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Effects of calcium polypeptides on wheat yield, grain quality and rhizosphere soil microbial community

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Calcium polypeptides (CPPs) prepared from agricultural wastes can provide nutrients to plants, promote plant growth, and reduce accumulation of heavy metals in vegetables. In this study, a field experiment including three treatments, normal fertilization control, reduced fertilization by 40% (RF-40), and reduced fertilization by 40% with dressing of 7.5 kg/666.67 m^2 CPPs (RF-40-CPPs), was conducted to investigate the effects of CPPs on wheat yields, grain properties, and rhizosphere soil microbial communities. Compared with the control, after reducing chemical fertilizer application by 40%, the yields, protein contents, wet gluten contents, and amino acid contents of RF-40 wheat grains were clearly decreased by 14.51%, 1.54%, 5.03%, and 19.58%, respectively. Interestingly, compared with RF-40, after CPP application, the yields, 1000-grain weights, protein contents, wet gluten contents, and amino acid contents of RF-40-CPPs were clearly increased by 11.53%, 20.28%, 1.66%, 5.39%, and 22.91%, respectively. Surprisingly, compared with the control grains, the RF-40-CPPs grains exhibited increases of 3.13%~70.00% in five essential amino acids for humans, including Leu, Val, Lys, Ile, and Met. Moreover, CPPs could alter the diversity of the rhizosphere soil bacterial community, and some bacteria, including *Bradyrhizobium*, *Bryobacter*, *Candidatus_Solibacter*, *Gemmatimonas*, and *Haliangium*, which are involved in soil carbon and nitrogen metabolism, were significantly enriched. CPPs can regulate rhizosphere soil microbial communities, reduce chemical fertilizer application, and improve the grain quality of wheat "Zhengmai 9023", resulting in its important agricultural application potential for green wheat production.
Keywords: Calcium polypeptides; wheat; reduction in chemical fertilizer application; wheat quality; rhizosphere soil microbial community.

INTRODUCTION

Wheat (*Triticum aestivum* L.) is the second largest cash crop cultivated in China and plays an important role in stabilizing the agricultural economy and ensuring food security (Habib *et al.*, 2020; Qamar *et al*., 2020; Wang *et al.*, 2020). In recent decades, to meet the nutritional needs of the rapidly growing population, people have established higher requirements for wheat yields, which has resulted in the widespread use of large quantities of chemical fertilizer to increase wheat production (Fan *et al.*, 2012). However, the repeated, excessive use of chemical fertilizers and the low efficiency of utilization (only 30–40%) have caused soil acidification and soil hardening, aggravated environmental water pollution, which has led to negative effects on soil quality and soil microbial community structure, and have even threatened human health (Ma *et al.*, 2012; Schmitt and de Vries, 2020). Therefore, there is an urgent need to develop an environmentally friendly strategy to reduce the application of chemical fertilizers, reduce the harm to the environment caused by chemical fertilizers, and achieve sustainable development of agriculture and the environment (Cheema *et al*., 2020).

As nontoxic high-nitrogen organic fertilizers, protein hydrolysates are prepared by recovering polluting waste proteins through acidic, alkaline, or enzymatic methods that can achieve secondary waste utilization (Schiavon *et al.*, 2008; Corte *et al.*, 2014; Gálvez *et al.*, 2016). Protein hydrolysates can improve rhizosphere soil microbial community structures, increase plant chlorophyll contents and photosynthetic performance, promote rapid plant growth, and enhance fruit quality in rice (Jie *et al.*, 2008), *Zea mays* L.

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(Schiavon *et al.*, 2008; Cholakova-Bimbalova *et al.*, 2019), *Brassica napus* L. (Popko *et al.*, 2015), tomato (Polo and Mata, 2017; Rouphael *et al.*, 2017), and sweet potato (Lindsey *et al.*, 2019). Similarly, protein hydrolysates could be used to supplement other fertilizers that can increase grain yields, grain protein contents and grain Zn and Fe concentrations in wheat (Gogos *et al.*, 2013). Moreover, protein hydrolysates can combine with the heavy metal-immobilizing bacterium *Enterobacter bugandensis* TJ6 to reduce the uptake of Cd^{2+} by wheat, increase the bacterial community diversity of wheat rhizosphere soil, and ensure the safe production of crops growing on heavy metal-polluted soils (Han *et al.*, 2020). Therefore, the application of protein hydrolysates in agricultural production will be a feasible strategy for wheat production to improve the efficiency of chemical fertilizer use and reduce the intensive use of inorganic fertilizers for wheat. Calcium polypeptides (CPPs), which are hydrolyzed from animal and plant waste proteins, such as soybean meal, animal hair and skin, hoof nails, skeletons, and rhizobia, are a new type of environmentally friendly protein hydrolysate organic fertilizer (Bhavsar *et al.*, 2016; Holkar *et al.*, 2016; Chen *et al.*, 2019). These polypeptides are molecular polymers (the average polypeptide molecular weight is less than 5,000 Daltons) between proteins and amino acids with ring structures generated by chelation reactions among peptides and calcium ions (Han *et al.*, 2020). CPPs have many advantages, such as good solubility, strong fluidity, thermal stability, low cost, good biocompatibility, and high nutrition, which result in their ability to promote plant growth, improve plant rhizosphere soil microbial diversity, and reduce heavy metal accumulations in plants (Han *et al.*, 2020; Liu *et al.*, 2020). CPPs prepared from waste vegetable meals by hydrolysis via the addition of quicklime under high temperatures not only could promote plant growth but also had an obvious competitive inhibition effect on heavy-metal Cd^{2+} enrichment in *Brassia campestris* L. (Chen *et al.*, 2019). In this study, under field conditions and after reducing the chemical fertilizer application by 40%, CPPs prepared from waste vegetable meals by mixing with quicklime were applied to wheat “Zhengmai 9023” (widely cultivated wheat cultivar in China (Wang *et al.*, 2020) during the wheat heading period. The aim of this study was to investigate the effects of CPPs on major agronomic indexes, grain quality, amino acid contents, wheat grain compositions, and rhizosphere soil microbial communities for wheat “Zhengmai 9023” after decreasing the amounts of chemical fertilizer application.

MATERIALS AND METHODS

***Plant material, fertilizer, and CPPs*:** Wheat “Zhengmai 9023” seeds were obtained from the Hubei Academy of Agricultural Sciences (Wuhan, Hubei, China). Basal fertilizer (N-P_2O_5-K_2O, 15-7-8) was obtained from the New Yangfeng Fertilizer Co. Ltd. (Jingmen, Hubei, China). CPPs were generated from waste vegetable meals by mixing with quicklime at high temperatures (Chen *et al.*, 2019). Briefly, waste vegetable meals (containing 40–45% protein) were mixed with quicklime (10–45% *wt./wt.* to waste vegetable meals) and were reacted for 1–6 h at 110–180°C. Ultimately, after spray drying, CPPs, which formed yellow powders, with an average polypeptide molecular weight of 5,000 Da were obtained. These CPPs contained 60.10% protein, 9.61% organic N, 0.35 g/kg P, 0.21 g/kg K, and 63.91 g/kg Ca.

***Wheat field experiments*:** Wheat field experiments were carried out from 26 October, 2019 to 28 May, 2020 (216 days) in a rice-wheat rotation area (Zhongxiang, Hubei, China). The basic physical and chemical properties of the experimental field soils were as follows: 27.31 $g\cdot kg^{-1}$ organic matter, 116.79 $mg\cdot kg^{-1}$ alkali-hydrolyzed nitrogen, 32.44 $mg\cdot kg^{-1}$ available phosphorus, 109.47 $mg\cdot kg^{-1}$ available potassium, and pH 7.20. The experimental field groups were established as follows: control, normal fertilization, no dressing fertilization; RF-40, fertilization reduced by 40%, no dressing fertilization; and RF-40-CPPs fertilization reduced by 40%, dressing with 7.5 kg/666.67 m^2 of CPPs. Every experimental set consisted of 3 replicates (666.67 m^2 per replicate). For sowing, the sowing rate was 15 kg/666.67 m^2. A total of 50 kg/666.67 m^2 of fertilizer (N-P_2O_5-K_2O, 15-7-8) was used as the basal fertilizer. Weeding was performed by spraying with 125 g/666.67 m^2 of 50% hyperpermeable isoprene wettable powder (Meifeng Agricultural Chemical Co. Ltd., Zhejiang, China). Disease control was achieved by spraying 100 g/666.67 m^2 of 50% carbendazim wettable powder (Sunong Biological Technology Co. Ltd., Anhui, China) during the wheat flowering period. Dressing with CPPs occurred during the wheat heading period (Figure 1).

***Determination of agronomic traits in wheat*:** Wheat yields, 1000-grain weights, plant heights, and numbers of effective wheat ears were determined according to Agricultural Trade Standards of the People's Republic of China NY/T 1301-2007 “Technical Procedures for Wheat Variety Regional Trials”.

***Determination of grain quality*:** To determine the grain quality, 500.0 g of wheat grains were analyzed by the Hubei Academy of Agricultural Sciences (Wuhan, Hubei, China). Moisture, protein content, wet gluten content, water absorption, and hardness of the wheat grains were determined by a DA7250 multifunctional near-infrared analyzer (Perten, Sweden).

***Determination of amino acid content of wheat grains*:** The amino acid contents of the wheat grains were analyzed by the Oil Crops Research Institute, Chinese Academy of Agricultural Sciences (Wuhan, Hubei, China) according to the State Standard of the People's Republic of China GB 5009.124-2016 “Determination of Amino Acids in Foods”. Briefly, wheat grain powder was digested in a hydrolyzing tube containing 6 mol/L HCl by using an electrothermostatic blast oven at 110 ± 1°C. Then, after filtration, the digested liquids were evaluated using an automatic amino acid

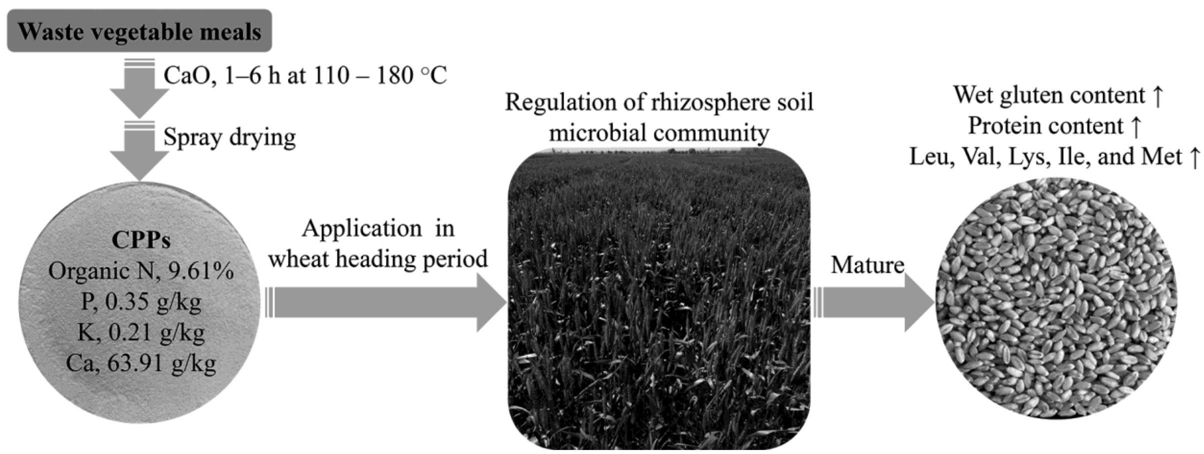

Figure 1. Schematic diagram of the preparation and application of CPPs.

analyzer L-8900 (Hitachi, Japan) according to the National Metrological Verification Regulations of the People's Republic of China JJG1064-2011 "Automatic Amino Acid Analyzer". The absolute difference between two independent measurements obtained under repeatable conditions must not exceed 12% of the arithmetic mean.

Analysis of bacterial community diversity for wheat rhizosphere soil**:** A total of 30.0 g of rhizospheric soil sample was collected during the grain filling stage and used for bacterial community analyses. Rhizospheric soil bacterial genomic DNA was extracted by a QIAamp ® Fast DNA Stool Mini Kit (Qiagen, Germany). The V3-V4 region of the 16S rRNA gene was sequenced by high-throughput sequencing using an Illumina HiSeq 2500 sequencing platform with the primers 338F (5'-ACTCCTACGGGAGGCAGCA-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3') and was analyzed by BMK Biotechnology Co., Ltd. (Beijing, China). Each sample consisted of three parallel repeats. The original sequencing sequences were assembled using FLASH v1.2.7 software and were filtered by Trimmomatic v0.33 software, and chimeras were removed by UCHIME v4.2 software. Then, the representative sequences of the operational taxonomic units (OTUs) were used for classification and phylogenetic analysis by Usearch software (Edgar, 2013). The alpha diversities of the rhizospheric soil microbial communities were reflected by the Chao1 and Shannon indexes by Mothur software (version v.1.30) (Tong *et al.*, 2018). Multidimensional analysis of community similarities was conducted by principal coordinate analysis (PCoA) that was based on weighted UniFrac distances (Ramette, 2007). The top 20 most-abundant genera were subjected to clustering analysis, and a heat map was drawn using R software. Significance analysis of groups was conducted using the LEfSe (Line discriminant analysis Effect Size) (Segata *et al.*, 2011).

Data analysis**:** The data were obtained from at least three assays and are presented as averages. SPSS 19.0 statistical software was used for statistical analysis. *P*-values were determined using an independent sample *T* test and $P < 0.05$ indicated statistically significant differences.

RESULTS

Effects of CPPs on major agronomic indexes of wheat "Zhengmai 9023"**:** To evaluate the effects of CPPs on the main agronomic indexes of wheat "Zhengmai 9023", the yields, 1000-grain weights, plant heights, and numbers of productive ears were determined after wheat harvest. As shown in Table 1, after decreasing the chemical fertilizer amount by 40%, the yield of RF-40 was 336.38 ± 12.83 kg/666.67 m^2, which was significantly lower than that of control (a 14.51% decrease, *P* value < 0.05). Fortunately, after application of CPPs during the wheat heading period, the yield of RF-40-CPPs reached 375.17 ± 21.35 kg/666.67 m^2, which was significantly higher than that of RF-40 (a 11.53% increase, *P* value < 0.05). However, the yield of RF-40-CPPs was still slightly lower than that of the control (a 4.65% decrease, *P* value > 0.05). For the 1000-grain weights, after decreasing chemical fertilizer amounts by 40%, RF-40 was not significantly different from the control (*P* value > 0.05). Interestingly, after applying CPPs during the wheat heading period, the 1000-grain weights of RF-40-CPPs reached 44.90 ± 2.87 g, which was significantly higher than that of the control (a 16.99% increase, *P* value < 0.05) and RF-40 (a 20.28% increase, *P* value < 0.05). Both plant heights and numbers of productive ears of RF-40 were lower than those of the control (decreases of 3.02% and 14.61%, respectively), and both plant heights and numbers of productive ears of RF-40-CPPs were greater than those of RF-40 (increases of 5.02% and 9.98%, respectively); however, there were no significant differences between these grains (*P* value > 0.05).

Effects of CPPs on grain quality of wheat "Zhengmai 9023**:** To evaluate the effects of CPPs on the grain quality of wheat "Zhengmai 9023", moisture contents, protein contents (wet

Table 1. The effects of calcium polypeptides on major agronomic indexes and wheat grain quality of wheat "Zhengmai 9023.

Test indexes		Control	RF-40	RF-40-CPPs
Major agronomic Indexes	Yield (kg/666.67 m^2)	393.48±12.42†	336.38±12.83*	375.17±21.35†
	1000-grains weight (g)	44.15±0.53†	37.33±3.21*	44.90±2.87†
	Plant height (cm)	93.08±3.83	90.27±2.24	94.80±2.51
	Number of productive ear (10^4/666.67 m^2)	30.26±3.15	25.84±2.30	28.42±1.47
Wheat grain quality	Moisture (*wt*%)	12.12±0.32%	12.00±0.05%	12.15±0.20%
	Protein content (wet basis *wt*%)	12.72±0.92%†	11.18±0.24%*	12.84±0.28%†
	Wet gluten content (wet basis *wt*%)	29.75±1.07%†	24.72±0.65%*	30.11±0.45%†
	Water absorption (fixed value = 14%)	65.70±0.64%	63.37±0.57%	65.19±1.38%
	Hardness (g/cm^2)	73.15±1.43	72.79±0.70	73.58±0.43

Means followed by different letters in a column were significantly different ($P < 0.05$). Control, normal chemical fertilizer application; RF-40, reduction of chemical fertilizer application by 40%; RF-40-CPPs, reduction of chemical fertilizer application by 40% and dressing with CPPs at 7.5 kg/666.7 m^2 during the wheat heading period.

basis), wet gluten contents (wet basis), water absorption, and hardness were determined after wheat harvest. As shown in Table 1, after reducing chemical fertilizer amounts by 40%, the protein contents (wet basis) and wet gluten contents (wet basis) of RF-40 grains were 11.18 ± 0.24% and 24.72 ± 0.65%, respectively, which were significantly lower than those of the control grains (decreases of 1.54% and 5.03%, respectively, P value < 0.05). Fortunately, after application of CPPs during the wheat heading period, the protein contents (wet basis) and wet gluten contents (wet basis) of the RF-40-CPPs grains were 12.84 ± 0.28% and 30.11 ± 0.45%, respectively, which were significantly higher than those of RF-40 grains (increases of 1.66% and 5.39%, respectively, P value < 0.05). In addition, the protein contents (wet basis) and wet gluten contents (wet basis) of the RF-40-CPPs grains were slightly higher than those of the control grains (increases of 0.12% and 0.36%, respectively, P values > 0.05). For the other indexes, there were no significant difference among all the experimental groups in terms of moisture (12.00% ~ 12.15%), water absorption (63.37% ~ 65.70%), and hardness (72.79 ~ 73.58 g/cm^2) (P value > 0.05).

***Effect of CPPs on the amino acid compositions of wheat "Zhengmai 9023 grains*:** To evaluate the effects of CPPs on the amino acid compositions of wheat "Zhengmai 9023" grains, the contents of sixteen amino acids were determined after the wheat harvest. As shown in Table 2, after decreasing chemical fertilizer amounts by 40%, the total amino acid content of the RF-40 grains was 7.64 mg/100 g, which was lower than that of the control grains (a 19.58% decrease). Except for Met and Arg, which showed a 20.00% increase and

Table 2. Effects of CPPs on amino acid compositions of wheat grains for wheat Zhengmai 9023.

Amino acids	Control*	RF-40*	RF-40-CPPs*	Relative differences		
				RF-40 *vs* Control	RF-40-CPPs *vs* RF-40	RF-40-CPPs *vs* Control
Total	9.50	7.64	9.39	-19.58%	22.91%	-1.16%
Met	0.10	0.12	0.17	20.00%	41.67%	70.00%
Ile	0.28	0.26	0.38	-7.14%	46.15%	35.71%
Lys	0.28	0.27	0.31	-3.57%	14.81%	10.71%
Val	0.45	0.40	0.48	-11.11%	20.00%	6.67%
Leu	0.64	0.54	0.66	-15.63%	22.22%	3.13%
Phe	0.49	0.38	0.49	-22.45%	28.95%	0.00%
Thr	0.30	0.26	0.30	-13.33%	15.38%	0.00%
Arg	0.37	0.37	0.43	0.00%	16.22%	16.22%
Ala	0.35	0.31	0.37	-11.43%	19.35%	5.71%
Pro	1.05	0.83	1.08	-20.95%	30.12%	2.86%
Gly	0.41	0.35	0.42	-14.63%	20.00%	2.44%
Asp	0.48	0.42	0.49	-12.50%	16.67%	2.08%
His	0.22	0.19	0.22	-13.64%	15.79%	0.00%
Glu	3.22	2.48	3.05	-22.98%	22.98%	-5.28%
Ser	0.50	0.40	0.46	-20.00%	15.00%	-8.00%
Tyr	0.16	0.06	0.08	-62.50%	33.33%	-50.00%

*, mg/100 g; Control, normal chemical fertilizer application; RF-40, reduction of chemical fertilizer application by 40%; RF-40-CPPs, reduction of chemical fertilizer application by 40% and dressing with CPPs at 7.5 kg/666.7 m^2 during the wheat heading period.

Table 3. Diversity indexes of the microbial communities in different domestication systems.

Sample ID		Effective Tags	OTUs		Chao1	Shannon
			phylum	genus		
RF-40	RF-40_A	73782	22	367	1505.45	6.07
	RF-40_B	74392	22	368	1537.93	5.98
	RF-40_C	64334	22	367	1507.24	6.33
RF-40-CPPs	RF-40-CPPs_A	72013	22	370	1534.18	6.40
	RF-40-CPPs _B	71944	22	368	1559.89	6.29
	RF-40-CPPs _C	73359	22	366	1549.94	6.28

OTUs, operational taxonomic unit; RF-40, reduction of chemical fertilizer application by 40%; RF-40-CPPs, reduction of chemical fertilizer application by 40% and dressing with CPPs at 7.5 kg/666.7 m^2 during the wheat heading period.

no change, respectively, all other fourteen amino acids in the RF-40 grains exhibited a 3.57% ~ 62.50% increase when compared with those of the control grains. Fortunately, after CPPs application during the wheat heading period, the total amino acid content of the RF-40-CPPs grains recovered to 9.39 mg/100 g, which was higher than for the RF-40 grains (a 22.91% increase), but it was still slightly lower than that of the control grains (a 1.16% decrease). Correspondingly, all sixteen amino acids in the RF-40-CPPs grains exhibited 14.81% ~ 46.15% increases when compared with those of RF-40 grains. Interestingly, five essential amino acids for humans, namely, Leu, Val, Lys, Ile, and Met, in the RF-40-CPPs grains showed 3.13% ~ 70.00% increases when compared with those in the control grains. The other two essential amino acids for humans, Thr and Phe, showed no differences between these grains. For other amino acids, the Asp, Gly, Pro, Ala, and Arg contents of the RF-40-CPPs grains exhibited 2.08% ~ 16.22% increases and Glu, Ser, and Tyr showed 5.28%-50.00% decreases when compared with those of the control grains; moreover, His showed no difference between these grains.

Effect of CPPs on the diversity of the rhizosphere soil bacterial community of wheat "Zhengmai 9023**:** The rhizosphere soils of the RF-40 and RF-40-CPPs wheats were collected during the grain filling stage for analysis of the rhizosphere soil bacterial community diversities of wheat "Zhengmai 9023". As shown in Table 3, there were 429824 (ranging from 64334 to 74392) total effective tags for the six samples. The OTUs in the six samples were all detected at the phylum level (22), and 366 to 370 were detected at the genus level. The Chao1 and Shannon diversity indexes of the RF-40-CPPs rhizosphere soil were higher than those of the RF-40 rhizosphere soil; however, there was no significant difference between these values (*P* value > 0.05). These results indicate that the microbial community diversity of the RF-40-CPPs rhizosphere soil during the grain filling stage was slightly enriched after application of CPPs during the wheat heading period compared to the RF-40 rhizosphere soil (Grice *et al.*, 2009).

Microbial communities were identified at the genus level, and the heat maps of flora composition after clustering analysis for those genera with the top 20 most abundant genera are shown in Figure 2. The dominant bacteria with the highest relative abundances in the RF-40 rhizosphere soil were *Mucilaginibacter* (6.70%), uncultured_ *Saccharimonadales* (6.27%), *Rhodanobacter* (5.16%), *Sphingomonas* (4.30%), *Lysinimonas* (2.50%), uncultured *Gaiellales* (2.29%), and uncultured *Micropepsaceae* (2.15%).

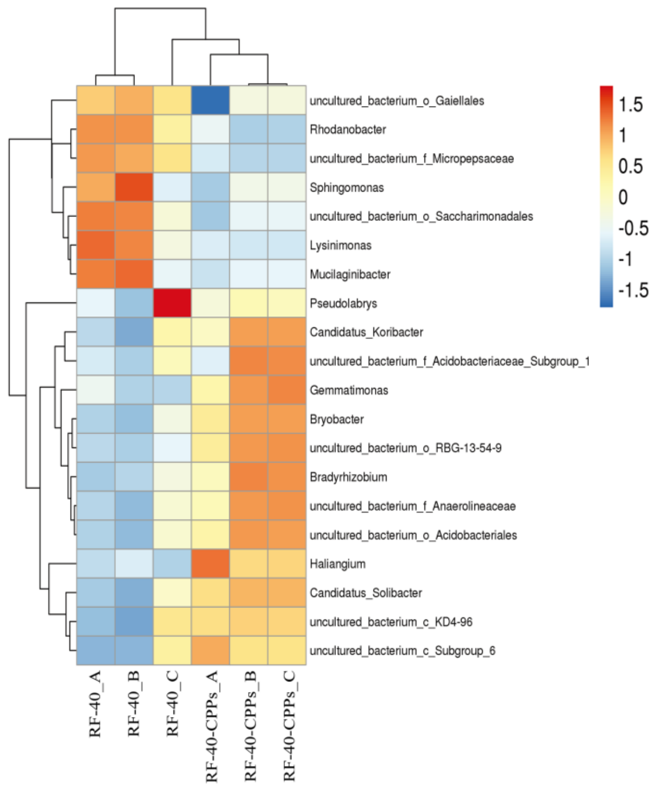

Figure 2. Effect of CPPs on heat maps of microflora compositions after clustering analysis for the top 20 most abundant genera in the rhizosphere soil of wheat "Zhengmai 9023". Genera with higher abundances in the corresponding samples are shown in red, while genera with lower abundances are shown in blue. RF-40, reduction in chemical fertilizer application by 40%; RF-40-CPPs, reduction in chemical fertilizer application by 40% and dressing with CPPs at

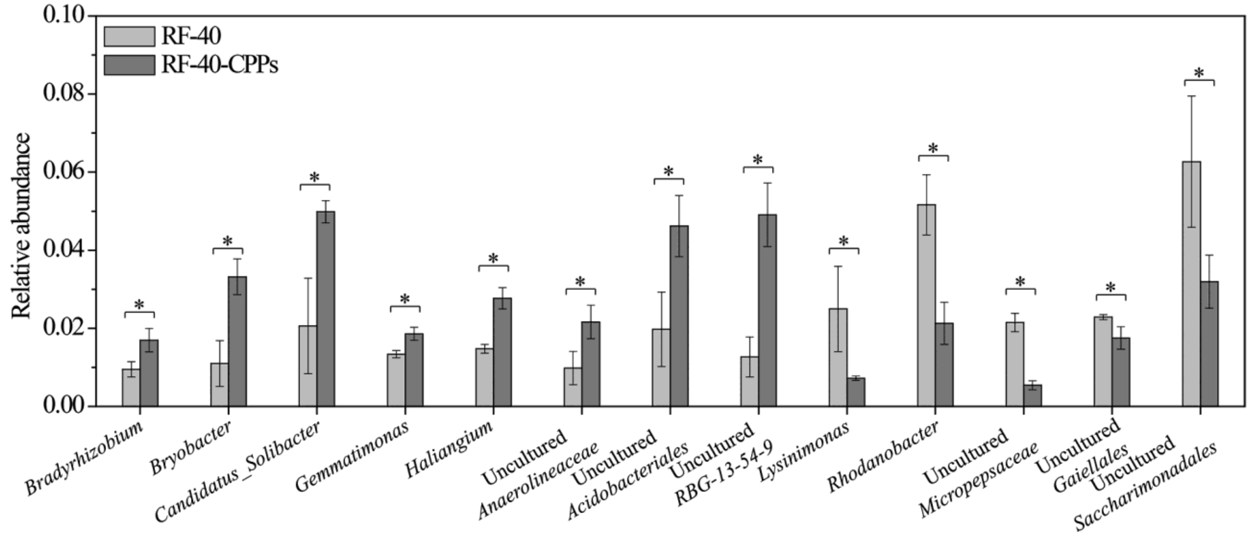

Figure 3. Comparison of bacterial communities at the genus level. *, Statistically significant difference in sample abundance in RF-40-CPPs compared to RF-40. RF-40, reduction in chemical fertilizer application by 40%; RF-40-CPPs, reduction in chemical fertilizer application by 40% and dressing with CPPs at 7.5 kg/666.7 m^2 during the wheat heading period.

After the application of CPPs, the top seven dominant bacteria in the RF-40-CPPs rhizosphere soil were *Candidatus_ Solibacter* (4.99%), uncultured *RBG-13-54-9* (4.91%), uncultured *Acidobacteriales* (4.62%), *Bryobacter* (3.32%), uncultured *Saccharimonadales* (3.19%), *Sphingomonas* (3.10%), and *Mucilaginibacter* (3.02%). Comparisons of the bacterial communities of rhizosphere soils at the genus level are shown in Fig. 3.

After the application of CPPs, the relative abundances of some bacteria, including *Bradyrhizobium* (*P* value = 0.023), *Bryobacter* (*P* value = 0.007), *Candidatus_Solibacter* (*P* value = 0.016), *Gemmatimonas* (*P* value = 0.009), *Haliangium* (*P* value = 0.002), uncultured *Anaerolineaceae* (*P* value = 0.028), uncultured *Acidobacteriales* (*P* value = 0.021), and uncultured *RBG-13-54-9* (*P* value = 0.003), significantly increased, whereas the relative abundances of certain other bacteria, including *Lysinimonas* (*P* value = 0.048), *Rhodanobacter* (*P* value = 0.005), uncultured *Micropepsaceae* (*P* value = 0.000), uncultured *Gaiellales* (*P* value = 0.034), and uncultured *Saccharimonadales* (*P* value = 0.043), significantly decreased.

In the PCoA based on weighted UniFrac distances, each point represents a sample, and the distances between two points were shorter, which indicate that the microbial communities among the samples were more similar (Sakaki *et al.*, 1994). As shown in Figure 4, the bacterial communities in the RF-40 rhizosphere soil showed differences in the parallel samples, whereas the bacterial communities of the RF-40-CPPs rhizosphere soil showed similarities in the parallel samples. Moreover, there were differences between these two experimental group samples, which indicated that the rhizosphere bacterial communities not only changed but were also enriched in the same direction after application of CPPs.

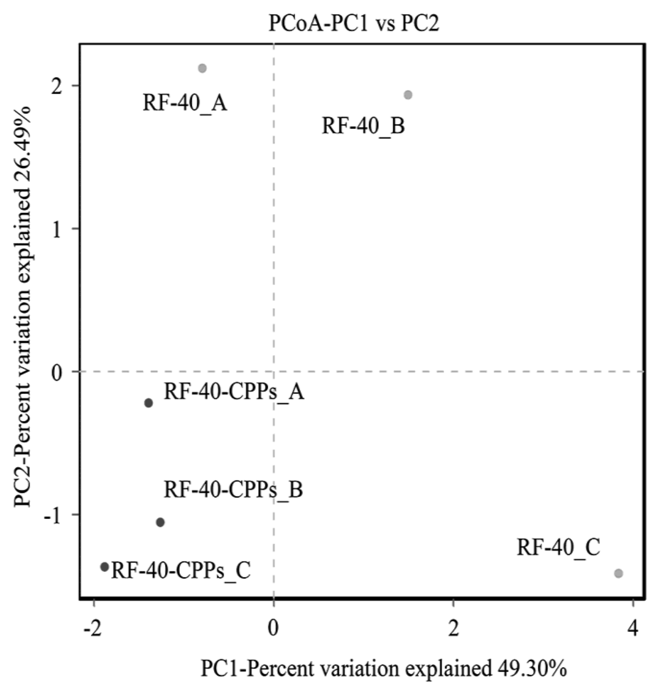

Figure 4. PCoA of rhizosphere soil community similarities of wheat "Zhengmai 9023" based on weighted UniFrac distances. RF-40, reduction in chemical fertilizer application by 40%; RF-40-CPPs, reduction in chemical fertilizer application by 40% and dressing with CPPs at 7.5 kg/666.7 m^2 during the wheat heading period.

7.5 kg/666.7 m^2 during the wheat heading period.

DISCUSSION

Partial replacement of chemical fertilizers with organics ones can reduce both environmental concerns and economic costs and provide greater soil health benefits (Jatav *et al.*, 2022). Previously, CPPs that were prepared by the recovery of waste vegetable meals by mixing with quicklime at high temperature have clearly been found to inhibit enrichment of heavy-metal Cd^{2+} in *B. campestris* L. and promote the growth of some vegetables (Chen *et al.*, 2019). In this study, the effects of CPPs on wheat yields, grain quality, and rhizosphere soil microbial communities after reducing chemical fertilizer application under field conditions were studied. In this study, after dressing CPPs by 7.5 kg (half a bag)/666.67 m^2 during the wheat heading period, the yield of the RF-40-CPPs also increased, which matched the corresponding 1000-grain weight increase. Similarly, a previous field experiment during 26 October, 2018 to 01 June, 2019 (218 days) showed that after dressing CPPs with 15.0 kg (one bag)/666.67 m^2 during the wheat heading period and after reducing the chemical fertilizer application by 20%, the wheat yield increased by 14.62% compared with the control (data not shown). This phenomenon may be similar to the results of previously reported that application of organic fertilizer (humic acid) can enhance wheat chemical fertilizer uptake, promote wheat growth, and increase 1000-grain weight and yield (Shafi *et al.*, 2020).

As one of the important indexes of wheat grain (Akhlaq *et al.*, 2022), the wet gluten content of wheat grains improved after application of CPPs (and only reached the strong flour level, wet gluten content $\geq$ 30.0%). After dressing with CPPs, the wheat grain protein contents and amino acid contents significantly increased, and these findings were similar to the results of previously reported that hydrolyzed wool application can increase wheat grain yields and grain protein contents (Gogos *et al.*, 2013). Moreover, we further found that application of CPPs can change the amino acid compositions of wheat grains, particularly because some amino acids that are essential for humans were increased, which indicated that CPPs can improve the quality and nutritional value of wheat.

The α-diversity indices Chao1 and Shannon reflected the richness and diversity of the bacterial community (Sun *et al.*, 2022). The application of CPPs did not notably change the richness and diversity of the rhizosphere soil bacterial community of wheat “Zhengmai 9023”. In our field experiment, *Bradyrhizobium* sp., *Gemmatimonas* sp., *Haliangium* sp., *Bryobacter* sp., and *Candidatus_Solibacter* were significantly enriched in wheat rhizosphere soils after application of protein hydrolysate CPPs. For the nitrogen-fixing bacteria *Bradyrhizobium* sp. (Amy *et al.*, 2022), a similar report showed that it was increased significantly in the sweet potato rhizosphere after the application of a protein hydrolysate that was prepared from organic fish fertilizer and poultry litter (Lindsey *et al.*, 2019). The plant-beneficial potential bacteria *Haliangium* sp. (Xue *et al.*, 2020) was reported to actively contribute to denitrification in anaerobic wetlands and to be involved in soil nitrogen cycling (McIlroy *et al.*, 2016; Lévesque *et al.*, 2020). *Gemmatimonas* sp. and *Bryobacter* sp. are known to contribute to the decomposition of organic matter in soil, such as cellulose and lignin (Guo *et al.*, 2016; Liu *et al.*, 2020). *Candidatus_Solibacter* are aerobic chemoorganotrophs that can hydrolyze several polysaccharides and organic acids (Chen *et al.*, 2020) and can also degrade cellulose substrates (Zhang *et al.*, 2019). Therefore, CPPs application to wheat can probably change soil microbial community compositions, contribute to cycling of carbon and nitrogen in the soil, and improve soil nutrient utilization efficiency.

The utilization of the plant growth-promoting rhizobacteria (PGPR) as an organic fertilizer additive can also help reduce the use of chemical fertilizers in plants (Azmat *et al.*, 2022; Kumawat *et al.*, 2022). In addition, *E. bugandensis* TJ6, which was isolated from heavy-metal-contaminated lettuce rhizosphere soil, exhibited synergistic effects with protein hydrolysates for safe wheat production in Cd^{2+}-contaminated soil (Han *et al.*, 2020). Therefore, the combined utilization of CPPs with PGPR for wheat production in the field deserves further studied in the future.

***Conclusions*:** The application of CPPs prepared from waste vegetable meals by mixing with quicklime at high temperatures can enrich the microflora involved in soil carbon and nitrogen metabolism and improve grain quality for wheat “Zhengmai 9023”, which indicates that CPPs have the potential for green wheat production and reducing chemical fertilizer application.

***Conflict of Interest*:** The Authors declare that there is no conflict of interest.

Authors’ Contribution Statements: XS, YX and SL executed the field research, XJ and XX few laboratory analyses, whereas HN and XS conceived the idea and supervised the work.

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REFERENCES

Akhlaq, M., S.W. Ali, A. Murtaza, M.A. Javed, A. Ahmad Khan and R.M. Amir. 2022. Impact of initial grain moisture, fumigation, and storage period on physicochemical characteristics of wheat in Pakistan.

Journal of Food Processing and Preservation. 46:e16387.

Amy, C., J.C. Avice, K. Laval and M. Bressan. 2022. Are native phosphate-solubilizing bacteria a relevant alternative to mineral fertilizations for crops? Part II: PSB inoculation enables a halving of P input and improves the microbial community in the rapeseed rhizosphere. Rhizosphere. 21:100480.

Azmat, A., Y. Tanveer, H. Yasmin, M.N. Hassan, A. Shahzad, M. Reddy and A. Ahmad. 2022. Coactive role of zinc oxide nanoparticles and plant growth promoting rhizobacteria for mitigation of synchronized effects of heat and drought stress in wheat plants. Chemosphere. 297:133982.

Bhavsar, P., M. Zoccola, A. Patrucco, A. Montarsolo, R. Mossotti, G. Rovero, M. Giansetti and C. Tonin. 2016. Superheated water hydrolysis of waste wool in a semi-industrial reactor to obtain nitrogen fertilizers. ACS Sustainable Chemistry & Engineering. 4:6722-6731.

Cheema, M.W.A., T. Rasool, H. Munir, M.M. Iqbal, T. Naz, M.I.U. Haq, A. Mustafa, M. Nadeem and S. Ullah. 2020. Weed control in wheat through different sorghum formulations as an organic herbicide. Asian Journal of Agriculture and Biology 8:129-137.

Chen, H., F. Shu, S. Yang, Y. Li and S. Wang. 2019. Competitive inhibitory effect of calcium polypeptides on Cd enrichment of *Brassia campestris* L. International Journal of Environmental Research and Public Health. 16:4472.

Chen, J., Q. Guo, D. Liu, C. Hu, J. Sun, X. Wang, G. Liang and W. Zhou. 2020. Composition, predicted functions, and co-occurrence networks of fungal and bacterial communities_ Links to soil organic carbon under long-term fertilization in a rice-wheat cropping system. European Journal of Soil Biology. 100:103226.

Cholakova-Bimbalova, R., V. Petrov and A. Vassilev. 2019. Photosynthetic performance of young maize (*Zea mays* L.) plants exposed to chilling stress can be improved by the application of protein hydrolysates. Acta Agrobotanica. 72:1769.

Corte, L., M.T. Dell'abate, A. Magini, M. Migliore, B. Felici, L. Roscini, R. Sardella, B. Tancini, C. Emiliani, G. Cardinali and A. Benedetti. 2014. Assessment of safety and efficiency of nitrogen organic fertilizers from animal-based protein hydrolysates--a laboratory multidisciplinary approach. Journal of the Science of Food and Agriculture. 94:235-245.

Edgar, R.C. 2013. UPARSE: highly accurate OTU sequences from microbial amplicon reads. Nature Methods. 10:996-998.

Fan, M., J. Shen, L. Yuan, R. Jiang, X. Chen, W.J. Davies and F. Zhang. 2012. Improving crop productivity and resource use efficiency to ensure food security and environmental quality in China. Journal of Experimental Botany. 63:13-24.

Gálvez, R.P., F.J.E. Carpio, E.M. Guadix and A. Guadix. 2016. Artificial neural networks to model the production of blood protein hydrolysates for plant fertilisation. Journal of the Science of Food and Agriculture. 96:207-214.

Gogos, A., M.W.H. Evangelou, A. Schäffer and R. Schulin. 2013. Hydrolysed wool: A novel soil amendment for zinc and iron biofortification of wheat. New Zealand Journal of Agricultural Research. 56:130-141.

Grice, E.A., H.H. Kong, S. Conlan, C.B. Deming, J. Davis, A.C. Young, G.G. Bouffard, R.W. Blakesley, P.R. Murray, E.D. Green, M.L. Turner and J.A. Segre. 2009. Topographical and temporal diversity of the human skin microbiome. Science. 324:1190-1192.

Guo, L., S. Zheng, C. Cao and C. Li. 2016. Tillage practices and straw-returning methods affect topsoil bacterial community and organic C under a rice-wheat cropping system in central China. Scientific Reports. 6:33155.

Habib, M., F.S. Awan, B. Sadia and M.A. Zia. 2020. Genetic dissection of yield enhancing traits in Pakistani spring wheat genotypes. Pakistan Journal of Agricultural Sciences. 57:1243-1251.

Han, H., X. Wu, L. Yao and Z. Chen. 2020. Heavy metal-immobilizing bacteria combined with calcium polypeptides reduced the uptake of Cd in wheat and shifted the rhizosphere bacterial communities. Environmental Pollution. 267:115432.

Holkar, C.R., A.J. Jadhav, P.S. Bhavsar, S. Kannan, D.V. Pinjari and A.B. Pandit. 2016. Acoustic cavitation assisted alkaline hydrolysis of wool based keratins to produce organic amendment fertilizers. ACS Sustainable Chemistry & Engineering. 4:2789-2796.

Jatav, S.S., S.K. Singh, M. Parihar, A.M. Alsuhaibani, A. Gaber and A. Hossain. 2022. Application of sewage Sludge in a rice (*Oryza sativa* L.)-wheat (*Triticum aestivum* L.) system influences the growth, yield, quality and heavy metals accumulation of rice and wheat in the northern gangetic alluvial plain. Life. 12:484.

Jie, M., W. Raza, Y.C. Xu and Q.R. Shen. 2008. Preparation and optimization of amino acid chelated micronutrient fertilizer by hydrolyzation of chicken waste feathers and the effects on growth of rice. Journal of Plant Nutrition. 31:571-582.

Kumawat, K.C., S. Nagpal and P. Sharma. 2022. Potential of plant growth-promoting rhizobacteria-plant interactions in mitigating salt stress for sustainable agriculture: A review. Pedosphere. 32:223-245.

Lévesque, V., P. Rochette, R. Hogue, T. Jeanne, N. Ziadi, M.H. Chantigny, M. Dorais and H. Antoun. 2020. Greenhouse gas emissions and soil bacterial community as affected by biochar amendments after periodic mineral fertilizer applications. Biology and Fertility of Soils. 56:907-925.

Lindsey, L., R. Shange, R.O. Ankumah, D.G. Mortley and S. Karki. 2019. Hydrolyzed organic fish fertilizer and poultry litter influence yield and rhizosphere ecology of sweetpotato. Hortscience. 54:941-947.

Liu, L., S. Li, J. Zheng, T. Bu, G. He and J. Wu. 2020. Safety considerations on food protein-derived bioactive peptides. Trends in Food Science & Technology. 96:199-207.

Liu, Z., J. Liu, Z. Yu, Q. Yao, Y. Li, A. Liang, W. Zhang, G. Mi, J. Jin, X. Liu and G. Wang. 2020. Long-term continuous cropping of soybean is comparable to crop rotation in mediating microbial abundance, diversity and community composition. Soil & Tillage Research. 197:104503.

Ma, L., G.L. Velthof, F.H. Wang, W. Qin, W.F. Zhang, Z. Liu, Y. Zhang, J. Wei, J.P. Lesschen, W.Q. Ma, O. Oenema and F.S. Zhang. 2012. Nitrogen and phosphorus use efficiencies and losses in the food chain in China at regional scales in 1980 and 2005. The Science of the Total Environment. 434:51-61.

McIlroy, S.J., A. Starnawska, P. Starnawski, A.M. Saunders, M. Nierychlo, P.H. Nielsen and J.L. Nielsen. 2016. Identification of active denitrifiers in full-scale nutrient removal wastewater treatment systems. Environmental Microbiology. 18:50-64.

Polo, J. and P. Mata. 2017. Evaluation of a biostimulant (pepton) based in enzymatic hydrolyzed animal protein in comparison to seaweed extracts on root development, vegetative growth, flowering, and yield of gold cherry tomatoes grown under low stress ambient field conditions. Frontiers in Plant Science. 8:2261.

Popko, M., R. Wilk, H. Górecka, K. Chojnacka and H. Górecki. 2015. Assessment of new NKSMg fertilizer based on protein hydrolysate of keratin in pot experiments. Polish Journal of Environmental Studies. 24:1765-1772.

Qamar, R., I. Anjum, A.U. Rehman, M.E. Safdar, H.M.R. Javeed, A. Rehman and Y. Ramzan. 2020. Mitigating water stress on wheat through foliar application of silicon. Asian Journal of Agriculture and Biology 8:1-10.

Ramette, A. 2007. Multivariate analyses in microbial ecology. FEMS Microbiology Ecology. 62:142-160.

Rouphael, Y., G. Colla, M. Giordano, C. El-Nakhel, M.C. Kyriacou and S. De Pascale. 2017. Foliar applications of a legume-derived protein hydrolysate elicit dose-dependent increases of growth, leaf mineral composition, yield and fruit quality in two greenhouse tomato cultivars. Scientia Horticulturae. 226:353-360.

Sakaki, T., T. Takeshima, M. Tominaga, H. Hashimoto and S. Kawaguchi. 1994. Recurrence of ICA-PCoA aneurysms after neck clipping. Journal of Neurosurgery. 80:58-63.

Schiavon, M., A. Ertani and S. Nardi. 2008. Effects of an alfalfa protein hydrolysate on the gene expression and activity of enzymes of the tricarboxylic acid (TCA) cycle and nitrogen metabolism in *Zea mays* L. Journal of Agricultural and Food Chemistry. 56:11800-11808.

Schmitt, E. and W. de Vries. 2020. Potential benefits of using Hermetia illucens frass as a soil amendment on food production and for environmental impact reduction. Current Opinion in Green and Sustainable Chemistry. 25:100335.

Segata, N., J. Izard, L. Waldron, D. Gevers, L. Miropolsky, W.S. Garrett and C. Huttenhower. 2011. Metagenomic biomarker discovery and explanation. Genome Biology. 12:R60.

Shafi, M.I., M. Adnan, S. Fahad, F. Wahid, A. Khan, Z. Yue, S. Danish, M. Zafar-ul-Hye, M. Brtnicky and R. Datta. 2020. Application of single superphosphate with humic acid improves the growth, yield and phosphorus uptake of wheat (*Triticum aestivum* L.) in calcareous soil. Agronomy. 10:1224.

Sun, Z., M. Lin, C. Du, Y. Hao, Y. Zhang and Z. Wang. 2022. The use of manure shifts the response of α-diversity and network while not β-diversity of soil microbes to altered irrigation regimes. Applied Soil Ecology. 174:104423.

Tong, X., M.U. Rehman, S. Huang, X. Jiang, H. Zhang and J. Li. 2018. Comparative analysis of gut microbial community in healthy and tibial dyschondroplasia affected chickens by high throughput sequencing. Microbial Pathogenesis. 118:133-139.

Wang, X., D. Liu, M. Wei and J. Man. 2020. Spraying 6-BA could alleviate the harmful impacts of waterlogging on dry matter accumulation and grain yield of wheat. Peer J. 8:e8193.

Xue, J., S. Yajing, W. Fengzhi, P. Kai and Z. Xingang. 2020. Intercropping of wheat changed cucumber rhizosphere bacterial community composition and inhibited cucumber *Fusarium* wilt disease. Scientia Agricola. 77:e2019000.

Zhang, X., Z. Zhong, F. Bian and C. Yang. 2019. Effects of composted bamboo residue amendments on soil microbial communities in an intensively managed bamboo (*Phyllostachys praecox*) plantation. Applied Soil Ecology. 136:178-183.