Fertilizer reduction with bio-organic fertilizer to regulate the root soil microbial community structure to improve Baby Chinese cabbage yield

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Abstract

Using high-throughput sequencing, this study aimed to explore the response of soil microbial community and Baby Chinese cabbage yield to the reduction of chemical fertilizers combined with bio-organic fertilizer in the Gansu plateau, China. Our experiments consisted of conventional fertilizer (CK), 30% chemical fertilizer reduction + 6,000 kg bio-organic fertilizer (T1), 30% chemical fertilizer reduction + 9,000 kg bio-organic fertilizer (T2), 40% chemical fertilizer reduction + 6,000 kg bio-organic fertilizer (T3), and 40% chemical fertilizer reduction + 9,000 kg bio-organic fertilizer (T4). Compared with CK, soil microbial diversity and richness were higher for all treatments with added bio-organic fertilizer. PCoA showed that the bacterial and fungal communities in T2 and T4 were similar to each other. Redundancy and Spearman's correlation analyses of microbial communities and soil physicochemical properties revealed that reductions in chemical fertilizer rate combined with bio-organic fertilizer had a stronger impact on the fungal than the bacterial community. They also increased the relative abundance of the dominant bacterial and fungal phyla. Baby Chinese cabbage yield was relatively higher under the combined bio-organic fertilizer plus reduced chemical fertilizer rate with T2 showing the highest yield. Therefore, this approach is feasible for sustainable agricultural, cost-effective and profitable crop production.

Keywords Baby Chinese cabbage; bio-organic fertilizer; microbial community; sustainable agriculture; yield

Importance

- Bio-organic + moderately reduced chemical fertilizer raised Chinese cabbage yield

- 33 • Bio-organic + chemical fertilizer was more efficacious than either one alone
- 34 • Presence of bio-organic fertilizer enhanced overall rhizosphere physicochemistry
- 35 • Bio-organic fertilizer improved beneficial bacterial & fungal abundance &
- 36 diversity
- 37 • Fertilizer combination sustainably & cost-effectively improves crop & soil quality

38 **1. Introduction**

39 Gansu Province, China is at high altitude, has abundant light, experiences wide
40 diurnal temperature differences, and has low summertime temperatures, which makes
41 cold-grown vegetable crops that are rich in nutrients and have excellent flavor. This
42 area is dry and has low precipitation rates and few diseases and insect pests. Pesticide
43 use is minimal in this region; thus, crop quality and safety have been assured. Gansu
44 Plateau summer vegetables have bright colors, appealing shapes and flavors, and high
45 national consumer demand. Baby Chinese cabbage (*Brassica rapa subsp. Pekinensis*)
46 is a subspecies of *Brassica oleracea* in the crucifer family. This crop prefers cold
47 climates and has become a mainstay of the summer vegetables in Gansu Plateau. Its
48 planting scale is expanding annually. To contend with growing crop demand and
49 improve yield, traditional agricultural systems have relied upon high application rates
50 of chemical fertilizer (Hartmann et al. 2015). Chemical fertilizer overuse is also
51 accompanied by a reduction in soil nutrient absorption efficiency, soil quality
52 deterioration, greenhouse gas emissions, and aquatic ecosystem eutrophication (Zhu
53 et al. 2016, Ajeng et al. 2020, Zhao xiang et al. 2020).

54 Under the advocacy of the sustainable development practice known as “zero
55 growth action plan for fertilizer use in China in 2020”, organic agriculture based on
56 natural products such as humic acid and natural fertilizer has been implemented to
57 improve soil quality. This practice is conducive to agricultural productivity and
58 ecosystem health (Semida et al. 2019, Huang et al. 2020). The application of plant
59 growth-promoting microorganisms to the soil is efficacious in organic agriculture.
60 This approach compensates for the low efficiency of synthetic fertilizer, improves
61 nutrient utilization efficiency and plant growth, reduces fertilizer investment by 50%,

62 and causes no yield loss(Hayat et al. 2010, Good and Beatty 2011, Da Costa et al.
63 2013). However, organic fertilizer input may also create competition for nutrients
64 between crops and soil microorganisms decomposing the fertilizer (Liu et al. 2009). I
65 To balance this competition, integrated agricultural systems are nonetheless
66 supplemented with chemical fertilizers to mitigate nutrient limitation (Harris 2009). I
67 Plant probiotic enrichment in the form of organic substrates, such as bio-organic
68 fertilizers, can help beneficial microorganisms thrive in the rhizosphere and improve
69 their function (Yang et al. 2011, Mukta et al. 2017, Xiong et al. 2018, Bubici et al.
70 2019). Diacono and Montemurro (Diacono and Montemurro 2011) conducted over
71 twenty long-term tests confirming that organic modifiers never lower crop yield.
72 Zhang et al. (Zhang et al. 2016) showed that replacing 30% of the total nitrogen
73 fertilizer (250 kg N/ha) with 3,000 kg/ha compost (equivalent to 60 kg N/ha)
74 improved maize yield, N uptake, and soil fertility, and reduced N loss. Hence, there is
75 a growing demand for bio-based organic fertilizers in agricultural production (Raja
76 2013).

77 Soil microorganisms regulate soil ecosystem functions and are indicators of soil
78 quality (Sharma et al. 2010). They promote nutrient cycling and organic matter
79 transformation, improve plant productivity, and control soil-borne diseases (Barrios
80 2007, Pieterse et al. 2016, Bakker et al. 2018). Microbial abundance, composition,
81 and activity largely determine sustainable agricultural productivity (Van Der Heijden
82 et al. 2008). The soil microbiome may be positively or negatively affected by soil
83 disturbances and management practices that alter soil microbiome classification and
84 function (Philippot et al. 2013, Cordovez et al. 2019). Long-term, large-scale
85 chemical fertilizer use leads to the deterioration of soil quality, nutrient imbalances,
86 reductions in soil microbial diversity, loss of microbial structural integrity, and a
87 decrease in sustainable farmland productivity (Savci 2012). Bio-organic fertilizers
88 contain beneficial microorganisms and organic components that directly or indirectly
89 promote soil nutrient mobilization, have a positive impact on plant health, and
90 improve crop yield (Tamreihao et al. 2016). Previous studies showed that bio-organic
91 fertilizers stimulate specific microbiota associated with plant disease inhibition such

as *Pseudomonas*, *Streptomyces*, *Flavobacterium*, and others (Mendes et al. 2011, Cha et al. 2016, Kwak et al. 2018). Therefore, strategies to stimulate the activities of these soil borne microbiota may be particularly effective in helping to inhibit plant diseases. Nevertheless, there are few reports on the changes that occur in the soil microbial diversity and root structure of Gansu Plateau summer vegetables in response to bio-organic fertilizer application.

In the present study, we conducted field trials on Baby Chinese cabbage under conventional fertilization, 30% chemical fertilizer reduction + 6,000 kg bio-organic fertilizer, 30% chemical fertilizer reduction + 9,000 kg bio-organic fertilizer, 40% chemical fertilizer reduction + 6,000 kg bio-organic fertilizer, and 40% chemical fertilizer reduction + 9,000 kg bio-organic fertilizer. We used directional sequencing of bacterial and fungal communities to analyze the response of the rhizosphere microbial community to the foregoing treatments. The aims of this study were to: 1) determine the changes in the physicochemical properties of the rhizosphere and the microbial community in response to various fertilizer treatments; 2) evaluate the impact of the different fertilizer treatments on Baby Chinese cabbage crop yield; 3) identify the correlations among rhizosphere microbial community composition, soil properties, and crop yield, 4) assess the benefits of combining bio-organic and chemical fertilizers, and 5) recommend the optimal amount of supplementary fertilizer required for Baby Chinese cabbage throughout its growth period. The results of this study will optimize crop productivity while minimizing the impact of chemical fertilization on the environment.

2. Materials and Methods

2.1 Experiment design and sampling

The experiment was conducted in Dachaigou Town (37°09'N, 102°99'E), Tianzhu County, Gansu Province between July 2019 and September 2020. This region has a continental plateau monsoon climate, altitude of ~2,630 m a.s.l., annual average temperature of -2 °C, annual average precipitation of 400–450 mm, and average annual sunshine hours of 2,500–2,700 h. The test site had a flat terrain and loam soil with medium, uniform fertility.

122 The Baby Chinese cabbage variety was “DeQin Golden Queen” produced by
123 Beijing De Runcheng Agricultural Science and Technology Development Co. Ltd.
124 (Beijing, China). The experiment was conducted over a 2-y period. In 2019, the Baby
125 Chinese cabbage was planted on July 10 and harvested in September. In 2020, the
126 Baby Chinese cabbage was planted in July and harvested in September. The fertilizers
127 were urea ($N \geq 46\%$), calcium superphosphate ($P_2O_5 \geq 16\%$), potassium sulfate (K_2O
128 $\geq 52\%$) and bio-organic fertilizer (effective strains: *Bacillus subtilis*, *Pseudomonas*
129 *stutzeri*, effective viable bacteria count ≥ 20 million/g, organic matter $\geq 40\%$). The
130 calcium superphosphate and bio-organic fertilizer were applied as basic fertilizer.
131 Thirty percent of the urea and potassium sulfate was applied as basal fertilizer,
132 another 30% was applied at the rosette stage, and the remaining 40% was applied at
133 early heading.

134 The experimental design comprised conventional fertilizer (CK), 30% chemical
135 fertilizer reduction + 6,000 kg bio-organic fertilizer (T1), 30% chemical fertilizer
136 reduction + 9,000 kg bio-organic fertilizer (T2), 40% chemical fertilizer reduction +
137 6,000 kg bio-organic fertilizer (T3), and 40% chemical fertilizer reduction + 9,000 kg
138 bio-organic fertilizer (T4). Each plot was 0.008 ha in area and the cultivation mode
139 was film-mulching ridge-furrow. The cultivation density was 59,550 plants/ha. The
140 ridge and furrow widths were 50 cm and 40 cm, respectively, and the planting and
141 row distances were 33 cm and 30 cm, respectively. Field management was consistent
142 with local traditional cultivation. The fertilization rates under each treatment are listed
143 in Table 1.

144 Soil samples were collected on the Baby Chinese cabbage harvest date
145 (September 11, 2020). For each plot, the edge effect was avoided. Thirty plants with
146 the same growth were selected per plot. Surface soil was removed from 0 to 5 cm
147 depth. Large soil clumps around the root systems were shaken off. The soil near the
148 root systems was brushed with a brush, sealed in bags, placed in ice boxes, and taken
149 to the laboratory. The soil samples were divided into two parts. One was air-dried and
150 used in the physicochemical property determinations. The other was sieved through a
151 2-mm screen and stored at -80°C until DNA extraction.

152 **2.2 Analysis of soil physical and chemical properties**

153 Soil physical and chemical properties were determined using the method of Lyu
154 et al.(Lyu et al. 2020). Soil pH and EC were measured with a pH Meter (PHS-3E;
155 Shanghai Jingke, Shanghai, China) and an EC Meter (DSJ-308A1 Shanghai Jingke)
156 (1:5 soil:water (w:v)), respectively. The soil organic matter content was measured by
157 the potassium dichromate method. Total N, P, and K were determined by the Kjeldahl,
158 molybdenum antimony colorimetry, and flame spectrophotometry (FP6410, Shanghai,
159 China) methods, respectively.

160 **2.3 DNA extraction and PCR amplification**

161 Microbial genomic DNA extraction from soil samples was performed with the
162 E.Z.N.A.® Soil DNA Kit (Omega Bio-tek, Norcross, GA, USA). DNA quality assays
163 were performed on 1% agarose gels. DNA concentration and purity were determined
164 with a NanoDrop 2000 UV-Vis spectrophotometer (Thermo Fisher Scientific,
165 Wilmington, DE, USA) for determination. The V3-V4 region of bacterial 16S rRNA
166 was amplified with primers s338F (5'-ACTCCTACGGGAGGCAGCAG-3') and 806R
167 (5'-GGACTACHVGGGTWTCTAAT-3'). The fungal ITS2 region was amplified with
168 primers ITS1F (5'-CTTGGTCATTTAGAGGAAGTAA-3') and ITS2R
169 (5'-GCTGCGTTCTTCATCGATGC-3'). PCR was performed using an ABI
170 GeneAmp® 9700 PCR thermocycler (Applied Biosystems, Foster City, CA, USA).
171 The PCR amplification process was as follows: initial denaturation at 95°C for 3 min,
172 denaturation at 95°C for 27 cycles of 30 s, annealing at 55°C for 30 s, extension at
173 72°C for 45 s, one-time extension at 72°C for 10 min, and termination at 4°C. A total
174 volume of 20 µL of PCR mixture includes 4 µL of 5× TransStart FastPfu buffer, 2 µL
175 of 2.5 mM dNTPs, 0.8 µL of 5 µM each of forward and reverse primers, 0.4 µL of
176 TransStart FastPfu DNA polymerase, 10 ng of template DNA, and sufficient ddH₂O.
177 PCR Reactions were performed in triplicate. PCR products were electrophoresed on a
178 2% agarose gel, then purified using the AxyPrep DNA Gel Extraction Kit (Axygen
179 Biosciences, Union City, CA, USA) and quantified using a Quantus™ fluorometer
180 (Promega, Madison, WI, USA).

181 **2.4 Illumina MiSeq sequencing**

182 Purified amplicons were pooled in equimolar quantities and paired-end

183 sequenced (2×300) according to standard protocols on an Illumina MiSeq platform
184 (Illumina, San Diego, CA, USA) by Majorbio Bio-Pharm Technology Co. Ltd.,
185 Shanghai, China.

186 **2.5 Processing of sequencing data**

187 The raw 16S rRNA and ITS gene sequencing reads were demultiplexed,
188 quality-filtered with Trimmomatic (<https://github.com/usadellab/Trimmomatic>), and
189 merged by FLASH (<https://sourceforge.net/projects/flashpage/files/>) using the
190 following criteria. (i) The 300-bp reads were truncated at any site with an average
191 quality score < 20 over a 50-bp sliding window. Truncated reads < 50 bp and reads
192 containing ambiguous characters were discarded. (ii) Only overlapping sequences $>$
193 10 bp were assembled. The maximum mismatch ratio of the overlap region was 0.2.
194 Reads that could not be assembled were discarded. (iii) Samples were distinguished
195 by their barcodes and primers and the sequence direction was adjusted. Exact barcode
196 matching was used and two nucleotide mismatches were permitted in the primer
197 matching.

198 The clean tags with sequence similarity greater than 97% were designated as an
199 OTU using UPARSE v. 7.1 (<http://drive5.com/uparse/>) and then clustering was
200 performed. The taxonomy of each OTU representative sequence (0.7 confidence
201 threshold) was analyzed using the RDP classifier (<http://rdp.cme.msu.edu/>) against the
202 fungal ITS (Unite 8.0) and bacterial 16S rRNA (Silva SSU128) databases.

203 **2.6 Determination of yield**

204 On September 16, 2019 and September 11, 2020, 30 plants were selected per plot
205 and the yield per hectare was calculated based on the plot area and total yield.

206 **2.7 Statistical analysis**

207 Microbiological data analyses were conducted in R v. 3.5.2 (Team 2018). The
208 Shannon, Simpson, and Chao indices were calculated with QIIME (<https://qiime2.org>).
209 A principal coordinate analysis (PCoA) was performed based on the Bray-Curtis
210 distance and it evaluated the similarities of the microbial community composition
211 among samples. A redundancy analysis (RDA) and Spearman's rank correlation heat
212 map analysis were used to study the relationships between soil physicochemical

properties and soil microbial communities. Permutational multivariate ANOVA (PerMANOVA) was used to assess the effects of different fertilization regimes on soil microbial communities. PCoA, RDA, and PerMANOVA was implemented using the functions in the R vegan package (Oksanen et al. 2020). The Circos graph was plotted with Circos-0.67-7 (<http://circos.ca/>) (Yan et al. 2020). SPSS v. 21.0 (SPSS Inc., Chicago, IL, USA) was used to perform basic statistical tests such as one-way analysis of variance (ANOVA) and Pearson's correlation analysis. Significant differences among treatments were indicated by $P < 0.05$ or other P values.

3. Result

3.1 Soil physicochemical properties under different fertilization regimes

The physicochemical properties of the soil significantly differed among fertilization treatments (Table 2). Compared with conventional fertilization (CK), the soil TN, TP, and TK increased with bio-organic fertilizer application rate. TN and TP were highest for the soil under T2 and were 23.08% and 39.76% greater than those under CK. Compared with CK, TP under T3 and T4 were 7.73% and 10.27% higher, and the differences were significant. The soil organic matter (SOM) content was lowest for CK and was significantly lower than the SOM under all other treatments. The SOM content increased sequentially from T1 to T4. All values were significantly higher than that of CK. The SOM content was highest under T4 and 48.08% greater than the SOM content under CK. The soil EC was highest under CK and was significantly higher than the soil EC under all other treatments. The soil EC under T1 through T4 were 37.49%, 29.28%, 24.36%, and 21.80% lower, respectively, than the soil EC under CK, and the differences were significant. The soil pH was highest under T2 and was 4.14% higher than the soil pH under CK. The difference was significant. The soil pH under all other treatments did not significantly differ from that under T2 but were significantly higher than that under CK.

3.2 Changes in soil microbial α -diversity under different fertilization regimes

We measured the Shannon, Simpson, and Chao indices of bacteria (Fig. 1 A-C) and fungi (Fig. 1 D-F) to evaluate the α -diversity of the soil microbial community in the Baby Chinese cabbage rhizosphere under different fertilization treatments. The

Shannon index is directly proportional to the diversity while the Simpson index is inversely proportional to it. The Chao index is directly proportional to the microbial abundance. Figure 1-A shows that after the various fertilization treatments, the Shannon index was 0.63% and 0.78% higher for the soil bacteria under T1 and T3, respectively, than it was for those under CK and the differences were significant. There was no significant difference between T4 and CK in terms of the Shannon index. However, the Shannon index was 1.10% lower under T2 than CK and the difference was significant. There were no significant differences among fertilization treatments in terms of their bacterial Simpson indices (Fig. 1-B). The bacterial Chao index was highest under T4 and was 34.20% higher than that under CK. However, there were no significant differences among the other treatments and CK in terms of the bacterial Chao index (Fig. 1-C). The fungal Shannon indices under T1 through T4 were 10.66, 14.42, 8.46, and 14.73% higher, respectively, than that under CK, and the differences were significant (Fig. 1-D). The fungal Simpson indices under T1 through T4 were 44.53, 53.13, 34.38 and 50.78% lower, respectively, than that under CK, and the differences were significant (Fig. 1-E). The fungal Chao indices under T2 and T4 were 23.40% and 23.49% higher, respectively, than that under CK, and the differences were significant (Fig. 1-F).

3.3 Changes in soil microbial α -diversity under different fertilization regimes

We applied PCoA based on the Bray-Curtis algorithm to analyze overall structural changes in the bacterial and fungal microbial communities and determine the impact of reducing chemical fertilizers and adding bio-organic fertilizers on their structure. The fertilization regimes significantly altered soil bacterial and fungal community structure in the Baby Chinese cabbage rhizospheres. The PCoA assigned the bacterial communities to two groups, namely, [CK, T1, and T3] and [T2 and T4] (Fig. 2-A). The T2 and T4 bacterial community structures were clustered together and were remote from those of the other treatment groups (Fig. 2-A). Hence, the soil bacterial community structure significantly changed with increasing bio-organic fertilizer application rate. The PCoA assigned the fungal communities to three groups, namely, [CK], [T1 and T3], and [T2 and T4] (Fig. 2-B). The T2 and T4 fungal

community structures were clustered together and were remote from that of CK (Fig. 2-B). There was a certain distance between T1 and T3 but both were nonetheless in the same quadrant. Thus, the change in the fungal community structure of the Baby Chinese cabbage rhizosphere depended on whether biological organic fertilizer was used and the quantity added. PERMANOVA revealed that each fertilization treatment significantly affected the bacterial ($F = 1.64$, $P = 0.01$) and fungal ($F = 6.66$, $P = 0.01$) community structures.

3.4 Associations between soil microbial communities and environmental factors

A redundancy analysis (RDA) was used to evaluate the influences of environmental factors on the soil microbial community composition in Baby Chinese cabbage rhizosphere (Fig. 3). The changes in the soil properties in response to the different fertilization treatments strongly affected the soil bacterial and fungal community structures. The axes explained that the variation in the soil bacterial (Fig. 3-A) and fungal (Fig. 3-C) communities were 19.63 and 81.56%, respectively. The first axis in Fig. 3-A was positively correlated with soil TN, TK, TP, pH, and SOM and negatively correlated with soil EC. The second axis was positively correlated with soil EC, TP, and pH and negatively correlated with soil TN, TK, and SOM. We also used Spearman's rank correlation to evaluate the relationships among the abundant bacterial phyla and soil physicochemistry (Fig. 3-B). Bacteroidota were significantly positively correlated with soil TN and TK, SAR324_cladeMarine_group_B and Firmicutes were significantly negatively correlated with soil EC, Acidobacteriota were significantly negatively correlated with soil TN and pH while Methyloirabillota and Latescibacterota were significantly negatively correlated with SOM. The first axis in Fig. 3-B was positively correlated with soil TN, TK, TP, pH, and SOM and negatively correlated with soil EC. The second axis was positively correlated with soil EC and SOM and negatively correlated with soil TN, TK, TP, and pH. Spearman's rank correlation between the soil fungal community and physicochemistry disclosed that Zoopagomycota, unclassified_k_Fungi, and Mortierellomycota were significantly positively correlated with soil TP, SOM, and TN, respectively, and Ascomycota were significantly positively correlated with soil TK, SOM, and TN and

very significantly positively correlated with soil pH. Mortierellomycota, Kickxellomycota, and Blastocladiomycota were significantly negatively correlated with soil EC while Olpidiomyota were significantly negatively correlated with SOM and soil pH.

3.5 Relative abundance of major bacterial and fungal taxa

The main bacterial phyla were Actinobacteriota, Proteobacteria, Chloroflexi, and Acidobacteriota (Fig. 4-A) and their average relative abundances were 35.14, 19.83, 16.39, and 10.56%, respectively. The subdominant bacterial phyla were Gemmatimonadota, Bacteroidota, Myxococcota, and Firmicutes. T1 through T4 had higher actinobacteria richness than that of CK. T2 and T4 had the highest and second highest Actinobacteriota abundance. Actinobacteriota abundance was higher under T1 than T3. T3 and T4 had higher Proteobacteria abundance than CK. However, Proteobacteria abundance did not significantly differ between T1 and T2. Compared with CK, Chloroflexi, Acidobacteriota, and Gemmatimonadota had higher abundance while Bacteroidota and Firmicutes had lower abundance under T1 through T4. The main fungal taxa were Ascomycota, Olpidiomyota, Mortierellomycota, Basidiomycota, and unclassified_k_Fungi (Fig. 4-B). T1 through T4 had higher Ascomycota abundance than CK. T2 had the highest Ascomycota abundance but relatively low Olpidiomyota abundance. T1 through T4 had relatively high Mortierellomycota abundance.

3.6 Effect of different fertilization regimes on Baby Chinese cabbage yield

We measured Baby Chinese cabbage yield in 2019 and 2020 and calculated the average for both years. Compared with CK, T1 through T4 significantly increased Baby Chinese cabbage yield by 6.39, 8.89, 3.94, and 5.14%, respectively. The yield was highest under T2 but did not significantly differ from that under T1 (Fig. 5).

4. Discussion

Widespread use of chemical fertilizers has led to losses of soil quality and organic carbon content (Kumar et al. 2017, Kumar et al. 2018). Therefore, fertilization must be optimized to ensure sustainable food production. Compared with conventional soil cultivation, organic soil cultivation enhances microbial functional

diversity. Long-term application of organic fertilizers or organic-inorganic compound fertilizers may increase microbial biomass and enzyme activity and improve SOM quantity and quality (Bending et al. 2004). In our study, compared with conventional fertilization, all four chemical fertilizer reduction and combined with bio-organic fertilizer application treatments significantly increased soil TP, TN, TK, pH, and SOM. Furthermore, both TK and SOM increased with bioorganic fertilizer application rate (Table 2). Therefore, the combined application of organic and chemical fertilizers increases soil fertility and supports sustainable productivity more effectively than chemical fertilizer application alone (Liu et al. 2014, Jiang et al. 2017). The addition of *Bacillus subtilis* and *Pseudomonas stutzeri* to the organic fertilizer further increased soil extracellular enzyme activity and nutrient content (Li et al. 2021). TP, TN, and pH were at their maxima under T2 (Table 2). For this reason, even moderate reductions in chemical fertilizer application rate followed by bio-organic fertilizer treatment effectively increase soil nutrient content and improve soil quality. By contrast, excessive reductions in chemical fertilizer application may decrease soil nutrient levels even when sufficient bio-organic fertilizer is used.

Several studies evaluated the effects of fertilization on soil microbes. Organic fertilizers, such as manure might have a positive effect on the soil microbial community. Compared with chemical or no fertilization, organic fertilization improves the resistance of the soil microbial community to perturbations (Francioli et al. 2016, Cui et al. 2018, Legrand et al. 2018). Our results showed that compared with conventional fertilization, T1 and T3 significantly increased bacterial diversity and T4 significantly increased bacterial richness (Fig. 1A-C). These findings were consistent with those of prior studies indicating that organic fertilizer addition increases bacterial community diversity (Chaudhry et al. 2012, Jannoura et al. 2013, Xiong et al. 2017). Compared with conventional fertilization, organic fertilization had a stronger effect on soil fungal communities (Cwalina-Ambroziak and Bowszys 2009, Wei et al. 2017). All treatments with bio-organic fertilizer increased soil fungal community diversity and richness. However, T2 and T4 were the most effective (Fig. 1D-F). That is, the effective enhancement of soil microbial diversity and richness may be a combined

effect of Bioorganic and chemical fertilizer applications (Ding et al. 2018).

The soil microbial community structure significantly differed between the bio-organic fertilizer and other treatments. The PCoA showed that the bacterial and fungal community structures under T2 and T4 and those under T1 and T3 were similar. In both cases, they were separated by conventional fertilization (CK) (Fig. 2A-B). These discoveries were consistent with previous observations that soil microbial community structures differ in response to organic and inorganic fertilizers (Marschner et al. 2003, Zhang et al. 2012). Moreover, fertilization regimes more strongly influence fungal than bacterial community structure (Wang et al. 2018b, Zhang et al. 2018).

A combination of PCoA and RDA demonstrated that fertilization may modulate the impact of environmental factors on fungal community structure. The variance between fungal community structure and environmental factors was 81.56% (Fig. 3-C). By contrast, the variance between bacterial community structure and environmental factors was only 19.63% (Fig. 3-A). Fertilizer application increases the abundance of oligotrophic organisms such as Bacteroidota which are significantly positively correlated with soil TN and TK (Fig. 3-B). Oligotrophic organisms tend towards K-selection (Berg and Smalla 2009). A high relative abundance of K-strategists promotes microbial community resistance and stability (Simonin et al. 2017). Soil pH is positively correlated with most bacterial communities but negatively correlated with Acidobacteriota (Fig. 3-B). In the present study, the soil pH was near neutrality and organic fertilizer application prevented soil acidification. In this manner, it buffered the potential effects of soil acidity on bacterial richness and diversity (Zhang et al. 2017). However, fungal communities are comparatively more strongly affected by the type of fertilization system. Fungal richness is positively correlated with soil N availability (Hu et al. 2017). Ascomycota are the most abundant fungi in the Baby Chinese cabbage rhizosphere and are significantly positively correlated with TN (Fig. 3-D). Hence, the fungal community is closely related to the soil nitrogen fertilizer application rate. SOM also plays an important role in fungal community composition (Yao et al. 2017, Dai et al. 2018). Most dominant fungal phyla such as

393 Ascomycota, Zoopagomycota, unclassified_k_Fungi, and Mortierellomycota are
394 positively correlated with SOM. Increases in EC are the result of increases in salinity
395 caused by continuous soil cropping. Soil salinity has negative effects on soil microbial
396 communities (Shen et al. 2016). The application of bio-organic fertilizer can reduce
397 EC. Most dominant fungal phyla are negatively correlated with this parameter (Fig.
398 3-D).

399 In all fertilization treatments, Actinobacteria, Proteobacteria, Chloroflexi, and
400 Acidobacteria were the dominant bacterial phyla (Fig. 4-A). This observation was
401 consistent with those of previous studies (Guo et al. 2018, Huang et al. 2019).
402 Actinobacteriota comprise a group of co-nutrients suitable for plant growth in high-C
403 environments (Fierer et al. 2012), Bio-organic fertilizers promote Actinobacteriota
404 proliferation because they create nutrient- and carbon-rich environments and their
405 abundance significantly increased in response to bio-organic fertilizer treatment and
406 was highest under T2. Composting is beneficial to the growth of Proteobacteria and
407 Firmicutes (Martínez-García et al. 2018). In this study, bio-organic fertilizer treatment
408 also increased Proteobacteria and Firmicutes abundance. Chloroflexi include
409 numerous acid-producing bacteria that anaerobically digest food waste and produce
410 methane gas (Wang et al. 2018a). Long-term chemical fertilizer application may
411 acidify the soil (Guo et al. 2010), thereby reducing soil microbial diversity and
412 community structure (Zhang et al. 2015). In the present study, Chloroflexi abundance
413 was higher under the conventional fertilization treatment. The addition of bio-organic
414 fertilizer increased the soil pH (Table 2) and reduced Chloroflexi abundance.
415 Acidobacteria are slow-growing oligotrophs that flourish under low-nutrient
416 conditions. Their growth is inhibited by N input (Trivedi et al. 2017). Bio-organic
417 fertilizer addition increased soil nutrient levels and inhibited Acidobacteria growth.
418 Nevertheless, further research is required to elucidate the mechanism(s) by which
419 bio-organic fertilizer addition increases bacterial abundance.

420 Ascomycota were the dominant fungi in the present study (Fig. 4-B). These
421 saprophytes can thrive in arid environments, have strong environmental adaptability,
422 degrade organic substrates, and are major decomposers of SOM containing cellulose,

lignin, and pectin (Yelle et al. 2008). The combination of bio-organic fertilizer plus chemical fertilizer reduction increased Ascomycota abundance. Ascomycota richness was highest under T2. Mortierellomycota are saprophytic and ubiquitous. They can dissolve phosphorus, increase crop yield, and form symbiotic relationships with plants (Fröhlich-Nowoisky et al. 2015, Grządziel and Gałazka 2019). In the present study, Mortierellomycota abundance increased in response to bio-organic fertilizer treatment and was significantly positively correlated with TP, SOM, and TN (Fig. 3-D). Therefore, improvements in the soil nutrients in Baby Chinese cabbage rhizosphere may be associated with higher relative Mortierellomycota abundance.

The production and integration of new bio-organic fertilizers with beneficial microorganisms and mature compost is an important way to increase crop yields and/or strengthen the control of soil-borne diseases (Zheng et al. 2020, Chen et al. 2021). It is feasible but difficult to reduce fertilizer application without causing a loss of productivity (Da Costa et al. 2013). The results of this experiment confirmed our hypothesis that bio-organic fertilizer significantly increased Baby Chinese cabbage yield compared with chemical fertilizer. T2 (30% chemical fertilizer reduction + 9,000 kg bio-organic fertilizer) realized the highest crop yield of all treatments. Ye et al. (Ye et al. 2020) showed that a combination of chemical fertilizer reduction and bio-organic fertilizer application could improve crop yield and quality.

These results provide a foundation for understanding how the addition of bioorganic fertilizers caused soil physicochemical improvement and then caused changes in soil microbial diversity and richness, which in turn enhanced Baby Chinese cabbage yield (Fig. 6).

5. Conclusion

Overall, the present study examined the effects of various fertilization regimes on soil microbial diversity and community structure in the rhizosphere of Baby Chinese cabbage grown on the Gansu Plateau. The potential value of bio-organic fertilization in Baby Chinese cabbage cultivation was assessed in terms of microbial structure and crop yield. Reduction of chemical fertilizers and addition of bio-organic

fertilizers improved rhizosphere physicochemistry, markedly altered the soil bacterial and fungal communities, increased their diversity and abundance, and improved the soil microenvironment. Compared with conventional fertilization, the combination of bio-organic fertilizer and reduced chemical fertilizer created a richer, more diverse microenvironment and realized higher Baby Chinese cabbage yield. Hence, this novel approach is relatively more efficient and desirable in terms of sustainable and high crop yield. Future research should investigate the changes that occur in the microbial profile of the rhizosphere and the effects of these microorganisms on the yield and quality of Baby Chinese cabbage subjected to long-term bio-organic fertilization.

Declaration of competing interest

The authors declare that they have no conflict of interest.

Data Availability Statement

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request. Sequencing data are stored in the NCBI database, access link: <https://identifiers.org/ncbi/insdc.sra:SRP359012>

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Table 1 Fertilization rates for each treatment

Treatments	Total amount of fertilization in the whole growth period (kg/ha)			
	Urea	Calcium superphosphate	Potassium sulphate	Bio-organic fertilizer
CK	1225	2012.5	250	0.0
T1	762.5	1037.5	375	6000
T2	762.5	1037.5	375	9000
T3	650	887.5	325	6000
T4	650	887.5	325	9000

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Table 2 Physicochemical properties of soils under different fertilization conditions

Treatments	TN (g/kg)	TP (g/kg)	TK (g/kg)	SOM (g/kg)	EC (uS/cm)	pH
CK	0.39±0.02c	0.83±0.02d	16.17±0.30b	33.38±0.58e	507.67±5.21a	7.25±0.07c
T1	0.48±0.01b	0.94±0.06c	17.17±0.44ab	35.78±0.55d	317.33±2.6d	7.49±0.01ab
T2	0.54±0.03a	1.16±0.03a	17.42±0.30ab	38.56±0.91c	359.00±5.86c	7.55±0.02a
T3	0.48±0.01b	1.06±0.05b	17.67±0.55a	43.74±0.33b	384.00±4.04b	7.43±0.12ab
T4	0.50±0.01ab	1.11±0.04ab	17.83±0.51a	49.43±0.77a	397.00±5.87b	7.48±0.08ab

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Values (mean ± standard deviation) followed by different lowercase letters within a column for the same factor indicate significant differences by Tukey test (P <0.05). TP, total phosphorus; TK, total potassium; TN, total nitrogen; pH, soil pH; EC, soil electrical conductivity; SOM, soil organic matter.

711

712 **Figure Legends**

713 **Fig. 1** The α -diversity of bacteria (A-C) and fungi (D-F) in the root soil of Baby
714 Chinese cabbage under different fertilization treatments. Different letters over
715 the plots indicate significant differences ($P < 0.05$).

716

717 **Fig. 2** Principal coordinate analysis (PCoA) of the bacteria (A) and fungi (B)
718 communities based on the Bray–Curtis distances.

719

720 **Fig. 3** Redundancy analysis (RDA) and Spearman's rank correlation heatmap ($P <$
721 0.05 *, $P < 0.01$ **), used to study the correlation between the microbial
722 communities of bacteria (A-B) and fungi (C-D) and soil physical and chemical
723 properties. TP, total phosphorus; TK, total potassium; TN, total nitrogen; pH, soil
724 pH; EC, soil electrical conductivity; SOM, soil organic matter.

725

726 **Fig. 4** The relative abundance of major taxonomic groups at the phylum level (Others
727 incorporated < 0.01) for bacteria (A) and fungi (B). The data were visualized by
728 Circos. The width of the bars from each phylum indicated the relative abundance
729 of the phylum.

730

731 **Fig. 5** Average yield of Baby Chinese cabbage under different fertilization systems in
732 2019–2020. Different letters over the plots indicate significant differences ($P <$
733 0.05).

734 **Fig. 6** A model of soil physical and chemical properties, microbial community and
735 yield changes after adding bio-organic fertilizer.

736

Figure 1

PCoA on OTU level

Figure 2 - A

PCoA on OTU level

Figure 2 - B

RDA on Phylum level

Figure 3 - A

Spearman Correlation Heatmap

Figure 3 - B

RDA on Phylum level

Figure 3 - C

Spearman Correlation Heatmap

Figure 3 - D

Circos

Figure 4 - A

Circos

Figure 4 - B

Figure 5

Figure 6