the Yulin Experimental Station of Northwest A&F University (109°46′26″E, 38°22′52″N) in 2021. The site is marked by the typical arid and semi-arid continental monsoon climate, receiving a mean annual precipitation of around 400 mm, over 60% of which occurs between June and September. The soil profile at a depth of 0–20 cm is characterized as sandy loam, containing 5.97 g kg^{-1} of organic matter, 0.25 g kg^{-1} of total nitrogen, 17.14 mg kg^{-1} of alkali-hydrolyzable nitrogen, 114.20 mg kg^{-1} of available potassium, 25.49 mg kg^{-1} of available phosphorus, and 8.76 of pH.

The experiment was conducted in 2022, comprising six treatments with three replicates per treatment: no fertilizer (CK), no fertilizer plus 10^4 kg ha^{-1} biochar (CK + B), traditional N fertilizer (TF, 150 kg N ha^{-1}), traditional N fertilizer plus 10^4 kg ha^{-1} biochar (TF + B), 30% organic manure replacing N fertilizer (OF), and 30% organic manure replacing N fertilizer plus 10^4 kg ha^{-1} biochar (OF + B). In the TF and OF, TF + B and OF + B treatments, equal amounts of N fertilizer were applied. All treatments included the same amounts of P_2O_5 (90 kg ha^{-1}) and K_2O (75 kg ha^{-1}). The organic fertilizer utilized in this study, primarily composed of sheep manure, was supplied by Anhui Lannong Technology Co., Ltd. (Hefei, China). The contents of total nitrogen, organic matter, and pH of this fertilizer were 1.02%, 70%, and 8.53, respectively. The biochar was produced by pyrolyzing wheat straw at 500 °C, which was obtained from Hubei Jinri Ecological Energy Co., Ltd. (Anlu, China). The contents of organic matter, total nitrogen, total potassium, total phosphorus, and pH in the biochar were 617.96 g kg^{-1}, 1.65 g kg^{-1}, 5.65 g kg^{-1}, 20.14 g kg^{-1}, and 10.39, respectively. Proso millet cultivar 'Shaanmi No. 2' was selected for this study, with sowing and harvest occurring on June 19 and October 4. The planting density was 600 000 plants ha^{-1}. Before sowing, biochar, nitrogen fertilizer, and organic fertilizer were evenly applied to the surface soil, and then they were spun into 0–15 cm deep soil using a rotary cultivator.

2.2 Soil sampling

Seventy-two soil samples at a depth of 0–20 cm were sampled using the five-point sampling method at the mature stage of proso millet, including 6 treatments, 4 aggregates and 3 repetitions. To prevent the disruption of soil aggregate structure, the soil in contact with the shovel was carefully removed, and the soil was delicately detached by hand following the natural break points on the surface, yielding small clumps of about 1 cm in size. Roots and rocks were picked out by sieving the samples with a 2 mm mesh, after which they were mixed to create bulk soils. The soil particle size fractions were classified using the dry sieving method (Zhang et al. 2019a). In brief, 100 g soil sample was subjected to manual sieving using a series of sieves with mesh sizes of 0.25 and 2 mm. The sieving process involved 300 shakes for 10 min with a 3-cm amplitude to effectively isolate the soil into various aggregate fractions. Specifically, (1) the soil sample with particle size more than 2 mm in diameter is defined as > 2 mm (large macro-aggregates); (2) the soil sample with particle size between 0.25 and 2 mm in diameter is defined as 0.25–2 mm (small macro-aggregates); (3) the soil sample with particle size smaller than 0.25 mm in diameter is defined as < 0.25 mm (microaggregates). A portion of the sieved soil was placed at − 80 °C for DNA extraction, while the remaining sieved soil was placed at − 20 °C for the analysis of soil properties. Total nitrogen (TN) content was assessed through the Kjeldahl digestion technique outlined by Purcell and King (1996). Soil organic matter (SOM) content was quantified following the potassium dichromate titrimetric method as described by Nelson and Sommers (1982). Soil samples were agitated for 1 h in a solution of 2 mol L^{-1} KCl, subsequently analyzed for nitrate (NO_3^--N) and ammonium (NH_4^+-N) concentrations with an auto analyzer (SEAL-AA3, Germany).

The extracellular enzymatic activities of soil, specifically C-acquiring enzymes [i.e., cellobiohydrolase (CBH), β-1,4-glucosidase (BG)], N-acquiring enzymes [i.e., β-*N*-acetylglucosaminidase (NAG), ʟ-leucine aminopeptidase (LAP)], and P-acquiring enzyme [i.e., alkaline phosphatase (AP)] were assessed with commercially available kits (Solarbio, China). Metabolic limitations were characterized through the evaluation of the length and angle of vectors representing extracellular enzymatic activities in relation to their proportional activities (Cui et al. 2021; Moorhead et al. 2016). In this model, the determination of vector angle is based on Eq. 1, while the calculation of vector length is derived from Eq. 2. In these equations, x and y denote the activities related to C-acquiring enzymes versus P-acquiring enzymes and C-acquiring enzymes versus N-acquiring enzymes, respectively. The enhancement of C limitation was observed with an increasing vector length. Vector angle exceeding 45° indicates a P limitation, while an angle below 45° signifies a N limitation. The degree of N limitation increases as the vector angle decreases, while P limitation intensifies as a larger vector angle.

$$\text{Angle}\left(^{\circ}\right) = \text{DEGREES}\left(\text{ATAN2}\left(x, y\right)\right) \tag{1}$$

$$\text{Length} = \text{SQRT}\left(x^2 + y^2\right) \tag{2}$$

2.3 DNA extraction and sequence analysis

Total DNA was extracted from 500 mg of soil in each sample using DNeasy 96 PowerSoil Pro QIAcube HT kit (QIAGEN, Germany). High-throughput sequencing of extracted DNA was performed using Illumina Miseq platform (Miseq-PE300, Majorbio Bio-pharm Technology Co., Ltd.). The bacterial 16S rRNA genes were amplified from the V3–V4 regions using the primer sets 338F/806R. Subsequently, the raw sequence was filtered by quality control, and USEARCH was employed to remove the chimeric sequences (Edgar et al. 2011). All sequences were consolidated and categorized into operational taxonomic units (OTUs) with 97% similarity adopting UPARSE algorithm (Edgar et al. 2011). Subsequently, the bacterial OTU sequences were matched against the Silva database (v138) (Quast et al. 2013).

2.4 Habitat specialists and generalists

The bacterial OTUs were categorized as either habitat specialists or generalists by assessing their specialization values falling below the lower 95% confidence interval or exceeding the upper 95% confidence interval, respectively, and this categorization was conducted using 1000 permutations with the EcolUtils package. Three methods were employed to estimate the specialization of bacterial OTUs, namely, Levins' niche breadth, occurrence, and Shannon diversity. The shared bacterial OTUs among the three methods were classified as either generalists or specialists (Additional file 1: Fig. S1). In total, we identified 1886 OTUs as habitat specialists, and 576 OTUs as habitat generalists within the bacterial community, respectively.

2.5 Statistical analysis

Statistical analyses were conducted utilizing the R program (v4.4.1). In this study, bacterial phyla with relative abundance exceeding 1% in all samples have been earmarked for further analysis. The alpha diversities (e.g. Shannon index and observed OTUs) were evaluated adopting the picante package. Principal Coordinate Analysis (PCoA) was employed to illustrate the clustering patterns among the treatments conducted using the vegan package. Permutational Multivariate Analysis of Variance (PERMANOVA) was employed to evaluate the impacts of biochar, fertilizer, and aggregate on the dissimilarity of bacterial communities using the ape package. Analysis of similarities (ANOSIM) was adopted to assess the effects of biochar, fertilizers and aggregates on the dissimilarity of bacterial communities employing the vegan package. To explore the influences of soil properties, C-acquiring enzymes, N-acquiring enzymes, and P-acquiring enzymes on the bacterial community within four aggregates, Redundancy Analysis (RDA) was employed adopting the vegan package. Meanwhile, the enfit function in the vegan package was also adopted to assess the soil properties, C-acquiring enzymes, which are the sum of CBH and BG, N-acquiring enzymes, (sum of CBH and BG), N-acquiring enzymes (sum of NAG and LAP), and P-acquiring enzymes (expressed by AP), which markedly influenced the bacterial communities within four aggregates. Partial least squares path modelling (PLS-PM) was adopted using plspm package. The analysis of co-occurrence networks for six fertilizer regimes and four aggregates was estimated adopting the Molecular Ecological Network Analysis Pipeline (MENAP) (Deng et al. 2012; Zhou et al. 2010) based on random matrix theory (RMT) applied to OTUs from a minimum of 8 out of the total 18 samples and 7 out of the total 12 samples, respectively. Data preparation and settings of RMT were executed using the default parameters of the MENAP platform, and the visualization of co-occurring network depended on the Gephi platform for analysis and presentation. Subsequently, the analysis included the calculation of the number of edges, nodes, degree, diameter, average path length (APL), and average clustering coefficient (ACC) adopting the igraph package.

The relative influence of assembly processes in fertilizer regimes and aggregates was assessed through null-model analysis (Stegen et al. 2013). Adjacency matrices of the beta-nearest taxon index (betaNTI) and Raup-Crick based on Bray–Curtis distance (RCbray) were employed for evaluating alterations in phylogenetic and taxonomic diversity. Values of betaNTI greater than 2 show variable selection, while values lower than -2 signify homogeneous selection in deterministic processes. When |betaNTI| is less than 2, RCbray falls 0.95 or exceeds below -0.95, it signals homogeneous dispersal or dispersal limitation in stochastic processes, respectively. If |RCbray| is less than 0.95, it is adopted to evaluate the impact of "undominated" assembly.

The variations in soil properties, enzymatic stoichiometry parameters, community composition, alpha diversities, and betaNTI values were evaluated using Duncan's test in SPSS software (SPSS, Inc., Chicago, IL, USA). Spearman correlation tests were employed to estimate the relationships between soil properties of four soil aggregates and enzymatic stoichiometry parameters versus alpha diversities, and these analyses were carried out utilizing the Hmisc package.

3 Results

3.1 Effects of biochar and fertilizer regimes on the soil properties and enzymatic stoichiometry under soil aggregates

For soil properties, regardless of the application of biochar, compared to no fertilization and addition of nitrogen fertilizer, the organic fertilizer substitution markedly enhanced the large macro-aggregate (> 2 mm) size distribution, had no effect on the small macro-aggregate (0.25–2 mm) size distribution, but markedly reduced the micro-aggregate (< 0.25 mm) size distribution. Furthermore, compared to non-biochar application, the addition of biochar significantly increased the large macro-aggregate (> 2 mm) size distribution, and decreased the micro-aggregate (< 0.25 mm) size distribution (Fig. 1). Apart from soil particles less than 0.25 mm, irrespective of the presence of biochar addition, the SOC content in TF and OF treatments showed varying degrees of increase compared to CK treatment. The SOC content in soils of various particle sizes exhibited a significant increase upon the addition of biochar compared to soils without biochar, and the soil particles more than 2 mm had the highest SOC content compared to other particle sizes. In the soil particles more than 2 mm, the TN content significantly increased in OF treatment compared to CK and TF treatments. The addition of biochar did not affect the TN content compared with the treatments without biochar application. After application of biochar in bulk soil and soil with particle size ranging from 0.25 to 2 mm, there was an obvious increase in NH_4^+-N and NO_3^--N contents in TF and OF treatments compared to CK treatment. This increase was particularly notable when organic fertilizer replaced nitrogen fertilizer (Table 1).

For extracellular enzyme activities of soil, in soils amended with biochar, OF treatment markedly enhanced the activities of CBH and BG compared with TF treatment, with the exception of particle size smaller than 0.25 mm, and LAP activity increased in all particle size fractions of the soil. ANOVA analysis indicated that the application of biochar had no effect on the activities of CBH and AKP (Additional file 1: Table S1). After applying biochar to the soil, TF and OF treatments increased the activities of C-acquiring enzyme and N-acquiring enzyme compared to CK treatment within the four aggregates, but ANOVA showed that fertilizer and biochar treatments had no effect on P-acquiring enzyme activity (Table 1).

In soil treated with biochar, OF treatment obviously improved the vector length of four aggregates compared to TF treatment (Fig. 2b, e, h, k). In soils without biochar application, compared with CK treatment, TF treatment showed no impact on vector angles in the bulk soil and three aggregates. However, OF treatment significantly reduced the vector angle of soil particles more than 2 mm and less than 0.25 mm (Fig. 2c, f, i, l).

3.2 Effects of biochar and fertilizer regimes on the bacterial community composition and diversities under soil aggregates

The prevalent phyla within the bacterial communities across samples were *Proteobacteria*, *Chloroflexi*, *Actinobacteriota*, and *Acidobacteriota*, observed within both the meta-community and habitat generalists (Additional file 1: Fig. S2A, B). However, the most prominent phyla present in the bacterial communities across samples included *Firmicutes*, *Proteobacteria*, *Bacteroidota*, and *Chloroflexi*, found in the specialists (Additional file 1: Fig.

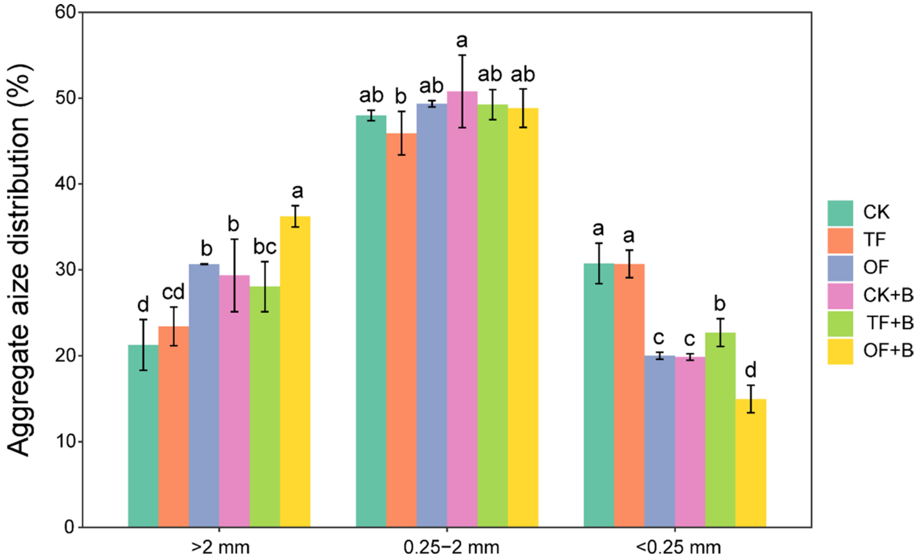


Fig. 1 The size distribution of soil aggregates under biochar amendment and fertilizer regimes. Different lowercase letters indicate significant differences ($p < 0.05$) in different treatments

Table 1 Soil properties and extracellular enzymatic stoichiometry within different aggregates under biochar and fertilizer regimes

Aggregate	Treatment	SOC (mg g^{-1})	TN (mg g^{-1})	NH_4^+-N (mg kg^{-1})	NO_3^--N (mg kg^{-1})	C-acquiring enzyme (nmol h^{-1} g^{-1})	N-acquiring enzyme (nmol h^{-1} g^{-1})	P-acquiring enzyme (nmol h^{-1} g^{-1})
Bulk soil	CK	2.97 ± 0.09f	0.248 ± 0.003c	4.23 ± 0.16bc	1.13 ± 0.02f	135.84 ± 13.33 cd	483.18 ± 3.54b	110.68 ± 1.82bc
	TF	3.23 ± 0.04e	0.245 ± 0.003c	4.62 ± 0.54ab	1.36 ± 0.04d	145.74 ± 8.07c	501.38 ± 13.26b	157.37 ± 36.54abc
	OF	3.83 ± 0.22d	0.246 ± 0.000c	4.90 ± 0.16a	1.46 ± 0.03c	362.51 ± 2.37a	526.03 ± 5.94a	198.59 ± 71.51a
	CK + B	4.15 ± 0.01c	0.245 ± 0.003c	3.88 ± 0.19c	1.27 ± 0.02e	98.73 ± 11.15e	327.38 ± 17e	100.37 ± 5.84c
	TF + B	5.18 ± 0.16a	0.293 ± 0.003a	4.45 ± 0.06ab	2.48 ± 0.03b	121.89 ± 5.16d	359.49 ± 11.17b	151.67 ± 5.15abc
	OF + B	4.59 ± 0.07b	0.261 ± 0.006b	4.52 ± 0.10ab	3.55 ± 0.05a	260.01 ± 13.12b	389.29 ± 6.49c	181.09 ± 48.23ab
> 2 mm	CK	3.81 ± 0.10e	0.267 ± 0.004d	5.25 ± 0.02a	3.72 ± 0.23a	184.22 ± 22.85a	450.51 ± 10.34a	177.56 ± 16.3a
	TF	4.33 ± 0.02d	0.291 ± 0.001c	4.50 ± 0.03c	2.95 ± 0.02b	112.11 ± 12.3c	342.66 ± 13.72b	136.26 ± 6.94bc
	OF	3.92 ± 0.10e	0.329 ± 0.012a	4.75 ± 0.12b	1.29 ± 0.14e	62.15 ± 18.37d	309.88 ± 17.62c	105.89 ± 7.47c
	CK + B	4.96 ± 0.02c	0.295 ± 0.006c	3.86 ± 0.04d	1.46 ± 0.03de	95.79 ± 7.43c	196.73 ± 6.47e	151.36 ± 9.82ab
	TF + B	6.49 ± 0.06a	0.295 ± 0.006c	4.70 ± 0.07b	1.63 ± 0.03 cd	100.92 ± 18.26c	265.11 ± 10.12d	162.46 ± 40.38ab
	OF + B	6.29 ± 0.18b	0.317 ± 0.005b	4.45 ± 0.13c	1.79 ± 0.10c	148.04 ± 9.72b	298.18 ± 7.37c	160.04 ± 23.78ab
0.25–2 mm	CK	3.57 ± 0.10b	0.243 ± 0.003b	5.78 ± 0.01a	0.84 ± 0.10c	132.58 ± 15.68b	235.99 ± 13.53d	110.21 ± 8.91b
	TF	3.83 ± 0.49b	0.259 ± 0.004a	4.43 ± 0.14c	1.06 ± 0.05b	223.58 ± 4.16a	284.64 ± 7.23b	113.80 ± 20.72b
	OF	3.7 ± 0.17b	0.246 ± 0.002b	4.54 ± 0.26c	1.36 ± 0.03a	200.98 ± 4.99a	309.61 ± 3.35a	142.23 ± 18.77a
	CK + B	3.98 ± 0.04b	0.237 ± 0.009b	3.03 ± 0.07e	1.02 ± 0.14b	78.72 ± 7.06c	114.2 ± 3.92e	94.87 ± 13.20b
	TF + B	5.74 ± 0.05a	0.243 ± 0.014b	3.68 ± 0.05d	1.31 ± 0.04a	116.26 ± 11.48b	230.71 ± 5.36d	108.29 ± 6.69b
	OF + B	5.66 ± 0.09a	0.240 ± 0.001b	5.02 ± 0.04b	1.36 ± 0.09a	217.35 ± 23.14a	255.4 ± 10.04c	144.62 ± 20.26a
< 0.25 mm	CK	3.11 ± 0.03a	0.233 ± 0.003a	4.72 ± 0.07a	1.72 ± 0.10b	182.27 ± 7.13a	223 ± 5.12c	164.72 ± 10.64a
	TF	3.12 ± 0.20a	0.218 ± 0.006b	4.09 ± 0.05b	1.45 ± 0.06c	159.25 ± 8.11b	240.81 ± 2.66ab	155.98 ± 10.21ab
	OF	2.68 ± 0.09b	0.22 ± 0.006b	3.32 ± 0.07c	1.37 ± 0.06c	165.1 ± 12.23b	247.24 ± 8.64a	92.46 ± 16.66c
	CK + B	3.18 ± 0.20a	0.187 ± 0.003c	2.65 ± 0.30d	1.62 ± 0.13b	77.67 ± 12.88d	221.54 ± 6.42c	135.11 ± 4.81b
	TF + B	3.19 ± 0.11a	0.209 ± 0.003b	3.31 ± 0.14c	2.17 ± 0.06a	109.31 ± 8.56c	225.07 ± 10.37c	147.16 ± 20.49ab
	OF + B	2.85 ± 0.13b	0.209 ± 0.013b	3.90 ± 0.24c	1.62 ± 0.10b	147.69 ± 6.54b	231.01 ± 3.49bc	135.67 ± 9.10b
ANOVA								
Fertilizer (F)		123.774***	37.824***	14.437***	33.872***	236.371***	105.794***	2.346
Biochar (B)		1084.774***	0.739	251.881***	42.532***	202.299***	1515.590***	0.010
Aggregate (A)		524.567***	596.264***	104.920***	419.424***	107.355***	1730.068***	6.359**
F*B		47.152***	7.789**	165.561***	301.580***	45.535***	113.932***	4.311*
F*A		25.746***	20.524***	12.419***	200.360***	118.702***	55.387***	9.343***
B*A		112.445***	40.854***	14.533***	469.313***	18.186***	167.076***	1.258
F*B*A		18.141***	18.119***	32.006***	106.962***	35.205***	59.178***	1.291

Values are the mean ± standard deviation (n = 3). C-acquiring enzymes, β-1,4-glucosidase and β-D-cellobiosidase; N-acquiring enzymes, β-1,4-Nacetylglucosaminidase and L-leucine aminopeptidase; P-acquiring enzyme, alkaline phosphatase. Different letters within the same column denote significant differences ($p < 0.05$, Duncan's HSD post-hoc analysis, n = 3) under biochar and fertilizer regimes

* represents $p < 0.05$; ** represents $p < 0.01$; *** represents $p < 0.001$

S2C). ANOVA analysis showed that fertilizer and biochar treatment had an obvious impact on the relative abundance of *Actinobacteriota*, *Firmicutes*, and *Nitrospirota* within the meta-community (Additional file 1: Table S2). Meanwhile, fertilizer treatment significantly influenced the relative abundance of taxa at the phyla, excluding *Actinobacteriota*. For instance, in soils without biochar addition, the relative abundances of *Firmicutes* and *Actinobacteriota* significantly increased, while the relative abundances of *Proteobacteria* showed a decrease in OF treatment compared to CK treatment (Additional file 1: Fig. S2C).

In the bulk soil, biochar addition markedly enhanced the observed OTUs compared with soils without biochar application in the whole community (Fig. 3a). OF treatment significantly increased the observed OTUs with particle size more than 2 mm and ranging from 0.25 to 2 mm in the specialists, compared to CK and TF treatments in both soils with and without biochar application (Fig. 3c). We also found that fertilizer and

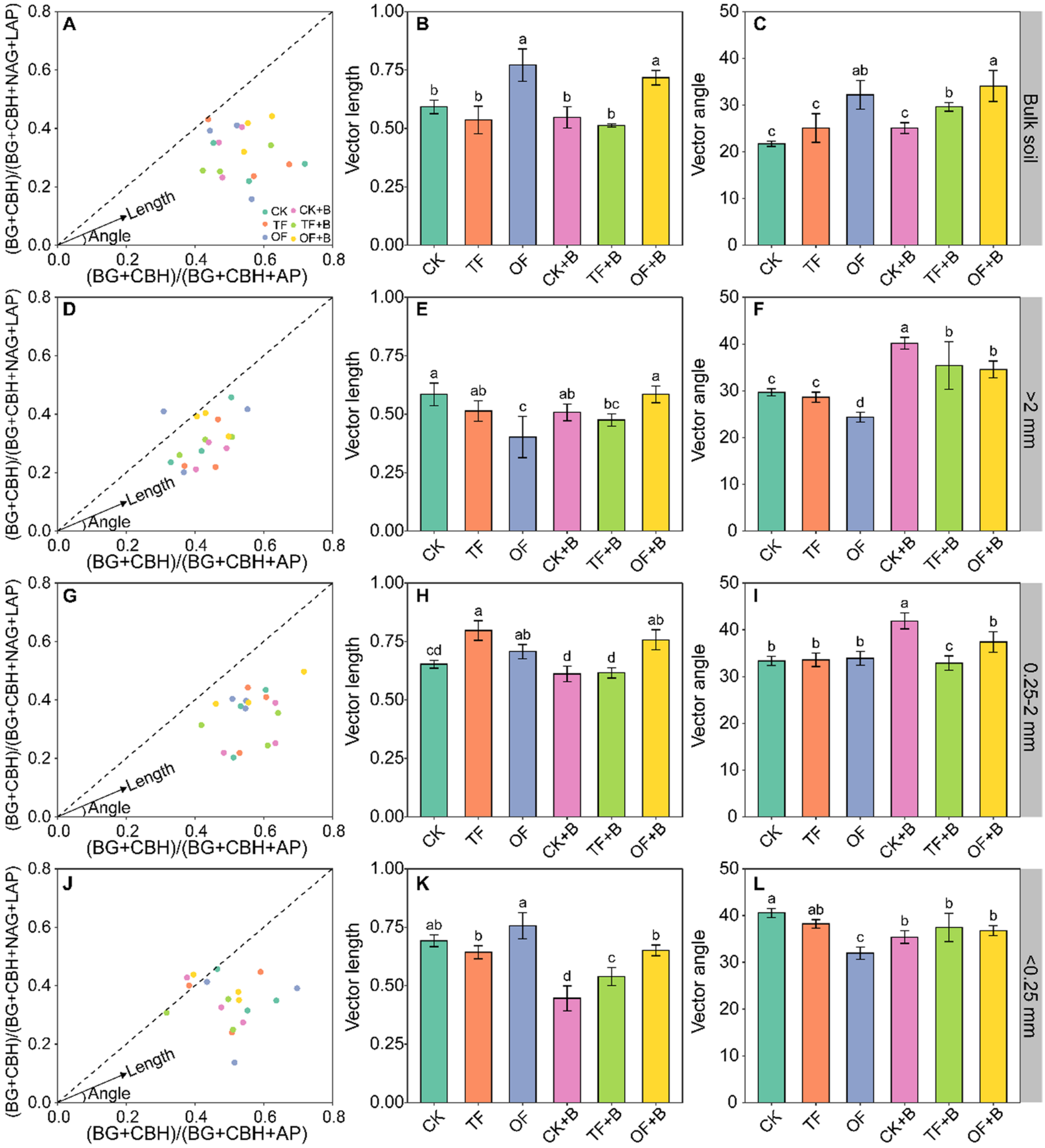


Fig. 2 Extracellular enzyme stoichiometry of the relative proportions of C to N acquisition versus C to P acquisition under biochar amendment and fertilizer regimes (**A**, **D**, **G**, **J**). Difference of vector length between different treatments and soil aggregates (**B**, **E**, **H**, **K**). Difference of vector angle between different treatments and soil aggregates (**C**, **F**, **I**, **L**). BG, β-1,4-glucosidase; CBH, β-D-cellobiosidase; NAG, β-1,4-Nacetylglucosaminidase; LAP, L-leucine aminopeptidase; AP, alkaline phosphatase; Vector length represents C limitation, Vector angle represents N limitation. Different lowercase letters indicate significant differences ($p < 0.05$) at the different treatments

biochar treatment had no effect on the observed OTUs and Shannon index in the generalists (Fig. 3b, e; Additional file 1: Table S3). The observed OTUs and Shannon index in soil particles smaller than 0.25 mm were significantly lower compared to the other particle sizes in the meta-community and generalists (Additional file 1: Fig. S3). Pearson correlation analysis indicated that soil properties have a stronger relationship with Shannon index and observed OTUs in bulk soil compared to other three aggregates (Additional file 1: Fig. S4).

PCoA results revealed that the community structure with particle size smaller than 0.25 mm was separated from that with other particle sizes along the axes

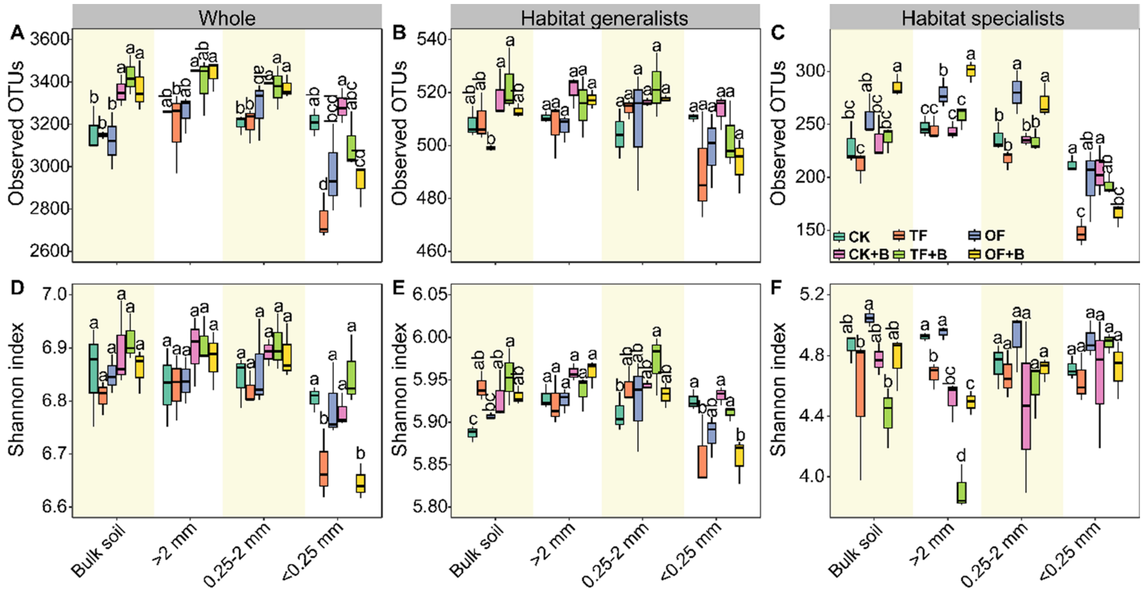


Fig. 3 The observed OTUs (**A**–**C**) and Shannon index (**D**–**F**) of bacterial meta-community and sub-community within soil aggregates under biochar amendment and fertilizer regimes. Different lowercase letters indicate significant differences ($p < 0.05$) at the different treatments

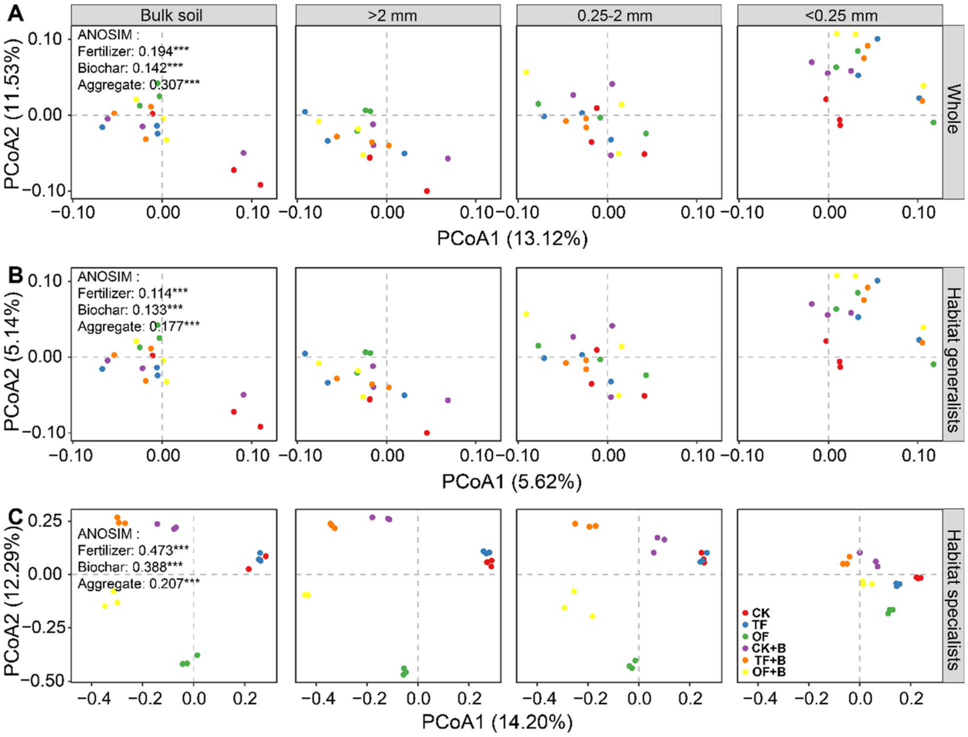


Fig. 4 The bacterial meta-community (**A**) and sub-community (**B**: Habitat generalists, **C**: Habitat specialists) structures under biochar amendment and fertilizer regimes revealed by Principal coordinates analysis (PCoA) based on Bray–Curtis distances. *** represents $p < 0.001$

2 in the meta-community and generalist community (Fig. 4a, b). More importantly, in the four aggregates, the community structure of habitat specialists in the six treatments was significantly separated along axis 1 and axis 2 (Fig. 4c). ANOSIM and PERMANOVA showed that aggregates had the greatest contribution in

explaining community structure of the meta-community and generalist community ($R^2 = 0.307$ and 0.177, $p < 0.001$) (Fig. 4a, b; Additional file 1: Table S3). The impacts of fertilizer and biochar on community structure were significant, with R^2 values of 0.473 and 0.388 ($p < 0.05$), respectively, followed by the influence of aggregates at an R^2 value of 0.207 ($p < 0.001$) (Fig. 4c). RDA analysis showed that the TN, SOC, NO_3^--N contents and N-acquiring enzymes activity were important variables affecting the bacterial community structure in bulk soil (Fig. 5a, e, i). The NH_4^+-N content and N-acquiring enzymes activity were important factors shaping the bacterial community structure in soil particles larger than 2 mm (Fig. 5b, f, j). The NO_3^--N, SOC, and NH_4^+-N contents were important factors shaping the community structure in soil particles ranging from 0.25 to 2 mm (Fig. 5c, g, k). The TN, NO_3^--N, NH_4^+-N contents and C-acquiring enzymes activity were important variables affecting the community structure in soil with particle size smaller than 0.25 mm (Fig. 5d, h, l). PLS-PM showed that the bacterial structure and richness of specialists, rather than generalists, were significantly affected by biochar and fertilizer regimes, as well as soil aggregates (Fig. 6a, c). Meanwhile, nutrient levels markedly influenced the bacterial structure of specialists ($\lambda = 0.797$, $p < 0.001$), and size distribution significantly impacted the bacterial richness of specialists ($\lambda = 0.321$, $p < 0.001$) (Fig. 6c).

3.3 Effects of biochar and fertilizer regimes on the co-occurrence networks and assembly processes under soil aggregates

We constructed eighteen ecological networks to examine the interactions among bacterial taxa (Fig. 7), and these networks exhibited a non-random pattern (Additional file 1: Fig. S6). In the soils amended with biochar, the whole bacterial network in OF + B treatment exhibited higher edge number, degree, ACC, and lower APL and diameter compared to CK + B treatment. However, the bacterial network of specialists showed the opposite trend. In soils without biochar application, the bacterial network of habitat specialists in TF treatment had greater edge number, degree, ACC, and lower APL and diameter compared to CK treatment. We also observed that in comparison to soils without biochar application, the bacterial network of specialists in soils treated with biochar showed higher edge number, degree, ACC, and lower APL, indicating the incorporation of biochar increased the stability and complexity of taxa interactions within the network of specialists (Table 2). Similar to the community composition, the major phyla of the network nodes in the meta-community and generalist community were *Proteobacteria*, *Actinobacteria*, *Acidobacteria*,

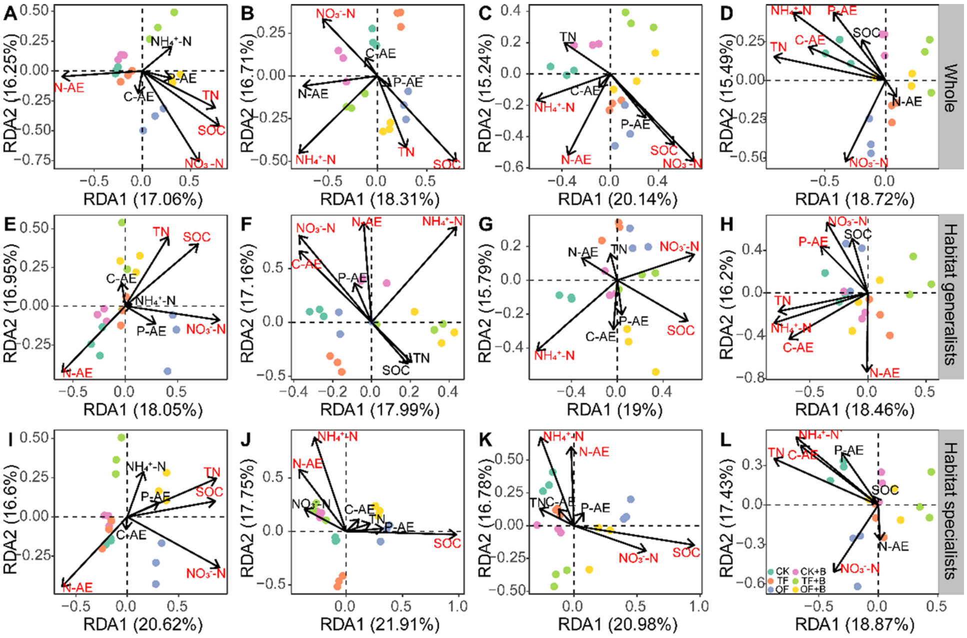


Fig. 5 Redundancy analysis (RDA) was used to clarify the relationships between the soil properties and extracellular enzymatic stoichiometry versus bacterial meta-community and sub-community. The red font represents environmental factors that significantly affected the bacterial meta-community and sub-community. N-AE represents N-acquiring enzymes; C-AE represents C-acquiring enzymes; P-AE represents P-acquiring enzyme

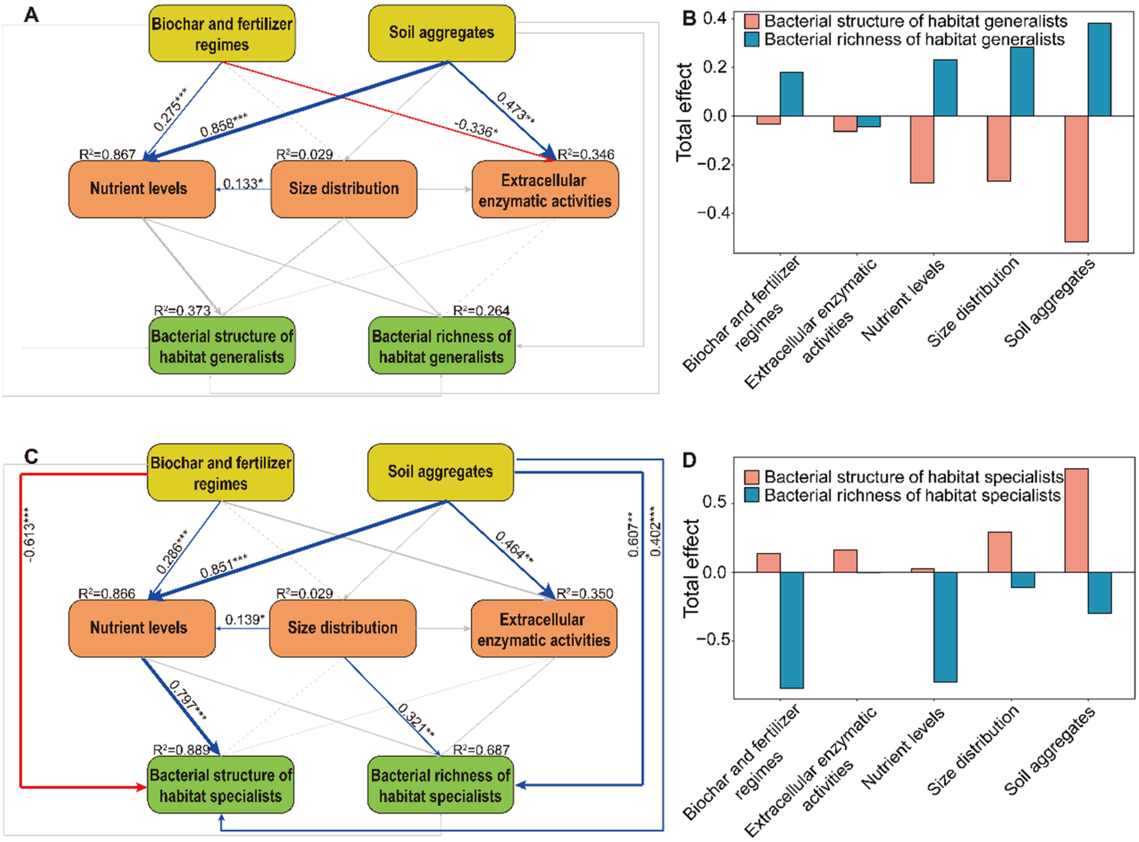


Fig. 6 Partial least squares path modeling (PLS-PM) showed the direct and indirect effects of biochar and fertilizer regimes, soil aggregates, nutrient levels, size distribution, extracellular enzymatic activities on bacterial structure and richness of habitat generalists (**A**) and specialists (**C**). Total effects of biochar and fertilizer regimes, soil aggregates, nutrient levels, size distribution, extracellular enzymatic activities on bacterial structure and richness of habitat generalists (**B**) and specialists (**D**). Nutrient levels are latent variable, which is expressed by SOC and TN. Extracellular enzymatic activities are latent variable, which is expressed by BG, LAP, NAG, and AKP. Bacterial richness and structure are expressed by observed OTUs and PCoA1 of bacterial community, respectively. The blue, red, and gray arrows indicate positive, negative, and unaffected flows of causality, respectively. Numbers on the arrowed lines and the thickness of the arrows indicate the path coefficient. R^2 beside the latent variables are the coefficients of determination. Asterisk (*) represents significant effects: *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$

and *Chloroflexi* (Additional file 1: Fig. S7A, B). In soils without the application of biochar, compared to CK treatment, OF treatment enhanced the relative proportions of *Acidobacteriota*, *Actinobacteriota*, *Chloroflexi*, and *Firmicutes* within the whole network (Additional file 1: Fig. S7A). In soils with and without the application of biochar, TF and OF treatments improved the relative proportions of *Actinobacteriota* and *Gemmatimonadota* in the bacterial network of specialist community compared to CK treatment (Additional file 1: Fig. S7C). We also constructed twelve ecological networks for the bacterial communities of four aggregates (Additional file 1: Fig. S8). The bacterial network in bulk soil, and specialist network in soil particles smaller than 0.25 mm exhibited more nodes, edges, degree, ACC, and smaller diameter and APL, revealing that the bacterial network in bulk soil, and specialist network in soil particles smaller than 0.25 mm had the complexity and stability of taxa interactions (Additional file 1: Table S5).

In soils without biochar application, OF treatment increased the betaNTI values of the meta-community, but decreased the betaNTI values of habitat-specific species compared to TF treatment (Fig. 8a, c). Meanwhile, biochar application reduced the betaNTI values of habitat specialists compared to soils without biochar application (Fig. 8c). We observed that the bacterial community in proso millet farmland soil was predominantly governed by stochastic process (Fig. 8d–f). Within the bacterial community of specialists, the application of biochar treatment decreased deterministic process compared to soils without biochar application (Fig. 7f). We also noted that with the decrease in soil particle size, the deterministic process governing the generalists became more pronounced (Additional file 1: Fig. S9E).

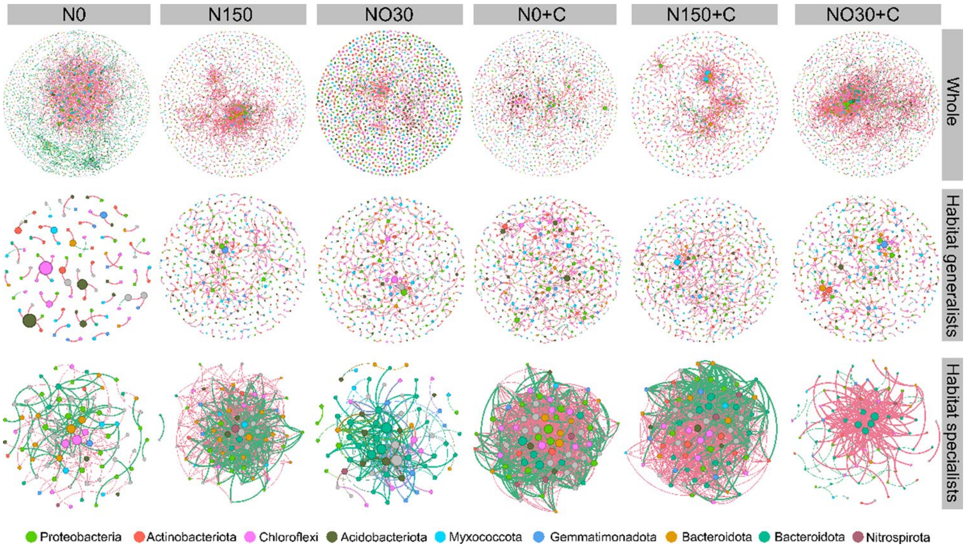


Fig. 7 Ecological networks of bacterial meta-community and sub-community within soil aggregates under biochar and fertilizer regimes. The node size is related to the node degree

Table 2 Topological properties of ecological networks of bacterial meta-community and sub-community under biochar and fertilizer regimes

	Treatment	Total node	Total link		Degree	Average clustering coefficient	Average path length	Diameter
			Positive	Negative				
Whole	CK	1571	616	3294	4.978	0.033	5.222	19
	TF	964	281	1541	3.78	0.073	5.355	16
	OF	1112	221	959	2.122	0.052	7.631	21
	CK + B	1277	640	710	2.114	0.063	10.15	19
	TF + B	631	143	676	2.596	0.109	5.405	15
	OF + B	895	487	2005	5.569	0.102	5.013	16
Habitat generalists	CK	100	9	50	1.18	0	1.337	3
	TF	371	92	287	2.043	0.058	7.673	20
	OF	379	87	318	2.137	0.088	9.68	23
	CK + B	423	95	457	2.61	0.072	10.22	25
	TF + B	428	101	389	2.29	0.06	9.714	25
	OF + B	366	65	356	2.301	0.07	7.784	23
Habitat specialists	CK	82	71	134	5	0.087	3.213	9
	TF	80	273	392	16.63	0.267	1.902	4
	OF	77	50	161	5.481	0.128	2.722	7
	CK + B	78	284	816	28.21	0.385	1.638	3
	TF + B	79	257	703	24.3	0.29	1.695	3
	OF + B	78	38	183	5.667	0.149	2.933	10

4 Discussion

4.1 Biochar and fertilizer regimes changed the physicochemical properties within soil aggregates

Soil aggregates are the crucial component in determining soil structure, providing the basis for the formation of ideal soil texture (Yan et al. 2018; Zhou et al. 2020). Biochar possessing porous structure and huge specific surface area can enhance cementing agents, thereby promoting the formation of soil aggregates (Du et al. 2017). In our study, biochar amendment enhanced the large macro-aggregate size distribution, whereas reduced the micro-aggregate size distribution, more importantly, the

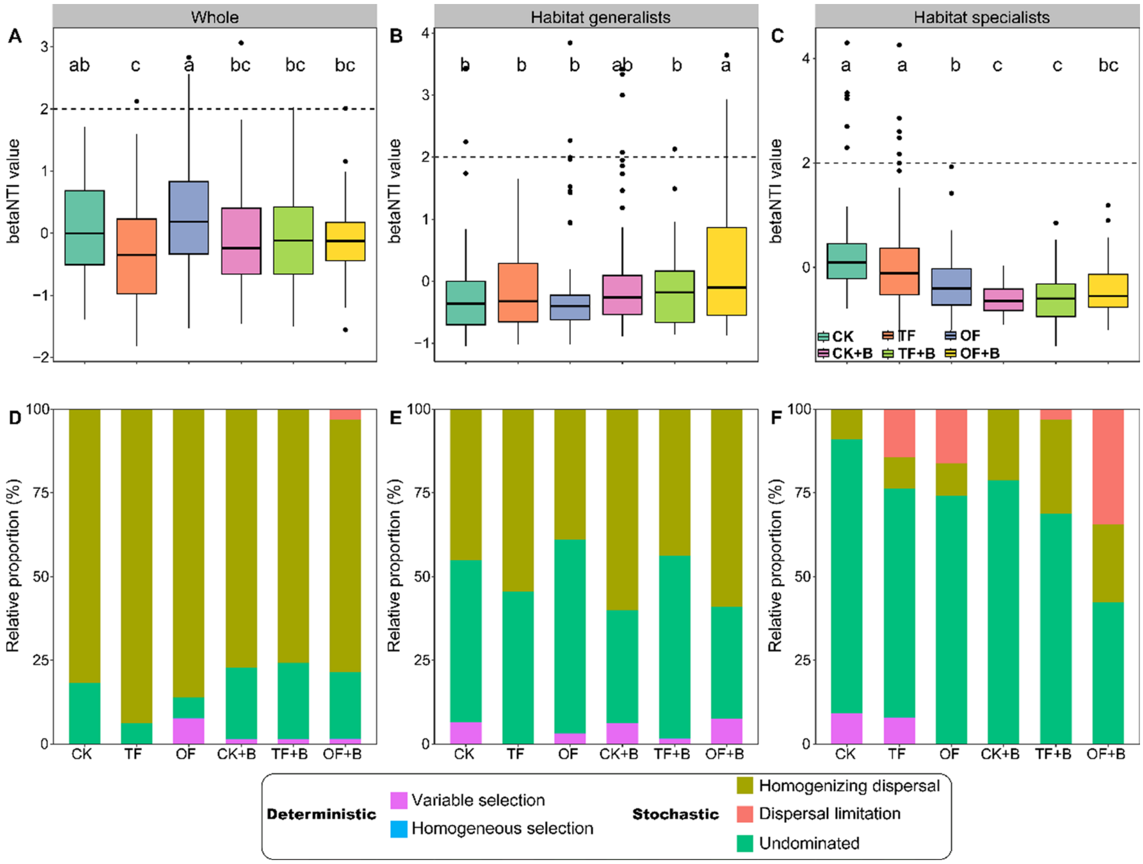


Fig. 8 The betaNTI value of bacterial meta-community (**A**) and sub-community assembly (**B**, **C**) under biochar and fertilizer regimes. Summary of ecological processes that affected the bacterial meta-community and sub-community assembly among six treatments (**D**–**F**). Relative proportion was given for the relative contribution of each process to the community assembly, as indicated by different colors. Different lowercase letters indicate significant differences ($p < 0.05$) at the different treatments

highest proportion of large macro-aggregate size distribution was observed at the OF + B treatment, which is in agreement with previous literature (Chen et al. 2021; Li et al. 2021; Zhang et al. 2021), revealing that application of biochar and chemical fertilizers is more beneficial for the formation of soil macroaggregates that consist of microaggregates held together by organic materials (Wang et al. 2023).

The storage capacity of soil nutrients varies among different soil aggregates (Hou et al. 2013). In our study, the greatest contents of SOC, TN, NH_4+-N, and NO_3^--N were observed in the soil particles larger than 2 mm, followed by 0.25–2 mm fraction and soil particles smaller than 0.25 mm. These results are similar to previous literature (Chen et al. 2020; Zhang et al. 2019b, 2021), suggesting that macro-aggregates contained greater levels of carbon, nitrogen, and particulate organic matter compared to micro-aggregates (Puget et al. 1995). Biochar addition into the soil has been well documented to enhance the accumulation of organic carbon within each aggregate size fraction (Cooper et al. 2020; Sun et al. 2023). There findings also corroborated that the biochar application alone, as well as the combined application of biochar and organic fertilizer, consistently augmented the SOC content in bulk soil, soil particles size larger than 2 mm, and 0.25–2 mm fraction. This improvement in SOC can be attributed to the introduction of an inert carbon source via biochar, and the recalcitrant carbon components in biochar also significantly restricted carbon mineralization, thereby increasing the soil's capacity for carbon sequestration (Liu et al. 2021; Mehmood et al. 2020). Furthermore, the combined addition of biochar and organic fertilizer obviously reduced the SOC level in soil particles smaller than 0.25 mm compared to sole biochar application, suggesting co-addition of biochar and organic fertilizer may accelerate the mineralization of organic carbon within this specific fraction (Zhang et al. 2021). Our observations indicated that compared to the soil without biochar application, the addition of biochar markedly enhanced the TN content in the bulk soil. This enhancement can be attributed to the nitrogen source from the biochar (Wang et al. 2015). Furthermore, in soils amended with biochar, substituting organic fertilizer for nitrogen fertilizer significantly increased the levels of TN, NO_3^--N, and NH_4^+-N in the bulk soil and three different aggregate fractions compared to the unfertilized control. These results are in line with the observations of Rivelli and Libutti (2022) and Oladele et al. (2019), who supported that the co-addition of fertilizer and biochar caused an obvious enhancement in total nitrogen and inorganic nitrogen, revealing that the addition of biochar may enhance nitrogen retention due to its porous structure, with the retained nitrogen partly derived from applied fertilizers (Li et al. 2019; Libutti et al. 2019). In short, our study indicated that the combination of biochar and substituting organic fertilizer for nitrogen fertilizer was more conducive to enhancing soil carbon and nitrogen storage, and is of great significance in decreasing soil nutrient loss in Loess Plateau.

Extracellular enzymes in soil have an important impact on the cycling and decomposition of soil nutrients (Sinsabaugh et al. 2009). In biochar-amended soils, compared with no fertilizer treatment, nitrogen fertilizer application significantly increased C-acquiring enzyme activities in the bulk soil and various aggregates. At the same time, substituting organic fertilizer for nitrogen fertilizer further significantly increased C-acquiring enzyme activity, enhanced the carbon limitation, and facilitated organic matter decomposition (Ye et al. 2022), which is consisted with the result of Liu et al. (2023a). We noted that the soil in this region is nutrient-poor, primarily impacted by nitrogen limitation, and the addition of nitrogen fertilizer or organic fertilizers enhanced the N-acquiring enzyme activities in the bulk soil, thus alleviating the nitrogen limitation. However, compared to soils without biochar application, the biochar addition reduced N-acquiring enzyme activities, thereby increasing the nitrogen limitation, particularly in macro-aggregates, which could be attribute to the fact that biochar has the potential to immobilize enzymes or their substrates within their pores, which could inhibit enzymatic reactions and result in an overall decrease in enzyme activities (Teutscherova et al. 2018).

4.2 Biochar and fertilizer regimes changed the bacterial community diversities with soil aggregates

The richness of soil bacterial communities is essential for preserving the stability and ecological functions within soil ecosystems, especially for different soil aggregates (Chen et al. 2023; Liu et al. 2023b). Our study observed that the large and small macro-aggregates exhibited significantly greater richness compared to the micro-aggregates in both the bacterial meta-community and subcommunity, which is in line with the previous reports (Liu et al. 2023b; Zhang et al. 2021). This might be due to the fact that microaggregates had the lowest SOC content with a slower turnover rate than macro-aggregates (Gupta and Germida 1988). Previous research also illustrated that significant heterogeneities existed within microbial communities across various size aggregate fractions due to the variations in physicochemical properties, and large macro-aggregates, which contained great nutrient contents, provided a favorable environment for microbial colonization (Liao et al. 2020). In the present study, regardless of the biochar application, substituting organic fertilizer for nitrogen fertilizer significantly enhanced the richness of specialists of bacteria in the large and small macro-aggregates compared to no fertilization, while had no impact on generalists. These observations are agree with a previous study (Liu et al. 2022b), revealing that organic fertilization has the potential to introduce numerous external bacteria into the soil, which could contribute to increase microbial richness diversity (Xu et al. 2020). Additionally, the biochar and fertilizer regimes had a higher influence on the bacterial community structure of specialists than generalists. Generally, generalists are defined by the capability to colonize a broad spectrum of habitats. Therefore, they can buffer anthropogenic interference. This also explains why the bacterial richness of generalists did not change significantly in the biochar and fertilizer treatments (Xu et al. 2020).

In our work, RDA results revealed that the NO_3^--N, SOC, TN, and N-acquiring enzymes were the essential role impacting the bacterial meta-community and sub-community structures in the bulk soils of proso millet farmland. Previous studies also confirmed that soil SOC, TN, DOC, and NO_3^--N were the important variables influencing the microbial community structure (Bai et al. 2020; Liu et al. 2023b) in paddy and wheat soils. This phenomenon also indicates that the growth and metabolism of microorganisms in bulk soil are greatly influenced by the labile nutrient pools through the incorporation of biochar and fertilizer (Li et al. 2017; Zhang et al. 2021). Furthermore, we also observed that inorganic nitrogen (i.e., NO_3^--N and NH_4^+-N) almost influenced the bacterial meta-community and sub-community structures within three aggregates. This may be due to the fact that the microbial decomposition and utilization of biochar matrix required more available nitrogen, and the addition of fertilizer to make up for the lack of nitrogen (Zhang et al. 2021). Therefore, the application of biochar and organic fertilizer in barren soil increased the soil nutrient levels, which is of great significance to increase the stability of soil microbial ecosystems and enhance crop yields (Mukhamed et al. 2023; Additional file 1: Fig. S10).

The distribution of microorganisms varies with the size of the aggregates as the aggregates constitute a complex environment for microorganisms (Bai et al. 2020). We found that the major phyla in the meta-community of the bulk soil and three aggregates were *Proteobacteria*, *Actinobacteria*, *Chloroflexi*, and *Acidobacteria*, aligning with findings from prior research (Liu et al. 2023b; Wei et al. 2020). Notably, the relative abundances of the predominant phyla of generalists were similar to those of the meta-community, whereas those of specialists are scarce. The notable differences in the composition of specialists and generalists may partly elucidate the reasons for their distinct diversity patterns (Luo et al. 2019). We also noted that the impacts of soil aggregates on the community composition of specialists were greater than those of generalists, and the impacts of biochar and fertilizer regimes on the relatively low-abundance phyla (*Myxococcota*, *Bacteroidota*, *Firmicutes*, and *Nitrospirota*) in specialists were greater than those in generalists, among which *Firmicutes* dominated in specialists. In the absence of biochar, the relative abundance of *Firmicutes* increased obviously when biochar and organic fertilizer were adopted to replace nitrogen fertilizer in the bulk soil and three aggregates, respectively. This result agrees with previous reports (Guo et al. 2022; Xu et al. 2021), illustrating *Firmicutes* can proliferate rapidly in a nutrient-rich soil environment and generate antibacterial compounds that enhance the crop growth (Kabisch et al. 2014; Taketani et al. 2013).

4.3 Biochar and fertilizer regimes changed the ecological network with soil aggregates

In this study, ecological network analyses were adopted to delineate the structure of bacterial interactions in response to the biochar and fertilizer regimes. Our findings revealed that the network of specialists was simpler than that of generalists, which is related to the composition and diversities of the both sub-communities. In the absence of biochar, nitrogen addition enhanced the bacterial network complexity and stability of specialists. Meanwhile, in the soils amended with biochar, substituting organic fertilizer for nitrogen fertilizer also increased the complexity and stability of whole bacterial network. These observations are in line with the recent results that the addition of nitrogen and organic fertilizer enhanced the complexity and tightness of the bulk soil and rhizosphere networks by increasing the interaction between species (Wang et al. 2022; Zhang and Shangguan 2023), suggesting that the addition of nitrogen and organic fertilizer enhanced the availability of soil nutrient resources, which in turn promoted the cooperation between microorganisms and improved the efficiency of resource transformation (Zhao et al. 2019). At the same time, it is plausible that the nutrient input constrained the growth of microorganisms to a certain degree. The proximity of microorganisms could potentially increase the network's stability by fostering resilience to adverse environmental conditions (Dong et al. 2022b). Our results also confirmed that the addition of biochar also increased the complexity and stability of the bacterial network of specialists compared with the soil without biochar application, indicating that microorganisms intensified interspecies competition in order to acquire nutrients from the pores of biochar, thereby enhancing the stability of the network. In addition, in comparison to large and small macro-aggregates, the network of specialists within micro-aggregates exhibited the highest number of edges, degrees, ACCs, and the smallest average path lengths, diameters. This assertion is confirmed by prior observations indicating that the bacterial network within micro-aggregates was tighter and more intricate than that of macro-aggregates (Sun et al. 2022; Zhang and Shangguan 2023). Compared to labile carbon in macro-aggregates, the decomposition of recalcitrant carbon in micro-aggregates could require microbial cooperation, thereby leading to the formation of a complex network (Fan et al. 2021). Overall, these findings provide evidences from the perspectives of the sustainable development of agro-ecosystems that biochar and fertilizer regimes, and soil aggregates can influence the interactions between soil microorganisms.

To our knowledge, this study is the first to analyze the assembly processes of bacterial meta-community and sub-community in response to biochar and fertilizer regimes on the Loess Plateau. Stochastic process had a greater influence on the bacterial communities of both generalists and specialists, with the undominated process significantly governing assembly processes within micro-aggregates compared to those within macro-aggregates. These findings align with previous studies, indicating that the generalists and specialists within microbial communities are primarily influenced by stochastic process (Zhang et al. 2020; Yan et al. 2022). Additionally, we also found that biochar and fertilizer either alone or co-application had no influence on the assembly process of the bacterial meta-community and sub-community, mainly determined by stochastic process, and the addition of nitrogen fertilizer, as well as the combined use of nitrogen and organic fertilizer, resulted in an increased proportion of dispersal limitations within the bacterial communities of specialists. However, several studies have indicated that biochar and fertilizer addition enhanced the deterministic process of the meta-microbial community (Liu et al. 2020, 2023c; Qiu et al. 2023), which contradicts this study. We speculated that soil aggregate structure plays a crucial regulatory role in the assembly processes of microbial communities (Liao et al. 2022), but the specific reasons still need further in-depth analysis.

5 Conclusions

In this study, the co-application of biochar and organic fertilizer substitution significantly reduced the structure of microaggregates, promoted the formation of macroaggregates, increased the nutrient levels and enzyme activities, and also increased the C limitation, declined the N limitation within the bulk and macro-aggregates. Whether or not biochar was added, organic fertilizer substitution markedly enhanced the richness of bacterial communities of specialists in macro-aggregates, while had no influence on generalists. The impacts of biochar, fertilizer regimes, and soil aggregates on the community structures and compositions of specialists were more pronounced compared to those of generalists. Our results confirmed that NH_4^+-N content significantly influenced the bacterial meta-community and sub-community structures within macro-aggregates and micro-aggregates. The application of biochar and organic fertilizer substitution improved the entire bacterial network complexity and stability compared to biochar alone, and the bacterial network of specialists with biochar application was more complex and tighter than that without biochar. Stochastic process plays a primary role in shaping the bacterial meta-community and sub-communities in bulk soil and three aggregates, but they were not impacted by biochar and fertilizer regimes. Overall, our work offers an essential reference for analyzing the improvement of soil quality on the Loess Plateau from the perspective of soil aggregates. Future studies could pay more attention to the ecological functions of bacterial-specific taxa to better understand the contribution of bacterial microorganisms to soil nutrient cycling under the combined application of organic fertilizer and biochar.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1007/s42773-024-00398-z.

Additional file 1.

Author contributions

Lixin Tian performed the experiments, analyzed the data, and write the original draft. Lixin Tian and Baili Feng conceived and designed the research. Lixin Tian collected soil samples with the help of Yawei Wang, Doudou Jin, Yulong Zhou, Bauyrzhan Mukhamed and Dan Liu. All authors contributed critically to manuscript writing and revisions. The author(s) read and approved the final manuscript.

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Availability of data and materials

The raw sequence data of bacteria presented in this study are accessible in the NCBI Sequence Read Archive under BioProject PRJNA849346.

Declarations

Competing interests

The authors state that they have no known competitive economic interests or personal relationships that may affect the work reported in this study.

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