



Remediation of organic amendments on soil salinization: Focusing on the relationship between soil salts and microbial communities

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ABSTRACT

Soil salinization has emerged as a major factor with an adverse influence on agricultural green development worldwide. It is necessary to develop high-efficiency and ecologically beneficial management measures for alleviating soil salinization. The experiment of application for cow manure (CM), biochar (BC), and bio-organic fertilizer (BIO) in soil with light salinity was conducted to investigate the remediation of organic materials on soil salinization with melon (*Cucumis melo* L.) by reducing the availability of saline ions and shifting the soil microbial community. Results showed that BC treatment significantly decreased the EC values of the soil and soil solution by 19.23% and 27.02% and the concentrations of Na⁺, K⁺, and Cl⁻ by 13.28%, 13.08%, and 15.21%, respectively, followed by CM and BIO treatments. High-throughput sequencing identified that organic amendments significantly improved the richness of the soil bacterial community and increased the relative abundances of Acidobacteria and Firmicutes by 33.11% and 111.2%, respectively, and the beneficial salt-tolerant bacterial genera *Flavobacterium*, *Bacillus* and *Arthrobacter* by 32.04%, 38.92% and 35.67%, respectively. Additionally, soil Na⁺, Ca²⁺, K⁺ and Cl⁻ were significantly negatively correlated with Acidobacteria and *Flavobacterium* and were also the most important factors driving the changes in the structure of the soil bacterial communities. The bacterial networks were more complex in the organic amendments treatments than in CK, reflecting through more nodes and links and a higher average clustering coefficient, density and modularity. This study provided a comprehensive understanding of the application of organic amendments in alleviating soil salinization and improving soil bacterial and fungal communities and provides scientific support for agriculture green development.

1. Introduction

Soil salinization is a serious land degradation problem in most coastal regions around the world due to the use of saline water for irrigation (Chi et al., 2021; Huang et al., 2022; Sun et al., 2020). It is well known that soil salt accumulation leads to lower soil microbial diversity and biological activity and lower fertilizer use efficiency, which further inhibits crop growth and even the green development of agriculture (Shrivastava and Kumar, 2015; Singh, 2015; Sun et al., 2020; Xiao et al., 2020). It is urgent to develop ecologically beneficial management

measures for ameliorating soil salinity in coastal regions.

Appropriate land management is an important prerequisite for improving the soil quality and productivity of saline soil. In general, a large amount of organic residue and waste are produced in the world every year; more importantly, the improper disposal of these organic residues has led to severe environmental pollution and nutrient losses (Bai et al., 2016; Hazrati et al., 2020; Jia et al., 2018). Recycling these organic residues to farmland is a common agricultural practice for increasing soil fertility and agricultural productivity during decades (Meena et al., 2016; Sun et al., 2015; Wu et al., 2021). Organic

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amendments, such as livestock manure, plant residue and waste, and bio-organic fertilizer are better practices to reclaim saline soil by alleviating soil salinity, improving soil fertility and promoting crop growth (Chen et al., 2021; Cui et al., 2021; Huang et al., 2019; Leogrande and Vitti, 2018; Wu et al., 2019). It was reported that the application of reed biochar could decrease the concentrations of K^+ , Na^+ , Ca^{2+} , Mg^{2+} , Cl^- , and SO_4^{2-} in soil but increase the levels of soil available phosphorus, potassium and organic matter in coastal saline-alkali soil with rotation of wheat and maize (Cui et al., 2021; Xiao et al., 2020). Animal manure, as the most common and cheap organic fertilizer or amendment, is normally applied in farmland, such as manure from chickens, pigs and cows. The manure of chickens and pigs is not considered to combine with saline water irrigation, which can easily aggravate salt accumulation (Ould Ahmed et al., 2010; Yao et al., 2007), but the manure of cows has been confirmed to be effective in remediating saline soil with wheat, rice and saffron (Su et al., 2021; Yarami and Sepaskhah, 2015). It was also found that applying bio-organic fertilizer combined with soil conditioners is an alternative pathway to reduce salinity and optimize the functions of the microbial community in saline soil with radishes (Zhu et al., 2021).

Furthermore, the addition of organic amendments could also improve agricultural soil health by altering the soil microbiome. For example, applying the organic manure increased bacterial richness and modified network topological patterns with corn (Tian et al., 2017; Wang et al., 2021), and applying biochar and bio-fertilizer could change the composition of soil microbial communities, suppress pathogenic fungi while increasing beneficial bacteria for vegetables under plastic shed conditions (Ji et al., 2022; Zhu et al., 2021). In addition, a recent survey illustrated that soil salinity decreased the bacterial richness and complexity of the bacterial network, changed its community composition, and even directly damaged soil microorganisms (Guan et al., 2020; Yan et al., 2021). Therefore, the variations in the soil microbiome are also closely related to the soil salinity. However, little is known whether the application of organic amendments might improve the diversity, composition and structure of soil microbial community in lightly saline soil.

The melon (*Cucumis melo* L.), an economically important Cucurbitaceae crop worldwide and is usually monocultured in plastic sheds, especially in northern China (Murakami et al., 2017; Zhang et al., 2021a). Qing County of Hebei Province, located in the coastal area of the Bohai Rim region, is well known as the most important melon-producing area in North China. Considering light soil salinization caused by seawater intrusion, coupled with closed plastic shed conditions, resulted in salt accumulation, which adversely affected the sustainable production system of melon (Zhang and Li, 2021; Zhang et al., 2021b). Hence, the application of organic amendments such as cow manure, biochar and bio-organic fertilizer may offer a feasible, highly efficient sustainable measure to reduce soil salinity and improve soil quality and the microbial community under plastic shed conditions. It was hypothesized that the application of organic amendments could mitigate soil salinity and then alter the soil microbial community, thus promoting the growth of melon. Therefore, a pot experiment was conducted to explore whether the application of cow manure (CM), biochar (BC), and bio-organic fertilizer (BIO) alleviates the adverse impact of soil salinity on melon growth and development through the change of soil microbial community. The objectives of this study were to clarify whether the application of organic amendments reduces soil salinity and promotes plant growth in saline soil, to investigate the application of organic amendments on the composition, diversity and structure of soil microbial communities in saline soil, and finally to ascertain the relationship between the application of organic amendments and the variation in soil microbial communities in saline soil cultivated with melon.

2. Materials and methods

2.1. Description of soil characteristics

Soil was collected from the top layer of 0–20 cm in a melon (*Cucumis melo* L.) plastic shed located in Qing County, Hebei Province, China (38°31'N, 116°44'E), which is the superior area of plastic shed melon with good quality fruits and is approximately 60 km from the coast of Bohai. The collected soil was thoroughly mixed, and the roots and stones were removed, air dried and ground to pass through a 5-mm sieve for the setup of the pot experiment. The soil type is classified as a typical fluvo-aquic soil, and the properties of 0–20 cm topsoil are shown in Table S1. The soil was slightly salinized soil with an average total dissolved salt of 2.15 g kg⁻¹ according to the classification of saline soil in China.

2.2. Pot experiment

A pot experiment was conducted with four treatments: (1) without application of any materials (CK), (2) application of cow manure at the rate of 6 t ha⁻¹ (CM), (3) application of biochar at the rate of 1.5 t ha⁻¹ (BC), and (4) application of biological organic fertilizer at the rate of 6 t ha⁻¹ (BIO).

The cow manure (Saiyuan Biotechnology Co., Ltd. Hebei Province, China) contained 45% organic matter, 3% N, 1% P₂O₅ and 1% K₂O. The biochar was derived from apricot shell at a temperature of 900 °C (Chengde Huajing Activated Carbon Co., Ltd., Hebei Province, China), which contained 54.81% total carbon, 0.75% total nitrogen, 348 mg kg⁻¹ available phosphorus and 6970 mg kg⁻¹ available potassium. The pH was 10.42, and the EC was 0.91 ms cm⁻¹. The bio-organic fertilizer (Runwo Biotechnology Co. Ltd. Hebei Province, China) contained *Bacillus subtilis* and *Paenibacillus mucilaginosus*, and the number of effective bacteria was more than 2 × 10⁷ CFU g⁻¹, containing more than 40% organic matter and a total nutrient of 5%, in which the ratio of N:P₂O₅:K₂O was 3:1:1. Pot experiment was established in a randomized block design with 12 replicates. Each plastic pot (15 cm × 19 cm) was packed with 1.5 kg (dry weight) soil. By adding NPK fertilizer, the contents of N, P₂O₅ and K₂O in each treatment were equal (Table S2). Organic amendments and fertilizer were adequately mixed before sowing. Soil solution were collected by a Rhizon soil solution sampling device (Rhizon MOM 10 cm length, 2.5 mm OD, Rhizosphere Research Products, Wageningen, The Netherlands).

Two seeds of melon were sown in each pot, and one strong seedling was kept per pot after two weeks. The melon seedlings were irrigated with saline water containing 2.98 mmol L⁻¹ Na⁺, 0.98 mmol L⁻¹ HCO₃⁻, 0.50 mmol L⁻¹ SO₄²⁻, and 1.00 mmol L⁻¹ Cl⁻, which were the same concentrations as the local irrigation water in Qing County. The melon was irrigated with 500 mL of water every 3–5 days depending on evaporation and transpiration. The pot experiment was carried out in a growth chamber with 280 μmol m⁻² s⁻¹ light intensity and 25 °C/20 °C day/night temperatures, and the photoperiod was 14 h.

2.3. Sampling and analyses of soil and plants

The soil samples were collected at week 2 and week 8 after sowing in each pot, and each sample was divided into two parts. A portion was immediately frozen with liquid nitrogen and stored at -80 °C for total DNA extraction. The other was air-dried and stored at room temperature and passed through a 2-mm sieve to analyze soil chemical properties. Concentrations of soil saline ions (K^+ , Na^+ , Ca^{2+} , Mg^{2+} , CO_3^{2-} , HCO_3^- , Cl^- , SO_4^{2-}) in soil extracts were determined according to the China forestry standard No. LYT 1251-1999 (Yue et al., 2020; Zhou et al., 2019). The EC of the soil (SEC) was measured using an EC meter (soil/water=1:5) (Han et al., 2015). Soil solution was collected from each pot at weeks 2 and 8 after sowing to determine the EC value (SSEC) (Wang et al., 2022).

The plant samples were collected at weeks 2 and 8 using destructive sampling methods in each pot for the measurement of the shoot height,

root length, and dry weight of shoot and root of melon. The shoot tolerance index (STI) was calculated by the ratio of the shoot height of the treatment to the control, and the root tolerance index (RTI) was calculated by the ratio of the root length of the treatment to the control (Zhang et al., 2018).

2.4. Soil DNA extraction and Illumina MiSeq sequencing

A total of 24 soil samples for week 2 and week 8 were used for DNA extraction, and the details were described as Zhao et al. (2021). Primers 338F (5'-ACTCCTACGGGAGGCAGCAG-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3') (Mori et al., 2014) were used to amplify the V4-V5 regions of bacterial 16S rRNA genes. Primers for ITS1F (5'-CTTGGTCATTTAGAGGAAGTAA-3') and ITS2R (5'-GCTGCGTTCCTCATCGATGC-3') (Adams et al., 2013) were used to amplify the fungal ITS genes. Polymerase chain reaction (PCR) and details of process of 16S rRNA/18S rRNA gene amplification and sequence analysis can be found in Zhao et al. (2020). The raw reads were deposited into the NCBI Sequence Read Archive (SRA) database (Accession Number: SRP369960). More details can be found in the supplementary files.

2.5. Statistical analysis

The statistical analysis of all data was tested using SPSS software (Version 19.1), and significant differences were followed by one-way ANOVA by Tukey's post-hoc test. Spearman's correlation analysis was performed in Origin 2021b to establish the relationship between plant growth and soil salt indicators. Soil microbial alpha diversity between treatments were analyzed by calculating the richness index (ACE) and diversity index (Shannon) using Mothur. Principal coordinate analysis (PCoA) was used to distinguish differences in microbial community structure between the samples. Redundancy analysis (RDA) was used to investigate the relationship between soil microbial communities based on the predominant phyla and soil environmental factors (EC, K^+ , Na^+ , Ca^{2+} , Mg^{2+} , CO_3^{2-} , HCO_3^- , Cl^- , SO_4^{2-}).

Co-occurrence network analyses based on Spearman's rank were performed to examine the relationship and interactions between different microbial taxa, using abundance data of microbial communities at the genus level. The relative abundances of bacterial and fungal genera were used to construct respective networks for the CK, CM, BC, and BIO treatments, and those genera with average relative abundances $>0.1\%$ were selected. The co-occurrence patterns were explored based on strong (Spearman's $r > |0.9|$) and significant correlations ($P < 0.01$). Gephi (<http://gephi.org/>) was used to visualize network graphs with a Fruchterman-Reingold layout, and the network analyzer tool was used to calculate network topology parameters (Ji et al., 2021).

3. Results

3.1. Plant growth

Organic amendments promoted the growth of melon in the early stage (week 2) after application, but no significant change was observed at week 8 (Table S3). At week 2, the BC, CM and BIO treatments significantly increased RDW by 80.63%–85.57%; additionally, the CM treatment significantly improved SDW by 65.09% compared with CK ($P < 0.05$) (Table S3).

The shoot/root tolerance index (TI) means the ratio of the melon shoot/root height of the organic amendments treatment to the control, which represents the tolerance of melon plants to different salinity soil after the application of organic amendments. The results showed that BIO treatment had the highest RTI, which was 1.22 and 1.18 at week 2 and week 8, respectively. In addition, the tolerance of shoots was also improved by applying organic amendments, and the effect was better at week 2 than at week 8 (Table S3). The results showed that the melon

under different organic amendments had different adaptation mechanisms of the tolerance index to salt stress.

3.2. Soil salinity

Compared with CK, organic amendments significantly decreased the SEC and SSEC. At week 2, the BC treatment significantly decreased the SEC and SSEC by 13.30% and 22.73%, respectively, while the CM and BIO treatments only decreased the SSEC by 17.23% and 7.13%, respectively ($P < 0.05$) (Fig. 1A & B). At week 8, all the organic amendments treatments significantly decreased the SEC and SSEC by 16.77%–25.15% and 15.56%–33.75%, respectively ($P < 0.05$) (Fig. 1A & B). Additionally, the SSEC in the CM and BC treatments was significantly lower than that in the BIO treatment at week 8 ($P < 0.05$) (Fig. 1B). The Spearman's correlation showed that there was a significant positive correlation between SEC and SSEC ($P < 0.01$) (Fig. 2 & Table S4).

Soil saline ions are the main index to evaluate the degree and characteristics of soil salinization. The addition of organic amendments decreased the concentrations of soil Na^+ , Ca^{2+} , K^+ , Mg^{2+} , SO_4^{2-} , and Cl^- (Fig. 1C-H). For the cations, Na^+ was dominant in all soil samples, accounting for 48.91% of the total cation content, followed by Ca^{2+} , K^+ and Mg^{2+} (Fig. 1C-F). At week 2, BC treatment significantly decreased the concentrations of all cations by 10.30%–19.52% compared with CK ($P < 0.05$). While, CM treatment only decreased the concentrations of Ca^{2+} and K^+ by 15.20% and 9.75%, and BIO treatment only decreased the concentrations of Na^+ and Ca^{2+} by 12.88% and 8.30%, respectively (Fig. 1C-F). At week 8, the three organic amendments significantly decreased the concentrations of Na^+ by 13.98%–19.97% and K^+ by 11.88%–13.13% ($P < 0.05$) (Fig. 1C & E).

Among the anions, SO_4^{2-} was the dominant anion in the soil, accounting for 52.52% of the total anion content, followed by HCO_3^- and Cl^- (Fig. 1G-I), and CO_3^{2-} was not found in any sample for easy hydrolysis to HCO_3^- . Compared with CK, BC treatment significantly decreased the concentrations of SO_4^{2-} and Cl^- by 21.08% and 12.50%, respectively, while the CM and BIO treatments only decreased the concentration of Cl^- by 18.18% and 22.73% at week 2, respectively. ($P < 0.05$) (Fig. 1G & H). At week 8, the CM and BC treatments significantly decreased the concentrations of Cl^- by 17.13% and 17.93%, respectively, while BIO treatment decreased the concentrations of SO_4^{2-} by 19.51%. ($P < 0.05$) (Fig. 1G & H). There was no significant difference in HCO_3^- between CK and treatments ($P < 0.05$) (Fig. 1I).

Spearman's correlation was conducted to explore the relationship between plant growth and soil salinization (Fig. 2 and Table S4). SH, RL, SDW and RDW were negatively correlated with the SEC and SSEC, all cations and Cl^- . This result indicated that the accumulation of soil saline ions inhibited the growth of melon in lightly saline soil. There was a significantly positive correlation between SSEC and SEC and Na^+ , Ca^{2+} , K^+ , Mg^{2+} , and Cl^- , which suggested that cations are predominant forms in soil salinity.

3.3. Diversities of soil bacterial and fungal communities

Based on the results that the application of organic amendments alleviated the soil salinity and improved plant growth, high-throughput sequencing was conducted to investigate the impact of organic material application on the diversities of soil microbial communities.

The bacterial and fungal community diversity (Shannon index) and richness (ACE index) were estimated to represent the α -diversities of the soil microbial communities. Compared with CK, the CM, BC and BIO treatments increased the Shannon index of bacteria by 3.23%–4.16% at week 2 but did not significantly change the Shannon index at week 8 (Table 1). Additionally, CM, BC and BIO treatments increased the ACE index by 8.42%–11.18% at week 8, and only BC treatment significantly increased the ACE index by 14.00% at week 2 ($P < 0.05$). For the fungal community, there was no significant difference in the Shannon and ACE

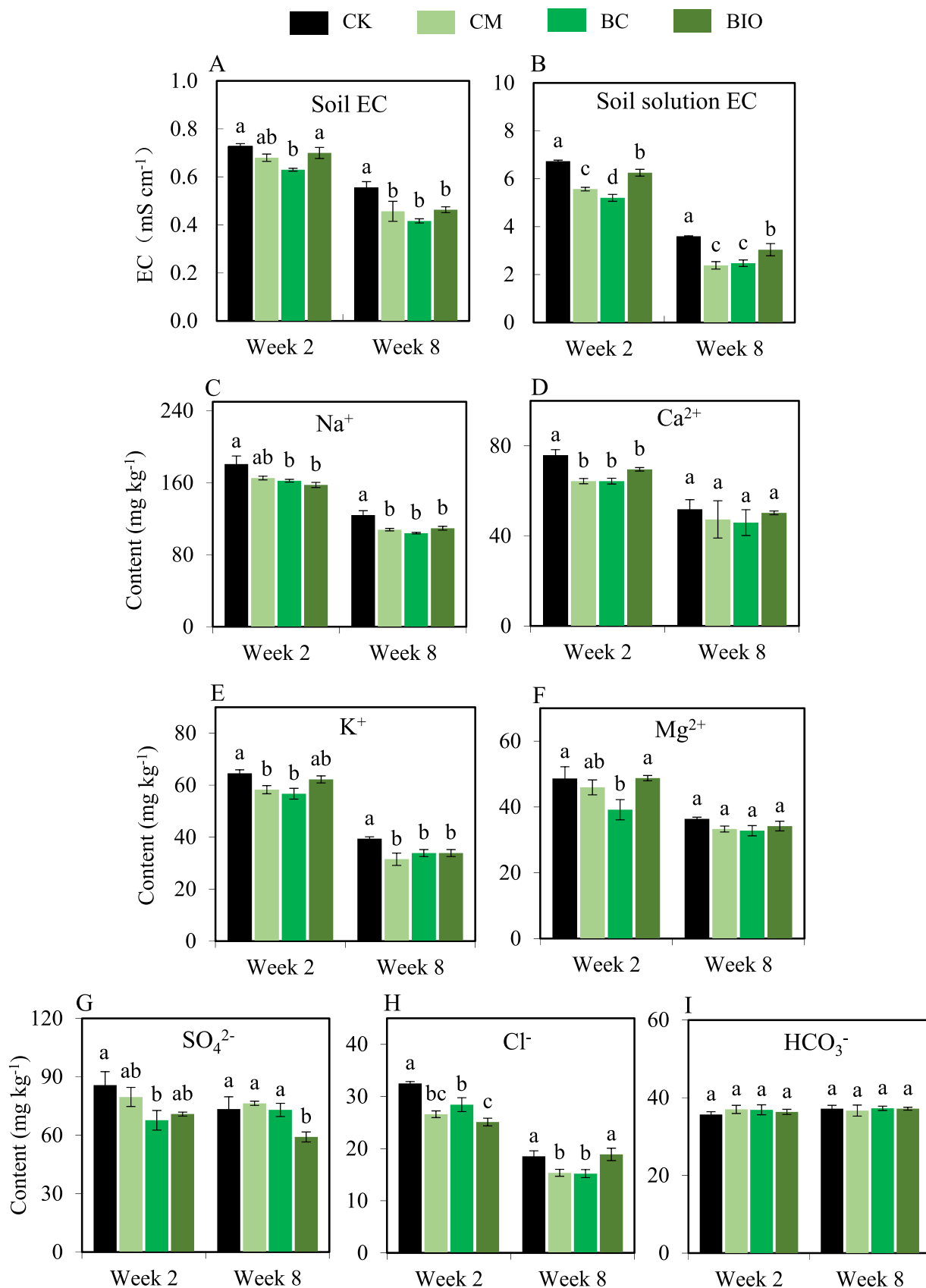


Fig. 1. Variations in soil EC (A), soil solution EC (B), soil Na⁺ (C), Ca²⁺ (D), K⁺ (E), Mg²⁺ (F), SO₄²⁻ (G), Cl⁻ (H), HCO₃⁻ (I) concentrations under the organic amendments treatments (means ± SE, n = 3). Different lowercase letters at each sampling time indicate significant differences among different treatments at P < 0.05 according to Tukey's post-hoc test.

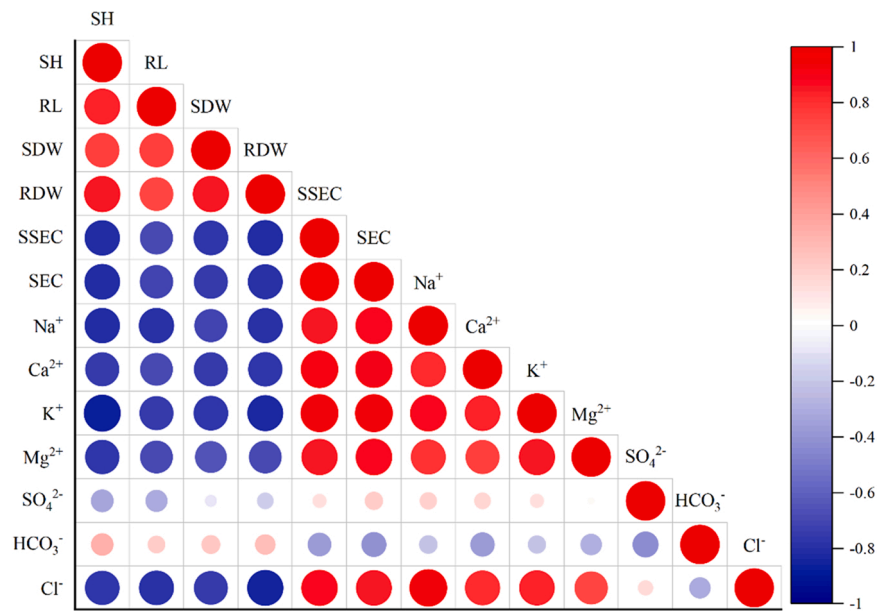


Fig. 2. Correlation analysis between plant growth and soil salt indicators. The size of the circle represents the size of the correlation coefficient. SH: shoot height; RH: root height; SDW: shoot dry weight; RDW: root dry weight; SSEC: soil solution electrical conductivity; SEC: soil electrical conductivity.

Table 1
The α -diversity indices of soil bacterial and fungal communities under organic amendments treatments (Means \pm SE, n = 3).

Time	Treatments	Bacterial		Fungal	
		Shannon	ACE	Shannon	ACE
Week 2	CK	6.20 \pm 0.09b	3466 \pm 84b	0.75 \pm 0.10b	148 \pm 14.5a
	CM	6.40 \pm 0.03a	3783 \pm 95ab	1.19 \pm 0.14a	168 \pm 13.1a
	BC	6.49 \pm 0.05a	3951 \pm 114a	1.07 \pm 0.13ab	147 \pm 15.7a
	BIO	6.48 \pm 0.03a	3658 \pm 109ab	0.80 \pm 0.08b	176 \pm 25.7a
Week 8	CK	6.37 \pm 0.13a	3471 \pm 108b	1.16 \pm 0.25a	151 \pm 15.3a
	CM	6.46 \pm 0.02a	3763 \pm 73a	1.20 \pm 0.09a	136 \pm 5.3a
	BC	6.48 \pm 0.08a	3859 \pm 75a	1.26 \pm 0.05a	163 \pm 18.9a
	BIO	6.41 \pm 0.12a	3776 \pm 103a	1.12 \pm 0.26a	157 \pm 7.6a

Different lowercase letters at each sampling time indicate significant differences among different treatments at $P < 0.05$ according to Tukey’s post-hoc test.

indices between CK and organic amendments treatments, except for the CM treatment, which increased the Shannon index at week 2 (Table 2).

Spearman’s correlation between soil saline indicators and α -diversity demonstrated that the ACE index of bacteria was significantly negatively correlated with K^+ and Mg^{2+} (Table S5). This result suggested that the addition of organic amendments could reduce the concentration of soil

K^+ and increase the soil bacterial diversity (Fig. 1 & Table 1). For the fungal diversity, the Shannon index was significantly negatively correlated with SSEC, SEC, Ca^{2+} , K^+ , Mg^{2+} , and Cl^- , although the application of organic amendments did not significantly change the soil fungal diversity except in the CM treatment at week 2.

Table 2
Spearman’s correlation coefficients between the concentrations of soil salts and the relative abundance of microbial taxa.

Microbiome	Phyla	Genera/ Order	SSEC	SEC	Na^+	Ca^{2+}	K^+	Mg^{2+}	SO_4^{2-}	HCO_3^-	Cl^-	
Bacteria	Acidobacteria	—	-0.562**	-0.53**	-0.53**	-0.49*	-0.65**	-0.43*	-0.15	0.18	-0.51*	
	Actinobacteria	—	0.051	0.07	-0.02	0.20	0.14	0.07	-0.26	0.02	0.03	
	Firmicutes	—	0.002	-0.03	-0.16	-0.03	-0.04	0.13	-0.45*	0.21	-0.12	
	Proteobacteria	<i>Sphingomonas</i>	-0.323	-0.19	-0.26	-0.07	-0.21	-0.11	-0.05	0.05	-0.22	
	Gemmatimonadetes	<i>Gemmatimonas</i>	-0.502*	-0.55**	-0.56**	-0.38	-0.57**	-0.57**	-0.06	0.34	-0.47*	
	Bacteroidetes	<i>Flavobacterium</i>	-0.525**	-0.88**	-0.91**	-0.82**	-0.84**	-0.77**	-0.35	0.21	-0.82**	
	Proteobacteria	<i>Lysobacter</i>	0.555**	0.55**	0.50*	0.57**	0.59**	0.57**	0.08	-0.08	0.54**	
	Proteobacteria	<i>MND1</i>	-0.147	-0.18	-0.09	-0.10	-0.19	-0.17	-0.02	0.14	-0.04	
	Firmicutes	<i>Bacillus</i>	-0.297	-0.18	-0.38	-0.14	-0.09	-0.17	-0.43*	0.26	-0.30	
	Actinobacteria	<i>Arthrobacter</i>	0.27	0.28	0.25	0.34	0.33	0.42*	-0.20	-0.12	0.28	
	Actinobacteria	<i>Streptomyces</i>	0.523**	0.50*	0.37	0.48*	0.53**	0.51*	0.00	-0.09	0.44*	
	fungi	Ascomycota	—	0.633**	0.550**	0.494*	0.480*	0.607**	0.533**	-0.263	-0.229	0.616*
		Mortierellomycota	—	-0.559**	-0.494*	-0.440*	-0.412*	-0.544**	-0.463*	0.171	0.166	-0.527**
		Ascomycota	Sordariales	0.425*	0.285	0.221	0.296	0.38	0.406*	-0.501*	0.028	0.313
Ascomycota		Hypocreales	-0.433*	-0.372	-0.223	-0.364	-0.418*	-0.411*	0.34	-0.063	-0.361	
Mortierellomycota		Mortierellales	-0.559**	-0.494*	-0.440*	-0.412*	-0.544**	-0.463*	0.171	0.166	-0.527**	

SSEC (Soil solution electrical conductivity); SEC (Soil electrical conductivity). ** $p < 0.01$, * $p < 0.05$.

3.4. Compositions of soil bacterial and fungal communities

For the soil bacterial community, the most dominant phylum (relative abundance >1% in at least one sample) in all treatments was Proteobacteria, which comprised 37.12% of the total sequences on average. Other dominant phyla included Acidobacteria (19.14%), Chloroflexi (10.88%), Gemmatimonadetes (7.49%), Actinobacteria (7.05%), Bacteroidetes (7.06%), Patescibacteria (2.44%), Planctomycetes (1.54%), Rokubacteria (1.47%) and Firmicutes (1.13%). These phyla accounted for over 88% of the bacterial sequences (Fig. S1A). The CM, BC and BIO treatments significantly increased the relative abundances of Acidobacteria by 42.62%–45.96%, 20.43%–27.87% and 22.53%–39.24%, as well as Firmicutes by 29.01%–155.62%, 71.80%–77.25% and 146.51%–186.90%, respectively ($P < 0.05$) (Fig. S1A). In addition, BC treatment significantly increased the relative abundance of Actinobacteria by 63.05% and 30.66% at weeks 2 and 8, respectively ($P < 0.05$) (Fig. S1A).

At the genus level, the application of organic amendments also significantly increased the relative abundances of the beneficial salt-tolerant bacterial genera *Flavobacterium* (Bacteroidetes), *Bacillus*

(Firmicutes) and *Arthrobacter* (Actinobacteria) (Fig. 3A & Table S6). BC treatment significantly increased the relative abundance of *Flavobacterium* by 11.46% and 88.97% at weeks 2 and 8, respectively. BIO treatment significantly increased the relative abundance of *Bacillus* and *Arthrobacter* by 21.84%–33.33% and 24.10%–47.25%, respectively ($P < 0.05$) (Fig. 3A & Table S6). In addition, the application of organic amendments increased the relative abundance of other growth-promoting and antagonistic pathogen genera, such as *Gemmatimonas* (Gemmatimonadetes), *Lysobacter* (Proteobacteria) and *Streptomyces* (Actinobacteria) (Fig. 3A & Table S6).

For the soil fungal community, Ascomycota was the dominant phylum, accounting for more than 70% of the fungal sequences, followed by Mortierellomycota (2.29%–26.14%) and Olpidiomycota (0–0.97%). Specifically, the CM treatment increased the relative abundance of Mortierellomycota by 2.89 and 10.40 times at weeks 2 and 8, respectively, compared with CK, which could defend against soil degradation. In contrast, the abundance of Ascomycota was lower in the CM treatment ($P < 0.05$) (Fig. S1B). At the order level, Mortierellales was the only order of the phylum Mortierellomycota and was the highest in the BC treatment, with a relative abundance of 10.85%–26.14%.

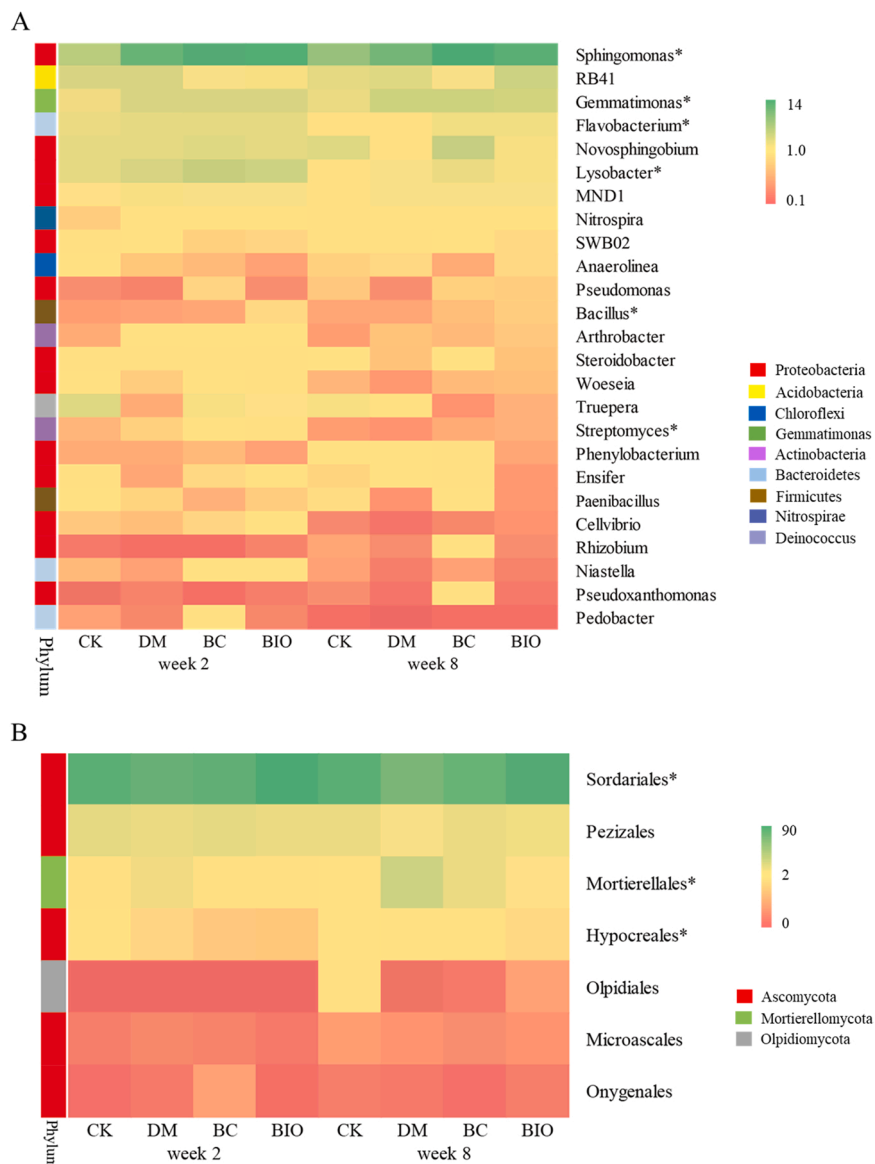


Fig. 3. Heatmaps of the dominant bacterial genera (relative abundance > 1% in at least one sample) (A) and fungal orders (relative abundance > 0.1% in at least one sample) (B) in the soils under different treatments. Asterisks (*) indicate significant differences ($P < 0.05$) among the different treatments.

Additionally, BC and BIO treatments significantly decreased the relative abundance of pathogenic fungal *Hypocreales* (Ascomycota) at week 2 and week 8, and the reduction effect at week 8 was less than that at week 2 ($P < 0.05$) (Fig. 3B & Table S7).

Furthermore, Spearman's correlation was used to examine the relationships between the relative abundance of predominant bacterial or fungal taxa and soil salts (Table 2). Within the bacterial community, the relative abundances of the dominant phylum Acidobacteria were negatively correlated with the levels of EC of the soil and soil solution,

Na^+ , Ca^{2+} , K^+ , Mg^{2+} , and Cl^- , which indicated that the application of organic amendments alleviating soil salinization was definitely related to the variation in Acidobacteria ($P < 0.05$) (Table 2). At the genus level, the relative abundances of *Lysobacter* and *Streptomyces* were positively correlated with the concentrations of SSEC, SEC, Ca^{2+} , K^+ , Mg^{2+} and Cl^- , whereas the relative abundances of *Flavobacterium* or *Gemmatimonas* were negatively correlated with SSEC, SEC, Ca^{2+} , K^+ , Mg^{2+} , Cl^- or SSEC, SEC, K^+ , Mg^{2+} , Cl^- ($P < 0.05$) (Table 2). This result suggested that the main phylum of Acidobacteria and these three genera were sensitive to

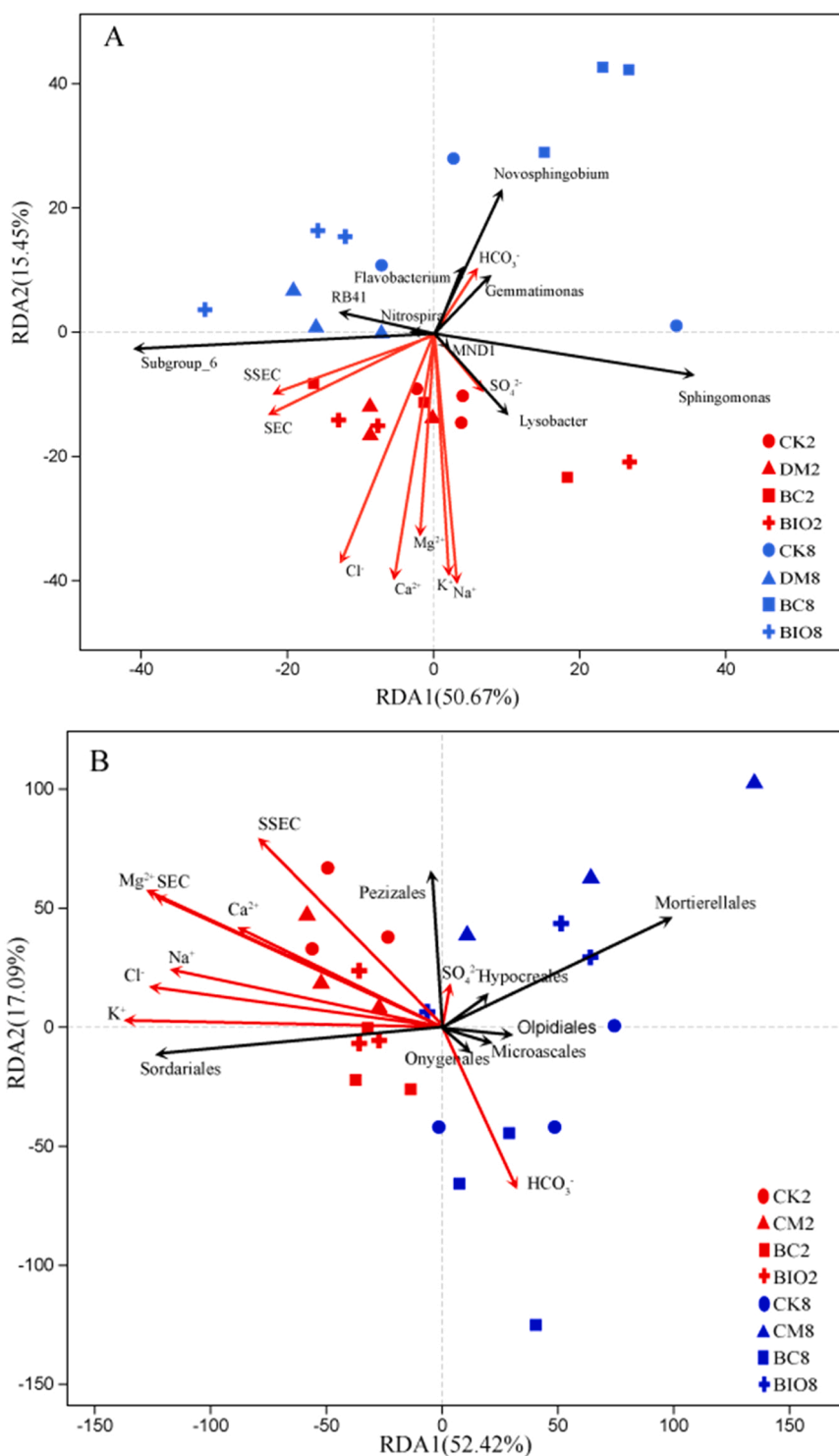


Fig. 4. Redundancy analysis (RDA) of soil bacterial (A) and fungal (B) communities and selected environmental variables for soil samples from organic amendments treatments.

the variations in soil salts. For the fungal community, the relative abundance of the phylum Mortierellomycota and the order Mortierellales were negatively correlated with SSEC, SEC, and concentrations of Na^+ , Ca^{2+} , K^+ , Mg^{2+} , and Cl^- , which was opposite to the results of Ascomycota ($P < 0.05$) (Table 2). In addition, all the main fungal phyla and orders significantly responded to the variations in SSEC and Mg^{2+} concentrations (Table 2). In short, these results suggested that the compositions of the soil microbial communities were closely related to soil salinization.

3.5. Structures of soil bacterial and fungal communities

PCoA showed the effect of organic amendments and different sampling times on soil bacterial and fungal communities (bacterial: $R^2 = 0.493$, $P = 0.001$; fungal: $R^2 = 0.773$, $P = 0.001$) (Fig. S2). The first two principal coordinates explained 52.4% (PC1) and 15.27% (PC2) of the variations in the bacterial community (Fig. S2 A). The bacterial community of the organic treatments, especially the BC and BIO treatments, was different from that of CK along the PC1 axis at weeks 2 and 8, and the difference was more significant at week 8 than week 2 (Fig. S2 A). This result indicated that the application of organic amendments significantly altered the structure of the soil bacterial community and changed dramatically with prolonged treatment time. In addition, the bacterial community structure from week 2 soil was in the down, which was distinctly separated from soil week 8 along PC2, which suggested that the structure of the soil bacterial community was also affected by the growth stages of melons (Fig. S2 A). Furthermore, the first two principal coordinates explained 55.1% (PC1) and 20.28% (PC2) of the variation in the fungal community (Fig. S2 B). The structure of the soil fungal community in the CM, BC and BIO treatments was different from that in CK along the PC2 axis at week 8, especially in the BIO treatment (Fig. S2 B). The fungal community from week 2 was distinctly separated from that of week 8 along PC1, indicating that the structure of the soil fungal community was totally affected by the growth stages of melons. Overall, the structure of the bacterial community was mainly affected by organic amendments treatments, while the structure of the fungal community was less affected by organic amendments treatments than growth stage.

Furthermore, RDA analysis was conducted to reveal the relationships

between soil salt indicators (SSEC, SEC, Na^+ , Ca^{2+} , K^+ , Mg^{2+} , SO_4^{2-} , Cl^- , CO_3^{2-}) and the structures of the soil microbial communities (Fig. 4). The soil salt indicators were limited to the first and second sorting axes, which explained 66.12% and 69.51% of the variation in bacterial and fungal structure, respectively (Fig. 4A). Among all the salt indicators, soil K^+ ($R^2=0.543$, $P < 0.01$), Na^+ ($R^2 = 0.591$, $P < 0.01$), Ca^{2+} ($R^2 = 0.558$, $P < 0.01$), Mg^{2+} ($R^2 = 0.373$, $P < 0.05$), and Cl^- ($R^2 = 0.557$, $P < 0.01$) were the most important factors in determining bacterial community, and SEC ($R^2=0.338$, $P < 0.05$), K^+ ($R^2=0.382$, $P < 0.01$), Na^+ ($R^2 = 0.257$, $P < 0.05$), Mg^{2+} ($R^2 = 0.359$, $P < 0.05$) and Cl^- ($R^2 = 0.321$, $P < 0.05$) were the most influential factors affecting the structure of the soil fungal community (Fig. 4B). The results illustrated that variations in the structures of soil microbial communities were closely related to the concentration of soil salts.

3.6. Microbial network analysis

Considering the difference in soil microbial community structure between the CK and treatments was larger in week 8 than in week 2. Bacterial and fungal cooccurrence networks at the genus level were constructed for the different treatments of organic amendments at week 8 (Fig. 5). For the bacterial community, the quantities of nodes and total links in the organic amendment networks were higher than those of CK, indicating that the networks of organic amendments were more complex than those of CK, especially for the BC treatment, and the nodes and total links increased 16.89% and 72.37% compared with CK, respectively (Fig. 5 & Table S6). The number of positive links in the CK, CM, BC and BIO treatments was 361, 424, 654 and 553, accounting for 51.94%, 52.22%, 54.59% and 53.33% of the total number of corresponding links, respectively, indicating that the application of organic amendments increased the positive co-occurrence patterns of the networks. In addition, the networks of organic amendments had a higher average clustering coefficient and network density than those of CK, indicating that the number of within-cluster links was higher than the number of between-cluster links, indicating that soil bacteria in similar niches were more closely linked than those in dissimilar niches after applying organic amendments. Compared with CK, the organic amendments networks were more obviously modular, indicating that the bacterial community structure is largely stable and ordered. For the fungal

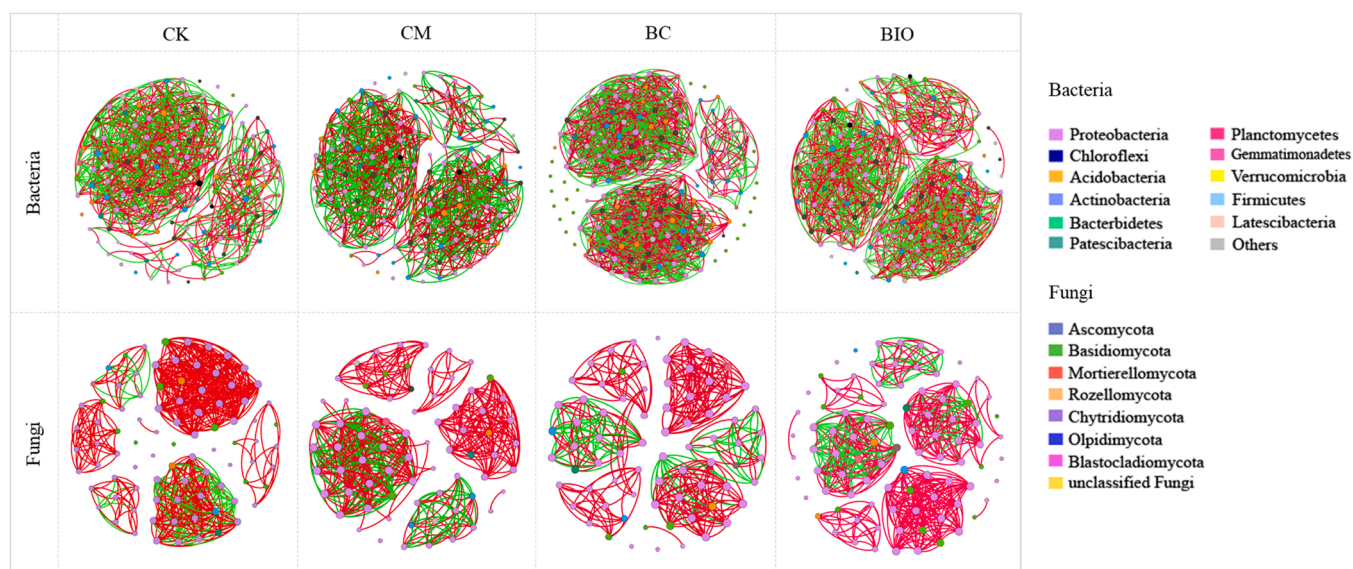


Fig. 5. Co-occurring network of bacterial and fungal communities at the genus level in response to organic amendments treatments on week 8. Nodes indicate the genera involved in the networks, and links indicate the relationships among the nodes. Red lines represent significant positive (Spearman's correlation, $r > 0.9$ and $P < 0.01$) relationships, and green lines denote negative (Spearman's correlation, $r < -0.9$ and $P < 0.01$) relationships. The different colored dots represent the different phyla to which the genera belong.

community, the modularity increased after applying organic amendments, and BIO treatment had a higher number of nodes and total links than the other three networks, suggesting that the BIO fungal network was the most complex network among the four networks (Fig. 5 & Table S8). The number of positive links of fungal networks comprised at least 73.0% of the total links, indicating that the fungal network was dominated by the positive co-occurrence patterns.

Keystone genera were defined as taxa interacting with many other members, which were thought to play crucial roles in the overall community (top 5% of interactions). Most keystone genera belonged to the phyla Proteobacteria, Actinobacteria, and Bacteroidetes in the bacterial networks, while the keystone genera all came from Ascomycota in fungal networks regardless of organic amendments or CK (Table S9 & S10). The keystone genera in bacterial and fungal networks were altered by the application of organic amendments (Table S9 & S10). This indicated that organic amendments had a significant influence on microbiome structure and function in the microbial network by altering the keystone genera of saline soil.

4. Discussion

Soil salinization is recognized as a significant problem for agricultural production. In this study, cow manure, biochar and bio-fertilizer were used for restoring soil salinization due to their high cost-effectiveness and ecological benefits. The three organic materials alleviated soil salinization, improved the soil microbiome, and promoted plant growth of melon, especially biochar treatment for the alleviation of soil salinization.

Biochar holds positive and negative charges, and the presence of various functional groups provides biochar with a suitable choice for the adsorption of various salts present in soils, thus mitigating the salinity of soil (Farhangi-Abri and Ghassemi-Golezani, 2021; Rajapaksha et al., 2016). It was found that applying biochar decreased the concentrations of Na^+ , Ca^{2+} , Mg^{2+} , SO_4^{2-} and Cl^- in coastal saline-alkali soil through a pot experiment with the crop maize (Zhang et al., 2022). Similar to the results observed in this study, the application of biochar significantly decreased the concentrations of Na^+ , K^+ and Cl^- during the whole experimental period, probably due to the absorption capacity of biochar to soil salts (Fig. 1).

In this experiment, CM significantly decreased the concentrations of K^+ and Cl^- and BIO treatment decreased the concentrations of Na^+ (Fig. 1). The application of cow manure is a traditional method of improving salinized soil, which is beneficial for promoting the stabilization of soil agglomerates. The formation and stabilization of soil agglomerates can improve soil structure, strengthen salt leaching, reduce surface evaporation and inhibit salt accumulation in surface soil (Liu et al., 2018; Meng et al., 2019). Bio-organic fertilizer consists of compost and specific microorganisms with unique functions, which have shown environmental friendliness and positive effects on soil (Li et al., 2021). Previous studies also illustrated that the application of bio-organic fertilizer could improve soil available nutrients and reduce soil EC and the concentrations of Na^+ and Ca^{2+} (Wu et al., 2018; Zhu et al., 2021).

In addition, soil biodiversity is considered an important indicator of the maintenance and sustainability of soil agroecosystem functions (Zhao et al., 2021). It was reported that the improvement in soil salinity was also related to the soil microbiome (Asgar et al., 2012; Mbarki et al., 2016; Raiesi and Sadeghi, 2019). This study shows that soil salinity is closely connected with soil microbial community diversity, composition and structure. Specifically, the application of different organic amendments significantly increased the Shannon and ACE indices of the soil bacterial community and the Shannon index of the soil fungal community (Table 1). This is likely due to organic amendments, especially biochar, which provide a habitat for microorganism to protect them from predators and increase the ecological niches to support more diversity (Lehmann et al., 2011). In addition, the negative correlation between soil saline indicators and α -diversity indicated that the increase

in diversity of the soil bacterial community was probably due to the alleviation of soil salinization by the application of organic amendments. However, the soil fungal community is not sensitive to the application of organic amendments. Moreover, higher microbial diversity is known to support the robustness and resilience of microbial communities to external disturbances (Chen et al., 2019). Therefore, the application of organic amendments enhancing the diversity of the soil bacterial community is also beneficial to the maintenance and even improvement of the multifunctionality and services of the soil ecosystem (Lupatini et al., 2016; Wagg et al., 2019).

Furthermore, the application of organic amendments altered the composition of the dominant microbial taxa (Fig. 3 and S1). On the one hand, these variations were mainly due to the labile and stubborn organic C in the organic modification, which can promote the growth of different microbial subgroups (Zhang et al., 2014). On the other hand, organic amendments can improve the soil environment by improving the contents of soil nutrients, water-holding capacity and soil aeration (Dong et al., 2021), which can also indirectly improve the diversity, structure, and composition of the soil microbial community (Yao et al., 2017). At the genus level, the addition of organic amendments significantly increased the relative abundance of *Flavobacterium*, *Bacillus* and *Arthrobacter*, indicating improvement of the growth and development of beneficial salt-tolerant bacteria in salinized soil (Ferrer et al., 1988; Han et al., 2014; Zhao et al., 2017). Additionally, Spearman's correlation analysis demonstrated that SSEC, SEC and soil saline ions were significantly correlated with the predominant soil bacterial and fungal populations (Table 2), which indicated that the application of organic amendments reduced the availability of soil salts and indirectly shifted the composition and structure of the soil microbial community (Fig. 3 & S2).

Microbial network analysis can be used to determine the interactions of specific microbial communities in ecological niches and to identify key groups that have the greatest impact on microbial communities (Liu et al., 2020a; Liu et al., 2020b; Zheng et al., 2018). Our results illustrated that the complexity of the bacterial networks (networks with a high number of nodes and links) increased with the application of CM, BC and BIO (Fig. 5 & Table S8). It has been shown that microbial communities with more complex co-occurrence networks are more resistant to environmental stresses than those with simpler networks (Banerjee et al., 2019; Santolini and Barabasi, 2018). These results demonstrated that the addition of organic amendments could enhance the potential resistance to environmental disturbance by increasing the complexity of soil microbial networks in saline soil.

5. Conclusions

The application of organic amendments is an effective approach to alleviating soil salinization, especially the biochar addition significantly decreased the EC values of the soil and soil solution by 19.23% and 27.02% and the concentrations of Na^+ , K^+ , and Cl^- by 13.28%, 13.08%, and 15.21%, respectively. Moreover, the application of organic amendments increased the diversity of the soil bacterial community, as well as the relative abundance of beneficial salt-tolerant microbial taxa, enhancing the interactions among microorganisms and therefore promoting the growth of melon. In general, this study illustrated the relationship between soil salts and microbial communities after applying organic amendments and provides new management for realizing agriculture green development.

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CRedit authorship contribution statement

Xiaoxi Mao: Investigation, Data curation, Writing – original draft. **Yang Yang:** Resources, Formal analysis. **Peibin Guan:** Investigation. **Liping Geng:** Investigation. **Li Ma:** Investigation. **Hongjie Di:** Supervision, Validation. **Wenju Liu:** Supervision, Conceptualization, Writing – review & Editing, Project administration, Funding acquisition, **Bowen Li:** Project administration, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.ecoenv.2022.113616](https://doi.org/10.1016/j.ecoenv.2022.113616).

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