




Effects of different types of microbial inoculants on available nitrogen and phosphorus, soil microbial community, and wheat growth in high-P soil

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Abstract

Irrational application of chemical fertilizers causes soil nutrient imbalance, reduced microbial diversity, soil diseases, and other soil quality problems and is one of the main sources of non-point pollution. The application of microbial inoculant (MI) can improve the soil environment and crop growth to reduce problems caused by irrational application of chemical fertilizers. Field experiments were carried out in high-phosphorus soils to study the effects of the addition of various MIs combined with chemical fertilizers on soil properties, wheat growth, and soil microbial composition and structure. The MIs consisted of one fungal agent: *Trichoderma* compound agent (TC) and five bacterial agents, namely soil remediation agent (SR), anti-repeat microbial agent (AM), microbial agent (MA), plant growth-promoting rhizobacteria (PG), and biological fertilizer agent (BF). The wheat yield increased by 15.2–33.4% with the addition of MIs, and PG with *Bacillus subtilis* as the core microorganism had the most obvious effect on increasing the production ($p < 0.05$). For the entire growth period of wheat, all MIs applied significantly increased the available nitrogen (AN) ($p < 0.05$) but did not significantly affect the available phosphorus (AP). BF has the best effect on increasing AN in the soil. The 16S rRNA sequencing results indicated that the dominant phyla of soil bacteria were Proteobacteria, Acidobacteria, Bacteroidetes, Actinobacteria, and Verrucomicrobia. The addition of MIs increased the relative abundance of Acidobacteria, Actinobacteria, Chloroflexi and decreased Proteobacteria and Bacteroidetes. The diversity of soil bacterial community (Chao1) was significantly higher in the soil added with TC than that added with BF ($p < 0.05$). All bacterial agents significantly enriched various genera ($p < 0.05$), while the fungal agent (TC) did not enrich the genera significantly. pH and AN, but not TP, were closely related to the dominant bacteria phylum in high-P soil. The application of MIs improved AN in soil, increased the wheat yield, and changed the relative abundance of the soil dominant phylum, and these changes were closely related to the type of MIs. The results provide a scientific basis for rational use of different types of MIs in high-P soil.

Keywords Microbial inoculant · Wheat growth · Soil properties · Bacterial and fungal agents · Soil bacterial community structure · High-P soil

Introduction

In recent years, China's grain production has increased significantly. In 2019, the total grain output was 663.84 million tons

with an increase of 0.9% compared with that in 2018. The huge increase in food production depends on the excessive use of chemical fertilizers (Gong et al. 2018). The application amount of chemical fertilizer in China is 531.9 kg hm⁻², which is about 3.9 times the world average level. The improper use of chemical fertilizer has brought many problems, such as soil acidification (Zhang et al. 2020), atmospheric nitrogen deposition (Hayashi and Yan 2010; Liu et al. 2020a), water quality deterioration (Fu et al. 2019; Hayashi and Yan 2010), decline in quality of agricultural products (Fu et al. 2019; Liu et al. 2004), and nitrogen (N) and phosphorus (P) imbalance (Long et al. 2018). Part of the current imbalance of N and P is due to excessive use of P fertilizer to maintain high crop yields, resulting in an increasing number of areas with soil

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having a very high-P level (Gilbert 2009; Xie et al. 2020). When the available P (AP) in soil exceeds the critical value of P leaching (60 mg kg^{-1}), total P (TP) emissions from the soil increase linearly (Hesketh and Brookes 2000). The discharged P will leach into the water and cause the water eutrophication (Wang et al. 2012). This situation is not only not conducive to the coordinated development of agricultural resources and the environment but also detrimental to the sustainable development of agriculture (Han et al. 2018; Li et al. 2019b). Therefore, new fertilizers with environmental protection should be used to improve soil quality and micro ecological environment in high-P soil.

Microbial inoculant (MI) is a new type of agricultural fertilizer containing living microorganisms, which can obtain a specific fertilizer effect when applied to agricultural production (Ge 2000; Gong et al. 2018). The combination of MIs and chemical fertilizers can achieve the coordination and supplement of chemical fertilizer by regulating the structure of the soil microbial community. According to the different functions of MIs, they could be divided into four types: plant growth promotion agents, plant resistance enhancer agents, soil remediation agents, and controlling greenhouse gas emission microbial agents. MIs could improve the nutrients of the plant rhizosphere and secrete growth hormones to promote crop growth. Some microorganisms have the function of biological nitrogen fixation (Kumar et al. 2007), which can increase the nitrogen content in the soil; meanwhile, some microorganisms can dissolve phosphorus (Sadeghi et al. 2012) and promote the absorption of phosphorus by plants (Kumar et al. 2007; Kumar et al. 2020; Vessey 2003). The grain yield of soybean increased by 51% with the treatment of compound MIs (*Bacillus coagulans*, *Azospirillum spp.*, *Pseudomonas fluorescens*, *Bacillus subtilis*) and modified biochar (Arabi et al. 2018). The use of MIs to improve crop stress resistance is also a research hotspot. The application of biological fertilizer containing *Bacillus* could control the stress resistance of banana by reducing the number of *Fusarium* and stimulating potential beneficial microorganisms (Shen et al. 2015). In addition, MIs can improve soil physical and chemical properties. The enhanced co-occurrence association in the bacterial network inoculated with plant growth-promoting bacteria (PGPB) might be the mechanism of PGPB in promoting plant growth and helping to remove heavy metals in contaminated soil (Kong et al. 2019). At present, many studies reported that MIs could improve soil conditions (Li et al. 2020; Schoebitz et al. 2014), change the structure of the rhizosphere microbial community (Ambrosini et al. 2016), and increase crop biomass (Mohamed et al. 2019). However, the effect of MIs on the growth of crops at different stages, on the improvement of soil nutrient status at various periods and on soil microorganisms, should be further explored.

Most previous studies focused on the effect of a single type of MIs on plant quality and soil nutrient status at maturity, but

few works compared the effect of different types of MIs applied to the same land at different growth stages of crops in high-P soil ($\text{AP} = 68.43 \text{ mg kg}^{-1}$). The colonization of microorganisms in the plant rhizosphere and the change in the soil microbial community structure and nutrient composition are the preconditions for the effect of MIs. Some studies indicated that soil inoculated with exogenous bacteria may affect indigenous microorganisms, leading to the increase (Bharti et al. 2016), decrease (Garcia et al. 2004), or maintenance of the native microbial activity (Li et al. 2018). Hence, scholars should focus on the effect of various MIs on the diversity and composition of the bacterial community in high-P soil. Recently, 16S rRNA high-throughput sequencing method has been used to explore changes in soil bacterial community (Li et al. 2019a; Xu et al. 2020). We hypothesized that the application of MIs could increase wheat yield and modify the soil microenvironment. Therefore, we conducted field in situ experiments with the following objectives: (1) to compare the effects of different MIs (including promoting crop growth, improving crop resistance, and soil remediation) on wheat growth and soil properties in different periods; (2) to discuss the effects of different MIs on bacterial community in high-P soil; (3) to reveal the relationship between soil bacterial community structure and environmental factors under the application of different MIs; (4) to evaluate the effect of all MIs comprehensively and find the best of all for wheat yield, soil nutrition, and microbial structure to provide a basis for the application of MIs in high-P soil.

Materials and methods

Experimental sites

A field study was conducted at Jining City ($116^{\circ} 88' \text{ E}$, $35^{\circ} 30' \text{ N}$) in Shandong Province, China from September 2018 to June 2019. Jining is located in the East Asian monsoon climate zone and experiences warm temperate monsoon climate. The annual average rainfall is 597–820 mm, the annual mean temperature is $13.3\text{--}14.1^{\circ} \text{C}$ and the annual average frost-free period is 199 days for the experimental field. The site is located in the storage area of the east line of the South to North Water Transfer Project, which is a typical representative of the agricultural intensive area in the North China Plain. N and P in soil are unbalanced due to the influence of local fertilization habits and river water quality. The detailed soil properties of the field were presented as follows: pH was 6.76 and the contents of soil organic matter (OM), available N (AN), $\text{NH}_4^+\text{-N}$, $\text{NO}_3^-\text{-N}$, AP, and TP were 15.25 mg kg^{-1} , $110.50 \text{ mg kg}^{-1}$, 12.83 mg kg^{-1} , 32.81 mg kg^{-1} , 68.43 mg kg^{-1} , and 0.66 g kg^{-1} , respectively. Combined with the soil fertility standard, the AP of this site was 68.43 mg kg^{-1} , which was higher than 40 mg kg^{-1} of the first-level standard.

Experimental design

A randomized block design was employed for wheat (Jimai22) during the fall season of 2018 with six MIs in three replicates. Wheat was grown in a 10 m × 5 m experimental plot under a conventional fertilization–irrigation system, and each cell was set with 2 m between the rows and 1.5 m between the columns and ridges to prevent cross-contamination. The following MIs were used in this study: (i) *Trichoderma* composite (TC); (ii) soil remediation agent (SR); (iii) anti-repeat microbial agent (AM); (iv) microbial agent (MA); (v) plant growth-promoting rhizobacteria (PG); and (vi) biological fertilizer function bacteria agent (BF). BF, PG, MA, and AM are microbial agents used to improve crop growth and resistance; BF, PG, and MA are plant growth promoters; AM is a plant resistance enhancer; and TC and SR are microbial agents for improving soil conditions. TC is a fungal agent, and the rest are bacterial agents. The details of these MIs per treatment are presented in Table 1. The MIs were applied at 30 kg ha⁻¹ before irrigation. Considering that the application amount of MI was less, it should be mixed with surface soil and fertilizer in advance for artificial fertilization. The rates of total nutrient addition were as follows: 195 kg N ha⁻¹, 210 kg P₂O₅ ha⁻¹, and 75 kg K₂O ha⁻¹.

Sample collection and analysis

Plant sample collection and analysis

Ten whole wheat plants were collected in each experimental plot at the regreening, flowering, and harvest stages for index tests and analysis. In addition, 1-m-long wheat should be cut continuously from two parallel rows in each plot to determine wheat yield at maturity.

The wheat plants were washed thoroughly with distilled water. The tissues were separated, inactivated in an oven at 105 °C for 15 min, and dried to constant weight at 70 °C. The following measurements were conducted for each plant: plant height, thousand grain weight, spike number, grain yield, and dry matter accumulation (DMA) of root and aboveground. The wheat grain was digested by H₂SO₄-H₂O₂ (Lu 2000) after

ground. The N content of the grain was determined by a continuous flow analyzer (SAN⁺⁺, SKALAR, Netherlands), and P content was determined by spectrophotometer (L5S, Shanghai Yidian Analytical Instrument Co., Ltd., China).

Soil sample collection and analyses

Topsoil (0–20 cm) was collected from each plot by a five-point sampling method as soil background value before wheat sowing. The 10 wheat plants collected in each plot were completely excavated, and loosely bound soil was shaken off; the tightly bound soil was retained as the rhizosphere soil sample (Jia et al. 2020) at the regreening, flowering, and maturity stages of wheat. The collected soil samples were transported to the laboratory with dry ice preservation. Part of the soil samples were dried naturally, ground, and sieved for determination of soil physical and chemical indicators. The other part of the soil samples were stored in a refrigerator at –80 °C for microbial diversity determination.

Soil properties were measured by the following methods (Lu 2000). The pH value of the soil was determined by pH meter (PHS-2F, Shanghai INESA, China) according to the ratio of 1:2.5 (soil: H₂O). The OM was determined by potassium dichromate oxidation–ferrous sulfate titration. The AN in soil was determined by the alkali diffusion method, AP and TP were measured by sodium bicarbonate extraction–molybdenum blue method. NH₄⁺-N and NO₃⁻-N were determined by potassium chloride leaching–automatic continuous flow analyzer (SAN⁺⁺, SKALAR, Netherlands).

The sampling of each plot before fertilization was used as the background value of each treatment to reduce the error caused by soil heterogeneity. The amount of change in the soil index at each stage was measured using the end value of this stage minus the initial value of the stage: $\Delta C = C_{\text{final}} - C_{\text{initial}}$.

DNA extraction, high-throughput sequencing, and data processing

PowerSoil extraction kit (Mo-Bio) was used to extract DNA from rhizosphere soil following the instructions (Liu et al.

Table 1 Details of the six microbial agents used in the field experiment

Microbial agent	Core microorganism	Manufacturer
TC	<i>Trichoderma</i>	Shandong Ivlong Co., Ltd.
SR	<i>Actinomycetes</i> , <i>Bacillus</i>	Shandong Jinyao Co., Ltd.
AM	<i>Bacillus subtilis</i> , <i>Trichoderma</i>	Zhongnong Ivkang Co., Ltd.
MA	<i>Bacillus subtilis</i> , <i>Bacillus cereus</i>	Zhongnong Ivkang Co., Ltd.
PG	<i>Bacillus subtilis</i> , <i>Paenibacillus polymyxa</i>	Shandong Ivlong Co., Ltd.
BF	<i>Bacillus subtilis</i> , <i>Bacillus licheniformis</i>	Shandong Ivlong Co., Ltd.

TC, *Trichoderma* composite; SR, soil remediation agent; AM, anti-repeat microbial agent; MA, microbial agent; PG, plant growth–promoting rhizobacteria; BF, biological fertilizer function bacteria agent

2020b). DNA concentration and purity were detected by NanoDrop One spectrophotometer (Thermo Fisher Scientific, MA, USA). The DNA fragments were amplified by 16S V4 (515f and 806r) primers, barcode specific primers, and PremixTaq (TaKaRa) (Turnbaugh et al. 2009). The length and concentration of the PCR products were verified by 1% agarose gel electrophoresis. The products were mixed by GeneTools Software (Version 4.03.05.0, SynGene) according to the equal quality principle and further purified by EZNA Gel Extraction Kit (Omega, USA) (Zhao et al. 2019a). The following procedures included the establishment of a library, processing of data, clustering of OTU, and annotation of species. Low-quality reads in the original reads were removed using the Mothur software (V1.35.1, <https://www.mothur.org/>) (Schloss et al. 2009). The Uchime software was used to eliminate the singleton OTU and chimeras, then the Usearch method was used to cluster valid sequences. Finally, the sequences with the highest frequency were selected as the representative sequences for analysis. Sequencing and microbial analysis were conducted by Illumina HiSeq. 2500 (Guangzhou Magigene Biotechnology Co. LTD., China) platform.

Statistical analysis

Alpha diversity including Chao1 and Shannon indices were calculated with QIIME (<http://qiime.org/>). SPSS software (version 22.0) was used to conduct one-way ANOVA to determine significant differences, and Spearman's correlation analysis was performed to determine the correlation and significance between environmental factors and species abundance. In addition, redundancy analysis (RDA) was displayed by Canoco (version 4.5), and heatmap was represented by the gplots, RColorBrewer, and vegan packages in R (version 4.0.3). Other figures were made by Origin (2018). The experimental data consisted of three replicates and reported as mean \pm standard deviation.

Results

Effect of MIs on crop growth

The DMA of winter wheat under different MI applications during regreening, flowering, and harvest stages is shown in Fig. 1. Among various MI treatments, a different increase in aboveground DMA was observed (Fig. 1a). At the regreening stage, the aboveground DMA of MI treatments was significantly higher than that of the control ($12.38 \text{ g} \cdot 10 \text{ plant}^{-1}$; $p < 0.05$). BF, PG, and SR significantly promoted the growth of wheat at the flowering stage, with increases of 48.0%, 37.9%, and 25.5%, respectively, compared with the control ($p < 0.05$). At the harvest stage, the aboveground DMA values of

wheat that received MA, AM, BF, and PG were significantly higher than those of wheat given with the control, with an increase ratio of 26.5–63.1% ($p < 0.05$). The results explained that MI combined with chemical fertilizers could promote crop growth, and at maturity, the promoting effect of all MIs on the DMA of wheat followed the order of MI aimed at crop (including MA, BF, PG, AM) > MI aimed at soil (including TC, SR) > control.

The growth of roots is vital before maturity and a key to nutrient uptake. All MI treatments, especially PG, AM and MA, enhanced the root growth at the regreening stage and flowering stage ($p < 0.05$) (Fig. 1b). Based on the ratio of the aboveground dry matter mass to the total dry matter mass, PG, BF, and MA were beneficial to the maturity, and the ratio increased to 97.06%, 96.21%, and 96.08%, respectively, compared with the control (95.75%).

Effect of MIs on crop nutrition and yield

The crop nutrition, yield, and growth characteristics of wheat treated with different microbial agents are shown in Table 2. At maturity, the grain P concentration decreased and the grain N concentration changed with MI compared with the control. The amount of nitrogen absorbed by grains with the addition of BF, AM, SR, and PG increased slightly by 6.0%, 1.1%, 0.5%, and 0.2% respectively. Compared with control, BF and TC were the maximum and minimum nitrogen uptake in grain, respectively. And TC significantly reduced nitrogen content in the grains compared with control ($p < 0.05$). This result indicated that the bacteria agents were better than fungi agents (TC) at promoting nitrogen uptake by wheat grain.

The yield of wheat increased by 15.2–33.4% compared with the control, indicating that MIs could promote grain growth. Compared with BF, AM, and SR, the treatments of PG, MA, and TC had higher yield ($9.38\text{--}10.38 \text{ t ha}^{-1}$) but relatively lower nitrogen content in grains ($15.16\text{--}16.56 \text{ g kg}^{-1}$). Although the content of AN in the soil increased, the yield increased, leading to the decrease in average concentration distributed in grains (Triboi et al. 2006). The plant height of wheat also increased in different degrees in the three periods. Spike number and thousand grain weight were significantly higher in PG treatments than in the control ($p < 0.05$). Considering the yield component factors (spike number and grain weight), MA, PG, BF, and AM, which promoted crop growth and stress resistance, were superior to SR and TC, which improved soil conditions; meanwhile, bacterial agents were more effective than fungal agents (TC).

Effect of MIs on soil AN and AP

The concentrations of AN and AP in the 0–20-cm soil layer of winter wheat in different periods under the application of different types of MIs are shown in Fig. 2. The application of all

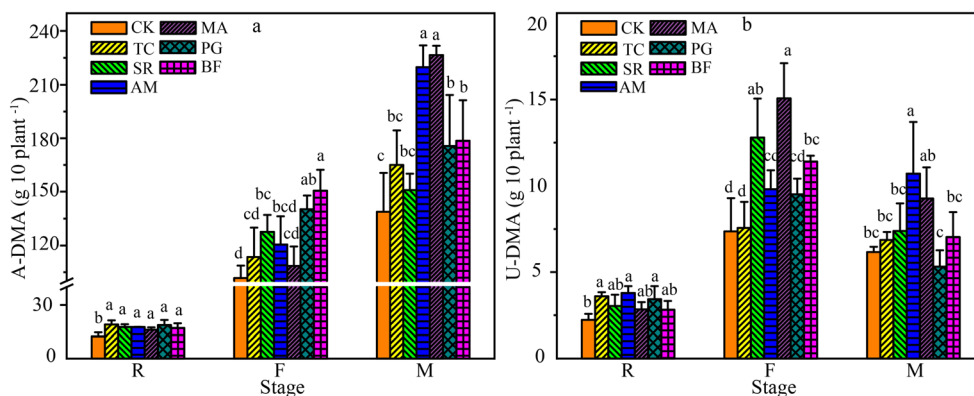


Fig. 1 DMA of aboveground (A-DMA) (a) and DMA of underground (U-DMA) (b) at the stage of regreening (R), flowering (F) and maturity (M) in the seven treatments: control (CK); *Trichoderma* composite (TC); soil remediation agent (SR); anti-repeat microbial agent (AM); microbial agent (MA); plant growth-promoting rhizobacteria (PG); biological

fertilizer function bacteria agent (BF). DMA means dry matter accumulation. Lower case letters indicate that the significant difference of DMA between different treatments at one stage is statistically significant ($p < 0.05$, Duncan's test)

MI increased the AN in soil significantly ($p < 0.05$) at the unfertilized-regreening (U-R) stage and regreening-flowering (R-F) stage compared with control (Fig. 2a). The MA increased the AN the most (21.5 g kg^{-1}) at the U-R stage, and PG increased the AN the most (25.67 g kg^{-1}) at the R-F stage (Fig. 2a). For the entire growth period, regardless of the type of MI added, the AN in soil increased significantly ($p < 0.05$) (Fig. 2b). In 0–20-cm soil, the order of the effect of different microbial agents on the increase in AN was as follows: BF > PG > MA > TC > SR > AM > CK (Fig. 2b). Hence, microbial agents with the aim of promoting growth (BF, PG, and MA) had better effect on increasing AN.

The AP increased first and then decreased in the entire growth period (Fig. 2c). At the U-R stage, all MI applications increased the P availability by 0.8% to 54% in comparison with the control (Fig. 2c). In the latter two stages, significant differences in P availability were found between the control and MI treatments ($p < 0.05$). In the entire period, the increase

in AP caused by all MI treatments was less than that of the control (except MA), but the difference was not significant (Fig. 2d).

Effect of MIs on soil bacterial community and diversity

Soil bacterial richness and diversity

The effect of MI application on the diversity of soil microbial communities should be understood. Compared with the control, there was no significant difference in Shannon index after the addition of MIs, indicating that there was no significant change in microbial diversity (Fig. 3a). However, the Shannon of soil added with TC was almost higher than other bacterial agents (except MA). For soil bacteria in the root zone of farmland, the order of Chao1 in the seven treatments was MA > TC > SR > CK > PG > AM > BF (Fig. 3b). The Chao1 of soil added with MA was significantly higher than control, and the

Table 2 The wheat height in three stages and the 1000 grain weight, grain number, yield and the P and N of grain at maturity

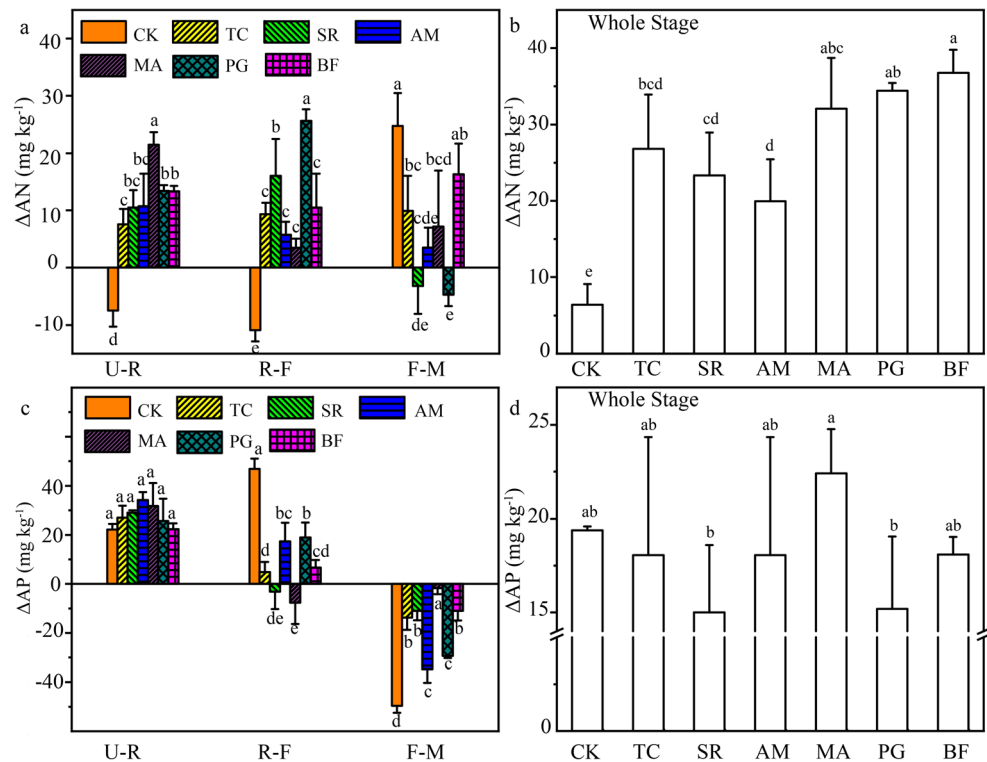
Treatment	Plant height (cm)			1000 grain weight (g)	Grain number	Yield (t ha^{-1})	Grain	
	R stage	F stage	M stage				P (g kg^{-1})	N (g kg^{-1})
CK	25.83±1.54de	69.45±3.67d	70.70±3.12c	41.99±0.05b	165±29b	7.78±1.27b	4.11±0.31a	16.53±0.84ab
TC	26.57±1.04c	71.60±3.05b	72.63±2.57b	42.88±0.10b	190±18ab	9.38±0.99ab	3.75±0.07a	15.16±0.46c
SR	26.36±0.65 cd	71.90±1.84b	71.01±2.10c	42.48±1.20b	195±14.43ab	8.96±1.04ab	3.54±0.51a	16.61±0.45ab
AM	27.61±1.25b	72.30±2.06b	73.82±3.31b	44.57±3.36ab	196±9.02ab	9.11±0.86ab	3.64±0.71a	16.71±0.10ab
MA	27.30±1.39b	69.83±2.61 cd	73.48±2.75b	43.56±1.86ab	200±16.70ab	9.93±0.49ab	3.61±0.24a	16.10±0.25bc
PG	28.40±0.74a	71.09±2.82bc	72.63±2.37b	46.34±1.63a	215±4.36a	10.38±0.28a	3.71±0.32a	16.56±0.31ab
BF	25.74±0.69e	74.50±2.48a	75.31±2.50a	43.99±1.57ab	217±24a	9.04±0.44ab	3.80±0.20a	17.51±1.01a

R, regreening; F, flowering; M, maturity

CK, control; TC, *Trichoderma* composite; SR, soil remediation agent; AM, anti-repeat microbial agent; MA, microbial agent; PG, plant growth-promoting rhizobacteria; BF, biological fertilizer function bacteria agent

Lower case letters indicate that the significant difference between different treatments is statistically significant ($p < 0.05$, Duncan's test)

Fig. 2 The change amount of AN in the soil (a) and the change amount of AP (c) in the soil at the stage of unfertilized–regreening (U–R), regreening–flowering (R–F), flowering–maturity (F–M) in the seven treatments: control (CK); *Trichoderma* composite (TC); soil remediation agent (SR); anti-repeat microbial agent (AM); microbial agent (MA); plant growth–promoting rhizobacteria (PG). The change amount of AN (b) and the change amount of AP (d) at the entire stage. AN, available nitrogen; AP, available phosphorus. Lower case letters indicate that the significant difference of AN, AP between different treatments at one stage is statistically significant ($p < 0.05$, Duncan’s test)



Chao1 of soil added with SR and TC was significantly higher than that of soil added with BF ($p < 0.05$), indicating that the addition of MIs had an effect on the richness of the bacterial community. Hence, soil with fungal agents (TC) had higher diversity and richness compared with soil with bacterial agents (except MA).

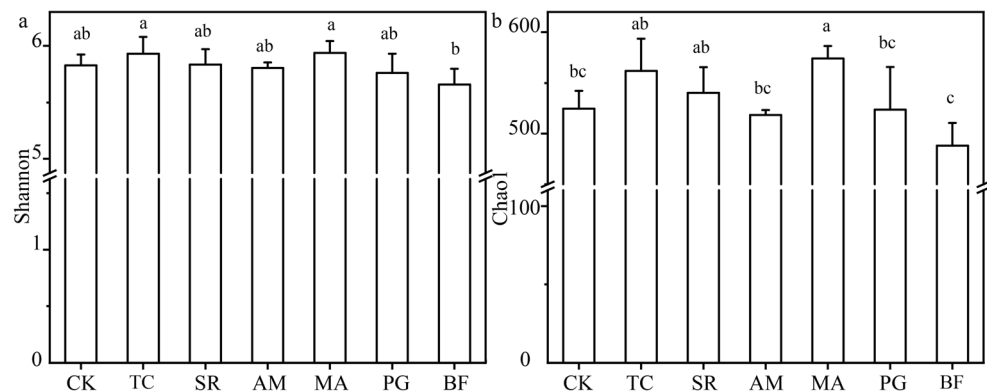
Differences in bacterial community structure

The dominant phyla (top five) were clustered into Proteobacteria (39.8–26.9%), Acidobacteria (23.7–12.0%), Bacteroidetes (20.9–8.5%), Actinobacteria (10.3–7.0%), and Verrucomicrobia (7.2–5.3%). The dominant phyla (> 5%) accounted for more than half of the total number of bacteria (Fig. 4a). Moreover, with the addition of microbial agents, the relative abundance of Acidobacteria, Actinobacteria, and

Chloroflexi increased by 23.7%, 10.3%, and 7.0%, respectively, and the relative abundance of Proteobacteria and Bacteroidetes decreased to 26.9% and 8.5%, respectively. The addition of SR led to the highest relative abundance of Acidobacteria and to the lowest abundance of Actinobacteria.

Proteobacteria was the most dominant phylum and consists of four major genera, namely *Sphingomonas*, *Pseudomonas*, *Variovorax*, and *Haliangium* (Fig. 4b). Compared with the control, the relative abundance of *Sphingomonas* decreased (except BF) and that of *Variovorax* increased. The relative abundance of *Haliangium* decreased with the addition of bacterial agents and increased with the addition of fungal agents compared with control. The relative abundance of *Haliangium* was significantly different between treatments with BF (bacterial agent) and TC (fungal agent) ($p < 0.05$). *Mucilaginibacter*

Fig. 3 Alpha diversity indices (97% similarity) included Shannon (a) and Chao1 (b) in soils with seven treatments: control (CK); *Trichoderma* composite (TC); soil remediation agent (SR); anti-repeat microbial agent (AM); microbial agent (MA); plant growth–promoting rhizobacteria (PG). Lowercase letters marked similarly between different treatments were not statistically significant ($p < 0.05$, Duncan’s test)



and *Pedobacter*, which belong to Bacteroidetes, decreased in MI treatments (except BF).

Bacterial composition with significant differences

LEfSe analysis was used to further explore bacterial constituents that differed significantly among the treatments (LDA value of 2, Fig. 5). *Mesorhizobium* (belongs to Proteobacteria) was enriched in CK. *Plantibacter* (belongs to Actinobacteria), *Lacibacter* (belongs to Bacteroidetes), and *Phyllobacterium* (belongs to Proteobacteria) were enriched in BF. *Actinoallomurus* (belongs to Actinobacteria) was enriched in PG. Oceanospirillales (belongs to Proteobacteria), Staphylococcaceae, and the genus *Staphylococcus* (belongs to Firmicutes) were enriched in SR. *Phytohabitans* (belongs to Actinobacteria), *Flaviumicrobium* (belongs to Bacteroidetes), Phaselicystidaceae, and its genus *Phaselicystis* (belongs to Proteobacteria) were enriched in MA. *Anaerococcus* (belongs to Firmicutes), *SMIA02* (belongs to Planctomycetes), Microcystaceae, and its genus *Microcystis_PCC* (belongs to Cyanobacteria) were enriched in AM. Different MIs enriched different bacterial compositions and treatment with the fungal agent (TC) exerted no significant enrichment effect on the bacterial composition.

Relationship between the composition of microbial community and environmental factors

RDA analysis was conducted to quantify the relative effect of environmental factors on bacterial community composition and abundance after MI application. The RDA results revealed that the weights explained for the first two axes were 39.8% and 1.7%, respectively (Fig. 6). These combinational environmental variables accounted for 41.5% of the variation in the bacterial community composition. In addition, the RDA diagram showed that pH had a significant effect on the abundance and composition of soil microbial community ($p = 0.01$).

Heatmap (Spearman's rank-order analysis) was further used to examine the relationship between environmental factors and soil bacterial community abundance at the phylum of the top 10 (Fig. 7). The correlations of the dominant phyla Proteobacteria and Bacteroidetes were basically consistent, and they were positively correlated with AN and AP and negatively correlated with pH significantly ($p < 0.01$). Other microbial species were positively correlated with pH and NH_4^+-N and showed a significant positive correlation with pH ($p < 0.05$) and a negative correlation with AN, AP, NO_3^--N , and TP. Planctomycetes was significantly positively correlated with pH and NH_4^+-N ($p < 0.01$) and significantly negatively correlated with AN ($p < 0.05$). Actinobacteria was significantly positively correlated with AN ($p < 0.01$). AP

was only significantly negatively correlated with Chloroflexi. No significant correlation was found between TP, OM, NO_3^- and soil bacterial community abundance at the phylum level. Hence, alterations in soil characteristics caused by MI addition would change the structure of the soil bacterial community.

Discussion

Different types of MIs improve the yield of wheat

Wheat yield is closely related to DMA. Under the condition of conventional tillage, the application of different microbial agents combined with chemical fertilizers increased the DMA of the aboveground and underground parts of winter wheat; as such, the combination of MI and chemical fertilizers had a synergistic effect. AM, BF, PG, and MA (*Bacillus* group as the core bacteria) exhibited more effective effects than soil remediation agents on promoting crop growth. In the pot experiments, the addition of compound microbial agent with *Bacillus* had a great promoting effect on pepper growth possibly due to the plant growth-stimulating substances released by inoculated *Bacillus* and that plant tissue absorbs soil nutrients released by *Bacillus* (Zhao et al. 2019b). Various microbial agents increased the plant height, spike number, and grain weight of wheat at maturity, consistent with previous studies (García de Salamone et al. 2012; Gong et al. 2018). Therefore, these factors combined to increase the yield of wheat with the addition of MI.

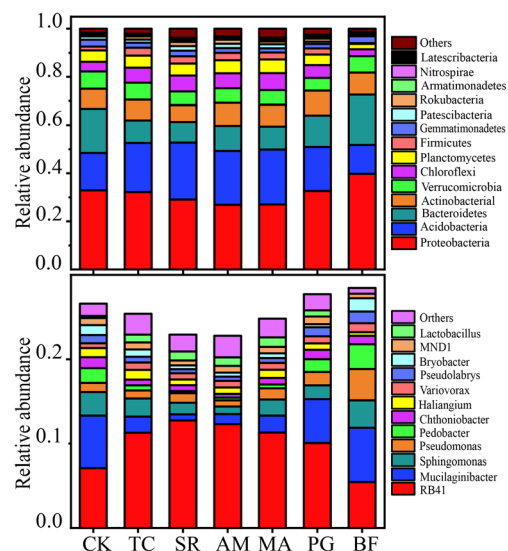
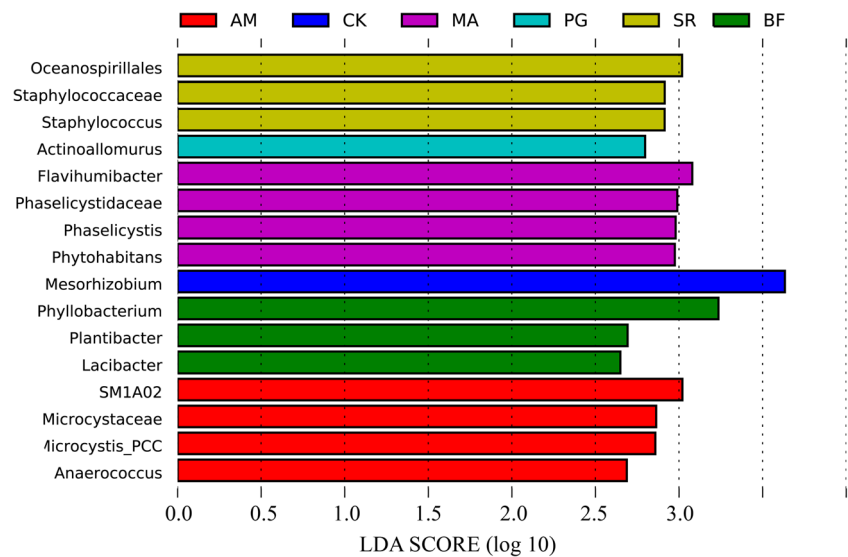


Fig. 4 The relative abundance of dominant phyla (a) and the relative abundance of dominant genera (b) in rhizosphere soil for the different treatments: control (CK); *Trichoderma* composite (TC); soil remediation agent (SR); anti-repeat microbial agent (AM); microbial agent (MA); plant growth-promoting rhizobacteria (PG) at maturity

Fig. 5 The relative abundance of bacterial community with significant differences among seven treatments by LEfSe analysis (LDA value of > 2). Seven treatments included control (CK), *Trichoderma* composite (TC), soil remediation agent (SR), anti-repeat microbial agent (AM), microbial agent (MA), plant growth-promoting rhizobacteria (PG)



Different types of MI increase the content of AN in high-P soil

The sustainable supply of nutrients in soil is an important factor that affects crop growth. In this study, the AN content significantly increased at maturity ($p < 0.05$). The increased AN confirmed the function of MI in changing soil properties. Chaudhary et al. (2020) found that inoculating halotolerant PGPR improved the availability of N in the soil because of the nitrogen fixation capacity of the strains isolated. The AN in soil inoculated with *Bacillus phosphide* and *Bacillus mucilaginosus* was also improved (Han et al. 2006). This finding could be explained by the fact that the successful colonization of the beneficial flora of microbial agents in the soil and the secretion of organic acids could dissolve and release N nutrients adsorbed by soil particles, thereby prolonging the effective supply of N to the soil (Lei et al. 2012). More than half of the MI treatments increased the nitrogen uptake of wheat grains. Rana et al. (2012) also found that inoculation with PGPR in the soil led to high-nitrogen absorption by the

crop. On the one hand, the addition of MI increases the activity of some microorganisms (Kuzyakov et al. 2000), accelerates the decomposition of organic matter, and increases the mineralization rate of nitrogen (Van der Heijden et al. 2008). On the other hand, inoculating MI (such as PGPR) plays a key role in dissolving, improving efficiency, and delivering insoluble nutrients to plants (Roesti et al. 2006).

Furthermore, inoculation of *Bacillus subtilis* and *Lysinibacillus fusiformis* in low P soil improved the AP content in the soil and the uptake of P by the crop (Rafique et al. 2017). The addition of MI (*Bacillus megaterium* and *Bacillus mucilaginosus*) in calcareous soil can improve the uptake of P by crops, which could be due to the solubilization function of MI (Zhao et al. 2019a). However, Assainar et al. (2018) showed that the addition of microbial agents had no effect on the concentration of P in wheat grains. In the present study, the addition of MIs did not significantly affect the content of AP in soil and the absorption of P in wheat grain. We hypothesized that P content was rich ($p > 60 \text{ mg kg}^{-1}$), so P was not a limiting factor for crop growth or microbial activity and did not affect the absorption by wheat grains.

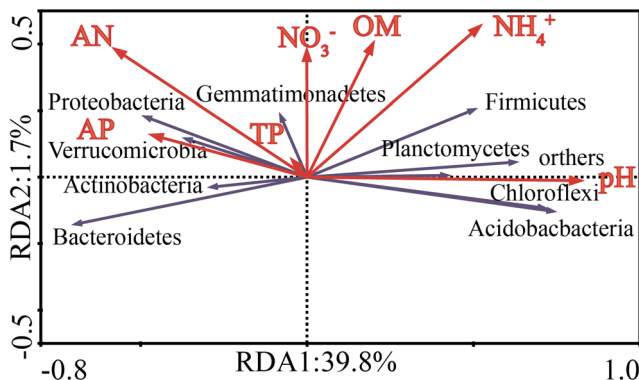


Fig. 6 Redundancy analysis (RDA) of the abundant phyla (top 10) and environmental variables. Organic matter (OM); available nitrogen (AN); available phosphorus (AP); total phosphorus (TP)

Different types of MI changed the composition and abundance of microorganisms

Soil microorganisms have the potential to enhance the function of sustainable ecosystem (S. Franz bender, 2019). In this study, the Shannon and Chao1 of fungal agents (TC) were higher than those of bacterial agents (except MA) and significantly higher than those of BF ($P < 0.05$). Zhao et al. (2018) found that the Simpson index decreased in the soil inoculated with *Trichoderma*. The Shannon value of soil inoculated with fungal agents (*Trichoderma*) was higher than that of bacteria agents (Wang et al. 2018), consistent with our results. Inoculants must be rooted in the soil or combined with the

host plant to promote growth. However, the persistence and stability of these inoculants in the soil may cause interference to the local microbial population (Gu et al. 2020). Although the application of fungal microbial agents might also affect the stability of the soil system, its impact and disturbance are not as significant as those of bacterial microbial agents (Wang et al. 2018). In general, the improvement of soil bacterial diversity with fungal agents indicated that they enhanced the anti-interference ability of the soil. Microbial agents are exogenous substances that can destroy the steady state of soil. Various microbial agents exhibit different levels of influence on microbial diversity and richness. Hence, MI types are important factors affecting soil microbial diversity (Kumar et al. 2007; Saldajeno and Hyakumachi 2011; Wang et al. 2019b).

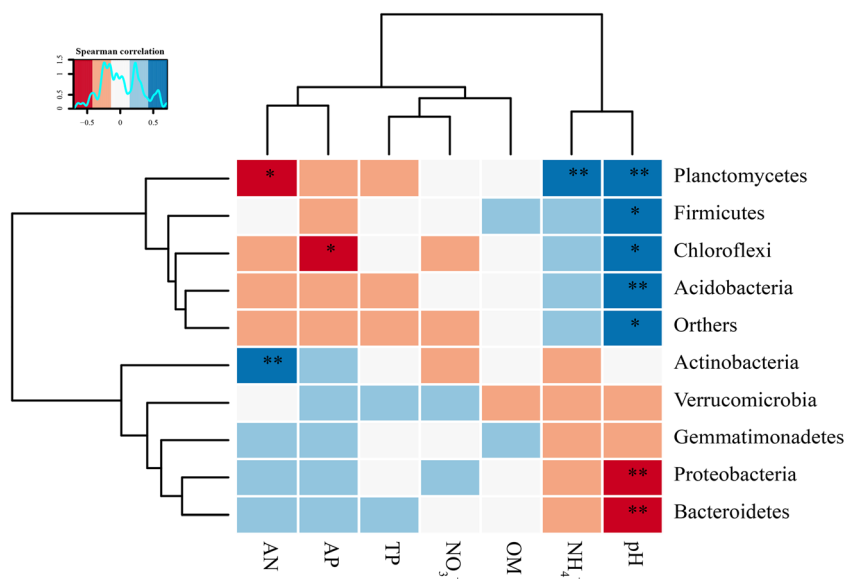
The addition of MI changed the composition of microorganisms in the soil, but the species of microorganisms contained were basically consistent. The decline in the major genera, namely *Sphingomonas* and *Haliangium*, explained the reduction in Proteobacteria. Proteobacteria was negatively correlated with TN and was more suitable for nitrogen fixation in low-nitrogen soils (Wang et al. 2017). However, AN was significantly enhanced after MI addition ($p < 0.05$). The addition of MI increased Acidobacteria and decreased Bacteroidetes, which may be due to the fact that the former is suitable for living in low-pH, high-nitrogen, and high-carbon environment (Sui 2017) in contrast to the latter. The decrease in the major genera of *Mucilaginibacter* and *Pedobacter* could better explain the reduction in Bacteroidetes. The LEfSe analysis further revealed that the genera in the soil bacterial community were significantly enriched by treatment with bacterial agents but not by treatment with fungal agents. The addition of BF significantly enriched *Phyllobacterium* and *Plantibacter*. They were closely related to plant roots and contained a large number of microorganisms

related to plant growth (Maquia et al. 2020; Wang et al. 2020). And *Plantibacter* had the function of increasing zinc and dissolving potassium (Costerousse et al. 2018). *Actinoalcomurus* was significantly enriched in PG, and it was proved to be a plant growth promoter (Mahnert et al. 2018).

pH is the main driving factor of microbial community composition (Wan et al. 2020; Wang et al. 2019a). Environmental parameters are often related to soil pH. Therefore, soil pH will further cause variation in the microbial community structure (Zhao et al. 2019b). We found that pH was significantly related to the abundance of Acidobacteria, Proteobacteria, Bacteroidetes, and so on ($p < 0.05$). Our result is consistent with the conclusions of Lauber et al. (2009) and Rousk et al. (2010). The application of MI resulted in the deviation of the composition of microbial communities in the soil and the changes in the proportion of dominant microbial communities probably due to the effect of MI addition on pH.

AN was significantly positively correlated with Actinobacteria and negatively correlated with Planctomycetes, NH_4^+ was significantly positively correlated with Planctomycetes. Planctomycetes was widely distributed in soil, including the bacteria with Anammox function (Nie et al. 2018). They could produce N_2 through NH_4^+ and NO_2^- (Van Teeseling et al. 2015), and play an important role in N removal in farmland soil with high-N (De Cocker et al. 2018). The increase in Planctomycetes might also be related to the participation of Planctomycetes in denitrification (Zhou et al. 2018). In this study, Planctomycetes was driven in a positive direction by NH_4^+ , suggesting that most of the bacteria from planctomycetes might have Anammox function. Similar to Anammox bacteria in river sediments, they were widely distributed in the condition of rich NH_4^+ (Sáenz et al. 2012). AN and NH_4^+ were significantly correlated with Actinobacteria and Planctomycetes ($p < 0.05$). TP had no significant

Fig. 7 Heatmap (Spearman's rank correlation) included a cluster analysis between different treatments and the relative abundances of dominant phylum (top 10). The horizontal variable is soil properties, vertical variable is the relative abundance of microorganisms, red and blue shows negative correlation and positive correlation respectively. Darker color expresses higher correlation, * and ** indicates $p < 0.05$ and $p < 0.01$ respectively. Organic matter (OM); available nitrogen (AN); available phosphorus (AP); total phosphorus (TP)



correlation with the dominant phylum, and only AP was significantly correlated with Chloroflexi. Shi et al. (2012) found that the reduction of P did not significantly affect the domain microbial phyla in the soil with a high threshold level of P; moreover, P did not significantly change the microbial community structure. Soil microbes were limited by carbon and nitrogen instead of AP even in soil with low AP content (Ehlers et al. 2010; Kuzyakov et al. 2000). This phenomenon could explain the absence of the significant effect of TP and AP on the soil microbial community structure (except Chloroflexi). The AP content in this field was speculated to be high, and the addition of MI did not evidently change the AP content, which is therefore not a limiting factor for microbial growth.

Conclusion

Scholars investigating the application of MIs should focus on soil properties and types of microbial agents. The compound microbial agent with *B. subtilis* as the core microorganism exhibited an evident effect on promoting wheat growth. The addition of MI significantly increased the AN in soil but had no significant effect on AP in high-P soil. Therefore, MI with the ability of fixing N might be more effective in high-P soil. Various types of microbial agents exerted different effects on soil microorganisms. Compared with bacterial agents, fungal agents could improve the diversity of bacterial communities in soil. pH and AN played an important role in shaping the microbial community. The changes in soil physical and chemical properties caused by different MIs further altered the microbial community structure. These findings suggest that appropriate MI and the effects of MI on soil properties and soil microorganisms should be paid attention to during the application of MI.

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Data availability The datasets generated and/or analyzed during the current study are not publicly available [REASON WHY DATA ARE NOT PUBLIC] but are available from the corresponding author on reasonable request.

Compliance with ethical standards

Competing interests The authors declare that they have no competing interests.

Ethics approval and consent to participate Not applicable.

Consent for publication Not applicable.

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