agriculture

Article

Addition of Exogenous Organic Ameliorants Mediates Soil Bacteriome and Microbial Community Carbon Source Utilization Pattern in Coastal Saline–Alkaline Soil

Binxian Gu [1,2], Tianyang Qin [1], Meihua Qiu [3], Jie Yu [3], Li Zhang [3] and Yunlong Li [1,2,4,*]

1 College of Environmental Science and Engineering, Yangzhou University, Yangzhou 225127, China; bxgu@yzu.edu.cn (B.G.)
2 State Key Laboratory of Organic Geochemistry, Guangzhou Institute of Geochemistry, Chinese Academy of Sciences, Guangzhou 510640, China
3 Department of Agriculture and Rural Affairs of Jiangsu Province, Jiangsu Cultivated Land Quality and Agro-Environment Protection Station, Nanjing 210003, China
4 Key Laboratory of Arable Land Quality Monitoring and Evaluation, Key Laboratory of Saline–Alkaline Soil Improvement and Utilization (Coastal Saline–Alkaline Lands), Ministry of Agriculture and Rural Affairs, Yangzhou University, Yangzhou 225127, China
* Correspondence: liyunlong@yzu.edu.cn

Abstract: Knowledge regarding how abiotic and biotic environmental factors operate in soil microbiome reassembly remains rudimentary in coastal saline–alkaline soils amended by different organic ameliorants. In this study, field trials were conducted to investigate the impacts and underlying mechanisms of sewage sludge (S) and sludge-based vermicompost (V) at the application amounts of 0, 50, and 100 t ha^{-1} on soil physicochemical characteristics, carbon source utilization pattern, and bacteriome in coastal saline–alkaline soils. Results revealed that impacts of the organic ameliorants on soil's physicochemical and microbial attributes were highly dependent upon the carbon types and amounts applied. Unsurprisingly, applying sewage sludge and vermicompost significantly alleviated environmental constraints, such as saline–alkaline stress and nutrient deficiency, with lower pH, salinity, and higher soil organic carbon content observed in organics-amended soils. Specifically, higher microbial substrate metabolic activity, but lower diversity was observed in saline–alkaline soils amended by organic ameliorants. In addition, reassembled bacteriomes harboring distinguishable core and unique community profiles were observed in reclaimed soils as compared to unamended saline–alkaline soil. Procrustes analysis showed that the soil microbial utilization pattern of carbon sources was significantly related to the alterations in their physicochemical property and bacterial core microbiome. Additionally, Redundancy Analysis (RDA) revealed that soil core bacteriome reassembly was dominated by the integrated impacts of soil salinity, successively followed by carbohydrates, amino acids, polymers, pH, soil organic carbon (SOC), and available nitrogen (AN). Overall, this study provides a comprehensive understanding of soil abiotic and biotic determinants in bacteriome assembly in coastal saline–alkaline soil remediation mediated by organic ameliorants.

Keywords: saline–alkaline soil; organic ameliorant amendment; core bacteriome; carbon source utilization pattern

check for updates

Citation: Gu, B.; Qin, T.; Qiu, M.; Yu, J.; Zhang, L.; Li, Y. Addition of Exogenous Organic Ameliorants Mediates Soil Bacteriome and Microbial Community Carbon Source Utilization Pattern in Coastal Saline–Alkaline Soil. *Agriculture* **2024**, *14*, 44. https://doi.org/10.3390/agriculture14010044

Academic Editor: Ryusuke Hatano

Received: 29 November 2023
Revised: 21 December 2023
Accepted: 25 December 2023
Published: 26 December 2023

1. Introduction

Coastal saline–alkaline soil is increasingly viewed as a promising reserve cultivated land resource with high agricultural potential [1–3]. However, due to long-term seawater immersion, newly reclaimed coastal saline–alkaline soil is characterized by high pH, high salinity, low soil organic carbon (SOC), and a nutrient-constrained soil microbiome accompanied by limited microbial metabolic functional diversity [4–6]. Therefore, the environmental constraints of alleviation and microbial community reassembly are broadly acknowledged to be crucial to the sustainable development of coastal saline–alkaline soils.

Among these abiotic and biotic constraints mentioned above, the soil microbiome has been widely recognized as an integral part of the soil ecosystem and sustains multiple ecosystem processes, e.g., nutrient cycling, organic matter decomposition, and humus formation, that are critical for soil productivity and sustainability, especially in coastal saline–alkaline soils [7,8]. Therefore, it is particularly important to understand the comprehensive impacts and underlying mechanisms of different agricultural practices on microbiomes in coastal saline–alkaline soils. Recently, the application of exogenous organic ameliorants has been proven to be conducive to the reassembly of the soil microbial community in coastal saline–alkaline areas [9–11]. However, contrasting findings regarding the impacts of exogenous organics on the soil microbiome were found due to the divergent experimental conditions (i.e., soil types, organic types, reclamation time, application methods, and additive amounts) [12–14]. Additionally, up to now, knowledge with respect to the underlying mechanisms of variations in soil microbiota induced by organic amendments combined with different organic substrates is still limited in coastal saline–alkaline soils. Therefore, it is necessary to comprehensively understand how ameliorants-amended agents operate in microbial community alterations in coastal saline–alkaline soils.

An increasing body of evidence suggests that soil metabolic activity and diversity are important ecological indicators to assess the nutrient transformation capabilities of a microbial community and are crucial for decoding the mechanisms of microbiome assemblage under different agriculture practices [15,16]. For example, Omirou et al. (2011) [17] found that organic amendment was capable of increasing soil microbial metabolic activity and diversity. However, most of these results have been found in farmland soil, and little is known about the response of soil microbial metabolic activity and diversity on different organic amendments in coastal saline–alkaline soils. Hence, disclosing the variations in the carbon source utilization pattern of the microbial community in response to organic amendments combined with different ameliorants will be beneficial for us to clarify the underlying mechanisms of microbiome reassembly and might be useful for fertilization strategy optimizations.

According to the findings mentioned above, we hypothesized that: (i) organic amendments would have significant impacts on the soil microbial community and these effects would be distinct in coastal saline–alkaline soils amended by different organic ameliorants; (ii) alterations in soil microbiomes would be dominated by concomitant alterations in physicochemical and microbial community carbon source utilization patterns, and the variations in these pivotal determinants would be highly dependent upon the carbon types applied. To test these hypotheses, field experiments were conducted in coastal saline–alkaline soils to investigate the influences of sewage sludge and vermicompost at the different application amounts on soil's physicochemical properties, metabolic activity and diversity, and compositional and structural diversity of the bacteriome.

2. Materials and Methods

2.1. Experimental Site and Experimental Layout

The experiment site selected was Tiaozini, Dongtai City, Jiangsu Province, China (120°56′51″ E, 32°49′56″ N). This region is characterized by the northern subtropical continental monsoon climate with a mean annual temperature and precipitation of 14.6 °C and 1417 mm, respectively. The experimental field was a newly (2-year-old) reclaimed mudflat by building an artificial seawall in 2017 to prevent seawater erosion combined with the planting of two salt-tolerant green manure crops, namely ryegrass (*Lolium perenne* L.) and sesbania (*Sesbania cannabina*). The majority of soil class in this area was typical saline–alkaline soil and belonged to the Aalaquepts group of Aquepts in Inceptisols. Sewage sludge and sludge-based vermicompost used in the current study, which apparently harbored different physicochemical characteristics (Table 1), were collected from the Jiangsu Chunguang earthworm cultivation company. Sewage sludge and vermicompost used in the current study met the requirements specified in the Chinese national standards.

Table 1. Physicochemical characteristics of the soil samples under different treatments and organic ameliorants used in this study.

Items	Treatments [§]					Organics	
	CK	**S50**	**S100**	**V50**	**V100**	**Sewage Sludge**	**Vermicompost**
BD (g cm^{-3})	1.34 ± 0.00 a	1.31 ± 0.00 b	1.28 ± 0.00 cd	1.29 ± 0.00 bc	1.26 ± 0.01 d	/	/
pH	9.33 ± 0.09 a	9.11 ± 0.04 b	8.78 ± 0.02 c	9.04 ± 0.06 b	8.96 ± 0.08 bc	6.32	6.33
Salinity (‰)	3.15 ± 0.16 ab	2.61 ± 0.16 c	2.79 ± 0.06 bc	3.41 ± 0.02 a	3.30 ± 0.03 a	32.9	8.43
SOC (g kg^{-1})	3.60 ± 0.32 b	11.27 ± 1.13 a	11.92 ± 1.83 a	11.73 ± 1.32 a	13.59 ± 3.44 a	216.2	464.9
TN (g kg^{-1})	0.33 ± 0.01 a	0.67 ± 0.06 a	0.70 ± 0.13 a	0.68 ± 0.03 a	0.90 ± 0.25 a	51.2	19.27
C/N	10.78 ± 3.19 a	16.90 ± 4.60 a	17.12 ± 6.35 a	17.27 ± 5.16 a	15.06 ± 5.56 a	4.22	24.33
TP (mg kg^{-1})	0.4 ± 0.02 b	0.61 ± 0.04 ab	0.68 ± 0.14 a	0.72 ± 0.06 a	0.82 ± 0.10 a	5.51	24.12
AN (mg kg^{-1})	37.98 ± 0.30 b	107.10 ± 14.8 a	82.82 ± 11.36 a	83.89 ± 7.43 a	112.39 ± 23.36 a	3440	15.87
AP (mg kg^{-1})	21.43 ± 1.77 b	84.50 ± 11.44 a	94.75 ± 21.61 a	84.96 ± 7.61 a	108.18 ± 15.38 a	813	2467

[§] Treatments: CK indicates unamended coastal saline–alkaline soil; S and V represent coastal saline soil amended by sewage sludge and sludge-based vermicompost, respectively. The "50" and "100" indicate coastal saline–alkaline soil amended by organic ameliorants at rates of 50 and 100 t ha^{-1}, respectively. Values (means ± standard error, n = 3) within each column followed by different letters indicate significant differences according to the Duncan post hoc test at 5% level.

The field plot experiment consisted of three treatment groups: (1) newly reclaimed coastal saline–alkaline soil without sewage and vermicompost amendments (CK), and (2–3) newly reclaimed coastal saline–alkaline soil amended by sewage sludge (SS) and vermicompost (SV), respectively. In order to investigate the effects of types of exogenous organic carbon on saline–alkaline soil abiotic and biotic properties, sewage sludge and vermicompost were applied to soil via one-time application at the application rates of 0, 50, and 100 t ha^{-1} on a dried basis, respectively. Each treatment comprised three randomly distributed experimental plots, and each plot was 4 m long and 4 m wide. Sewage sludge and vermicompost were applied once to the test plots, and mixed with topsoil in October 2019. Barley (*Hordeum vulgare* L.) and maize (*Zea mays* L.) were successively cultivated and rotated from 2019 to 2020. During the whole experimental period, all plants were only fed by rainwater instead of irrigation or fertilization. Surface soil was collected with a soil auger up to a depth of 0~20 cm after removing the uppermost layer in October 2020. A total of forty-five samples were collected (five soil samples at each micro plot, which were mixed to form a composite individual sample) from fifteen sampling plots. The collected soils were divided into three subsamples and stored at room temperature, 4 °C, and −80 °C for subsequent physicochemical, enzymatic, and molecular determinations, respectively.

2.2. Soil Physicochemical and Biolog Microplate Assays

Soil bulk density (BD), pH, salinity, SOC, total nitrogen (TN), total phosphorus (TP), available N (AN), and available P (AP) were determined in this study according to the established methods described by Bai et al. (2017) [18] and Li et al. (2021) [19]. The BIOLOG plate technique was used to determine the allelopathic potential of different species of ginsenosides on the soil microbial community carbon source utilization patterns [20,21]. Briefly, 10 g of treated soil samples was added into 90 mL 0.85% sterile sodium chloride (NaCl) solution immediately; then, the suspension was shaken for 20 min at 200 rpm (25 °C). After standing for 30 min, 1 mL of the supernatant was used for gradient dilution until 10^{-3} cell suspensions were obtained. Then, 125 µL of the organism suspension was added into each well of the ECO Microplate™ (Biolog, Inc., Hayward, CA, USA). The microplates were incubated in the dark at an environmental temperature of 25 °C. The average well color development (AWCD) was recorded every 24 h at 590 nm for 168 h using an automated microplate reader (BioTek Instruments Inc., Winooski, VT, USA).

AWCD values to denote the metabolic activity of the soil microbial community were calculated according to a previous report [20], i.e., $AWCD = \sum (C_{it} - C_{0t})/31$, where C_{it} and C_{0t} represent the absorbance values of the sole substrate well and the control well at the time t, respectively. The number 31 represents the species of substrates used in the ECO Microplate. To estimate the influences of different organic ameliorants on

the functional diversity of the soil microbial community, the Shannon's diversity (H) and substrate richness (species of carbon source that were utilized by the soil microbial community, S) were calculated using the final AWCD values (OD > 0.2) at 168 h [22], i.e., $H = -\sum (P_i \times lnP_i)$, where $P_i = (C_i - C_0)/\sum(C_i - C_0)$, where meanings of C_i and C_0 are described above. Then, 31 carbon sources were grouped into six categories including carbohydrates, carboxylic acids, amino acids, phenolic acids, amines, and polymers, and then the AWCD values of theses substrate categories at 168 h were calculated as detailed in the previous study by Feigl et al. (2017) [23]. Additionally, a principal component analysis (PCA) was performed using the normalized AWCD values to visualize the impacts of organic amendments on the soil microbial functional diversity.

2.3. DNA Extraction and Real-Time Quantitative PCR

For each soil sample, cell lysis and total genomic DNA were separately isolated from 500 mg of fresh soil using a FastDNA® SPIN Kit (MP Biomedicals, Santa Ana, CA, USA) following the manufacturer's instructions. DNA extracts were dissolved into 100 µL DES (DNase/pyrogen-free water). The quality and quantity of DNA samples were determined based on the ratios of A260/A280 nm and A260/A230 nm using a NanoDrop ND-1000 spectrophotometer (ThermoFisher Scientific, Waltham, MA, USA). Finally, DNA samples were stored at −80 °C for subsequent molecular analyses.

The plasmid copies were determined using a CFX-48 Optical Real-time Detection System (Bio-Rad Laboratories Inc., Hercules, CA, USA) with the 341-F (5′-CCT ACG GGA GGC AGC AG-3′) and 518-R (5′-ATT ACC GCG GCT GCT GG-3′) primer set. Each 20 µL reaction mixture contained 10 µL of SYBR Premix Ex Taq (Takara, Kyoto, Japan) with 1 µL DNA template and 0.4 µL each forward and reverse primers, and 8.2 µL of nuclease-free water. The real-time PCR run started with an initial denaturation and enzyme activation step for 2 min at 95 °C, followed by 39 cycles of 5 s at 95 °C and 30 s at 60 °C, and then the fluorescence signal was recorded. The amplification specificity of PCR products was visualized via melting curve analysis.

2.4. Miseq Sequencing and Bioinformatics Analysis

For PCR amplification, the primers targeting the V4 region of bacterial 16S rDNA were amplified using primer pairs 515-F (5′-GTG CCA GCM GCC GCG GTA A-3′) and 806-R (5′-GGA CTA CHV GGG TWT CTA AT-3′). The PCR reaction mixture and protocol used in this study was according to the established method detailed in the study by Zhao et al. (2014) [24]. PCR products were polled in equimolar and then purified using AgencourtAMPure XP beads (Beckman Coulter, Brea, CA, USA). Paired-end sequencing was performed on an Illumina Miseq platform (Illumina, San Diego, CA, USA) at Genesky Biotechnology Co., Ltd., Shanghai, China, according to the standard protocols.

Raw sequences obtained from Miseq sequencing were quality-controlled, merged, and analyzed as described by Li et al. (2022) [25]. All samples were rarefied to a unified depth (70,000) for subsequent community diversity analysis. Shannon diversity was calculated to characterize the compositional differences of soil bacterial communities in whole. Principal coordinates analysis (PCoA) with permutational multivariate analysis of variance (PERMANOVA) was performed to compare the dissimilarity of structural diversities of the bacterial communities across different treatments based on Bray–Curtis distance matrices. In this study, OTUs presented in all replicates of each treatment were retained and defined as core OTUs, and OTUs that appeared only in three replicates of one treatment were retained and defined as unique OTUs.

2.5. Statistical Analyses

Transformation-based redundancy discriminant analysis (tb-RDA) was chosen and performed on these environmental factors and Hellinger-transformed OTUs using the decorana and rda functions of the vegan package. The individual effects of each environmental variable on the alterations in bacterial community structure and soil aggregation

were computed using the rdacca.hp function [26]. Additionally, Procrustes analysis was performed to compare the PCoA ordinations between soil aggregates, physicochemical properties, and bacteriome. The significant differences in soil physicochemical properties, bacterial abundances (Lg-transformed), Shannon diversities, and community taxonomic compositions across all treatments were tested using one-way ANOVA followed by a Duncan test at a 5% level.

3. Results

3.1. Soil Physicochemical Characteristics

Overall, organic ameliorant applications significantly ($p < 0.05$) improved soil physicochemical attributes (Table 1). In particular, both sewage sludge and vermicompost reduced soil BD and pH with the lowest values of BD and pH observed in the V100 and S100 treatments, respectively. Compared with CK (3.15), significantly ($p < 0.05$) lower soil salinity was found in the S50 treatment (2.61). Additionally, vermicompost rather than sewage sludge exhibited more profound effects on the improvement of soil nutritional contents with the maximum values of SOC, TN, TP, AN, and AP observed in the V100 treatment, respectively (Table 1).

3.2. Soil Microbial Metabolic Utilization of Carbon Sources

In general, the application of organic ameliorants significantly ($p < 0.05$) improved AWCD values when compared to CK soil after 168-h incubation (Figure 1a). Particularly, metabolic activities in soils treated by S100, V50, and V100 were significantly ($p < 0.05$) higher than CK. Likewise, applications of sewage sludge and vermicompost notably increased substrate utilization capacities (i.e., carbohydrates, followed by carboxylic acids, amino acids, and amines) of soil microbial community in coastal areas as compared to CK. In particular, the metabolic activities of amines, carbohydrates, and carboxylic acids in soils treated by V100 were significantly ($p < 0.05$) increased compared to those in other treatments (Figure 1b).

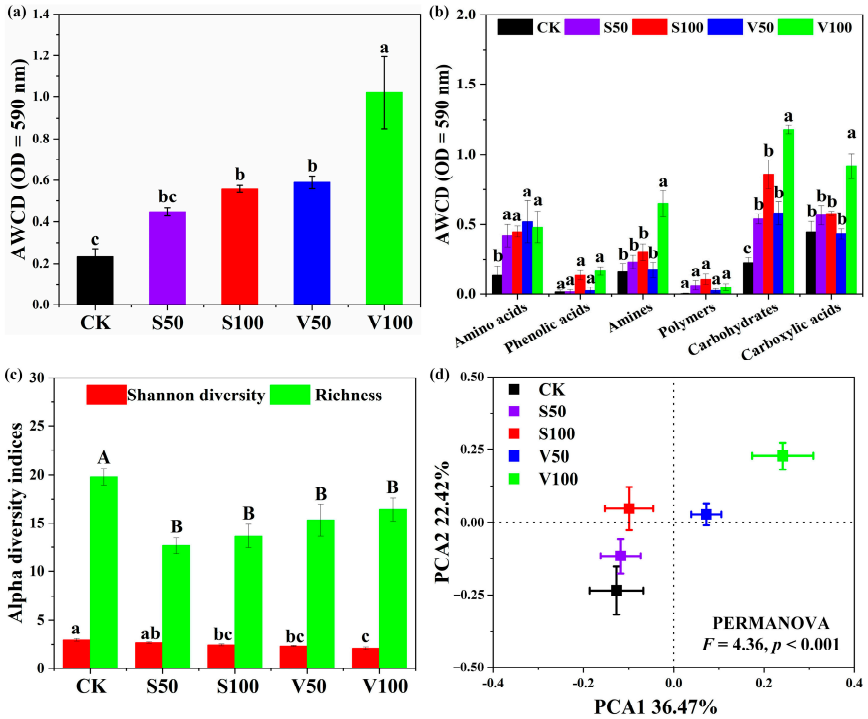

Figure 1. Microbial carbon source utilization in different treatments. AWCD values (**a**), utilization capacities of categorized substrates (**b**), Shannon diversity (red) and richness (green) of carbon source utilization (**c**), and principal component analysis of carbon source utilization patterns (**d**). Error bars indicate the standard errors of the means of three replicates. Different letters represent significantly

different at $p < 0.05$ according to Duncan's multiple range test. Treatments: CK indicates unamended coastal saline–alkaline soil; S and V represent coastal saline soil amended by sewage sludge and sludge-based vermicompost, respectively. The "50" and "100" indicate coastal saline–alkaline soil amended by organic ameliorants at rates of 50 and 100 t ha^{-1}, respectively.

In addition, the metabolic diversity and substrate utilization pattern of the soil microbial community were significantly altered as a result of sewage sludge and vermicompost application (Figure 1c,d). Specially, substrate diversity and richness significantly ($p < 0.05$) decreased in soils treated by organics when compared to those in CK, with the lowest Shannon diversity and richness observed in V100- and S50-amended soils, respectively (Figure 1c). Principle component analysis was carried out to visualize the influences of sewage sludge and vermicompost on the substrate utilization pattern of the microbial community in saline–alkaline soil. Obviously, soils amended by different types of exogenous organic carbon harbored a distinct (PERMANOVA, $p < 0.001$) substrate utilization pattern compared to CK (Figure 1d). The first and second principal components of PCA analysis expressed 36.47% and 22.42% of the overall variance across the treatments, respectively. Apparently, substrate utilization patterns of sewage sludge-related treatments were similar to CK, but clearly different from the vermicompost-related amendments in the multivariate space (Figure 1d).

3.3. Compositional and Structural Diversities of Bacterial Community

Organic ameliorants significantly ($p < 0.05$) altered the compositional and structural diversities of bacterial communities in coastal saline–alkaline soil (Figure 2). Overall, significantly ($p < 0.05$) higher bacterial abundances were observed in soils amended by sewage sludge and vermicompost when compared to CK. Particularly, bacterial populations in soils amended by sewage sludge significantly ($p < 0.05$) increased with the application amount of sewage sludge, whereas opposite variations in bacterial abundance in vermicompost-amended soils were observed (Figure 2a).

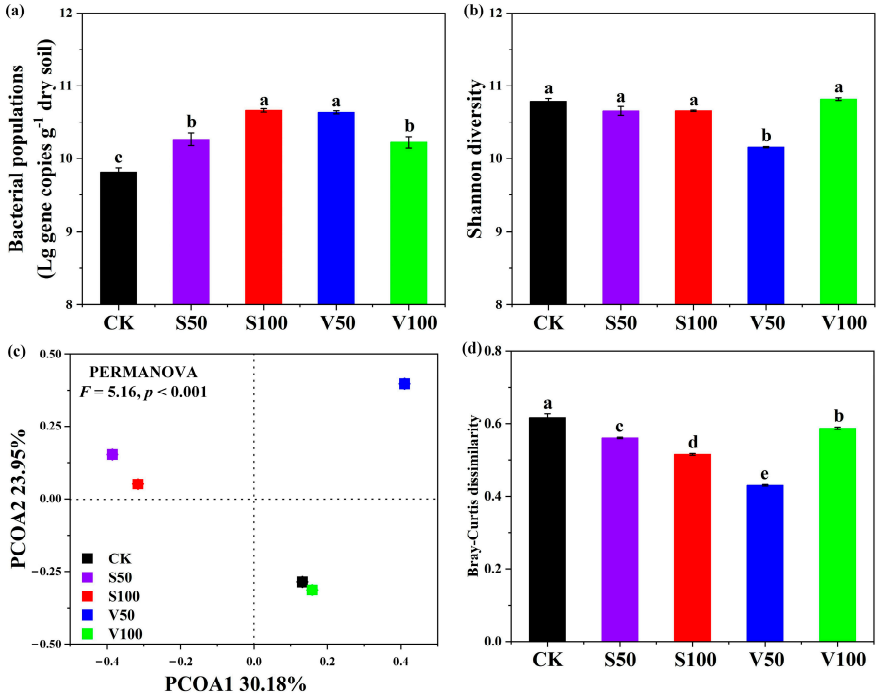

Figure 2. Effects of organic amendments on compositional and structural diversity of bacterial communities in coastal saline–alkaline soils. (**a**), soil bacterial populations; (**b**), Shannon diversity; (**c**,**d**) indicate principal coordinate analysis (PCoA) and Bray–Curtis dissimilarity analysis, respectively. Error bars denote standard errors of the means of three replicates. Different letters

represent significantly different at $p < 0.05$ according to Duncan's multiple range test. Treatment abbreviations are defined in Figure 1.

As for the compositional diversity of the soil bacterial community, applications of sewage sludge and vermicompost reduced the parameters to an extent, with significantly ($p < 0.05$) lower Shannon diversity observed in the V50 treatment as compared to CK (Figure 2b). Additionally, organic ameliorants significantly (PERMANOVA, $p < 0.001$) changed the soil bacterial community structure (Figure 2c,d). The first and second principal components of PCoA ordination plot expressed 30.2% and 24.0% of the overall variance across the five treatments, respectively. Unlike the soil bacterial community structure in V100, soils amended by S50, S100, and V50 harbored distinguishable bacterial community structure as compared to CK (Figure 2c). Meanwhile, Bray–Curtis dissimilarity analysis further revealed the effects of organic ameliorants on soil bacterial community structures across different treatments, with significantly ($p < 0.05$) lower values observed in all organics-amended soils as compared to CK treatment (Figure 2d).

3.4. Soil Bacterial Community Composition

Organic ameliorants significantly ($p < 0.05$) modulated the bacterial community at multiple taxonomic levels as compared to the CK treatment (Figure 3a,b). At the phylum level, *Proteobacteria*, followed by *Chloroflexi*, *Bacteroidetes*, *Acidobacteria*, and *Actinobacteria* were dominant bacterial phyla across different treatments. In particular, significantly higher ($p < 0.05$) relative abundances of *Chlorobi* and *Cyanobacteria* were observed in all amended soils as compared to CK, while RAs of *Chloroflexi* and *Acidobacteria* showed opposite patterns (Figure 3a). At the family level, *Xanthomonadaceae*, followed by *Pelobacteraceae*, *Sinobacteraceae*, *Cytophagaceae*, and *Hyphomicrobiaceae* were dominant bacterial families across different treatments. Particularly, significantly higher ($p < 0.05$) RA of *Sphingomonadaceae* were observed in all amended soils as compared to CK, while RAs of *Pelobacteraceae*, *Bacillaceae*, and *Alteromonadaceae* showed opposite patterns (Figure 3b).

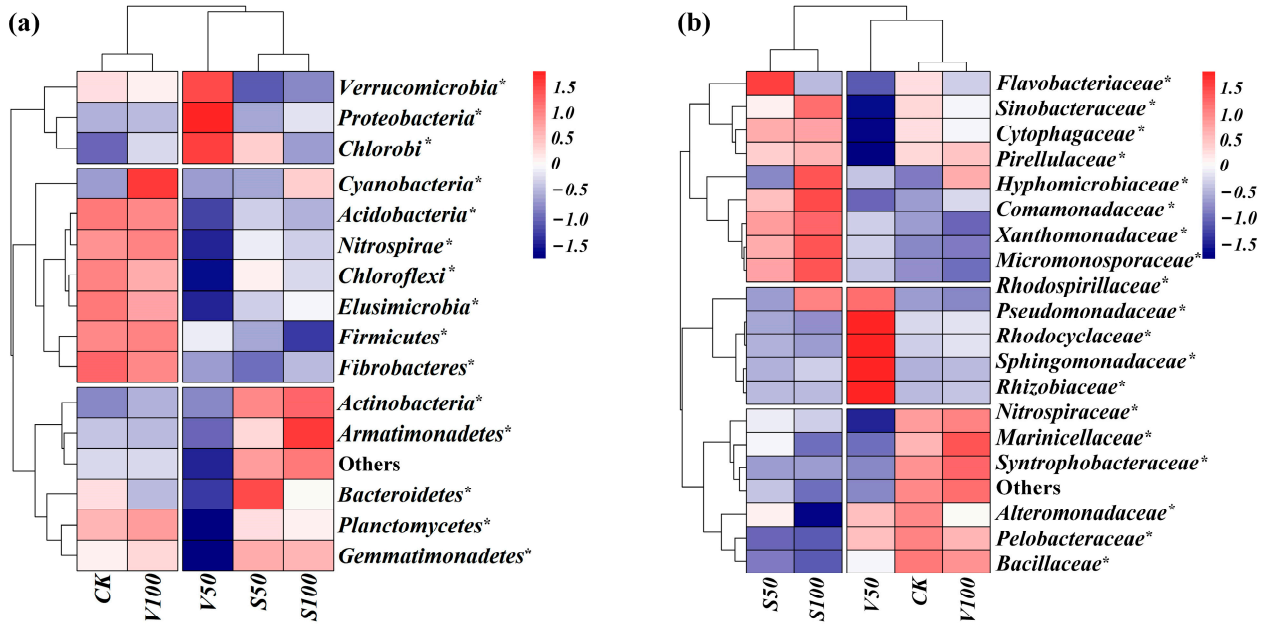

Figure 3. *Cont.*

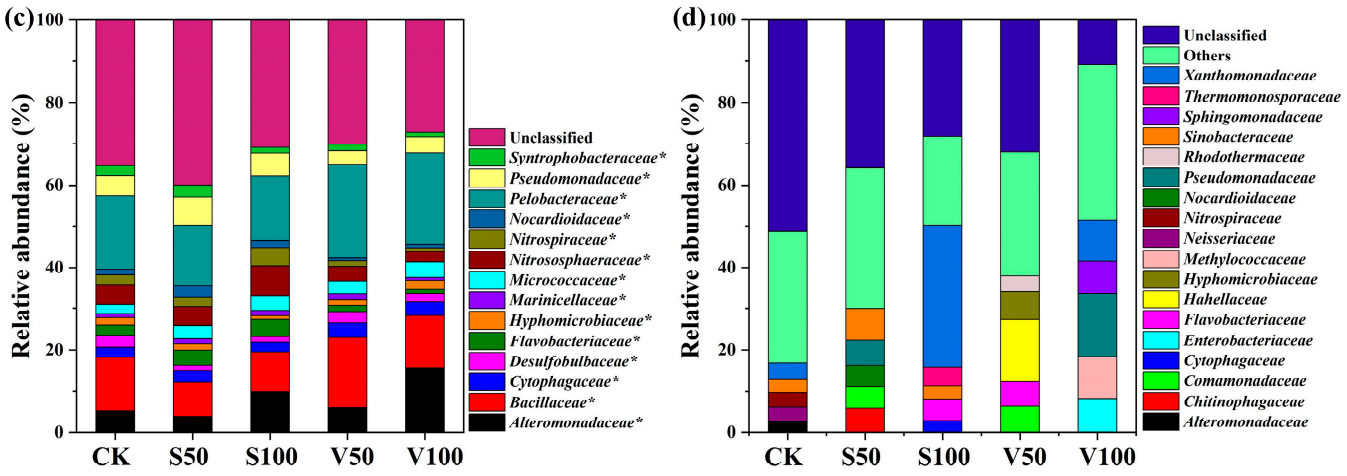

Figure 3. Bacterial community compositions at multiple taxonomic levels in coastal saline–alkaline soils after organic amendments. (**a**,**b**) indicate dominant bacterial phyla and families with higher relative abundances across different treatments, respectively; (**c**,**d**), relative abundances of dominant core and unique OTUs in different treatments. The key from blue to red represents the least abundant to most abundant for a given bacterial family in each row in the heatmap. Asterisks (*) at the upper right of the taxon indicate significant differences according to Duncan's post hoc test at 5% level. Treatment abbreviations are defined in Figure 1.

Unsurprisingly, results in this study showed distinct influences of different species of ginsenosides on the bacterial core and unique microbiome (Figure 3c,d). Core OTUs affiliated with *Pelobacteraceae* were the predominant bacterial family across different treatments. Significantly higher ($p < 0.05$) RAs of core family *Micrococcaceae* were observed in all amended soils as compared to CK, while RAs of *Desulfobulbaceae* showed opposite patterns (Figure 3c). For unique OTUs, the most abundant unique family changed from *Xanthomonadaceae* (CK) to *Sinobacteraceae* (S50), *Xanthomonadaceae* (S100), *Hahellaceae* (V50), and *Pseudomonadaceae* (V100), respectively (Figure 3d).

3.5. Correlating the Microbial Function with Soil Physicochemical Property and Microbial Community

Procrustes analysis showed that the ordination of soil property ($M^2 = 0.7012$, $p = 0.023$) and core bacteriome ($M^2 = 0.5843$, $p = 0.043$) significantly correlated with the ordination of microbial function, namely microbial carbon metabolic activity (Figure 4a,b). Furthermore, Spearman's rank–order correlation analysis showed that substrate utilization capabilities significantly ($p < 0.05$) and positively related to soil nutrient status, whereas significantly ($p < 0.05$) negative correlations were observed among these carbon sources and soil bulk density, pH, and salinity (Figure 4c). Similarly, soil bacteriome exhibited significant ($p < 0.05$) correlations with microbial substrate utilization, with significant correlations observed among these carbon sources and most of the bacterial core families, except for families *Hyphomicrobiaceae* and *Marinicellaceae* (Figure 4c).

Additionally, RDA results indicated that seven environmental factors, namely, carbohydrates, amino acids, polymers, pH, salinity, SOC, and AN, were strongly related to the core bacteriome reassembly in coastal saline–alkaline soil amended by organic ameliorants (Figure 5a). These physicochemical and microbial attributes completely explained 65.4% ($p = 0.007$) of the soil core bacteriome variations, with the explanatory powers of the first two axes accounting for 50.8% (RDA1, $p_{adj} = 0.003$) and 22.7% (RDA2, $p_{adj} = 0.002$), respectively. In particular, soil salinity, accompanied by carbohydrates, amino acids, and polymers were the main agents driving the variations in core bacteriome in coastal saline–alkaline soils amended by organic ameliorants, which jointly explained a total of 90.9% of the variations in the core bacteriome (Figure 5b).

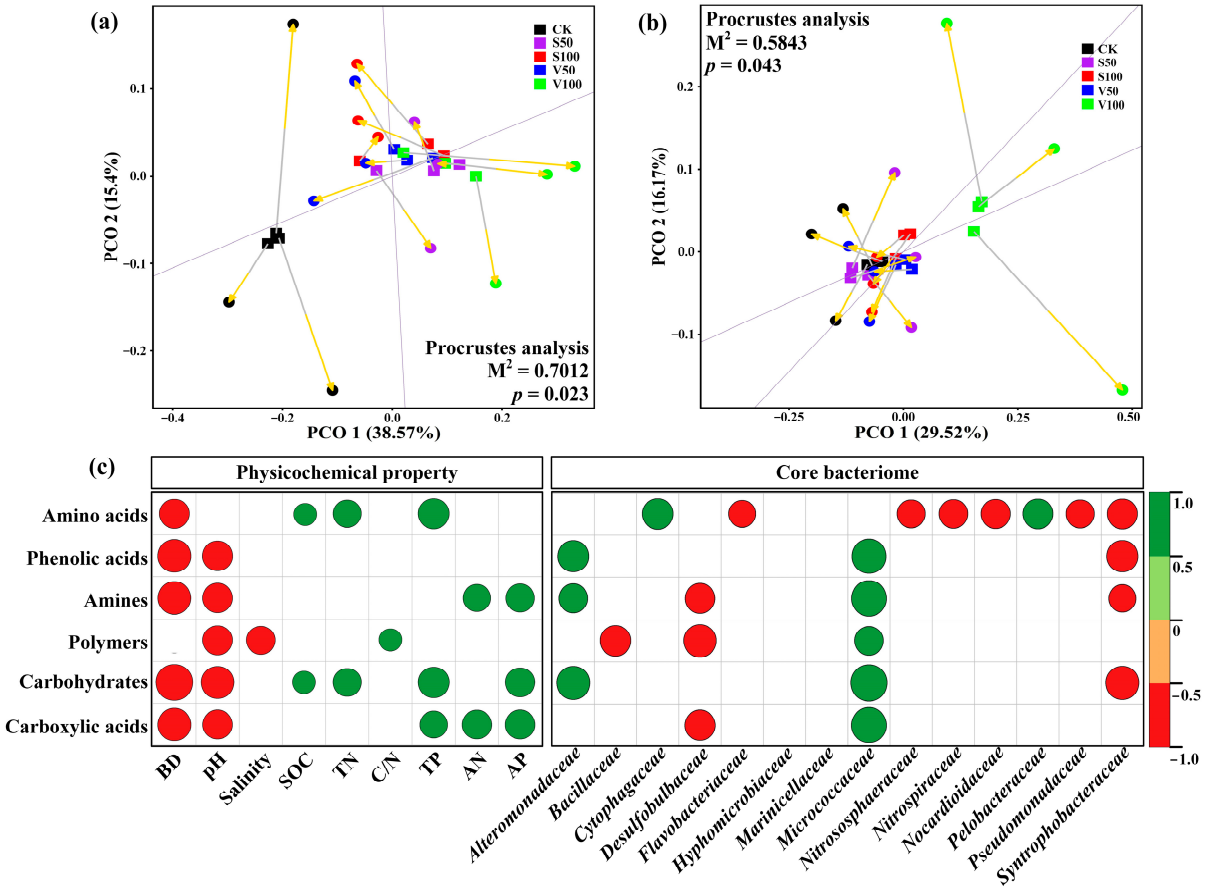

Figure 4. Correlating the microbial community carbon utilization pattern with physicochemical property and bacterial community in coastal saline–alkaline soil. (**a**,**b**) indicate Procrustes analysis of PCoA ordination plots between the microbial metabolic activities of carbon sources and soil physicochemical property and core bacteriome based on the Bray–Curtis distance matrices, respectively. (**c**), Spearman's rank–order correlation analysis between substrate utilization soil physicochemical property and core bacterial families. Only significant correlations are presented. The circles in red and green represent negative correlation and positive correlation. Treatment abbreviations are defined in Figure 1.

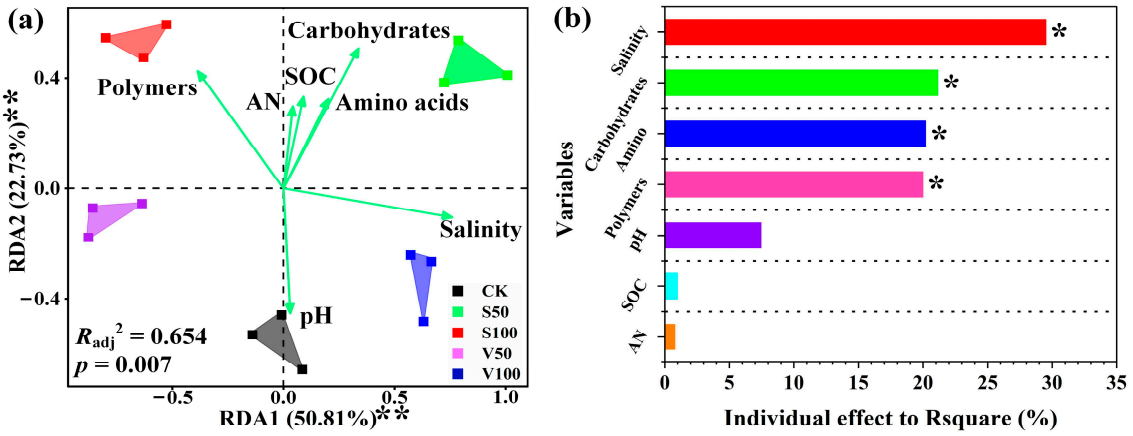

Figure 5. Relationships between soil core bacteriome and physicochemical and microbial metabolic determinants. (**a**), RDA of abiotic and biotic drivers in alterations of soil core microbiome; (**b**), values of relative importance (%) of each environmental variable on soil core bacteriome variations. * $p < 0.05$, ** $p < 0.01$. Treatment abbreviations are defined in Figure 1.

4. Discussion

It has been increasingly confirmed that organic amendments are conducive to soil quality improvement. Particularly, applying sewage sludge and vermicompost has been demonstrated to be capable of saline–alkaline stress alleviation and nutrient availability promotion in coastal saline–alkaline soils [1,27,28]. In the current study, significantly alleviated soil environmental constraints were found in organics-amended soils (CK vs. S50, S100, V50, and V100) (Table 1), which was in line with the findings of Bai et al. (2017) [18] and Xie et al. (2021) [1], who also found the positive effects of the two organic ameliorants on the soil environment modification in coastal saline–alkaline soils. Specifically, significantly lower BD observed in all amended soils as compared to initial soil may be due to the dilution effects of less dense organic ameliorants, as well as the improved aggregation status caused by organic material applications [14]. Consistent with our previous findings, reduced pH values in all amended soils might be explained by the dilution of sewage sludge and vermicompost with lower pH (Table 1), as well as the neutralization caused by acid compounds derived from the biodegradation of organic matter [19]. Considerably increased soil organic carbon content lies in the fact that applying sewage sludge and vermicompost with abundant organic matter into mudflat salt-affected soil can rapidly elevate soil organic carbon stocks [29]. Additionally, significantly elevated SOC in amended soils might be primarily responsible for the reduction in soil salinity because of their blocking effects on the capillary rise of upward-moving salt [30,31].

Community-level physiological profiles (CLPP) analysis is a sensitive biological approach to indicate the variations in the metabolic activities of soil microbial communities [32–34]. In the current study, significantly higher substrate metabolic activity in soils amended by sewage sludge and vermicompost indicated that organic ameliorant applications were conducive to substrate utilization capacity enhancement of the microbial community in coastal saline–alkaline soil, as also described by Pascual et al. (2008) [35]. One possible explanation is that alleviated environmental constraints (i.e., lower pH and more available substrates) in soils amended by organics are capable of promoting the flourishment of microbial populations possessing versatile potentials in carbon source utilization [19]. Interestingly, lower α-diversity indices and distinct utilization patterns of carbon sources were observed in sewage sludge- and vermicompost-amended soils as compared to CK treatment (Figure 1c). This was likely attributed to the relatively specific carbon source types in exogenous organic ameliorants (sewage sludge and vermicompost) since they could selectively promote or inhibit specific microorganism's links to distinct carbon source utilization strategies [36,37].

It has been increasingly confirmed that the compositional and structural diversities of the soil microbial community are deeply influenced by the variations in their microhabitats harboring different environmental attributes [1,27,28]. Significantly positive responses of bacterial populations observed in all amended soils as compared to CK (Figure 2a) were similar to the previous observations of Latare et al. (2018) [38] and Bhat et al. (2018) [39], in which both sewage sludge and vermicompost significantly increased soil bacterial populations in different soil types. This is attributed to the facilitation of abundant nutrient components contained in the organic inputs on the microbial proliferations. Similar to the findings of Jiang et al. (2019) [5] and Zheng et al. (2022) [40], sewage sludge and vermicompost decreased the Shannon diversity of the bacterial community in amended soils as compared to CK soil (Figure 2b). This was likely due to the selective promotion or inhibition of specific microbial species induced by unique carbon sources contained in distinct organic ameliorants [41,42]. This assumption was supported by the significantly shifted bacterial families occupying the predominant niche, distinctively enriched and depleted OTUs, and differentiated core and unique bacterial microbiomes across different treatments (Figure 3), which indicated that the compositional diversity of the bacterial community in coastal saline–alkaline soil is deeply dependent upon both the types and amounts of organic ameliorants used. Apart from compositional diversity, distinct bacterial community structures observed in amended soils (Figure 2c,d) were consistent with

previous findings by Jiang et al. (2019) [5] and Li et al. (2021) [19], who suggested that distinct carbonaceous compounds derived from organic amendments with different application types and amounts were pivotal agents in restructuring the bacterial microbiome. RDA showed that soil salinity and pH were significantly and negatively associated with variations in bacterial communities in most organic ameliorant-amended soils (Figure 5), suggesting that reduced soil pH and salinity as a result of organic amendments favoring bacterial reassembly in coastal saline–alkaline soils, as most microbes are narrowly pH- and salt-tolerant [43–45]. Interestingly, carbohydrates, followed by amino acids and polymers, exhibited more pronounced influences on bacterial community variations when compared with SOC, which further validated that specific carbonaceous compounds, rather than the content of SOC, may be even more important determinants driving bacterial community reassembly in newly reclaimed coastal areas.

5. Conclusions

Results in this study demonstrated that organic amendments can promote soil bacteriome reassemblage, mainly through the organics-mediated integrated impacts of environmental constraints alleviation and carbon source utilization patterns alteration in coastal saline–alkaline soil. Specifically, the impacts of sewage sludge and vermicompost on soil physicochemical and microbial properties were highly dependent upon the carbon types and amounts applied, with vermicompost amendment exhibiting more pronounced effectiveness on the improvement of physicochemical characteristics and reconstructions of the carbon source utilization pattern and bacteriome than sewage sludge treatments. In particular, positive impacts of vermicompost amendment at the rate of 100 t ha^{-1} on soil microbial substrate metabolic activity was more significant than other organic ameliorant treatments, which might be the suitable exogenous organic ameliorant type and application amount for the amendment of coastal saline–alkaline soil. Statistical analysis indicated that alterations in soil bacteriomes in coastal saline–alkaline soils were dominated by abiotic factors (e.g., salinity, followed by SOC and pH) and microbial utilization capabilities of carbon sources (e.g., carbohydrates, followed by amino acids and polymers). These outcomes are beneficial for us to comprehensively understand the impacts and underlying mechanisms of the organics-facilitated fertility improvement of coastal saline–alkaline soil.

Author Contributions: Conceptualization, Y.L. and B.G.; methodology, T.Q.; data curation, M.Q., J.Y. and L.Z.; writing—review and editing, Y.L. and B.G. All authors have read and agreed to the published version of the manuscript.

Funding: This research was supported by the National Natural Science Foundation of China (41977089), State Key Laboratory of Organic Geochemistry, GIGCAS (SKLOG202118), State Key Laboratory of Pollution Control and Resource Utilization (PCRRF21036), and Jiangsu Agricultural Industry Technology System (JATS[2023]317 and JATS[2023]318) for Yanchao Bai, the National Natural Science Foundation of China (31872179) for Yuhua Shan.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are available upon request from the corresponding author.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Xie, X.F.; Pu, L.J.; Zhu, M.; Meadows, M.; Sun, L.C.; Wu, T.; Bu, X.G.; Xu, Y. Differential effects of various reclamation treatments on soil characteristics: An experimental study of newly reclaimed tidal mudflats on the east China coast. *Sci. Total Environ.* **2021**, *768*, 144996. [CrossRef] [PubMed]
2. Wang, F.; Wall, G. Mudflat development in Jiangsu Province, China: Practices and experiences. *Ocean Coast. Manag.* **2010**, *53*, 691–699. [CrossRef]

3. Cao, W.Z.; Wong, M.H. Current status of coastal zone issues and management in China: A review. *Environ. Int.* **2007**, *33*, 985–992. [CrossRef] [PubMed]
4. Dahlawi, S.; Naeem, A.; Rengel, Z.; Naidu, R. Biochar application for the remediation of salt-affected soils: Challenges and opportunities. *Sci. Total Environ.* **2018**, *625*, 320–335.
5. Jiang, S.Q.; Yu, Y.N.; Gao, R.W.; Wang, H.; Zhang, J.; Li, R.; Long, X.H.; Shen, Q.R.; Chen, W.; Cai, F. High-throughput absolute quantification sequencing reveals the effect of different fertilizer applications on bacterial community in a tomato cultivated coastal saline soil. *Sci. Total Environ.* **2019**, *687*, 601–609. [CrossRef]
6. Ritz, K.; Young, I.M. Interactions between soil structure and fungi. *Mycologist* **2004**, *18*, 52–59. [CrossRef]
7. Goldford, J.E.; Lu, N.; Bajić, D.; Estrela, S.; Tikhonov, M.; Sanchez-Gorostiaga, A.; Segrè, D.; Mehta, P.; Sanchez, A. Emergent simplicity in microbial community assembly. *Science* **2018**, *361*, 469–474. [CrossRef]
8. Palmer, M.A.; Zedler, J.B.; Falk, D.A. *Ecological Theory and Restoration Ecology*; Foundations of Restoration Ecology; Island Press: Washington, DC, USA, 2016; pp. 3–26. [CrossRef]
9. Bai, Y.C.; Tao, T.Y.; Gu, C.H.; Wang, L.; Feng, K.; Shan, Y.H. Mudflat soil amendment by sewage sludge: Soil physicochemical properties, perennial ryegrass growth, and metal uptake. *Soil Sci. Plant Nutr.* **2013**, *59*, 942–952. [CrossRef]
10. Wu, L.P.; Wang, Y.D.; Zhang, S.R.; Wei, W.L.; Kuzyakov, Y.; Ding, X.D. Fertilization effects on microbial community composition and aggregate formation in saline-alkaline soil. *Plant Soil* **2021**, *463*, 523–535. [CrossRef]
11. Yao, R.J.; Yang, J.S.; Wang, X.P.; Xie, W.P.; Zheng, F.L.; Li, H.Q.; Tang, C.; Zhu, H. Response of soil characteristics and bacterial communities to nitrogen fertilization gradients in a coastal salt-affected agroecosystem. *Land Degrad. Dev.* **2021**, *32*, 338–353. [CrossRef]
12. Sastre-Conde, I.; Lobo, M.C.; Beltrán-Hernández, R.I.; Poggi-Varaldo, H.M. Remediation of saline soils by a two-step process: Washing and amendment with sludge. *Geoderma* **2015**, *247*, 140–150. [CrossRef]
13. Zhang, T.; Wang, T.; Liu, K.; Wang, L.; Wang, K.; Zhou, Y. Effects of different amendments for the reclamation of coastal saline soil on soil nutrient dynamics and electrical conductivity responses. *Agr. Water Manag.* **2015**, *159*, 115–122. [CrossRef]
14. Aksakal, E.L.; Sari, S.; Angin, I. Effects of vermicompost application on soil aggregation and certain physical properties. *Land Degrad. Dev.* **2016**, *27*, 983–995. [CrossRef]
15. Moorhead, D.L.; Sinsabaugh, R.L. A theoretical model of litter decay and microbial interaction. *Ecol. Monogr.* **2006**, *76*, 151–174. [CrossRef]
16. Sinsabaugh, R.L.; Lauber, C.L.; Weintraub, M.N.; Ahmed, B.; Allison, S.D.; Crenshaw, C.; Contosta, A.R.; Cusack, D.; Frey, S.; Gallo, M.E. Stoichiometry of soil enzyme activity at global scale. *Eol. Lett.* **2008**, *11*, 1252–1264. [CrossRef] [PubMed]
17. Omirou, M.; Rousidou, C.; Bekris, F.; Papadopoulou, K.K.; Menkissoglou-Spiroudi, U.; Ehaliotis, C.; Karpouzas, D.G. The impact of biofumigation and chemical fumigation methods on the structure and function of the soil microbial community. *Microb. Ecol.* **2011**, *61*, 201–213. [CrossRef] [PubMed]
18. Bai, Y.C.; Zuo, W.G.; Yan, Y.Y.; Gu, C.H.; Guan, Y.X.; Mei, L.J.; Xue, W.J.; Shan, Y.H.; Feng, K. Sewage sludge amendment combined with green manuring to a coastal mudflat salt-soil in eastern China: Effects on soil physicochemical properties and maize yield. *Int. J. Agron.* **2017**, *2017*, 8526598. [CrossRef]
19. Li, Y.L.; Wang, Y.M.; Shen, C.; Xu, L.; Yi, S.Q.; Zhao, Y.L.; Zuo, W.G.; Gu, C.H.; Shan, Y.H.; Bai, Y.C. structural and predicted functional diversities of bacterial microbiome in response to sewage sludge amendment in coastal mudflat soil. *Biology* **2021**, *10*, 1302. [CrossRef]
20. Garland, J.L.; Mills, A.L. Classification and characterization of heterotrophic microbial communities on the basis of patterns of community-level sole-carbon-source utilization. *Appl. Environ. Microbiol.* **2017**, *57*, 2351–2359. [CrossRef]
21. Choi, K.H.; Dobbs, F.C. Comparison of two kinds of Biolog microplates (GN and ECO) in their ability to distinguish among aquatic microbial communities. *J. Microbiol. Meth.* **1999**, *36*, 203–213. [CrossRef]
22. Fisk, M.C.; Ruether, K.F.; Yavitt, J.B. Microbial activity and functional composition among northern peatland ecosystems. *Soil Biol. Biochem.* **2003**, *35*, 591–602. [CrossRef]
23. Feigl, V.; Ujaczki, É.; Vaszita, E.; Molnár, M. Influence of red mud on soil microbial communities: Application and comprehensive evaluation of the Biolog EcoPlate approach as a tool in soil microbiological studies. *Sci. Total Environ.* **2017**, *595*, 903–911. [CrossRef] [PubMed]
24. Zhao, J.; Ni, T.; Li, Y.; Xiong, W.; Ran, W.; Shen, B.; Shen, Q.; Zhang, R.F. Responses of bacterial communities in arable soils in a rice-wheat cropping system to different fertilizer regimes and sampling times. *PLoS ONE* **2014**, *9*, e85301. [CrossRef] [PubMed]
25. Li, Y.L.; Wang, Y.M.; Gu, C.H.; Shen, C.; Xu, L.; Zhao, Y.L.; Yi, S.Q.; Zuo, W.G.; Shan, Y.H.; Zhang, Z.Q.; et al. Differential effects of organic ameliorants on the reassembly of bacterial communities in newly amended coastal mudflat salt-affected soil. *Agronomy* **2022**, *12*, 2525. [CrossRef]
26. Lai, J.; Zou, Y.; Zhang, J.; Peres-Neto, P.R. Generalizing hierarchical and variation partitioning in multiple regression and canonical analyses using the rdacca. *Methods Ecol. Evol.* **2022**, *13*, 782–788. [CrossRef]
27. Jorenush, M.H.; Sepaskhah, A.R. Modelling capillary rise and soil salinity for shallow saline water table under irrigated and non-irrigated conditions. *Agr. Water Manage.* **2003**, *61*, 125–141. [CrossRef]
28. Wu, W.; Huang, H.L.; Biber, P.; Bethel, M. Litter decomposition of *Spartina alterniflora* and *Juncus roemerianus*: Implications of climate change in salt marshes. *J. Coastal Res.* **2017**, *33*, 372–384. [CrossRef]

29. Kacprzak, M.; Neczaj, E.; Fijakowski, K.; Grobelak, A.; Grosser, A.; Worwag, M.; Rorat, A.; Brattebo, H.; Almås, Å.; Singh, B.R. Sewage sludge disposal strategies for sustainable development. *Environ. Res.* **2017**, *156*, 39–46. [CrossRef]
30. Bai, Y.C.; Zuo, W.G.; Shao, H.B.; Mei, L.J.; Tang, B.P.; Gu, C.H.; Wang, X.K.; Guan, Y.X. Eastern China coastal mudflats: Salt-soil amendment with sewage sludge. *Land Degrad. Dev.* **2018**, *29*, 3803–3811. [CrossRef]
31. Tian, X.M.; Fan, H.; Wang, J.Q.; Ippolito, J.; Li, Y.B.; Feng, S.S.; An, A.J.; Zhang, F.H.; Wang, K.Y. Effect of polymer materials on soil structure and organic carbon under drip irrigation. *Geoderma* **2019**, *340*, 94–103. [CrossRef]
32. Nannipieri, P.; Ascher, J.; Ceccherini, M.T.; Landi, L.; Pietramellara, G.; Renella, G. Microbial diversity and soil functions. *Eur. J. Soil Sci.* **2003**, *68*, 12–26. [CrossRef]
33. Gomez, E.; Ferreras, L.; Toresani, S. Soil bacterial functional diversity as influenced by organic amendment application. *Bioresour. Technol.* **2006**, *97*, 1484–1489. [CrossRef] [PubMed]
34. Govaerts, B.; Mezzalama, M.; Unno, Y.; Sayre, K.D.; Luna-Guido, M.; Vanherck, K.; Dendooven, L.; Deckers, J. Influence of tillage, residue management, and crop rotation on soil microbial biomass and catabolic diversity. *Appl. Soil Ecol.* **2006**, *37*, 18–30. [CrossRef]
35. Pascual, I.; Avilés, M.; Aguirreolea, J.; Sánchez-Díaz, M. Effect of sanitized and non-sanitized sewage sludge on soil microbial community and the physiology of pepper plants. *Plant Soil.* **2008**, *310*, 41–53. [CrossRef]
36. Liu, X.; Guo, K.L.; Huang, L.; Ji, Z.Y.; Jiang, H.M.; Li, H.; Zhang, J.F. Responses of absolute and specific enzyme activity to consecutive application of composted sewage sludge in a Fluventic Ustochrept. *PLoS ONE* **2017**, *12*, e0177796. [CrossRef] [PubMed]
37. Calleja-Cervantes, M.E.; Aparicio-Tejo, P.M.; Villadas, P.J.; Irigoyen, I.; Irañeta, J.; Fernández-González, A.J.; Fernández-López, M.; Menéndez, S. Rational application of treated sewage sludge with urea increases GHG mitigation opportunities in Mediterranean soils. *Agric. Ecosyst. Environ.* **2017**, *238*, 114–127. [CrossRef]
38. Latare, A.M.; Singh, S.K.; Kumar, O. Impact of sewage sludge application on soil fertility, microbial population and enzyme activities in soil under rice-wheat system. *J. Indian Soc. Soil Sci.* **2018**, *66*, 300–309. [CrossRef]
39. Bhat, S.A.; Singh, J.; Vig, A.P. Earthworms as organic waste managers and biofertilizer producers. *Waste Biomass Valori.* **2018**, *9*, 1073–1086. [CrossRef]
40. Zheng, J.; Wang, L.; Zhao, J.; Niu, Y.; Xiao, H.; Wang, Z.; Yu, S.; Shi, Z. Forty-year-old orchards promote carbon storage by changing aggregate-associated enzyme activities and microbial communities. *Catena* **2022**, *213*, 106195. [CrossRef]
41. Li, F.Q.; Xue, C.; Qiu, P.F.; Liu, Y.X.; Shi, J.X.; Shen, B.; Yang, X.M.; Shen, Q.R. Soil aggregate size mediates the responses of microbial communities to crop rotation. *Eur. J. Soil Biol.* **2018**, *88*, 48–56. [CrossRef]
42. Six, J.; Paustian, K.; Elliott, E.T.; Combrink, C. Soil structure and organic matter I. Distribution of aggregate-size classes and aggregate-associated carbon. *Soil Sci. Soc. Am. J.* **2018**, *64*, 681–689. [CrossRef]
43. Zhao, S.; Liu, J.J.; Banerjee, S.; Zhou, N.; Zhao, Z.Y.; Zhang, K.; Tian, C.Y. Soil pH is equally important as salinity in shaping bacterial communities in saline soils under halophytic vegetation. *Sci. Rep.* **2018**, *8*, 4550. [CrossRef] [PubMed]
44. Li, H.Y.; Luo, N.Y.; Ji, C.L.; Li, J.; Zhang, L.; Xiao, L.; She, X.L.; Liu, Z.; Li, Y.L.; Liu, C.S.; et al. Liquid organic fertilizer amendment alters rhizosphere microbial community structure and co-occurrence patterns and improves sunflower yield under salinity-alkalinity stress. *Microb. Ecol.* **2021**, *84*, 423–438. [CrossRef] [PubMed]
45. Luo, S.S.; Wang, S.J.; Tian, L.; Shi, S.H.; Xu, S.Q.; Yang, F.; Li, X.J.; Wang, Z.C.; Tian, C.J. Aggregate-related changes in soil microbial communities under different ameliorant applications in saline-sodic soils. *Geoderma* **2018**, *329*, 108–117. [CrossRef]