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The biocompatible soil amendment boosts the winter wheat yield and modulates soil microorganisms under saline-alkaline stress

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Abstract

Nowadays, the globally increasing salinization of land is causing a growing shortage of food. To alleviate the saline and alkaline stress and enhance the crop yield, a biocompatible and low-cost soil amendment was developed. This was the combination of seaweed extract and poly- γ -glutamic acid broth at a ratio of 50:50% (w:w). The amendment was evaluated in a soil column and winter wheat field experiments. The soil microorganisms were analysed by 16S rRNA high throughput sequencing. The results indicated that the soil amendment reduced the increment of salt content by 43.3% and 30.3% for topsoil and subsoil, respectively. The soil amendment enhanced the winter wheat yield by 10.7%. Meanwhile, the biocompatible soil amendment modified the composition, structure and diversity of soil bacteria, which contributed to the plant growth and crop yield. There was no significant effect on the fungi community. The soil amendment played a beneficial role in alleviating the salt stress and enhancing the winter wheat yield in a mildly saline and alkaline soil.

Key words: salinization, seaweed, poly- γ -glutamic acid, biodiversity, microbiota.

Introduction

Nowadays, the global scarcity of water resources, increased salinization of land, loss of biodiversity and environmental degradation are the major threats to humans, which are caused by the rapid growth of the world's population (Sakr, El-Metwally, 2009; Myers et al., 2017; Egamberdieva et al., 2019). Among them, the salinization of land around the world is getting more and more serious. It has resulted from long-term natural processes of accumulation of salts in the soil and human activity such as pollution, irrigation of agricultural land with saline water and poor management practices (Flowers, Yeo, 1995). It has been estimated that more than 50% of the arable agricultural land will be salinized by the year 2050 (Moin et al., 2017; Kumar et al., 2019).

According to the definition in literature (Wood, 2001; Çavuşoğlu, Kabar, 2010), salinity is the excessive concentration of soluble salts and mineral elements in water and soil solution or excessive accumulation of

soluble anions and cations in the root zone, which results in the difficulty in absorbing enough water from the soil solution and affects the growth of plants. It is reported that the productivity and quality of most of the crop plants are limited under the salinity stress, because most of the crop plants are sensitive to high concentrations of salts in the soil (Shrivastava, Kumar, 2013). As a result, if the salinity of soil is not effectively remediated, it will become increasingly difficult to meet the world's ever-increasing food demand (Ladha et al., 1998; Etesami, Maheshwari, 2018).

To alleviate the salinity stress and increase crop productivity in these soils, hydraulic, physical, chemical and biological methods have been developed (Lu et al., 2017; Deng et al., 2019). However, the application of these methods is often limited because of cost, unavailability of good soil conditioner and good irrigation water, etc. In other words, such strategies are time-consuming and costly.

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It was reported that poly- γ -glutamic acid (γ -PGA) could absorb moisture, enhance the apparent fertilizer utilization efficiencies and increase the relative abundance of potential plant-growth-promoting bacteria in soil (Zhang et al., 2017; Liang, Shi, 2021). Some studies indicated that γ -PGA bioproduct improved the saline soil mainly by assisting nitrogen conservation and reducing cadmium (Cd) uptake of lettuce (Wang et al., 2020; Chen et al., 2021).

Seaweed extract is commonly used as one of the plant biostimulants, which are defined as any substance or microorganism applied to plants with the aim of enhancing nutrition efficiency, abiotic stress tolerance and/or crop quality traits, regardless of their nutrient content. This also acted positively on soil retention, remediation and soil microflora (Boukhari et al., 2020). A commercial seaweed extract was researched, and the results showed that it structured microbial communities associated with tomato and pepper roots and significantly increased crop yield (Renaut, 2019; Anjos Neto et al., 2020). The seaweed extracts enhanced the growth, secondary metabolites and bioactivity of *Calibrachoa* cultivars by soil drench or foliar spray (Elansary et al., 2016).

According to the above background information, it was expected to develop a kind of effective and low-cost amendment to alleviate soil salinity stress and enhance crop yield. In this study, to prepare a kind of biocompatible soil amendment, γ -PGA and seaweed extract were employed. The soil amendment was evaluated by the soil column experiment, winter wheat field experiment and sequence of soil microorganisms; it showed a significant positive effect. Fortunately, the soil amendment is inexpensive and widely available, and expected to be widely applied and increase crop plant productivity in mildly saline and alkaline soil.

Materials and methods

The preparation of the biocompatible soil amendment. The soil amendment is a mixture of seaweed extract and poly- γ -glutamic acid (γ -PGA) broth. The seaweed extract was prepared by enzymatic hydrolysis using pectinase and cellulase at pH 8.0, 55°C temperature for 2 hours. The content of seaweed polysaccharide was 1.5%. The γ -PGA was fermented by *Bacillus licheniformis* strain A2-10, which was isolated in State Key Laboratory of Biobased Material and Green Papermaking, Qilu University of Technology, China. The fermentation was performed at 37°C temperature for 40 h, using glucose 60 g L⁻¹, sodium glutamate 30 g L⁻¹, peptone 5 g L⁻¹, yeast powder 5 g L⁻¹, sodium chloride (NaCl) 2 g L⁻¹ and dipotassium hydrogen phosphate 0.5 g L⁻¹. After fermentation, the acidity (pH) of the broth was adjusted to 6.5, which had a viscosity of 4000 mPa.s, and the γ -PGA content was 25 g L⁻¹. Then the seaweed extract and the γ -PGA broth were mixed at room temperature with a different proportion. The pH was adjusted to 6.5 with phosphoric acid (H₃PO₄).

Soil column experiment. To evaluate the role of the combined soil amendment in alleviating the salt accumulation and optimize the ratio of seaweed extract and γ -PGA broth, the soil column experiment was designed and conducted. In detail, the electrical conductivity (EC) variation of soil extract was measured with the different composition of soil amendment. The composition of soil amendment was set as: (1) 100% seaweed extract, (2) 70% seaweed extract and 30% γ -PGA broth, (3) 50% seaweed extract and 50% γ -PGA broth, (4) 30% seaweed extract and 70% γ -PGA broth, and (5) 100% γ -PGA broth; control – without soil amendment. The soil in a depth of 10 cm was collected from the test plot in the Yellow River Delta (Binzhou) National Agricultural Science and Technology Park. Then it was air-dried and passed through a sieve of

2 mm to simulate the soil surface roughness before the soil was loaded into the container. The bulk density of the soil was 2.57 g cm⁻³.

The schematic diagram of the device for the soil column experiment is shown in Figure 1. The container was made of a PVC (polyvinyl chloride) pipe with a 60 cm height and 14 cm inner diameter. At the bottom, the quartz sand filter was set and the NaCl solution of 3 g L⁻¹ was supplied through a flexible pipe, which simulated the salty water in the deeper soil layer. An infrared lamp (250 W) was installed at 20 cm above the PVC pipe, which was used to simulate the sunshine exposure.

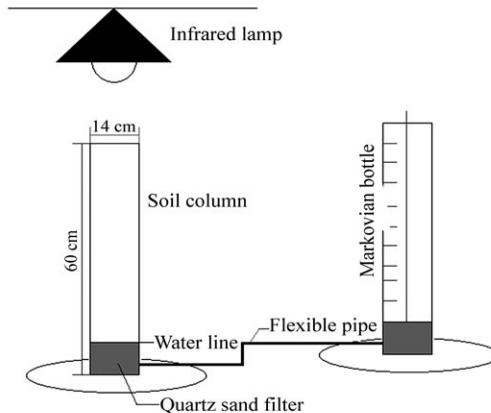


Figure 1. The soil column device used to study the effects of the soil amendment on saline soil

The lamp at the top of the soil column continued to shine on it. The compatible soil amendment was dissolved in 2 L NaCl solution (3 g L⁻¹). The soil was irrigated by the obtained solution. Five soil samples were collected every 8 hours for a total of 16 times in a depth of 10 cm. Every time, the samples were mixed, dried at 105°C temperature, ground and dissolved by double distilled water 1:5 (w:w), and the EC of extract solution was measured.

The measurement of the salt content of soil from the experiment field. The field experiment was carried out in the Yellow River Delta (Binzhou) National Agricultural Science and Technology Park, which is located in Shandong Province, Northern China (118.0882° E, 37.5792° N) and has a typical Monsoon climate of medium latitudes with a mean annual temperature of 12.7°C and a mean annual rainfall of 564.8 mm.

The salt content, that is EC of soil from the mildly saline and alkaline field, was measured before and after treatment with the biocompatible soil amendment. In detail, the mildly saline and alkaline field was divided into 10 plots and ploughed. The 5 plots were selected randomly and used as control plots; the other plots were treated plots. At the beginning of the experiment (on the 23rd of November, 2018), the control soil samples were obtained from the control plots in topsoil (depth of 0–15 cm) and subsoil (depth of 15–30 cm) by sampling in five locations. The samples were labelled as topsoil control group at beginning of experiment (BTCG) and subsoil control group at beginning of experiment (BSCG); by the same way, the treated soil samples from treated plots were obtained and labelled as topsoil treated group (BTTG) and subsoil treated group (BSTG) at beginning of experiment. During the experiment, no soil amendment was applied to the control plots. The treated plots were sprayed with the compatible soil amendment solution at a dose of 210 kg ha⁻¹ and mixed with soil as thoroughly as possible with a wooden rake. At the end of experiment (on the 23rd of May, 2019), all the soil samples were collected again, including the topsoil control group at end of experiment (ETCG), subsoil control group at

end of experiment (ESCG), topsoil treated group at end of experiment (ETTG) and subsoil treated group at end of experiment (ESTG). All the samples were sieved (2 mm diameter) to remove soil fauna, fine roots and rock fragments and stored at -80°C temperature for the measurement of salt content and sequencing of soil microorganisms. The soil samples were dried and ground and dissolved in double distilled water adequately at a ratio of 1:10 (w:w). The EC of the solutions was measured carefully, and the salt content was calculated.

The winter wheat yield in the field. The effect of biocompatible soil amendment on wheat yield was investigated in field experiment. The study was carried out in the mildly saline and alkaline field as mentioned above. The field was ploughed with a rotary cultivator HX-SL (Hongxin Machinery, China) to a depth of over 20 cm after maize harvest on the 20th of September, 2018, and then randomly divided into 10 plots for the experiment. Each plot was 10 × 10 m in area. No soil amendment was used in the 5 control plots. In 5 treated plots, the amendment was sprayed on the soil surface at a dose of 210 kg ha⁻¹ and mixed with soil as thoroughly as possible with a wooden rake. Then winter wheat (*Triticum aestivum* L., cultivar 'Jimai 20') seeds were directly sowed to plots by a seed planter 2BXF-9 (Dezhou Jiatian Machinery Co. Ltd., China) on the 22nd of September, 2018 and harvested manually on the 6th of June, 2019. The chemical nitrogen (N) input totalled 320 kg ha⁻¹ N with a proportion of 80 kg ha⁻¹ N as compound of N: P₂O₅:K₂O (15:15:15) fertilizer, the other 240 kg ha⁻¹ N as urea, 30% of which was allocated as basal fertilizer, 45% as elongation fertilizer and the other 25% as booting fertilizer. The harvested grain of each plot was counted and weighed at about 35% grain moisture, and the process was repeated three times independently. The winter wheat yield was calculated and statistically analysed.

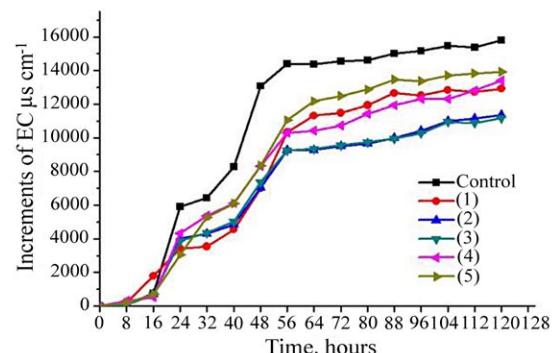
High throughput sequencing of soil microorganisms. To find out the effect of the biocompatible soil amendment on the soil microorganisms, the soil samples were collected. The DNA was extracted and sequenced. In detail, the microbial total DNA from soil samples was extracted using the commercial soil DNA Kit (Omega Bio-Tek, USA). The quality and quantity of the DNA was confirmed using a Nanodrop 1000 (Thermo Fisher Scientific, Germany). Illumina MiSeq high-throughput sequencing was done by Personal Biotechnology Co. Ltd. (China). The data of raw DNA sequence was analysed using software *QIIME2*, version 2019.4. Classification of 16S data used a trained classifier with the Greengenes 97% alignment taxonomy (McDonald et al., 2012). Classification of ITS (Internal Transcription Spacer) data used the UNITE database (<https://unite.ut.ee/>) for molecular identification of fungi (Nilsson et al., 2019). Phylogenetic trees were constructed using 'MAFFT' for alignment 'mask' to remove high variability regions and 'Fast Tree' to build the phylogeny (Katoh et al., 2002).

Statistical analysis. The relative abundance of bacteria and fungi, α -diversity were tested for differences using analysis of variance (ANOVA) and post hoc Tukey's test ($n = 5$, $p < 0.05$). Taxonomic principal coordinate analysis (PCoA) plots (Bray-Curtis dissimilarity) were generated using *QIIME2*'s phylogenetic core metrics (Bray, Curtis, 1957).

Results and discussion

Soil column assays. Because salt from deeper soil layer moves upward with the evaporation of salty water and accumulates on the soil surface, the salt content of surficial soil will increase. The results showed that the surficial salt content of all the samples increased as time

went on, which was in accordance with the above point (Figure 2).



Control – without soil amendment, 1 – 100% seaweed extract, 2 – 70% seaweed extract and 30% γ -PGA broth, 3 – 50% seaweed extract and 50% γ -PGA broth, 4 – 30% seaweed extract and 70% γ -PGA broth, 5 – 100% γ -PGA broth; EC – electrical conductivity

Figure 2. The effect of soil amendments with different compositions on soil salinization process

Compared with the control samples, the lower EC increments of soil samples treated by the amendment with different compositions were observed, which showed that the soil amendments had a beneficial effect on decreasing the salt content. Among the amendments of different compositions, the amendment carbon (3: 50% seaweed extract and 50% γ -PGA broth) displayed the smallest increment of EC. In other words, the amendment C showed the most significant effect on alleviating the salt stress. Therefore, amendment C was employed in the following experiment.

Salt content of soil sample from the mildly saline and alkaline field. The salt content is one of the basic soil properties and main obstacle influencing the normal growth of plants. In this study, to show the alleviated effects of the biocompatible soil amendment on the salt stress of the mildly saline and alkaline field, the salt content of soil samples from the mildly saline and alkaline field was measured and analysed (Figure 3A).

At the beginning of the experiment, in the topsoil, the average salt content of control and treated plots was 0.286 and 0.346 g L⁻¹; at the end of the experiment, the average salt content of control and treated plots was 0.830 and 0.650 g L⁻¹, respectively. There was a salt content increment of 0.546 g L⁻¹ for the topsoil control plots through the experiment. However, the salt content increment of topsoil treated plots was 0.304 g L⁻¹, which was 43.3% less than that of control plots. Similar results were obtained for the subsoil control and treated plots (Figure 3B). The increment of salt content in subsoil treated plots was 30.3% less than that in the subsoil control plots. The results indicated that the soil amendment still worked in the subsoil.

Soil salinity and alkalinity is a process of soil colloid adsorbing sodium ion and cation exchanging between solid and liquid phases (Mullissa et al., 2017). Through the experiment, the water (liquid phase) in deeper soil (solid phase) contained a wide range of salt and minerals. The water was moving up and evaporating at the soil surface. The salt and minerals were also carried to the topsoil and accumulated. Therefore, the salt content increased in the control and treated plots. For the treated plots, both the seaweed extract and γ -PGA broth played an important role in keeping moisture and decreasing water vaporization. Then the accumulation of the salt and

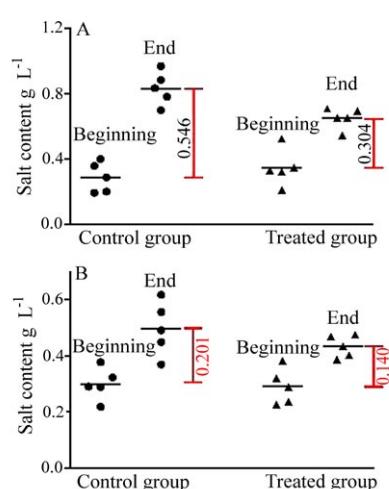


Figure 3. The salt content of topsoil (0–15 cm) (A) and subsoil (15–30 cm) (B) samples from the mildly saline and alkaline field before and after treatment with the soil amendment

Table 1. The effect of the biocompatible soil amendment on the yield of winter wheat

Soil	Spikes per hectare ($\times 10^6$)	Kernels per spike	Thousand kernel weight g	Average grain weight kg ha ⁻¹	Increase of yield %
Control	4.73 ± 0.23 a	33 ± 2 b	430 ± 11 a	5705	—
Treated	4.80 ± 0.41 a	36 ± 3 a	430 ± 05 a	6315	10.7

Note. Spikes per hectare, kernels per spike and a thousand kernel weight were analysed for differences using ANOVA ($p < 0.05$) and post hoc Tukey's test ($n = 3$); the data are shown by mean ± SD; different letters in a single column indicate significant difference between groups.

Both seaweed extract and γ -PGA broth can enhance the fertilizer utilization efficiencies and increase the relative abundance of potential plant growth-promoting bacteria in the soil. Therefore, the yield of winter wheat was increased by about 10.7%.

Effect of the biocompatible soil amendment on soil microbial composition. DNA samples were extracted from 30 samples for 16S rRNA gene amplification. For bacteria and fungi, a total of 2884949 and 3404579 high-quality sequences were obtained, respectively. The sequence length ranged from 260 to 442 and 113~407 base pairs for bacteria and fungi, respectively. The rarefaction curves had 97% sequence similarity, and all curves nearly reached gentle line, which showed that the sequencing numbers of 30 samples were reasonable, and most microorganisms had been detected by MiSeq sequencing (Huang et al., 2017). Finally, 1899 bacterial and 157 fungal operational taxonomic units (OTUs) were identified. In total, 27 phyla, 127 families, 95 genus and 34 species of bacteria as well as 22 phyla, 33 families, 59 genera and 130 species of fungi were discovered.

minerals was decreased, and the increment of salt content reduced. It could be concluded that the biocompatible soil amendment alleviated the soil salinity and played an obvious beneficial role in the soil remediation.

The winter wheat yield in the mildly saline and alkaline field. The results of the field experiment conducted in the Yellow River Delta (Binzhou) National Agricultural Science and Technology Park are shown in Table 1. Compared with the control plots, the spikes per hectare, the kernels per spike and the yield of grain increased significantly ($p < 0.05$) by 1.5, 9.1 and 10.7 % in treated plot, which was applied with 210 kg ha⁻¹ soil amendment at the beginning of wheat growing. The average yield of grain in control and treated plots was 5705 and 6315 kg ha⁻¹, respectively.

In general, the salt will accumulate in the root zone and the plant encounters difficulty in absorbing enough water from the soil solution. As a result, the soil salinity and alkalinity are widely blamed for decreased crop yield and usable agricultural land (Egamberdiyeva, 2007; Manchanda, Garg, 2008). In this study, the use of the biocompatible soil amendment alleviated the saline stress and reduced the salt content of topsoil.

The biocompatible soil amendment affected soil microbial composition at phylum level. The compositions of bacterial and fungal communities at phylum level are shown in Table 2. The predominant phyla were *Proteobacteria*, *Actinobacteria*, *Acidobacteria*, *Bacteroidetes*, *Chloroflexi*, *Rokubacteria*, *Gemmamimonadetes*, *Planctomycetes*, *Firmicutes* and *Nitrospirae* for bacteria; the top 8 abundant fungal phyla were classified as *Ascomycota*, *Basidiomycota*, *Mortierellomycota*, *Mucoromycota*, *Chytridiomycota*, *Glomeromycota*, *Blastocladiomycota* and *Zygomycota*.

For bacteria, the phyla with relative abundance above 1% in all samples were selected for analysis of the significant differences in microbial taxonomic composition (Table 2). The phyla that did not show a significant difference were not shown. Comparing the abundance of phyla in BTG and BSCG, it was possible to see an obvious compositional variation with the depth of soil. At the beginning of experiment, the relative abundance of *Actinobacteria* in BTG was 21.10%, which is 4.65% more than in BSCG; the relative

Table 2. The relative percentage abundance of phyla with significant difference at different conditions for bacteria

Phyla	BTG	BSCG	ETCG	ESCG	ETTG	ESTG
<i>Proteobacteria</i>	26.06 ± 0.15 a	27.67 ± 1.45 a	27.49 ± 2.11 a	27.60 ± 2.37 a	31.58 ± 1.90 b	30.44 ± 1.60 b
<i>Acidobacteria</i>	8.81 ± 1.02 ab	9.80 ± 0.55 ac	8.43 ± 1.36 b	9.43 ± 1.28 ab	8.28 ± 0.44 ad	10.97 ± 0.65 c
<i>Actinobacteria</i>	21.10 ± 2.10 a	16.45 ± 1.87 b	22.96 ± 4.46 a	17.46 ± 2.72 b	17.21 ± 1.66 b	12.40 ± 0.66 c
<i>Chloroflexi</i>	4.72 ± 0.33 a	4.57 ± 0.28 a	3.70 ± 0.31 bd	3.51 ± 0.30 b	4.03 ± 0.64 cd	4.05 ± 0.27 cd
<i>Bacteroidetes</i>	2.84 ± 0.50 a	2.21 ± 0.47 a	5.97 ± 1.37 b	4.86 ± 1.66 b	6.91 ± 1.10 b	3.91 ± 0.81 c
<i>Planctomycetes</i>	1.37 ± 0.22 a	1.26 ± 0.35 a	0.63 ± 0.30 b	1.17 ± 0.39 a	0.68 ± 0.23 b	0.69 ± 0.42 b
<i>Rokubacteria</i>	1.27 ± 0.28 a	2.20 ± 0.61 bc	1.63 ± 0.80 ac	2.35 ± 0.59 b	1.53 ± 0.09 a	2.72 ± 0.37 b
<i>Gemmamimonadetes</i>	1.23 ± 0.11 a	1.25 ± 0.11 a	1.89 ± 0.29 b	1.68 ± 0.07 bc	1.52 ± 0.34 c	1.17 ± 0.12 a

Note. BTG – topsoil control group at beginning of experiment, BSCG – subsoil control group at beginning of experiment, ETCG – topsoil control group at end of experiment, ESCG – subsoil control group at end of experiment, ETTG – topsoil treated group at end of experiment, ESTG – subsoil treated group at end of experiment; the data are shown by mean ± SD; different letters in a single row indicate significant difference between groups at $p < 0.05$.

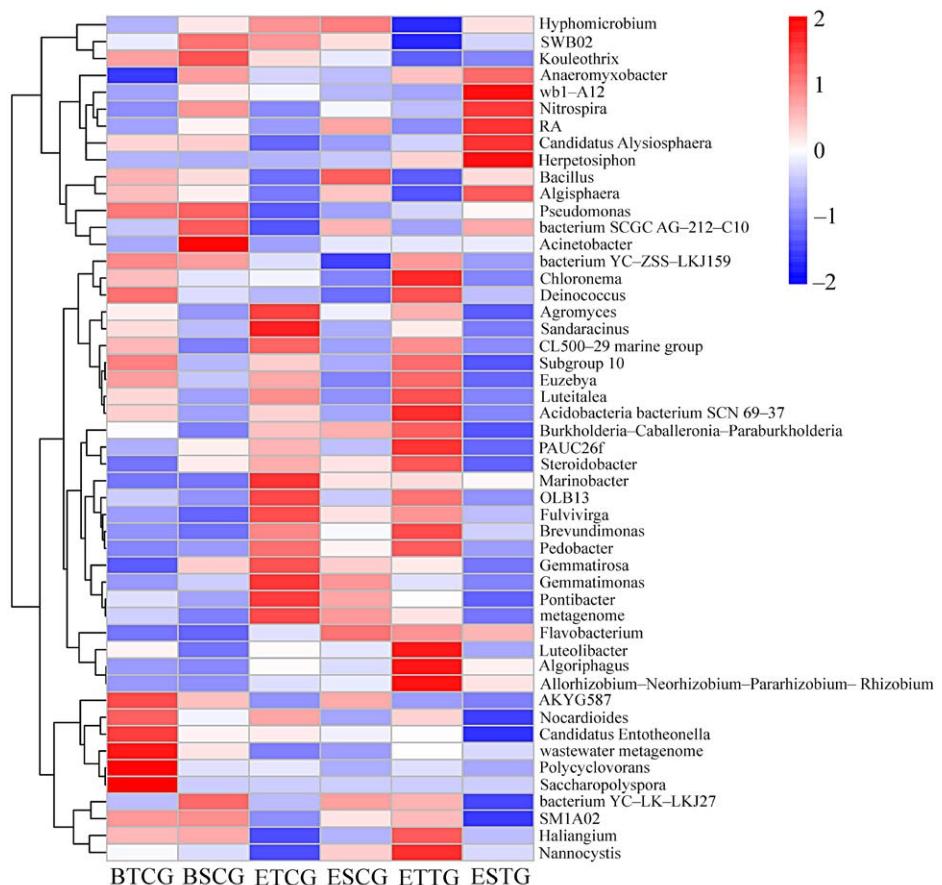
abundance of *Rokubacteria* in BTCG was 0.93% less than in BSCG. At the end of experiment, adding of the biocompatible soil amendment made almost all the phyla composition varied distinctly in ETTG and ESTG; the composition of *Actinobacteria* decreased from 22.96% in BTCG to 17.46% in BSCG, and the composition of *Rokubacteria* increased from 1.63% in BTCG to 2.35% in BSCG. Whether in topsoil or subsoil, the results revealed that the composition of bacteria would change as the time went on. For example, from the beginning to the end of experiment, the abundance of *Chloroflexi* and *Planctomycetes* decreased significantly, and the abundance of *Bacteroidetes* and *Gemmamimonadetes* distinctly increased in topsoil. The same results were obtained in subsoil samples.

The use of the biocompatible soil amendment obviously affected the abundance of phyla. Compared with ETCG, the composition of *Proteobacteria* and *Chloroflexi* in ETTG was improved significantly by 15% and 9.8%, respectively. The composition of *Acidobacteria*, *Actinobacteria* and *Gemmamimonadetes* was reduced obviously. In a similar way in ESTG, the biocompatible soil amendment also influenced the composition of *Proteobacteria*, *Acidobacteria* and *Chloroflexi* positively and of *Actinobacteria*, *Bacteroidetes*, *Planctomycetes* and *Gemmamimonadetes* negatively.

Proteobacteria are Gram-negative aerobic or facultative bacteria, many of which have a broad range of metabolic capabilities, including the ability to fix nitrogen (Dworkin et al., 2007). An increase in this phylum suggests that the soil amendment could hypothetically increase nutrient bioavailability for both the plant and the surrounding microbial communities. The amendment reduced the relative abundance of *Actinobacteria*. The two studies (Takahashi et al., 2014; Deng et al., 2019) have shown that water stress in crop roots systems is correlated with an increase in the abundance of many *Actinobacterial* lineages. The observed decrease in *Actinobacteria* may be a result of the observed increase in water uptake in amendment-treated plants.

Effect of soil amendment on soil microbial composition at genus level. For bacteria, the average abundance of the top 50 genera in every group was analysed and shown by heatmap (Figure 4). The results showed that the relative abundance of most genera was affected remarkably by the depth of soil, sampling date and the biocompatible soil amendment.

The effect of soil depth and sampling date at genus level is similar as phylum one. For ETTG, the biocompatible soil amendment had obviously positive influence on the abundance of *Chloronema*, *Deinococcus*, *Euzebya*, *Luteitalea*, *Steroidobacter*,



Note. Explanation of acronyms under Table 2; the heatmap visualization with hierarchical clustering of the top 50 most abundant genera was generated according to the similarity among their constituents, and they were arranged in a horizontal order according to the clustering results; red represents the more abundant and blue represents the less abundant genera in the corresponding sample.

Figure 4. The taxonomic analysis at bacteria genus level through heat map

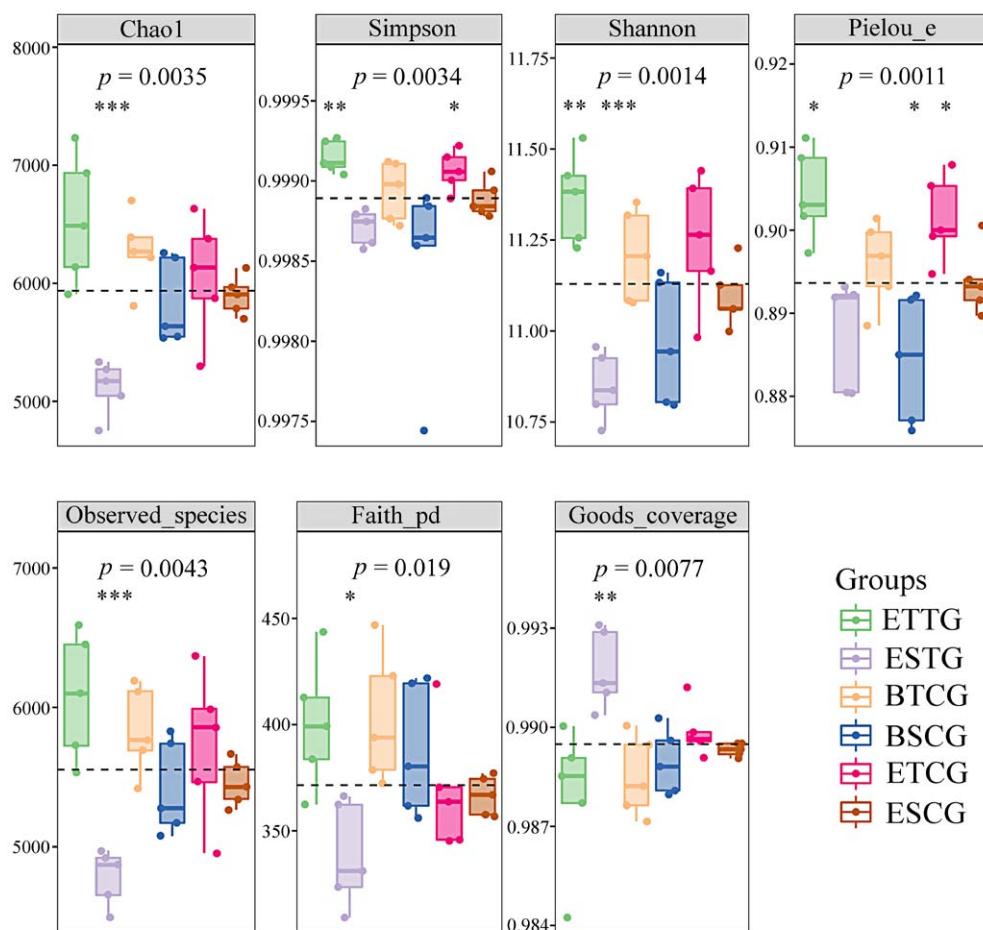
Luteolibacter, *Algoriphagus*, *Rhizobium*, *Haliangium*, *Nannocystis*, etc., and it had remarkably negative influence on the composition of *Hyphomicrobium*, *Kouleothrix*, *Gemmatirosa*, *Gemmamimonas*, *Pontibacter*, etc. For ESTG, the biocompatible soil amendment increased the relative abundance of *Anaeromyxobacter*,

Wb1-A12, *Nitrospira*, *Candidatus*, *Alysiosphaera*, *Herpetosiphon*, *Algispaea*, etc., and it clearly reduced the composition of *Hyphomicrobium*, *Kouleothrix*, *Agromyces*, *Paraburkholderia*, *PAUC26f*, *Steroidobacter*, *Gemmatirosa*, *Gemmamimonas*, *Pontibacter*, *Nocardioides*, *Candidatus*, *Entotheonella*, etc.

For fungi, the same analysis at genus level was done. The results indicated that the depth of soil, sampling date and the biocompatible soil amendment also caused the community structure shift of soil microorganisms at genus level. For ETTG, the percentage of *Myrmecridirm*, *Sapergillus*, *Rhizophlyctis*, *Zopfilella*, *Sarocladium*, *Septoria* and *Cladosporium* was increased distinctively, and the percentage of *Schizothecium*, *Bipolaris*, *Gibberella*, *Plectosphaerella*, *Cephalotrichum*, *Mortierella* and *Pseudogymnosascus* was decreased clearly by the biocompatible soil amendment. ESTG obviously improved the relative abundance of *Podospora*, *Acremonoum*, *Chaetomium*, *Botryotrichum*, *Talaromyces*, *Nectria*, *Teetracladium*, *Verticillium*,

Acaulium, *Malassezia*, *Umbilicaria*, *Lecanicillium* and *Waitea*, and while inhibiting *Cephalotrichum*, *Mortierella*, *Pseudogymnoascus*, *Dichotomopilus*, *Cyphellophora*, *Chrysosporium*, *Humicola*, *Magnaportheopsis*, *Rhizopus*, *Preussia* and *Stachybotrys* could be seen that the biocompatible soil amendment affected the microbial composition and structures obviously. Further studies should be done to figure out the influence mechanism on the soil microorganism by the biocompatible soil amendment.

Effect of the biocompatible soil amendment on α -diversity. The soil microbial α -diversities of different conditions for bacteria were characterized and analysed (Figure 5).



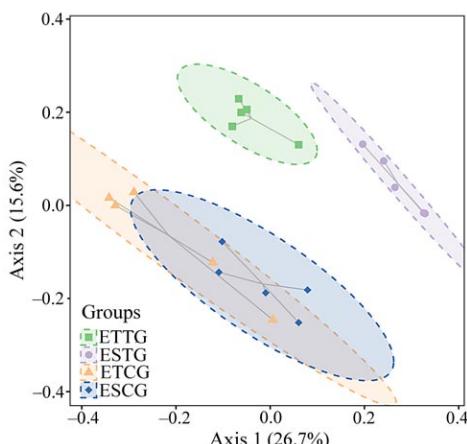
Note. Explanation of acronyms under Table 2; the medians are represented by the centre lines; the box limits represent the 25th and 75th percentiles; whiskers extend 1.5 times the interquartile range from the 25th and 75th percentiles, outliers are represented by dots.

Figure 5. The box plots of α -diversity indices for bacteria

For bacteria, all Good's coverage indices were higher than 0.988 and could reflect the coverage rate of sequencing results. The lower Chao1 and Observed_species indices of ESTG indicated that the microbial community richness was reduced significantly by the biocompatible soil amendment in subsoil. The higher Simpson and Shannon indices of ETTG represented that the biocompatible soil amendment significantly improved the microbial community diversity in topsoil. Meanwhile, it significantly reduced the evolutionary diversity in topsoil according to the Faith_pd index. The higher Pielou_e index of ETTG meant that the amendment increased the evenness of microbial community. In conclusion, the biocompatible soil amendment reduced the microbial community richness of subsoil and significantly increased the microbial community diversity and evenness of topsoil.

For fungi, the soil amendment lowered the Chao1 and Observed_species indices of ESTG, which indicated that the microbial community richness was reduced significantly by the biocompatible soil amendment in subsoil. The other indices did not show significant difference. It was microbial community structure and abundance to be responsible for the α -diversity changes. Besides the soil amendment, the microbes would interact with each other. As far as is known, there was no clear explanation for the change in α -diversity.

Effect of the biocompatible soil amendment on β -diversity. β -diversity reflects the difference or similarity in the microbial community. For bacteria, principal coordinate analysis (PCoA) was employed to show the difference in microbial communities with the 1st two axes explaining 42.3% of variation in the data (Figure 6).



Note. Explanation of acronyms under Table 2; the same colour points belong to the same soil group, and the same soil group points are marked by ellipses; the long distance among ellipses mean the greater difference in microbial community structure, the shorter – the more similar.

Figure 6. The β -diversity analysis by multi-dimensional ranking of the principal coordinate analysis (PCoA)

Without treatment by the biocompatible soil amendment, the ETCG and ESCG showed a community similarity. It represents that in natural conditions there is no clear difference between the microbial community in topsoil and subsoil. With treatment by the biocompatible soil amendment, there was a long distance between ETCG and ETTG. A similar phenomenon was observed between ESCG and ESTG, ETTG and ESTG.

It can be seen that the biocompatible soil amendment shifted the microbial β -diversity in the topsoil and subsoil. However, the mechanism by which the biocompatible soil amendment affects the soil microbial community is unknown and would necessitate more research.

Conclusions

1. The biocompatible soil amendment, which is the combination of 50% seaweed extract and 50% γ -PGA (poly- γ -glutamic acid) broth, was developed and employed to alleviate the salinity stress.

2. In the soil column experiment, the biocompatible soil amendment could significantly alleviate the salt accumulation. The increment of the salt content for the topsoil treated group (TTG) was 43.3% less than that of the treated control group (TCG), and for the subsoil treated group (STG) it was 30.3% less than that of the subsoil control group (SCG).

3. In the field experiment, the winter wheat yield increased by 10.7% after the biocompatible soil amendment was employed at a dose of 210 kg ha⁻¹.

4. The biocompatible soil amendment changed the composition and structure of microbial community. For bacteria, in the topsoil treated group at end of experiment (ETTG), it improved the composition of *Proteobacteria* and *Chloroflexi* by 15% and 9.8%, respectively. In the subsoil treated group at end of experiment (ESTG), the soil amendment influenced the phyla composition of *Proteobacteria*, *Acidobacteria*, *Chloroflexi* positively and of *Actinobacteria*, *Bacteroidetes*, *Planctomycetes*, *Gemmimonadetes* negatively. The α -diversity and β -diversity were also changed by the biocompatible soil amendment.

The next study would explore the correlation between the alleviating salt stress, increase of winter wheat yield and the composition and diversity of microbial community.

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References

- Anjos Neto A. P. D., Oliveira G. R. F., Mello S. D. C., Silva M. S. D., Gomesjunior F. G., Novembre A. D. D. L. C., Azevedo R. A. 2020. Seed priming with seaweed extract mitigate heat stress in spinach: effect on germination, seedling growth and antioxidant capacity. *Bragantia*, 79 (4): 337–346. <https://doi.org/10.1590/1678-4499.20200127>
- Boukhari M. E. B., Barakate M. Bouhia Y., Lyamlouli K. 2020. Trends in seaweed extract based biostimulants: manufacturing process and beneficial effect on soil-plant systems. *Plants*, 9 (3): 359–371. <https://doi.org/10.3390/plants9030359>
- Bray J. R., Curtis J. T. 1957. An ordination of the upland forest communities of southern Wisconsin. *Ecological Monographs*, 27: 325–349. <https://doi.org/10.2307/1942268>
- Çavuşoğlu K., Kabar K. 2010. Effects of hydrogen peroxide on the germination and early seedling growth of barley under NaCl and high temperature stresses. *Eurasian Journal of BioSciences*, 4 (1): 70–79. <https://doi.org/10.5053/ejobios.2010.4.0.9>
- Chen L., Su W., Xiao J., Zhang C., Zheng J., Zhang F. 2021. Poly- γ -glutamic acid bioproduct improves the coastal saline soil mainly by assisting nitrogen conservation during salt-leaching process. *Environmental Science and Pollution Research International*, 28 (7): 8606–8614. <https://doi.org/10.1007/s11356-020-11244-7>
- Deng S., Wipf H. M.-L., Pierroz G., Raab T. K., Khanna R., Coleman-Derr D. 2019. A plant growth-promoting microbial soil amendment dynamically alters the strawberry root bacterial microbiome. *Scientific Reports*, 9(1): 17677–17691. <https://doi.org/10.1038/s41598-019-53623-2>
- Dworkin M. et al. (eds). 2007. *Proteobacteria: alpha and beta subclasses*. The Prokaryotes, vol. 5.
- Egamberdieva D., Li L., Ma H., Wirth S., Bellingrath-Kimura S. D. 2019. Soil amendment with different maize biochars improves chickpea growth under different moisture levels by improving symbiotic performance with *Mesorhizobium ciceri* and soil biochemical properties to varying degrees. *Frontiers in Microbiology*, 10: 2423–2436. <https://doi.org/10.3389/fmicb.2019.02423>
- Egamberdiyeva D. 2007. The effect of plant growth promoting bacteria on growth and nutrient uptake of maize in two different soils. *Applied Soil Ecology*, 36 (2–3): 184–189. <https://doi.org/10.1016/j.apsoil.2007.02.005>
- Elansary H. O., Norrie J., Ali H. M., Salem M. Z. M., Mahmoud E. A., Yessoufou K. 2016. Enhancement of Calibrachoa growth, secondary metabolites and bioactivity using seaweed extracts. *BMC Complementary Medicine and Therapies*, 16 (1): 341. <https://doi.org/10.1186/s12906-016-1332-5>
- Etesami H., Maheshwari D. K. 2018. Use of plant growth promoting rhizobacteria (PGPRs) with multiple plant growth promoting traits in stress agriculture: action mechanisms and future prospects. *Ecotoxicology and Environmental Safety*, 156: 225–246. <https://doi.org/10.1016/j.ecotox.2018.03.013>
- Flowers T. J., Yeo A. R. 1995. Breeding for salinity resistance in crop plants: where next? *Australian Journal of Plant Physiology*, 22 (6): 875–884. <https://doi.org/10.1071/PP9950875>

- Huang X., Dong W., Wang H., Feng Y. 2017. Role of acid/alkali-treatment in primary sludge anaerobic fermentation: insights into microbial community structure, functional shifts and metabolic output by high-throughput sequencing. *Bioresource Technology*, 249: 943–952. <https://doi.org/10.1016/j.biortech.2017.10.104>
- Katoh K., Misawa K., Kuma K., Miyata T. 2002. MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. *Nucleic Acids Research*, 30 (14): 3059–3066. <https://doi.org/10.1093/nar/gkf436>
- Kumar M., Etesami H., Kumar V. (eds). 2019. Saline Soil-based Agriculture by Halotolerant Microorganisms. Springer, 253. <https://doi.org/10.1007/978-981-13-8335-9>
- Ladha J. K., Tirolpadre A., Punzalan G. C., Castillo E., Singh U., Reddy C. K. 1998. Nondestructive estimation of shoot-nitrogen in different rice genotypes. *Journal of Agronomy*, 90(1): 33–40. <https://doi.org/10.2134/agronj1998.00021962009000010007x>
- Liang J., Shi W. 2021. Poly- γ -glutamic acid improves water-stable aggregates, nitrogen and phosphorus uptake efficiency, water-fertilizer productivity, and economic benefit in barren desertified soils of Northwest China. *Agricultural Water Management*, 245: 1–6. <https://doi.org/10.1016/j.agwat.2020.106551>
- Lu S., Zhang X., Xue Y. 2017. Application of calcium peroxide in water and soil treatment: a review. *Journal of Hazardous Materials*, 337: 163–177. <https://doi.org/10.1016/j.jhazmat.2017.04.064>
- Manchanda G., Garg N. 2008. Salinity and its effects on the functional biology of legumes. *Acta Physiologiae Plantarum*, 30 (5): 595–618. <https://doi.org/10.1007/s11738-008-0173-3>
- McDonald D., Price M. N., Goodrich J., Nawrocki E. P., DeSantis T. Z. 2012. An improved Greengenes taxonomy with explicit ranks for ecological and evolutionary analyses of bacteria and archaea. *The ISME Journal*, 6 (3): 610–618. <https://doi.org/10.1038/ismej.2011.139>
- Moin M., Bakshi A., Madhav M. S., Kirti P. B. 2017. Expression profiling of ribosomal protein gene family in dehydration stress responses and characterization of transgenic rice plants overexpressing RPL23A for water-use efficiency and tolerance to drought and salt stresses. *Frontiers in Chemistry*, 5: 97–112. <https://doi.org/10.3389/fchem.2017.00097>
- Mullissa A. G., Tolpekin V., Stein A., Perissin D. 2017. Polarimetric differential SAR interferometry in an arid natural environment. *International Journal of Applied Earth Observation and Geoinformation*, 59: 9–18. <https://doi.org/10.1016/j.jag.2017.02.019>
- Myers S. S., Smith M. R., Guth S., Golden C. D., Vaitla B., Mueller N. D., Dangour A. D., Huybers P. 2017. Climate change and global food systems: potential impacts on food security and undernutrition. *Annual Review of Public Health*, 38: 259–277. <https://doi.org/10.1146/annurev-publhealth-031816-044356>
- Nilsson R. H., Larson K. H., Taylor A. F. S., Bengtsson-Palme J., Jeppesen T. S., Schigel D., Kennedy P., Picard K., Glöckner F. O., Tedersoo L. 2019. The UNITE database for molecular identification of fungi: handling dark taxa and parallel taxonomic classifications. *Nucleic Acids Research*, 47 (D1): D259–D264. <https://doi.org/10.1093/nar/gky1022>
- Sakr M. T., El-Metwally M. A. 2009. Alleviation of the harmful effects of soil salt stress on growth, yield and endogenous antioxidant content of wheat plant by application of antioxidants. *Pakistan Journal of Biological Sciences*, 12 (8): 624–630. <https://doi.org/10.3923/pjbs.2009.624.630>
- Renaut S., Masse J., Norrie J. P., Blal B., Hijri M. 2019. A commercial seaweed extract structured microbial communities associated with tomato and pepper roots and significantly increased crop yield. *Microbial Biotechnology*, 12 (6): 1346–1358. <https://doi.org/10.1111/1751-7915.13473>
- Shrivastava U. P., Kumar A. 2013. Characterization and optimization of 1-amino cyclopropane-1-carboxylate deaminase (ACCD) activity in different rhizospheric PGPR along with *Microbacterium* sp. strain ECI-12A. *International Journal of Applied Sciences and Biotechnology*, 1 (1): 11–15. <https://doi.org/10.3126/ijasbt.v1i1.7921>
- Takahashi S., Tomita J., Nishioka K., Hisada T., Nishijima M. 2014. Development of a prokaryotic universal primer for simultaneous analysis of Bacteria and Archaea using next-generation sequencing. *PLoS ONE*, 9 (8): 2369–2375. <https://doi.org/10.1371/journal.pone.0105592>
- Wang X., Dong G., Liu X., Zhang S., Li C., Lu X., Xia T. 2020. Poly- γ -glutamic acid-producing bacteria reduced Cd uptake and effected the rhizosphere microbial communities of lettuce. *Journal of Hazardous Materials*, 398: 398–408. <https://doi.org/10.1016/j.jhazmat.2020.123146>
- Wood N. T. 2001. Nodulation by numbers: the role of ethylene in symbiotic nitrogen fixation. *Trends in Plant Science*, 6 (11): 501–502. [https://doi.org/10.1016/S1360-1385\(01\)02128-8](https://doi.org/10.1016/S1360-1385(01)02128-8)
- Zhang L., Yang X., Gao D., Wang L., Li J., Wei Z., Shi Y. 2017. Effects of poly- γ -glutamic acid (γ -PGA) on plant growth and its distribution in a controlled plant-soil system. *Scientific Reports*, 7: 6090–6102. <https://doi.org/10.1038/s41598-017-06248-2>

Biologinis dirvožemio gerinimo produktas didina žieminių kviečių derlingumą ir moduliuoja dirvožemio mikroorganizmų kiekį druskos bei šarmo streso sąlygomis

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Santrauka

Šiuo metu dėl visame pasaulyje didėjančio žemės druskėjimo vis labiau trūksta maisto produktų. Siekiant sumažinti druskų ir šarmų sukeliamą stresą ir padidinti pasėlių derlių, buvo sukurtas biologiškai suderintas ir nebrangus dirvožemio gerinimo produktas, kurį sudaro jūros dumblių ekstraktas ir poli- γ -gliutamo rūgšties nuoviras santiui 50:50 % (w:w). Jis buvo įvertintas atliekant bandymą su dirvožemio monolitais laboratorijoje ir vykdant žieminių kviečių lauko eksperimentą. Dirvožemio mikroorganizmai buvo tirti 16S rRNA didelio našumo sekoskaitos metodu. Tyrimo rezultatai parodė, kad biologinis produktas dirvožemio viršutiniame sluoksnyje ir podirvyje druskų kiekį sumažino atitinkamai 43,3 ir 30,3 %. Dirvožemio gerinimo produktas žieminių kviečių derlingumą padidino 10,7 %. Biologinis produktas taip pat pakeitė dirvožemio bakterijų sudėtį, struktūrą ir įvairovę, o tai turėjo įtakos augalų augimui ir derlingumui; grybų bendrijai didelės įtakos nebuvu nustatyta. Biologinis produktas buvo naudinges mažinant druskos stresą ir didinant žieminių kviečių derlingumą silpnai druskingame ir šarmingame dirvožemyje.

Reikšminiai žodžiai: biologinė įvairovė, druskėjimas, jūros dumbliai, mikrobiota, poli- γ -gliutamo rūgštis.