

# Fungal alpha-diversity drives the stochasticity of bacterial and fungal community assembly in soil aggregates in the apple orchard

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## Research

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# Abstract

**Background** In soil ecosystems, bacteria and fungi always co-exist in the same niche and interact with each other, especially in different sized soil aggregates. The bacterial and fungal community assembly process and bacteria-fungi interactions in soil aggregates, which is important for bacterial and fungal community diversity and composition, is still unclear.

**Methods** We examined bacterial and fungal community assembly in soil macroaggregate (> 0.25 mm), microaggregate (0.053–0.25 mm) and smaller microaggregate (silt + clay, < 0.053 mm) in an apple orchard. The microbial community assembly processes were analyzed by normalized stochasticity ratio index (NST).

**Results** Bacterial community diversity, composition and assembly were more affected by agricultural practice and aggregate than fungal community. Bacterial community assembly was more stochastic in silt + clay than in macroaggregate, and was more stochastic (NST > 50%) than fungal community in soil aggregates. Meanwhile, bacterial NST was negatively correlated with fungal diversity, and fungal NST was positively correlated with fungal diversity. Co-occurrence network suggested that the bacteria and fungi were less strongly interacting in the network of silt + clay, compared to macroaggregate. The results indicated that fungi impact on the bacterial community assembly in soil aggregate, and the stochasticity of bacterial community assembly was increased with the decrease of interaction between bacteria and fungi in soil aggregates.

**Conclusions** This study enhances our understanding of the mechanism of bacterial and fungal community assembly and co-exists pattern of bacteria and fungi in soil aggregates.

## Introduction

Soil bacteria and fungi are the largest reservoirs of biodiversity in terrestrial ecosystems and play important roles in soil functions, such as decomposition of soil organic matter and soil nutrient cycles (carbon, nitrogen, phosphorus, sulfur, etc.) (Falkowski et al., 2008; Tedersoo et al., 2014; Wagg et al., 2014). In soil, bacterial and fungal community diversities and compositions are generally affected by many biotic and abiotic factors (e.g. soil pH, organic carbon quality and quantity, temperature and predation and viral lysis) (Fierer, 2017). Meanwhile, the bacterial and fungal communities also positively and negatively interact with each other, thereby influence their respective diversities and compositions. Bacteria and fungi naturally coexist in various environments, and their cooperation or antagonistic interactions are widely demonstrated for humans, plants, aquatic and soil ecosystems and significantly impact their functions (Das et al., 2012; Gomes et al., 2019). Since soil bacteria and fungi are the major components of soil ecosystems, exploration of the bacterial and fungal community diversities and compositions and their co-exist patterns in different sizes of spatial habitats in the soil is primarily important for revealing the mechanism of change of bacterial and fungal diversity and composition in the whole soil.

Assessment and prediction of the dynamics of microbial communities in soil under variable conditions needs a proper understanding of the mechanisms of microbial community assembly and is an eternal theme of microbial ecology (Yan et al., 2019). In community ecology, the turnover of microbial community composition was governed by ecological assembly (deterministic and stochastic) processes, and the contributions of deterministic and stochastic processes in the assembly of microbial communities are debated all the time (Stegen et al., 2012; Ferrenberg et al., 2013; Dini-Andreote et al., 2015; Graham et al., 2016; Graham and Stegen, 2017). The basic ecological assembly processes include selection, drift, diversification, and dispersal (Vellend, 2010; Zhou and Ning, 2017). Ecological selection with biotic and abiotic factors is a deterministic process, leading to similar (dissimilar) community compositions in heterogeneous environments (Mac Arthur and Wilson, 2001). Drift (random changes in species relative abundances) is thought to be wholly stochastic and strongly affects community assembly when communities are small or have recently been released from selection imposed by stresses (Chase and Myers, 2011; Vellend et al., 2014). Both diversification and dispersal have stochastic and deterministic components (Zhou and Ning, 2017). Deterministic processes impact the fitness of microbial communities, thereby influence the abundance and composition of microbial communities, and stochastic processes lead to unpredictable changes in community compositions (Gao et al., 2020). Subsequently, both deterministic and stochastic processes affect the microbial community functions (Tilman, 2004; Zhang et al., 2016). Therefore, it is essential to explore the community assembly in agricultural soil ecosystem to better understand the impacts of agricultural practices on soil microbial communities and functions.

The geographic scale can influence the effects of deterministic and stochastic processes on soil microbial communities (Martiny et al., 2006). Recently, many researches have studied the effects of deterministic and stochastic processes on distributions of microbial communities from large to small scales, such as global scale, continental scale, regional scale, local scale (Hollister et al., 2010, Caruso et al., 2011; Graham et al., 2017, Liu et al., 2017; Zhao et al., 2019). However, little is known about deterministic and stochastic processes on soil microbial communities at soil aggregate scale. Bacteria and fungi always co-exist in the soil aggregate and interacted with each other. Different sizes of aggregate provide spatially heterogeneous habitats for soil microbes with various nutrient availability, water potential, predation pressure and oxygen concentration (Ranjard and Richaume, 2001; Jiang et al., 2017). Macroaggregates (> 0.25 mm) generally contain more labile substrates mainly derived from plant residues (Bronick and Lal, 2005), whereas the microaggregates (< 0.25 mm) contains higher contents of recalcitrant organic carbon and provide a protective microenvironment for microbial growth (Six et al., 2000). Previous studies have shown that each aggregate represents a different ecological niche for bacterial and fungal colonization (Trivedi et al., 2015; Li et al., 2019). Conversely, bacteria and fungi can also improve aggregate formation and stability through releasing mucilaginous exudates (Rashid et al., 2016). Meanwhile, fungal hyphae is also a key factor for aggregate formation due to hyphal movement, entanglement and compression of soil particles and/or microaggregates (Lehmann and Rillig, 2015; Rillig et al., 2017). However, the difference of assembly processes between the bacterial and fungal community

in different sized soil aggregates, and the interactive effect of bacterial and fungal communities on their assembly processes in each sized aggregates are still unclear.

The objectives of this study were to 1) evaluate the relative importance of stochastic and deterministic processes in shaping soil bacterial and fungal communities of different sized aggregates in soil with different agricultural practices (chemical fertilizer and cover crop); and 2) study the interactive effects of bacterial and fungal communities on their assembly processes. We performed a field (apple orchard) experiment which was begun in 2008 under four soil managements (control, chemical fertilizer, cover crop, chemical fertilizer and cover crop). Soil samples were separated into three aggregate sizes, and bacterial and fungal community compositions were examined in both whole soil and different sized aggregates. Compared to many bacterial species, the colonization and penetration abilities of fungal hyphae make fungi more capable of searching for the heterogeneously distributed nutrient resources (de Bore et al., 2005). Considering about this, we hypothesize that 1) soil management and aggregates have more effects on bacterial community diversity, composition as well as the assembly process, compared to the fungal community; 2) the bacterial community assembly process was affected by fungi, and the increase of fungal community diversity would decrease the stochasticity of bacterial community assembly; 3) the effect of fungal community on bacterial community assembly process would decrease with the decrease of soil aggregate size.

## Materials And Methods

### Experimental site and design

The experiment was conducted in a Fuji apple orchard located at the Weibei Dryland Experimental Station of Northwest A&F University (109°56'E, 35°21'N; altitude of 838 m) in Baishui County, Shaanxi Province, China. The soil in the apple orchard was silt loam and classified as Haplustalfs (USDA textural classification system). In the study area, the summer is hot and moist, while the winter and early spring are always cold and dry. The average annual precipitation of this area was 570 mm and 60% occurred from July to September. Apple trees were planted in 2005 (1200 plants per hectare). The experiment was established in 2008 with a split-plot design (two main plots and two sub plots). In total, four plots were included with control, chemical fertilizer, cover crop, chemical fertilizer and cover crop. For control, no fertilizer was applied and cover crop was also not planted with weeds were controlled manually by farmers. For the chemical fertilizer treatment, urea, calcium superphosphate, and potassium sulfate were used and 192 kg N ha<sup>-1</sup>, 108 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup>, and 168 kg K<sub>2</sub>O ha<sup>-1</sup> were applied each year. For cover crop treatment, crown vetch (*Coronilla varia* L.) was sown in each inter-row of the apple trees in the orchard at a depth of 1.5 cm with the sowing rate of 6.0 kg per hectare. The cover crop was mowed in early July, August, and September, where the residues were left on the ground as mulch. The chemical fertilizer and cover crop treatment was the combination of chemical fertilizer treatment and cover crop treatment.

### Soil sampling and aggregate fractionation

Soil samples were collected in late September after nine years of fertilization and cover cropping. Six soil cores were randomly collected from the inter-rows in each plot at a depth of 20 cm and composited to one soil sample. Then the soil samples were placed on ice before they were immediately transported to the laboratory. Soil aggregate fractionation was performed using the wet-sieving technique according to Davinic et al. (2012). Three fractions were obtained for each sample: macroaggregates (> 0.25 mm), microaggregates (0.053–0.25 mm), and silt and clay (< 0.053 mm). The soil organic carbon and total nitrogen content of whole soil and each sized aggregate was determined using the K<sub>2</sub>CrO<sub>7</sub>-H<sub>2</sub>SO<sub>4</sub> oxidation and Kjeldahl method, respectively.

## **Illumina sequencing**

Soil bacterial and fungal communities were analyzed using high-throughput sequencing. The DNA of the whole soil and aggregates was extracted using MoBio PowerSoil™ DNA Isolation Kits (Mo Bio Laboratories, Carlsbad, CA, USA). The V4 region of the 16S rRNA gene was amplified for bacteria (515F/806R primer), and the ITS1 region was amplified for fungi (ITS2/ITS5 primer). Sequencing was performed using the Illumina HiSeq2500 platform. The acquired sequences were filtered for quality control as previously described (Zheng et al., 2018). Any chimeric sequences were removed using the USEARCH tool based on the UCHIME algorithm. Sequences with  $\geq 97\%$  similarity were assigned to the same operational taxonomic unit (OTU). For each representative sequence, the SILVA (bacteria) and UNITE (fungi) databases were used to annotate taxonomic information.

## **Statistical analysis**

After sequencing and bioinformatics analysis, bacterial and fungal alpha diversity (observed species and Chao1, ACE, Shannon, and Simpson indices) of whole soil and aggregates was calculated. Statistical analyses were conducted in the R environment (v3.5.3; <http://www.r-project.org/>). The “limma” package was used to analyze the difference of relative abundance of OTUs between different treatments and sizes of aggregate. PERMANOVA was used to assess the effect of fertilizer, cover crop and their interaction on bacterial and fungal community composition with the default 999 permutations (based on the Bray-Curtis distance using the vegan package). Normalized stochasticity ratio (NST), which showed high accuracy and precision, was utilized to estimate and determinacy and stochasticity in the bacterial and fungal assembly process (Ning et al. 2019). The NST index was evaluated with taxonomic beta-diversity metrics and used 50% as the boundary point between more deterministic (< 50%) and stochastic (> 50%) assembly. The effects of cover crop, fertilizer and their interaction on NST was tested by two-way ANOVA after normality (Shapiro–Wilk test) and variance homogeneity (Bartlett test) test. The difference of NST between different sizes of aggregate was analyzed by the Kruskal-Wallis test. The importance of impact factors and taxonomy for the bacterial and fungal NST were identified by a classification random forest (RF) analysis using “randomForest” package. In these RF models, bacterial and fungal alpha-diversity

and taxonomy were served as predictors for the bacterial and fungal NST. Increased in mean squared error (IncMSE%) and increased node purity (IncNodePurity) were used to estimate the importance of variables: higher IncMSE% and IncNodePurity values imply more important variables. Based on the inferred taxonomy, the functional group of each fungal OTU was inferred using the program FUNGuild 1.0 (Nguyen et al., 2016).

Co-occurrence network of bacteria and fungi was constructed by OTU abundance using the routine CoNet in Cytoscape 3.4. The OTUs with a relative abundance of < 0.01% were removed to reduce rare OTUs in the data set. To build the network, data of bacterial and fungal OTU abundance was normalized, then the Pearson and Spearman correlation coefficients, and the Bray-Curtis (BC) and Kullback-Leibler (KLD) dissimilarity indices were combined to estimate the correlations between OTUs. The threshold for edge selection was set to 1000 top-score and bottom-score. During randomization, 100 iterations were calculated for edge scores. In the following bootstrap step, 100 iterations were calculated, and unstable edges were filtered out (p-level threshold of 0.05). The Brown method was chosen as the P-value merging method, and the Benjamini–Hochberg procedure was selected for multiple test correction. The network was analyzed by NetworkAnalyzer in Cytoscape. At last, the network and its sub modules were visualized by Gephi 0.9.2.

## Results

### Microbial community alpha-diversities and compositions

Bacterial and fungal alpha diversities were affected by fertilization and cover crop in different sized aggregates, and they showed significant differences among different sized aggregates, especially for bacterial alpha diversity (Figure 1 and S1). The bacterial Chao1, observed species and Simpson index could be impacted by fertilization and cover crop in macroaggregate, microaggregate or silt+clay, and they were showed higher in silt+clay than in other sized aggregates (Figure 1 and S2). However, fungal Chao1, observed species and Simpson index were generally affected by fertilization and cover crop in macroaggregate, and they were not significantly different between macroaggregate, microaggregate and silt+clay.

After compared the differences of abundance between treatments and control, cover crop (1490 OTUs with  $p < 0.05$ ) and fertilizer + cover crop (1234 OTUs with  $p < 0.05$ ) have more effects on soil bacterial OTU abundances than fertilizer (774 OTUs with  $p < 0.05$ ), but this was not found for fungal OTU abundances (Figure 2a). For bacteria, OTUs in Proteobacteria and Planctomycetes are more sensitive to fertilization and cover crop, compared to OTUs in other phyla (Figure 2c and Table S1). For fungi, OTUs in Ascomycota and Basidiomycota are more sensitive to fertilization and cover crop, compared to OTUs in other phyla (Figure 2c and Table S1). Furthermore, bacterial OTU abundances were also more influenced

by soil aggregate, compared to fungal OTU (Figure 2b). Compared to macroaggregate and microaggregate, silt+clay enriched more bacterial OTUs (348 OTUs), but this was not found for fungal OTUs. PERMNOVA analysis indicated that cover crop had more effects on bacterial and fungal community compositions in the whole soil and different sized aggregates than fertilization (Figure 2d).

### **Microbial community assembly processes and co-existence**

The fertilization and the cover crop could both influence the NST of bacteria and fungi (Figure 3). However, the bacterial NST in whole soil, macroaggregate and silt + clay was significantly impacted by fertilization or cover crop ( $p < 0.05$ ), but the fungal NST was only impacted by cover crop in macroaggregate ( $F = 14.533$ ,  $p = 0.019$ ). For bacteria, the NST indexes in whole soil and aggregates were generally higher than 50%, indicated that the community assembly was stochastic (Figure 3a). Cover crop increased the bacterial NST in macroaggregate, and fertilization decreased and increased bacterial NST in whole soil and silt + clay, respectively (Figure 3a and 3c). Meanwhile, the bacterial NST in silt+clay was higher than in macroaggregate. However, the assembly of the fungal community showed more deterministic than bacterial community (the NST in some treatments and aggregates was lower than 50%). The fungal NST was decreased by cover crop in macroaggregate, and the fungal NST in cover cropping treatments (cover crop and cover crop + fertilizer treatment) showed the increasing trend with the decrease of the size of aggregate (silt+clay > microaggregate > macroaggregate).

Relationship analysis suggested that bacterial and fungal NST were significantly correlated to the fungal Shannon and Simpson index, but not correlated to bacterial Shannon and Simpson index (Figure 4a). Bacterial NST was negatively correlated to the fungal Shannon and Simpson index ( $p < 0.01$ ), and fungal NST was positively correlated to the fungal Shannon ( $p = 0.0207$ ) and Simpson ( $p = 0.0153$ ) index. Both IncMSE% and IncNodePurity indicated that the fungal alpha-diversity were the most important factors for the change of bacterial NST, while TN and fungal alpha-diversity were the most important factors for the change of fungal NST (Figure 4b).

Subsequently, we constructed the bacterial-fungal co-occurrence network for the whole soil and different sized aggregates based on the Pearson and Spearman correlation coefficients, and the Bray-Curtis (BC) and Kullback-Leibler (KLD) dissimilarity indices using OTUs with relative abundance  $\geq 0.01\%$ . There were 938, 938, 876 and 786 OTUs with abundance higher than 0.01% in the networks of whole soil, macroaggregate, microaggregate and silt+clay, respectively (Figure 5). The number of edges, clustering coefficient and the average number of neighbours showed that the OTUs in macroaggregate were more interconnected than in microaggregate and silt + clay (Table S2). The topological features of bacterial and fungal OTUs were calculated (Figure 5i and S3). In the networks of macroaggregate, microaggregate

and silt+clay, betweenness centrality of bacterial OTUs were higher than fungal OTUs (Figure 5i and S4). In the network of macroaggregate, the degree of fungal OTUs was higher than bacterial OTUs. Meanwhile, the top 5 degree of OTUs in silt + clay was averagely 26 and was much lower than in macroaggregate (averagely 103) and microaggregate (averagely 102) (Table S3).

In macroaggregate, bacterial and fungal OTUs with a high degree were co-occurred in the same module (e.g. OFU-379, OTU-3034, OTU-3767, OTU-4676 and OTU-2666) (Figure 5f and Table S5), and this trend was also found in microaggregate (e.g. OFU-28, OFU-596, OFU-706, OFU-1564 and OFU-424) (Figure 5g and Table S5). Furthermore, the number of fungal OTUs was higher than bacterial OTUs in the largest module of networks in macroaggregate (OFU vs. OTU was 106 vs. 48) and microaggregate (OFU vs. OTU was 143 vs. 31). However, in the network of silt + clay, the number of fungal OTUs was lower than bacterial OTUs in module 2 and 21 (OFU vs. OTU was 4 vs. 94 for module 2; OFU vs. OTU was 1 vs. 70 for module 21) and was higher than bacterial OTUs in module 5 (OFU vs. OTU was 97 vs. 11) (Table S5).

To make the keystone species in the network clearer, bacterial-fungal networks were split and showed as phylum network (Figure 6). For the bacterial network of whole soil, the OTUs with a high degree were belonged to Proteobacteria and Bacteroidetes. However, the OTUs with a high degree were belonged to Proteobacteria and Actinobacteria in aggregates (Figure 6 and Table S3). For fungal network, the OTUs with high degree were belonged to Ascomycota, Zygomycota, Basidiomycota and Glomeromycota (Table S3). Especially, many fungal OTUs with high degree in the network of aggregate were saprotrophic.

## Discussion

The mechanism of microbial community assembly had been investigated in numerous terrestrial habitats (Nemergut et al., 2011). In the agricultural field, a typical human-managed terrestrial ecosystem, unravelling the drivers of microbial community diversity and composition in response to different agricultural practice managed by human is a major goal in ecology. Based on the large-scale soil survey, Jiao et al. (2020) revealed microbial community assembly in adjacent pairs of maize and rice fields across different habitats and regions throughout Eastern China. Based on the field experiment, Feng et al. (2018) suggested that long-term fertilization significantly influenced the community assembly processes of soil diazotrophs. Gao et al. (2020) also indicated that fungal community assembly in drought-stressed sorghum shows stochasticity, selection and universal ecological dynamics. However, the microbial community assembly process in soil aggregate in agricultural ecosystem seems to be neglected. The soil aggregate and its nutrient contents could be affected by agricultural practices, such as cover crop and fertilization (Pramanik et al., 2014; Murilo et al., 2019; Nkanyiso et al., 2019). Different from the soil, fungi and bacteria were generally co-existed in soil aggregates and cooperated and competed with each other (Lehmann and Rillig, 2015). Thus, the interaction of bacterial and fungal communities may impact their assembly processes. The NST index was used to estimate the determinacy and stochasticity of the

bacterial and fungal community assembly process. Since established the experiment in 2008, the soil was annually managed by cover crop and fertilization for many years, and the bacterial and fungal community assembly process was mainly stochastic (NST > 50%) in the whole soil and aggregates (Fig. 3), which were consistent with other researches (Gao et al., 2020; Jiao et al., 2020). A long-term history of cultivation could positively select microorganisms with fitness advantages under relatively constant environmental conditions, resulting in less environment filtering (Jiao et al., 2020). For the bacterial community, the assembly process was more stochastic in silt + clay than macroaggregate (Fig. 3). Bacteria decompose organic material to form organo-mineral products that are associated with soil particles to form silt + clay (< 53  $\mu\text{m}$  diameter) (Tisdall and Oades, 1982; Tisdall, 1994), and these small microaggregates are in turn bound by bacterial and saprophytic fungi products to form slightly larger microaggregates (53–250  $\mu\text{m}$  diameter). The silt + clay could offer protective growth habitats for bacteria and fungi. Many researches have reported that nutrients in smaller aggregates were less impacted by environmental changes or agricultural practices (Neumann et al., 2013; Nie et al., 2014; Trivedi et al., 2015). Jiang et al. (2017) also suggested that microaggregate could protect bacteria from the predation of nematode due to its interior is not accessible (Jiang et al., 2017). Meanwhile, this was also found for the fungal community in cover crop treatment, but the assembly process of the fungal community was less stochastic than bacterial community and even deterministic in some treatments and aggregates. The different responses of bacterial and fungal community assembly processes to agricultural practice and aggregates was probably attributed to their different physiological traits and existing patterns in soil and aggregates. The relative effects of deterministic and stochastic processes on microbial communities depend on the organism types, such as body size and dispersal mode (Hanson et al., 2012; Kou et al., 2018). Bacterial cells do possess mobility, and can move towards, and perhaps to a limited extent, also into aggregates (Dechesne et al., 2010), but the vast majority of the aggregate interior is blocked from dispersal (Rillig et al., 2017). However, the fungi hypha (especially for saprobic fungi) generally penetrate or entangle the soil aggregate (Fig. 7), and the dispersal of the fungal community could be both deterministic and stochastic (Zhou and Ning, 2017), thus the fungal community assembly was deterministic and stochastic in macroaggregate of cover crop and control, respectively (Fig. 3).

In soils, especially in soil aggregate, bacteria and fungi generally co-exist in the same niche. In our study, we found that fungal alpha-diversity was positively correlated to the fungal NST, which consistent with other research (Gao et al., 2020). Interestingly, fungal alpha-diversity negatively impacted on bacterial NST, indicated that higher fungal diversity would cause lower stochasticity of bacterial community assembly, and this was not found for bacterial alpha-diversity (Fig. 4). Furthermore, both IncMSE% and IncNodePurity showed the fungal diversity was the most important factors for bacterial NST change. Based on this, we supposed that the fungal community was more dominate in soil aggregates than bacterial community, and the bacterial community assembly process was greatly influenced by the interaction of fungi and bacteria in soil aggregates.

Determination of the relationships between microbial community assembly and species coexistence is fundamental for the revealing of mechanisms of community diversity (Vályi et al., 2016; Zhou and Ning, 2017). The co-occurrence ecological network often used to demonstrate the potential biotic interactions

between the microbial taxa (Barberan et al., 2012; Ma et al., 2016), and further reflected the niche partitioning of microbial communities in soil ecosystem (Dini-Andreote et al., 2014). The network analysis had been adopted to disentangle deterministic and stochastic processes on biotic community assembly in various ecosystems (Yu et al., 2019). Because of the complex soil processes are jointly driven by both soil bacterial and fungal communities as well as their interactions, research about soil microbiomes has increasingly focused on all the microbial members as a whole (Ma et al., 2017; Morrien et al., 2017; Jiao et al., 2018). Compared to macroaggregate, the microbial interactions were decreased in silt + clay, especially for the fungal-bacterial interactions (Fig. 5 and Table S2), and stochastic assembly processes were more dominated in the bacterial community in silt + clay (Fig. 3) due to the fewer effects from the fungal community. Module analysis also partially supported this result, and bacteria and fungi were always co-occurred in the same largest module in the network of whole soil, macroaggregate and microaggregate with more fungal taxa than bacterial taxa (Table S5). However, in the network of silt + clay, the fungal and bacterial taxa were less co-occurrence in the same module and formed respective module (module 2 and 21 was dominated by bacteria and module 5 was dominated by fungi) (Fig. 5). Meanwhile, saprobic fungi played a key role in bacterial-fungal interactions in soil aggregates, because most of fungal OTUs with a high degree were saprobic fungi (Table S3), and saprobic fungi occupied a large proportion in soil aggregates in our study (Figure S5). Saprobic fungi was related to the aggregate formation, stabilization and disintegration (Lehmann and Rillig, 2015; Rillig et al., 2017), and the fungal taxa belonged to saprotroph and saprotroph-symbiotroph have high importance for the bacterial community assembly process (Figure S6).

## Conclusions

The bacterial and fungal community assembly process and their interactions in different sized soil aggregates were still unclear. Both bacteria and fungi can contribute to the formation of soil aggregate, and bacteria and fungi always co-exist in the same aggregate (Fig. 7). Differently, bacteria were generally adhered and enfolded by soil particles during aggregate formation, and fungi (especially for saprobic fungi) penetrates or entangles the soil aggregate by hypha and promotes the formation of aggregate. Thus, the bacterial community assembly process was more stochastic in silt + clay, compared to macroaggregate, due to less affected by fungi community. Network analysis suggested that interaction between bacteria and fungi was decreased in silt + clay, compared to macroaggregate. Although there may be other reasons for the change of stochasticity of bacterial community assembly, such as nutrient change and nematode grazing, our study suggested that the interaction between fungal and bacterial communities significantly impacted the bacterial community assembly, and the increase of fungal community diversity would decrease the stochasticity of bacterial community assembly.

## Declarations

### Ethics approval and consent to participate

Not applicable.

## **Consent for publication**

Not applicable.

## **Availability of data and material**

All of the sequence data were deposited in the NCBI database under accession number PRJNA392375.

## **Competing interests**

The authors declare that they have no conflict of interest.

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## **Author's contributions**

Wei Zheng performed the statistical analysis and helped to draft the manuscript; Fenglian Lv and Yanan Yin designed and coordinated the study and drafted the manuscript. Zhiyuan Zhao performed bioinformatic analysis; Zhaohui Wang and Zhengyang Zhao coordinated the study; Wei Zheng, Ziyang Li and Bingnian Zhai conceived of the study, and participated in its design and coordination and helped to draft the manuscript. All authors read and approved the final manuscript.

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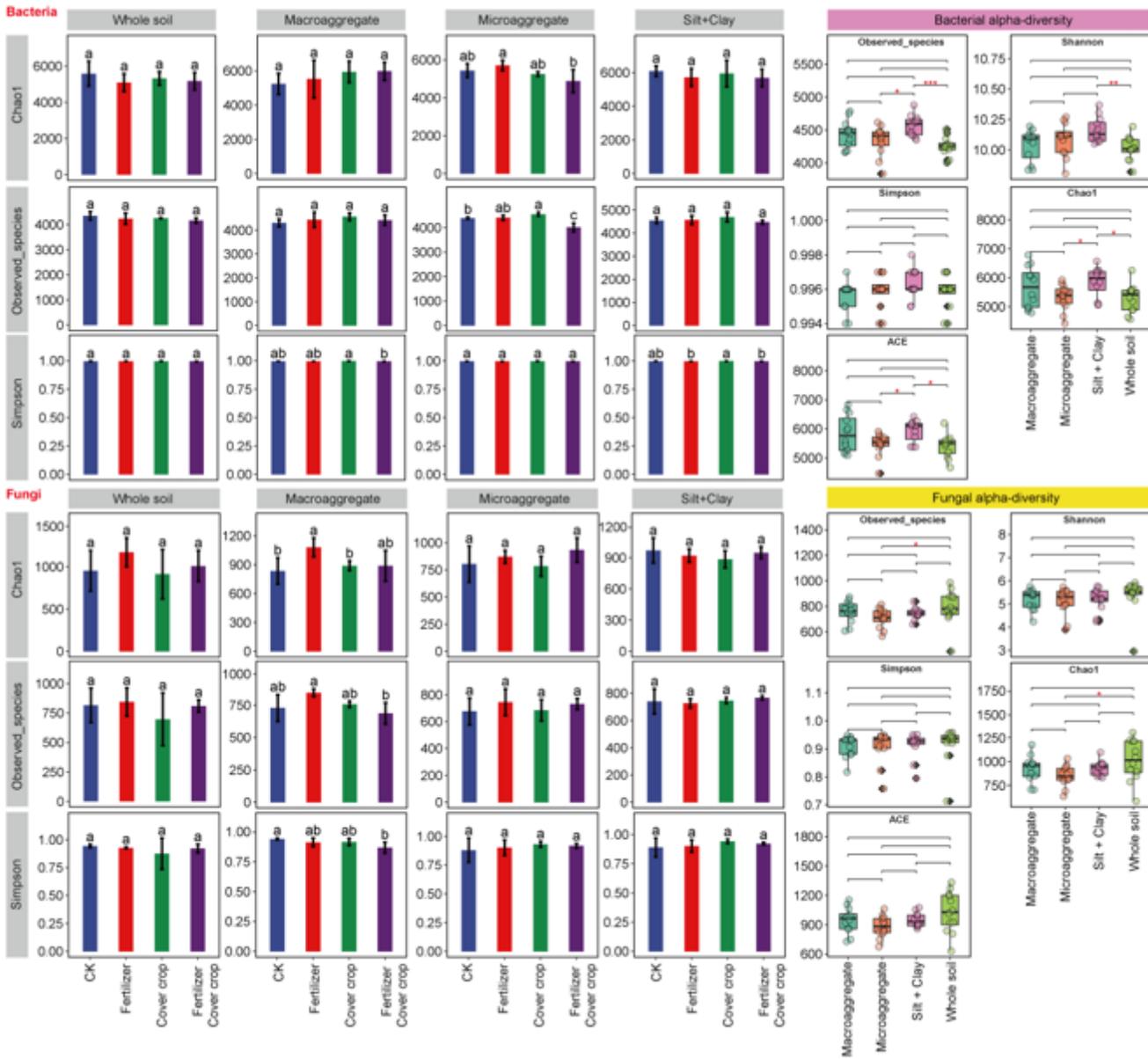
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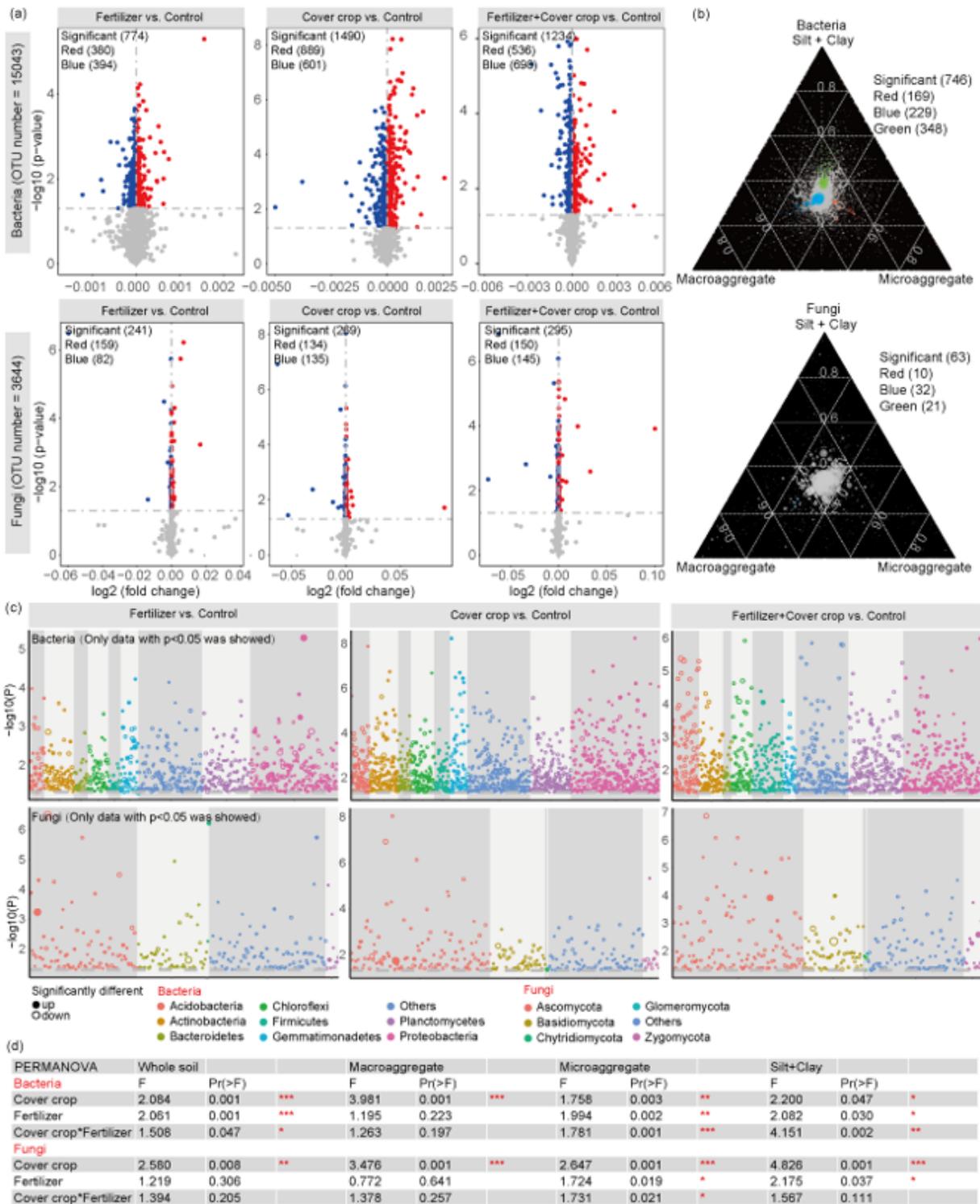
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## Figures



**Figure 1**

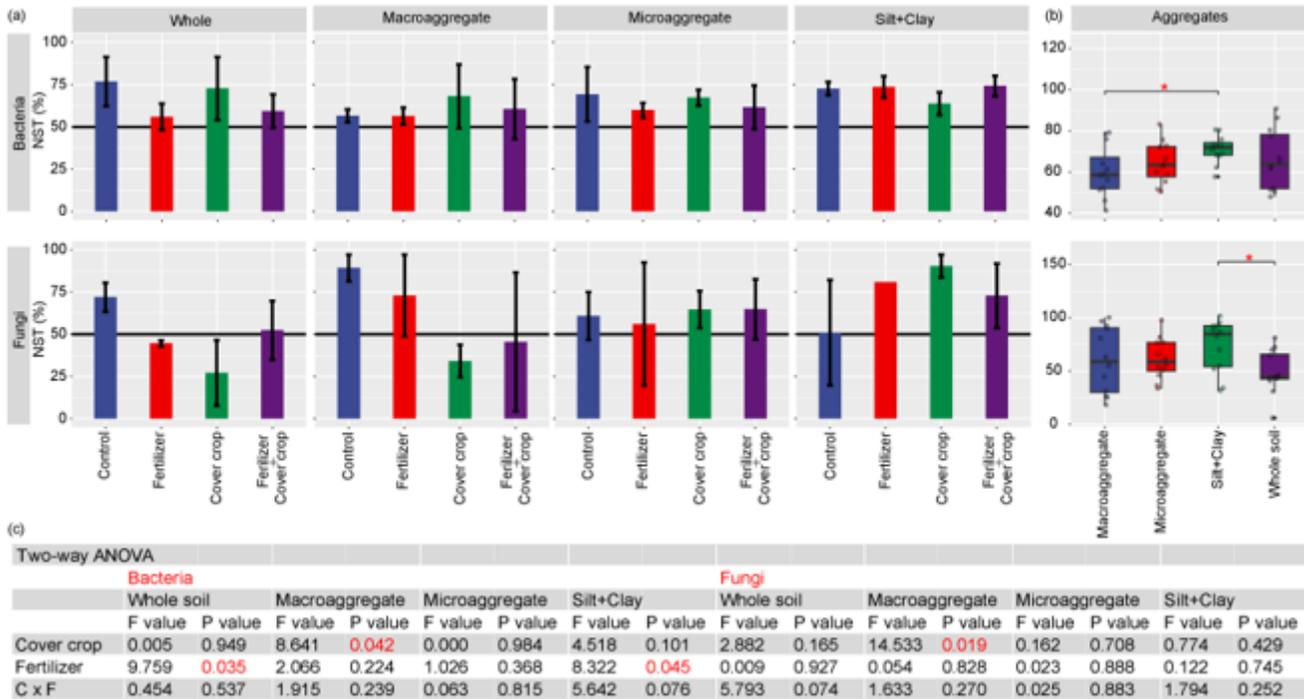
Bacterial and fungal alpha diversity between different treatments and aggregates. Differences of alpha diversity between different treatments and aggregates were tested by Kruskal-Wallis test. \*,  $p < 0.05$ ; \*\*,  $p < 0.01$ ; \*\*\*,  $p < 0.001$ .



**Figure 2**

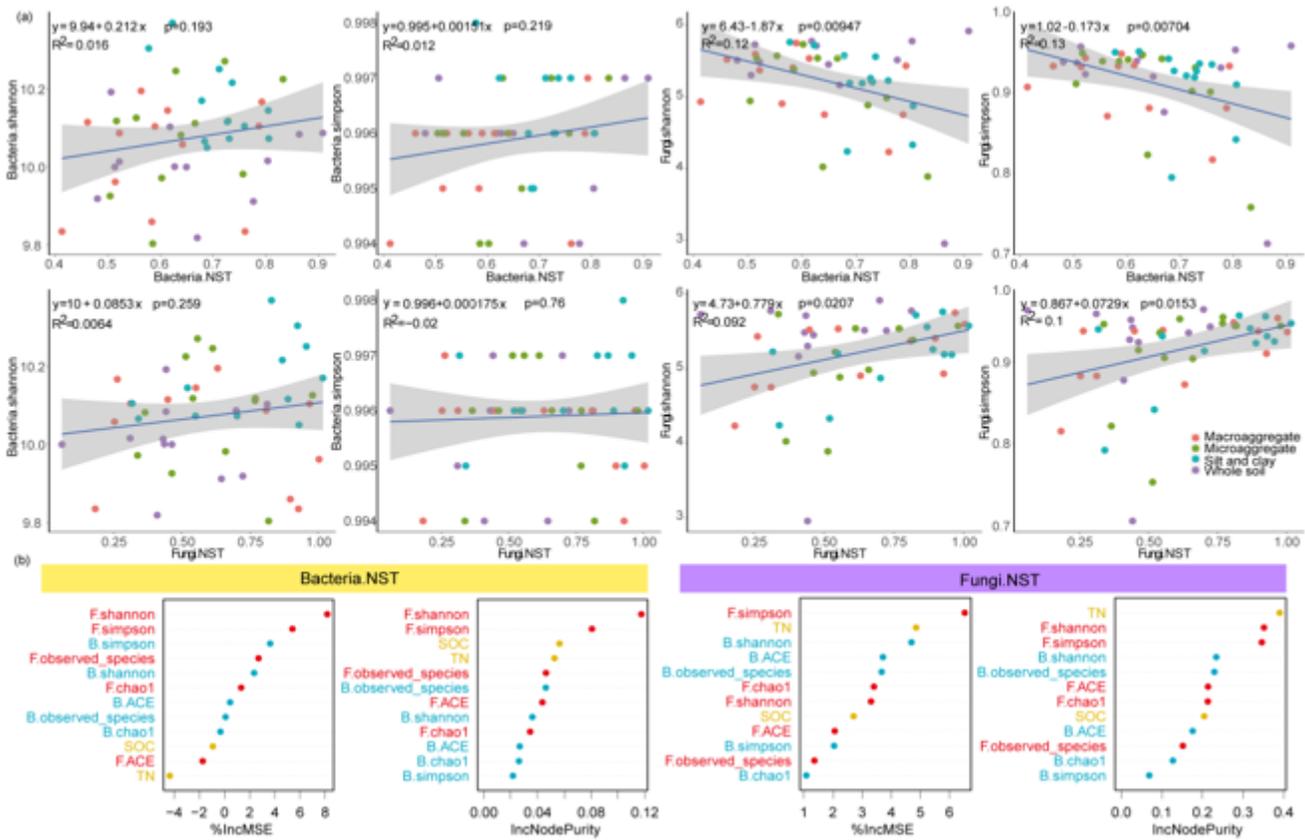
Difference of relative abundance of OTUs between different treatments and sizes of aggregate. a) Significant difference of relative abundance of OTUs between different treatments. Red (blue) points indicated OTU abundance of treatments with fertilizer or cover crop were significantly higher (lower) than control ( $P < 0.05$ ). b) Significant difference of relative abundance of OTUs between different sizes of aggregate. Blue, red and green points indicated OTU abundances were significantly higher in

macroaggregate, microaggregate and silt+clay than in other sizes of aggregate, respectively ( $P < 0.05$ ). c) Manhattan plots showed the OTUs (relative abundances were significantly different between different treatments) for different phyla. Points (circles) indicated OTU abundance of treatments with fertilizer or cover crop were significantly higher (lower) than control ( $P < 0.05$ ). The size of points or circles was related to the relative abundance of OTUs. d) PERMANOVA tests (999 permutations based on Bray-Curtis distances) showed the effects of cover crop and fertilizer on bacterial and fungal community compositions in the whole and aggregates.



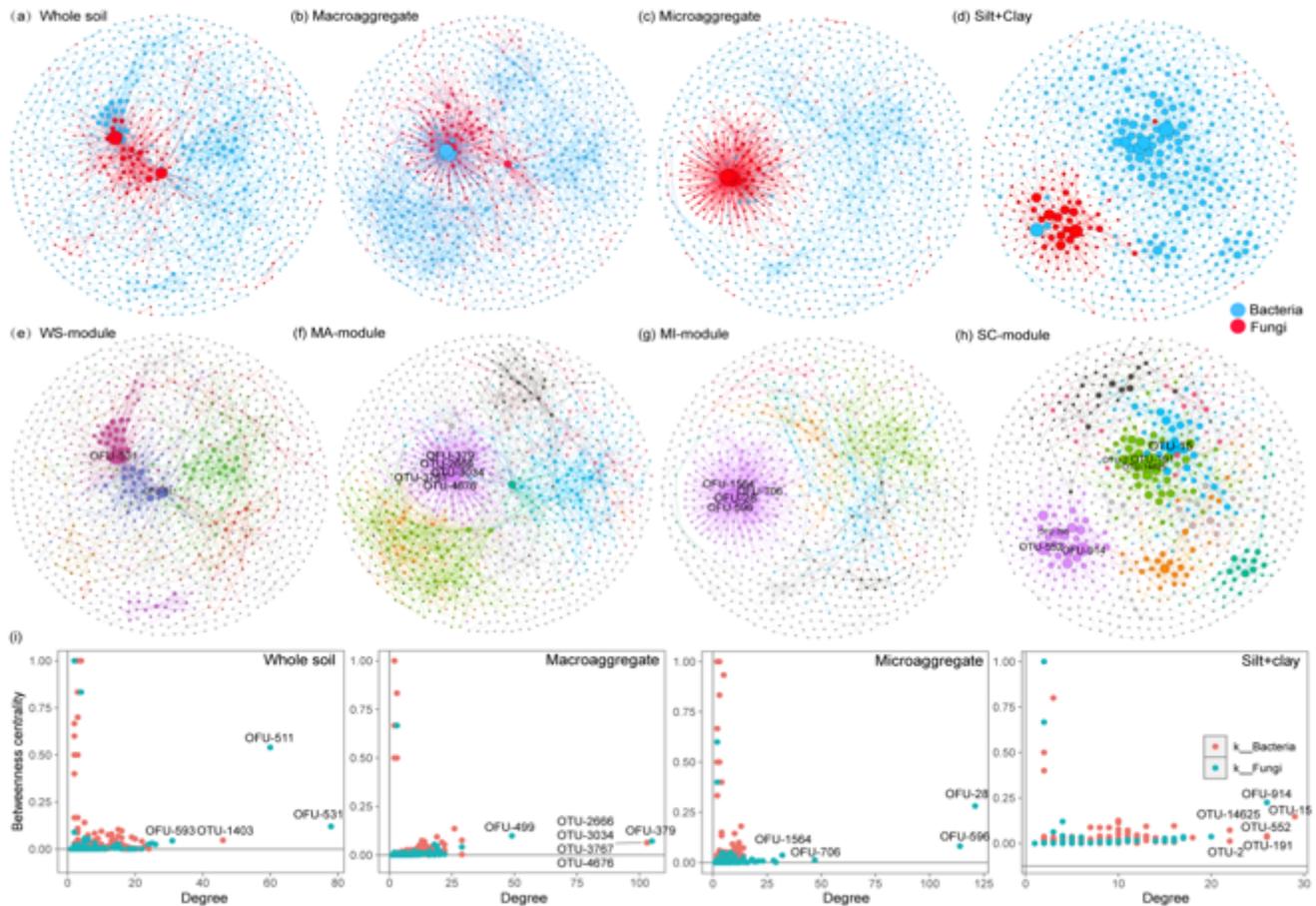
**Figure 3**

The estimated normalized stochasticity ratio (NST) of bacteria and fungi in different treatments and sizes of aggregate. a) Bar plots showed the difference of bacterial and fungal NST in different treatments. b) Box plots showed the difference of bacterial and fungal NST in different sizes of aggregate. Stars indicated  $p < 0.05$ . c) Two-way ANOVA analysis showed the effects of cover crop, fertilizer and their interaction on bacterial and fungal NST in whole soil and different sizes of aggregate.



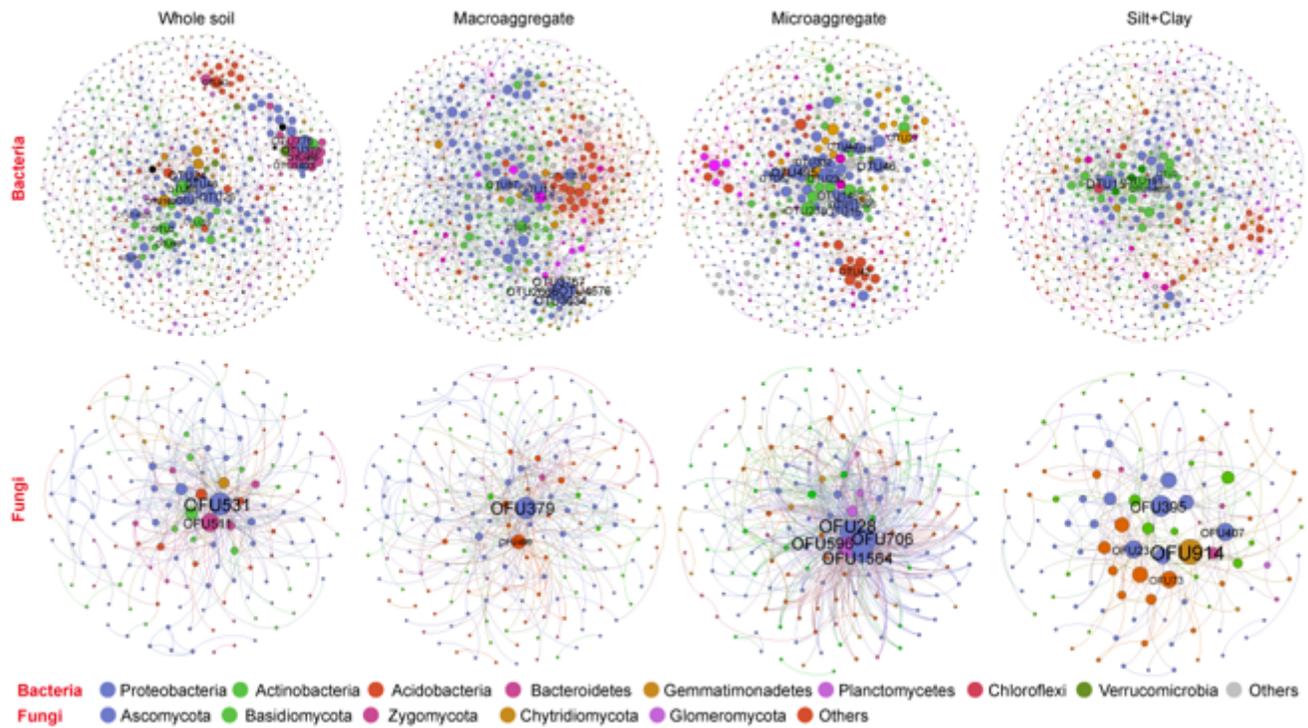
**Figure 4**

Relationships between bacterial and fungal alpha-diversity and NST. a) Linear relationship between alpha-diversity and NST. b) Random forest (RF) analysis showed importance (IncMSE% and IncNodePurity) of impact factors for the bacterial and fungal NST.



**Figure 5**

Co-occurrence network of bacterial and fungal OTUs in whole soil (a), macroaggregate (b), microaggregate (c) and silt+clay (d). The node size was related to the node degree. Modular analysis of network for the whole soil (e), macroaggregate (f), microaggregate (g) and silt+clay (h). Different colors indicated different modules, and the modules with low number of nodes were showed with gray. Nodes with high degree were marked with OTU and OFU for bacterial and fungal OTUs, respectively. i) Betweenness centrality and degree of each OTUs of bacteria and fungi in whole soil, macroaggregate, microaggregate and silt+clay. WS, whole soil; MA, macroaggregate; MI microaggregate; SC, silt+clay.



**Figure 6**

Sub network of networks in Figure 5. Nodes with high degree were marked with OTU and OFU for bacterial and fungal OTUs, respectively.

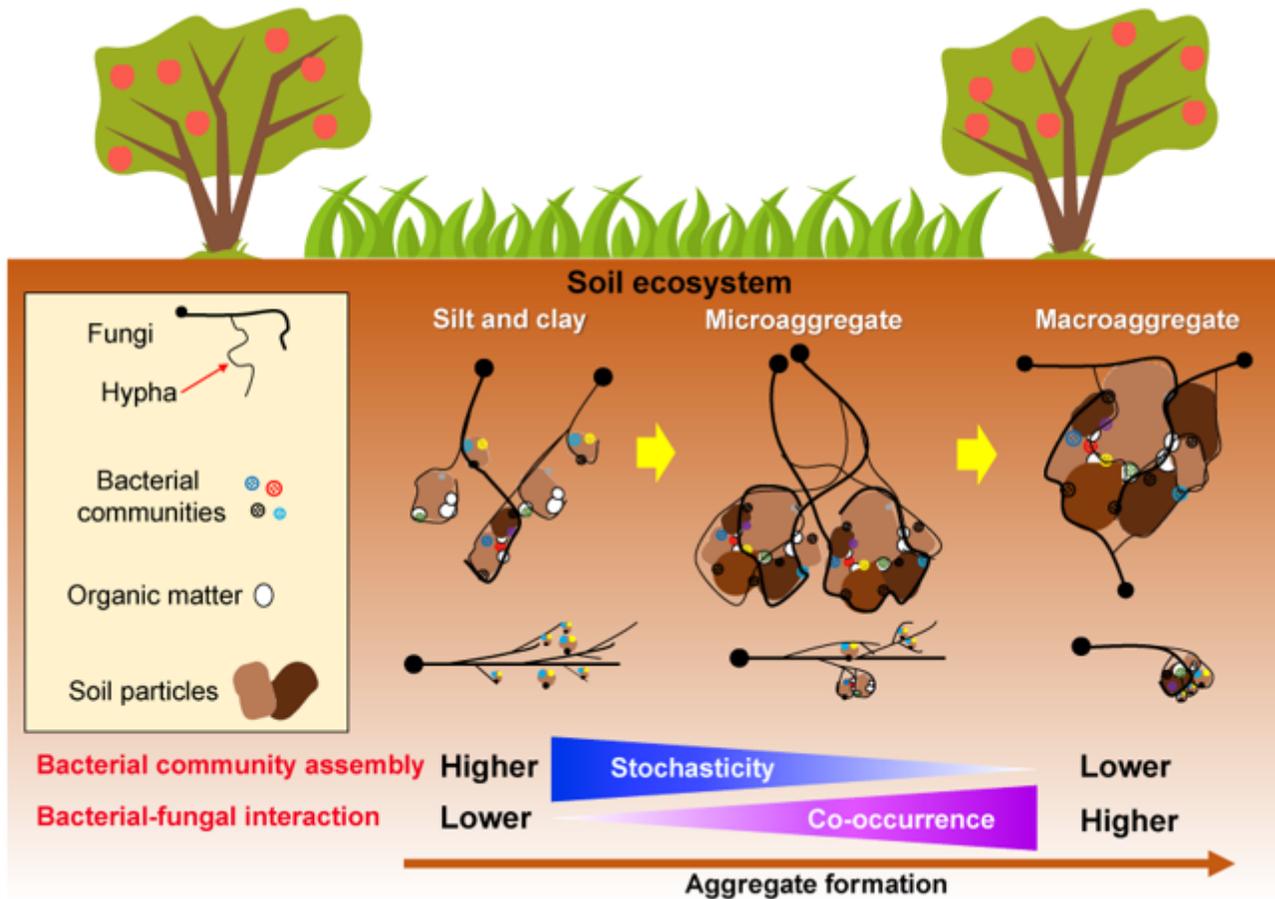


Figure 7

Schematic diagram showed the co-existence model of bacteria and fungi in different sizes of aggregates.

## Supplementary Files

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