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Manure and *Trichoderma harzianum* increase cotton yield via regulating soil bacterial community and physicochemical properties

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ABSTRACT: A field experiment with four treatments (chemical fertilizer alone, CK; manure plus chemical fertilizer, CM; *Trichoderma harzianum* plus chemical fertilizer, CF; *T. harzianum* plus manure plus chemical fertilizer, CMF) was conducted to investigate changes in soil bacterial community and cotton yield in 2020 and 2021. Soil aggregate stability and economic feasibility of the four treatments were evaluated. Manure and *T. harzianum* application significantly improved soil organic carbon (SOC) and total nitrogen and increased soil aggregate stability. Increases of *Proteobacteria* abundance were 31.62% and 23.51% in 2020 and 2021, respectively, while the increases of *Actinobacteria* were increased by 21.65% in 2020 and 19.19% in 2021. Manure effects contributed to 46.15% variation of soil bacterial community while *T. harzianum* contributed to 40.67% variation. Relative abundances of *Acidobacteria* and *Actinobacteria* were negatively correlated with SOC. Differently, *Chloroflexi* was positively correlated with SOC. Concerning the economic feasibility, maximum appreciation was also obtained when manure combined with *T. harzianum*, which was better than sole manure or *T. harzianum*. Co-application of manure and *T. harzianum* improved soil fertility and regulated soil bacterial community which contributed to increases of cotton yield. Thus, it can be applied as a promising measure for promoting cotton production.

KEYWORDS: soil physicochemical properties, soil bacterial community, economic feasibility, cotton yield

INTRODUCTION

Chemical fertilizer has been applied to increase crop yield worldwide, and its application has increased fourfold in recent decades, which would continue to increase by approximately 2% annually in the next several years especially in China [1]. Cotton received an average N rate of 300 kg/ha in China [2], causing serious environmental problems and decrease in crop yield as well as soil quality [3]. To minimize the negative effect of chemical fertilizer, organic fertilizer rich in organic matter and beneficial microbes is widely accepted [4]. Therefore, partial replacement of chemical fertilization with organic materials or functional stain has been introduced to agricultural production [5], which makes it possible to take advantages of both chemical fertilizer and organic materials [6].

Decreases in soil bulk density and increases in soil aggregate stability and porosity after manure application indicated improved soil physical properties [7]. Moreover, manure application could increase soil nutritional levels and elevate soil pH which was reduced by excess chemical fertilization [8]. Additionally, *T. harzianum* could also promote soil nutrient availability, which was considered an effective strategy to manage nutrients in low fertility soil and to reduce the use of chemical fertilizer [9, 10]. Playing a vital role in soil ecosystem, soil microorganisms could affect nutrient decomposition and sequestration as well as crop growth. It has been reported that manure and *T. harzianum* could regulate soil microbial community composition and meliorate soil microenvironment in different regions and soil types [11]. Long-term manure application increased abundances of *Terrisporobacter* and *Clostridium butyricum* [11] but decreased abundance of ammonia-oxidizing archaea (AOA) [12]. Similarly, the probiotic application of *T. harzianum* in the rhizhosphere soil could also impact population dynamics of soil bacteria, archaea, and eukaryotes [13], indicating changes in soil microbial community composition [14].

Studies have demonstrated that combination of chemical fertilizer and organic material or functional strain is an effective measure to increase crop yield via promoting soil quality [6, 15, 16]. Zhu et al [5] also showed that chemical fertilizer partially substituted by biofertilizer (rich in *T. harzianum*) significantly improved soil structure and increased carbon sequestration. Organic fertilizer could regulate cotton growth and promote soil available nutrients, which contribute to increases in cotton yields. Obviously, after colonization of *T. harzianum*, potassium manganate accumulated in crop and soybean yield were also elevated [9]. Thus, increases in crop yield induced by fertilization can be attributed to changes in soil nutrients as well as micro-ecology.

However, effect of co-application of manure and *T. harzianum* in the cotton field is not well evaluated.

Table 1 The amounts of fertilizer nutrient input for each treatment.

Treatment[†]	Chemical fertilizer nutrient (kg/ha)			Manure nutrient (kg/ha)			*T. harzianum* (kg/ha)
	N	P	K	N	P	K	
CK	220	105	105	0	0	0	0
CM	110	50	63.75	110	55	41.25	0
CF	220	105	105	0	0	0	15
CMF	110	50	63.75	110	55	41.25	15

[†] CK, chemical fertilizer alone; CM, chemical fertilizer + manure; CF, chemical fertilizer + *T. harzianum*; CMF, chemical fertilizer + manure + *T. harzianum*.

The interaction effect of manure and *T. harzianum* on soil microbial community remains unclear. Additionally, the North China Plain, the largest and most important agricultural area in China, is a major production area for cotton accounting for about 31% of China's total [17]. Thus, based on a positioning experiment on the North China Plain, this study was conducted to determine effects of manure and *T. harzianum* on cotton yield, soil physicochemical properties, and bacterial community in a long-term field experiment. We hypothesized that co-application of manure and *T. harzianum* can increase cotton yield via regulating soil bacterial community composition and improving soil physicochemical properties.

MATERIAL AND METHODS

Experimental site and location

A field experiment was established in 2016 in Xunxian County (114°40′ E, 34°40′ N; 72.3 m above mean sea level), Henan Province, China. Wheat-cotton succession is the typical cropping patterns in this region. The site is located in a region with a mean annual temperature of 13.7 °C and an average precipitation of 648 mm in the last 50 years. Approximately 60% of the rainfall is concentrated from June to August each year, which could meet the water requirement of cotton growth. Classified as Fluvo-Aquic soil, the soil is calcareous with a pH of 7.88, and it is a typical soil in the region with a profile of sandy loams with a texture of 15% sand, 75% silt, and 10% clay, containing 9.5 g/kg organic carbon, 1.1 g/kg total nitrogen, 15.9 mg/kg Olsen-phosphorus (P), and 109.1 mg/kg anmonium acetate-potassium (K) within the top 20 cm layer.

Experimental design

The field experiment was laid out in a randomized complete block design in 3 replicates of 60 m^2 (10 m × 6 m) plots. All the plots were under a pattern of winter wheat (*Triticumaestivum* L.) and summer cotton (*Gossypiumhirsutum* L.) for each experimental year, which was set following "three rows of wheat and one row of cotton". The wheat and cotton varieties were Aikang 58 and Lumian 2387, respectively. The cotton hybrid was sowed on 15 May 2020 and 13 May 2021 at densities of 5.25×10^4 plants/ha, harvested on 24 October 2020 and 27 October 2021, respectively. Three seeds were put per hole, and seedlings were thinned to the given densities at the three-leaf stage. Four treatments included chemical fertilizer (CK), chemical fertilizer plus organic manure (CM), chemical fertilizer plus *T. harzianum* (CF), and chemical fertilizer plus organic manure plus *T. harzianum* (CMF). Chemical fertilizers were once applied at the bud stage each year in the forms of urea (46% N), super phosphate (12% P_2O_5), and potassium chloride (45% K_2O). Organic manure was applied in the form of cow manure, which had an average content of 320 g/kg organic carbon, 4.0 g/kg total N, 2.0 g/kg total P, and 1.5 g/kg total K. Manure provided 110 kg N/ha, and contents of P and K were deducted and supplemented with corresponding chemical fertilizer (Table 1). *T. harzianum*, isolated from the soybean root rhizosphere, was applied at a rate of 15 kg/ha in the corresponding treatment. The *T. harzianum* powder, containing effective live fungi more than 2×10^9 cfu/g, was prepared by solid-state fermentation and low-temperature drying. Chemical fertilizer and organic manure were spread evenly on the surface of corresponding plot and thoroughly mixed with the top 20 cm layer by rotary cultivator, a week before cotton sowing. *T. harzianum* powder was spread in strip trenches near cotton and immediately covered, one week after cotton sowing. The other managements were conducted according to the local practice. The details of fertilizer managements were listed in the Table 1.

Plant sampling

Cotton biomass was determined at the boll opening stage. Five successive plants at each plot were harvested in October 2020 and 2021 by hand. For experimental years, the boll weight and boll numbers per plant were determined. Bolls and seeds were dried to a constant weight in an oven at 75 °C and weighed after a quick cell killing at 105 °C for 30 min. The lint was weighed after air-dried, and the lint percentage was calculated by lint yield and total yield (lint yield plus seed yield).

Soil sampling and analysis

Soil samples were collected after cotton harvest from 3 plots in each plot in 0–20 cm layer. The subsamples collected from the same plot were homogenized and placed in plastic bags after removing visible materials. Then, the soil samples were transported to laboratory, stored in a cooler, and divided into 2 sets: one set stored at 4 °C and used to assess soil basic properties and another set stored at −80 °C for soil DNA extraction.

Soil organic carbon (SOC) was determined by chemical oxidation as reported by Mebius [18]. Soil total nitrogen (TN) was assessed by the Kjeldahl method using an automatic Kjeltec 2300 analyzer unit (FOSS, Hoeganaes, Sweden). The available P (AP) was extracted with 0.5 mol/l sodium bicarbonate solution at pH 8.5 and measured with a colorimetric method [19]. Available K (AK) was extracted with 1.0 mol/l ammonium acetate solution (pH 7.0) and determined with flame photometer. Soil aggregate was determined by wet sieving method according to Zhu et al [5]. Namely, subsamples of 300 g were shaken on a Motorized Vibratory Sieve-Shaker for 3 min with a mesh size of 2, 0.25, and 0.053 mm to obtain 3 size fractions: > 2, 0.25–2, 0.053–0.25, and < 0.25 mm.

Soil DNA was extracted with a Power Soil DNA Isolation Kit (MoBio Laboratories Inc., Carlsbad, USA) according to Wang et al [20]. The qualified DNA was stored at −20 °C until analysis. The V3–V4 regions of bacterial 16S rDNA gene were amplified using the universal primers 347F (5′-GGAGGCAGCAGTRRGGAAT-3′) and 531R (5′-CTNYGTMTTACCGCGGCTGC-3′). Amplification was performed with a thermal profile, which included a predenaturation at 95 °C for 3 min, followed by 30 cycles of 3 s (95 °C), 30 s (60 °C), and 45 s (75 °C), with a final extension for 10 min at 72 °C. Then, the specificity of amplicons was determined with 1% (w/v) gels, and the PCR products were sequenced by Illumina MiSeq (Illumina Inc., California, USA). The sequencing data were processed using MOTHUR 1.29.2. Unoise 3 was used to separate the operational taxonomic units (OTUs) at a similarity threshold of 97%. A representative sequence was selected from each OTU, and the Ribosomal Database Project (RDP) classifier was used to assign taxonomic information. The remaining high-quality chimera-free sequences were used for downstream analysis. The Chao index (richness) was calculated using Mothur software package (version 1.29.2) and the "summary. single" command.

Statistical analysis

Normality of all the data was evaluated using the Kolmogorov-Smirnov normality test. The data was expressed as means of 3 replicates. Two-way analysis of variance (ANOVA) by applying organic manure and *T. harzianum* as 2 fixed factors was applied to evaluate treatment effects on the tested parameters within different experiment years. Difference between treatments were considered significant compared to the least significant difference at $p < 0.05$. The α diversity and β diversity of soil microbial community were calculated with Mothur software package and Quantitative Insights Into Microbial Ecology (QIME) software, respectively. Permutational multivariate analysis of variance (PERMANOVA) was used to separate and quantitatively evaluate effects of fertilization and sampling years on soil bacterial community structure using the "adonis" function in the "vegan" package of R. The Pearson correlations between microbial diversity and environmental factors were calculated using Hmisc package in R software, and a correlation matrix was established. The correlation heatmap was drawn with (Toolkit for Biologists integrating various biological data-handling (TB) tools (http://ww.tbtools.com).

RESULTS

Cotton yields

Manure and *T. harzianum* significantly affected cotton yield and its components except for lint percentage, and sampling year significantly affected seed yield and lint yield. The synergistic effect of manure and *T. harzianum* on seed yield was significant. The triple interaction of manure, *T. harzianum*, and sampling year significantly affected bolls, boll weight, and lint yield (Table 2). CMF achieved more bolls and boll weight than CK across 2 sampling years. CM and CF showed no difference in both years, but both increased bolls and boll weight by 7.59%–14.92% and 1.94%–2.65%, respectively, relative to CK. Combined manure with *T. harzianum*, CMF increased seed and lint yields over CK in the 2 years. Compared with CK, no difference was observed between CM and CF, but both increased seed and lint yields in both years. The Lint percentage was unaffected by manure or *T. harzianum* alone in 2020 but significantly increased by 1.88% and 1.66% in CM and CF, respectively, in 2021. CMF treatment significantly increased lint percentage over CK by 1.79% and 3.34% in 2020 and 2021, respectively.

Soil aggregation and chemical properties

Soil aggregate distribution was significantly affected by manure and *T. harzianum*, while sampling year slightly affected the quantity of aggregate (Table 3). The synergistic effect of manure and *T. harzianum* on 0.25–2 mm ($p < 0.01$) and 0.053–0.25 mm ($p < 0.001$) were also significant. However, only 0.053–0.25 mm aggregate was affected by the triple interaction of manure, *T. harzianum*, and sampling year (Table 3). Large macro-aggregates (> 2 mm) accounted for 23.60–31.39 g/100 g of the total water stable aggregates and increased significantly in the 3 treatments compared with CK. There was no difference of large macro-aggregate in CM and CF relative to CK, while increases over CK were observed in CMF in both years. Small macro-aggregate (0.25–2 mm) were significantly increased in CM, CF, and CMF over CK in both years, while no difference was recorded among the 3 treatments. Differently, micro-aggregate (0.053–0.25 mm) was significantly decreased in CM, CF, and CMF compared with CK. Dominated the soil water stable aggregate, silt (< 0.053 mm) accounted for 32.02–44.10% in the two years. CK had the largest proportion of silt (43.80% and 44.10% in 2020 and

Table 2 Effects of manure and *T. harzianum* on cotton yield and its components in 2020 and 2021.

Year	Treatment	Boll (m^{-2})	Boll weight (g)	Lint percentage (%)	Seed yield (kg/ha)	Lint yield (kg/ha)
2020	CK	70.27 ± 1.90^{c}	5.11 ± 0.05^{c}	42.52 ± 0.40^{b}	2662.64 ± 4.06^{c}	1132.05 ± 10.08^{c}
	CM	76.04 ± 0.59^{b}	5.22 ± 0.03^{b}	42.55 ± 0.39^{b}	2795.52 ± 7.05^{b}	1189.47 ± 8.83^{b}
	CF	76.38 ± 0.45^{b}	5.24 ± 0.01^{b}	42.61 ± 0.43^{b}	2812.84 ± 21.67^{b}	1198.56 ± 2.95^{b}
	CMF	79.40 ± 0.82^{a}	5.39 ± 0.03^{a}	43.28 ± 0.83^{a}	2926.39 ± 10.66^{a}	1266.41 ± 22.02^{a}
2021	CK	72.18 ± 1.53^{c}	5.16 ± 0.03^{c}	42.36 ± 0.09^{c}	2762.69 ± 4.16^{c}	1170.33 ± 2.02^{c}
	CM	77.66 ± 1.07^{b}	5.26 ± 0.02^{b}	43.16 ± 0.29^{b}	2904.93 ± 11.55^{b}	1253.74 ± 9.30^{b}
	CF	78.39 ± 1.42^{b}	5.30 ± 0.02^{b}	43.07 ± 0.27^{b}	2905.62 ± 23.13^{b}	1251.29 ± 8.84^{b}
	CMF	82.95 ± 1.05^{a}	5.47 ± 0.05^{a}	43.78 ± 0.30^{a}	3060.71 ± 45.23^{a}	1339.84 ± 11.30^{a}
Source of variation						
Manure (M)		93.26^{***}	101.89^{***}	3.58	262.39^{***}	83.07^{***}
T. harzianum (T)		115.43^{***}	150.47^{***}	3.35	299.31^{***}	90.66^{***}
Year (Y)		2.07	1.71	1.33	6.41^{*}	5.62^{*}
M × T		3.52	3.67	0.22	0.04^{*}	0.23
M × T × Y		6.02^{**}	5.54^{**}	0.63	43.16	12.95^{***}

Different letters within a column in the same year indicate significant difference ($p < 0.05$). * indicates significant difference at $p < 0.05$, ** indicates significant difference at $p < 0.01$ and *** indicates significant difference at $p < 0.001$. CK, CM, CF, and CMF are defined as in Table 1.

Table 3 Distribution of soil aggregates among different treatments in 2020 and 2021.

Year	Treatment	Quantity of soil water stable aggregate (g/100 g)				MWD (mm)
		>2 mm	0.25–2 mm	0.053–0.25 mm	<0.053 mm	
2020	CK	23.60 ± 2.04^{b}	24.62 ± 0.27^{b}	7.99 ± 0.18^{a}	43.80 ± 1.96^{a}	1.72 ± 0.12^{c}
	CM	26.48 ± 2.07^{b}	29.21 ± 1.32^{a}	5.97 ± 0.42^{b}	38.33 ± 0.63^{b}	1.94 ± 0.11^{bc}
	CF	27.56 ± 2.06^{ab}	28.83 ± 1.17^{a}	5.42 ± 0.26^{b}	38.18 ± 1.28^{b}	2.00 ± 0.13^{b}
	CMF	31.39 ± 2.56^{a}	30.91 ± 1.21^{a}	5.68 ± 0.62^{b}	32.02 ± 2.36^{c}	2.25 ± 0.15^{a}
2021	CK	26.70 ± 0.95^{b}	23.06 ± 2.43^{b}	6.14 ± 0.17^{a}	44.10 ± 1.65^{a}	1.88 ± 0.03^{c}
	CM	28.28 ± 1.19^{b}	30.20 ± 0.09^{a}	5.74 ± 0.10^{b}	35.77 ± 1.21^{b}	2.05 ± 0.07^{b}
	CF	28.51 ± 1.62^{ab}	29.64 ± 1.29^{a}	5.49 ± 0.12^{c}	36.36 ± 2.22^{b}	2.06 ± 0.10^{b}
	CMF	29.71 ± 0.42^{a}	31.86 ± 1.20^{a}	5.41 ± 0.45^{c}	30.01 ± 1.58^{c}	2.34 ± 0.04^{a}
Source of variation						
Manure (M)		19.27^{***}	56.58^{***}	16.43^{**}	64.37^{***}	29.12^{***}
T. harzianum (T)		28.20^{***}	44.09^{***}	48.58^{***}	60.20^{***}	37.69^{***}
Year (Y)		6.37^{*}	0.32	17.23^{**}	3.45	6.86^{*}
M × T		1.56	12.19^{**}	22.27^{***}	0.15	0.67
M × T × Y		0.43	1.35	9.77^{**}	0.59	0.22

MWD, mean weight diameter. Values are means ± standard deviation ($n = 3$); different letters followed by values within a column in the same year indicate significant difference at $p < 0.05$. * indicates significant difference at $p < 0.05$, ** indicates significant difference at $p < 0.01$ and *** indicates significant difference at $p < 0.001$. CK, CM, CF, and CMF are defined as in the Table 1.

2021, respectively) while CMF shared the lowest value (32.02 and 30.01 g/100 g in 2020 and 2021, respectively). There was no difference between CM and CF in the silt proportion, but both decreased by 12.48% and 12.82% compared with CK in 2020, respectively. CMF significantly decreased the silt proportion by 31.94% in 2021. Compared with CK, CF and CMF significantly increased soil aggregate stability, i.e. MWD, which was increased by 16.30% and 30.97% in 2020, respectively. Differently, compared with CK, CM and CMF significantly increased MWD by 9.56% and 24.18%, respectively, in 2021, while no difference was observed between CF and CM.

The application of manure and *T. harzianum* had significant effects on soil fertility parameters; sampling year also affected the soil fertility except for TN. Similarly, TN was not affected by the synergistic effect of manure and *T. harzianum* or the triple interaction of the 3 factors (Fig. 1). Compared to CK, CM and CF led to small but significant increases on SOC and TN in both years. In CMF, the highest SOC and TN were recorded with the increases of both by 16.49%–17.99% and 15.86%–19.47% in 2020 and 2021, respectively, compared to CK. No difference was recorded between

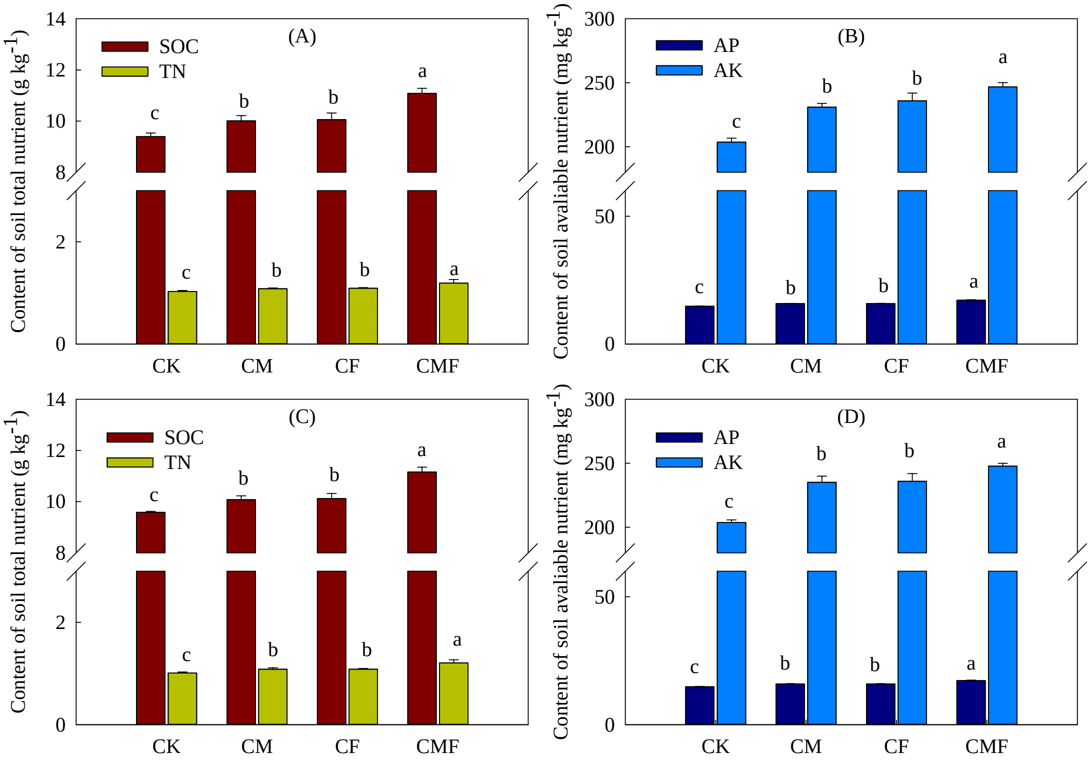

Fig. 1 Influence of different treatments on soil fertility parameters in 2020 and 2021. (A) and (C) content of soil organic carbon (SOC) and total nitrogen (TN) in 2020 and 2021, respectively. (B) and (D) content of soil available P (AP) and available K (AK) in 2020 and 2021, respectively. Different lowercase letters above the bars within an index indicate significant difference at $p < 0.05$. CK, CM, CF, and CMF are defined as in Table 1.

CM and CF, but both significantly increased available P over CK in both years. Significant increases of 16.01% in 2020 and 16.68% in 2021 were recorded for available P in CMF compared with CK. Similarly, CM, CF, and CMF significantly increased available K in both years relative to CK.

Soil bacterial community

The soil bacterial community structure changed significantly when soil was applied with manure and *T. harzianum* in different experimental years (Fig. 2A). The first two principal component analysis (PCA) axes explained 60.3% and 26.0% of the total variation, respectively. Additionally, PERMANOVA was carried out to quantify the relative contributions of manure and *T. harzianum* on soil bacterial community structure (Fig. 2B). Difference of soil bacterial community structure could be explained by manure and *T. harzianum* but not their interaction effects, of which manure exhibited 46.15% variation of bacterial community structure ($p < 0.001$) followed by sampling *T. harzianum* (40.67%, $p < 0.001$).

The principal bacterial phylum in different treatments was similar each year. The top 10 phyla that were higher than 1% relative abundance in both years were shown in Fig. 3. *Proteobacteria* dominated the soil bacterial community regardless of treatments and sampling years with the abundance in an order of CMF>CM>CF>CK. The increases of *Proteobacteria* abundance across treatments were 31.62%–38.73% and 23.51%–35.41% in 2020 and 2021, respectively. *Actinobacteria*, as the third largest bacteria, showed a similar trend to *Proteobacteria* in both years. Though abundance of *Acidobacteria* showed no difference among treatments, the ratio of *Proteobacteria*/*Acidobacteria* was significantly increased after manure and/or *T. harzianum* introduction, and it was highest in CMF treatment in both years (1.78 and 1.77 in 2020 and 2021, respectively). Similarly, abundance of *Actinobacteria* was significantly increased in both years. However, abundances of *Chloroflexi*, *Bacteroidetes*, and *Firmicutes* were significantly decreased in CM, CF, and CMF treatments relative to CK. The relationship was employed to evaluate relationships between basic soil properties and abundant bacterial phyla (Fig. S1). Relative abundances of *Acidobacteria* and *Actinobacteria* were negatively correlated with SOC. Differently, *Chloroflexi* was positively correlated with SOC. *Chloroflexi* was positively correlated with SOC and TN. *Firmicutes* was negatively correlated

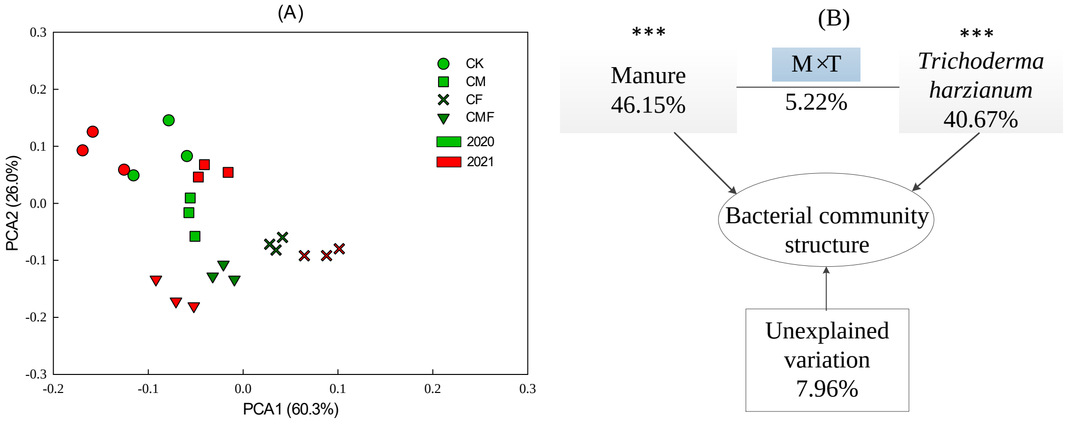

Fig. 2 Soil bacterial community structure of soil treated with different treatments. (A) Principal component analysis (PCA) of the soil bacterial data for different treatments in two experimental years. (B) Permutational multivariate analysis of variance (PERMANOVA) comparing effects of manure and *T. harzianum* on soil bacterial community. The letters M and T indicate manure and *T. harzianum*, respectively. *** indicates significant differences at $p < 0.001$. CK, CM, CF, and CMF are defined as in Table 1.

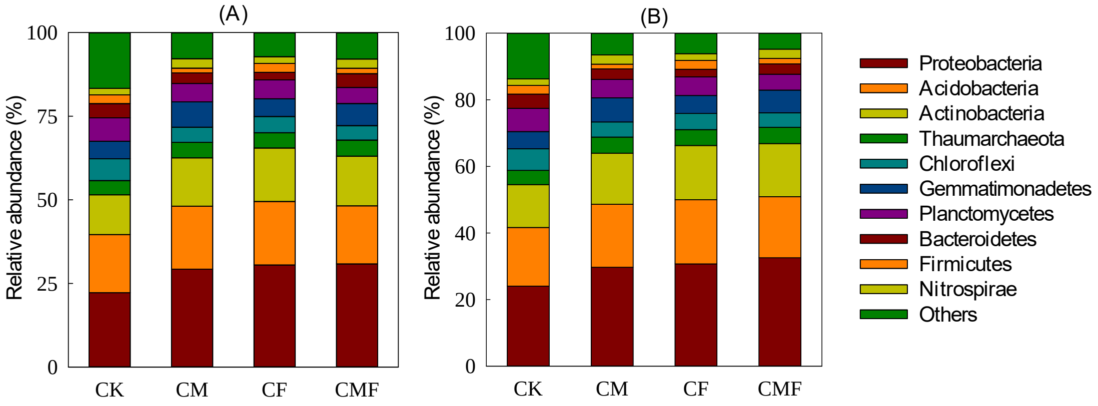

Fig. 3 Soil bacterial community composition at phylum level in 2020 (A) and 2021 (B). CK, CM, CF, and CMF are defined as in Table 1.

with soil microbial biomass carbon. Generally, relative abundances of *Acidobacteria*, *Actinobacteria*, *Chloroflexi*, and *Firmicutes* were positively or negatively correlated with basic soil properties.

Economic feasibility

Since the irrigation and weed and pest control across treatments were identical, the costs of different treatments only included fertilization. The average costs of urea, super phosphate, and potassium chloride used in this study were 1.8, 1.5, and 2.8 yuan/kg, respectively, and average costs of manure and *T. harzianum* were 0.5 and 20 yuan/kg, respectively. The average cotton (lint and seed) price was 7.0 yuan/kg. Considering the average cost and income, economic benefits among treatments in the 2 experimental years were listed in Table 4. The output values of each treatment were significantly increased, which ranged from 5.02% to 10.49% in 2020 and ranged from 5.74% to 11.89% in 2021. As for the economic benefit, the most value was recorded in CMF in 2021, and the lowest was recorded in CK in 2021. The appreciations in CM, CF, and CMF were 3508.99, 1377.03, and 4823.64 yuan/ha over CK in 2020, respectively. However, higher appreciation in each treatment in 2021 was observed with the highest appreciation of 5309.51 yuan/ha.

DISCUSSION

Effects of manure and *T. harzianum* on soil physicochemical properties

Manure and *T. harzianum* increased the accumulation of organic C in soil due to large amounts of C introduction and returning to soil. Liu et al [6] showed that crop yield tended to increase with increasing of soil organic matter, which was verified by our results. These findings confirmed that organic matter in the

Table 4 Effects of different treatments on the economic benefit in 2020 and 2021.

Year	Treatment	Input (yuan/ha)	Output (yuan/ha)	Economic benefit (yuan/ha)	Appreciation (yuan/ha)
2020	CK	4654.27	26562.78^c	21908.52^d	–
	CM	2477.42	27894.93^b	25417.51^b	3508.99^b
	CF	4794.27	28079.85^b	23285.58^c	1377.06^c
	CMF	2617.42	29349.58^a	26732.16^a	4823.64^a
2021	CK	4654.27	27531.14^c	22876.87^d	–
	CM	2477.42	29110.69^b	26633.27^b	3756.40^b
	CF	4794.27	29098.35^b	24304.08^c	1427.21^c
	CMF	2617.42	30803.80^a	28186.38^a	5309.51^a

Different letters within a column in the same year indicate significant difference ($p < 0.05$). * indicates significant difference at $p < 0.05$, and *** indicates significant difference at $p < 0.001$. CK, CM, CF, and CMF are defined as in Table 1.

soil might be a vital factor in regulating crop yield. In the present study, both manure and *T. harzianum* application significantly increased TN, AK and AP, while increases of nutrients were more significant in soil receiving both additions. These results indicated that increases of soil nutrients would be essential for crop production. Cai et al [24] also demonstrated increases in soil TN, AK and AP, which were beneficial to increase crop yield. Previous studies confirmed that chemical fertilizer had no significant effect on soil TN and soil nutrients might be more affected by manure application and *T. harzianum* introduction which contributed to high and stable crop yields [6, 9]. Moreover, *Trichoderma* could enhance iron solubility especially P availability in soil [22, 25], while increasing of soil available P and K could well increase crop yield [26]. Manure application and *T. harzianum* might promote the positive effects by increasing soil pH compared with chemical fertilization [10, 24].

Long-term application of chemical fertilizer might be harmful for macro-aggregate formation, while manure and *T. harzianum* had positive effects on soil aggregation [5, 27]. The highest value of mean weight diameter (MWD) was observed in treatment with co-application of manure and *T. harzianum* in the present study relative to the control. Manure contained abundant nutrients and binding agents that could increase soil fertility and improve soil structure [24, 27]. Macro-aggregates are formed by binding of micro-aggregates through roots or other organic acids generated by decomposition processes of organic materials [28, 29]. The abundant polyvalent metal cations in manure also promoted the formation of macro-aggregates [30]. It has been demonstrated that fungal preparation enhanced soil aggregation [31] and hyphal development beyond the rhizosphere (particularly by mycorrhizal fungi) provided nutrients for microorganisms, thereby promoting microorganism development and soil aggregate stability. The improved soil structure allowed soil to retain more water and nutrients, which in turn contributed to crop productivity [22]. The above results clearly highlighted the importance of manure and *T. harzianum* in promoting soil quality which is an important reason for higher cotton yield. In the present study, total and available nutrients as well as soil aggregate distribution were significantly improved in CM, CF, and CMF treatments relative to CK, indicating that manure and/or *T. harzianum* could be used to comprehensively improve soil environment in terms of soil physicochemical properties, which helped to promote crop yield.

Effects of manure and *T. harzianum* on soil bacterial traits

Soil microbes could increase soil nutrient availability and promote crop growth and productivity by improving soil fertility, of which soil bacterial diversity and abundance had been demonstrated to be essential for achieving stable soil productivity and soil ecological balance. However, soil bacterial traits were often regulated by different fertilization. Generally, chemical fertilization was harmful to soil bacterial diversity [32], whereas organic fertilization contributed to higher bacterial diversity [33]. It has been found that soil nutrient concentrations were responsible for alterations in soil bacterial abundance and diversity. SOC could stimulate growth of soil microorganisms and caused higher soil bacterial abundance and diversity [6], which was verified by our results of positive correlations between SOC with soil bacterial phyla (Fig. S1).

Though the structure of soil microbial community was sensitive to fertilization, the most frequent phyla were *Proteobacteria*, *Chloroflexi*, *Acidobacteria*, *Actinobacteria*, and *Bacteroidetes* in agricultural soils [33]. High abundances of *Proteobacteria* and *Acidobacteria* could promote cycling of essential nutrients, which were beneficial to improving soil fertility for sustainable agriculture. *Proteobacteria* were generally adapted to an environment with abundant nutrients [34], whereas *Acidobacteria* were generally considered to prefer oligotrophic environments with limited nutrients [33]. Clearly, soil bacterial community shifted considerably because of manure and/or *T. harzianum* application. Clear distinction was also observed among treatments and sampling years, which agreed with previous studies [20]. In the present study, composition of soil bacterial community was detected at the phylum level, and the predominant phylum *Proteobacteria* was increased by manure and *T. harzianum* application. Additionally, ratios of *Proteobacteria*/*Acidobacteria* in treatments applied with manure and/or *T. harzianum* were significantly larger relative to CK, especially in CMF. These results indicated that a preferred habitat with an intermediate level between the copiotrophic and oligotrophic conditions for soil bacteria after manure and *T. harzianum* application was achieved. It was known that *Actinobacteria* were functionally diverse and contributed to decomposition of organic matter [35]. Increases of *Actinobacteria* in the current study indicated that manure and *T. harzianum* application might contribute to nutrient cycling in soil. Numerous previous studies have found that SOC, pH, and N availability all contributed to changes in soil bacterial community [20], which was supported by our result. Moreover, soil microbial biomass carbon was also closely correlated with soil bacterial community since it was vital for soil C cycling and could affect soil C mineralization processes [36]. The highest abundance of bacterial population relative to the control and the improved soil physicochemical properties might explain positive effects of manure and *T. harzianum* on cotton production.

Effects of manure and *T. harzianum* on cotton yield and economic feasibility

In the present study, significantly positive effects of manure and *T. harzianum* on cotton yield were recorded. Co-application of manure and *T. harzianum* enhanced cotton yield more than either alone across experiment years. This might be due to the synergistic effect of manure and *T. harzianum* exerting a considerable promotion over the control. The results indicated that manure and *T. harzianum* are important factors affecting cotton yield, which were consistent with previous findings [21]. Manure directly added nutrients in soil and increased nutrient bioavailability, whereas *Trichoderma* has been reported to increase nutrient availability, uptake, and assimilation in crops. *Trichoderma* spp. may also promote plant growth and crop yield by improving the root system architecture and nutrition absorption [22]. Therefore, the co-application of manure and *T. harzianum* might have synergistically facilitated cotton growth and then would be beneficial for achieving higher cotton yield. Additionally, various proteins associated with photosynthesis enhanced by *Trichoderma* would increase the photosynthesis ability of plants, leading to higher cotton yield [22, 23].

It is important to assess large economic benefits with sustainable agricultural production. In this study, manure and/or *T. harzianum* introduction increased the total output in cotton production in both experimental years. Co-application of manure and *T. harzianum* (CMF treatment) produced the maximum economic benefit for cotton production in this region, which might be suitable for most smallholder farmers. Since manure combined with chemical fertilizer (CM treatment) could also achieve better economic benefit, most farmers might first choose this practice, for it was more labor-saving and convenient. However, given the fact that Chinese government emphasizes the importance of sustainable production and soil fertility improvement in agriculture [37], co-application of manure and *T. harzianum* in cotton production will not only increase economic benefits, but also contribute greatly to improvements in soil quality and agricultural productivity.

CONCLUSION

Manure and/or *T. harzianum* introduction significantly increased cotton yield and improved soil physicochemical properties as well as soil bacterial traits compared with soil applied with chemical fertilizer alone across years. The soil bacterial community shifted as a result of manure and *T. harzianum* application, and a distinction was observed among treatments and across years. The predominant phylum *Proteobacteria* was increased by manure and *T. harzianum* application. Additionally, ratios of *Proteobacteria*/*Acidobacteria* in treatments applied with manure and/or *T. harzianum* were significantly larger relative to CK, especially in CMF. The findings suggested that co-application of manure and *T. harzianum* might be a feasible practice from perspectives of economic and ecological benefits.

Appendix A. Supplementary data

Supplementary data associated with this article can be found at http://dx.doi.org/10.2306/scienceasia1513-1874.2024.031.

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Appendix A. Supplementary data

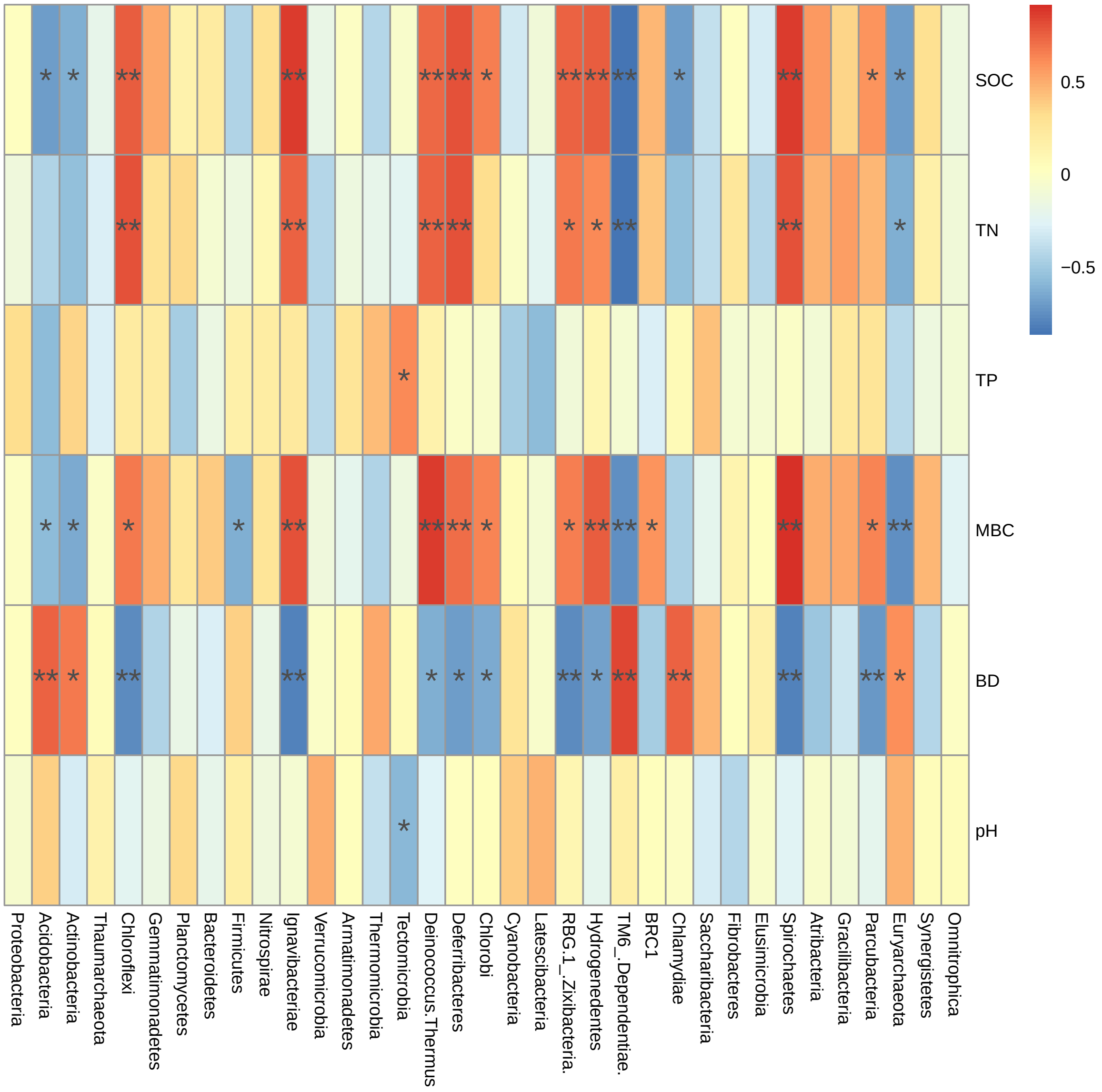

Fig. S1 Pearson correlation heat map between soil basic properties and bacterial phyla across two years. SOC, soil organic carbon; TN, total nitrogen; TP, total phosphorus; MBC, soil microbial biomass carbon; and BD, bulk density. *, **, and *** indicate significant difference at $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively.