Maize residue retention shapes soil microbial communities and co-occurrence networks upon freeze-thawing cycles (#96003)

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Maize residue retention shapes soil microbial communities and co-occurrence networks upon freeze-thawing cycles

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Maize residue retention is an effective agricultural practice for improving soil fertility in black soil region, where suffered from long freezing-thawing periods and intense freeze-thaw cycles (FTCs). However, very few studies have examined the influence of maize residue retention on soil microbial communities under FTCs. We investigated the response of soil microbial communities and co-occurrence networks to maize residue retention at different FTCs intensities across 12 cycles using microcosm experiment. Our results indicated that maize residue retention induced dramatic shifts in soil archaeal, bacterial and fungal communities towards copiotroph-dominated communities. Maize residue retention consistently reduced soil fungal richness across all cycles, but this effect was weaker for archaea and bacteria. Normalized stochastic ratio analysis revealed that maize residue retention significantly enhanced the deterministic process of archaeal, bacterial and fungal communities. Although FTC intensity significantly impacted soil respiration, it did not induce profound changes in soil microbial diversity and community composition. Co-occurrence network analysis revealed that maize residue retention simplified prokaryotic network, while did not impact fungal network complexity. The network robustness index suggested that maize residue retention enhanced the fungal network stability, but reduced prokaryotic network stability. Moreover, the fungal network in severe FT treatment harbored the most abundant keystone taxa, mainly being cold-adapted fungi. By identifying modules in networks, we observed that prokaryotic Module #1 and fungal Module #3 were enhanced by maize residue retention and contributed greatly to soil multifunctionality. Together, our results showed that maize residue retention exerted stronger influence on soil microbial communities and co-occurrence network patterns than FTCs and highlighted the potential of microbial interactions in improving soil functionality.

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Abstract

Maize residue retention is an effective agricultural practice for improving soil fertility in black soil region, where suffered from long freezing-thawing periods and intense freeze-thaw cycles (FTCs). However, very few studies have examined the influence of maize residue retention on soil microbial communities under FTCs. We investigated the response of soil microbial communities and co-occurrence networks to maize residue retention at different FTCs intensities across 12 cycles using microcosm experiment. Our results indicated that maize residue retention induced dramatic shifts in soil archaeal, bacterial and fungal communities towards copiotroph-dominated communities. Maize residue retention consistently reduced soil fungal richness across all cycles, but this effect was weaker for archaea and bacteria. Normalized stochastic ratio analysis revealed that maize residue retention significantly enhanced the deterministic process of archaeal, bacterial

and fungal communities. Although FTC intensity significantly impacted soil respiration, it did not induce profound changes in soil microbial diversity and community composition. Co-occurrence network analysis revealed that maize residue retention simplified prokaryotic network, while did not impact fungal network complexity. The network robustness index suggested that maize residue retention enhanced the fungal network stability, but reduced prokaryotic network stability. Moreover, the fungal network in severe FT treatment harbored the most abundant keystone taxa, mainly being cold-adapted fungi. By identifying modules in networks, we observed that prokaryotic Module #1 and fungal Module #3 were enhanced by maize residue retention and contributed greatly to soil multifunctionality. Together, our results showed that maize residue retention exerted stronger influence on soil microbial communities and co-occurrence network patterns than FTCs and highlighted the potential of microbial interactions in improving soil functionality.

Keywords: Maize residue retention; freezing-thawing cycles; black soil; microbial community; soil multifunctionality

1. Introduction

As one of the most precious soil resources in China, black soil is faced with serious soil erosion and fertility deterioration over the past several decades (Liu and Diamond, 2005;Yao et al., 2017). Maize residue retention is an advocated agricultural practice for improving soil fertility and crop yield in black soil region (Mitchell et al., 2016). It enables the utilization of straw resources while effectively ameliorate soil water use efficiency, prevent soil erosion and enhance soil fertility (Dai et al., 2017). Moreover, the benefits of maize residue retention on soil fertility

were also reflected at its effect on soil microorganisms (Wu et al., 2023).

Soil microorganisms are crucial component of soil ecosystem and contribute greatly to the process of straw decomposition (Fierer, 2017;Yang et al., 2021). Maize residue retention provides large amounts of substrate for soil microbes and improves soil nutrient availabilities, and thereby may enhance soil microbial biomass, activity and diversity (Yaoand, 2017). However, the effects of maize residue retention on soil microorganisms would depend on various factors including the climate, application time and types of straw. In cold regions, where low temperature and frequent freeze-thaw cycles (FTCs) are limiting factors for the crop residue decomposition, there is still uncertainty of maize residue retention on soil microbial communities (Gu et al., 2020;Guan et al., 2022).

FTCs is a common phenomenon in black soil region during winter (Groffman et al., 2010;Wei et al., 2016), and it encompasses two physical processes: soil freezing and melting. Previous microcosm and field studies have shown that FTCs would impose complex effects on soil microbial communities in several ways (Haei et al., 2011;Han et al., 2018;Yanai et al., 2011). Firstly, FTCs may directly disrupt soil microbial communities through lysis of microbial cells due to ice crystal formation (Yanaiand, 2011), and 7% of soil microorganisms may die by repeated FTCs (Ji and Wang, 2022). Secondly, the releasing nutrients from dead microbial cells and disruption of aggregates together lead to a rapid increase in soil available nutrients, which trigger the growth of soil microbes and induce changes in their community composition after thawing (Haeiand, 2011;Hanand, 2018). These changes may further influence soil enzyme activities, as well as the straw decomposition process. Consequently, understanding how soil microbial communities respond to FTCs would offer a more comprehensive insight into the performance of maize residue retention in cold regions.

In agricultural soils, the myriad of microbes lives together and form complex interconnected microbial networks, where microbes associate with each other directly or indirectly through processes, such as competition, predation, and mutualism (de Vries et al., 2018;Wagg et al., 2019). It is theoretically expected that microbial communities with more complex associations will have more active metabolic processes and faster growth rates, resulting in improved community performance (Brown et al., 2004;Chen et al., 2023;Jordan, 2009). Previous researchers have tried to link microbial network complexity to ecosystem multifunctionality (Chen et al., 2022;Waggand, 2019), and Chen et al (2022) reported that soil microbial network complexity contributed more to multifunctionality than diversity. Therefore, elucidating the complexity and stability of these microbial associations based on network analysis would provide more meaningful information than community analysis (Deng et al., 2012;Yuan et al., 2021). In recent years, a few studies have reported the effect of organic input (e.g., compost, crop residue) on the microbial co-occurrence network patterns. For instance, Xu et al. (2023) reported that maize residue retention complicates and stabilizes the soil microbial networks. However, the effects of FTCs on soil microbial networks are far less understood than that of maize residue retention, especially lacking the interactive effects of maize residue retention and FTCs. More importantly, very little is known of whether differences in the microbial networks have consequences for microbiome function upon maize residue retention.

Sanjiang Plain is located in the seasonal frozen soil area in Northeast China, suffering from long freeze-thaw periods and intense freeze-thaw cycles (Ouyang et al., 2013). Maize residue retention is an advocated agricultural practice to increase the contents of soil available nutrients in this region (Shen et al., 2018), and it will be crucial to emphasize the interactive effect of maize residue retention and FTCs on the soil microbial communities. Therefore, we conducted a

microcosm experiment and hypothesized that: (1) Soil microbial communities and co-occurrence networks would be affected by maize residue retention and FTCs; (2) Maize residue retention would exert a stronger effect on soil microbes than FTCs; (3) Maize residue retention would improve soil multifunctionality, and this effect would be mediated through soil microbial network properties.

2. Materials and methods

2.1. Soil collection and experimental design

The study was conducted at Institute of Environment and Sustainable Development in Agriculture, Chinese Academy of Agricultural Sciences, Beijing in 2022. The experimental design was a fully-factorial experimental design with three factors. One factor is maize residue retention, containing two treatments: no maize residue (control), maize residue retention. To stimulate the maize residue retention in field, the amount of maize residue incorporated was approximately equal to the application rate (13000 kg/hm^2) in Sanjiang Plain. The second factor is FTC intensity, containing three treatments: constant at 4℃ (no FTC), -4℃/ 4℃ (moderate FTCs), and -10℃/ 4℃ (severe FTCs). The soil was frozen at -4 ℃ or -10℃ for 2 h and then thawed at 4℃ within 12 h, repeat twice, which was regarded as a freeze–thaw cycle. The third factor is the number of FTCs, with 1, 3, 6, and 12 FTCs included. Each treatment was replicated four times, resulting in 96 pots (capacity: 4 cm in diameter, 7 cm in height) in total.

Soils used in the present study was collected from maize cropland in Sanjiang Plain (30°63′ N, 116°60′E) in August 30th, 2022. The soil at this study site is classified as Mollisol (according to USDA soil taxonomy). The dry maize straw generated in the same year was cut into pieces of ca. 1 cm length manually, its water content was 92.48%. Soil samples were evenly mixed or not

mixed with maize straw after sieved through 2 mm mesh, and incubated at 25℃ for 30 days. We added ca. 200 g soils in each pot and placed all pots in incubators. After each cycle, the pots were taken out without being put back, the filed water holding capacity was maintained at 24% during the period. Soils in each pot were divided into three parts, and stored at 4°C, -80°C and room temperature, respectively.

2.2. Soil physiochemical variables and enzyme activities determination

The soil moisture content was measured by the drying method. Soil pH was measured by potentiometer according to the ratio of soil to water 2.5:1. Soil total carbon (TC) and nitrogen (TN) were determined by elemental analyser. Soil nitrate N (NO_3^--N) and ammonia N (NH_4^+-N) were determined by flow analyzer. The available phosphorus (AP) was determined by the molybdenum-antimony resistance colorimetric method. The available potassium (AK) was determined by flame atomic absorption spectrometry. Soil microbial respiration was determined by LI-850 CO_2/H_2O analyzer (Bao, 2000).

In this study, six soil enzymes related to C, N, and P metabolism were selected, including β-D-glucosidase (BG), β-D-xylosidase (XYL), urease (UE), leucine aminopeptidase (LAP), polyphenol oxidase (PPO) and acid phosphatase (APE) activities. The above six enzymes were all determined by micro method with enzyme labeling apparatus (DeForest, 2009).

2.3. DNA extraction and Miseq sequencing

Total DNA was extracted from soil using PowerSoil DNA isolation kit (Qiagen, Crawley, UK). Prokaryotic 16S rDNA region was amplified with primer 515F/806R (Wasimuddin et al., 2020), and fungal ITS2 fragment was amplified with primer gITS7/ITS4 (Ihrmark et al., 2012). PCR products were detected by electrophoresis and purified. The DNA concentration of purified PCR product was determined using Nanodrop2000 (Thermoscientific, USA), 50ng DNA was

taken from each DNA sample and corrected to 10 ng μL^{-1}. The corrected samples were then sequenced using IlluminaMiseq platform. The raw sequence data have been deposited on the NCBI SRA, with accession number PRJNA1045363(SRR26950863-SRR26951054).

QIIME PipelineVersion1.8.0 (Caporaso et al., 2010) was used to remove sequences that contained incorrect primers, fuzzy bases, the same continuous base > 8 or average quality values < 25. The "chimera.uchime" command in Mothur software was used to remove potential chimera sequences. Prokaryotic sequences were then error-filtered and grouped into amplicon sequence variants (ASVs) using the Deblur software (Amir et al., 2017). The ASVs were blasted against the silva 16s database and UNITE database to annotate their taxonomy, and ASVs that are not assigned as prokaryotes or fungi were removed. The number of sequences per sample was rarefied to 32,582 and 11,975 for prokaryotes and fungi using the "vegan" package, respectively. Furthermore, the archaeal and bacterial ASVs were picked from prokaryotic ASV tables and rarefied, respectively.

2.4. Data analysis

Archaeal, bacterial and fungal diversity indices were calculated for each treatment using the "vegan" software package. Fungal trophic modes (pathotroph, saprotroph, symbiotroph) were annotated using FUNGuild (Nguyen et al., 2016). The effects of residue retention and FTC on soil physicochemical properties, enzyme activities, respiration, and prokaryotic/fungal diversity and fungal trophic modes were analyzed by mixed effect model (random =~ 1|Block/Plot, correlation = corCAR1(form =~ Cycle|block/plot). The prokaryotic and fungal community compositions were ordinated by principal co-ordinate analysis (PCoA) based on bray-curtis dissimilarity. Then the effect of maize residue retention and FTCs were examined using permanova analysis with 999 permutations. Mantel test in "ecodist" software package was used to analyze the relationship between soil prokaryotic/fungal community composition and soil physiochemical properties. The

normalized stochasticity ratio (NST) was calculated to examine the community assembly processes of archaea, bacteria and fungi using the “NST” package (Ning et al., 2019).

Co-occurrence networks were constructed for soil prokaryotic and fungi based on all soil samples using the package "igraph". ASVs with relative frequency >50% are retained for network construction. The spearman correlation coefficient among different ASVs was calculated using the "psych" software package. After the P value was corrected by FDR, the correlations with $P > 0.01$ and $r < 0.6$ were removed. Nodes with a value of among-module connectivity > 0.625 or within-moduleconnectivity >2.5 are identified as keystone species (Guimerà and Amaral, 2005). The modules with more than 5 nodes were identified, and the topological properties including edges, connectedness and robustness were calculated. We also constructed sub-networks for each treatment to compare the different network patterns.

Soil multifunctionality index is a synthetic parameter calculated from the average value of z-score transformation of APE, BG, XYL, LAP, AP, TN and TC, which could reflect the soil function comprehensively. The random forest model was used to explore the contribution of soil microbial diversity indices, network modules, topological properties and the positive edges/negative edges (P/N) ratio to soil multifunctionality. All analysis were conducted using R 3.6.0 (R core Team, 2014).

3. Results

3.1. Soil physiochemical properties, enzyme activities and respiration

The soil physiochemical variables, enzyme activities and respiration among treatments were presented in Table S1. Mixed effect model revealed that maize residue retention significantly enhanced soil nutrient availabilities (e.g., TN, AK and AP), but FTC intensity exhibited no effect on these variables (Table 1). Among the six soil enzyme indices, BG, XYL and LAP were

188 increased by maize residue retention, while PPO was reduced by maize residue retention (Table
189 S1). Soil respiration was significantly impacted by FTC intensity and its interaction with maize
190 residue retention. Moderate and severe FTC significantly reduced soil respiration without maize
191 residue retention, but did not impact soil respiration under maize residue retention (Fig. S1).

192 **3.2. Soil prokaryotic and fungal communities**

193 A total of 32,582 prokaryotic ASVs and 11,975 fungal ASVs were obtained after quality
194 control and flattening. Among prokaryotic ASVs, 32,355 ASVs belonged to bacteria, and 227
195 ASVs belonged to archaea. Actinobacteriota (25.60% in total abundance) was the dominant
196 phylum for bacteria, followed by Proteobacteria (21.08%), Verrucomicrobiota (12.22%) and
197 Acidobacteriota (12.19%). Crenarchaeota (99.91%) was the predominant phylum for archaea,
198 while other phyla only occupied a minor fraction of archaeal communities. For fungi,
199 Ascomycota, Zygomycota and Basidiomycota dominate their communities and occupied 95.49%
200 of the total abundance (Fig. 1D-F).

201 Maize residue retention consistently reduced soil fungal richness across all cycles, but this
202 effect was weaker for archaea and bacteria (Table 1, Fig. 2A-C). At the phylum level, Bacteroidota
203 harbored significantly higher richness in maize residue retention than in control, while
204 Acidobacteriota, Actinobacteriota, Chloroflexi, Myxococcota, Planctomycetota,
205 Verrucomicrobiota, Crencrchaeota, Ascomycota and Basidiomycota exhibited opposite pattern
206 (Fig. S2A-C). Although the total archaeal, bacterial and fungal richness were not affected by FTC
207 intensity, the richness of phylum Acidobacteriota, Firmicutes and Crenarchaeota were slightly
208 reduced by moderate or severe FTC intensity (Fig. S2D-F).

209 Soil bacterial, archaeal and fungal community composition were ordinated using PCoA based
210 on Bray-Curtis dissimilarity. The ordination plots clearly indicated that they were all separated by

maize residue retention (Fig 1A-C), which was also supported by permanova analysis (Table S2). However, FTC intensity did not significantly impact soil microbial community composition, except a marginal effect on archaeal community composition (Table S2). The shift of soil microbial communities was also reflected at the phylum level. The relative abundance of Acidobacteriota, Chloroflexi, Basidiomycota were reduced by maize residue retention, while Actinobacteriota, Firmicutes and Zygomycota were enriched. (Fig. 1）

3.3 Predicted fungal function

Soil fungal community was assessed in terms of fungal guilds, and 34.10 % of fungal ASVs were assigned to a fungal guild. ANOVA analysis revealed that the abundance of saprotroph and symbiotroph (pathotroph) were all significantly impacted by maize residue retention and its interaction with the number of FTCs. The relative abundance of saprotroph was enhanced by maize residue retention in the 6^{th} FTCs and 12^{th} FTCs, but the pathotroph was only enhanced in the 1^{st} FTC. Moreover, the relative abundance of symbiotroph were consistently reduced by maize residue retention (Fig. S3).

3.4 Community assembly process

NST analysis revealed that both of bacterial and archaeal communities were dominated by stochastic process (the average NST value was 64.92% and 63.79%, respectively), and fungal community was dominated by deterministic process (the average NST value was 38.30%, Fig 2D-F). Maize residue retention significantly enhanced the deterministic process of archaeal, bacterial and fungal communities. Moreover, FTC intensity did not impact the archaeal, bacterial and fungal community assembly process (Fig 2D-F).

3.5. Prokaryotic and fungal co-occurrence networks

Prokaryotic and fungal co-occurrence networks were firstly constructed based on all samples

(Fig. 3A, C). As shown in Fig. 3, prokaryotic network was larger and more connected than fungal network. We then visualized modules with more than five nodes in networks, and focused on the top 4 modules for both prokaryotes and fungi. For prokaryotic network, Module #1 was the largest module and composed of multiple phyla, mainly including Actinobacteriota, Crenarchaeota, Proteobacteria and Firmicutes. Interestingly, Module #2, #3 and #4 were composed of single phylum, that is, Verrucomicrobiota, Chloroflexi, and Crenarchaeota, respectively (Fig. 3B). The relative abundance of Module #1 in maize residue retention treatment was significantly higher than in control, whereas, Module #2, #3, and #4 were unaffected by maize residue retention (Fig. 3E-H). For fungal network, the different modules contained distinct fungal phyla. Ascomycota was the dominant phylum in the Module #1, #2 and #4, and Basidiomycota dominated the Module #3 (Fig. 3D). Maize residue retention significantly enhanced the relative abundance of Module #3, but reduced the abundance of Module #1, #2 and #4 in the fungal network. However, FTC intensity exhibited no effect on these modules (Fig. 3I-L).

The visualized prokaryotic networks were smaller and less connected in maize residue retention than in control treatment, while the fungal networks did not display marked difference between treatments (Fig. 4A-D). These patterns were further supported by the topological properties (e. g. connectedness) calculated based on the whole prokaryotic and fungal networks. Maize residue retention significantly reduced the robustness and connectedness of prokaryotic network, suggesting that the complexity and stability of prokaryotic network decreased after maize residue retention (Fig. 4C, D). Although maize residue retention did not affect the connectedness of fungal network, it significantly increased the network robustness. We then inferred the interaction relationships among prokaryotes and fungi by calculating the number of positive and negative links in their networks. The positive/negative links (P/N) ratio of prokaryotic network

was significantly increased by maize residue retention, but the fungal network exhibited opposite trend (Fig. 4E-H).

Keystone prokaryotes and fungi were identified based on Pi and Zi value in each treatment. The keystone prokaryotes and fungi were different among control, moderate and severe FTC treatments. Notably, fungal network in severe FTC treatment harbored more keystone taxa (18) than control (5) and moderate FTC treatment (3). The annotation of each keystone taxa is shown in Supplementary Table S4.

3.6. Soil multifunctionality

Soil multifunctionality was comprehensively assessed using multiple variables including soil nutrient availabilities and enzyme activities. Independent-sample t test analysis revealed that soil multifunctionality was significantly improved by maize residue retention (Fig. 5A). Random forest model was performed to identify the key factors in predicting soil multifunctionality, and explained 40 % of the variations in soil multifunctionality. Fungal Module #3 was the most important determinant for soil multifunctionality, followed by fungal P/N ratio, prokaryotic Module #1, fungal richness, fungal Module #1, Module #2 and prokaryotic Module #4 (Fig. 5B). Especially, soil multifunctionality had strong and positive correlations with the relative abundance of fungal Module #3 and prokaryotic Module #3 (Fig. 5C, D).

4. Discussion

4.1. Maize residue retention altered soil microbial communities

Our study is a short-term microcosm study which simulate maize residue retention under different FTC intensity across 12 FTCs. Our results indicated that soil archaeal, bacterial and fungal community compositions were significantly impacted by maize residue retention, which

was supported by a large number of field studies (Liu et al., 2021). For instance, Xu et al. (2023) reported that maize residue retention induced significant shift in soil microbial communities across a latitudinal gradient (Xu et al., 2023). Maize straw is rich in labile and recalcitrant organic carbon, thus provide substrates for soil microbes and reshape their community compositions (Wuand, 2023). Alternatively, maize residue retention would possibly alter the soil microbial communities through the change in soil physiochemical characteristics. As revealed by Mantel test, soil microbial communities were correlated with a series of soil physiochemical variables (e.g., AK, AP, TN) in the present study.

Maize residue retention induced a shift from oligotrophic-dominated community to copiotrophic-dominated community. The copiotrophic groups including Firmicutes and Bacteroidota, which have high growth rates under resource-rich conditions (Fierer et al., 2007), were enriched by maize residue retention. It is true that there will be dead cells in the soil after FTC treatment, but this will only affect the identification of the presence or absence of species, and will not have much effect on the relative abundance comparison. In contrast, Acidobacteria, Chloroflexi, Verrucomicrobiota, which have oligotrophic attributes, were reduced by maize residue retention. The shift of fungal community was also reflected on the functional guilds. The saprotroph was enriched in maize residue retention treatment, and this pattern was increasingly obvious along with the incubation time. This result confirmed the importance of saprotroph in straw decomposition, and implied saprotroph would be increasingly important during the straw decomposition. One concern for farmers in adopting maize residue retention practice is its potential increase in incidence of plant disease (Tang et al., 2011). However, our results indicated that maize residue retention only briefly increased the pathotroph abundance in the first FTC, which suggested that maize residue retention practice will not threaten crop health.

Contrary to previous studies (Guanand, 2022;Muhammad et al., 2021), maize residue retention depressed soil archaeal, bacterial and fungal diversity in the current study. The lower soil microbial diversity in maize residue retention treatment can be attributed to the increased importance of deterministic process. Because in communities with large populations, the assembly processes are more susceptible to deterministic process (Xun et al., 2019). Specifically, the decreased richness mainly belonged to the phylum that defined as oligotroph. Therefore, the maize residue retention may act as a selection pressure, and probably caused the decrease in the microbial diversity via the disfavour of the oligotrophic groups.

4.2 FTCs decreased soil microbial activity without affecting their community compositions

FTCs is a common phenomenon and important factor that leads to soil degradation in black soil region. Our study indicated that moderate and severe FTCs significantly reduced soil respiration. FTCs may depress soil microbial activity directly by lysis of soil microbes or indirectly by disturbance of soil aggregates (Ji et al., 2022;Zong et al., 2023). However, the effect of FTCs on soil respiration was not detectable under maize residue retention, indicating that maize residue retention would alleviate the adverse effect of FTC on soil microbial activity.

In contrary to our first hypothesis, we found that FTCs had no significant effect on soil microbial diversity and community composition. This finding was consistent with some studies, which find minor or no detectable effects of FTCs on soil microbial communities (Männistö et al., 2009;Meisner et al., 2021). Firstly, although repeated FTCs would directly reduce soil microbial biomass and diversity, it also release nutrients to soils from dead microbial cells which would trigger the growth of soil microbes after thawing (Haeiand, 2011;Hanand, 2018). These effects may offset each other. Secondly, soil microbial communities developed in high-altitude or high-

latitude regions are reported to be cold-tolerant (Koponen et al., 2006; Yergeau and Kowalchuk, 2008) and resistant to repeated FTCs (Pastore et al., 2023). Alternatively, the shift of soil microbial communities under FTCs may be reflected at the gene expression level but not at the DNA replication level. Because soil microorganisms can enter a dormant state under FTCs, and their 16s rDNA or ITS fragments can still be detected by amplicon sequencing (Woodcroft et al., 2018).

4.3. Soil microbial network was affected by maize residue retention rather than FTCs

Since organic inputs provide a substantial supply of substrates and nutrients for soil microbes, previous studies indicated that organic inputs generally increased the complexity of soil microbial networks (Yang et al., 2019). However, we observed that maize residue retention simplified soil prokaryotic network, reflected by the greater number of nodes, links and connectedness. The simplified prokaryotic network is not likely due to the increased nutrient availabilities, but more likely to be the consequence of disturbed microhabitats and fragmented niches after maize straw incorporated in soils. Simple networks with smaller connectivity are generally less resistant to environmental perturbations than complex networks (Xuand, 2023). Herein, the robustness of prokaryotic network was also reduced by maize residue retention. Fungal network exhibited different pattern as compared with prokaryotic network. Although maize residue retention did not affect fungal network complexity, it dramatically enhanced the network stability. This result also collaborated with the finding that maize residue retention decreased fungal P/N ratio. As proposed by Coyte et al. (2015), the negative interactions among members might stabilize co-oscillation in communities and promote stability of networks (Coyte et al., 2015). Taken as a whole, our findings suggested that soil fungal community would be resistant against environmental stresses under maize residue retention.

Although FTC intensity did not impact the network pattern of soil prokaryoates and fungi, it altered the keystone taxa in network. The keystone prokaryotes and fungi were totally different among control, moderate and severe FTC treatments. Especially, the fungal network in severe FTC treatment harbored the most abundant keystone taxa. Among these keystone taxa, *Pseudogymnoascus roseus* and *Pseudeurotium hygrophilum* were reported to be cold-adapted fungi (Ramasamy et al., 2023), and thereby may stabilize fungal network under repeated severe FTCs.

4.4. Potential roles of network modules in driving soil multifunctionality

The effect of maize residue retention on soil function is still a subject of considerable debate (Wuand, 2023). Our results proved that maize residue retention would improve soil multifunctionality (Fig.5). The multifunctionality was a composite index combined by APE, BG, XYL, LAP, AP, TN and TC, which could properly reflect the soil function. We then explored the key factors that contribute to soil multifunctionality. Recently, a large number of research have recorded that soil multifunctionality is positively correlated with soil microbial diversity. The current study, however, proposed that network modules were more important than microbial diversity in predicting soil multifunctionality. Random forest analysis indicated that the relative abundance of fungal Module #3 and prokaryotic Module #1 were the most important determinants for soil multifunctionality, suppporting our third hypothesis.

Modules identified in the network represent a group of microbial taxa that potentially interact or share similar niches (Wiens, 2010), and contribute to specific ecological processes. We found that the main modules exhibit different strategies to maize residue retention. Module #1 in prokaryotic network, which consists of multiple phylum, positively responded to maize residue retention. Members in this module are capable of celloluse degradation (e.g. *Bacillus* and

372 *Cellulosimicrobium*), lignin degradation (e.g. *Streptomyces* and *Paenibacillus*), participate in N
373 cycling (e.g. *Burkholderia*). These members interacted with each other and would be efficient in
374 straw degradation. Likewise, Module #3 in fungal network also exhibited a great preference to
375 maize residue retention. Interestingly, more than half members in fungal Module #3 belonged to
376 genus *Chaetomium*, *Rhizopus* and *Mucor*, which are typical cellulose-degrading fungi (Ferreira et
377 al., 2013;Liuand, 2021). These results indicated that members in these two modules would involve
378 in processes that related to maize residue retention. However, the relative abundance of fungal
379 Module #1, 2, and 4 were sharply decreased by maize residue retention. These modules possibly
380 either be depressed by the unfavorable condition created by maize residue retention or due to the
381 aggravated competition.

382 ## 5. Conclusions

383 In conclusion, our results indicated that maize residue retention induced pronounced changes
384 in soil microbial communities and significantly reduced their richness. Although FTC intensity did
385 not impact soil microbial diversity and community composition, it depressed soil respiration
386 without maize residue retention. Moreover, maize residue retention reduced the complexity and
387 stability of soil prokaryotic network, while improved fungal network stability, indicating a high
388 resistance of fungal communities to maize residue retention. Taken as a whole, our results
389 indicated that maize residue retention is a stronger determinant than FTCs in shaping soil microbial
390 communities in black soil region. Another contribution of the present study was the finding that
391 the network modules contributed more to soil multifunctionality than microbial diversity.

392 ## Acknowledgments

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Table 1(on next page)

The mixed effect models with FTC cycles (Cycle) autocorrelation were used to evaluate the influence of residue retention (RR), freeze-thawing cycle (FTC), and interaction between RR and FTC on each index.

(random =~ 1|Block/Plot, correlation = corCAR1(form =~ Cycle|block/plot). The data in the table are *P* values.

Table 1
The mixed effect models with FTC cycles (Cycle) autocorrelation were used to evaluate the influence of residue retention (RR), freeze-thawing cycle (FTC), and interaction between RR and FTC on each index. (random =~ 1|Block/Plot, correlation = corCAR1(form =~ Cycle|block/plot).The data in the table are *P* values.

Variables	Cycle	RR	FTC	RR*FTC
AK	**0.043**	**<0.001**	0.588	0.346
AP	0.065	**<0.001**	0.974	0.667
pH	0.603	0.157	0.316	0.969
NH_4^+-N	**0.036**	0.135	0.603	0.091
NO_3^--N	0.087	0.260	0.428	0.999
TN	0.094	**<0.001**	0.214	0.738
TC	**<0.001**	0.098	0.381	0.427
APE	0.332	0.138	0.610	0.690
BG	0.204	**<0.001**	0.247	0.580
XYL	**<0.001**	**0.018**	0.734	0.522
LAP	**<0.001**	**<0.001**	0.523	0.940
PPO	**<0.001**	**0.025**	0.073	0.091
UE	**0.077**	0.825	0.667	0.999
Respiration	**<0.001**	0.151	**0.015**	**0.011**
MBC	0.054	**<0.001**	0.491	0.746
BAC_S	**0.018**	**<0.001**	0.231	0.882
FUN_S	**0.002**	**<0.001**	0.386	0.750
ARCH_S	**<0.001**	**<0.001**	0.162	0.316

1

Figure 1

Principal coordinate analysis of soil archaeal (A), bacterial (B) and fungal (C) community compositions among treatments. Relative abundance of archaeal (D), bacterial (E) and fungal (F) phyla.

Abbreviations: CK, control; RR, residue retention; FTC, freezing-thawing cycle; FTC1, constant 4 °C; FTC2, -4℃/ 4℃ (moderate FTCs), and -10℃/ 4℃ (severe FTCs). 1FTC, 3FTCs, 6FTCs and 12FTCs represents for one, three, six and 12 freezing-thawing cycles, respectively.

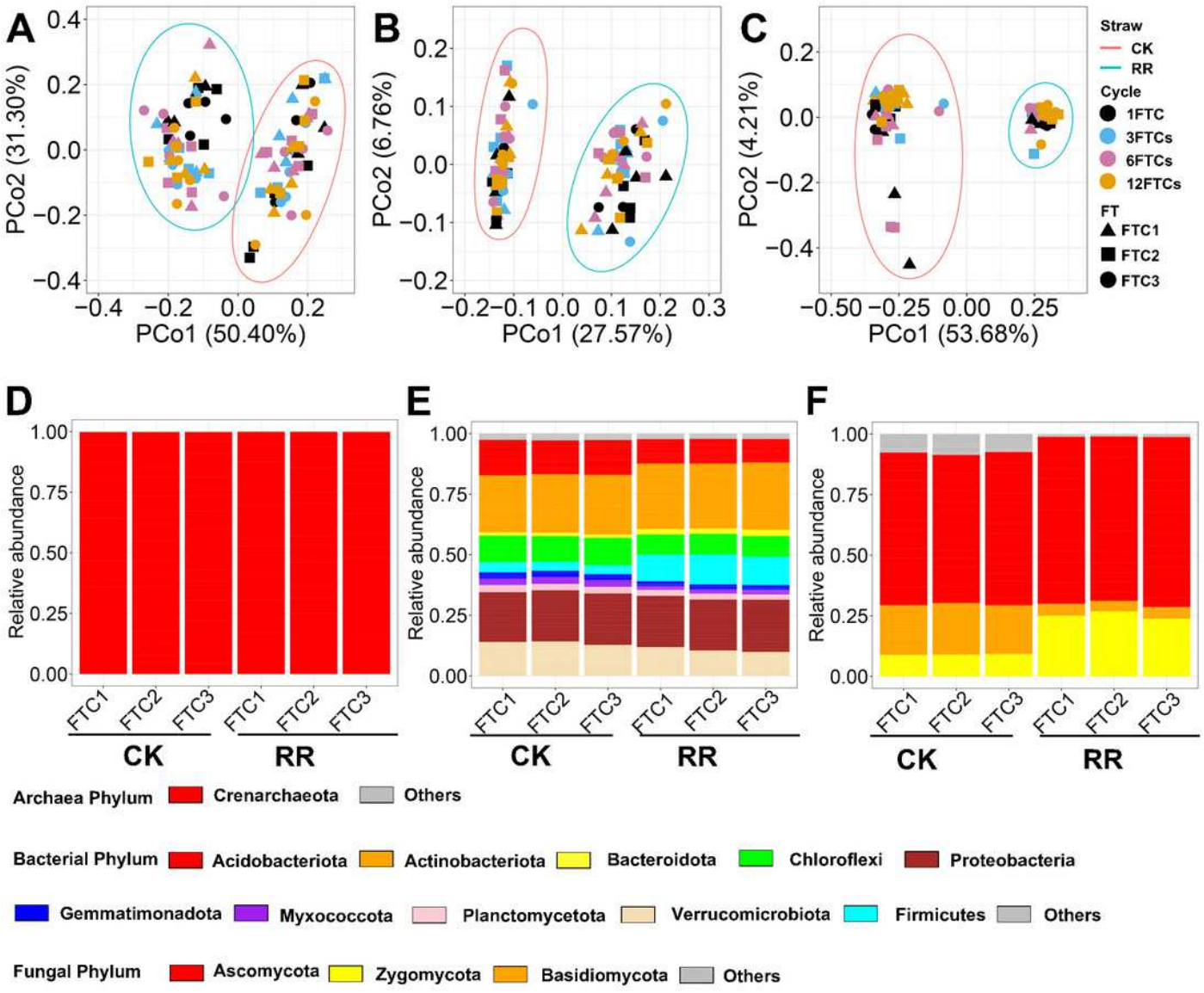


Figure 2

Box plot showing the archaeal (A), bacterial (B) and fungal (C) richness among treatments in 1FTC, 3FTCs, 6FTCs and 12FTCs. Bar plots showing the normalized stochastic ratio of archaeal (D), bacterial (E) and fungal (F) community assembly.

Abbreviations: CK, control; RR, residue retention; FTC, freezing-thawing cycle; FTC1, constant 4 °C; FTC2, -4℃/ 4℃ (moderate FTCs); FTC3, -10℃/ 4℃ (severe FTCs). 1FTC, 3FTCs, 6FTCs and 12FTCs represents for one, three, six and 12 freezing-thawing cycles, respectively. In A-C, bars without shared letters indicate significant difference at $P < 0.05$. In D-F, symbols indicate the P values from t test: *, $0.01 < P < 0.5$; **, $0.001 < P < 0.01$; ***, $0.0001 < P < 0.001$; **** $P < 0.0001$; ns, not significant.

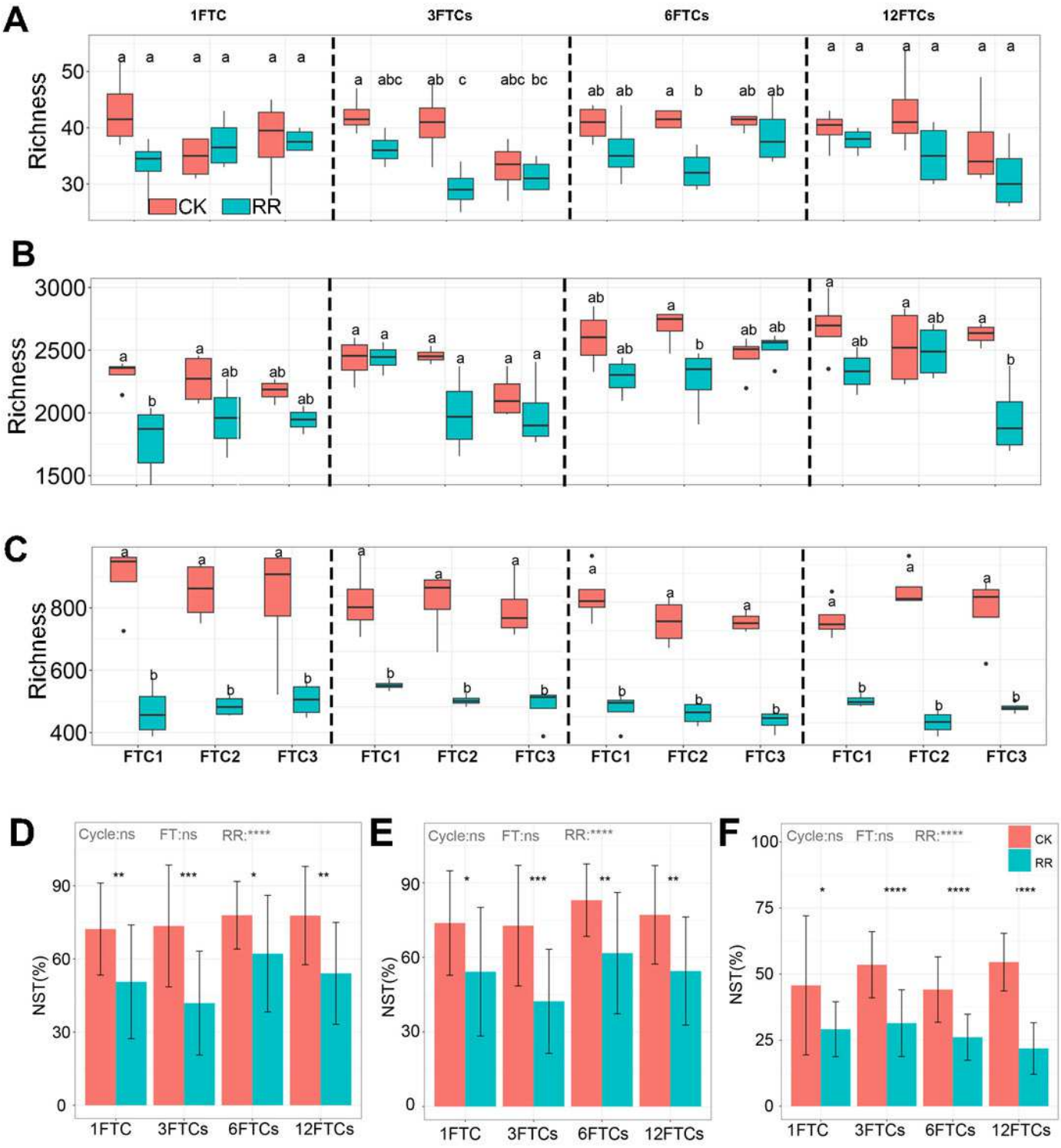

A
1FTC
3FTCs
6FTCs
12FTCs
Richness
CK
RR
B
Richness
C
Richness
FTC1
FTC2
FTC3
D
Cycle:ns
FT:ns
RR:****
NST(%)
1FTC
3FTCs
6FTCs
12FTCs
E
Cycle:ns
FT:ns
RR:****
NST(%)
F
Cycle:ns
FT:ns
RR:****
CK
RR
NST(%)

Figure 3

Soil prokaryotic (A) and fungal (C) networks with nodes colored according to each of the four main modules. The relative abundance of the prokaryotic (B) and fungal (D) phyla in the four modules.

The relative abundance (z-score) of Module #1, Module #2, Module #3 and Module #4 (prokaryotic modules: E, I, G and H; fungal modules: I, J, K and L) among treatments. Abbreviations: CK, control; RR, residue retention; FTC, freezing-thawing cycle; FTC1, constant 4 °C; FTC2, -4℃/ 4℃ (moderate FTCs); FTC3, -10℃/ 4℃ (severe FTCs). 1FTC, 3FTCs, 6FTCs and 12FTCs represents for one, three, six and 12 freezing-thawing cycles, respectively.

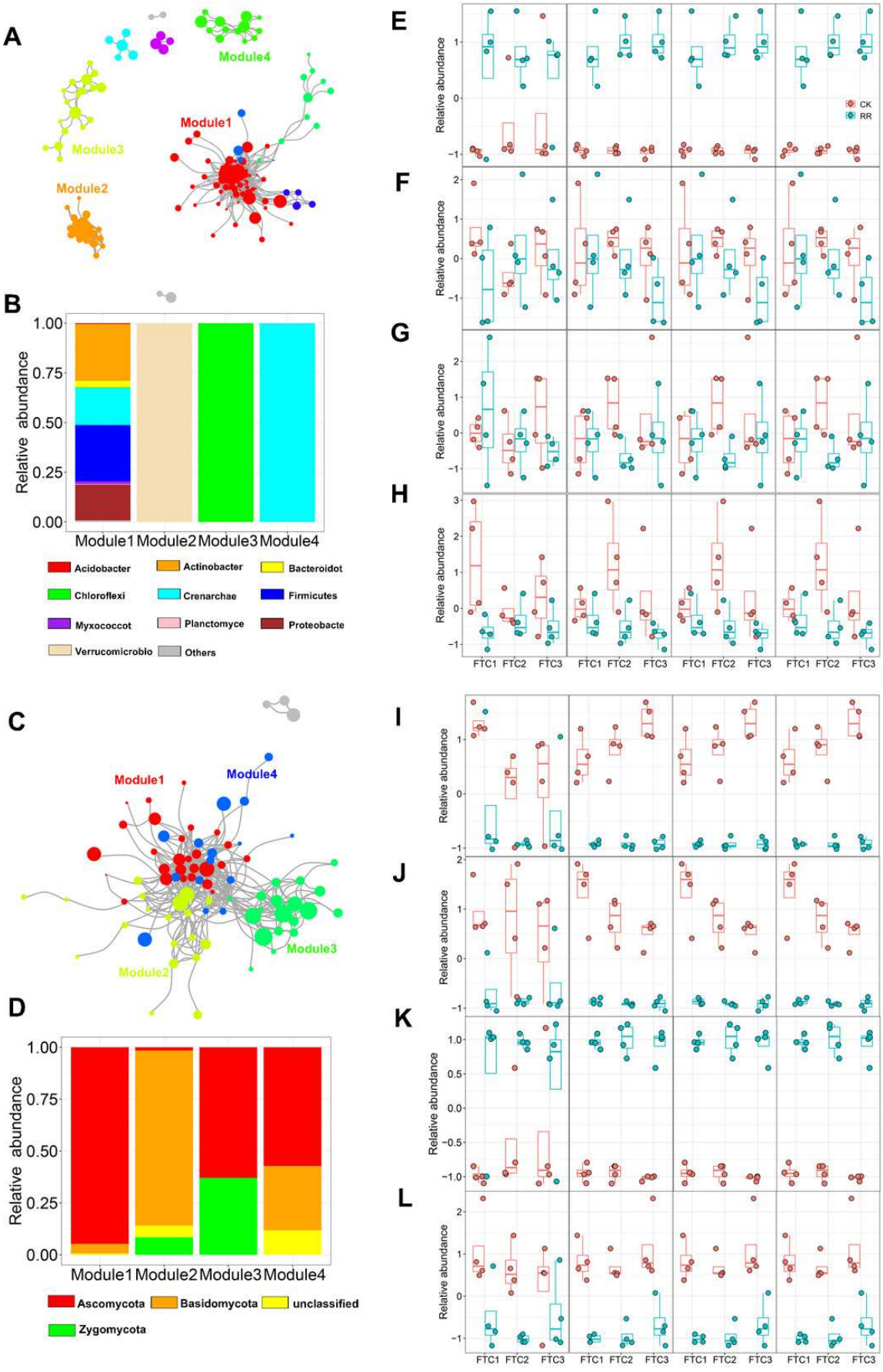
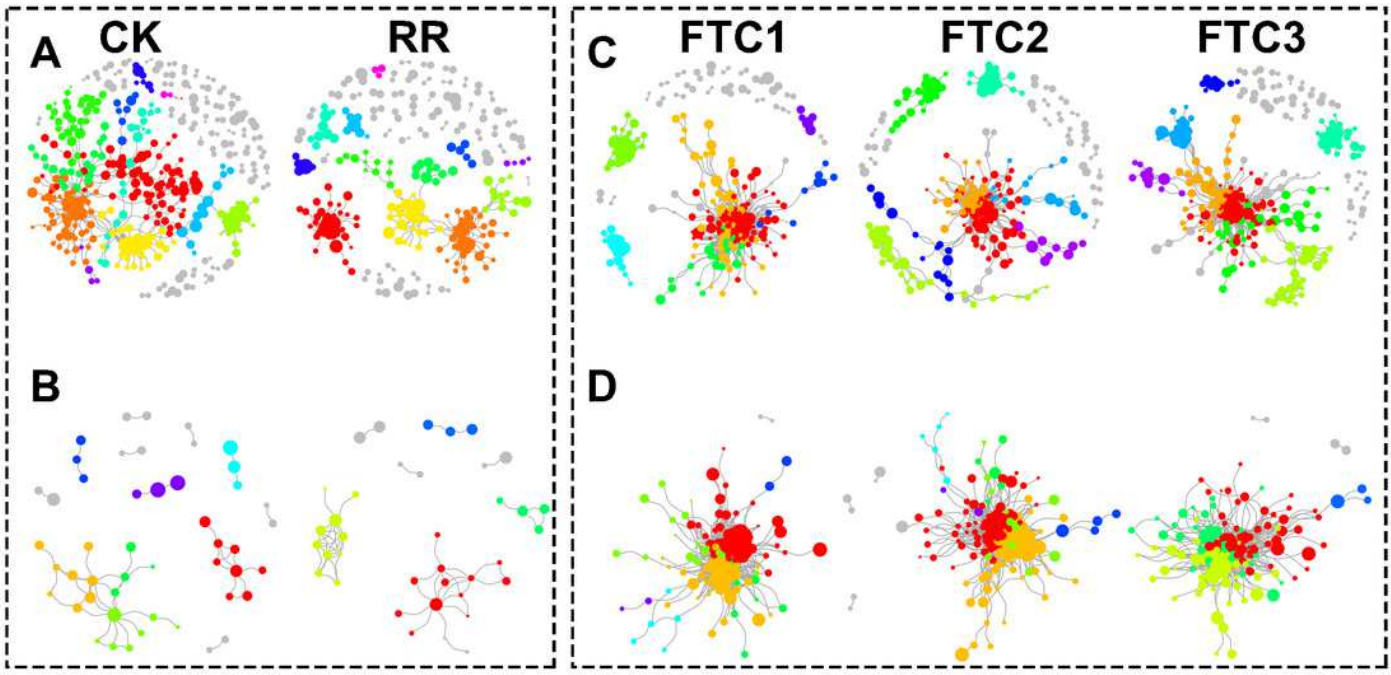
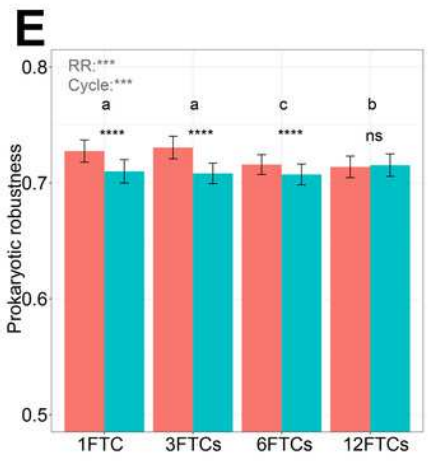

A
Module4
Module1
Module3
Module2
B
Relative abundance
1.00
0.75
0.50
0.25
0.00
Module1 Module2 Module3 Module4
Acidobacter
Actinobacter
Bacteroidot
Chloroflexi
Crenarchae
Firmicutes
Myxococcot
Planctomyce
Proteobacte
Verrucomicrobio
Others
C
Module1
Module4
Module3
Module2
D
Relative abundance
1.00
0.75
0.50
0.25
0.00
Module1 Module2 Module3 Module4
Ascomycota
Basidomycota
unclassified
Zygomycota
E
F
G
H
I
J
K
L
Relative abundance
CK
RR
FTC1 FTC2 FTC3 FTC1 FTC2 FTC3 FTC1 FTC2 FTC3 FTC1 FTC2 FTC3

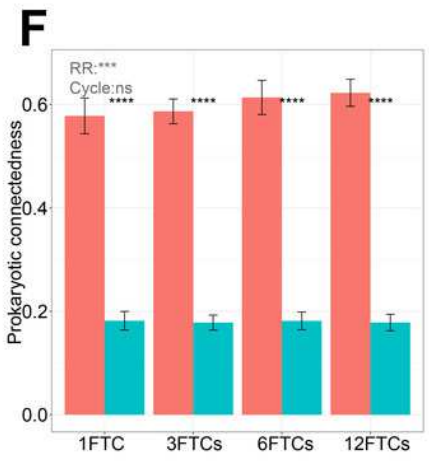
Figure 4

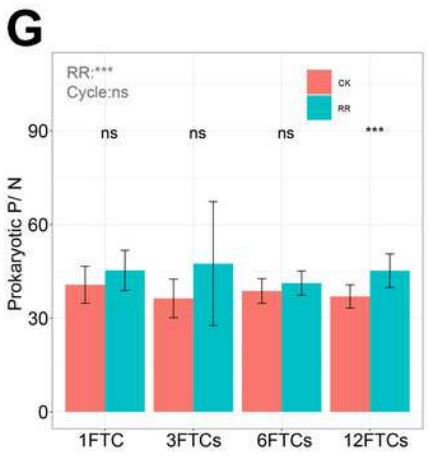
Prokaryotic co-occurrence network in control (A) and residue retention (B) treatments; fungal co-occurrence network in control (C) and residue retention (D) treatments.

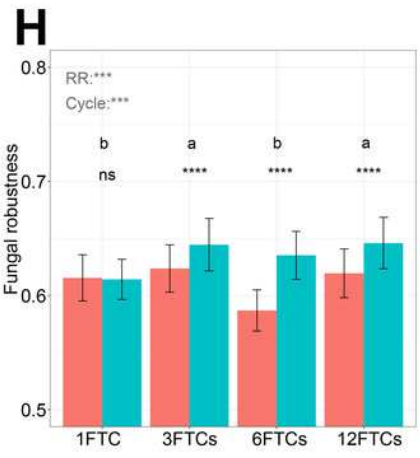
Robustness (E), connectedness (F) and positive links to negative links ratio (P/N ratio, G) of prokaryotic network among treatments; fungal robustness (H), connectedness (I) and positive links to negative links ratio (P/N ratio, J) of fungal network among treatments. In E-J, symbols indicate the *P* values from t test: ns, not significant; *, $0.01 < P < 0.5$; **, $0.001 < P < 0.01$; ***, $0.0001 < P < 0.001$; ****, $P < 0.0001$. Bars without shared letters indicate significant difference among cycles at $P < 0.05$. Abbreviations: P/N, positive links/negative links ratio; CK, control; RR, residue retention; FTC, freezing-thawing cycle; FTC1, constant 4 °C; FTC2, -4℃/ 4℃ (moderate FTCs); FTC3, -10℃/ 4℃ (severe FTCs). 1FTC, 3FTCs, 6FTCs and 12FTCs represents for one, three, six and 12 freezing-thawing cycles, respectively.

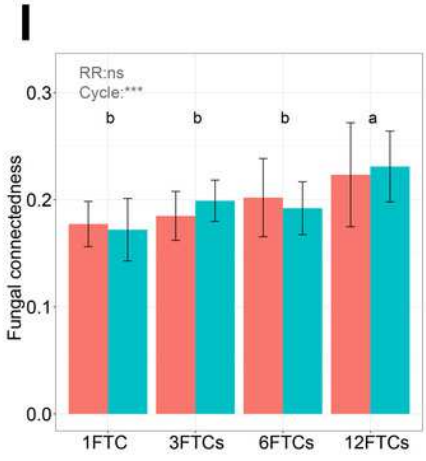

A
CK
RR
C
FTC1
FTC2
FTC3
B
D


E
RR:***
Cycle:***
a
a
c
b
****
****
****
ns
Prokaryotic robustness
0.8
0.7
0.6
0.5
1FTC
3FTCs
6FTCs
12FTCs


F
RR:***
Cycle:ns
****
****
****
****
Prokaryotic connectedness
0.6
0.4
0.2
0.0
1FTC
3FTCs
6FTCs
12FTCs


G
RR:***
Cycle:ns
CK
RR
ns
ns
ns
***
Prokaryotic P/ N
90
60
30
0
1FTC
3FTCs
6FTCs
12FTCs


H
RR:***
Cycle:***
b
a
b
a
ns
****
****
****
Fungal robustness
0.8
0.7
0.6
0.5
1FTC
3FTCs
6FTCs
12FTCs


I
RR:ns
Cycle:***
b
b
b
a
Fungal connectedness
0.3
0.2
0.1
0.0
1FTC
3FTCs
6FTCs
12FTCs

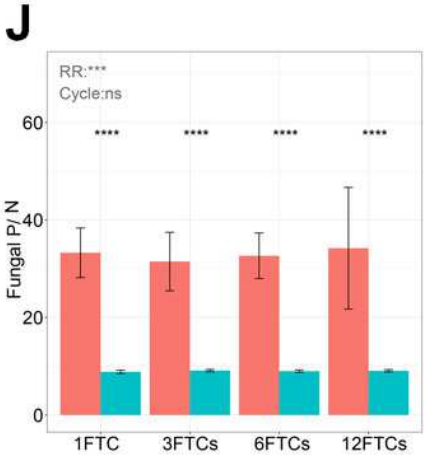

J
RR:***
Cycle:ns
****
****
****
****
Fungal P/ N
60
40
20
0
1FTC
3FTCs
6FTCs
12FTCs

Figure 5

Bar plots showing the multifunctionality in control (CK) and residue retention (RR) treatments (A). Random forest mean predictor importance (percentage of increase of mean square error) of archaeal, bacterial and fungal alpha-diversity and network indices

symbols indicate the *P* values: ns, not significant; *, 0.01 < *P* < 0.05; **, 0.001 < *P* < 0.01; ***, 0.0001 < *P* < 0.001. Abbreviations: F, fungal; B, bacteria; A, archaeal; S, richness; P/N, positive links/negative links ratio.