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Influence of Organic Amendments Based on Garden Waste for Microbial Community Growth in Coastal Saline Soil

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Abstract: Garden waste compost (GWC) has been applied as an amendment to improve the desalination efficiency, nutrient availability and diversity of the microbial community in coastal saline soil. Understanding the response of the microbial community to garden waste compost application is of great significance in coastal ecological restoration. Four treatments were established: CK, nonamended control; T1, application of 68 kg·m^{-3} garden waste compost; T2, application of 15 kg·m^{-3} bentonite; and T3, a mixture of garden waste compost and bentonite. In addition, soil physicochemical properties, soil enzymes, microbial biomass carbon and the soil microbial community were measured. The results show that T3 had a more significant effect on increasing soil enzymes, as well as microbial biomass carbon and nitrogen, urease, sucrase and dehydrogenase activities. Based on the relative abundance, microbial diversity and linear discriminant effect size (LEfSe) analyses, the amendments can be seen to have increased the microbial abundance and alpha diversity of the bacterial structure and also altered the microbial community structure. RDA and Pearson correlation analysis at the phylum level indicated that available nitrogen, total porosity, hydraulic conductivity, bulk density and EC were the primary determinants of microbial communities associated with this amendment. In conclusion, the application of garden waste compost enables more microorganisms to participate in the soil material cycle, indicating that garden waste composting is beneficial to the restoration of coastal soils.

Keywords: garden waste compost; coastal saline soil; microbial community structure

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1. Introduction

Garden waste is an important component of urban solid waste and generally refers to organic wastes such as dead branches, bark, fallen leaves and grass clippings generated in the process of urban greening and garden maintenance. With the growth of urban green areas in China, the amount of garden waste has also increased as a result of landscape maintenance. In the decade from 2011 to 2019, the amount of garden waste in China surged from 22.453 million tons in 2011 to 33.134 million tons in 2019 [1]. However, the recycling rate of garden waste in China is less than 10%. Therefore, an effective and ecofriendly treatment method is urgently needed to address garden waste. Garden waste composting is an efficient method of recycling organic matter and nutrients by converting garden waste into stable humus via microorganisms [2]. Compost products can be used as soil amendments or as organic fertilizers to maintain soil moisture, improve soil fertility and improve soil structure [3].

Recently, compost has been applied to enhance the desalination of reclaimed coastal saline soil [4,5]. Many coastal regions in China have been reclaimed and developed into aquaculture, agricultural land and construction land [6]. Human activities have gradually become the most active and dominant driving factors that change the natural coastline and tidal flats [7]. Meanwhile, coastal reclamation inevitably creates some negative environmental and ecological issues, such as ecosystem collapse, biodiversity reduction and

habitat degradation [6,8]. In coastal areas, salinization is a common and serious problem for crop cultivation and the ecological restoration of degraded wetlands. The soil salinity ranged from 0.11 to 10.50 $dS \cdot m^{-1}$ (1:5 dilution $EC_{1:5}$), and the salinity in the topsoil was greater than that in the subsoil [9,10]. Soil desalination is difficult when there is shallow terrain and slow internal drainage [5].

Changes in soil properties and soil improvement processes in reclaimed coastal areas have been extensively analyzed and discussed by researchers [10–12]. The application of organic and inorganic amendments to reclaimed soils can significantly adjust a range of soil properties, the C, N and P contents and stoichiometry and extracellular enzyme activities [13]. The addition of gypsum and rice straw compost resulted in a reduction in soil bulk density and a corresponding increase in soil porosity in highly saline–sodic soils [3]. Most of these studies focused on soil texture, salinity and nutrient changes, and few studies have systematically analyzed the impact of compost products on the soil microbial community structure in coastal areas [14–16]. Soil microbial diversity is critical for maintaining soil fertility and function, as microorganisms regulate most soil biological processes and geochemical cycles.

However, foundational knowledge is still lacking with respect to how garden waste interacts with local microbial populations, as well as how the microbial utilization pathways of C in garden waste compost (GWC) organic amendments affect community characteristics, leading to changes in the development of microbial taxa [17–19]. Therefore, exploring the interaction between microbial populations and clarifying the main controlling factors of garden waste affecting the distribution of microbial communities are crucial for improving coastal ecological resilience and resource recycling.

2. Materials and Methods

2.1. Experimental Site and Material

The study was carried out in July 2016 at the Future Technology City (39°7′ N, 117°32′ E), Binhai New Area, Tianjin, China, where a wide-ranging coastal saline land greening project was in progress (Figure 1). Tianjin has a typical warm temperate semi humid continental monsoon climate with an average annual maximum temperature of 18.6 °C, an average annual minimum temperature of 9.7 °C and an average annual precipitation of 516 mm. The long-term average surface water evaporation is 1625 mm, and the evaporation amount is approximately three times higher than the amount of precipitation [20]. The research soil was a silt-filled soil that had been deposited over 5 years, and the soil type was clay. The general properties of the soil (0–40 cm depth) collected from the research site are shown in Table 1.

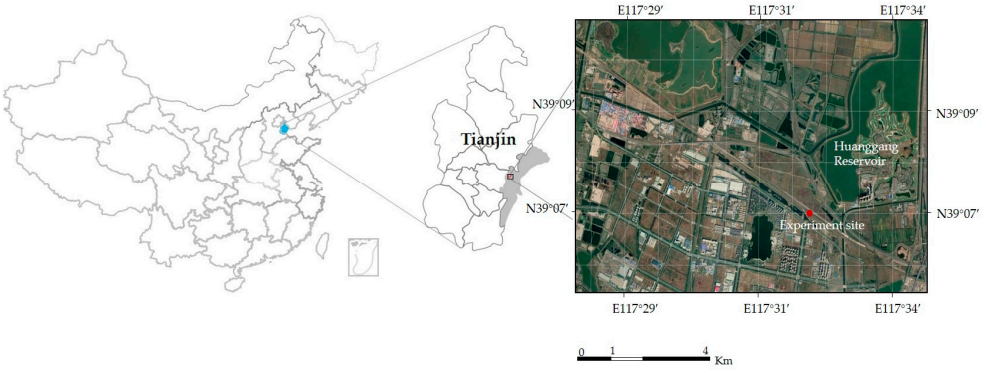


Figure 1. Location of the experimental site.

Table 1. Properties of the saline soil [a].

Index	Value
pH	7.69 ± 0.42
$EC_{1:5}$ ($mS \cdot cm^{-1}$)	4.89 ± 0.35
Salinity ($g \cdot kg^{-1}$)	16.73 ± 1.27
Bulk density ($g \cdot cm^{-3}$)	1.53 ± 0.08
Total porosity (%)	39.37 ± 2.41
Hydraulic conductivity ($cm \cdot s^{-1}$)	$0.63 \times 10^{-5} \pm 0.10 \times 10^{-5}$
Organic matter ($g \cdot kg^{-1}$)	1.73 ± 0.01
Available nitrogen ($mg \cdot kg^{-1}$)	41.21 ± 1.22
Available phosphorus ($mg \cdot kg^{-1}$)	4.53 ± 0.39
Available potassium ($mg \cdot kg^{-1}$)	58.2 ± 2.41
CO_3^{2-} ($g \cdot kg^{-1}$) [b]	0.03 ± 0.01
HCO_3^- ($g \cdot kg^{-1}$) [b]	0.17 ± 0.01
Cl^- ($g \cdot kg^{-1}$) [b]	4.75 ± 0.02
Ca^{2+} ($g \cdot kg^{-1}$) [b]	0.83 ± 0.01
Mg^{2+} ($g \cdot kg^{-1}$) [b]	0.68 ± 0.01
K^+ ($g \cdot kg^{-1}$) [b]	0.37 ± 0.01
Na^+ ($g \cdot kg^{-1}$) [b]	6.48 ± 0.15
SO_4^{2-} ($g \cdot kg^{-1}$) [b]	2.86 ± 0.18

Values are the averages ± standard deviations (n = 5). [a] The properties of the saline soil were determined according to the methods described by Li [5]. [b] Soluble salt ions.

Garden waste comprises fallen leaves, grass clippings and branches (*Sophora japonica*, *Rhus typhina* and *Festuca ela ta*) collected during greening-related maintenance. The properties of green waste are listed in Table 2. The entire composting experiment was conducted at Tianjin Beilin Xinyuan Greening Engineering Co., Ltd. (Tianjin, China). The waste was broken down by a brush chipper (BC700XL, Vermeer, Pella, IA, USA) to a particle size of approximately 0.5–2 cm. The moisture content of these raw materials was adjusted to approximately 50–60%, and urea was added to adjust the initial C/N ratio of the compost material to 25–30 to optimize the microbial activity. A commercial microbial inoculant, effective microorganisms (EM, from Zhengzhou Nongfukang Biotechnology Co., Ltd., Zhengzhou, China), was added to the compost material to accelerate microbial fermentation. To ensure an adequate oxygen supply, an automatic compost mixing system was used to turn the compost pile for a total of 60 min each day. The composting process lasted approximately 40 days. When the temperature of the pile was equal to the ambient temperature, the composting process ended. After the compost had naturally dried for 3 days, the final product was transported to the experimental site for application. All the indices indicate that the final garden waste compost used was stable and mature.

2.2. Experiment

To explore the effect of garden waste compost on the soil microbial community, inorganic modified bentonite commonly used in coastal saline soil was used for the comparative analysis. The application of the soil amendments in each treatment was as follows: (1) a nonamended control (CK), (2) the addition of 68 $kg \cdot m^{-3}$ garden waste compost (T1), (3) the addition of 15 $kg \cdot m^{-3}$ bentonite (T2) and (4) the addition of 68 $kg \cdot m^{-3}$ garden waste compost plus 15 $kg \cdot m^{-3}$ bentonite (T3). The amount of bentonite and garden waste compost added in the field experiment was based on previous experimental results [5] and studies by Zhou [21].

Table 2. The properties of raw materials and garden waste compost.

Index	Value [a]
Raw materials	
pH	7.17 ± 0.31
EC (mS·cm^{-1})	1.21 ± 0.05
Bulk density (g·cm^{-3})	0.15 ± 0.01
Total carbon (%)	42.93 ± 3.72
Total nitrogen (%)	0.82 ± 0.04
C/N ratio	52.34 ± 3.21
Water content	40.17 ± 2.55
Garden waste compost	
pH	6.65 ± 0.22
EC (mS·cm^{-1})	1.62 ± 0.14
Bulk density (g·cm^{-3})	0.34 ± 0.02
Total carbon (%)	68.13 ± 2.71
Total nitrogen (%)	2.57 ± 0.18
Available phosphorus (mg·kg^{-1})	498.266 ± 9.46
Available potassium (mg·kg^{-1})	1078.654 ± 8.08
Germination index (%)	98.99 ± 1.31

The values indicate the averages ± standard deviations (n = 5). [a] The properties of the raw materials were determined according to the methods described by Tong [20].

Each treatment was repeated three times and was randomly arranged in 12 blocks. Each block was 54 m^2 (6 m × 9 m), surrounded by 50 cm-wide protection lines. A drainage desalination system with a depth of 1 m and a spacing of 5 m was installed underground. All the blocks were separated by plastic sheets to a depth of 1 m. The drainage pipe consisted of a perforated polyvinyl chloride (PVC) pipe with a diameter of 0.06 m and a slope decrease of 2‰. To prevent soil particles from entering the pipe and causing blockage, crushed stones were laid on top of the pipe as a filter material, the thickness of which was 20 cm. Considering the engineering cost and the depth of the plant roots, the amendments and soil within the 0–40 cm layer were thoroughly mixed. The salt in the soil was removed by leaching; microspray irrigation was applied 5 times from July to October for rinsing. Three-year-old *Robinia pseudoacacia* cv. Idaho trees were planted at a spacing of 3 m in August. Trees displaying similar height, basal diameter and growth potential were obtained from the saline–alkali-tolerant botanical garden. These trees are shallow-rooted, and most of the roots are found in the top 30–40 cm layer of the soil. Because of its tolerance to salt stress, the tree is planted in coastal saline soil [22].

2.3. Soil Sampling and Physicochemical Analysis

One year after planting, soil samples were collected around randomly selected trees. Briefly, the radial area around each torso was divided into six 60° segments, two of which were randomly selected along the diagonal for sampling. At 100–140 cm from the level of the trunk, 0–20 cm of the soil was dug. The soil was then mixed evenly and collected by quartering. All samples were collected in sterile zip-lock bags, stored in an ice box at 4 °C and transported to the laboratory. A portion of the collected soil samples was sieved through a 2 mm sieve and stored in a −80 °C freezer for determination of the soil microbial community. The other part was air-dried, crushed, uniformly mixed and sieved through a 2 mm sieve to determine the following physicochemical properties: soil samples were prepared as soil leachate extracted from a 1:5 (w/v) soil-distilled water solution to measure pH and EC; soil bulk density and total porosity were measured according to the cutting-ring method; saturated hydraulic conductivity was determined by a Guelph permeameter (Model 2800K, Armidale, Australia) [23]; and soil pH and salinity were measured via a multiparameter water quality meter (Model DZS-706, Beijing, China). The organic matter was determined using the dry combustion method [24]; alkali solution diffusion was used to measure the available nitrogen content [25]; the available phosphorus

content was measured using the Olsen method [26]; and the available potassium content was subsequently measured via ammonium acetate digestion–flame photometry [27].

2.4. Soil Microbial Biomass

The chloroform fumigation extraction method was used to determine the soil microbial biomass carbon (MBC) and nitrogen (MBN) contents [28–30]. Both fumigated and unfumigated soil samples were extracted with 0.5 M K_2SO_4. The content of MBC was determined by titrating the extract with $FeSO_4$ after hot digestion with $K_2Cr_2O_7$-H_2SO_4, and MBN was determined calorimetrically using a spectrophotometer (570 nm) to calculate the NH_3-N content with ninhydrin reagent. MBC was calculated as MC/KC, where MC = (organic C extracted from fumigated soils) − (organic C extracted from nonfumigated soils), and KC was 0.45. MBN was calculated as MN/KN, where MN = (total N extracted from fumigated soils) − (total N extracted from nonfumigated soils), and KN was 0.54.

2.5. Soil Enzyme Activities

The determination of soil enzyme activity was based on the method of Guan et al. [31], including the assessment of urease activity by the indophenol blue method [32]; dehydrogenase activity was assessed by the triphenyl tetrazolium chloride (TTC) colorimetric method [33]; alkaline phosphatase activity was assessed by the phenyl disodium phosphate colorimetric method [29]; and sucrase activity was assessed by the 3,5-dinitrosalicylic acid ratio colorimetric method [34].

2.6. Soil Microbial Community

Bacterial community structural diversity: DNA was extracted from soil samples and garden waste compost products using the FastDNA™ SPIN kit. DNA purity and concentration were assessed by agarose gel electrophoresis and spectrophotometry. Primers (338F-5′ ACTCCTACGGGAGGCAGCAG-3′ and 806R-5′ GGACTACHVGGGTTWTCTAAT-3′) were designed to amplify the V3–V4 hypervariable region of the 16S rRNA gene. PCRs were performed using TransGen AP221-02: TransStartFastpfu DNA polymerase, 20 μL reaction system, with each 20 μL PCR mix containing 4 μL of buffer (5× Transgen), 2 μL of 2.5 mM dNTPs, 0.8 μL of forward primer (5 μM), 0.8 μL of reverse primer (5 μM), 0.4 μL of FastPfu polymerase, 0.2 μL of BSA, 10 ng of template DNA and 20 μL of ddH2O. The PCR thermal cycling conditions were as follows: initial denaturation at 95 °C for 3 min, followed by 30 cycles of denaturation at 95 °C for 30 s, annealing at 55 °C for 30 s, primer extension at 72 °C for 45 s and a final 10 min for 30 cycles at 72 °C and 10 °C until being stopped by the user.

Fungal community structure: DNA purity and concentration were assessed by agarose gel electrophoresis and spectrophotometry, and primers were designed with ITS1 as the target region to amplify the ITS1 region of the ITS gene. The reaction system was as follows: TransStartFastpfu DNA polymerase, 20 μL of reaction system for PCR, with each 20 μL PCR mix containing 4 μL of buffer (5× Transgen), 2 μL of 2.5 mM dNTPs, 0.8 μL of forward primer (5 μM), 0.8 μL of reverse primer (5 μM), 0.4 μL of FastPfu polymerase, 0.2 μL of BSA, 10 ng of template DNA and20 μL of ddH_2O. The PCR thermal cycling conditions were as follows: initial denaturation at 95 °C for 3 min, followed by 30 cycles of denaturation at 95 °C for 30 s, annealing at 55 °C for 30 s, primer extension at 72 °C for 45 s and a final 10 min for 30 cycles at 72 °C and 10 °C until stopped by the user.

Amplification products were detected by 2% agarose gel electrophoresis and washed with Tris-HCl. The sequencing of PCR products was performed by Beijing Saiogino Biotechnology Co., Ltd., and the diversity and composition of microbial communities were measured using an Illumina HiSeq 2500 (San Diego, CA, USA). All sequences were deposited in NCBI under the accession numbers SUB12502561 and SUB12502442.

2.7. Statistical Analysis

The calculation of microbial α-diversity was conducted using Mothur (version v.1.30). One-way analysis of variance (ANOVA) with Duncan's test was performed to analyze the differences in soil properties, microbial biomass, enzyme activities and microbial diversity indices (SPSS software, version 19.0, Chicago, IL, USA). Correlations among soil properties, soil enzyme activities and soil microbial compositions were assessed using redundancy analysis (RDA) (CANOCO 4.5) and Pearson analysis [35]. A nonmetric multidimensional scaling (NMDS) approach was employed to assess differences in soil microbial communities across all soil sample types based upon Bray–Curtis distances (R, vegan), with similarities between samples being displayed via hierarchical clustering analyses (R, vegan, ape and ggtree). Linear discriminant analysis (LDA) with effect size (LEfSe) was employed to identify land use type related biomarkers [36].

3. Results

3.1. Soil Physicochemical Properties

Table 3 shows that, after leaching, the salinity of the CK group was still greater than 3 $dS \cdot m^{-1}$, indicating that the desalting effect was weak. A significant decrease in EC was observed for T1 (1.96 $mS \cdot cm^{-1}$), T2 (1.87 $mS \cdot cm^{-1}$) and T3 (1.22 $mS \cdot cm^{-1}$) compared with the CK. T3 yielded the best desalination effect, which was significantly better than that of the other treatments ($p < 0.05$).

Table 3. Soil physicochemical properties.

Treatment	CK	T1	T2	T3
pH	7.69 ± 0.20 a	7.60 ± 0.22 a	7.58 ± 0.31 a	7.54 ± 0.24 a
$EC_{1:5}$ ($mS \cdot cm^{-1}$)	3.21 ± 0.33 a	1.96 ± 0.32 b	1.87 ± 0.14 b	1.2 ± 0.22 c
BD ($g \cdot cm^{-3}$)	1.53 ± 0.02 a	1.31 ± 0.04 bc	1.49 ± 0.03 ab	1.37 ± 0.01 c
TP (%)	43.35 ± 0.91 a	51.13 ± 1.14 c	44.38 ± 1.65 ab	49.30 ± 1.06 c
Ksat ($cm \cdot s^{-1}$)	$0.74 \times 10^{-5} \pm 0.30 \times 10^{-5}$ a	$8.67 \times 10^{-4} \pm 0.29 \times 10^{-4}$ b	$1.18 \times 10^{-5} \pm 0.10 \times 10^{-5}$ a	$6.63 \times 10^{-4} \pm 0.13 \times 10^{-4}$ c
OM ($g \cdot kg^{-1}$)	1.77 ± 0.10 a	2.22 ± 0.20 b	1.81 ± 0.20 a	3.63 ± 0.58 c
AN ($mg \cdot kg^{-1}$)	39.72 ± 6.52 a	61.45 ± 8.80 b	41.18 ± 5.92 a	80.00 ± 8.79 c
AP ($mg \cdot kg^{-1}$)	4.00 ± 0.94 a	6.21 ± 0.97 b	4.46 ± 0.87 a	7.12 ± 0.50 c
AK ($mg \cdot kg^{-1}$)	84.83 ± 8.67 a	113.15 ± 14.04 b	91.73 ± 10.38 a	133.33 ± 12.08 b

BD: bulk density; TP: total porosity; Ksat: saturated hydraulic conductivity; OM: organic matter; AN: available nitrogen; AP: available phosphorus; AK: available potassium. Mean value ± standard deviation (n = 15). Significant differences were analyzed via Duncan's test, with different letters indicating significant differences between treatments ($p < 0.05$).

The pH of the original soil in the experimental area was 7.69 (CK), and the pH decreased slightly after adding the amendment, but the difference was not significant. T1 showed the best improvement in soil physical properties; the total porosity and hydraulic conductivity were 51.13% and 8.67×10^{-4} ($cm \cdot s^{-1}$), respectively, which were significantly greater than those of the other treatments.

T3 manifested the best improvement in soil nutrient content; the soil OM (3.63 $g \cdot kg^{-1}$), AN (80.00 $g \cdot kg^{-1}$) and AP (7.12 $g \cdot kg^{-1}$) in T3 were significantly higher than those in the other treatments.

3.2. Soil Enzymes and Microbial Biomass

Table 4 shows that there was no significant difference in soil phosphatase activity between the four treatments, with values ranging from 0.15 to 0.16 $mg \cdot g^{-1} \cdot d^{-1}$. The urease and sucrase levels of the T1 and T3 treatments with compost were significantly higher than those of the control; the urease and sucrase levels of the T2 treatment with inorganic bentonite were not significantly different from those of the control, indicating that organic matter can significantly promote soil urease and sucrase activity. The dehydrogenase activities of the T1, T2 and T3 treatments were significantly higher than those of the control, indicating that both organic and inorganic amendments could significantly increase the activity of dehydrogenase, among which the mixed application of organic and

inorganic amendments (T3) had the most obvious promotion effect on soil dehydrogenase. The urease, sucrase and dehydrogenase levels were 10.1, 9.0 and 5.4 times those of the control, respectively.

Table 4. Soil enzymes and microbial biomass.

Treatment	CK	T1	T2	T3
Phosphatase ($mg \cdot g^{-1} \cdot d^{-1}$)	0.15 ± 0.01 a	0.16 ± 0.01 a	0.16 ± 0.02 a	0.16 ± 0.01 a
Urease ($mg \cdot g^{-1} \cdot d^{-1}$)	0.08 ± 0.02 a	0.69 ± 0.01 c	0.08 ± 0.02 b	0.81 ± 0.01 d
Sucrase ($mg \cdot g^{-1} \cdot d^{-1}$)	0.18 ± 0.03 a	1.45 ± 0.04 b	0.20 ± 0.04 a	1.63 ± 0.12 c
Dehydrogenase ($mg \cdot kg^{-1} \cdot d^{-1}$)	0.34 ± 0.03 a	1.15 ± 0.03 c	0.89 ± 0.03 b	1.85 ± 0.04 d
Microbial biomass nitrogen ($mg \cdot kg^{-1}$)	4.49 ± 0.56 a	7.30 ± 0.59 c	5.49 ± 1.10 ab	7.99 ± 0.81 d
Microbial biomass carbon ($mg \cdot kg^{-1}$)	32.12 ± 2.09 a	38.13 ± 1.73 c	34.38 ± 2.46 b	40.07 ± 1.38 d

Mean value ± standard deviation (n = 15). Significant differences were analyzed via Duncan's test, with different letters indicating significant differences between treatments ($p < 0.05$).

After the amendment was applied, the contents of soil microbial biomass carbon and soil microbial biomass nitrogen showed an increasing trend. The soil microbial biomass carbon of each treatment was significantly higher than that of the control, among which the soil microbial biomass carbon and soil microbial biomass nitrogen of the T3 group were significantly higher than those of the other treatments. Studies have shown that adding garden waste can increase the pores of compost products, providing a better growth environment for microorganisms, and microorganisms can transfer carbon and nitrogen into themselves through assimilation [18].

3.3. Composition of the Soil Microbial Community

In order to analyze the effect of garden waste on the soil microbial composition, the relative abundance was assessed at two different taxonomic levels: phyla and genus. At the phyla level, Proteobacteria, Actinobacteria, Bacteroidetes, Chloroflexi, Gemmatimonadetes and Acidobacteria were the most dominant bacterial phyla in all treatment groups (Figure 2a). The abundance of Actinobacteria in garden waste compost was only 2.03%, while it was from 16.7% to 24.8% in other treatments.

Figure 2b shows that the most dominant genera identified were Sphingomonas, Pontibacter, Bryobacter and Salinimicrobium. The application of garden waste compost reduced the abundance of Salinimicrobium. Compared with CK and T2 without garden waste compost (5.57% and 3.03%), the Salinimicrobium abundance levels in T1 and T3 were almost zero.

At the fungal phylum level, Ascomycota had the highest fungal abundance, accounting for 59.6–80.7% of the total community, and the others were Basidiomycota, Chytridomycota and Mortierellomycota (Figure 2c). The T1 and T3 treatments with garden waste increased the abundances of Basidiomycota, which were 3.51- and 1.27-fold greater than those of the controls, respectively. The addition of garden waste and bentonite reduced the abundances of Chytridomycota and Mortierellomycota, which were much lower than those of the controls at the phylum level. The abundance of fungi at the genus level varied greatly (Figure 2d). Coprinus was the dominant genus in garden waste compost, and its abundance was 19.85%, which is significantly higher than that in the other treatments. Pseudogymnoas was the most important fungal genus in CK, with an abundance of 8.20%.

3.4. Soil Microbial Diversity

Diversity indices (Shannon and Simpson) and richness indices (ACE and Chao1) were compared between different treatments in the bacterial and fungal communities, respectively (Table 5). The bacterial Shannon and Simpson indices, ACE richness index and Chao1 richness index were significantly increased by the soil amendments compared with CK ($p < 0.05$). The bacterial diversity index did not change significantly between the treatments, except for CK. Garden waste compost and bentonite did not significantly

increase the richness and diversity of fungi. The ACE, Chao, Simpson and Shannon indices were slightly higher than those of the control group, but the difference was not significant.

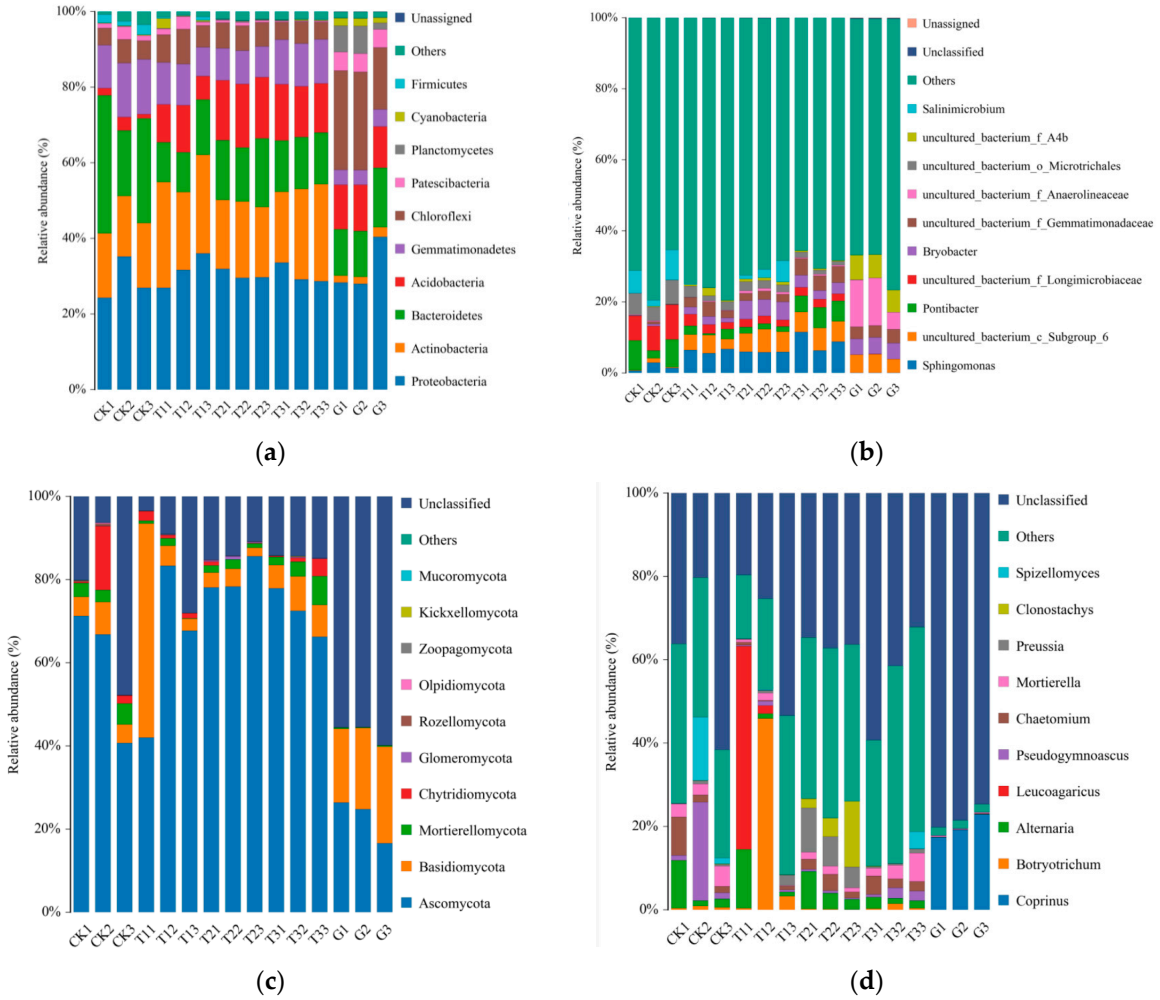


Figure 2. Relative abundance: (**a**) bacterial phylum level; (**b**) bacterial genus level; (**c**) fungal phylum level; and (**d**) fungal genus level.

Table 5. Bacterial and fungal diversity index analysis.

Treatment	ACE	Chao1	Simpson	Shannon
Bacteria				
CK	1102.60 ± 189.69 a	1100.59 ± 191.63 a	0.02 ± 0.01 b	5.06 ± 0.59 a
T1	1432.63 ± 13.93 b	1446.77 ± 6.90 b	0.01 ± 0.00 a	6.07 ± 0.03 b
T2	1310.49 ± 17.97 b	1349.46 ± 30.75 b	0.01 ± 0.00 a	6.03 ± 0.08 b
T3	1433.11 ± 59.71 b	1459.90 ± 69.04 b	0.01 ± 0.00 a	6.10 ± 0.15 b
Fungi				
CK	372.82 ± 65.29 a	359.17 ± 56.03 a	0.10 ± 0.07 a b	3.96 ± 0.33 b
T1	386.18 ± 73.02 a	395.30 ± 79.93 a	0.18 ± 0.10 b	3.19 ± 0.52 a
T2	449.73 ± 18.77 a	475.79 ± 45.16 a	0.03 ± 0.01 a	4.49 ± 0.34 b
T3	400.67 ± 48.62 a	421.02 ± 56.34 a	0.04 ± 0.02 a	4.23 ± 0.31 b

Mean value ± standard deviation (n = 3). Significant differences were analyzed via Duncan's test, with different letters indicating significant differences between treatments ($p < 0.05$).

The NMDS ranks for bacteria and fungi were 0.017 and 0.096, respectively, where a value of <0.2 is consistent with good analytical results. The closer the samples' coordinates are, the higher the similarity. Figure 3a,b shows that the three replicates of each treatment were close together, indicating that the samples in this study had good repeatability. NMDS showed that T1, T2 and T3 were significantly separated from CK, indicating that the improvement in the bacterial community after garden waste and bentonite application was significantly different from that of CK. T1 and T3 were relatively close, indicating that the bacterial communities of the two treatments with the addition of garden waste had a certain degree of similarity. Following the hierarchical clustering analyses in Figure 3c,d, it was also found that the T1 and T3 samples were closer together, resulting in a more similar species composition between the two treatments. The separation of CK from the other three treatments also confirmed the NMDS results.

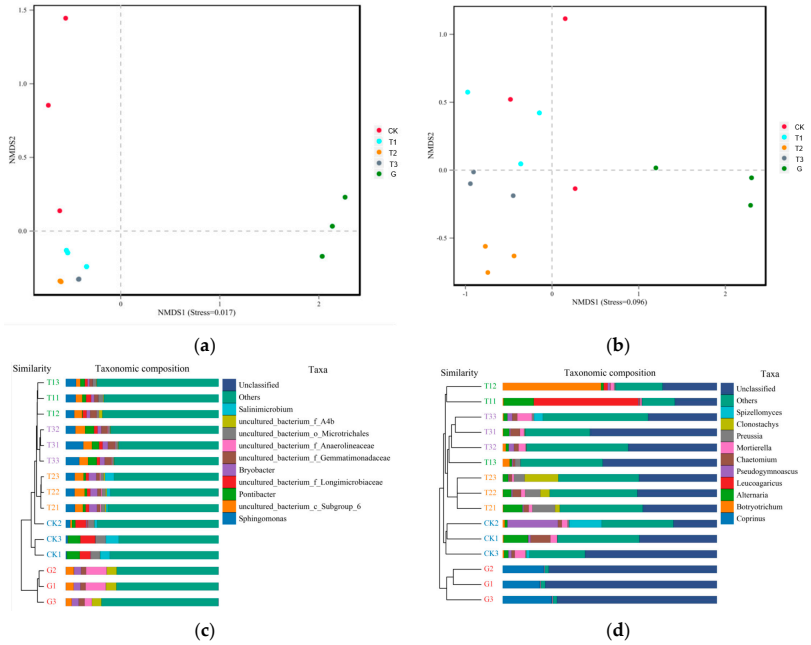


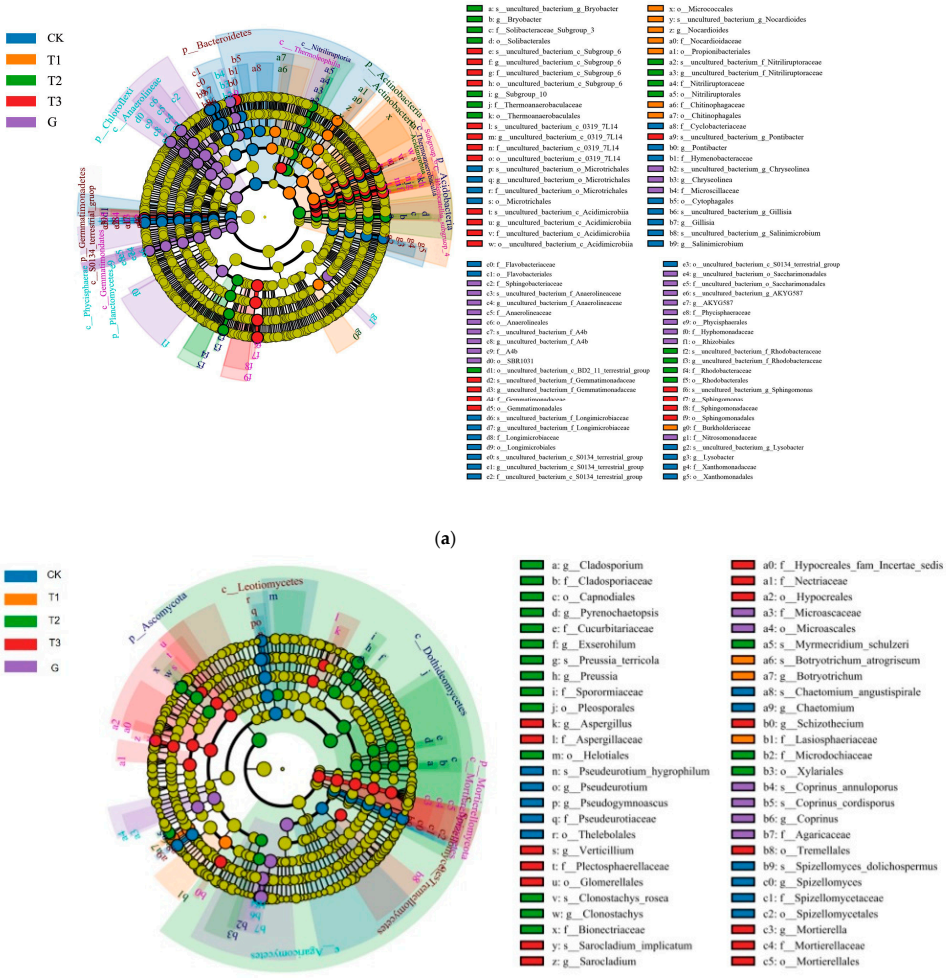
Figure 3. The NMDS and hierarchical clustering analysis of bacterial and fungal communities: (**a**) NMDS plot of bacteria; (**b**) NMDS plot of bacterial fungi; (**c**) hierarchical clustering analysis of bacteria; and (**d**) hierarchical clustering analysis of fungi.

The NMDS and hierarchical clustering analysis results for fungi are different from those for bacteria, with the CK sample's results close to those of G3. In addition, the three treatments T1, T2 and T3 were relatively separated, suggesting that there were also certain differences in the fungal community among the three treatments.

3.5. Soil Microbial Community Structure

Linear discriminant effect size (LEfSe) analysis was conducted to identify and compare the unique microbial taxa significantly related to each treatment [15]. The biomarker bacterial and fungal groups have been depicted in cladograms, and linear discriminant analysis (LDA) scores of $\geq$4.0 were then assessed (Figure S1). The taxa for each treated group are displayed in Figure 4. The indicator bacteria of the CK group were of the family Longimicrobiaceae (belonging to phylum Gemmatimonadetes) and Xanthomonadaceae (belonging to phylum Proteobacteria). Those of the inorganic amendments (T2) group were of the family Thermoanaerobaculaceae (belonging to phylum Firmicutes) and Rhodobacteraceae (belonging to phylum Proteobacteria). Those of the organic amendments (T1, T3 and G) group were the phyla Bacteroidetes (including families Microscillaceae Chitinophagaceae and Sphingobacteriaceae) and Proteobacteria (including families Burkholderiaceae, Nitrosomonadaceae and Hyphomonadaceae). Other significantly abundant biomarkers found in

the garden waste compost (G) belonged to the phyla Chloroflexi (including family Anaerolineae) and Planctomycetes (including family Phycisphaeraceae), which are predominant bacterial taxa present during composting [37].



(**a**)

(**b**)

Figure 4. LEfSe analysis of bacterial and fungal communities: (**a**) bacteria and (**b**) fungi.

The fungal community LEfSe analysis results are shown in Figure 4b. The indicator fungi of the CK group were of the family Pseudeurotiaceae (belonging to the phylum Ascomycota) and family Spizellomycetaceae (belonging to the phylum Chytridiomycota). Those of T2 group were of the families Bionectriaceae, Sporormiaceae, Cucurbitariaceae and Cladosporiaceae (belonging to the phylum Ascomycota). Those of the organic amendments (T1, T3 and G) groups were of the families Lasiosphaeriaceae, Microascaceae, Nectriaceae, Plectosphaerellaceae and Aspergillaceae (belonging to the phylum Ascomycota). Other significantly abundant biomarkers in garden waste compost (G) belonged to the phylum Basidiomycota (including the family Agaricaceae), which is consistent with the results of fungal community abundance.

3.6. Associations between Soil Microbial Communities, Enzymes and Soil Properties

The soil microbial community structure and the activity of soil enzymes have been regarded as appropriate indicators for evaluating ecological phytoremediation [38]. Figure 5 shows the correlation among soil physicochemical properties, enzyme activities and microbial biomass; since the contribution rate of RDA1 reached 70.77%, the relationship between the vectors of each physicochemical property and the RDA1 axis could explain its effect on soil enzyme activity and microbial biomass. Among them, salinity, pH and bulk density

point in the negative direction of the RDA1 axis, indicating that the increase in these three indicators has an inhibitory effect on soil enzyme activity and microbial biomass, while the four indicators of available nitrogen, available phosphorus, available potassium and organic matter have the opposite effect. It can be seen from the RDA that CK and T2 without garden waste are concentrated in the second and third quadrants, while T1 and T3 with garden waste are concentrated in the first and fourth quadrants, and there is no overlap between the two groups, indicating that the addition of garden waste compost plays an important role in the increase in soil enzyme activity and microbial biomass.

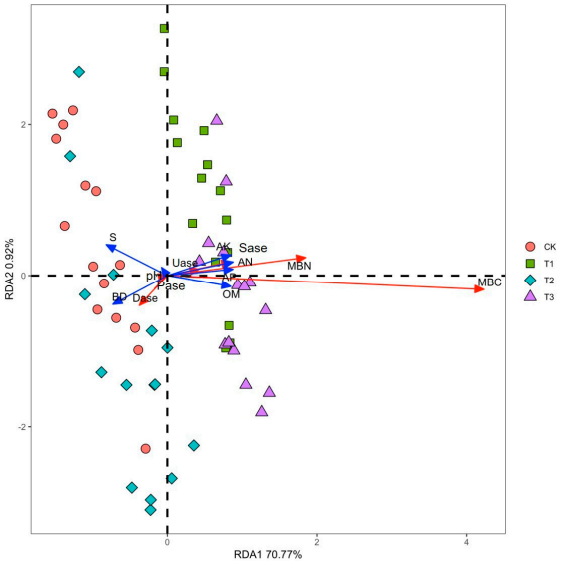


Figure 5. Redundancy analysis (RDA) for the correlation among physical and chemical soil characteristics, enzyme activities and microbial biomass. S: $EC_{1:5}$; BD: bulk density; TP: total porosity; Ksat: saturated hydraulic conductivity; OM: organic matter; AN: available nitrogen; AP: available phosphorus; AK: available potassium; Pase: phosphatase; Uase: urease; Sase: sucrase; Dase: dehydrogenase; MBN: microbial biomass nitrogen; and MBC: microbial biomass carbon.

To explore the effects of soil physicochemical factors on the microbial community, RDA and Pearson correlation analysis were performed at the phylum level. Figure 6a shows that the first principal axis explained 84.05% of the variation, and the second principal axis explained 15.95% of the variation. The axis can reflect most of the information on the relationship between the bacterial phylum level and soil environmental factors. Actinobacteria were significantly positively correlated with available potassium, extremely significantly positively correlated with available phosphorus, total porosity and hydraulic conductivity and extremely significantly negatively correlated with bulk density. Bacteroidetes were significantly negatively correlated with available N, total porosity and hydraulic conductivity, extremely significantly correlated with EC and significantly correlated with bulk density. Acidobacteria and Planctomycetes had a very significant and significant negative correlation with EC, respectively. Firmicutes had a very significant positive correlation with EC.

The RDA and Pearson correlation analysis of the fungal phyla are shown in Figure 6b,d. Figure 6b shows that the first principal axis explained 89.79% of the variation, and the second principal axis explained 10.21% of the variation. Fungi were less affected by soil physicochemical factors, and only two fungal phyla showed significant correlations with physicochemical factors. Among them, Rozellomycota was significantly positively correlated with EC, and Kickxellomycota was significantly positively correlated with available nitrogen and organic matter.

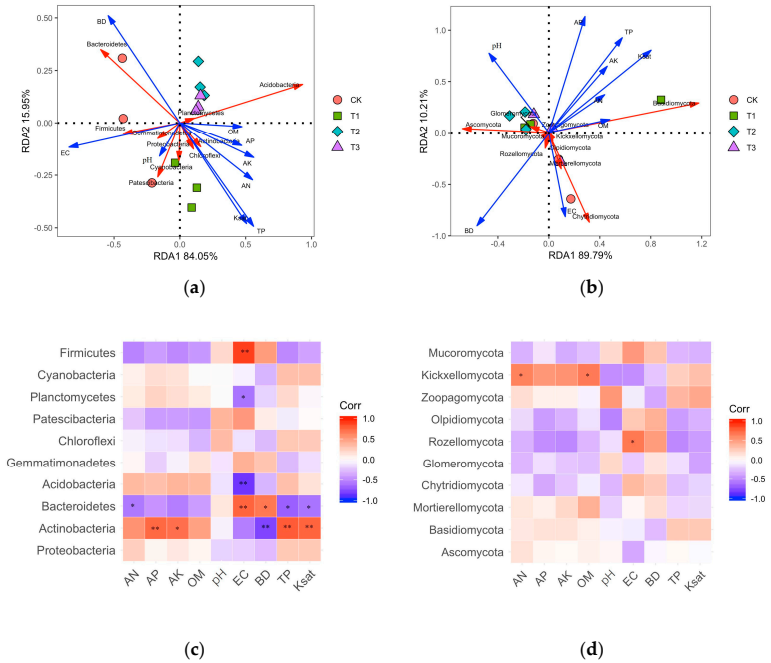


Figure 6. Redundancy analysis (RDA) of (**a**) bacterial and (**b**) fungal community, and Pearson correlation analysis of (**c**) bacterial and (**d**) fungal community. *: Significance at $p < 0.05$; **: significance at $p < 0.01$.

4. Discussion

4.1. Responses of Soil Properties to Garden Waste

Coastal saline soil has the characteristics of heavy texture, low nutrients and high salt content, which makes soil desalination difficult, air permeability poor and plant mortality high. The coastal area is generally improved by the engineering method of underground pipe drainage, but due to the low-lying terrain and limited nutrient space, secondary salinization may occur. Therefore, the saline soil should be ameliorated by the synergistic improvement of amendments and drainage engineering [39–41].

The analysis of soil physical and chemical properties showed that the original soil conductivity value was 4.89 mS·cm^{-1}, and the salinity class was extreme. One year later, the EC of the CK group was 3.21 mS·cm^{-1}, and the salinity class was still severe [42]. The salinity class of the T1–T3 group with the addition of garden waste and bentonite was moderate, which effectively alleviated the degree of salt damage. Studies have shown that organic amendments significantly improve soil nutrient accumulation compared with inorganic amendments [43], and our results confirm this. Compared with T1, BD decreased, and porosity increased in the garden waste treatment group, while the effective nutrient contents of OM, TN, TK and TP were also improved. This result occurred due to the fertilization of the soil with organic amendments, resulting in increased humic acid concentrations, soil aeration, weakened capillary action, dilution of the denser soil mineral fraction and possibly greater porosity and soil structure stability, thereby further improving nutrient supply to the rhizosphere [35,44].

Interestingly, T3 exhibited a better promotion effect in reducing soil salinity and increasing soil nutrients. This may be due to the interaction between organic and inorganic amendments, thereby enhancing the improvement effect [45]. The use of compost products directly increases the content of soil organic matter, and the microorganisms generated during the fermentation process also play a role in activating soil nutrients. However, the ion exchange effect of bentonite improves the soil physical structure and water and fertilizer retention performance, which play a synergistic role.

4.2. Responses of Soil Enzymes to Garden Waste

Soil enzymes are biologically active substances secreted by animal and plant residues, plant roots and microorganisms in the soil. They participate in the circulation of almost all organic matter and nutrient elements in the soil, and they are important indicators of soil biological characteristics [46]. Among them, sucrase is the key enzyme of soil carbon cycle transformation, while urease is used to characterize the soil nitrogen supply [47]. The results of our study show that the addition of organic amendments can significantly promote the activities of soil urease, sucrase and dehydrogenase. Microorganisms are in a low-nutrient state in coastal saline soil. The addition of organic amendments provides additional energy for microorganisms. However, fermented garden waste itself also results in many microorganisms, which further increases the secreted amount of soil microorganisms and enzymes. The addition of inorganic amendments can also increase the activities of soil invertase and dehydrogenase, while bentonite has a large adsorption capacity, thereby increasing soil moisture and water storage [3], and this provides more water and nutrients to plants. In turn, greater plant growth leads to more root biomass and exudates returned to the soil as energy sources for soil microorganisms, so in addition to microbial activity, bentonite would also likely affect soil microbial community structure [48]. A study that added inorganic modifiers such as gypsum, sand and gravel to saline–alkali soil also revealed improved soil enzyme activity, which is consistent with the results of our study [49].

4.3. Responses of the Soil Microbial Community to Garden Waste

The composition and diversity of the soil microbial community are the basis of the soil's ecological function. They are important indicators reflecting the soil ecological mechanism and stability [29]. A good microbial community has better adaptability, contributing to maintaining soil fertility and external factors. Amendments significantly increased bacterial alpha diversity (Table 5). The dominant bacterial phyla in this study were Proteobacteria, Actinobacteria, Bacteroidetes, Chloroflexi, Gemmatimonadetes and Acidobacteria. Compost has a high content of microbial organic resources, which can provide supplementary metabolic nutrients for soil microorganisms and support the regulation of the soil microbial community structure. After adding compost, the abundance of Acidobacteria and Bacteroidetes significantly increased. At the same time, Bacteroidetes are also a representative species, highlighting the differences between groups, and can be used as biomarkers. Agricultural practices and soil genesis have a central role in determining Bacteroidetes abundance [50]. Chryseolinea, Microscillaceae Chitinophagaceae and Sphingobacteriaceae in Bacteroidetes are the dominant flora during straw fermentation and also acted as biomarkers at the genus and family levels in this study. This result indicates that the bacteria in garden waste compost also played a role in regulating the structure of the soil microbial community.

Acidobacteria, an oligotrophic group, are widely distributed throughout agricultural soils, and their relative abundance is enriched in soils with low resource availability [51], mainly decomposing refractory carbon. Consistent with the results of Ren et al., there was a significant negative correlation between the relative abundance of Acidobacteria and EC in this study [35]. Since the addition of garden waste compost and bentonite reduced the EC value, the relative abundance of Acidobacteria in the treatment was significantly higher than that in CK.

The dominant phyla of the fungal community included Ascomycota, Mortierella, Basidiomycota and Zygomycota. Unlike bacteria, these fungal phyla did not respond strongly to changes in soil physicochemical properties caused by organic and inorganic amendments. The α-diversity of the fungal community associated with each treatment also did not increase significantly compared with that of the control, confirming that fungal communities were less sensitive to amendments than bacterial communities. Bacteria are generally more sensitive than fungi to the availability of C substrates because their turnaround times are much shorter than those of fungi [35,52]. The abundance of Mortierellomycota in the treatment with garden waste was lower than that in CK. Studies have shown that the relative abundance of

Mortierellomycota is significantly negatively correlated with soil total carbon content, and garden waste composting products can significantly increase soil total carbon. This content may be responsible for the decrease in the abundance of Mortierella [17].

5. Conclusions

In conclusion, we found that the mixed application of garden waste compost and bentonite can significantly reduce soil salinity, improve soil physical and chemical properties and increase soil enzyme activity. The improvement effect here was significantly better than that of the other treatments. Although inorganic amendments can improve soil physicochemical properties, promoting the activity of some soil enzymes and microbial biomass carbon, garden waste compost can provide more nutrients, such as nitrogen, phosphorus and potassium, reduce bulk density, improve soil structure and significantly increase soil enzyme activity and microbial biomass carbon and nitrogen, which are more conducive to the improvement of soil ecological function. Compost can provide supplementary metabolic nutrients for soil microorganisms and support the regulation of soil microbial community structure. The dominant bacterial phylum of Bacteroides in garden waste is different between groups and can be used as a biomarker. Key soil properties, including available nitrogen, total porosity, hydraulic conductivity, bulk density and EC, were the primary determinants of microbial communities associated with this amendment. Overall, this study showed that compost used as a soil amendment could represent an alternative fertilizer source due to its potential to improve soil nutrients and positively affect microbial community, especially bacterial ecosystems. Harnessing these potentials will also make a positive contribution to the ecology of coastal saline areas. In addition, the α-diversity of the fungal community was less sensitive to amendments, and this also suggests that further research is necessary to explore the mechanisms and make improvements.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/su15065038/s1, Figure S1: Linear discriminant analysis (LDA) of bacterial and fungal communities (**a**) Bacteria; (**b**) Fungi.

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