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Effect of fertilisation on fungal community in topsoil of winter wheat field

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Abstract: Soil fungi played important roles in the maintenance of soil fertility and soil sustainable development. In this study, the effects of different fertilisers (i.e. bacterial fertiliser (BF), composed of organic matters and bacteria; mineral fertiliser (MF), composed of N, P and K) on soil fungi in wheat field were analysed. The results showed that the yield of winter wheat with BF was 4 788.52 kg/ha, which was significantly higher than that with term MF. Chao 1 and Shannon indexes and principal coordinates analysis showed that fertilisation increased the richness of soil fungi to varying degrees and changed the fungal community structure of soil compared with no fertiliser control (NF). The soil fungal community was mainly composed of Ascomycota, Basidiomycota and Mortierellomycota, with Ascomycota as the main species (62.67–65.08%). Compared with MF, the relative abundance of potential beneficial fungi *Talaromyces* in BF increased 4.44 times. Compared with no fertiliser control, the relative abundance of potential beneficial fungi *Chrysosporium* in BF increased 4.11 times. The abundance of potential soil pathogenic fungi ($P < 0.01$), like *Stachybotrys*, *Acrocalymma*, *Achroiostachys*, *Arachnomyces* and *Setophoma*, significantly decreased in BF treatment, which was beneficial to the maintenance of crop health and the sustainable development of the environment. Moreover, the network analysis showed that the interspecific relationship of soil fungi in BF was more intimate than MF and NF and fungi were inclined to adopt cooperative manner to adapt ecological niches in BF treatment. The improvement of wheat yield might be due to the optimisation of soil fungal community structure by applying BF, which strengthened the transformation of nutrients in soil, increased some biocontrol microorganism, and reduced the crop disease. The results explain the improvement of wheat yield by BF to a certain extent, and provided theoretical basis for high-yield cultivation of wheat.

Keywords: *Triticum aestivum* L.; microbial community; high-throughput sequencing; soil activity; nutrient cycling

Wheat is one of the most important crops, and the planting area accounts for about 30% of the total planting area in China. The application of term mineral fertiliser (MF) is an effective way to improve the yield of crops in traditional agriculture. While the long-term application of MF could damage the soil structure and cause the loss of soil nutrients,

which is not conducive to the maintenance of soil health and quality (Paungfoo-Lonhienne et al. 2015). Increased nitrogen fertiliser dosage had potential negative impacts on carbon cycling in soil and promoted fungal genera with known pathogenic traits (Paungfoo-Lonhienne et al. 2015). In recent years, the application of bacterial fertiliser (BF) performed

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quite well. BF was rich in beneficial microorganisms, which could participate in the transformation of substance and energy, the formation and decomposition of humus, and the release of nutrients in soil. The results of Ansari and Mahmood (2017) showed that the application of *Rhizobium* sp. (Rh) + municipal waste produced more crop growth and yield of pigeon pea than untreated control.

Microorganisms in soil are important index of soil activity, and they are important providers of soil ecological functions. Fungi community in soil were diverse and could be divided into symbiotic, endophytic and pathogenic fungi (Fan et al. 2020). They played important roles as decomposers, plant symbionts or pathogens in soil (Paungfoo-Lonhienne et al. 2015). Soil fungi had strong abilities to degrade organic matter in soil, such as cellulose, hemicellulose, pectin, starch, fat and lignin, to provide nutrients for plant growth. Soil fungi could also form aggregates with soil, and then increase the permeability of soil. Some fungi played essential roles in the formation of humus (Kara and Bolat 2007). The quantity and structure of soil fungi community changed with the change of vegetation type, soil fertility, pH, precipitation and other environmental factors (Siciliano et al. 2014, Yang et al. 2020). There were studies showed that the application of different types of fertilisation, such as organic and inorganic fertilisation, could affect the yield of crops and change the microbial community in soil (Wang et al. 2017).

In this study, the wheat field soil samples with BF, MF and no fertiliser control (NF) were collected. High-throughput sequencing technique was employed to analyse the effect of different fertilisation treatments on soil fungal community. The fungal diversity, fungal community composition and interspecific relationship of fungal community were analysed. This study could provide theoretical basis and technical support for the sustainable development of soil and high-yield cultivation of wheat.

MATERIAL AND METHODS

Site description. The experimental wheat field is located in Dongliang Village, Liujiayuan town, Shanxi province, China (36°22'N, 111°35'E, 648 m a.s.l.). The active accumulated temperature in this area is 3 327 °C days, the annual average rainfall is 364–441 mm, the frost-free period is 180–210 days, and the annual mean sunshine duration is 2 419 h. The area of experimental plot was 0.23 ha. The

experimental soil was calcareous cinnamon soil. Physicochemical properties of the soil were as follows: pH 8.00, total nitrogen content 0.74 g/kg, total phosphorus content 0.14 g/kg, total potassium content 39.88 g/kg, organic carbon content 11.12 g/kg, and cation exchange capacity 10.75 cmol₊/kg.

Experimental design and soil sampling. Monitoring fertilisation was used in this experiment. The amount of fertiliser was determined according to the contents of nutrients in the soil to be tested. The different fertilisation treatments were as follows: (1) no fertiliser control; (2) mineral fertiliser, which was composed of N, P and K, and the fertiliser rate was: 134.00 kg N/ha, 59.00 kg P/ha, 35.95 kg K/ha; (3) bacterial fertiliser, which was composed of organic matters ($\geq 40\%$) and bacteria (mainly *Bacillus licheniformis* 2×10^7 CFU (colony forming unit)/g), and the fertiliser rate was 1 400 kg/ha with addition of 105.00 kg N/ha, 39.00 kg P/ha. The total supply of N, P and K (from soil and exogenous fertiliser) in BF and MF treatments was the same. Different fertilisation treatments were carried out in October 2020 and the soil samples were collected in December 2020 during the over-wintering period. Four parallel experiments were conducted for each treatment. Wheat was harvested in May 2021 and the wheat yield under different fertilisation treatments was counted. The wheat yield under NF, BF and MF treatments were 3 524.23, 4 788.52 and 4 530.37 kg/ha, respectively.

Soil total DNA extraction and PCR amplification. Soil DNA was extracted with DNA Isolation Kits (Fast DNA™ SPIN Kit for Soil, MP Bio, California, USA). The quantity and quality of DNA extracts were determined by agarose gel electrophoresis (AGE). Finally, the DNA samples were dissolved by sterile water to 1 ng/ μ L. The PCR was conducted under the following conditions: denaturation at 98 °C for 60 s, followed by 30 cycles of 98 °C for 10 s, 0 °C for 30 s and 72 °C for 30 s with the final extension for 5 min at 72 °C. The primers were ITS1F (5'-CTTGGTCATTTAGAGGAAGTAA-3') and ITS2R (5'-GCTGCGTTCTTCATCGATGC-3') (Adams et al. 2013).

Processing of sequencing data. PCR amplification products were recovered using AxyPrepDNA gel recovery kit (Solarbio Company, Beijing, China) and quantified by Quantus™ Fluorometer. Tru Seq® DNAPCR-Free Sample Preparation Kit (ThermoFisher Company, Shanghai, China) was used for library construction, and real-time fluorescence quantification

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Table 1. Sequencing data of fungal communities in soil samples with no fertilisation (NF), mineral fertilisation (MF) and bacterial fertilisation (BF)

Treatment	Effective tags	Mean length	OTUs number	Coverage (%)
NF	53 200	246 ± 1.29	388 ± 33	99.92 ± 0.02
MF	53 200	245 ± 8.68	395 ± 41	99.91 ± 0.04
BF	53 200	247 ± 3.27	431 ± 18	99.89 ± 0.04

OTU – operational taxonomic unit

was performed by FTC-3000TM Real-Time PCR. After the library was qualified, high-throughput sequencing data was deposited by Shanghai Majorbio BioPharm Technology Company (Shanghai, China).

Statistical analysis. The sequencing information statistics of fungal communities in soil under different fertilisation treatments were analysed by Excel 2007 (Redmond, USA). Software SPSS Statistics 2.2 (New York, USA) was used for inter-group difference analysis (one-way ANOVA and *T*-test). The fungal diversity indices (Chao1 richness, Shannon diversity index, principal coordinates) at OTU (operational taxonomic unit)-level were analysed. Origin 2019 combined with Magi Bio Cloud platform were employed to plot. Spearman correlation analysis of fungi at genus level across different soil was performed by SPSS Statistics 2.2, then the genera with significant correlation ($P < 0.05$) were screened to perform Network association analysis by employing Cytoscape 3.7.1 (Washington, California, USA; Paris, France).

RESULTS AND DISCUSSION

Soil fungal diversity. The sequencing information statistics of fungal communities under different fertilisation treatments were listed in Table 1. There were 12 samples in this experiment (3 treatments × 4 parallel experiments), and 159 600 effective sequences were obtained, with an average of 53 200 reads for each sample. There were 388 OTUs, 431 OTUs and 395 OTUs in NF, BF and MF samples, respectively. The coverages of all treatments were higher than 99% (Table 1), combined with rarefaction curves (Figure 1), which indicating that the amount of sequencing data was reasonable and could reflect the status of soil fungal community actually.

Chao1 and Shannon indexes were analysed to reflect the richness and diversity of fungi in soil after different fertilisation treatments. As shown in Figure 2, compared with NF treatment, Shannon indexes of MF and BF treatments decreased respectively 1.65%

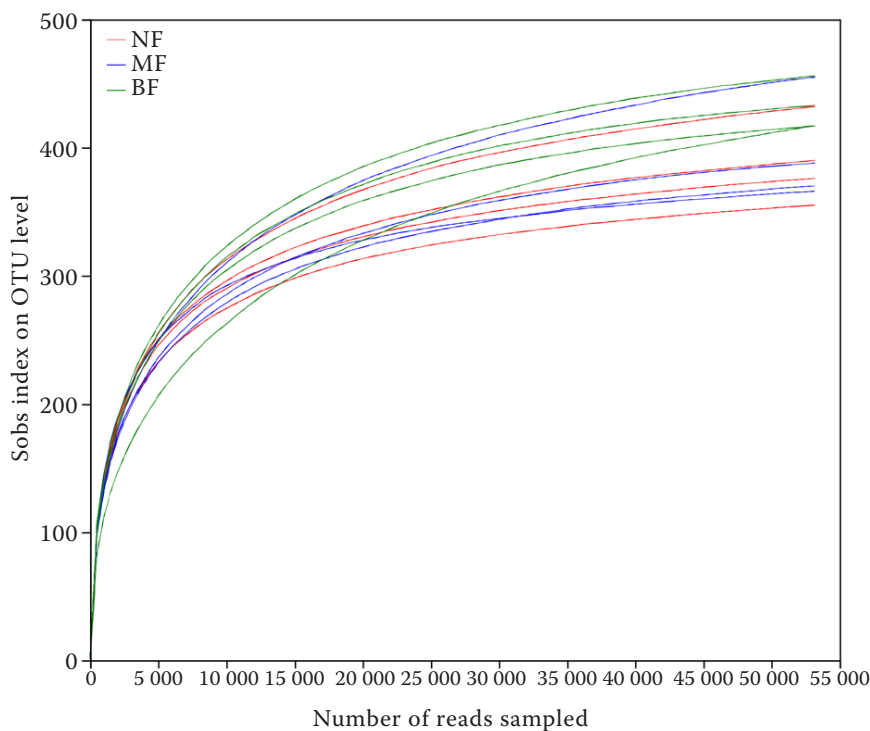


Figure 1. Rarefaction curves of soil samples with no fertilisation (NF), mineral fertilisation (MF) and bacterial fertilisation (BF). OTU – operational taxonomic unit

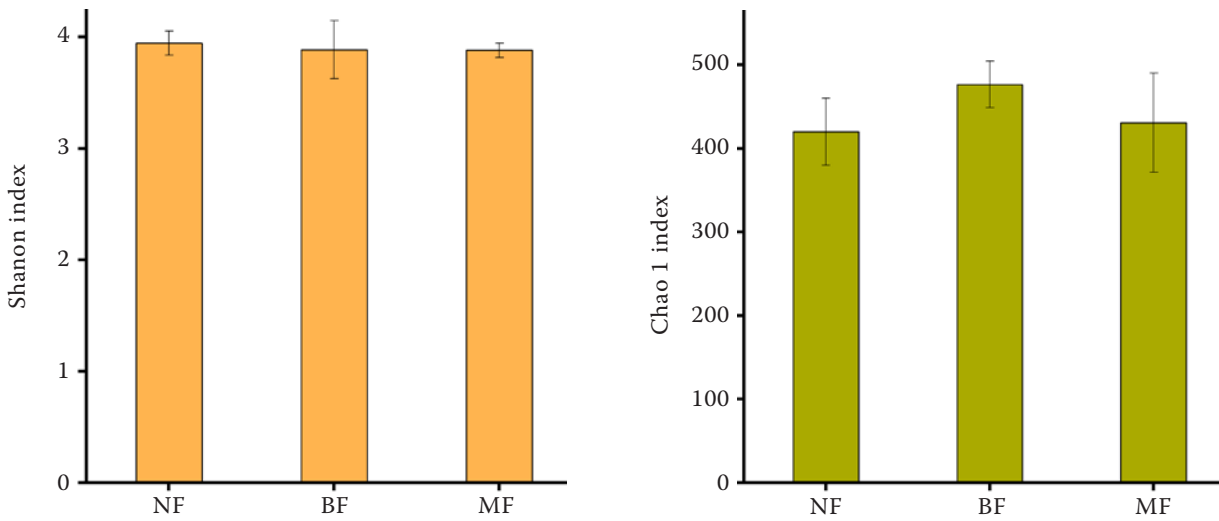


Figure 2. Chao1 and Shannon indexes of soil fungi in soil samples with no fertilisation (NF), mineral fertilisation (MF) and bacterial fertilisation (BF)

and 1.01%, whereas Chao1 indexes increased respectively 2.62% and 4.76%. It reflected that fertilisation resulted in selective enrichment of fungi in soil, and the application of BF was more obvious.

The principal coordinates (PCoA) of fungal communities on the OTUs classification level were analysed by employing Bray-Curtis distance algorithm (Figure 3). The results showed that the total explanatory degree of the two ordination axes was 51.88%. The clustering results showed that the fungal communities of

different treatments clustered together, respectively. ANOSIM was used for further analysis, the results showed that the difference between groups was significant ($P < 0.01$), which meant that the structure of soil fungal community was changed significantly in MF and BF treatments.

Soil fungal community composition. The abundance of fungal community at phylum level (top 5) in different treatments were analysed and the results were listed in Figure 4A. The results showed that the

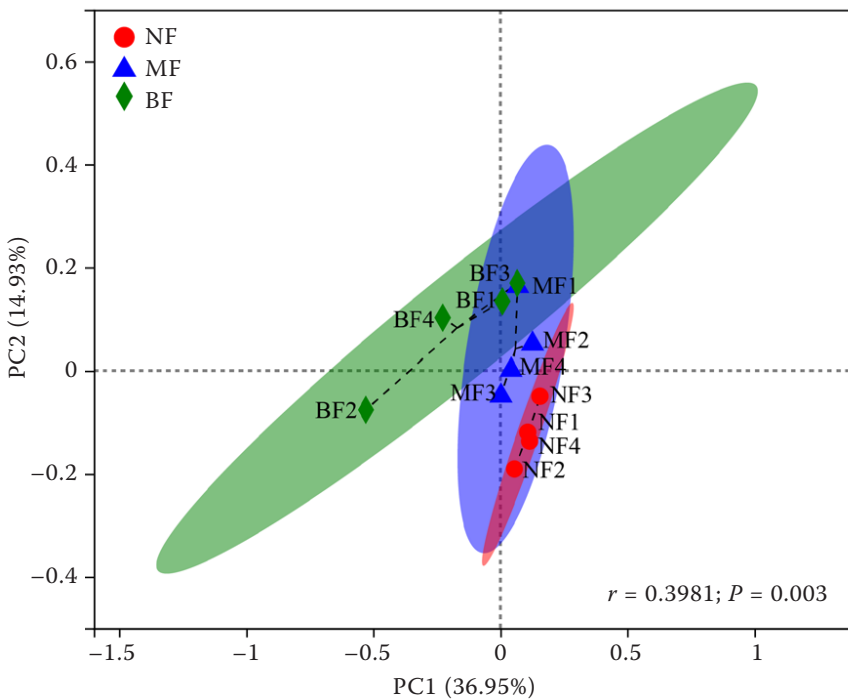


Figure 3. Principal coordinates analysis (PCoA) of fungal community in soil samples with no fertilisation (NF), mineral fertilisation (MF) and bacterial fertilisation (BF)

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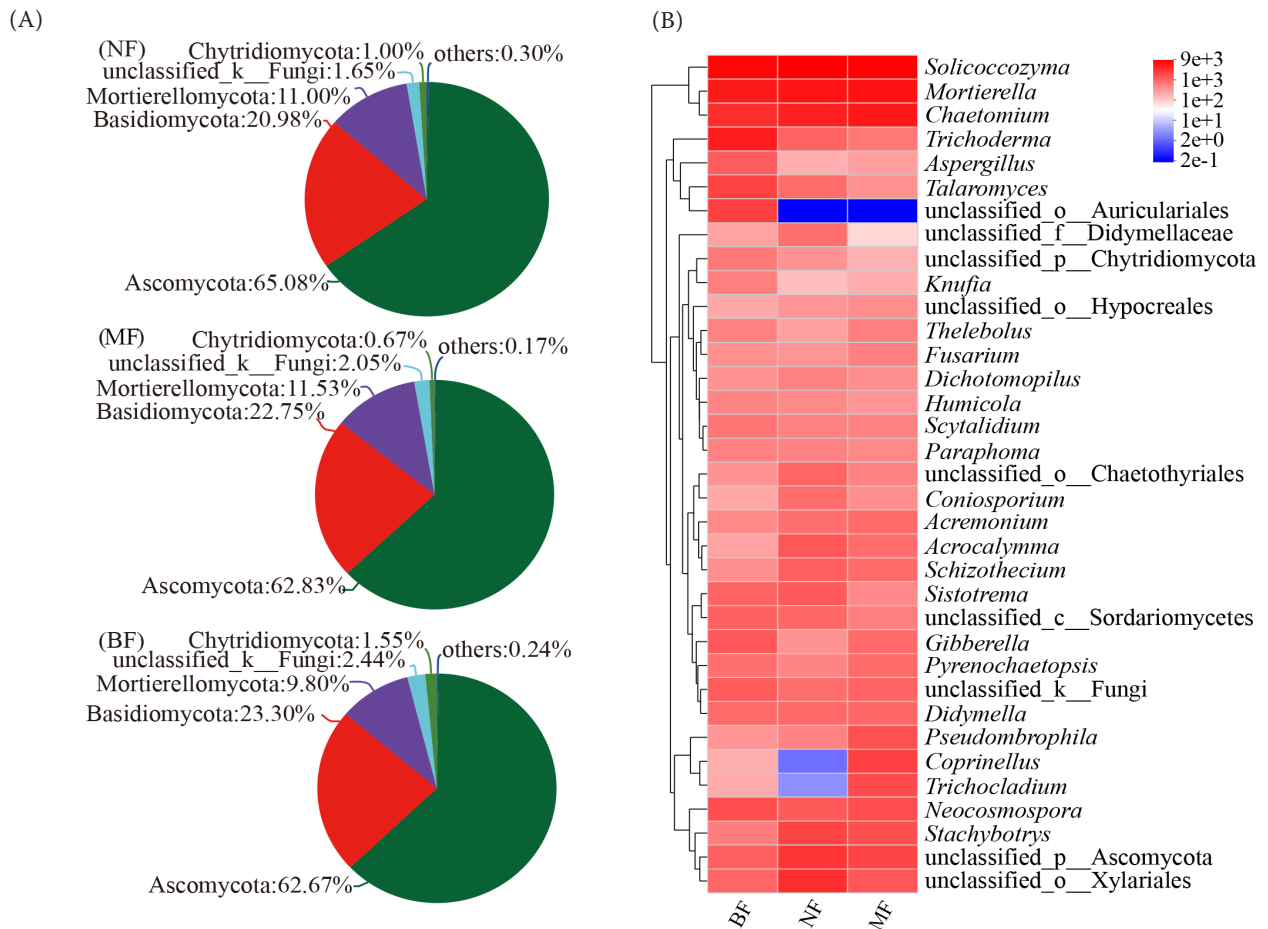


Figure 4. Relative abundances of fungal community at phyla level (top 6) (A) and genus level (top 35) and (B) in soil samples with no fertilisation (NF), mineral fertilisation (MF) and bacterial fertilisation (BF)

top 5 abundant phyla were as follows: Ascomycota, Basidiomycota, Mortierellomycota, unclassified_k_Fungi and Chytridiomycota, with Ascomycota as the main species (62.67–65.08%). The relative abundances of Basidiomycota and unclassified_k_Fungi in soil increased significantly ($P < 0.05$) in BF and MF treatments compared to NF. The relative abundances of Basidiomycota in MF and BF treatments increased by 8.44% and 11.06%, respectively. Compared with NF, the relative abundances of Mortierellomycota in BF significantly decreased by 10.91%, while the relative abundances of Chytridiomycota significantly increased by 55.00% ($P < 0.05$). There was no significant difference of the relative abundances of Chytridiomycota and Mortierellomycota between NF and MF treatments. Compared with NF, the relative abundances of Basidiomycota and unclassified_k_Fungi in MF significantly increased by 8.44% and 24.24%, respectively ($P < 0.05$).

The relative abundance of fungal community at genus level (top 35) (Figure 4B) and the significant

differences between the three samples were analysed (Figure 5). The results showed that the top 2 abundant genera of fungi in NF, MF and BF were respectively *Solicoccozyma* and *Mortierella*, they accounted for 23.90–27.50% of the entire fungal community. Compared with NF, the abundances of unclassified_o_Xylariales, *Chaetothyriales*, *Periconia*, *Setophoms*, *Orbiliaceae*, *Stachybotrys*, *Acrocalymma*, and *Achroiostachys* in BF significantly decreased by 68.25, 61.25, 67.50, 65.00, 98.29, 70.73, 76.92 and 86.13%, respectively, while those of *Pyrenochaetopsis*, *Chrysosporium*, *Sarocladium* and *Collarina* significantly increased by 60.00, 664.70, 260.00, and 260.00%, respectively ($P < 0.05$). Compared with MF, the abundances of *Talaromyces*, *Aspergillus* and *Didymellaceae* in BF significantly increased 4.25, 3.00 and 2.06 times, respectively, while those of *Arachnomyces*, *Ophiocordyceps* and *Corynascella* in BF significantly decreased by 87.50, 54.89 and 71.08%, respectively ($P < 0.05$).

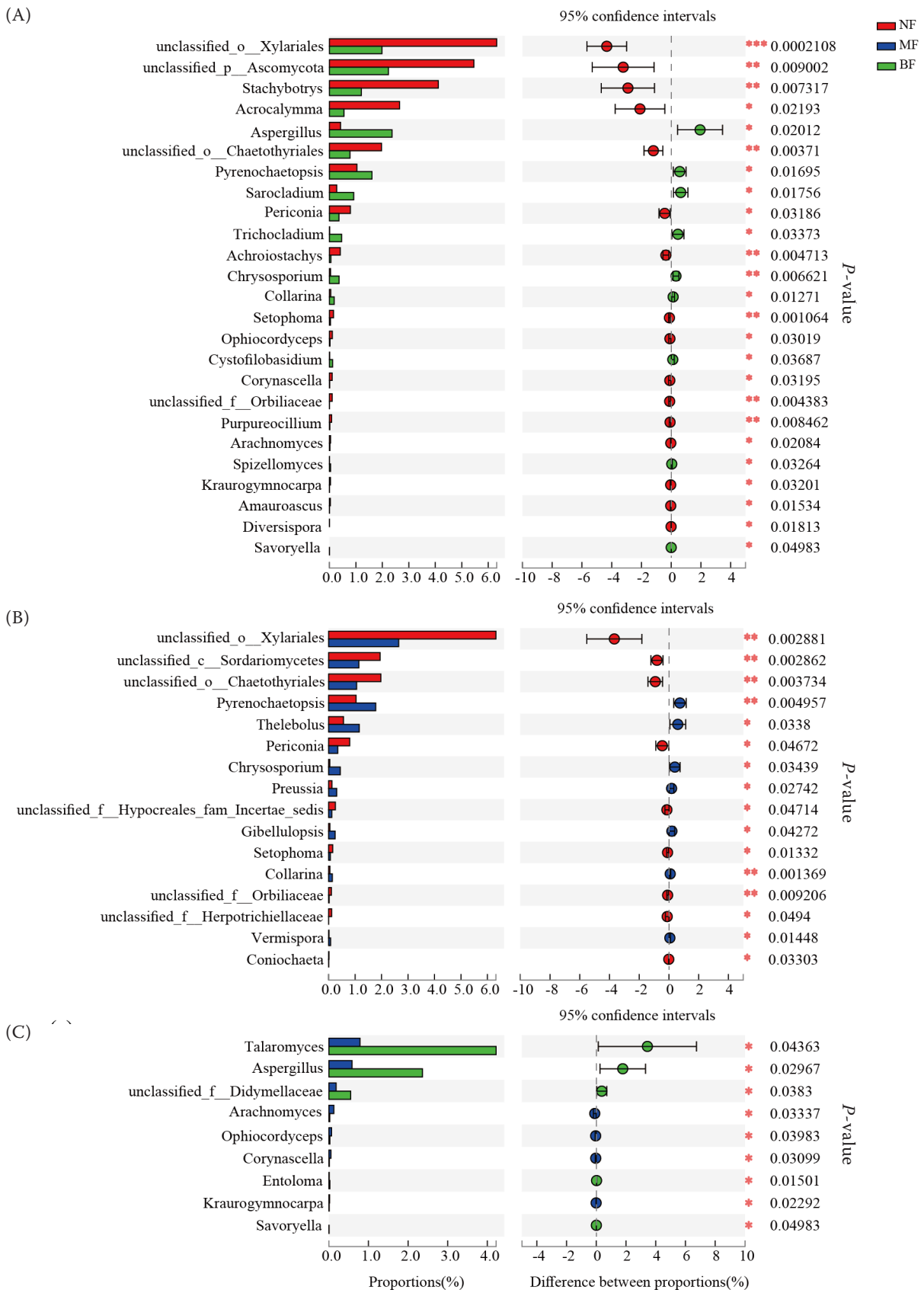


Figure 5. Significance analysis of relative abundance of fungal community at genus level in soil samples with no fertilisation (NF), mineral fertilisation (MF) and bacterial fertilisation (BF)

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Soil beneficial fungi were important for the maintenance of plant health and soil fertility. On one hand, these fungi provided soluble nutrients for plant growth, and on the other hand, some of the soil beneficial fungi inhibited the growth of pathogenic microorganisms to protect plants. Based on this, they could be used as biocontrol agents. The relative abundance of *Sarocladium* in BF increased by 193.10% compared with that in NF (Figure 5A). The genus *Sarocladium* included about 10 species. Among them, *S. oryzae*, *S. attenuatum* and *S. sinense* were reported as pathogen of rice (Giraldo et al. 2015, Prasannakumar et al. 2021). However, *S. oryzae* could synthesis cerulenin ((2R, 3S, E)-2,3-Epoxy-4-oxo-7,10-dodecadienamide), which was a potent antifungal agent and could be used as biocontrol agents (Côrtes et al. 2021). The result of Côrtes et al. (2021) showed that the genetic improved *S. oryzae* had a good control effect on root rot and white mold disease (Côrtes et al. 2021). The role of the genus *Sarocladium* in wheat soil needs to be further studied, so that relevant measures could be taken in the early stage of wheat planting, in order to better provide basis for subsequent wheat planting.

Compared with MF treatment, the relative abundance of *Talaromyces* in BF treatment increased by 443.69% ($P < 0.01$) (Figure 5C). *Talaromyces* spp. were the main decomposers of plant residues in soil and important antagonistic fungi. Some species secrete powerful cellulases that could decompose lignocellulose effectively, like *T. pinophilus* and *T. verruculosus* (Fujii et al. 2014, Goyari et al. 2015). Some of *Talaromyces* spp. secreted organic acids and phosphatase, which could dissolve inorganic calcium phosphate and phosphate ester in soil and promote the absorption of phosphorus by plants, like *T. pinophilus* and *T. purpurogenus* (Yadav and Tarafdar 2011, Maity et al. 2014). What's more, *T. wortmanni* synthesised volatile organic compounds in the rhizosphere, which could promote plant growth and enhance the resistance to pathogens (Yamagiwa et al. 2011). *T. flavus* (Klocker) was one of the most important species of antagonistic fungi (Naraghi et al. 2012). It had inhibitory effects on many plant pathogens, such as *Sclerotinia sclerotiorum*, *Rhizoctonia solani*, and *Verticillium daliae* (Naraghi et al. 2010). The result of Miyake et al. (2012) showed that *T. flavus* prevented the growth of rice seedling pathogens (i.e. *Rhizopus oryzae*, *Pythium graminicola*, and *Gibberella fujikuroi*) by parasitising and dissolving their mycelium (Miyake et al. 2012). *T. flavus* could

also against the causal agent of wilt diseases of cotton and potato (Naraghi et al. 2012).

Chrysosporium was widely distributed, which was related to the production of a large number of enzymes and abundant metabolites (Yamashita et al. 1984, da Silva et al. 2010). *Chrysosporium* spp. could synthesize cellulase and keratinase, produce some secondary metabolites with physiological activity, which could inhibit the growth of some microorganisms and parasites (Yamashita et al. 1984, Tsipouras et al. 1997, Kohno et al. 2001). Compared with NF, the relative abundance of *Chrysosporium* in BF and MF treatments increased significantly ($P < 0.01$), especially in BF treatment, it increased by more than 4.11 times (Figure 5).

Those results above showed that, compared with NF and MF, BF treatment significantly increased the relative abundance of some beneficial fungi in soil, which was beneficial to the maintenance of crop health and the sustainable development of the environment. However, there were many harmful fungi in soil, which could cause crop diseases, livestock diseases and even human infections.

The genus *Stachybotrys* was placed in the family Stachybotryaceae (Lombard et al. 2016). Compared with NF, the relative abundance of *Stachybotrys* in BF and MF soil samples was significantly reduced, especially in BF soil samples, which decreased by 70.73%. *Stachybotrys* spp. widely existed in plant materials (like herbs, straw, and hay) and building materials (like wallpaper, plasterboard, or wooden lining) due to their high cellulolytic potential (Nielsen et al. 2004, Biermaier et al. 2015). Many of the species reported in *Stachybotrys* genus were associated with toxin production (Došen et al. 2016, Ulrich et al. 2020). The toxins that produced by *S. chartarum* caused negative health effects to humans, and might be linked to idiopathic pulmonary haemosiderosis in babies (Jarvis et al. 1998, Etzel 2007). In addition, there was literature suggesting that feed contaminated with *Stachybotrys* spp. caused animal poisoning (Jarvis et al. 1986). *Arachnomyces* spp. were placed in the family Arachnomycetaceae. Some of the *Arachnomyces* spp. were associated with nail infections in humans. *A. nodosetosus* and *A. kanei* were reported to cause nails and skin infection and nondermatophytic onychomycosis (Sun et al. 2019). What's more, several isolates of *A. glareosus* were obtained from nail or skin scrapings of patients (Gibas et al. 2002). In the present experiment, the relative abundance of *Arachnomyces* decreased by 122.22% by applying BF compared with MF. The

results of the present experiment showed that fertilisation treatment significantly reduced the relative abundance of *Stachybotrys* and *Arachnomyces* in soil, and the reduction degree of BF treatment was more significant. It had potential significance for the health of human and livestock, as well as for environment protection.

The genus *Achroiostachys* was also placed in the family Stachybotryaceae (Lombard et al. 2016). The relative abundance of *Achroiostachys* in soil decreased 301.02% by applying BF compared with NF. *Achroiostachys* spp., as plant saprotroph, widely existed in plant rhizosphere soil, such as sorghum. The result of Gao et al. (2020) showed that the presence of *Achroiostachys* spp. affected the genotypes of sorghum roots, and then affect some metabolic characteristics. Compared with NE, the relative abundance of *Acrocalymma* in BF and MF soil samples was significantly reduced, especially in BF soil samples, which decreased by 76.92%. There were about 7 species of *Acrocalymma* were identified to the present. They widely existed in many places, such as leaf litter, freshwater stream, soil and some plants (Jayasiri et al. 2019, Das et al. 2020). Some of *Acrocalymma* spp. were phytopathogens. The results of Alcorn and Irwin showed that *A. medicaginis* was a plant pathogen that caused root and crown rot disease of lucerne (*Medicago sativa* L.) in Australia (Alcorn and Irwin 1987). The relative abundance of *Setophoma* also decreased significantly ($P < 0.01$) by applying BF and MF compared with NF, especially in BF treatment, it decreased by 137.50%. *Setophoma* spp. were reported as plant pathogen. They were biotroph fungus, which could infect the roots of some plants. *S. terrestris* was one of the most severe pathogens in soils of tropical and subtropical climates, which infected plant roots or parasitizes the root tissues of onion and it led to severe crop losses (Orio et al. 2015, Rivera-Méndez et al. 2021). The relative abundance of *Periconia* decreased more than double by applying BF and MF compared with NF. *Periconia* genus widely distributed in various habitats, most of them were regarded as terrestrial saprobes and endophytes. Some of *Periconia* genus were plant pathogenic fungi, which could cause various roots and leaves diseases, like *P. circinate* and *P. macrospinosa* (Markovskaja and Kačergius 2014).

The results of the present experiment showed that compared with NF and MF, BF treatment significantly reduced the relative abundance of the potential plant saprotroph or pathogen *Stachybotrys*, *Acrocalymma*, *Achroiostachys*, *Arachnomyces*, *Setophoma* and

Periconia. Overall, BF treatment significantly increased the relative abundance of some beneficial fungi in soil, and decreased the relative abundance of potential pathogenic fungi.

Interspecific relationship of soil fungal community. The result of Network association analysis was showed in Figure 6. By comparing the network topology parameters, it could be found that the number of nodes in the network of NF, MF and BF was similar, respectively 38, 37 and 35. Compared with NF and MF, however, the fungi links in BF respectively increased by 46.15% and 22.58%. The average number of neighbors in the network of BF increased by 58.39% and 29.33% compared with that of NF and MF. Furthermore, the network density of BF was higher than that of NF and MF. Those network topology parameters indicated that BF further improved the interaction relationships among fungi. In addition, the positive links among species in BF accounted for 73.68% of the total links, which respectively increased by 52.26% and 9.46% compared with those in MF and NF. The above results suggested that fungi tended to adopt cooperative approaches to adapt to the ecological niches in BF treatment.

In order to further reflect the interspecific relationship of soil fungi, submodules were analysed (Figure 6), which could identify closely related genera in a network. Two submodules were found in each treatment, and the highest submodule score (8.0) was found in BF. Among the 8 genera in the submodule, only *Paraphoma* was negatively correlated with other fungi, the other 7 genera were positively correlated. The other submodule in BF also got a high score (score 7.0). Among the 7 genera in the submodule, only *Knufia* and *Trichoderma* were negatively correlated with other five genera, while there were positive correlations between the other five genera. A similar relationship was observed in the other submodule from the network of MF (score 6.0), only *Paraphoma* and *Thelebolus* were negatively correlated with the other five genera, while there were positive correlations between the other five genera. However, in the other submodule from the network of MF (score 6.0), the 6 genera could be divided into two groups, *Periconia*, *Humicola* and *Scytalidium* as one group, *Coprinellus*, unclassified_f_Didymellaceae and *Schizothecium* as the other group. The genera between groups were negatively correlated, while the genera within groups were positively correlated. Those results indicated that the interspecific relationship of BF was closer than that of MF.

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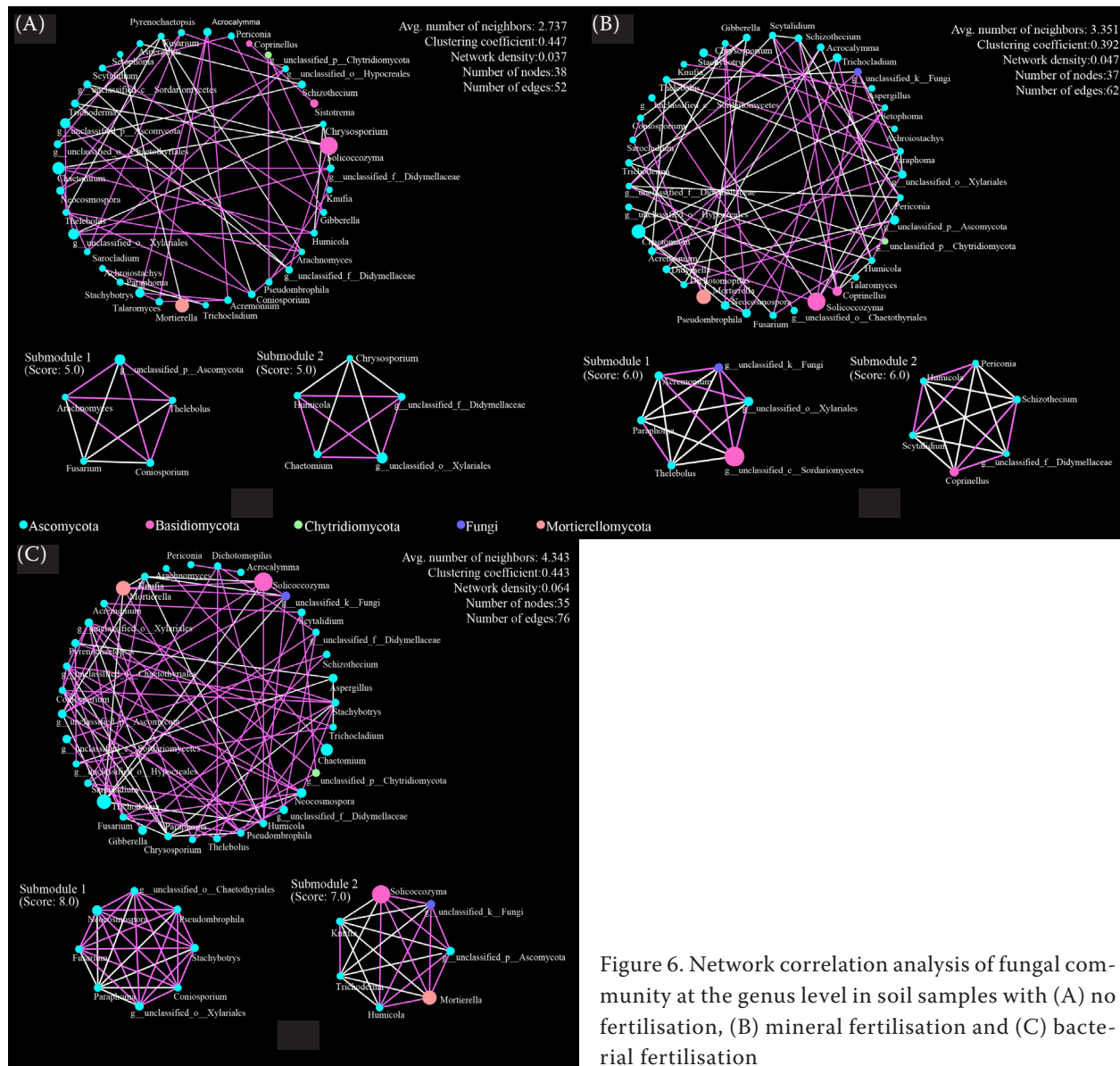


Figure 6. Network correlation analysis of fungal community at the genus level in soil samples with (A) no fertilisation, (B) mineral fertilisation and (C) bacterial fertilisation

Soil microbial communities played essential roles in soil organic matter dynamics and nutrient cycling in agroecosystems. The changes in composition of soil microbial communities (e.g., community types) or in microbial biomass could lead to changes in soil quality (Yao et al. 2006, Ashworth et al. 2017). And it may further affect the yield of crops. Most Basidiomycota in soil were saprophytes, which could promote soil material circulation. The relative abundances of Basidiomycota in MF and BF treatments increased by 8.44% and 11.06%, respectively (Figure 4A). Further analysis showed that fertilisation changed the relative abundance of fungi in soil at genus level (Figure 4B, Figure 5). In addition, the network analysis showed that BF enhanced the interaction between

soil fungi, evidenced by more edges (Figure 6). Fungi in BF tended to adapt to the ecological niches in cooperative approaches, which could be demonstrated by more positively links.

The results of the submodule in Figure 6 showed that the interspecific relationship in BF was more complex than MF and NF. For example, the genus *unclassified_o_Xylariales* was connected with 7 nodes in the submodule of BF, while it was connected with respectively 4 and 5 nodes in the submodules of NF and MF. In addition, the results of Figure 6 also showed that fertilisation changed the interspecific relationship of soil fungi. For example, *Humicola* and *Didymellaceae* were positively correlated in NF treatment, while the two genera were negatively correlated

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in MF treatment. *Fusarium* and *Coniosporium* were negatively correlated in NF treatment, while the two genera were positively correlated in BF treatment.

In this paper, the effects of BF on soil fungal community composition of wheat field were analysed. In subsequent studies, we will pay continuous attention to the effects of BF on other factors, such as soil physical and chemical properties, bacterial composition, etc., to provide sufficient support to fully explain the effects of BF on wheat soil, so as to provide theoretical basis for high-yield planting of wheat and sustainable development of soil.

In conclusion, BF had unparalleled advantages in regulating microbial community structure and interspecific relationship, which is ultimately conducive to wheat yield. The details are summarised as follows: (1) Fertilisation increased the richness of soil fungi to varying degrees and changed the fungal community structure of soil; (2) compared with NF and MF, the BF treatment significantly increased the relative abundance of some beneficial fungi, like *Talaromyces* and *Chrysosporium*; (3) the BF treatment significantly decreased the relative abundance of some potential soil pathogenic fungi ($P < 0.01$), like *Stachybotrys*, *Acrocalymma*, *Achroiostrictis*, *Arachnomyces* and *Setophoma*; (4) the interspecific relationship of soil fungal community was tenser by applying BF; (5) fungi were inclined to adopt cooperative manner to adapt ecological niches in BF treatment.

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