agronomy

Article

Effect of Multi-Walled Carbon Nanotubes on the Carbon and Nitrogen Cycling Processes in Saline Soil

Yutian Zuo [1,2], Chenchen Wei [3], Yue Hu [4], Wenzhi Zeng [1,5], Chang Ao [1,*] and Jiesheng Huang [1,*]

[1] State Key Laboratory of Water Resources Engineering and Management, Wuhan University, Wuhan 430072, China; zuoyutian@whu.edu.cn (Y.Z.); zengwenzhi1989@whu.edu.cn (W.Z.)
[2] Nanjing Hydraulic Research Institute, Nanjing 210029, China
[3] Agricultural Water Conservancy Department, Changjiang River Scientific Research Institute, Wuhan 430010, China; weichenchen@cau.edu.cn
[4] Central-Southern Safety and Environment Technology Institute Co., Ltd., Wuhan 430072, China; hongdoulvdoucmd@163.com
[5] College of Agricultural Science and Engineering, Hohai University, Nanjing 210098, China
* Correspondence: aochang@whu.edu.cn (C.A.); sdjshuang@whu.edu.cn (J.H.)

Abstract: Soil salinization is a pressing issue that needs to be addressed in current agricultural production. In this study, we utilized novel materials, unfunctionalized multi-walled carbon nanotubes (MWCNT) and functionalized multi-walled carbon nanotubes (MWCNT-OH), to explore the effects of soil carbon and nitrogen cycles in saline soil. We set up four treatments, which were exposed to two exposure doses of 1 g/kg and 1 μg/kg and two MWCNT types of functionalized MWCNT-OH and unfunctionalized MWCNT. Our results demonstrate that exposure of saline soil to 1 g/kg functionalized MWCNT-OH significantly increased the soil inorganic nitrogen ($p < 0.05$), while also promoting the soil microbial biomass. This exposure can also potentially enhance greenhouse gas emissions from saline soil. Moreover, exposure to MWCNTs significantly increased the proportion of Actinobacteria and Proteobacteria, two dominant phyla ($p < 0.05$), which in turn improved their contribution to the carbon and nitrogen cycling processes within saline soil. High exposure dose treatments (1 g/kg) significantly increased the abundance of functional genes associated with carbon metabolism, carbon fixation, methane metabolism, and nitrogen cycling processes within saline soil. In contrast, low exposure dose treatments (1 μg/kg) had no significant effect on the abundance of functional genes related to nitrogen cycling, but significantly increased the abundance of special functional genes related to carbon cycling. Redundancy analysis revealed that the microbial community composition within saline soil was significantly impacted by the soil total carbon, total nitrogen, and nitrate nitrogen content. Furthermore, it was observed that over 80% of the carbon and nitrogen cycling processes within the saline soil were contributed by the dominant phyla. In summary, our research confirms the potential applicability of MWCNTs within saline soil. Notably, exposure of saline soil to 1 g/kg functionalized MWCNT-OH exhibited the most significant promoting effect on the carbon and nitrogen cycles.

Keywords: functionalized multi-walled carbon nanotubes; saline soil; carbon and nitrogen cycling; metagenomics; microbial community

Citation: Zuo, Y.; Wei, C.; Hu, Y.; Zeng, W.; Ao, C.; Huang, J. Effect of Multi-Walled Carbon Nanotubes on the Carbon and Nitrogen Cycling Processes in Saline Soil. *Agronomy* **2023**, *13*, 2455. https://doi.org/10.3390/agronomy13102455

Academic Editor: Baskaran Stephen Inbaraj

Received: 26 August 2023
Revised: 13 September 2023
Accepted: 19 September 2023
Published: 22 September 2023

1. Introduction

Soil salinization has become one of the main factors limiting agricultural productivity and farmland use efficiency [1]. Currently, there are approximately 11 million square kilometers of saline soil worldwide, and its total area is still expanding at a rate of 10% annually [2]. Soil salinization is caused not only by climatic factors such as drought and intense evaporation but also by human factors such as improper cultivation practices, leading to secondary soil salinization [2]. Soil salinization suppresses normal crop growth, reduces crop yields, changes the structure and function of cell membranes, and ultimately decreases soil microbial activity [3]. Furthermore, the decreased microbial activity within saline soil inhibits the carbon and nitrogen cycling processes, which can lead to the accumulation of salts such as nitrates and nitrites, exacerbating soil salinization. Current methods to address soil salinization include irrigation and drainage, improving crop salt tolerance, and applying chemical agents such as gypsum and calcium sulfite to reduce soil salinity [2,3]. While these methods can effectively improve the environment of saline soil and promote nutrient cycles in farmlands, they still have limitations such as high costs, low efficiency, and potential damage to local ecosystems. Therefore, there is a pressing need to identify efficient, cost-effective, and sustainable agricultural development approaches, improving the living environment for soil microorganisms, and promoting carbon and nitrogen cycles to alleviate the pressure of soil salinization in farmlands.

In recent years, multi-walled carbon nanotubes (MWCNTs) have emerged as an efficient and novel material with enormous development potential in many fields, owing to their unique mechanical properties and thermal and chemical stability [4]. Prior research has demonstrated the beneficial effects of MWCNTs on saline farmlands. Specifically, according to Carmen Martinez-Ballesta et al. [5], MWCNTs can enter plant cells, enhance water absorption, mitigate salt stress-induced damage, and ultimately promote cauliflower growth in saline soil. Li et al. [6] have discovered that MWCNTs can enhance the salt tolerance of grape seeds and seedlings. This novel approach not only significantly increases the root length and seed germination rate of crops, but also maintains the antioxidant capacity of grape seedlings under high salt stress. Chen and Wang [7] suggested that MWCNTs can promote the growth of alfalfa in saline–alkaline soil while regulating crop physiological characteristics such as photosynthesis and antioxidant systems. Ahmadian et al. [8] have found that applying MWCNTs in agriculture has a more beneficial effect than other plant growth regulators. MWCNTs not only directly change crop physiological characteristics, but also alter microbial activity, thereby influencing the elemental cycling process in soil systems. The effects of MWCNTs extend beyond immediate changes to crop physiological characteristics, as it also alters microbial activity, thereby influencing the elemental cycling process in soil systems. Soil carbon and nitrogen cycles are crucial components of the global biogeochemical cycle, with vast implications for land use and ecological balance on a worldwide scale. Therefore, a comprehensive understanding of agricultural soil carbon and nitrogen cycling processes is of utmost importance for improving crop productivity, increasing agricultural yields, and promoting sustainable land management practices. Sekhon [9] indicates that compared to traditional fertilizers, carbon nanotubes can enhance soil nutrients, promote soil carbon and nitrogen cycling processes, and prevent nutrient loss caused by soil eutrophication or runoff.

Furthermore, alterations to soil carbon and nitrogen cycling processes have the potential to impact the soil affected by greenhouse gas emissions, including carbon dioxide (CO_2), nitrous oxide (N_2O), and methane (CH_4) [10]. However, the use of MWCNTs in agricultural production often faces some challenges. Some researchers have shown that exposure to single-walled carbon nanotubes negatively affects soil nutrient cycling by affecting soil microbial communities [11,12]. Wang et al. [13] indicated that the exposure of MWCNTs exposure doses of 1 or 10 mg/kg for 100 days led to a significant reduction in soil microbial diversity, altered soil microbial community composition and metabolic pathways, and caused a decrease in specific microbial groups and functional genes. Ultimately, these changes significantly impacted soil carbon and nitrogen cycling processes. Thus, the investigation of nutrient cycling in soil systems is inextricably linked to the exploration of soil microbial communities. The effects of carbon nanotube exposure on soil carbon and nitrogen cycling processes are still a matter of debate. Specifically, future studies are required to evaluate the effect of carbon nanotubes on carbon and nitrogen cycling processes in saline farmlands, where the soil environment is more intricate.

Furthermore, the effectiveness of carbon nanotubes is contingent upon multiple factors, including material type, exposure dose and time, functionalization type, and more [14]. Previous research finds that the application of unfunctionalized carbon nanotubes in agricultural farmlands results in problems such as poor mixing with soil, as well as potential toxicity to crops and microorganisms, due to hydrophobicity and unsatisfactory biocompatibility [15]. Thus, several studies have focused on the development of functionalized carbon nanotubes to enhance their positive impact on crops and agricultural soil, while minimizing their potential hazards [16,17]. Kerfahi et al. [18] shows that functionalized carbon nanotubes have a milder impact on soil microbial community structures compared to unfunctionalized ones, with a smaller effect on microbial community diversity as well. Su et al. [19] indicates that functionalized carbon nanotubes possess a larger number of hydrophilic groups on their surface, making them more easily utilized by microorganisms via a lipid-assisted mechanism. Additionally, they exhibit improved dispersibility in water, which facilitates their application in agriculture. However, some researchers have suggested that functionalized carbon nanomaterials, despite having stronger biocompatibility compared to unfunctionalized carbon nanomaterials, exhibit high toxicity towards soil microorganisms at relatively low exposure levels, leading to a reduction in soil microbial biomass [12]. Despite the potential benefits of functionalized MWCNTs in agricultural soil, research on their application in saline farmlands still needs to be completed. Given the potential negative impacts of MWCNTs on soil health, it is crucial to explore the disparities in the efficacy of functionalized and unfunctionalized MWCNTs in saline soil, with the aim of utilizing nanotechnology more effectively to mitigate salinity stress in agricultural production.

Based on the research background, the objective of our study is to shed light on the effects of distinct types and exposure doses of MWCNTs on the carbon and nitrogen cycling process in saline soils. To accomplish this aim, we carried out experiments into the carbon–nitrogen cycling process of saline soils that were subjected to two exposure doses (1 g/kg and 1 μg/kg) of both functionalized MWCNT-OH and unfunctionalized MWCNTs. The objective of the study is (1) to investigate the suitability of MWCNTs in saline soils, (2) to determine whether functionalized MWCNT-OH exhibit greater enhancement effects on the carbon–nitrogen cycling process in saline soil than unfunctionalized MWCNTs, and (3) to investigate the effects of multi-walled carbon nanotube exposure on the structure and function of microbial communities in saline soils by metagenomics.

2. Materials and Methods

2.1. Test Materials

Experimental soil was collected from the autonomous county of Yanqi Hui, Bayingol Mongol Autonomous Prefecture, Xinjiang, China, at a geographical location of approximately 41°45′ N, 85°44′ E. The experimental area experiences a climate that results in serious soil salinization. Soil samples were collected at the sampling site using a stratified multi-point sampling method, and the undisturbed soil samples were placed in sealed bags and stored for later use. Soil particle size characteristics were measured by a particle size analyzer (S3500, Microtrac Inc., Pittsboro, NC, USA). The soil texture was determined to be sandy loam, comprising 52.23% sand, 39.31% silt, and 8.46% clay. A conductivity meter (HQ40d, HACH Co., Loveland, CO, USA) was used to measure the conductivity of the 1:5 soil–water extract was 841 μS/cm, and the pH value was 8.02.

The selected unfunctionalized MWCNT was purchased from the Chengdu Organic Chemical Co., Ltd., Chinese Academy of Sciences (http://www.cocc.cn/, accessed on 27 October 2022). The purity was >98%, the outer diameter was 5–15 nm, the inner diameter was 2–5 nm, the length was 0.5–2 μm, and the surface area was >350 m^2/g. Functionalized MWCNT-OH purity was >98%, the outer diameter was 5–15 nm, the inner diameter was 2–5 nm, the length was 0.5–2 μm, the surface area was >380 m^2/g, and the -OH content was 5.58%. Functionalization treatment significantly increased the material's surface area and -OH content. To prepare a uniform suspension, both MWCNTs were soaked in sterile deionized water at 38 °C for 12 h, then subjected to 15 h of ultrasonic treatment in a high-frequency 40 kHz, 100 W ultrasonic water bath. The resulting suspensions were mixed evenly with soil by spraying, and the soil was adjusted to a uniform moisture content of 20%.

2.2. Experimental Scheme

The experiment was conducted in the microbial culture box of the Irrigation and Drainage Test Field at the State Key Laboratory of Water Resources and Hydropower Engineering Science at Wuhan University. Prior to the experiment, the soil was air-dried, crushed, and sieved through a 2 mm screen mesh. The soil samples were then layered and compacted using the layering method based on a bulk density of 1.4 g/cm^3. The soil was then filled into 650 mL culture bottles with a filling volume of 200 mL. The filled soil columns were placed in a constant temperature chamber at 25 °C and 50% humidity for 7 days to recover the soil condition for pre-incubation. During the experiment, the filled soil columns were incubated for 56 days under the same constant temperature and humidity conditions as during the pre-incubation. The samples were exposed to artificial lamps to simulate the sunlight (light intensity: 1700 $\mu mol/m^2 \cdot s$, light period: 12 h/12 h, day/night). The culture bottles were weighed every 24 h, and the soil moisture content was adjusted to ensure that the moisture content was consistent across all treatments during the experiment. Four treatments were set up in the experiment: MW1 treatment (soil exposed to 1 g/kg of unfunctionalized MWCNTs), MW2 treatment (soil exposed to 1 mg/kg of unfunctionalized MWCNTs), HMW1 treatment (soil exposed to 1 g/kg of functionalized MWCNT-OH), and HMW2 treatment (soil exposed to 1 mg/kg of functionalized MWCNT-OH). In addition to the four treatments, a control group was set up and labeled as CK treatment. Each treatment was replicated six times, resulting in a total of 30 culture bottles.

2.3. Research Methods

Soil samples were collected after 7 and 56 days of incubation, and soil characteristics related to the carbon–nitrogen cycling process were measured. Soil total organic carbon content (TOC) was measured using the K2Cr2O7 oxidation-spectrophotometric method (UV8000, Metash Instruments Co., Shanghai, China). Soil microbial biomass carbon and nitrogen content (MBC and MBN) were determined using the chloroform fumigation-K2SO4 extraction method (multi N/C 3100, Analytik Jena AG, Jena, Germany) [20]. Soil nitrate nitrogen and ammonium nitrogen (NO_3^- and NH_4^+) content were measured using an Ultraviolet-visible Spectrophotometer (UV8000, Metash Instruments Co, Shanghai, China). Soil nutrient cycling indices for each treatment were calculated using the method proposed by Delgado-Baquerizo et al. [21] to evaluate the soil nutrient cycling capacity. In addition, on days 7, 9, 11, 14, 17, 21, 28, 35, 42, 49, and 56 of the experiment, gas-tight caps with sampling ports were used to seal the culture bottles. At 0 and 45 min after sealing, 20 mL of gas was extracted from the headspace of the bottles using a syringe with a rotating three-way valve. The extracted gas was used to measure soil greenhouse gas flux and calculate cumulative emissions. The collected gas samples were immediately analyzed for greenhouse gas concentrations using a gas chromatograph (GC2010, Shimadzu Corporation, Japan) equipped with a thermal conductivity detector (TCD) and an electron capture detector (ECD). Soil greenhouse gas emission flux and cumulative greenhouse gas emissions, were calculated according to the methods described in Cai et al. [22]. In addition, the microbial metabolic quotient (qCO_2), developed by Anderson and Domsch [23], was also calculated to represent soil microbial efficiency. A high qCO_2 value indicates low microbial efficiency and can be used as an indicator of microbial stress.

$$qCO_2 = r/MBC \tag{1}$$

where r represents the mean respiration rate of CO_2-C from the soil till on the sampling day and MBC is microbial biomass carbon in the soil.

2.4. Metagenomic Analysis

The differences in soil microorganisms among all treatments were analyzed by metagenomic sequencing and were detected immediately after rapid freezing of liquid nitrogen during sampling. Microbial community DNA was extracted from the soil (0.5 g). After microbial community DNA extraction, the extracted genomic DNA was detected by 1% agarose gel electrophoresis. High quality DNA was sheared into 400-bp fragments using an ultrasonicator (M220, Covaris, Woburn, MA, USA), and metagenomic shotgun sequencing proceeded on the NovaSeq Reagent Kits. Fifteen soil samples of DNA were analyzed in triplicate by whole-genome shotgun sequencing. The original sequencing data were obtained using Fastp (https://github.com/OpenGene/fastp, accessed on 30 December 2022) and MEGAHIT (https://github.com/voutcn/megahit, accessed on 30 December 2022) for quality control and assembly. The open reading frames of spliced contigs were predicted using Prodigal (https://github.com/hyattpd/Prodigal, accessed on 30 December 2022), and a nonredundant gene catalog was constructed using CD-HIT (http://weizhongli-lab.org/cd-hit/, accessed on 30 December 2022). Non-redundant gene sets were compared with the Non-Redundant Protein Sequence (NR) Database (https://ftp.ncbi.nlm.nih.gov/blast/db/FASTA/, accessed on 30 December 2022) using DIAMOND (http://ab.inf.uni-tuebingen.de/software/diamond/, accessed on 30 December 2022). The non-redundant gene set sequences were compared with the Kyoto Encyclopedia of Genes and Genomes (KEGG) (https://www.genome.jp/kegg, accessed on 30 December 2022), and the abundance of functional classes was calculated based on the sum of gene abundance corresponding to Kegg Orthology (KO), Pathway, Read numbers, and Module.

2.5. Data Analysis

All data processing, analysis, and plotting were performed using R. One-way ANOVA was used to test for significant differences among the treatments, and the least significant difference (LSD) test was used to compare the differences between different treatments. The mean values and standard errors of all parameters were calculated from at least three replicates. Bias-corrected 95% confidence interval were non-overlapping, and $p < 0.05$ were considered statistically significant.

3. Research Results

3.1. Soil Characteristics and Greenhouse Gas Emissions Related to Carbon and Nitrogen Cycles in Saline Soil

Based on the data presented in Table 1, both short-term and long-term exposure to MWCNTs significantly affect the characteristics of saline soil and greenhouse gas emissions related to the carbon and nitrogen cycle in saline soil.

After 7-day exposure to MWCNTs, the differences between the various treatments and the CK treatment were relatively small, except the HMW1 treatment. Notably, the HMW1, HMW2, and MW1 treatments significantly increased the total carbon content of the saline soil ($p < 0.05$). Furthermore, the HMW1 treatment greatly increased the MBC and MBN content of the saline soil ($p < 0.05$). Compared to the CK treatment, the MBC and MBN content of the HMW1 treatments were increased by 93.60% and 218.92%, respectively, with the CK treatment soil containing 10.62 mg/kg and 0.74 mg/kg of MBC and MBN, respectively. Additionally, the HMW1 treatment significantly increased the total nitrogen content of the saline soil ($p < 0.05$).

After a 56-day exposure to MWCNTs, significant changes in the saline soil characteristics occurred, with increasing differences between the various treatments and the control group. The exposure to MWCNTs significantly increased the total nitrogen content, nitrate nitrogen, and ammonium nitrogen in the soil and greatly promoted the nitrogen cycling process in saline soil ($p < 0.05$). With the increased exposure dose to MWCNTs, the MBC content of the soil also significantly increased ($p < 0.05$). The HMW1 and MW1 treatments increased the soil MBC content compared to the HMW2 and MW2 treatments. Notably, the HMW1 treatment significantly increased the soil MBC and MBN content compared to other treatments ($p < 0.05$). Compared to the CK treatment, the MBC and MBN content of the HMW1 treatments were increased by 25.16% and 220.99%, respectively, with the CK treatment soil containing 12.52 mg/kg and 0.81 mg/kg of MBC and MBN, respectively. However, the exposure to MWCNTs significantly decreased the organic carbon content of the saline soil ($p < 0.05$).

Table 1. Changes of soil characteristics and cumulative greenhouse gas emissions related to the carbon and nitrogen cycle in saline soil.

	TOC (g/kg)	Total C (g/kg)	MBC (mg/kg)	Total N (g/kg)	MBN (mg/kg)	NO_3^- (mg/kg)	NH_4^+ (mg/kg)	N_2O (kg/ha)	CO_2 (kg/ha)	CH_4 (kg/ha)	Exposure Time
CK	4.64 ± 0.53 a	14.88 ± 0.28 c	10.62 ± 1.23 b	23.71 ± 0.15 b	0.74 ± 0.04 a	58.02 ± 0.32 ab	1.01 ± 0.22 a	-	-	-	7 day
HMW1	3.75 ± 0.28 a	26.69 ± 1.59 a	20.56 ± 1.37 a	29.39 ± 0.60 a	2.36 ± 0.98 a	56.47 ± 1.23 ab	1.00 ± 0.07 a	-	-	-	
HMW2	3.76 ± 0.20 a	19.70 ± 1.39 b	16.21 ± 3.72 ab	23.75 ± 0.78 b	1.50 ± 0.79 a	60.76 ± 3.80 a	1.10 ± 0.05 a	-	-	-	
MW1	5.37 ± 1.20 a	20.41 ± 0.76 b	17.57 ± 2.71 ab	24.44 ± 0.40 b	2.83 ± 1.71 a	53.16 ± 1.40 b	1.26 ± 0.16 a	-	-	-	
MW2	3.85 ± 0.24 a	18.43 ± 1.40 bc	14.62 ± 3.45 ab	23.26 ± 0.36 b	1.10 ± 0.68 a	61.17 ± 2.32 a	1.35 ± 0.16 a	-	-	-	
CK	5.08 ± 0.27 a	13.22 ± 0.25 b	12.52 ± 0.48 b	25.03 ± 0.97 b	0.81 ± 0.42 b	72.47 ± 4.53 b	0.32 ± 0.05 b	0.58 ± 0.02 a	757.94 ± 27.82 b	1.18 ± 0.26 ab	56 day
HMW1	3.68 ± 0.27 b	17.20 ± 0.23 a	15.67 ± 0.53 a	27.95 ± 0.61 a	2.60 ± 0.43 a	86.73 ± 3.72 a	0.48 ± 0.05 a	0.56 ± 0.01 a	841.21 ± 48.83 a	1.08 ± 0.08 b	
HMW2	3.36 ± 0.33 b	16.15 ± 0.58 b	12.08 ± 2.46 b	27.85 ± 0.32 a	1.39 ± 0.76 b	90.33 ± 3.86 a	0.53 ± 0.07 a	0.56 ± 0.01 a	735.33 ± 176.75 ab	1.09 ± 0.21 b	
MW1	4.29 ± 0.41 ab	15.88 ± 0.55 b	15.33 ± 1.84 ab	28.72 ± 0.38 a	2.15 ± 0.99 ab	87.25 ± 0.96 a	0.55 ± 0.03 a	0.58 ± 0.00 a	726.06 ± 130.19 ab	0.63 ± 0.35 b	
MW2	3.49 ± 0.15 b	15.73 ± 1.01 b	12.28 ± 3.36 ab	28.75 ± 0.82 a	1.69 ± 0.47 b	81.49 ± 2.11 a	0.60 ± 0.02 a	0.57 ± 0.01 a	716.41 ± 73.59 b	1.65 ± 0.28 a	

Note: different lowercase letters represent significant differences between different treatments ($p < 0.05$).

The exposure to MWCNTs significantly reduced the soil MBC/MBN ($p < 0.05$), with the HMW1 treatment showing the smallest value. After 56 days of exposure to MWCNTs, the MBC/MBN content of the CK treatment was 15.51, which was significantly reduced by 61.19% in the HMW1 treatment as compared to the CK treatment. With the exposure time increased, the MBC/MBN content of all treatments, except for the CK treatment, exhibited a decline. This suggests that the promotion of nitrogen cycling by MWCNTs exposure surpassed that of carbon cycling. In addition, exposure to MWCNTs significantly reduced the loss rate of soil ammonium nitrogen ($p < 0.05$) and promoted the increase in nitrate nitrogen, demonstrating that exposure to MWCNTs can significantly increase the soil mineralization rate ($p < 0.05$). After 56 days of cultivation, CK treatment resulted in a decrease of 68.79% in soil ammonium nitrogen, while the other treatments, HMW1, HMW2, MW1, and MW2, showed reductions of 52.24%, 51.71%, 56.32%, and 55.41%, respectively. In addition, except for the CK treatment, all other treatments showed a decrease in soil organic carbon content compared to the 7-day exposure. Furthermore, it was observed that higher soil microbial biomass was associated with higher soil organic carbon content.

As the exposure time of MWCNTs increased, the greenhouse gas emission flux from saline soil gradually stabilized, and the changes in greenhouse gas emissions from each treatment were small after 56 days of exposure. The exposure of MWCNTs did not have a significant effect on the emission flux of N_2O from saline soil but had a significant effect on the emissions of CO_2 and CH_4. Among them, the exposure of functionalized MWCNT-OH had a significantly greater effect on CO_2 emissions than unfunctionalized MWCNT. The HMW1 treatment significantly increased the CO_2 emissions from saline soil ($p < 0.05$), and the cumulative CO_2 emission flux from the CK treatment after 56 days was 757.94 kg/ha. Compared with the CK treatment, the HMW1 treatment increased by 10.99%. In addition, the MW2 treatment significantly promoted the emission of CH_4 from saline soil ($p < 0.05$), while the other three treatments had no significant effect on CH_4 emissions. By calculating the comprehensive greenhouse effect index of the soil, the HMW1 treatment showed a substantial increase of 7.98% compared to the CK treatment ($p < 0.05$). In contrast, other treatments showed no significant difference compared to the CK treatment.

3.2. Changes in Microbial Community Structure in Saline Soil

The metagenomic sequencing generated a total of 246 million raw reads, with an average of 49.11 million raw reads per treatment. Strict read and quality control measures resulted in an average of 97.34% high-quality sequences, which were subsequently used for more accurate downstream analyses.

The microbial community structure in saline soil was mainly annotated by comparing against the NR database, and the soil samples included 5 domains, 12 kingdoms, 233 phyla, 440 classes, 847 orders, 1577 families, 4887 genera, and 29,909 species. Figure 1a displays the microbial community composition at the phylum level. Among them, the CK treatment phylum level microbial community composition includes Actinobacteria 29%, Proteobacteria 28%, Chloroflexi 10%, Gemmatimonadetes 9.6%, Acidobacteria 9.4%, and Others 14%. HMW1 treatment significantly increased the proportion of Actinobacteria and Proteobacteria ($p < 0.05$), especially Proteobacteria, which significantly increased by 11.14% compared to CK treatment. The MW1 treatment significantly increased the proportion of Actinobacteria ($p < 0.05$) but had no significant impact on the proportion of other species and had a smaller impact on the microbial community composition than the HMW1 treatment. However, the effects of low exposure dose, including MW2 and HMW2 treatment, on soil microbial community composition were insignificant. We further analyzed the top 30 relative abundances of microbial genera in different treatments, created a heatmap, and conducted cluster analysis between different treatments (Figure 1b). Among them, the exposure of MWCNTs significantly increased the proportion of Methylibium ($p < 0.05$), and compared to CK treatment, HMW1 treatment significantly increased by 343.78%. According to cluster analysis, the exposure dose of MWCNTs is the main factor affecting the soil microbial community composition. HMW1 treatment and MW1 treatment are divided into one group, while MW2 treatment and HMW2 treatment are divided into one group. Furthermore, the dissimilarity in soil microbial community composition between the CK treatment and the low exposure dose treatments is comparatively smaller than that observed between the high exposure dose treatments. In addition, we analyzed the effects of exposure to MWCNTs on microbial α-diversity in saline soil (Figure 1c). Except for the HMW1 treatment, there was no significant difference in soil microbial diversity between the CK treatment and the other three treatments. Among them, HMW1 treatment significantly reduced the Simpson index (the smaller the Simpson index, the greater the microbial diversity) and increased the soil Shannon index and Chao index ($p < 0.05$). The Simpson index of CK treatment was 0.026, which decreased by 6.62% compared to CK treatment. HMW1 treatment significantly increased soil microbial community diversity and richness. Finally, we investigated the differences between soil microbial communities (Figure 1d). The dissimilarity in soil microbial community follows a similar pattern to the changes in community composition mentioned above, indicating that MWCNTs' exposure dose significantly impacts soil microbial diversity. Under low exposure doses, the soil microbial community composition in the HMW2 and MW2 treatments showed little difference compared to the CK treatment. There were no significant differences between the two treatments. However, under high exposure doses, the soil microbial community composition in the HMW1 and MW1 treatments showed significant differences compared to the CK treatment. There were also noticeable differences between the two treatments.

3.3. Changes of Microbial Carbon and Nitrogen Cycles Function in Saline Soil

3.3.1. Changes of Functional Genes Related to the Carbon Cycles in Saline Soil

Given the intricate nature of carbon cycling processes in soil systems, this research delved into the modules associated with three key carbon cycling processes, including carbon metabolism, carbon fixation, and methane metabolism in saline–alkaline soil, based on the KEGG database.

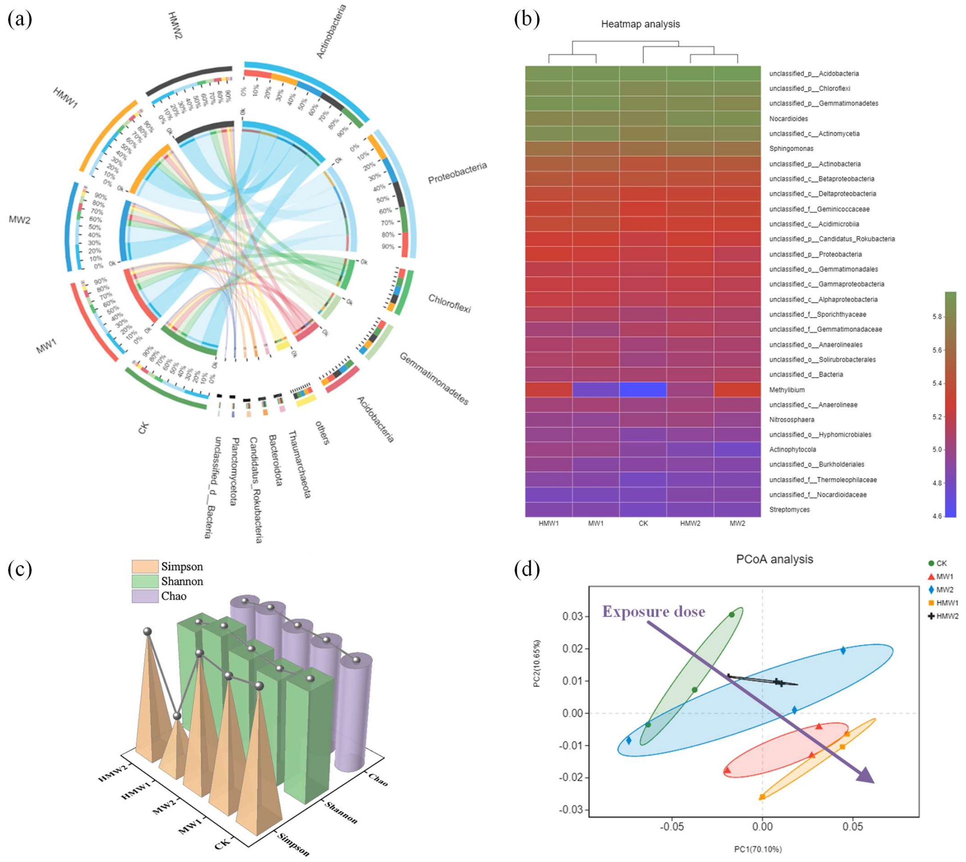

Figure 1. Effects of MWCNTs exposure on the microbial community structure in saline soil. (**a**) Microbial community composition at the phylum level. (**b**) Microbial community composition at the genus level (color depth represents the relative abundance of genera). (**c**) Microbial $\alpha-$diversity (Simpson index, Shannon index, Chao index). (**d**) Microbial $\beta-$diversity. (Purple arrow indicates increasing MWCNTs exposure dose from low to high).

From Figure 2a, the exposure of MWCNTs significantly affects the carbon metabolism process in saline soil, and 14 related modules were significantly affected by the exposure of MWCNTs. Overall, the exposure of MWCNTs significantly increased the number of functional genes related to carbon metabolism in saline soil, including M00001 (Glycolysis, glucose => pyruvate), M00002 (Glycolysis, core module involving three-carbon compounds), M00003 (Gluconeogenesis, oxaloacetate => fructose-6P), M00004 (Pentose phosphate pathway (Pentose phosphate cycle)), M00009 (Citrate cycle (TCA cycle, Krebs cycle)), M00010 (Citrate cycle, first carbon oxidation, oxaloacetate => 2-oxoglutarate), M00011 (Citrate cycle, second carbon oxidation, 2-oxoglutarate => oxaloacetate), M00012 (Glyoxylate cycle), M00307 (Pyruvate oxidation, pyruvate => acetyl-CoA), M00373 (Ethylmalonyl pathway), M00532 (Photorespiration), M00620 (Incomplete reductive citrate cycle, acetyl-CoA => oxoglutarate), M00740 (Methylaspartate cycle), and M00741 (Propanoyl-CoA metabolism, propionyl-CoA => succinyl-CoA) ($p < 0.05$). The effectiveness of HMW1 treatment is the most significant, and the high exposure dose of MW1 treatment has a more robust promoting effectiveness than the low exposure dose of HMW2 treatment and MW2 treatment. Therefore, exposure dose is one of the main factors affecting the abundance of soil microbial carbon metabolism function genes. Specifically, the exposure of MWCNTs promotes Glycolysis, Pentose phosphate cycle, Citrate cycle, Glyoxylate cycle, Pyruvate oxidation, Incomplete reductive citrate cycle, Propanoyl-CoA metabolism to promote carbon metabolism in saline soil. From Figure 2b, it can be seen that exposure to MWCNTs significantly affects the carbon fixation process in saline soil, and six modules were significantly affected by the MWCNTs. Among them, HMW1 treatment significantly promoted the carbon fixation process of saline soil, including M00173 (Reductive citrate cycle (Arnon–Buchanan cycle)), M00167 (Reductive pentose phosphate cycle, glyceraldehyde-3P => ribulose-5P), M00165 (Reductive pentose phosphate cycle (Calvin cycle)), M00376 (3-Hydroxypropionate bi-cycle), M00375 (Hydroxypropionate-hydroxybutylate cycle), M00374 (Dicarboxylate-hydroxybutyrate cycle) ($p < 0.05$). The other three treatments only significantly promoted the processes of M00173 and M00374 in saline soil ($p < 0.05$) and had no significant impact on the carbon fixation process in most soil systems. Figure 2c shows that exposure to MWCNTs significantly affects the methane metabolism process in saline soil, and six modules were significantly affected by the MWCNTs. The exposure of MWCNTs significantly promoted the M00346 and M00357 processes in saline soil ($p < 0.05$). In addition, high exposure dose treatments (HMW1 and MW1 treatments) also significantly promoted saline soil M00563 (Methanogenesis, methylamine/dimethylamine/trimethylamine => methane) and M00567 (Methanogenesis, CO_2 => methane) processes ($p < 0.05$).

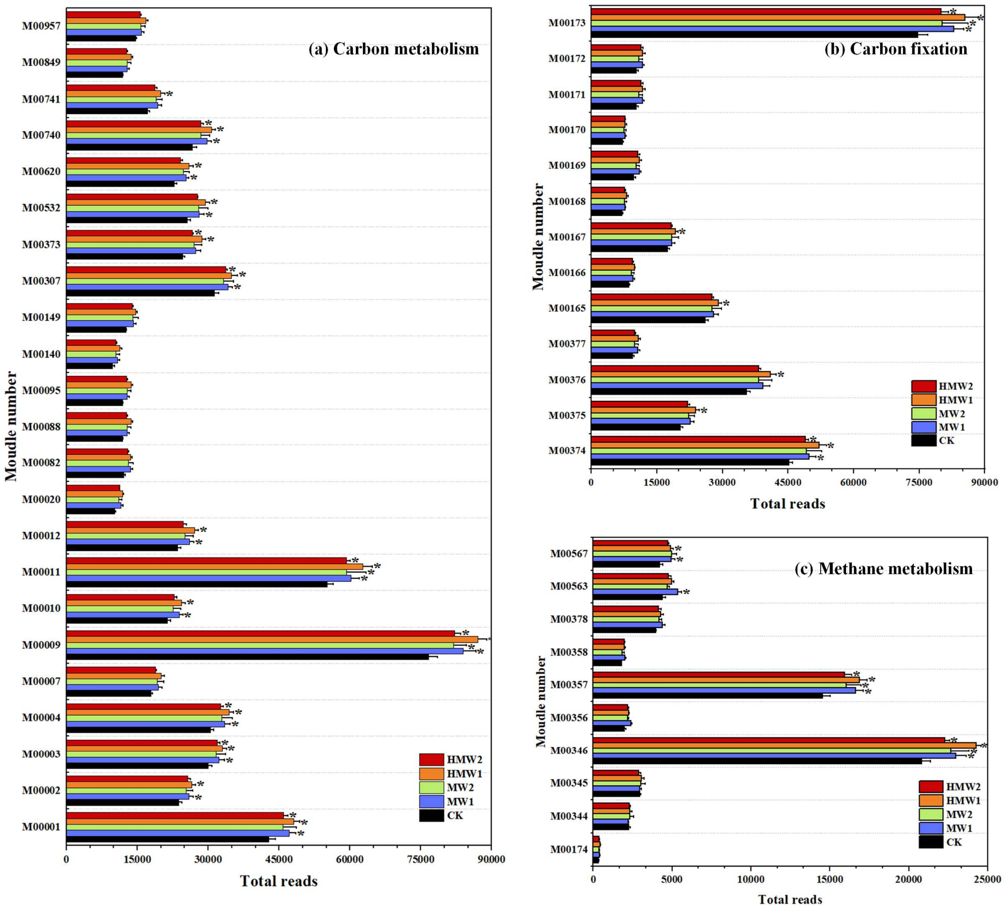

Figure 2. Effects of MWCNTs exposure on carbon cycle-related functional genes in saline soil (asterisk indicates that there is a significant difference between this treatment and CK treatment, $p < 0.05$).

3.3.2. Changes of Functional Genes Related to the Nitrogen Cycle in Saline Soil

The nitrogen cycle in the soil system is an integral part of the global biogeochemistry cycle. It can be seen from Figure 3 that exposure to MWCNTs significantly affects the nitrogen cycle process of saline soil.

Notes: the digits in the figures show the relative content changes of nitrogen cycle functional genes in MWCNTs exposure treatment and CK treatment, the arrows in different colors indicate the process of the soil nitrogen cycle, and the asterisk indicates that the process in this treatment is significantly different from that in CK treatment, $p < 0.05$.

In general, the high exposure dose treatments (HMW1 treatment and MW1 treatment) significantly promoted the nitrogen cycle process of saline soil, while the low exposure dose treatment (HMW2 treatment and MW2 treatment) had no significant effect. Moreover, the improvement effects of different types of MWCNTs were also significantly different, and the promotion effect of HMW1 treatment on the nitrogen cycle of saline soil was more substantial than that of MW1 treatment. HMW1 treatment significantly promoted the transformation of NO_3^- to NO_2^- during denitrification, nitrogen fixation, NH_4^+ to NH_2OH, and NO_2^- to NO_3^- during nitrification in saline soil, and significantly promoted the mutual transformation of organic nitrogen and ammonium nitrogen in the soil ($p < 0.05$). In addition, MW1 treatment also considerably affected the nitrogen cycle process of saline soil, significantly promoted the transformation of NO_2^- to NO_3^- during nitrification, NO_3^- to NO_2^- during assistant nitrate reduction, and NO_3^- to NO_2^- during soil denitrification, and also significantly promoted the mutual transformation of organic nitrogen and ammonium nitrogen in the soil ($p < 0.05$). However, the effect of MW1 treatment on the nitrogen cycle of saline soil was less than that of HMW1 treatment.

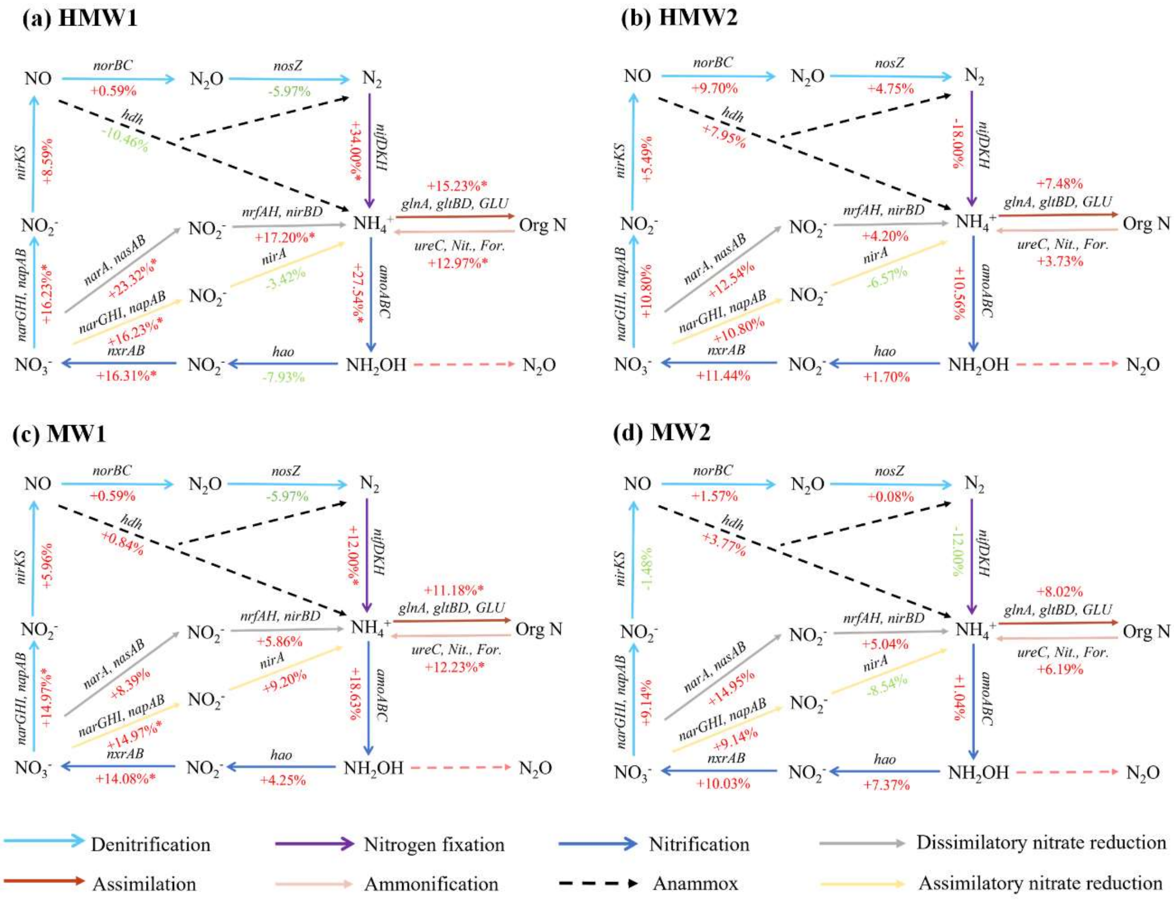

Figure 3. Effects of MWCNTs exposure on the nitrogen cycle−related functional genes in saline soil (asterisk indicates that there is a significant difference between this treatment and CK treatment, $p < 0.05$).

3.3.3. Responses of Biotic and Abiotic Factors to Carbon and Nitrogen Cycles in Saline Soil

The exposure of MWCNTs significantly affects the functional contribution of soil microorganisms in saline soil. Figure 4a shows the functional contribution of dominant phyla, including carbon metabolism, carbon fixation, methane metabolism, and nitrogen metabolism in saline soil. Our results show that more than 80% of the carbon and nitrogen cycling processes in saline soil can be contributed by four dominant phyla, Actinobacteria, Proteobacteria, Chloroflexi, and Gemmatimonadetes. Among them, Actinobacteria and Proteobacteria contributed significantly more to the carbon and nitrogen cycles of saline soil than Chloroflexi and Gemmatimonadetes. Under the exposure of MWCNTs, the proportion of Actinobacteria and Proteobacteria in saline soil is the highest, thereby making the most significant contribution to the biological function of saline soil. In addition, the exposure of MWCNTs can promote the contribution of dominant phyla to the carbon and nitrogen cycles in saline soil. Actinobacteria contribution to the nitrogen cycle of saline soil was significantly higher than that to the carbon cycle. At the same time, Proteobacteria's contributes to the carbon cycle of saline soil was considerably higher than that of the nitrogen cycle. HMW1 treatment significantly increased the proportion of Actinobacteria and Proteobacteria (Figure 1) and further promoted the carbon and nitrogen cycling processes in saline soil.

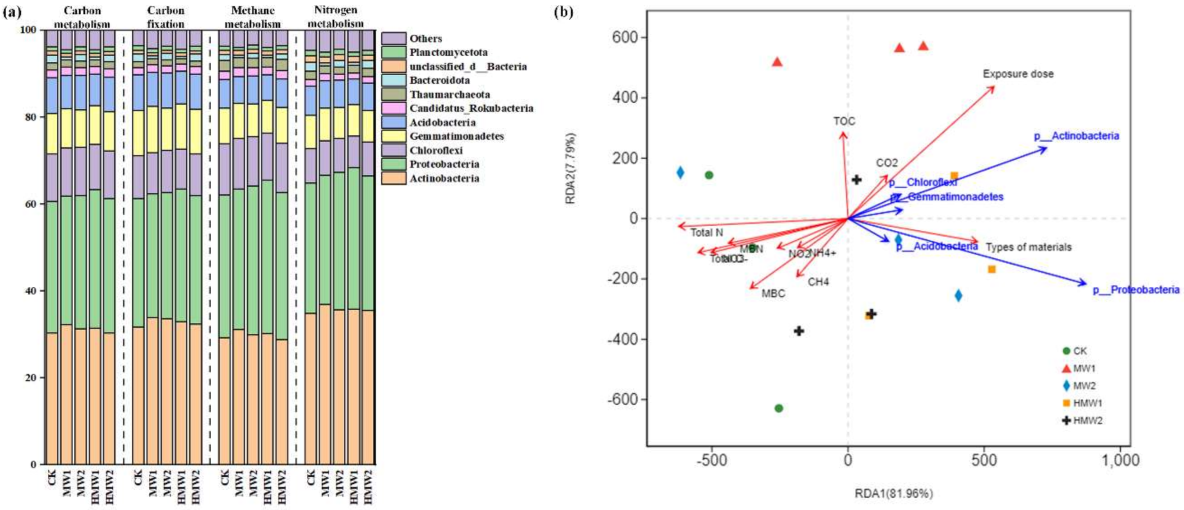

Figure 4. (**a**) The functional contribution of dominant phyla (%); (**b**) RDA analysis of the relationship between soil microbial community and environmental factors (RDA, redundancy analysis). Red represents environmental factors, and blue represents soil microbial community.

Exposure of MWCNTs significantly affects the response relationship between soil microbial community and environmental factors. Figure 4b shows the response relationship between dominant phyla and saline soil characteristics and greenhouse gas emissions based on RDA analysis. The cumulative contributions of the first and second axes reach 89.75%, so the response mechanisms of most microorganisms to environmental factors can be determined through RDA1 (81.96%) ($p < 0.05$). RDA analysis showed that soil total carbon ($p = 0.024$), total nitrogen ($p = 0.012$), and nitrate nitrogen ($p = 0.046$) significantly affected the microbial community composition in saline soil ($p < 0.05$). There is no significant response relationship between other soil characteristics and the microbial community composition in saline soil, while there is a strong correlation between different saline soil characteristics. Apart from the direct effects of MWCNTs in saline soil microorganisms, the difference in soil environment caused by the diverse exposure doses and material types of MWCNTs also indirectly influences the carbon and nitrogen cycles in saline soil ($p < 0.05$). Furthermore, a significant positive correlation exists between the exposure dose of MWCNTs and the dominant phyla within saline soil ($p < 0.05$).

4. Discussion

4.1. Effects of MWCNTs Exposure on Carbon and Nitrogen Cycles in Saline Soil

The HMW1 treatment significantly increased the microbial biomass (MBC and MBN) in saline soil ($p < 0.05$), and the positive effect of the HMW1 treatment increased gradually with exposure time (Table 1). Compared to the CK treatment, the HMW1 treatment demonstrated a notable 9.03% increase in metagenomic read length, suggesting a substantial enhancement in the soil microbial biomass. Furthermore, the HMW1 treatment significantly increased the microbial community diversity in saline soil ($p < 0.05$). The enhancement of MBC and diversity, which can effectively regulate the ecosystem functioning and services [24]. The significant reduction in soil MBC/MBN by HMW1 treatment ($p < 0.05$) indicates that exposure to functionalized MWCNT-OH increases bacterial community and decreases fungal community in saline soil, representing a more robust ability of soil to resist adverse factors [25]. The HMW1 treatment significantly enhances the total carbon and total nitrogen content in the soil by promoting the carbon fixation and nitrogen fixation processes ($p < 0.05$). This can effectively mitigate nutrient leaching losses in the soil during crop growth [25]. Furthermore, the HMW1 treatment significantly increases the ammonium nitrogen and nitrate nitrogen content in saline soil ($p < 0.05$), increasing soil nutrient content and reducing the reliance on traditional fertilizers used in saline farmlands to a certain extent. In addition, the soil nutrient cycling index, as calculated by Delgado-Baquerizo et al. [21], indicates that applying MWCNTs significantly enhances the carbon and nitrogen cycling processes in saline soil, with the HMW1 treatment demonstrating the most notable improvement effect. The soil nutrient cycling process of the high exposure dose treatments (HMW1 and MW1 treatments) is more robust than that of the low exposure dose treatments (HMW2 and MW2 treatments). In contrast, the low exposure dose has no significant effect on most of the soil carbon and nitrogen cycling processes in saline farmlands. Furthermore, Dharni et al. [26] indicated that as the exposure dose of carbon nanomaterials increases, inhibited soil microbial activity, reduced soil microbial diversity, and the development of some fungi and bacteria may be hindered. Jin et al. [27] indicated that the exposure dose of single-walled carbon nanotubes is negatively correlated with soil microbial biomass, and the exposure of MWCNTs has a particular impact on the soil microbial community composition. The divergent conclusions of these studies from the present study may be attributed to variations in the application mode of carbon nanomaterials and soil environmental conditions. Moreover, discrepancies in the definition of high and low exposure doses of carbon nanomaterials across different studies may also contribute to these differences. The exposure of MWCNTs also significantly affects greenhouse gas emissions in saline soil ($p < 0.05$), with CO_2 emission flux often positively proportional to soil microbial activity. This finding also indicates that the HMW1 treatment can effectively increase microbial activity in saline soil. Moreover, the exposure of MWCNTs reduces qCO_2, with the HMW1 treatment significantly increasing qCO_2 by 11.33% compared to the CK treatment, considerably enhancing the functional biological efficiency of saline soil ($p < 0.05$). The microbial community composition and functional structure drive the most critical soil element cycling processes. Moreover, the utilization of metagenomic offers a powerful means of elucidating the underlying mechanisms by which exposure to MWCNTs impacts carbon and nitrogen cycling processes in saline soil.

Several studies have shown that the major phyla in the soil ecosystems have higher competitive potential and growth rates and are significantly correlated with soil biological function. In contrast, species with lower proportions contribute less to soil biological function [28]. In this study, the dominant bacterial phyla Actinobacteria and Proteobacteria in saline soil also contribute the most to soil biological function (Figure 4). Therefore, the HMW1 treatment in this study significantly increases the number of dominant phyla in saline soil, thereby considerably enhancing the biological function of saline soil ($p < 0.05$). Moreover, from the perspective of soil microbial functional genes, the exposure of MWCNTs significantly increases the abundance of functional genes related to soil carbon and nitrogen cycling ($p < 0.05$), indicating the carbon and nitrogen cycling process in saline soil. According to previous studies, a rich set of functional genes often leads to higher soil microbial activity. However, Yang et al. [29] showed that the abundance of functional genes in soil microorganisms is not significantly correlated with soil microbial activity but rather represents the primary energy flow and material cycling processes in the soil ecosystems. Consistent with the findings of this study, the HMW1 treatment has a rich microbial community structure and functional gene abundance, representing a more frequent carbon and nitrogen cycling process within the soil system and greater exchange with the external environment. The exposure of MWCNTs not only increases soil nutrients in saline soil but also provides TOC to soil microorganisms. This has the potential to reduce the consumption of traditional fertilizers used in saline farmlands, further mitigating the increase in soil salinity and promoting sustainable agricultural development. Furthermore, we indicated that soil microbial communities could optimize soil system function by regulating their ecosystem functioning and services under appropriate exposure doses of MWCNTs. However, beyond a certain threshold, they exhibit toxicity in certain critical carbon and nitrogen cycling processes [30,31]. This research found that the toxicity threshold of MWCNTs was increased under low exposure doses or in saline soil. Specifically, the highest exposure dose of 1 g/kg of MWCNTs did not exhibit toxicity in saline soil.

4.2. Applicability Analysis of MWCNTs in Saline Soil

In previous studies, MWCNTs have often exhibited toxicity in soil. However, the main reasons for MWCNTs showing predominantly positive effects in saline soil in the present research might be explained in the following four aspects:

Firstly, the electrical charge strength of the soil can affect the surface charge of MWCNTs, which in turn controls the degree of cellular absorption of carbon nanotubes [32]. Therefore, the high electrical charge and high pH soil conditions modulated the biocompatibility of MWCNTs, enhancing their effectiveness. Secondly, along with soil nitrification, hydrogen ions are produced, which weaken the alkalinity of saline soil. Therefore, the exposure of MWCNTs promotes the nitrification process in saline soil, thereby mitigating the detrimental effects of saline soil. Thirdly, the exposure of saline–alkali soil to acidic carbon nanotubes can neutralize the alkalinity of the soil, lower the soil pH, and improve the soil environment. Fourthly, in saline–alkali soil environments, along with insufficient soil aeration and other conditions, the activity of nitrobacteria is inhibited, leading to an increase in the accumulation of nitrate and nitrite. The accumulation of nitrate and nitrite in the soil will produce toxic effects on soil microorganisms (inhibiting microbial activity and even death), crops (burning seeds, rotten buds, rotten roots, and seedling death), and even human bodies (carcinogen). Promoting the nitrogen cycle of saline soil will help alleviate the toxic effects of salt accumulation in saline soil [33]. The exposure of MWCNTs has also been found to mitigate the toxic effects of salt accumulation in saline soil on soil microorganisms by promoting the nitrogen cycle and reducing the rate of ammonium nitrogen loss.

4.3. Advantages of Functionalized MWCNT-OH in Saline Soil

The direct effects of carbon nanomaterials on soil microorganisms and crops are primarily attributed to their inherent chemical structures and biological properties [1]. This study suggests that functionalized MWCNT-OH has a more significant promoting effect on the carbon and nitrogen cycling processes in saline soil than unfunctionalized MWCNT. The main reasons are as follows:

At first, the electronegative functional groups present in functionalized MWCNT-OH can provide negative charges to moderately active nutrient ions and biological macromolecules, thereby improving soil properties and enhancing the effectiveness of soil nutrients [34]. Secondly, heteroaggregation may occur due to the interaction between positively charged sites on soil components and negatively charged nanomaterials [34]. Under alkaline soil conditions, unfunctionalized MWCNTs with strong negative charges tend to have a stronger aggregation effect with soil compared to functionalized MWCNT-OH, which can weaken their bioavailability. Moreover, functionalized MWCNT-OH has a higher positive charge, which can better interact with negatively charged soil microorganisms and improve the utilization efficiency of MWCNTs [35,36]. Then, the nanoscale structure and richer hydrophilic functional groups of functionalized MWCNT-OH make it a better transport carrier for providing water and nutrients to soil microorganisms and crop cells [37]. Next, soil nutrient availability is crucial in determining soil microbial biomass. Under conditions of nutrient deficiency, promoting microbial nutrient retention can lead to more available nutrients being stored within the microorganisms, thereby increasing nutrient effectiveness [38,39]. Compared to unfunctionalized MWCNT, functionalized MWCNT-OH has a higher surface area structure, which can retain more nutrients in conditions of soil nutrient deficiency, alter the habitat of soil microorganisms, regulate the soil microbial communities' structure, and enhance the effectiveness of soil carbon and nitrogen elements [36]. Furthermore, the high surface area of functionalized MWCNT-OH can effectively reduce the availability of unstable organic carbon and the decomposition of existing soil organic carbon by adsorbing available organic carbon, thereby further strengthening the limiting effect of carbon on nitrogen denitrification. Additionally, as a carbon source, MWCNTs have a high carbon–nitrogen ratio, which can further inhibit microbial denitrification [40]. Eventually, functionalized MWCNT-OH altered its hydrophobicity, making it more dispersible in suspension and soil and increasing its chances of contact with crops and microorganisms, thereby enhancing the bioavailability of MWCNTs [19].

Although the above results have demonstrated the positive effects of MWCNTs exposure on the carbon and nitrogen cycles in saline soil, there are still many issues that need to be addressed:

Firstly, exposure to MWCNTs cause changes in soil microbial communities and higher microbial diversity, which pose risks to ecological stability and weaken the soil to resist adverse effects. Subsequently, the longest exposure time set in this study was 56 days. After 56 days of exposure, the greenhouse gas emission fluxes from each treatment showed only a marginal change, indicating the effects of the MWCNTs on soil microbial activity and nutrient cycling had reached a relatively stable state. However, Ge et al. [41] suggested that soil exposed to MWCNTs and graphene can still alter the soil microbial communities' structure after one year. Therefore, future research should explore the long-term effects of carbon nanotube exposure in the saline soil and select appropriate application modes to the peculiarities of the local soil environment. At last, exposure to functionalized MWCNT-OH (HMW1 treatment) significantly increased the microbial biomass in saline soil and greenhouse gas emissions ($p < 0.05$), exacerbating global greenhouse effects. Nevertheless, this should not be considered a limitation of carbon nanotube materials since nearly all fertilizers utilized in agricultural production may yield comparable adverse consequences. Therefore, in future research, we intend to contrast the dissimilarities between the impacts of MWCNT and conventional fertilizers on greenhouse gas emissions within saline soil.

Based on this study, the exposure to MWCNTs positively impacts the carbon and nitrogen cycling processes in saline soil. MWCNTs can improve the quality and productivity of saline farmlands. Moreover, functionalized MWCNT-OH is more suitable for saline soil as it has a more pronounced promoting effect on the carbon and nitrogen cycling processes in saline soil than unfunctionalized MWCNT ($p < 0.05$). This article highlights the potential of MWCNTs as a novel material for employment in saline soil. Controlling the exposure time and dose is necessary for the large-scale application of MWCNTs in saline farmlands in the future. However, it is crucial to regulate the exposure time and dose as prerequisites for the widespread application of MWCNTs in saline farmlands in the future.

5. Conclusions and Prospects

This study carries significant scientific implications for enhancing soil quality, boosting crop productivity, and promoting sustainable agricultural development in saline farmlands. It provides a robust theoretical basis for the future large-scale application of MWCNTs in saline farmlands. Based on the results obtained, the conclusions are as follows:

Firstly, short-term exposure of saline soil to MWCNTs and HMW1 treatment significantly increased the total carbon and nitrogen content of the soil ($p < 0.05$). In contrast, other treatments did not have significant effects on the saline soil characteristics. Under long-term exposure conditions, MWCNTs significantly increased saline soil's total nitrogen content, nitrate nitrogen content, and ammonium nitrogen content ($p < 0.05$), promoting the nitrogen cycling process in saline soil. Moreover, the HMW1 treatment significantly increased the MBC and MBN in saline soil, promoting greenhouse gas emissions from saline soil ($p < 0.05$).

Secondly, high exposure doses of MWCNTs significantly altered the microbial community structure in saline soil. The HMW1 treatment significantly increased the proportion of Actinobacteria and Proteobacteria ($p < 0.05$). It increased the microbial diversity in saline soil, while other treatments did not have a significant impact on microbial diversity. The MW1 treatment significantly increased the proportion of Actinobacteria ($p < 0.05$), while not having a significant impact on the proportion of other phyla. Low exposure doses of MWCNTs did not significantly impact the microbial community composition in saline soil. The exposure dose and material type of MWCNTs are important factors affecting the microbial community structure in saline soil. The microbial community structure in saline soil is significantly influenced by the type and exposure dose of MWCNTs.

Then, exposure to MWCNTs significantly affects the quantity of functional genes in saline soil. High exposure doses of MWCNTs increase the abundance of functional genes related to carbon metabolism, carbon fixation, methane metabolism, and nitrogen cycling processes in saline soil. The HMW1 treatment was observed to be more efficacious in stimulating the aforementioned promotion effects compared to the MW1 treatment. However, low exposure doses of MWCNTs had no significant impact on the quantity of most nitrogen cycling related functional genes in saline soil.

Next, more than 80% of the carbon and nitrogen cycling processes are contributed by four dominant phyla, including Actinobacteria, Proteobacteria, Chloroflexi, and Gemmatimonadetes in saline soil. Furthermore, exposure of MWCNTs can increase the contribution of these dominant phyla to the carbon and nitrogen cycling processes in saline soil. Within the dominant phyla, Actinobacteria play a more prominent role in nitrogen cycling as compared to carbon cycling, whereas Proteobacteria exhibit a greater contribution to carbon cycling than nitrogen cycling within saline soil.

Eventually, the RDA analysis revealed that the microbial community composition in saline soil is significantly influenced by the total carbon, total nitrogen, and nitrate nitrogen content of the soil ($p < 0.05$), while other soil characteristics do not exert a significant impact. Furthermore, a significant positive correlation ($p < 0.05$) was observed between the exposure dose of MWCNTs and the proportions of dominant phyla within saline soil.

It is recommended that future studies focus on the following ideas: (1) To set the most suitable exposure dose for multi-walled carbon nanotubes according to different soil and climate conditions. (2) To probe the interaction mechanism between CNMs and microbial cells. (3) To investigate the soil environment in which MWCNTs are suitable.

In conclusion, applying MWCNTs in saline soil exhibits immense potential for future development. Functionalized MWCNT-OH has a more pronounced promoting impact on the carbon and nitrogen cycling processes in saline soil as compared to unfunctionalized MWCNT. The exposure dose and material type significantly influence the effectiveness of MWCNTs in saline soil, and therefore, precise control of the exposure dose of different MWCNTs under varying environmental conditions is imperative. Notably, exposure of saline soil to 1 g/kg functionalized MWCNT-OH exhibited the most significant promoting effect on the carbon and nitrogen cycles.

Author Contributions: Conceptualization, Y.Z. and C.A.; methodology, Y.Z. and W.Z.; formal analysis, C.A.; investigation, Y.Z., C.W. and Y.H.; resources, C.A. and J.H.; data curation, Y.Z.; writing—original draft preparation, Y.Z.; writing—review and editing, C.A.; visualization, Y.Z.; supervision, J.H.; project administration, J.H. and C.A.; funding acquisition, C.A., J.H. and W.Z. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the National Key Research and Development Program of China (Grant No. 2021YFD1900805-03), the National Natural Science Foundation of China (NSFC) (Grant Nos. 52179039 and 52009093) and and the Fundamental Research Funds for the Central Universities (Grant No. 2042023kf0158).

Data Availability Statement: No new data were created or analyzed in this study. Data sharing is not applicable to this article.

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

References

1. Chen, Q.; Cao, X.; Li, Y.; Sun, Q.; Dai, L.; Li, J.; Guo, Z.; Zhang, L.; Ci, L. Functional Carbon Nanodots Improve Soil Quality and Tomato Tolerance in Saline-Alkali Soils. *Sci. Total Environ.* **2022**, *830*, 154817. [CrossRef] [PubMed]
2. Cui, Q.; Xia, J.; Yang, H.; Liu, J.; Shao, P. Biochar and Effective Microorganisms Promote Sesbania Cannabina Growth and Soil Quality in the Coastal Saline-Alkali Soil of the Yellow River Delta, China. *Sci. Total Environ.* **2021**, *756*, 143801. [CrossRef] [PubMed]
3. Qu, Y.; Tang, J.; Liu, B.; Lyu, H.; Duan, Y.; Yang, Y.; Wang, S.; Li, Z. Rhizosphere Enzyme Activities and Microorganisms Drive the Transformation of Organic and Inorganic Carbon in Saline–Alkali Soil Region. *Sci. Rep.* **2022**, *12*, 1314. [CrossRef]
4. Hochella, M.F.; Mogk, D.W.; Ranville, J.; Allen, I.C.; Luther, G.W.; Marr, L.C.; McGrail, B.P.; Murayama, M.; Qafoku, N.P.; Rosso, K.M.; et al. Natural, Incidental, and Engineered Nanomaterials and Their Impacts on the Earth System. *Science* **2019**, *363*, eaau8299. [CrossRef] [PubMed]
5. Carmen Martinez-Ballesta, M.; Zapata, L.; Chalbi, N.; Carvajal, M. Multiwalled Carbon Nanotubes Enter Broccoli Cells Enhancing Growth and Water Uptake of Plants Exposed to Salinity. *J. Nanobiotechnol.* **2016**, *14*, 42. [CrossRef]
6. Li, Y.; Liu, M.; Yang, X.; Zhang, Y.; Hui, H.; Zhang, D.; Shu, J. Multi-Walled Carbon Nanotubes Enhanced the Antioxidative System and Alleviated Salt Stress in Grape Seedlings. *Sci. Hortic.* **2022**, *293*, 110698. [CrossRef]
7. Chen, Z.; Wang, Q. Graphene Ameliorates Saline-Alkaline Stress-Induced Damage and Improves Growth and Tolerance in Alfalfa (*Medicago sativa* L.). *Plant Physiol. Biochem.* **2021**, *163*, 128–138. [CrossRef]
8. Ahmadian, K.; Jalilian, J.; Pirzad, A. Nano-Fertilizers Improved Drought Tolerance in Wheat under Deficit Irrigation. *Agric. Water Manag.* **2021**, *244*, 106544. [CrossRef]
9. Sekhon, B.S. Nanotechnology in Agri-Food Production: An Overview. *Nanotechnol. Sci. Appl.* **2014**, *7*, 31–53. [CrossRef]
10. Esser, G.; Kattge, J.; Sakalli, A. Feedback of Carbon and Nitrogen Cycles Enhances Carbon Sequestration in the Terrestrial Biosphere. *Glob. Change Biol.* **2011**, *17*, 819–842. [CrossRef]
11. Chung, H.; Son, Y.; Yoon, T.K.; Kim, S.; Kim, W. The Effect of Multi-Walled Carbon Nanotubes on Soil Microbial Activity. *Ecotoxicol. Environ. Saf.* **2011**, *74*, 569–575. [CrossRef] [PubMed]
12. Rodrigues, D.F.; Jaisi, D.P.; Elimelech, M. Toxicity of Functionalized Single-Walled Carbon Nanotubes on Soil Microbial Communities: Implications for Nutrient Cycling in Soil. *Environ. Sci. Technol.* **2013**, *47*, 625–633. [CrossRef]
13. Wang, Q.; Feng, X.; Liu, Y.; Cui, W.; Sun, Y.; Zhang, S.; Wang, F. Effects of Microplastics and Carbon Nanotubes on Soil Geochemical Properties and Bacterial Communities. *J. Hazard. Mater.* **2022**, *433*, 128826. [CrossRef]
14. Hu, P.; An, J.; Faulkner, M.M.; Wu, H.; Li, Z.; Tian, X.; Giraldo, J.P. Nanoparticle Charge and Size Control Foliar Delivery Efficiency to Plant Cells and Organelles. *Acs Nano* **2020**, *14*, 7970–7986. [CrossRef] [PubMed]
15. Jin, L.; Son, Y.; Yoon, T.K.; Kang, Y.J.; Kim, W.; Chung, H. High Concentrations of Single-Walled Carbon Nanotubes Lower Soil Enzyme Activity and Microbial Biomass. *Ecotoxicol. Environ. Saf.* **2013**, *88*, 9–15. [CrossRef] [PubMed]
16. Han, P.; Zhou, D.; Tong, M.; Kim, H. Effect of Bacteria on the Transport and Deposition of Multi-Walled Carbon Nanotubes in Saturated Porous Media. *Environ. Pollut.* **2016**, *213*, 895–903. [CrossRef]
17. Zhang, M.; Bradford, S.A.; Simunek, J.; Vereecken, H.; Klumpp, E. Roles of Cation Valance and Exchange on the Retention and Colloid-Facilitated Transport of Functionalized Multi-Walled Carbon Nanotubes in a Natural Soil. *Water Res.* **2017**, *109*, 358–366. [CrossRef]
18. Kerfahi, D.; Tripathi, B.M.; Singh, D.; Kim, H.; Lee, S.; Lee, J.; Adams, J.M. Effects of Functionalized and Raw Multi-Walled Carbon Nanotubes on Soil Bacterial Community Composition. *PLoS ONE* **2015**, *10*, e0123042. [CrossRef]
19. Su, Y.; Zheng, X.; Chen, A.; Chen, Y.; He, G.; Chen, H. Hydroxyl Functionalization of Single-Walled Carbon Nanotubes Causes Inhibition to the Bacterial Denitrification Process. *Chem. Eng. J.* **2015**, *279*, 47–55. [CrossRef]
20. Wu, J.; Joergensen, R.; Pommerening, B.; Chaussod, R.; Brookes, P. Measurement of Soil Microbial Biomass C by Fumigation Extraction—An Automated Procedure. *Soil Biol. Biochem.* **1990**, *22*, 1167–1169. [CrossRef]
21. Delgado-Baquerizo, M.; Maestre, F.T.; Reich, P.B.; Jeffries, T.C.; Gaitan, J.J.; Encinar, D.; Berdugo, M.; Campbell, C.D.; Singh, B.K. Microbial Diversity Drives Multifunctionality in Terrestrial Ecosystems. *Nat. Commun.* **2016**, *7*, 10541. [CrossRef] [PubMed]
22. Cai, Y.; Ma, T.; Wang, Y.; Jia, J.; Jia, Y.; Liang, C.; Feng, X. Assessing the Accumulation Efficiency of Various Microbial Carbon Components in Soils of Different Minerals. *Geoderma* **2022**, *407*, 115562. [CrossRef]
23. Anderson, T.; Domsch, K. The Metabolic Quotient for Co2 (Qco2) as a Specific Activity Parameter to Assess the Effects of Environmental-Conditions, Such as Ph, on the Microbial Biomass of Forest Soils. *Soil Biol. Biochem.* **1993**, *25*, 393–395. [CrossRef]
24. Yang, Q.; Zhang, M. Effect of Bio-Organic Fertilizers Partially Substituting Chemical Fertilizers on Labile Organic Carbon and Bacterial Community of Citrus Orchard Soils. *Plant Soil* **2023**, *483*, 255–272. [CrossRef]
25. Prayogo, C.; Jones, J.E.; Baeyens, J.; Bending, G.D. Impact of Biochar on Mineralisation of C and N from Soil and Willow Litter and Its Relationship with Microbial Community Biomass and Structure. *Biol. Fertil. Soils* **2014**, *50*, 695–702. [CrossRef]
26. Dharni, S.; Sanchita; Unni, S.M.; Kurungot, S.; Samad, A.; Sharma, A.; Patra, D.D. In Vitro and in Silico Antifungal Efficacy of Nitrogen- Doped Carbon Nanohorn (NCNH) against Rhizoctonia Solani. *J. Biomol. Struct. Dyn.* **2016**, *34*, 152–162. [CrossRef]
27. Jin, L.; Son, Y.; DeForest, J.L.; Kang, Y.J.; Kim, W.; Chung, H. Single-Walled Carbon Nanotubes Alter Soil Microbial Community Composition. *Sci. Total Environ.* **2014**, *466*, 533–538. [CrossRef]
28. Ma, Y.; Wang, Y.; Chen, Q.; Li, Y.; Guo, D.; Nie, X.; Peng, X. Assessment of Heavy Metal Pollution and the Effect on Bacterial Community in Acidic and Neutral Soils. *Ecol. Indic.* **2020**, *117*, 106626. [CrossRef]

29. Yang, Y.; Wu, L.; Lin, Q.; Yuan, M.; Xu, D.; Yu, H.; Hu, Y.; Duan, J.; Li, X.; He, Z.; et al. Responses of the Functional Structure of Soil Microbial Community to Livestock Grazing in the Tibetan Alpine Grassland. *Glob. Change Biol.* **2013**, *19*, 637–648. [CrossRef]
30. Qin, F.H.; Zhou, Y.; Yuan, Z.; Jin, F.; Xi, L.C.; Mei, W.Y.; Qing, P.S.; Dong, J.X. Subchronic Oral Toxicity Evaluation of Lanthanum: A 90-Day, Repeated Dose Study in Rats. *Biomed. Environ. Sci.* **2018**, *31*, 363–375. [CrossRef]
31. Su, H.; Zhang, D.; Antwi, P.; Xiao, L.; Zhang, Z.; Deng, X.; Lai, C.; Zhao, J.; Deng, Y.; Liu, Z.; et al. Adaptation, Restoration and Collapse of Anammox Process to La(III) Stress: Performance, Microbial Community, Metabolic Function and Network Analysis. *Bioresour. Technol.* **2021**, *325*, 124731. [CrossRef] [PubMed]
32. Pillai, P.P.; Huda, S.; Kowalczyk, B.; Grzybowski, B.A. Controlled PH Stability and Adjustable Cellular Uptake of Mixed-Charge Nanoparticles. *J. Am. Chem. Soc.* **2013**, *135*, 6392–6395. [CrossRef] [PubMed]
33. Liu, S.; Schloter, M.; Brueggemann, N. Accumulation of NO_2- during Periods of Drying Stimulates Soil N_2O Emissions during Subsequent Rewetting. *Eur. J. Soil Sci.* **2018**, *69*, 936–946. [CrossRef]
34. You, T.; Liu, D.; Chen, J.; Yang, Z.; Dou, R.; Gao, X.; Wang, L. Effects of Metal Oxide Nanoparticles on Soil Enzyme Activities and Bacterial Communities in Two Different Soil Types. *J. Soils Sediments* **2018**, *18*, 211–221. [CrossRef]
35. Cornelis, G.; Hund-Rinke, K.; Kuhlbusch, T.; van den Brink, N.; Nickel, C. Fate and Bioavailability of Engineered Nanoparticles in Soils: A Review. *Crit. Rev. Environ. Sci. Technol.* **2014**, *44*, 2720–2764. [CrossRef]
36. Song, B.; Zeng, Z.; Zeng, G.; Gong, J.; Xiao, R.; Chen, M.; Tang, X.; Ye, S.; Shen, M. Effects of Hydroxyl, Carboxyl, and Amino Functionalized Carbon Nanotubes on the Functional Diversity of Microbial Community in Riverine Sediment. *Chemosphere* **2021**, *262*, 128053. [CrossRef]
37. Saleem, H.; Zaidi, S.J. Recent Developments in the Application of Nanomaterials in Agroecosystems. *Nanomaterials* **2020**, *10*, 2411. [CrossRef]
38. Schmidt, S.K.; Costello, E.K.; Nemergut, D.R.; Cleveland, C.C.; Reed, S.C.; Weintraub, M.N.; Meyer, A.F.; Martin, A.M. Biogeochemical Consequences of Rapid Microbial Turnover and Seasonal Succession in Soil. *Ecology* **2007**, *88*, 1379–1385. [CrossRef]
39. Yang, X.; Liu, D.; Fu, Q.; Li, T.; Hou, R.; Li, Q.; Li, M.; Meng, F. Characteristics of Greenhouse Gas Emissions from Farmland Soils Based on a Structural Equation Model: Regulation Mechanism of Biochar. *Environ. Res.* **2022**, *206*, 112303. [CrossRef]
40. Laird, D.A.; Brown, R.C.; Amonette, J.E.; Lehmann, J. Review of the Pyrolysis Platform for Coproducing Bio-Oil and Biochar. Biofuels Bioprod. *Biorefining Biofpr.* **2009**, *3*, 547–562. [CrossRef]
41. Ge, Y.; Priester, J.H.; Mortimer, M.; Chang, C.H.; Ji, Z.; Schimel, J.P.; Holden, P.A. Long-Term Effects of Multiwalled Carbon Nanotubes and Graphene on Microbial Communities in Dry Soil. *Environ. Sci. Technol.* **2016**, *50*, 3965–3974. [CrossRef] [PubMed]