https://doi.org/10.17221/456/2020-PSE

Trichoderma asperellum improves soil microenvironment in different growth stages and yield of maize in saline-alkaline soil of the Songnen Plain

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Citation: Fu J., Xiao Y., Liu Z.H., Zhang Y.F., Wang Y.F., Yang K.J. (2020): *Trichoderma asperellum* improves soil microenvironment in different growth stages and yield of maize in saline-alkaline soil of the Songnen Plain. Plant Soil Environ., 66: 639–647.

Abstract: The Songnen Plain is an important agricultural base in China and one of the important areas of distribution of saline-alkaline soils in the cold region. Saline-alkaline soils severely restrict maize growth. This study was to potentially promote the soil nutrient in the maize rhizosphere, microbes diversity, and maize yield by *Trichoderma asperellum* in saline-alkaline soil of the cold region. In the present study, we applied different amounts of *T. asperellum* in field experiments for three consecutive years. High-throughput sequencing was used to analyse the impact of *Trichoderma* on microbes diversity in maize rhizosphere soils. Changes in crop yield and soil nutrients were also monitored. *T. asperellum* treatment significantly increased the relative abundance of beneficial microbes genera. In the control treatment, the pathogenic microbes were the dominant genera. Pearson's correlation analysis revealed that changes in the soil microbial community composition were closely related to soil nutrients and were highly correlated with *T. asperellum* treatment concentration. Further, *T. asperellum* treatment increased crop yield by 4.87–20.26%. These findings suggest that *T. asperellum* treatment optimised the microenvironment of the maize rhizosphere soil, alleviated microbial community degeneration in cold region saline-alkaline soil, and promoted maize growth.

Keywords: bioremediation; saline-alkaline; microorganism; nutrient cycle; cold climate

Soil salinisation is one of the most serious problems affecting agricultural production globally. Excessive Na_2CO_3 and $NaHCO_3$ in the agricultural soil of northeastern China's Heilongjiang province afflicts the crops in this area with Na^+ toxicity and high pH stress, causing more crop damage than NaCl

Supported by grants from the Initiation Foundation for Introduced Talent Person of Heilongjiang Bayi Agricultural University, Grant No. XYB201901; by the Key Laboratory of Saline-Alkali Vegetation Ecology Restoration, Ministry of Education (Northeast Forestry University), Project No. 20200522-4; by the Project funded by China Postdoctoral Science Foundation, Grant No. 2020M670930; by the Heilongjiang Bayi Agricultural University Support Program for San Heng San Zong, Project No. ZRCQC202002; by the National Science and Technology Support Plan of China, Project No. 2018YFD0300101, and by the Daqing Guiding Science and Technology Plan Project, Project No. zd-2019-24 and Heilongjiang Bayi Agricultural University Postdoctoral Science Foundation.

https://doi.org/10.17221/456/2020-PSE

(Luo et al. 2018). In the cold region of Heilongjiang province, soil total organic carbon content is low due to various natural environmental conditions (e.g., long-term drought and uneven distribution of seasonal precipitation) and inadequate land management. Poor physical and chemical conditions lead to the low fertility of saline-alkali soil. The high content of soluble salt together with the high pH limits the growth and reproduction of microorganisms, thereby limiting microbial species diversity and abundance (Tripathi et al. 2007), which in turn affects the nutrient cycle and eventually reduces crop yield. Therefore, questions regarding how to alleviate the stress of saline-alkali soil on crop growth, how to better understand mechanisms of saline-alkali regulation, and how to optimise the use of saline-alkali soil resources in the cold region are of great significance to the sustainable development of agriculture in Heilongjiang province.

As an important biocontrol fungus, *Trichoderma* can improve plant resistance to pathogens and promote plant growth due to its strong colonisation ability around the rhizosphere (Harman et al. 2004). Some studies have shown that *Trichoderma* has a significant inhibitory effect on pathogenic fungi in the soil and that it can effectively improve soil structure, promote the establishment and maintenance of beneficial microbial colonies in soil (Fontenelle et al. 2011), increase soil nutrient availability and plant nutrient utilisation rate (Saravanakumar et al. 2013). The metabolites secreted by *Trichoderma* interact with the plant root system, which affects the chemical properties and nutrients of the soil (Zhang et al. 2013). In these ways, *Trichoderma* improves the microecological structure of soil and effectively promotes plant growth. However, the long-term effects of *T. asperellum* on the soil microenvironment and crop yield in different growth stages of maize under saline-alkali soil conditions have, to our knowledge, not been previously studied.

To do this, we applied *T. asperellum* at different concentrations to farmlands in Heilongjiang province for three consecutive years and analysed soil samples to examine the effects of *T. asperellum* on the rhizosphere of maize at different growth stages. We hypothesised that the soil microorganism community structure would be affected by long-term *Trichoderma* treatment, aiming to effectively improve maize rhizosphere soil microorganism abundance, nutrient, and ultimately improve crop yield. This study provides the theoretical basis for the alleviation of saline-alkaline soil stress on maize plants in cold climates and appropriate utilisation of cold-region saline-alkaline soil resources. Understanding how these *T. asperellum* treatments will alter the soil microorganism communities is critical if we accurately predict saline-alkaline soil responses and identify approaches for ameliorating the negative effects.

MATERIAL AND METHODS

Experimental site and cultivar. Field trials were conducted during 2015, 2016, and 2017 at the Experimental Station of Heilongjiang Bayi Agricultural University, Daqing (46°37'N, 125°11'E), Heilongjiang province, China. A high-yield maize cultivar NY525, exhibiting saline and alkali tolerance and suitable for local planting, was screened by the laboratory for field experiments at the experimental site during these 3 years. The experimental field contained alkaline meadow soil, which soil pH value, alkaline nitrogen, available potassium, available phosphorus, and organic carbon with 8.39 ± 0.05, 134.70 ± 8.37 mg/kg, 126.80 ± 6.89 mg/kg, 25.69 ± 3.88 mg/kg and 29.42 ± 2.02 g/kg, respectively.

Experimental design. Maize seeds were manually sowed at a planting density of 82 500 plants/ha, with exact 0.7 m line spacing. Planting was carried out on May 20, 2015, May 18, 2016, and May 15, 2017. The planting area was divided into three treatment plots. An area of 56 m^2 with eight ridges (0.7 m × 10 m) was allotted to each treatment group, and a randomised block design with three replicates was used.

For the treatments, 0 (Con), 0.7 (T1), and 1.4 (T2) g of *T. asperellum* conidium powder mixed with 200 mL of water were applied to each seedling in the respective treatment groups. *T. asperellum* (Gene bank accession: KJ541741) was first activated in PDA media 28 ± 2 °C at 185 r/min, and then prepared as a spore suspension (1×10^9 colony forming units/mL), which was then inoculated onto a sterilised solid matrix (1:20, *v*/*w*) and incubated at 28 °C for 10 days. *T. asperellum* inoculum times and root-irrigation method: *T. asperellum* treatments were inoculated into the soil at the roots of maize seedlings in the form of a solution on days 25 following maize seedling emergence. Maize was cultivated in accordance with the regular agronomic practices in the northeast China.

Sampling. The five-point sampling method was used to soil sample from each treatment plot in 2015, 2016, and 2017 when the maize plants were at the vegetative 6^{th} leaf stage (V6), tasseling stage (VT),

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20 days after silking stage (DAS 20), and physiologically mature stage (R6). The rhizosphere soil was shaken off, and 50 g soil was collected as a sample, three replicates for each treatment. A portion of each soil sample was placed in a 50-mL centrifuge tube and stored at –80 °C for soil DNA extraction. The remaining soil was air-dried for analysis of soil nutrients. However, considering that the microbial community differences would become more apparent between treatment groups with an increased number of years exposed to *Trichoderma* application, we determined soil microbial diversity in the rhizosphere soil at VT and R6 in 2017.

Determination of the soil nutrient. The soil pH was determined using a pH meter (Thermo Orion-868; Thermo Orion Co., Waltham, USA). Soil total organic carbon (TOC) was determined by the $K_2Cr_2O_7$-H_2SO_4 digestion method (Bao 2000). Available nitrogen (AN) was extracted with 1 mol/L KCL and analyses using the cadmium reduction method (Bao 2000). Available phosphorus (AP) was extracted with a 0.5 mol/L $NaHCO_3$ solution, adjusted to pH 8.5 (Bao 2000). Available potassium (AK) was extracted with neutral 1 mol/L NH_4OAc (Bao 2000).

High-throughput sequencing analysis of soil bacterial and fungal communities. Total soil genomic DNA was extracted using a Power Soil DNA Isolation Kit (MoBio Laboratories Inc., Carlsbad, USA) following the manufacturer's instructions. The concentration and quality (A260/A280 ratio) of the DNA samples were determined using a NanoDrop 2000 spectrophotometer (Thermo Scientific, Waltham, USA). Pyrosequencing analyses of the 16S rRNA gene and ITS region were performed to determine the diversity and composition of bacterial and fungal communities, respectively. The V3–V4 region of the bacterial 16S rRNA gene was PCR-amplified using primers 338F (5'-ACTCCTACGGGAGGCAGCAG-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3'), while the ITS1 region of the fungal ITS was targeted by the primers ITS1-F (5'-CTTGGTCATTTAGAGGAAGTAA-3') and ITS2R (5'-TGCGTTCTTCATCGATGC-3'). The obtained PCR products were purified using a PCR Purification Kit (Axygen Bio, Union City, USA) and quantified with PicoGreen® dsDNA reagent (Promega, Madison, USA). The purified amplicons were then pooled in equimolar concentrations as a single aliquot and employed for library construction using the NEB Next® Ultra™ DNA Library Prep Kit for Illumina (New England Biolabs, Ipswich, UK). All library preparation was performed on the Illumina MiSeq platform.

The sequences retained for each sample were analysed following the UPARSE pipeline, using USEARCH and Perl scripts to generate a table of operational taxonomic units (OTUs) and to select representative sequences. MOTHUR (version 1.25.1, Michigan, USA) standard operating procedure was employed for further analyses of pyrosequencing data. To correct the sampling effort, the lowest sequencing number of 11 616 and 44 294 sequences was randomly selected per sample and used for further bacterial and fungal community analyses.

Maize yield. To determine the yield, maize ears (14 m^2) from each treatment group were harvested by hand from each plot at crop maturity on September 30, 2015; September 29, 2016, and September 27, 2017. All harvested areas were surrounded by five guard rows. Grain yield and thousand kernel weight were converted to yield using a fixed grain water content of 14%.

Data analysis. The data were organised using Microsoft Excel (Redmond, USA). One-way analysis of variance (ANOVA) and correlation analysis were performed using SPSS 22.0 software (SPSS Inc., Chicago, USA), and tables were generated using Microsoft Office Excel 2010. Sequencing depth was calculated using MOTHUR.

RESULTS AND DISCUSSION

Effects of *T. asperellum* treatment on rhizosphere soil nutrient and maize yield

Our results showed that the *T. asperellum* treatments significantly improved the rhizosphere soil nutrient under different growth stages, compared to the control, with significant year × treatments interaction effects, in addition to total organic carbon ($P < 0.05$) (Table 1). As crop yield acts as a general reflection of soil nutrients, the effects of treatments at different concentrations of *T. asperellum* on soil nutrients must be reflected in changes in crop yield. Compared with the Con treatment, maize yield significantly increased under *T. asperellum* application at different concentrations for three consecutive years, with significant year × treatments interaction effects ($P < 0.05$) (Table 1). These studies indicate, the application of *Trichoderma* increases soil nutrients, and stimulates microbial activity in the soil, damage to maize growth from saline-alkali soil, as well as from continuous cropping, were reduced, all of which contributed to an increase in maize yield in the pres-

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Table 1. Influence of *Trichoderma asperellum* on nutrients concentration of maize rhizosphere soil

Year (Y)	Treatment (T)	TOC (g/kg)	AN	AP	AK	Yield (kg/ha)
			(mg/kg)			
2015	V6-Con	20.74 ± 1.36^{gh}	109.29 ± 4.22^{f}	50.97 ± 2.23^{h}	148.77 ± 25.56^{f}	–
	V6-T1	24.33 ± 2.02^{e}	122.07 ± 2.42^{de}	60.08 ± 6.34^{f}	197.62 ± 20.21^{d}	–
	V6-T2	26.42 ± 3.09^{d}	126.68 ± 9.72^{d}	69.63 ± 5.02^{e}	217.73 ± 14.05^{cd}	–
	VT-Con	25.28 ± 3.47^{de}	125.11 ± 6.53^{de}	76.89 ± 3.97^{d}	157.07 ± 17.56^{f}	–
	VT-T1	31.29 ± 2.61^{b}	147.41 ± 8.83^{b}	82.66 ± 3.41^{bc}	234.66 ± 13.13^{b}	–
	VT-T2	35.23 ± 2.85^{a}	154.93 ± 6.79^{a}	88.22 ± 2.66^{a}	244.13 ± 16.11^{a}	–
	DAS 20-Con	20.22 ± 2.36^{h}	123.46 ± 3.05^{de}	63.39 ± 1.36^{f}	144.10 ± 14.84^{f}	–
	DAS 20-T1	24.64 ± 1.55^{e}	125.24 ± 3.69^{de}	78.24 ± 9.48^{c}	185.19 ± 18.38^{e}	–
	DAS 20-T2	28.64 ± 3.65^{c}	134.70 ± 5.51^{c}	85.52 ± 6.69^{b}	206.10 ± 12.02^{cd}	–
	R6-Con	22.43 ± 5.89^{fg}	118.90 ± 2.89^{e}	55.39 ± 3.06^{g}	140.97 ± 14.02^{f}	9 597.12 ± 284.63^{c}
	R6-T1	27.75 ± 1.32^{c}	137.55 ± 6.83^{c}	71.00 ± 1.14^{e}	202.93 ± 13.00^{d}	10 065.36 ± 28.29^{b}
	R6-T2	32.93 ± 3.74^{b}	147.38 ± 4.91^{b}	76.86 ± 4.42^{d}	225.81 ± 11.72^{b}	10 647.72 ± 283.56^{a}
2016	V6-Con	23.42 ± 1.82^{e}	114.42 ± 3.96^{i}	54.87 ± 1.91^{f}	156.57 ± 11.31^{e}	–
	V6-T1	26.83 ± 1.56^{d}	127.37 ± 2.41^{gh}	68.64 ± 2.73^{e}	238.94 ± 22.72^{b}	–
	V6-T2	29.86 ± 3.11^{bc}	138.57 ± 3.11^{d}	77.53 ± 1.83^{d}	249.84 ± 12.60^{b}	–
	VT-Con	26.35 ± 3.32^{d}	136.04 ± 9.59^{e}	79.70 ± 3.42^{d}	161.00 ± 19.83^{e}	–
	VT-T1	33.27 ± 2.53^{b}	156.02 ± 5.41^{b}	89.27 ± 3.77^{b}	245.64 ± 18.59^{b}	–
	VT-T2	39.56 ± 2.93^{a}	165.00 ± 3.29^{a}	96.76 ± 3.12^{a}	270.12 ± 10.74^{a}	–
	DAS 20-Con	22.54 ± 2.34^{f}	131.33 ± 4.01^{f}	68.04 ± 1.36^{e}	153.21 ± 15.45^{e}	–
	DAS 20-T1	28.16 ± 1.72^{cd}	133.22 ± 3.71^{ef}	83.32 ± 5.32^{c}	194.59 ± 18.51^{d}	–
	DAS 20-T2	31.96 ± 3.00^{b}	142.65 ± 5.61^{d}	92.45 ± 5.15^{b}	215.27 ± 12.02^{c}	–
	R6-Con	23.81 ± 4.94^{e}	124.43 ± 3.08^{h}	56.33 ± 2.79^{f}	144.04 ± 19.83^{e}	9 879.45 ± 98.79^{c}
	R6-T1	31.67 ± 2.33^{b}	149.83 ± 6.16^{c}	79.74 ± 2.04^{c}	216.02 ± 19.05^{c}	10 447.83 ± 205.48^{b}
	R6-T2	36.94 ± 3.73^{a}	161.02 ± 7.56^{b}	89.73 ± 1.89^{b}	243.49 ± 10.50^{b}	11 105.57 ± 228.14^{a}
2017	V6-Con	24.91 ± 1.74^{g}	118.94 ± 7.79^{h}	55.20 ± 1.89^{g}	164.83 ± 18.33^{e}	–
	V6-T1	30.35 ± 1.57^{e}	133.35 ± 2.32^{f}	72.12 ± 3.27^{f}	243.29 ± 14.28^{c}	–
	V6-T2	33.71 ± 2.67^{cd}	143.39 ± 2.80^{d}	82.47 ± 3.04^{e}	267.11 ± 8.22^{b}	–
	VT-Con	27.23 ± 3.36^{g}	137.53 ± 8.03^{ef}	80.83 ± 2.21^{e}	167.18 ± 13.22^{e}	–
	VT-T1	36.56 ± 1.81^{b}	166.87 ± 4.33^{b}	93.82 ± 4.97^{bc}	268.65 ± 14.40^{b}	–
	VT-T2	43.31 ± 1.99^{a}	176.39 ± 4.35^{a}	100.79 ± 5.20^{a}	296.20 ± 8.89^{a}	–
	DAS 20-Con	23.93 ± 1.16^{g}	133.03 ± 3.99^{f}	69.26 ± 0.69^{f}	163.37 ± 16.53^{e}	–
	DAS 20-T1	30.80 ± 1.89^{d}	141.39 ± 5.56^{d}	87.67 ± 2.38^{d}	203.89 ± 10.38^{d}	–
	DAS 20-T2	34.00 ± 2.78^{cd}	153.51 ± 3.44^{c}	96.04 ± 5.54^{b}	229.08 ± 14.8^{4c}	–
	R6-Con	25.11 ± 5.20^{g}	125.54 ± 3.05^{g}	56.61 ± 2.05^{g}	147.02 ± 13.93^{f}	9 907.75 ± 182.14^{c}
	R6-T1	34.55 ± 3.56^{c}	155.95 ± 2.74^{c}	81.56 ± 1.07^{e}	235.78 ± 12.67^{c}	10 885.36 ± 351.98^{b}
	R6-T2	39.70 ± 2.00^{b}	165.10 ± 3.81^{b}	90.60 ± 3.73^{c}	260.95 ± 13.00^{b}	11 914.66 ± 520.07^{a}
ANOVA	Y	**	**	**	**	**
	T	**	**	**	**	**
	Y × T	ns	*	**	*	*

V6 – vegetative 6th leaf stages; VT – tasselling stage; DAS20 – 20 days after silking; R6 – physiologically mature stage; Con – control; T1 – 0.7 g *Trichoderma* treatment; T2 – 1.4 g *Trichoderma* treatment. Values are mean (standard deviation) (n = 5). Values followed by a different small letter within a column are significantly different at the 5% probability level. Differences between treatments were calculated within the different growth stages for each particular year. ns – not significant. *P < 0.05; **P < 0.01

https://doi.org/10.17221/456/2020-PSE

ent study. In previous studies indicate, *Trichoderma* metabolites can inhibit soil-borne pathogens, promote crop growth (Fontenelle et al. 2011).

Venn diagram for soil samples collected from different treatment

Between the VT and R6 periods, a total of 1 377 and 1 372 bacterial and 830 and 723 fungal OTUs, respectively, were retained from all three treatment groups (Figure 1). As shown in the Venn diagram (Figure 1A), 862 OTUs (62.6%) existed in all the growth stages of plants in VT. And 81 OTUs (5.9%) only observed in Con, 45 OTUs (3.3%) in T1, 68 OTUs (4.9%) in T2; 807 OTUs (58.8%) existed in all the growth stages of plants in R6. And 58 OTUs (4.2%) were only observed in Con, 101 OTUs (7.4%) in T1, 60 OTUs (4.4%) in T2. In fungal (Figure 1B), 309 OTUs (37.2%) existed in all the growth stages of plants in VT. And 68 OTUs (8.2%) only observed in Con, 114 OTUs (13.7%) in T1, 86 OTUs (10.4%) in T2, 282 OTUs (39.0%) existed in all the growth stages of plants in R6. And 80 OTUs (11.1%) were only observed in Con, 60 OTUs (8.3%) in T1, 106 OTUs (14.7%) in T2, which indicated that the number of OTUs existing in various developmental stages different with plant maturity.

Bacterial and fungal community composition in soil samples collected from different treatments

Bacterial and fungal genera. In comparing bacterial diversity in treatment *versus* control groups, we found that the relative abundance of *Nitrospira*, *Steroidobacter*, and *Sphingomonas* was greater under different treatment concentrations (Figure 2).

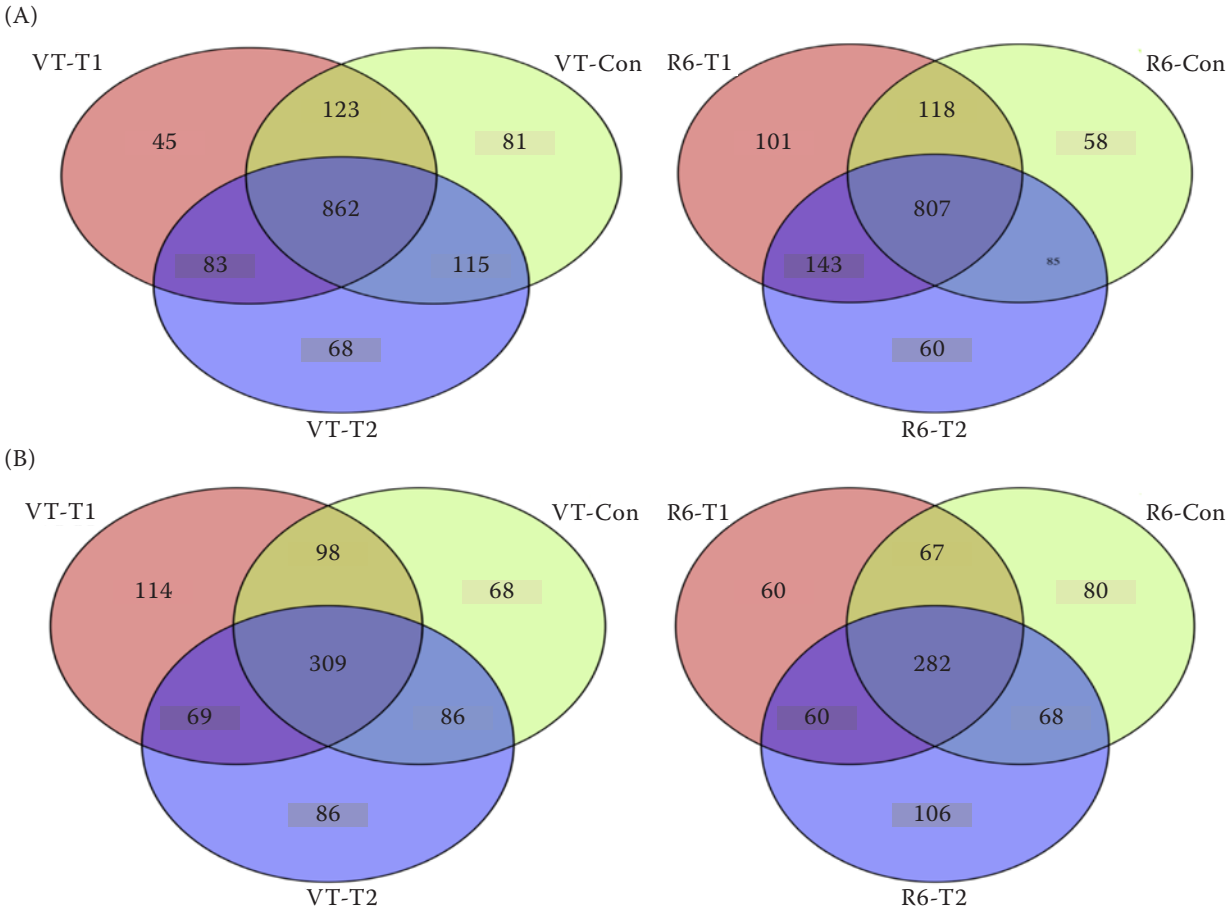

Figure 1. Venn diagram of bacteria and fungi showing the unique and shared operational taxonomic units (OTUs) (97% sequence similarity) under different *Trichoderma* treatment conditions (A – bacteria; B – fungi). VT – vegetative tasseling stage; R6 – physiologically mature stage. Con – control; T1 – 0.7 g *Trichoderma* treatment; T2 – 1.4 g *Trichoderma* treatment

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Under a high *T. asperellum* concentration, the relative abundance of *Nitrospira* was the highest in the VT stage and the lowest in the R6 stage, whereas the opposite condition prevailed under a low treatment concentration. Treatment with a low concentration of *T. asperellum* resulted in a higher relative abundance of *Steroidobacter* and *Sphingomonas* than that under a high-concentration treatment. *Nitrospira* and *Steroidobacter* play key roles in nitrogen metabolism, with the former acting as an integral component of the soil nitrogen cycle (Daims et al. 2015), in addition to producing acidic substances that alleviate soil salinity (Canfora et al. 2014). *Steroidobacter* is vital to soil denitrification (Wang et al. 2017), and *Sphingomonas* can degrade toxic substances in the soil, thereby improving soil quality (Adhikari et al. 2001). The relative abundance of *Haliangium*, which is halophilic, was significantly higher in the control treatment than that under *T. asperellum* treatment. Likewise, *Gemmatimonas*, *Altererythrobacter*, and *Bryobacter* all showed higher relative abundance under the control treatment than under *T. asperellum* in the VT stage, whereas the opposite was true in the R6 stage. *Gemmatimonas* and *Altererythrobacter* influence soil phosphorus metabolism, and *Bryobacter* promote the soil carbon cycle. The presence of *T. asperellum* can therefore promote phosphorus and carbon absorption in growing plants, which reduces the relative abundance of these nutrients, and promote the transformation of insoluble soil elements during the full ripening stage, thus increasing their relative abundance. *Stenotrophomona*, a fungus which we found only under treatment with *T. asperellum*, has the ability to degrade pesticide residues in the soil, while *Blastocatella* can decompose complex organic hydrocarbons and nitrogen-containing substances into small molecular substances (Foesel et al. 2013). Our results show that the application of *T. asperellum* at different concentrations has different effects on the relative abundance of *Blastocatella* during different growth periods. This indicates that different mechanisms are associated with *T. asperellum* at various stages of plant growth, and these require further study.

The comparison of fungal diversity under different treatments showed that the relative abundance of *Trichoderma*, *Mortierella*, *Myrmecridium*, and *Ceratobasidium* increased significantly under the *T. asperellum* treatment (Figure 3). The relative abundance of *Myrmecridium* increased under a high concentration of *T. asperellum* compared to its abun-

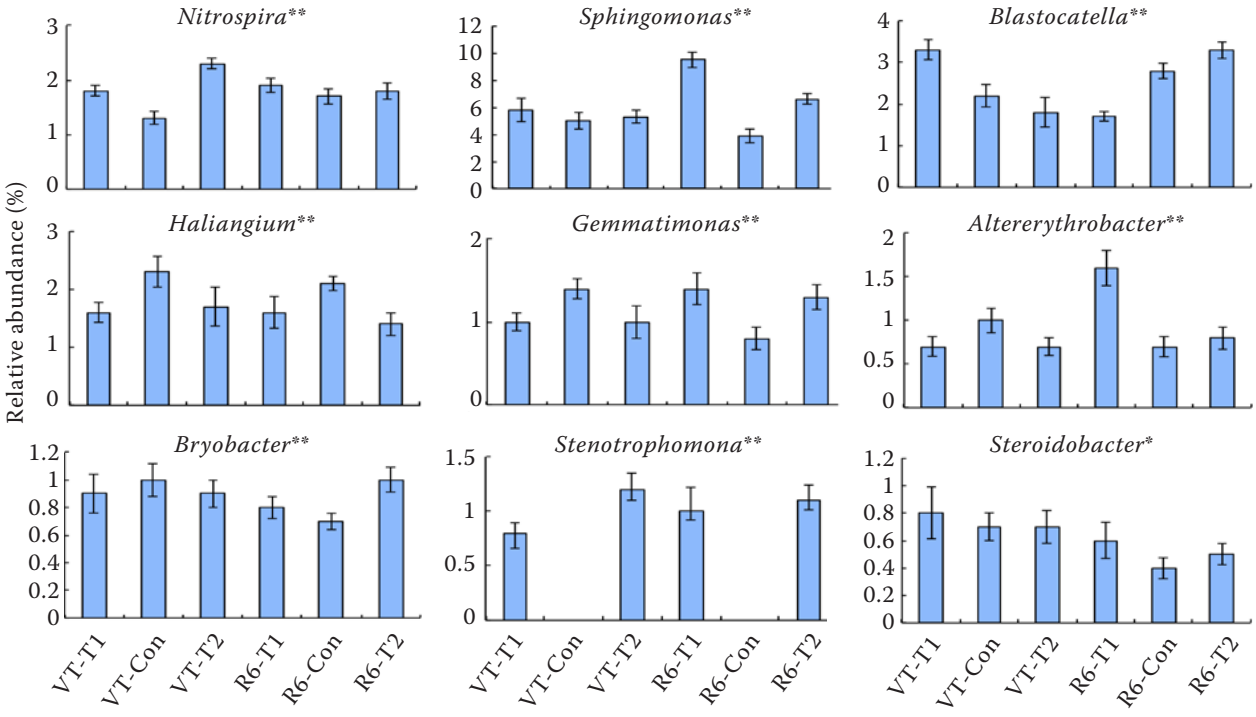

Figure 2. Relative abundances of bacterial abundant genera under *Trichoderma asperellum* treatments. VT – vegetative tasseling stage; R6 – physiologically mature stage. Con – control; T1 – 0.7 g *Trichoderma* treatment; T2 – 1.4 g *Trichoderma* treatment; *$P < 0.05$; **$P < 0.01$

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dance under a low concentration in the VT stage, whereas the relative abundance of *Ceratobasidium* was opposite to that of *Myrmecridium* during the R6 stage. The relative abundance of *Trichoderma* was higher under a high treatment concentration, whereas the relative abundance of *Mortierella* showed the opposite pattern. *Trichoderma* is able to proliferate on the surface of plant roots and in the soil surrounding them, where it stimulates a pathogen resistance response in the host plant and thereby participates in promoting crop growth and tolerance to environmental stress (Harman et al. 2004). *Ceratobasidium* (Basidiomycota) is able to compete with the pathogenic bacteria in the soil for nutrients and living space (Abo-Elyousr et al. 2009). *Trichoderma* spp. can also improve the antioxidant capacity and enable pathogen resistance of plants, thus promoting their growth (Li et al. 2019). *Mortierella* and *Acremonium* are antagonistic plant pathogenic fungi and are therefore crucial in controlling plant diseases (Sathiyabama and Balasubramanian 2018). *Myrmecridium* has a strong protease-producing ability. The pathogenic fungi *Gibberella*, *Neonectria*, and *Fusarium* were significantly higher under *T. asperellum* treatment than under control treatment. It has been found that *Neonectria* fungus contains a number of plant pathogens that are seriously harmful to agriculture and forestry production (Zhuang 2010). *Fusarium* is an important pathogenic soil fungus; *Gibberella* produces toxins that cause serious crop diseases, and *Psathyrella* is a bacterial genus that was unique to the control treatment. We found that the abundance of *Trichoderma* under the control treatment was very low, indicating that the saline-alkali soil in the cold region is unlikely to benefit from the biological control properties of this genus. This shows that the application of *T. asperellum* can effectively inhibit the abundance of pathogenic microorganisms in the rhizosphere of maize in saline-alkali soil in the cold region, and increase the abundance of beneficial microorganisms in the soil, thereby improving the rhizosphere microenvironment and promoting optimum growth and yield in maize.

Relationships between bacterial and fungal communities and soil nutrient

According to previous studies, bacterial and fungal diversity correlates with soil nutrient content

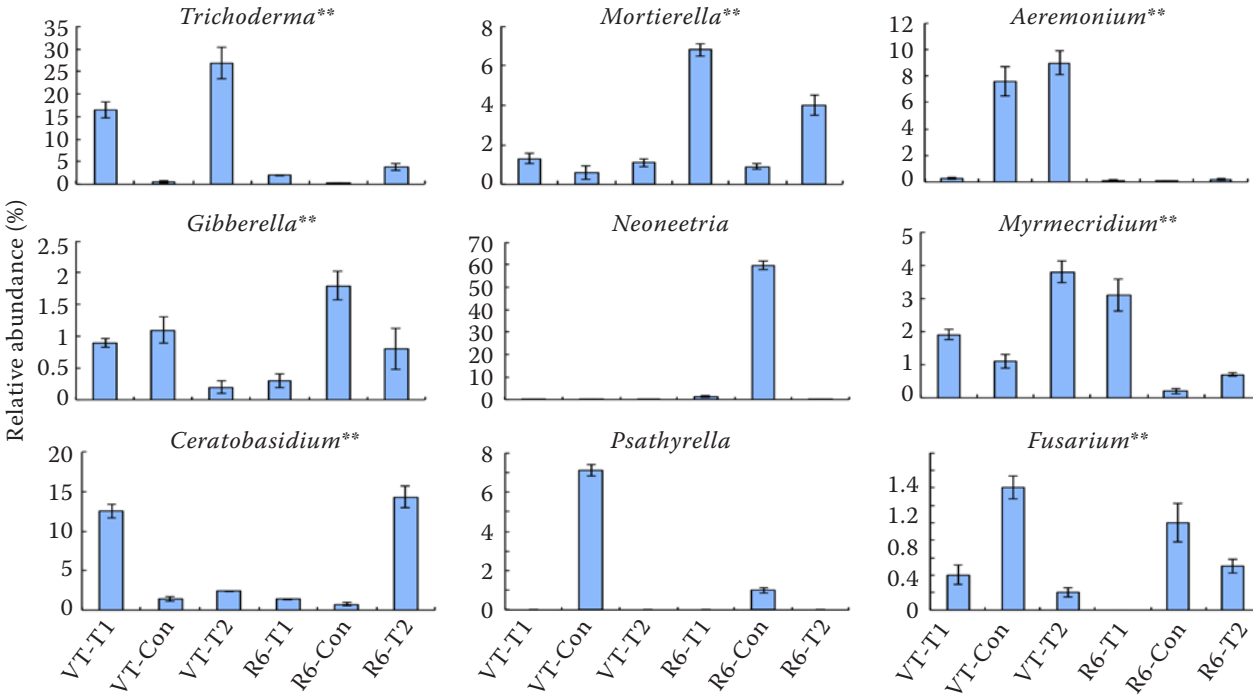

Figure 3. Relative abundances of fungal abundant genera under *Trichoderma asperellum* treatments. VT – vegetative tasseling stage; R6 – physiologically mature stage. Con – control; T1 – 0.7 g *Trichoderma* treatment; T2 – 1.4 g *Trichoderma* treatment; $^{*}P < 0.05$; $^{**}P < 0.01$

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Table 2. Pearson correlations between bacterial and relative fungal abundance of abundant genera and soil nutrient

Genus		TOC	AN	AP	AK	Yield
Bacteria	*Nitrospira*	0.616*	0.72*		0.765*	0.888**
	Sphingomonas	0.603*				0.915**
	Blastocatella					
	Haliangium	–0.941**	–0.794*		–0.826*	–0.778*
	Gemmatimonas	0.839**	0.929**			
	Altererythrobacter					
	Bryobacter			0.674*		
	Stenotrophomona	0.921**	0.928**	0.777*	0.943**	0.849**
	Steroidobacter			0.718*		0.791*
Fungus	*Trichoderma*	0.789*	0.771*	0.738*	0.777*	0.606*
	Mortierella	0.638*				–0.828**
	Acremonium					0.715*
	Gibberella	–0.668*	–0.829*	–0.807*	–0.81*	
	Neonectria		–0.738*	–0.877*		
	Myrmecridium		0.684*	0.645*	0.682*	
	Ceratobasidium	0.581*	0.524*		0.525*	0.771*
	Psathyrella	–0.688*	–0.545*		–0.618*	
	Fusarium	–0.771*	–0.757*		–0.799*	–0.680*

Soil factors indicated include: TOC – total organic carbon; AN – available nitrogen; AP – available phosphorus; AK – available potassium.*$P < 0.05$; **$P < 0.01$

(Rousk et al. 2010, Tedersoo et al. 2015). In the current study, many of the most abundant genera were significantly correlated with these environmental factors, while only the genera *Altererythrobacter* and *Blastocatella* were not (Table 2). The genera *Haliangium*, *Gibberella*, *Neonectria*, *Psathyrella*, *Fusarium*, exhibited a negative correlation with soil nutrient, whereas the others genera exhibited a positive correlation with soil nutrient (Table 2). Indeed, in the current study, levels of soil nutrients increased after *T. asperellum* treatment.

In the present study, the potential mechanisms that allow *T. asperellum* to maintain a better soil microbiome for plant growth in saline-alkali soil can be explained as follows: plant growth might initially be improved by enhanced plant root growth, as reported in our previous study (Fu et al. 2018). Then, better root growth influences soil microbes through the supply of more root exudates or rhizodeposition, and soil microbes, in turn, alter plant performance through higher diverse microflora and more frequent interactions, resulting in more available nutrients. This process continues in a cycle. More bioavailable nutrients cause better root growth, facilitating more microbial colonisation in the rhizosphere, and finally promotes greater nutrient uptake by the inoculated plants.

In conclusion, bioremediation of the diseased soil with the *T. asperellum* effectively suppressed pathogenic soil microbial. The introduction of *T. asperellum* significantly altered the microbial community in the maize rhizosphere soil, enhance soil nutrients and promoted maize plant growth. *T. asperellum* applied at a level of 1.4 g per plant had the greatest yield, suggesting that this treatment should be adopted for application in maize fields in the region. These results provide important insight into the microbial community structure in this distinct ecosystem and identify the major factors shaping the microbial community.

Acknowledgment. We thank the *Trichoderma* research team of the Forestry College of Shenyang Agricultural University.

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Received: September 2, 2020
Accepted: November 16, 2020
Published online: November 25, 2020