

Article

Evolution of Physicochemical and Microbial Characteristics During Natural Precipitation Leaching in Dongying's Moderate–Severe Saline–Alkaline Soil and Its Ecological Significance

Yu Bai ¹, Xueli Lu ², Xiaobin Li ³, Feng Jiang ⁴, Rong Ma ⁴, Yue Liu ⁴, Zongchang Xu ^{2,*}  and Meng Wang ^{1,*}

¹ College of Agronomy, Qingdao Agricultural University, Qingdao 266109, China; bmyl1009@163.com

² Marine Agriculture Research Center, Tobacco Research Institute of Chinese Academy of Agricultural Sciences, Qingdao Key Laboratory of Coastal Saline-Alkali Land Resources Mining and Biological Breeding, Qingdao 266100, China; luxl100@163.com

³ Institute of Agricultural Resources and Regional Planning, Chinese Academy of Agricultural Sciences, Beijing 100081, China; lixiaobin01@caas.cn

⁴ College of Agricultural Science and Engineering, Hohai University, Nanjing 211100, China; 13216569718jf@gmail.com (F.J.); 231310010029@hhu.edu.cn (R.M.); liuyue021124@163.com (Y.L.)

* Correspondence: xuzongchang@caas.cn (Z.X.); wangmeng@qau.edu.cn (M.W.)

Abstract

Soil salinization threatens agricultural production, and increasing extreme rainfall may alter natural leaching processes in coastal saline–alkaline soils. However, the relationships among salt ion migration, alkalinity changes, nutrients, and bacterial communities under natural rainfall leaching remain unclear. Therefore, a phased natural rainfall leaching experiment was conducted from June to September 2025 using moderate to severe NaCl-type saline–alkaline soil from Dongying in the Yellow River Delta. The results showed that natural rainfall leaching significantly reduced soluble salt ions, especially Na^+ , Cl^- , and SO_4^{2-} , and rapidly alleviated early salt stress. However, soil pH did not decline continuously with salt reduction, but fluctuated under the buffering effect of the carbonate system, indicating that desalination was not necessarily accompanied by alkalinity alleviation. Available nutrients showed stage-dependent changes, with HN and AK increasing around the middle leaching stage. Bacterial community composition and co-occurrence networks also changed during leaching, and these changes were more closely associated with salt ions and HCO_3^-/pH than with available nutrients. These results suggest that post-rain management of saline–alkaline soils should not rely only on total salinity, but should also consider major salt ions, pH/HCO_3^- , and nutrient availability.

Keywords: physicochemical properties; bacterial community; co-occurrence network; alkalinity gradient; Yellow River Delta; soil nutrient activation



Academic Editor:
Antonio Paz-González

Received: 27 April 2026
Revised: 28 May 2026
Accepted: 5 June 2026
Published: 9 June 2026

Copyright: © 2026 by the authors.
Licensee MDPI, Basel, Switzerland.
This article is an open access article distributed under the terms and conditions of the [Creative Commons Attribution \(CC BY\)](https://creativecommons.org/licenses/by/4.0/) license.

1. Introduction

Soil salinization has become a serious land degradation problem in the world. It is estimated that approximately 1.1×10^9 ha of land is affected by salinization [1], which severely restricts crop growth and leads to yield reduction [2]. In China, saline–alkaline land is also large in area and widely distributed. The intensification of salinization will reduce cultivated land productivity and increase the risk of limited land use and local abandonment, thereby threatening food security [3].

Global warming is driving the intensification of the water cycle, which in turn causes systematic changes in rainfall patterns on global and regional scales [4]. Based on the rainfall observation records in China over the past 40 years, the maximum hourly rainfall intensity in summer has generally shown an upward trend with an average increase of about 11.2% [5]. This indicates that in monsoon regions such as North China and the Yellow River Delta, the infiltration-leaching process caused by natural rainfall may become more frequent and intense [6]. Therefore, the impact of natural rainfall on the redistribution of water and salt and ecological processes in saline–alkaline land needs to be re-evaluated.

In saline–alkaline soils, rainfall infiltration and leaching can promote the downward migration of soluble salt ions in the soil, thereby reducing surface salt content and changing the ionic strength of the soil solution [7]. At the same time, the wetting–drying cycle often triggers the transformation and release of some nutrients from the solid phase to the available state in the short term [8]. Previous studies have shown that this can affect soil bacterial communities by changing nutrient availability [9]. However, under continuous leaching conditions, nutrients may also migrate downward or even be lost along with salts [10].

Notably, the “desalination” caused by salt ion migration does not necessarily lead to a synchronous decrease in soil alkalinity. Under the buffering background of the carbonate system, alkalinity indicators such as pH/HCO_3^- may still be strongly constrained in the later stage of desalination, resulting in environmental changes characterized by the parallel features of “attenuation of salt ion gradient and prominence of alkalinity gradient” [11,12]. Due to the different preferences of different microbial communities for salinity and moisture, changes in the soil physicochemical environment will drive community turnover: halophilic groups are relatively dominant under high-salt conditions, but their dominance may weaken with the progress of desalination; when the increase in water content leads to limited oxygen diffusion, facultative anaerobic or strictly anaerobic communities are usually more likely to increase. Therefore, natural rainfall leaching affects the changes in bacterial community diversity and composition structure by regulating salt migration [13,14].

Previous studies have shown that rainfall can promote the migration of soluble salts in soil, reduce surface salt accumulation, and affect nutrient availability and microbial community structure [7,15]. However, most related studies have been based on irrigation leaching [16] or artificially controlled moisture experiments [15], whereas the staged responses of water–salt dynamics in saline–alkaline soils to natural rainfall, especially under continuous or phased heavy rainfall conditions, remain insufficiently understood. In addition, previous studies have commonly used total salinity or electrical conductivity to characterize salinity changes [17], with less attention paid to the separate dynamics of major salt ions, such as Na^+ , Cl^- , and SO_4^{2-} , and alkalinity-related indicators, such as pH and HCO_3^- . Therefore, it remains unclear whether salt reduction during natural rainfall leaching is necessarily accompanied by alkalinity alleviation, and how changes in salt ions and alkalinity background are associated with soil bacterial community composition.

Based on these knowledge gaps, this study selected moderate to severe saline–alkaline soils from Dongying in the Yellow River Delta as the research object. Relying on a phased natural rainfall leaching process, we comprehensively analyzed the dynamic changes in total water-soluble salts, major water-soluble ions, pH/HCO_3^- , nutrient availability, and bacterial community structure. In combination with RDA, Mantel analysis, and co-occurrence network analysis, we further explored the relationships between changes in soil physicochemical properties and bacterial community responses during natural rainfall leaching. By distinguishing salt ion migration from alkalinity background changes, this study aimed to clarify the staged characteristics of “desalination–alkalinity response–microbial community succession” in saline–alkaline soils under natural rainfall leaching,

and to provide a basis for stage-specific management of saline–alkaline land after extreme rainfall. The specific objectives were to: (1) determine the changes in total salinity, major salt ions, and pH/HCO₃[−] during phased natural rainfall leaching; (2) analyze the relationships between changes in salinity, major salt ions, and alkalinity indicators and bacterial community composition, dominant taxa replacement, and microbial co-occurrence network structure; and (3) identify key soil indicators that should be prioritized in the agricultural utilization of saline–alkaline soils after natural rainfall, thereby providing a basis for post-rainfall stage-specific management, nutrient regulation, and saline–alkaline constraint diagnosis in the coastal saline–alkaline land of the Yellow River Delta.

2. Materials and Methods

2.1. Study Area Overview

The natural precipitation leaching experiment of saline–alkaline soil was carried out from 26 June to 7 September 2025 in the moderate to severe saline–alkaline land experimental demonstration area of the National Technology Innovation Center for Comprehensive Utilization of Saline–Alkaline Land in Lijin County, Dongying City, Shandong Province (37°47′ N, 118°38′ E). This region belongs to a warm temperate continental monsoon climate, with dry winters and springs and rainy summers. The average annual rainfall is about 540.9 mm, most of which is concentrated in summer, accounting for more than 65% of the annual rainfall. The interannual variation is large, and droughts and floods are prone to occur [18]. The soil salt content ranges from 0.4% to 2.4%, and the pH ranges from 8.0 to 9.5, which is a typical NaCl-type saline–alkaline soil [19].

2.2. Experimental Design

Topsoil (0–20 cm) was collected from the experimental demonstration area, sieved through a 1 cm aperture iron sieve, and thoroughly mixed to ensure uniform properties of the soil used in the experiment. The total soil salt content was 5.53 g·kg^{−1}, and the pH was 9. Plastic flowerpots with the same specifications (upper diameter 17.5 cm, lower diameter 14.5 cm, height 19 cm) were selected. Each flowerpot was filled with the same weight of uniformly mixed saline–alkaline soil. A total of 5 treatments (T1–T5) were set up, with 4 replicates for each treatment. A total of 2 kg of unplanted soil was used as the control treatment (CK), also with 4 replicates. Each replicate was divided into two parts immediately: one part of fresh soil was stored in a −80 °C refrigerator for soil microbial community detection; the other part was naturally air-dried for the determination of soil physicochemical properties.

The 20 flowerpots were placed synchronously in an open field to ensure that each flowerpot was exposed to the same natural environmental conditions such as light, temperature, and rainfall.

During the experimental period, when the precipitation exceeded 100 mm, 4 flowerpots were randomly selected and brought back to the laboratory for sampling on the 2nd day after rainfall, recorded as T1. The 0–15 cm deep flowerpot soil was collected, and each replicate was divided into two parts, which were processed in the same manner as the CK. Soil samples were collected 5 times on 3 July, 24 July, 6 August, 18 August, and 6 September (Table S1), recorded as T1–T5, respectively.

2.3. Indicator Detection Methods

2.3.1. Soil Physicochemical Properties Testing Methods

The air-dried soil samples were passed through a 2 mm sieve for further analysis. Soil total water-soluble salt content (TWSS) was extracted using a soil-to-water ratio of 1:5. The extract was filtered, and an aliquot of the filtrate was evaporated and oven-dried at 105 °C

to constant weight. The dried residue was then used to calculate TWSS [20]. The soil pH value was measured using a glass electrode meter (PHS-3E, Leici, Shanghai, China), with a soil-to-water (w/v) ratio of 1:5 [21].

The organic matter content was determined by the high-temperature external heating potassium dichromate oxidation–volumetric method [22]. The content of soil total nitrogen (TN), total phosphorus (TP), and total potassium (TK) was measured using the Kjeldahl method, the molybdenum blue colorimetric method, and the flame photometry method, respectively [23]. Soil hydrolyzable nitrogen (HN), available phosphorus (AP) and available potassium (AK) were determined using the alkaline hydrolysis diffusion method, the Olsen method, and the neutral ammonium acetate extraction–flame photometry method, respectively [24]. The composition of soil water-soluble ions was determined from the extract: K^+ and Na^+ were measured by flame photometry, whereas the other ions (Ca^{2+} , Mg^{2+} , CO_3^{2-} , HCO_3^- , Cl^- and SO_4^{2-}) were determined using titrimetric methods [25].

2.3.2. Soil Microbial Community Detection

Microbial DNA was extracted from soil samples using the E.Z.N.A.[®] Soil DNA Kit (Omega Bio-Tek, Norcross, GA, USA) according to the manufacturer's instructions. The quality of the extracted genomic DNA was assessed using 1% agarose gel electrophoresis, and the DNA concentration and purity were determined using a NanoDrop2000 (Thermo Scientific, Waltham, MA, USA). The V3–V4 variable region of the 16S rRNA gene was amplified with the upstream primer 338F (5'-ACTCCTACGGGAGGCAGCAG-3') and downstream primer 806R (5'-GGACTACHVGGGTWTCTAAT-3'), carrying Barcode sequences [26]. The PCR reaction system was: 5×TransStart FastPfu buffer 4 µL, 2.5 mM dNTPs 2 µL, upstream primer (5 µM) 0.8 µL, downstream primer (5 µM) 0.8 µL, TransStart FastPfu DNA polymerase 0.4 µL, template DNA 10 ng, and ddH₂O to make up to 20 µL. The amplification program was as follows: pre-denaturation at 95 °C for 3 min, 27 cycles (denaturation at 95 °C for 30 s, annealing at 55 °C for 30 s, extension at 72 °C for 30 s), then final extension at 72 °C for 10 min, and storage at 4 °C (PCR instrument: ABI GeneAmp[®] 9700, Carlsbad, CA, USA). Library construction of the purified PCR products was performed using the NEXTFLEX Rapid DNA-Seq Kit 2.0 (NOVA-5188-02, Revvity, Waltham, MA, USA). Sequencing was performed on the Illumina NextSeq 2000 platform (Majorbio Bio-pharm Technology Co., Ltd., Shanghai, China). The paired-end raw reads were quality-controlled using fastp software (<https://github.com/OpenGene/fastp>, accessed on 8 November 2025, version 0.19.6) [27] and assembled using FLASH software (<https://www.cbcb.umd.edu/>, accessed on 25 November 2025, version 1.2.11) [28]. Based on the default parameters, the optimized sequences after quality control and assembly were denoised using the DADA2 [29] plugin in the QIIME 2 pipeline [30]. Sequences annotated to chloroplasts and mitochondria in all samples were removed. To minimize the impact of sequencing depth on subsequent alpha and beta diversity data analysis, the number of sequences for all samples was normalized to 24,044. Based on the SILVA 16S rRNA gene database (v 138), the Naive Bayes classifier in QIIME 2 was used for taxonomic analysis of ASVs.

2.4. Statistical Analysis

Excel 2019 was used for data collation, and the significance of differences in soil physicochemical properties and microbial diversity indicators under different leaching times was tested ($p < 0.05$ was considered significant). Relevant graphs were drawn using Origin 2021, and microbial community structure analysis and correlation analysis were performed using R 4.2.0 software. Alpha diversity, PCA, community composition,

and Mantel analysis were performed using the Majorbio Cloud Platform (<https://cloud.majorbio.com>, accessed on 2 March 2026).

3. Results

3.1. Effects of Different Leaching Times on Soil Physicochemical Properties

3.1.1. Changes in Soil pH Value and Total Salt Content

As shown in Figure 1A, the overall effect of rainwater leaching on soil pH was not significant ($p > 0.05$), but it showed a “decrease–increase–decrease” trend with the increase in leaching times. Compared with CK, the soil pH of T1 and T2 decreased by 0.50 and 0.34, respectively, while a temporary recovery occurred in the T3–T4 stage. However, the effect of rainwater leaching on soil salt content was extremely significant ($p < 0.01$; Figure 1B). The soil salt content generally showed a trend of first decreasing and then increasing. In the early stage of leaching, the salt content decreased rapidly, reaching a minimum of $1.03 \text{ g} \cdot \text{kg}^{-1}$ at T2; then it began to recover from T3 and increased significantly in the later stage. Compared with CK, the soil salt contents of T4 and T5 were significantly higher, at 1.51 times and 2.47 times those of CK, respectively.

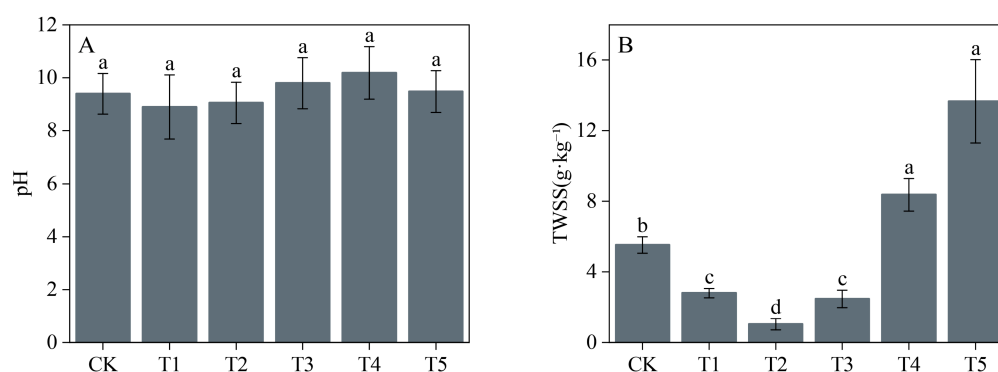


Figure 1. Changes in soil pH value and salt content under natural rainfall leaching at different stages. (A) pH; (B) Total water-soluble salt content (TWSS); CK: control (no rain addition); T1–T5: soil samples collected on 3 July, 24 July, 6 August, 18 August, and 6 September, respectively. Columns represent mean $\pm$ standard deviation. Different lowercase letters above the bars indicate significant differences among treatments ($p < 0.05$), whereas the same letter indicates no significant difference.

3.1.2. Changes in Soil Organic Matter and Nutrient Content

Rainwater leaching significantly affected soil HN, AP, and AK ($p < 0.05$; Figure 2A–C). Compared with CK, soil HN significantly increased with the increase in leaching times, reaching the maximum value ($23.53 \text{ mg} \cdot \text{kg}^{-1}$) at the T3 stage, which was 2.24 times that of CK. Subsequently, HN slightly decreased in the T4 and T5 stages but was still significantly higher than that of CK (Figure 2A). The soil AP of T1, T2, and T3 decreased by 19.34–21.18% compared with CK, but recovered in the later stage (Figure 2B). Soil AK peaked at the T3 stage, reaching 3.6 times the level of the control, and all other treatments were also higher than the control to varying degrees (Figure 2C). In contrast, TN showed only minor fluctuations across the leaching stages and did not differ significantly among treatments ($p > 0.05$; Figure 2D). TP, TK, and SOM varied slightly but showed significant differences among some treatments ($p < 0.05$; Figure 2E–G), with the magnitude of variation becoming more pronounced around T3.

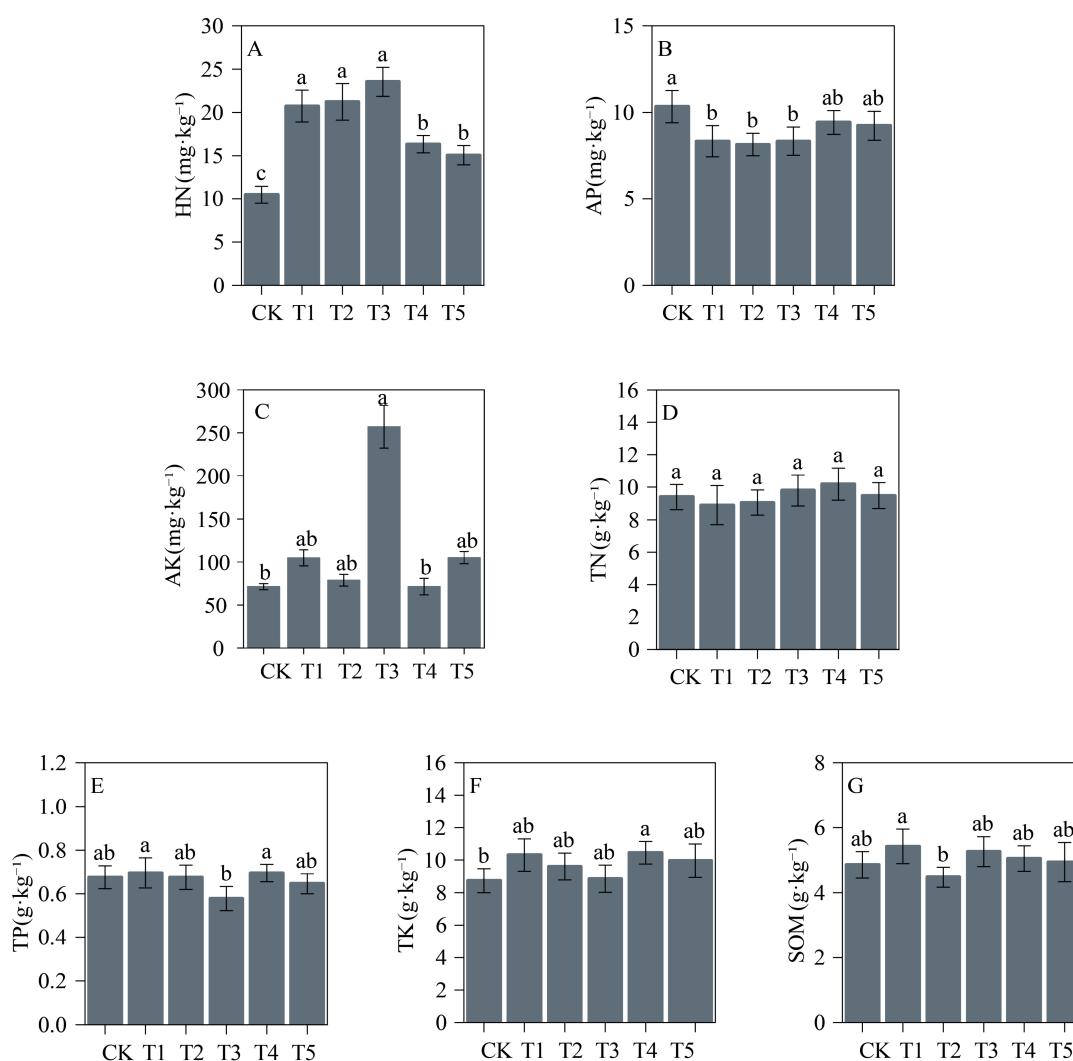


Figure 2. Changes in soil nutrient content under natural rainfall leaching at different stages. (A) Hydrolyzable nitrogen (HN); (B) Available phosphorus (AP); (C) Available potassium (AK); (D) Total nitrogen (TN); (E) Total phosphorus (TP); (F) Total potassium (TK); (G) Soil organic matter (SOM). CK: control (no rain addition); T1–T5: soil samples collected on 3 July, 24 July, 6 August, 18 August, and 6 September, respectively. Columns represent mean $\pm$ standard deviation. Different lowercase letters indicate significant differences between treatments ($p < 0.05$).

3.1.3. Changes in Soil Ion Content

Rainwater leaching significantly reshaped the composition of soil water-soluble ions ($p < 0.05$). Except for K^+ , the major salt ions (Na^+ , Ca^{2+} , Mg^{2+} , Cl^- , and SO_4^{2-}) generally decreased during leaching and reached their lowest levels at T4 (Figure 3). Among them, Cl^- showed the greatest decline, with an average decrease of 80.92% (Figure 3A); Na^+ decreased from $765.55 \text{ mg}\cdot\text{kg}^{-1}$ in CK to $173.04 \text{ mg}\cdot\text{kg}^{-1}$ at T4 (Figure 3B), and Ca^{2+} and Mg^{2+} decreased by 64.81% and 72.73%, respectively (Figure 3C,D). SO_4^{2-} also declined, from $0.212 \text{ mg}\cdot\text{kg}^{-1}$ in CK to $0.067\text{--}0.185 \text{ mg}\cdot\text{kg}^{-1}$ across the leaching treatments, and further decreased to $0.056 \text{ mg}\cdot\text{kg}^{-1}$ at later stages (Figure 3E). In contrast, K^+ did not exhibit a monotonic decrease, reaching its minimum at T3 ($7.68 \text{ mg}\cdot\text{kg}^{-1}$) and then increasing markedly at T4 ($19.35 \text{ mg}\cdot\text{kg}^{-1}$) (Figure 3F). In addition, carbonate system ions (CO_3^{2-} and HCO_3^-) generally remained at relatively high levels or showed an increasing trend (Figure 3G,H).

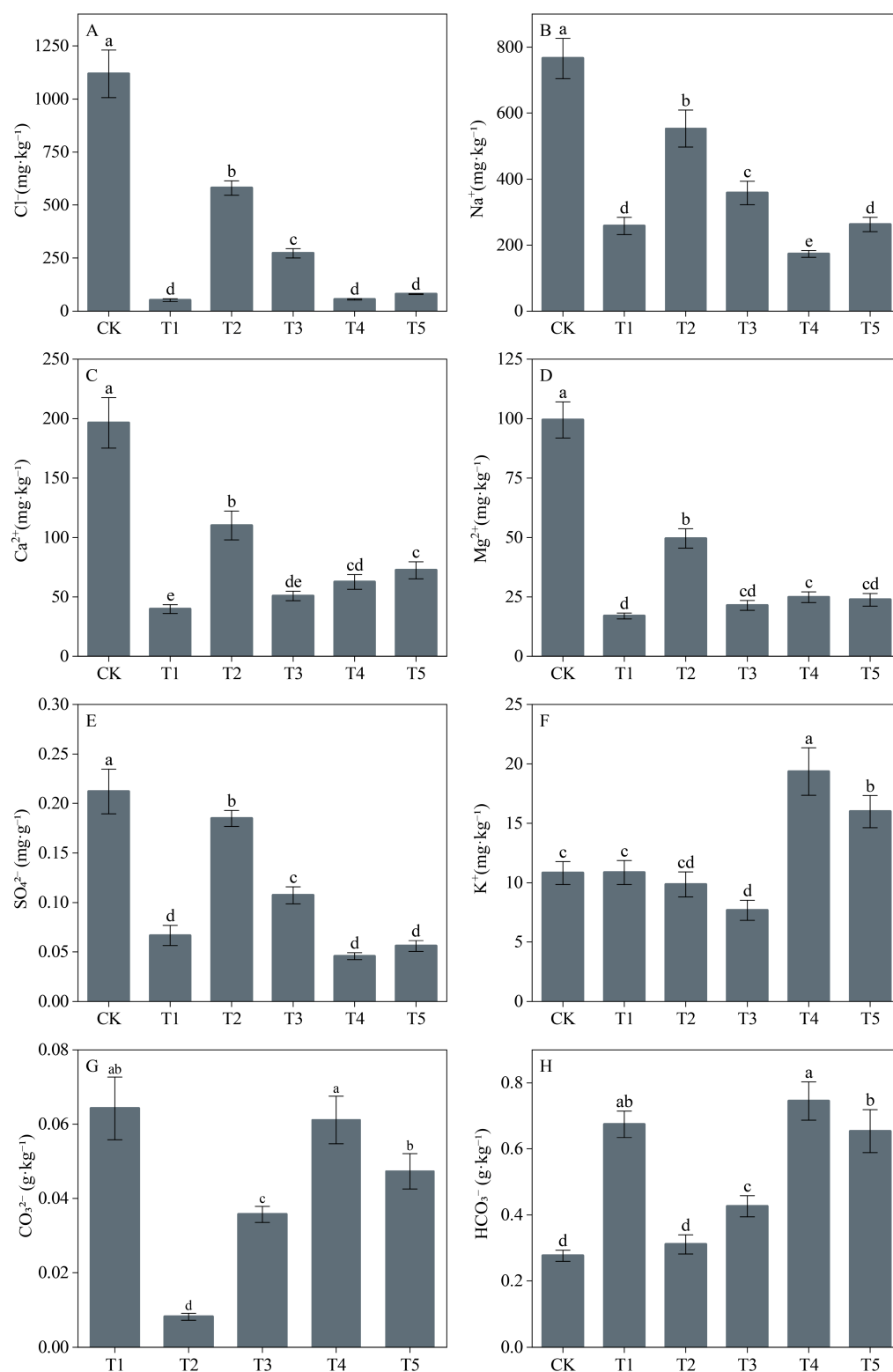


Figure 3. Changes in soil water-soluble ion content under natural rainfall leaching at different stages. (A) Chloride ion (Cl^-); (B) Sodium ion (Na^+); (C) Calcium ion (Ca^{2+}); (D) Magnesium ion (Mg^{2+}); (E) Sulfate ion (SO_4^{2-}); (F) Potassium ion (K^+); (G) Carbonate ion (CO_3^{2-}); (H) Bicarbonate ion (HCO_3^-). CK: control (no rain addition); T1–T5: soil samples collected on 3 July, 24 July, 6 August, 18 August, and 6 September, respectively. Columns represent mean $\pm$ standard deviation. Different lowercase letters indicate significant differences between treatments ($p < 0.05$).

3.2. Relationships Among Rainfall, Soil Physicochemical Properties, and Water-Soluble Ions

3.2.1. Correlations Between Rainfall and Soil Physicochemical Properties

Spearman correlation analysis was used to study the relationship between soil physicochemical properties and rainfall (Table 1). Rainfall was extremely significantly negatively correlated with Na^+ , SO_4^{2-} , and Cl^- ($r = -0.593$ to -0.787 , $p < 0.01$), and significantly positively correlated with K^+ , TK, and HCO_3^- ($r = 0.549$ – 0.705 , $p < 0.05$), while the correlations with other indicators were not significant ($p > 0.05$).

Table 1. Correlation coefficients between rainfall and soil physicochemical properties during natural rainfall leaching.

	pH	TWSS	TN	TP	TK	HN	AP	AK	SOM	K^+	Na^+	Ca^{2+}	Mg^{2+}	SO_4^{2-}	Cl^-	HCO_3^-	CO_3^{2-}
Rainfall	0.160	0.398	0.078	0.204	0.549 *	−0.034	−0.041	−0.154	−0.091	0.705 **	−0.743 ***	−0.135	−0.172	−0.787 **	−0.593 **	0.693 **	0.327

Note: Calculated using Spearman's rank correlation coefficient. *, ** and *** indicate significant correlations at $p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively. Abbreviations: TWSS, Total water-soluble salt content; TN, Total nitrogen; TP, Total phosphorus; TK, Total potassium; HN, Hydrolyzable nitrogen; AP, Available phosphorus; AK, Available potassium; SOM, Soil organic matter.

3.2.2. Correlation Analysis Between Soil Physicochemical Properties and Water-Soluble Ions

The results of Spearman correlation analysis showed that there were generally significant correlations between soil nutrient indicators and water-soluble ions (Figure 4). Among soil nutrient indicators, TN was significantly negatively correlated with pH ($r = -0.61$, $p < 0.05$) and significantly positively correlated with HN ($r = 0.62$, $p < 0.05$), while TWSS was significantly negatively correlated with HN ($r = -0.77$, $p < 0.001$); meanwhile, TK was significantly positively correlated with TP ($r = 0.62$, $p < 0.05$), and AK was significantly negatively correlated with TP ($r = -0.62$, $p < 0.05$).

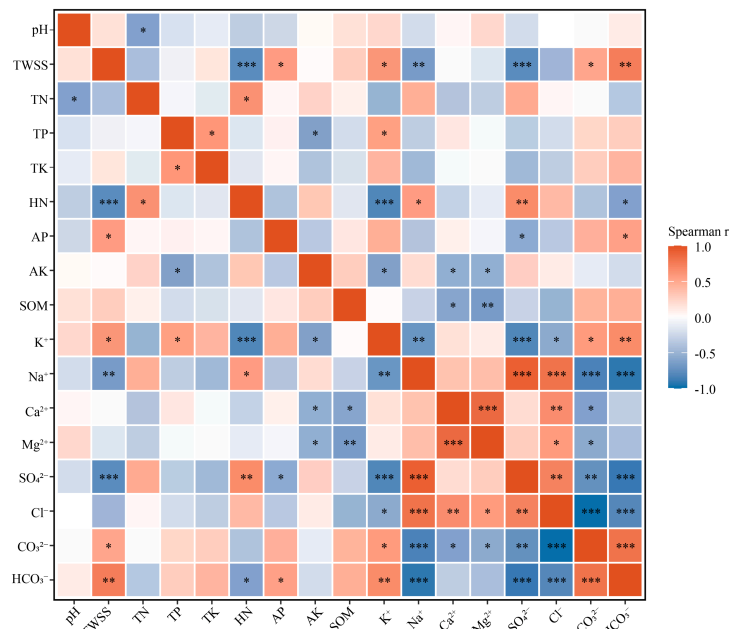


Figure 4. Pairwise relationships and correlation matrix of soil physicochemical properties during natural rainfall leaching. The diagonal shows the distribution of each physicochemical indicator (kernel density curve); the lower left corner shows the scatter plots and fitted trend lines of pairwise variables; the upper right corner shows the Spearman correlation coefficient (r). *, **, and *** indicate significant correlations at $p < 0.05$, $p < 0.01$, and $p < 0.001$ levels, respectively. TWSS, total water-soluble salt content; TN, total nitrogen; TP, total phosphorus; TK, total potassium; HN, hydrolyzable nitrogen; AP, available phosphorus; AK, available potassium; SOM, soil organic matter; K^+ , Na^+ , Ca^{2+} , Mg^{2+} , SO_4^{2-} , Cl^- , CO_3^{2-} , and HCO_3^- are water-soluble ions.

Among the main water-soluble ions, Na^+ , SO_4^{2-} , and Cl^- were extremely significantly positively correlated ($r = 0.81$ to 0.93 , $p < 0.01$), while K^+ , CO_3^{2-} , and HCO_3^- were significantly positively correlated ($r = 0.57$ to 0.66 , $p < 0.05$) and significantly negatively correlated with SO_4^{2-} and Cl^- ($r = -0.56$ to -0.81 , $p < 0.05$). In addition, there was an extremely significant positive correlation between Ca^{2+} and Mg^{2+} ($r = 0.86$, $p < 0.01$). TWSS was significantly positively correlated with K^+ , CO_3^{2-} , and HCO_3^- ($r = 0.51$ to 0.75 , $p < 0.05$) and significantly negatively correlated with Na^+ and SO_4^{2-} ($r = -0.66$ to -0.77 , $p < 0.01$).

The correlations between ions and nutrients showed certain differentiation characteristics: HN , Na^+ , and SO_4^{2-} were significantly positively correlated ($r = 0.57$ to 0.92 , $p < 0.05$), while they were significantly negatively correlated with K^+ and HCO_3^- ($r = -0.61$ to -0.92 , $p < 0.05$); TP was significantly positively correlated with K^+ ($r = 0.55$, $p < 0.05$). Overall, there were significant but differently directed correlations between soil nutrients and ion composition.

3.3. Effects of Different Leaching Times on Soil Microbial Characteristics

3.3.1. Changes in Alpha Diversity of Soil Microbial Communities

There were certain differences in the alpha diversity indices of soil bacterial communities under different leaching times (Figure 5). With the increase in leaching times, the Chao index generally showed a decreasing trend, with a slight recovery at the T4 stage, but CK was still significantly higher than other treatments (Figure 5A). The Shannon index significantly decreased at the T1 stage and recovered slightly at the T4 stage, but was still lower than CK (Figure 5B). The Good's coverage of all samples exceeded 0.99 (Supplementary Figure S1A), and the ACE richness estimator (Supplementary Figure S1B) indicated differences in bacterial richness among treatments. In addition, the rarefaction curves tended to be flat, suggesting that the sequencing depth was adequate for subsequent analyses (Supplementary Figure S2).

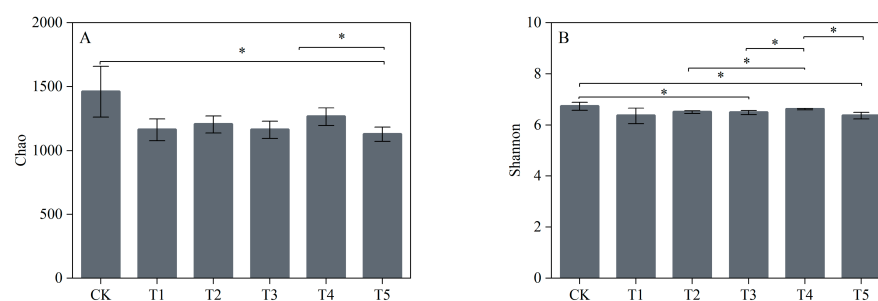


Figure 5. Soil alpha diversity indices under natural rainfall leaching at different stages. (A): Chao richness index; (B): Shannon diversity index. CK: control (no rain addition); T1–T5: soil samples collected on 3 July, 24 July, 6 August, 18 August, and 6 September, respectively. Columns are mean $\pm$ standard deviation. Horizontal brackets and “*” indicate significant differences between treatments ($p < 0.05$).

3.3.2. Changes in Soil Microbial Community Structure

Rainwater leaching not only had a significant impact on the differentiation of bacterial community structure but also on the changes in the species composition of bacterial communities. In all treatment samples, the soil bacterial community was mainly composed of phyla such as Pseudomonadota, Actinomycetota, Chloroflexota, Acidobacteriota, Gemmatimonadota, Bacteroidota, Cyanobacteriota, and Thermodesulfobacteriota (Figure 6A), which accounted for relatively high relative abundances in each treatment. Pseudomonadota was the most abundant species in all treatments, accounting for 20.41–27.69%, and was relatively stable without significant changes. Actinomycetota, Chloroflexota, Acidobacteriota, Gemmatimonadota, Bacteroidota, Cyanobacteriota, and Thermodesulfobacteriota

showed significant responses to rainwater leaching. Among them, Actinomycetota, Bacteroidota, and Thermodesulfobacteriota had relatively high relative abundances in CK and the early stage of leaching (T1), and significantly decreased with the increase in leaching times ($p < 0.05$). In contrast, Chloroflexota, Acidobacteriota, and Cyanobacteriota gradually increased in multiple leaching treatments, and their relative abundances were significantly higher than CK in T3–T5 ($p < 0.05$). The relative abundance of Gemmatimonadota fluctuated among different treatments, ranging from 4.84% to 7.73%, and the difference was extremely significant ($p < 0.01$).

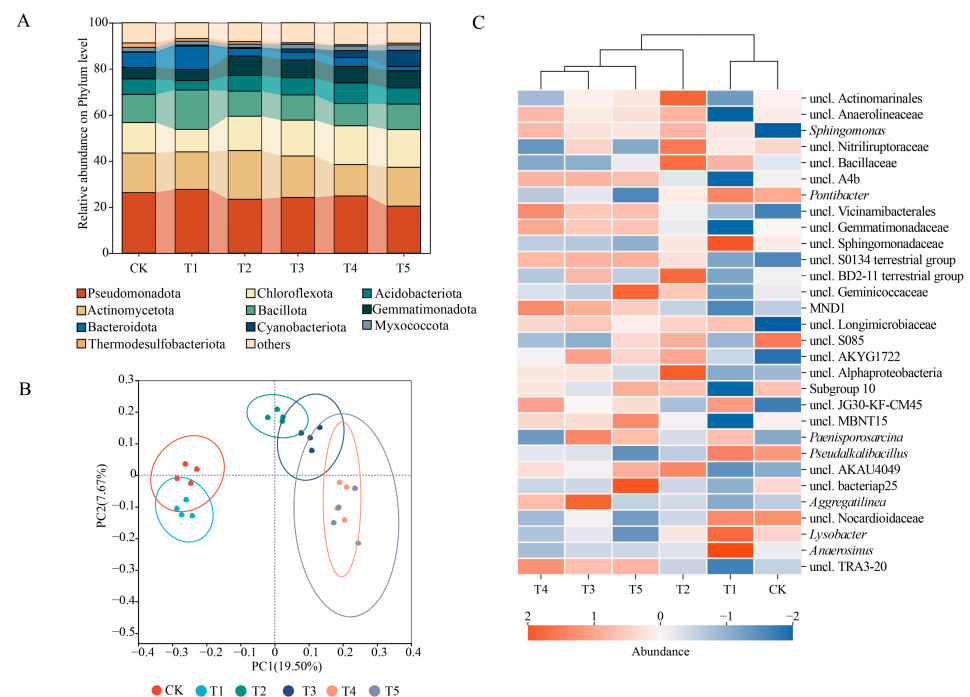


Figure 6. Succession of soil bacterial communities across natural rainfall leaching stages. (A) Relative abundance of dominant bacterial phyla in CK and T1–T5 treatments; (B) Principal coordinate analysis (PCoA) of bacterial community composition at the genus level, showing separation among treatments along PC1 (19.50%) and PC2 (7.67%); (C) Hierarchical clustering heatmap of the top 30 genera across six samples, with dendrograms for both samples and genera; color scale indicates log₁₀-transformed standardized abundance (blue to red, low to high). CK represents the initial soil before leaching, and T1–T5 represent soils collected at successive rainfall-leaching stages.

The PCoA ordination showed separation among treatments, with PC1 and PC2 explaining 19.50% and 7.67% of the total variation, respectively (Figure 6B). ANOSIM confirmed significant differences in bacterial community composition among treatments ($R = 0.878$, $p = 0.001$, 999 permutations). The grouping pattern in the PCoA was generally consistent with the genus-level hierarchical clustering heatmap (Figure 6C). In the heatmap based on the treatment-averaged abundances of the top 30 genus-level taxa, CK and T1 clustered together, whereas T2, T3, T4, and T5 were grouped in the same major branch. These results indicate that repeated leaching changed bacterial community composition, and the later treatments showed relatively similar genus-level abundance patterns.

At the genus level, Kruskal–Wallis H tests showed that several genus-level taxa differed significantly among treatments after FDR correction ($H = 13.18$ – 21.44 , FDR-adjusted $p < 0.05$; Supplementary Table S2). These taxa were mainly assigned to Pseudomonadota, Bacteroidota, Actinomycetota, Chloroflexota, Acidobacteriota, and Gemmatimonadota. According to their abundance changes across different leaching stages, the significantly different taxa could be broadly divided into two patterns. One group showed relatively

higher abundance during the middle-to-late leaching stages. For example, MND1 and uncl. TRA3-20, affiliated with Pseudomonadota, had lower mean abundance at T1 but higher abundance at T4; the Gemmatimonadota-affiliated taxa uncl. S0134 terrestrial group and uncl. AKAU4049 also showed relatively higher abundance in the later treatments. The other group showed a decreasing trend under repeated leaching, with higher abundance in CK or T1 and lower abundance in subsequent treatments. For example, *Pontibacter* and *Lysobacter* were relatively abundant in CK or T1 but decreased toward T5. In addition, the Actinomycetota-affiliated taxon uncl. Nitriliruptoraceae showed a stage-specific pattern, with relatively higher abundance at T2 and lower abundance at T4–T5.

3.4. Soil Microbial Community Co-Occurrence Network and Assembly

Based on the preceding genus-level analysis, co-occurrence networks were constructed at the genus level to further reveal changes in potential interactions among microbial taxa ($|r| > 0.6$, $p < 0.05$). The genus-level co-occurrence network analysis showed that the network structure under CK and T1 was relatively complex, with 50 nodes and 442 edges, and both positive and negative correlations were observed (Positive/Total = 53.39%) (Figure 7A). In contrast, the network scale under T2–T5 was markedly reduced, with the number of nodes and edges decreasing to 36 and 118, respectively, while the proportion of positive edges increased to 69.49% (Figure 7B). Further topological analysis showed that, compared with CK–T1, degree centrality ($p < 0.01$; Figure 8A) and closeness centrality ($p < 0.05$; Figure 8B) decreased significantly in the T2–T5 network, whereas betweenness centrality showed a slight increasing trend (Figure 8C).

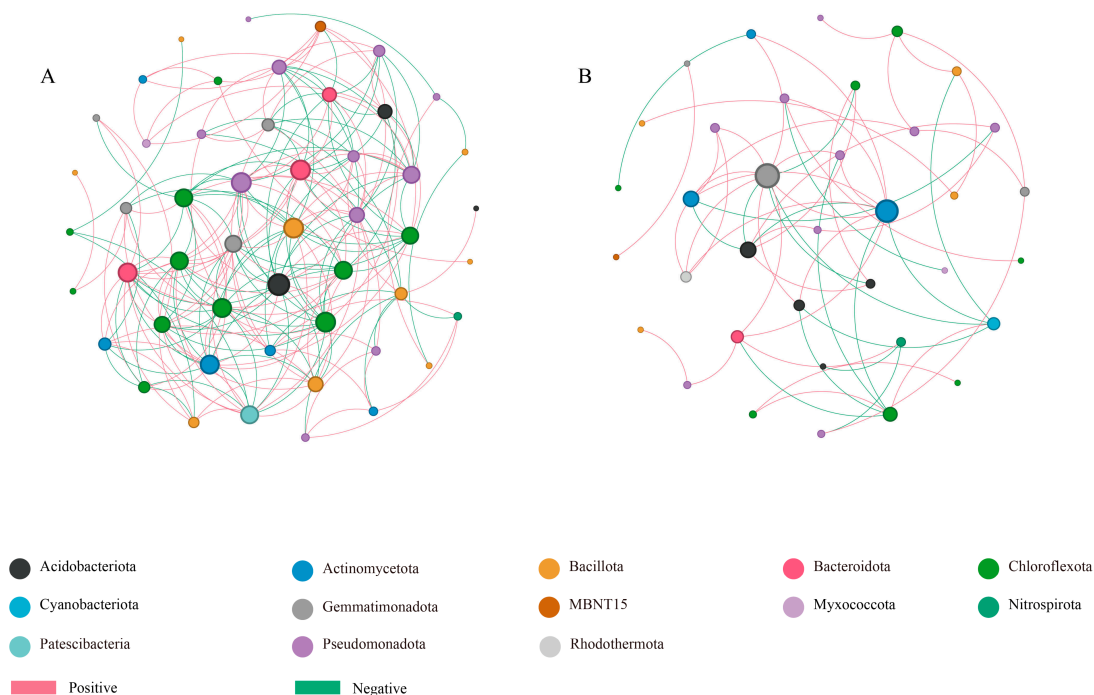


Figure 7. Genus-level co-occurrence network structure of bacterial communities at different natural rainfall leaching stages. (A) Genus-level co-occurrence network at the CK–T1 stage; (B) Genus-level co-occurrence network at the T2–T5 stage. Network nodes represent bacterial genera, and node colors represent the phylum to which they belong; edges represent significant correlation relationships among genera, with red and green indicating positive and negative correlations, respectively.

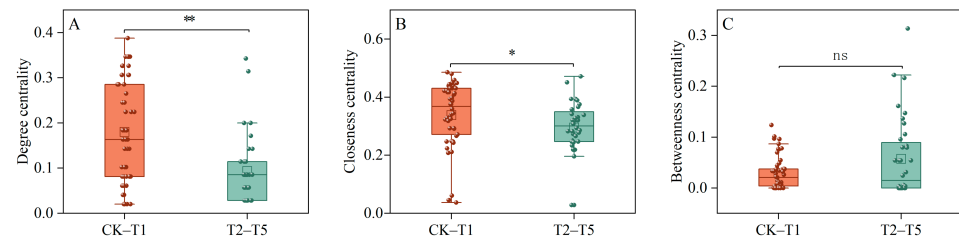


Figure 8. Comparison of node topological indicators of bacterial community co-occurrence networks at different natural rainfall leaching stages. **(A)** Degree centrality; **(B)** Closeness centrality; **(C)** Betweenness centrality. Boxplots represent the median and interquartile range (IQR), whiskers represent the $1.5 \times \text{IQR}$, and scatter points represent the indicator values of individual nodes. Brackets and asterisks indicate significant differences between the two groups. “*” indicates $p < 0.05$, “***” indicates $p < 0.01$, and ns indicates no significant difference.

3.5. Correlation Analysis Between Soil Physicochemical Properties and Microbial Characteristics

RDA indicated that pH, Na^+ , SO_4^{2-} , Cl^- , and HCO_3^- were significantly correlated with genus-level community structure based on permutation tests ($r^2 = 0.306\text{--}0.428$, $p < 0.05$), while HN, AP, and AK were not significant ($p > 0.05$). In addition, K^+ and Mg^{2+} showed marginally significant trends ($p = 0.065$ and 0.063 , respectively) (Supplementary Table S3). The first two RDA axes explained 41.67% of the variation, with RDA1 and RDA2 accounting for 28.68% and 12.99%, respectively (Figure 9A). These results suggested that soil physicochemical factors were associated with differences in bacterial community structure at the genus level. In terms of the ordination direction, the vectors of Na^+ , Cl^- , and SO_4^{2-} , accompanied by Mg^{2+} , generally pointed to the negative direction of RDA2, while pH and HCO_3^- pointed to the positive direction of RDA2. Among the three available nutrients, the HN vector was biased towards the positive direction of RDA2, the AP vector was closer to the direction of salt ions and biased towards the negative direction of RDA2, and AK was mainly along the positive direction of RDA2, consistent with the pH direction (Figure 9A). This suggested that community variation was distributed along the “salt ion–alkalinity (HCO_3^-/pH)” gradient. In the early leaching stage, community composition was more closely associated with salt ions, whereas in the later stage, it was more closely related to the carbonate buffering and alkalinity background represented by pH/ HCO_3^- , accompanied by changes in the available nutrient background. Different treatments showed a separation trend along this gradient: CK and T1–T2 were generally biased towards the negative direction of RDA2, corresponding to relatively higher salt ion levels and AP, while T4–T5 were closer to the positive direction of RDA2 and were more related to higher pH/ HCO_3^- conditions, accompanied by HN and AK. The Mantel test showed that the overall bacterial community composition (at the genus level) was significantly positively correlated with Na^+ , SO_4^{2-} , and HCO_3^- ($r = 0.182\text{--}0.210$, $p < 0.05$; Figure 9B).

The Spearman correlation heatmap showed that, among the top 30 genus-level taxa, some taxa were closely associated with salt ions and alkalinity-related indicators (Figure 9C). Among them, uncl. Actinomarinales, uncl. Nitriliruptoraceae, and uncl. BD2-11 terrestrial group were significantly positively correlated with Na^+ , SO_4^{2-} , and Cl^- ($r = 0.47\text{--}0.84$, $p < 0.05$), but significantly negatively correlated with HCO_3^- ($r = -0.51$ to -0.81 , $p < 0.05$). uncl. Nitriliruptoraceae and uncl. BD2-11 terrestrial group were also significantly negatively correlated with K^+ ($r = -0.61$ to -0.74 , $p < 0.01$). In addition, uncl. S085 was significantly positively correlated with AP and salt ions, including Na^+ , Mg^{2+} , SO_4^{2-} , and Cl^- ($r = 0.49\text{--}0.59$, $p < 0.05$), whereas it was significantly negatively correlated with HN, AK, and HCO_3^- ($r = -0.49$ to -0.58 , $p < 0.05$). In contrast,

Anaerostinus was significantly negatively correlated with AP ($r = -0.52, p < 0.05$), while uncl. Anaerolineaceae was significantly positively correlated with pH ($r = 0.55, p < 0.05$).

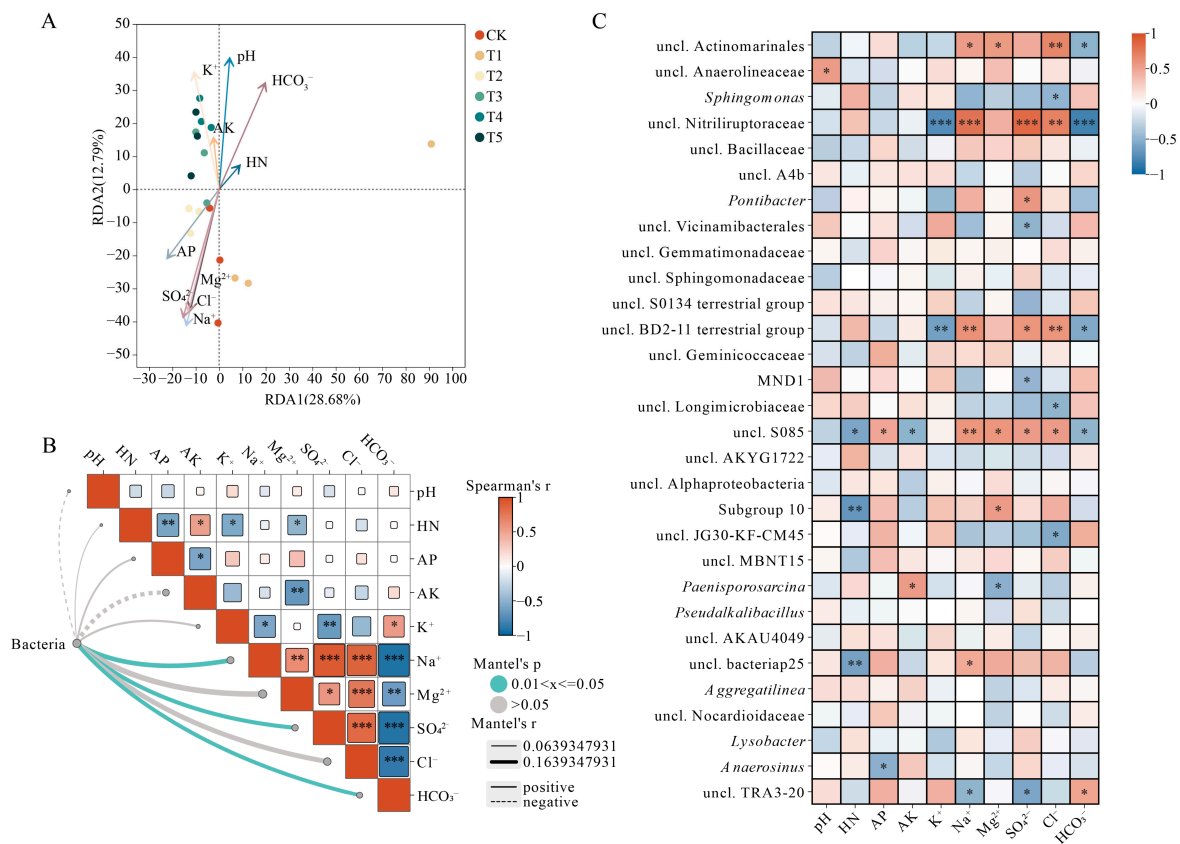


Figure 9. Correlation analysis between soil physicochemical factors and bacterial community structure and key taxa. **(A)** Redundancy analysis (RDA) based on the genus-level community matrix, showing the ordination distribution of samples at different leaching stages (CK, T1–T5) along environmental gradients. Arrows represent environmental variables, including pH, soil nutrients, and soluble ions; arrow direction indicates the correlation direction, and arrow length reflects the strength of association with community variation. **(B)** Integrated diagram of Mantel test results and correlations among environmental factors. Square colors indicate pairwise Spearman correlation coefficients (r) among environmental variables. Lines from “Bacteria” to each environmental variable indicate Mantel correlations; line thickness represents the magnitude of Mantel’s r , and line color indicates significance. Solid and dashed lines indicate positive and negative correlations, respectively. **(C)** Spearman correlation heatmap between the top 30 genus-level taxa and environmental variables. Colors indicate the direction and magnitude of correlation coefficients, and asterisks indicate significance levels (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$). Abbreviations: HN, hydrolyzable nitrogen; AP, available phosphorus; AK, available potassium; all other ion indicators are treated as soluble ions.

4. Discussion

4.1. Effects of Rainfall Leaching on Soil Ion Migration and pH Regulation Mechanism

The improvement and utilization of saline–alkaline soils have become an urgent global issue [31]. Dongying Lijin is located in the Yellow River Delta, which is dominated by chloride-type saline–alkaline soils, with Cl⁻ and Na⁺ accounting for approximately 60–88% of the salt composition, seriously restricting plant growth [32]. In this study, natural rainfall leaching markedly reduced the content of soluble salt ions such as Na⁺, Cl⁻, and SO₄²⁻ during the early leaching stage (Figure 3A,B,E), indicating that natural rainfall plays an important role in the desalination process of saline–alkaline soils. Previous studies

have also reported that the concentrations of salinity-related ions gradually decrease with increasing leaching frequency [33].

However, TWSS decreased rapidly during the early leaching stage but increased again in the later stage (Figure 1B). This phenomenon may be related to the redistribution of soluble ions under repeated wetting–drying cycles. Changes in soil pore CO_2 partial pressure during wetting and drying can affect carbonate equilibrium and promote the relative accumulation of $\text{HCO}_3^-/\text{CO}_3^{2-}$ components [34] (Figure 3G,H). Meanwhile, wetting–drying cycles may enhance the release of K^+ into the soil solution [35] (Figure 3F). These processes may partly explain the later increase in TWSS, suggesting that the increase in total water-soluble salts during the later leaching stage was not simply a reversal of salt leaching, but was also related to ion redistribution and carbonate-system regulation under wetting–drying alternation.

Moreover, soil pH did not continuously decrease with decreasing salinity (Figure 1A), which is consistent with previous studies suggesting that carbonate buffering can maintain alkaline soil conditions even after partial desalination [36]. This phenomenon may be related to differences in the migration and buffering characteristics of different salt components during the leaching process. During rainfall leaching, readily soluble neutral salts such as NaCl and Na_2SO_4 can migrate rapidly with water; therefore, soluble salt ions such as Cl^- and SO_4^{2-} usually respond clearly to leaching processes [37]. In contrast, carbonate-related components do not simply migrate synchronously with water, but are more strongly controlled by the $\text{CO}_2\text{--HCO}_3^-\text{--CO}_3^{2-}$ equilibrium, Ca/Mg carbonate dissolution–precipitation reactions, and soil pCO_2 conditions [38,39]. Therefore, even when soluble salt ions such as Na^+ , Cl^- , and SO_4^{2-} undergo marked migration, the $\text{HCO}_3^-/\text{CO}_3^{2-}$ -related alkaline background may still influence soil pH through carbonate buffering, thereby causing pH to fluctuate during the later stage of leaching.

4.2. Accumulation of Nutrients by Rainfall Leaching

Soil nutrient status is closely related to soil fertility and crop productivity and is an important indicator for evaluating the agricultural utilization potential of saline–alkali land [40]. In this study, the available nutrient indicators HN and AK increased during rainfall leaching and reached their maximum values at the middle leaching stage T3 (Figure 2A,C). This pattern suggests that natural rainfall leaching not only promotes the migration of soluble salt ions but may also alter soil nutrient availability in the short term.

Previous studies have shown that salt reduction can weaken ionic competition and modify soil exchange conditions, thereby increasing the availability of some nutrients. In saline–alkali soils, high concentrations of Na^+ and soluble salts may limit nutrient availability by occupying exchange sites or altering nutrient retention processes. Therefore, the rapid decrease in major salt ions during the early leaching stage may provide a short-term window for nutrient activation [41,42]. In addition, repeated drying–rewetting cycles caused by natural rainfall can promote the release of nutrients from solid-phase pools into the soil solution, which may also explain the increases in HN and AK during the middle leaching stage [35,43].

However, the increase in available nutrients did not persist throughout the entire leaching process. After T3, available nutrient content showed a decreasing trend, which may be attributed to the redistribution of some activated nutrients or their downward migration with water [44]. In our study, AP decreased rapidly during the early leaching stage and then gradually increased again in the later stage (Figure 2B). Similar patterns have been reported previously, possibly because drying–rewetting cycles can enhance the P adsorption capacity of some soils, thereby temporarily reducing P availability. After

rewetting, however, part of the adsorbed or immobilized P may be released again, allowing AP levels to gradually increase [45,46].

In contrast, soil organic matter and most nutrient indicators changed only within a limited range during rainfall leaching (Figure 2D–G), indicating that short-term natural rainfall did not substantially alter the total pools of soil nitrogen, phosphorus, potassium, and organic matter. This result is consistent with the slow accumulation of organic matter and the relatively stable nutrient pools commonly observed in saline–alkali soils [18,47].

4.3. Evolution of Soil Microbial Community Diversity and Structure Driven by Rainwater Leaching

Alpha and Beta diversity respectively reflect the diversity level within microbial communities and the differences in community composition among different samples and are important indicators for evaluating community responses to environmental changes [48]. In this study, during phased natural rainfall leaching, the ACE, Chao, Shannon, and Coverage indices of bacterial communities all decreased significantly (Figures 5 and S2), indicating that rainfall leaching may have exerted a short-term filtering effect on soil bacterial communities. This change may be related to the rapid alteration of soil moisture conditions after heavy rainfall, limited gas diffusion, and changes in oxygen supply, because previous studies have shown that microbial richness and diversity may temporarily decrease after soil water content increases [16,49]. Meanwhile, PCoA analysis showed that CK/T1 was clearly separated from T2–T5 in the ordination space (Figure 6B), indicating that the bacterial community structure may have undergone a relatively obvious stage-specific change after the early stage of leaching.

To further explain the observed differentiation in community structure, this study analyzed changes in community composition at the phylum and genus levels. Previous studies have shown that, in saline soils under halophytic vegetation, both salinity and pH significantly affect bacterial community composition, and taxa such as Pseudomonadota, Actinomycetota, and Bacteroidota are the main dominant groups in saline soils [50]. The results of this study also showed that natural rainfall leaching did not substantially alter the overall pattern of dominant bacterial phyla but was mainly reflected in the redistribution of genus-level taxa within dominant phyla, as well as stage-specific changes in some taxa sensitive to salinity or moisture conditions. For example, we found that Pseudomonadota remained dominant across all treatments, but its relative abundance varied only slightly (Figure 6A). However, at the genus level, MND1 and uncl. TRA3-20 increased significantly in the middle and late stages of leaching, whereas uncl. Sphingomonadaceae showed a decreasing trend during the middle and late stages (Figure 6C, Supplementary Table S2). This indicates that the relative stability of Pseudomonadota at the phylum level may mask the redistribution of genus-level taxa within this phylum.

In addition to the redistribution of taxa within dominant phyla, some bacterial groups that are more sensitive to salinity and moisture conditions also showed stage-specific changes. In this study, Bacteroidota and its dominant genus *Pontibacter* showed relatively high abundances in CK and the early stage of leaching but decreased in the subsequent treatments (Figure 6A,C; Supplementary Table S2). Previous studies have shown that halotolerant taxa exist within the genus *Pontibacter* and can adapt to relatively high-salinity environments [51]. Therefore, the decreasing trend of *Pontibacter* during the leaching process was generally consistent with the decline in soluble salts observed in this study, indicating that early dominant salinity-related taxa may gradually decrease during natural rainfall leaching. In contrast to the decline in Bacteroidota and *Pontibacter*, Chloroflexota and its related genus uncl. A4b were relatively enriched in the middle and late stages of leaching (Figure 6A,C; Supplementary Table S2). Previous studies have shown that some lineages within Chloroflexota are commonly found in low-oxygen or anaerobic environ-

ments and are associated with organic matter decomposition [52,53]. Considering that rainfall infiltration may increase soil water content, limit gas diffusion, and form localized low-oxygen microenvironments within soil aggregates [49], the increase in Chloroflexota-related taxa may reflect changes in soil moisture status and microregional oxygen conditions after leaching.

Overall, changes in bacterial communities under natural rainfall leaching were not simply characterized by the increase or decrease in a single dominant taxon but rather by a combined process involving the redistribution of taxa within dominant phyla, the decrease in salinity-related taxa, and the increase in taxa that may be adapted to moist or low-oxygen microenvironments. These results suggest that changes in salinity and soil moisture conditions during rainfall leaching may be associated with the stage-specific restructuring of bacterial community structure.

4.4. Reorganization of Microbial Interaction Networks

Natural rainfall leaching not only affected bacterial community composition but also changed the potential co-occurrence relationships among bacterial taxa. In this study, compared with CK–T1, the T2–T5 network had fewer nodes and edges, together with lower degree centrality and closeness centrality (Figures 7 and 8). This change may be related to the previously observed decrease in salinity, changes in soil moisture conditions, and the decline of some bacterial taxa. Previous studies have also shown that changes in soil salinity and moisture can affect microbial community composition and further alter the co-occurrence relationships among taxa [54,55]. Other studies have found that soil moisture conditions can affect microbial community structure and co-occurrence networks, indicating that microbial networks are relatively sensitive to changes in moisture [56]. These studies are generally consistent with the reduction in network size observed in this study, suggesting that the association structure of bacterial communities may have changed during natural rainfall leaching.

Along with the decrease in network size during T2–T5, the proportion of positive edges increased (Figure 7). Similar studies have also found that the proportion of positive edges in microbial co-occurrence networks can change under different salinity conditions, indicating that the salinity background can affect the association patterns among microbial taxa [55]. However, an increase in the proportion of positive edges does not directly indicate enhanced interactions among bacteria, because correlations in co-occurrence networks may arise from shared environmental preferences, similar niches, or indirect associations [57]. In this study, the increase in the proportion of positive edges more likely reflects that, after the decrease in salinity and changes in moisture conditions, some of the remaining bacterial taxa had similar adaptive responses to the changed environment, rather than an increase in overall network complexity. Overall, natural rainfall leaching may have shifted the bacterial community from an early-stage co-occurrence structure that was relatively complex and had more associations to a middle-to-late-stage association structure with a smaller network size, weaker connectivity, and a higher proportion of positive edges.

4.5. Coupling Relationship Between Soil Physicochemical Properties and Microorganisms

As described above, salt ion migration and the buffering effect of the carbonate system constituted an important physicochemical background during natural rainfall leaching. The RDA, Mantel analysis, and genus-level correlation heatmap jointly showed that changes in bacterial community structure were more closely associated with salt ions and HCO_3^-/pH than with available nutrients (Figure 9A–C; Table S3). Moreover, different bacterial genera showed inconsistent correlation directions with environmental factors.

Previous studies have suggested that, in saline–alkaline environments, microbial responses to increased salinity are often related to osmotic regulation, ion homeostasis maintenance, and metabolic adaptation, and that differences in these adaptive capacities among taxa may be reflected in changes in community composition [17,58,59]. Meanwhile, pH is commonly regarded as an important soil factor related to bacterial community structure [60]. Wu et al. [61] and Cai et al. [62] also reported close relationships between microbial community changes and salt ions or alkaline indicators such as HCO_3^- /pH in saline–alkaline soils, which is generally consistent with the results of this study. Compared with previous studies, our results further suggest that under natural rainfall leaching conditions, even when soluble salts migrated markedly, HCO_3^- and pH remained closely associated with bacterial community structure. Therefore, bacterial community changes under natural rainfall leaching should not be interpreted only from the perspective of desalination; the carbonate system and its related alkaline indicators should also be considered.

4.6. Implications for Agricultural Production Management Based on the Phased Characteristics of Natural Leaching

Based on the results of this study, post-rain management of saline–alkaline land should not evaluate the improvement effect only based on total salt content or a single measurement; instead, it should also comprehensively consider changes in major salt ions, pH, HCO_3^- , and nutrients.

In the early stage of leaching, the rapid decline in soluble salts may provide a relatively favorable short-term window for post-rain sowing and seedling growth [63]. However, the use of this period should also be combined with drainage and aeration management, because short-term waterlogging after heavy rainfall may have adverse effects on the crop rhizosphere environment [64].

In the middle stage of leaching, available nutrients such as HN, AP, and AK showed a phased increase or a turning trend, indicating that soil nutrient supply after rainfall may change in the short term. If topdressing is still carried out according to a fixed fertilization scheme, it may not fully match the actual soil nutrient status. Therefore, it is recommended to remeasure available nutrients such as HN, AP, and AK after rainfall and adjust the topdressing intensity and fertilizer formulation accordingly. This may help avoid excessive fertilization when available nutrients temporarily increase, thereby improving fertilizer use efficiency and reducing the risk of nutrient migration or loss with water [65].

In the later stage of leaching, management should not focus only on reducing salinity, but should also pay attention to changes in the soil alkaline environment. Since pH/ HCO_3^- may still have important effects on the soil environment and microbial community after soluble salts have migrated, pH and HCO_3^- should be included in post-rain soil testing. This may help avoid the misjudgment that desalination is equivalent to dealkalization, which may otherwise lead to limited nutrient availability or inhibited rhizosphere growth [66].

In addition, post-rain management should also consider two issues related to ion changes. First, SO_4^{2-} is both a salt ion and a source of sulfur nutrition. While heavy rainfall reduces salt stress, it may also increase the risk of SO_4^{2-} migration with water. Therefore, in fields with a potential sulfur deficiency risk, or when crops with high sulfur demand are planted, post-rain sulfur testing may be considered, followed by appropriate supplementation based on the results [67]. Second, although Na^+ decreased significantly with leaching and Na^+ stress was alleviated to some extent, Ca^{2+} and Mg^{2+} also decreased, which may affect the relative balance of Na–Ca–Mg and soil structural stability. Therefore, when evaluating the post-rain desalination effect, attention should also be paid to the sodium adsorption ratio (SAR), soil infiltration performance, and Ca^{2+} / Mg^{2+} supply. If necessary, Ca^{2+} and Mg^{2+} can be included in post-rain remeasurement, and fertilization

or amendment application can be adjusted accordingly to maintain soil environmental stability and improve the improvement effect [68].

Overall, natural rainfall leaching provides an important opportunity to alleviate salinity stress in saline–alkaline soils, but post-rain management should not only focus on whether total salinity has decreased. In practical production, salt ion migration, pH/HCO₃[−] changes, available nutrient supply, and the balance of major cations should be considered together. This may help more reasonably evaluate the post-rain status of saline–alkaline soils and reduce the adverse effects of later-stage alkaline constraints and nutrient imbalance on crop growth. However, the management suggestions proposed here should be adjusted according to crop type, rainfall intensity, drainage conditions, and the initial salinity–alkalinity status of the soil, rather than being applied as a fixed scheme.

5. Conclusions

This study clarified the changes in soil physicochemical properties and bacterial communities during phased natural rainfall leaching in moderate to severe saline–alkaline soils from Dongying, Yellow River Delta. Natural rainfall leaching promoted the migration of soluble salt ions and rapidly alleviated salt stress in the early stage. However, soil pH did not decrease continuously with desalination, but fluctuated under the buffering effect of the carbonate system, indicating that salt reduction was not necessarily accompanied by alkalinity alleviation. With the progress of leaching, HCO₃[−]/pH remained important indicators for evaluating the soil alkaline environment after soluble salt migration.

Bacterial community composition and potential co-occurrence networks also changed during natural rainfall leaching. The RDA, Mantel analysis, and genus-level correlation heatmap showed that bacterial community structure was more closely associated with salt ions and HCO₃[−]/pH than with available nutrients, while different bacterial genera showed different correlation patterns with environmental factors. These results suggest that bacterial community changes under natural rainfall leaching were related not only to salt ion migration, but also to pH/HCO₃[−] changes and the redistribution of specific bacterial taxa. Meanwhile, changes in the co-occurrence network indicated that the potential association structure among bacterial taxa also differed between the early and middle-to-late leaching stages.

Overall, natural rainfall leaching provides an important opportunity for reducing salt stress in saline–alkaline soils, but post-rain management should not rely only on total salt content or a single measurement. In practical production, major salt ions, pH, HCO₃[−], available nutrients, and key cation balance should be considered together when evaluating soil conditions after rainfall. Future studies should combine more intensive field observations with functional validation to further clarify how bacterial community changes are related to soil function improvement and crop growth in saline–alkaline land. However, because this study was conducted under pot conditions, the results may not fully represent field conditions, especially in terms of soil profile depth, pore connectivity, spatial heterogeneity, natural aggregate structure, and biological disturbance. Therefore, long-term in situ field experiments are still needed to further verify the applicability of these findings under natural field conditions.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/agriculture16121276/s1>, Figure S1: Changes in sequencing coverage and richness index of soil bacterial communities under natural rainfall leaching treatments at different stages. (A) Sequencing coverage; (B) ACE richness index. Bars represent mean ± standard deviation. Horizontal brackets and “*” indicate significant differences among treatments ($p < 0.05$); Figure S2: Rarefaction curves of soil bacterial communities under natural rainfall leaching treatments at different stages. (A) Observed ASVs (Sobs/Observed ASVs); (B) Chao richness index. The x-axis

represents the number of sequencing reads sampled, and the y-axis represents the corresponding richness estimate; Table S1: Sampling dates and rainfall characteristics at different stages of natural rainfall leaching; Table S2 Bacterial genera showing significant differences across natural rainfall leaching stages among the top 30 genera; Table S3: Fitting results of environmental factors in genus-level RDA ordination of the bacterial community.

Author Contributions: Y.B.: Writing—original draft, Writing—review and editing, Methodology, Software. X.L. (Xueli Lu): Writing—review and editing, Methodology, Validation, Data curation. X.L. (Xiaobin Li): Writing—review and editing, Conceptualization, Supervision. F.J.: Writing—review and editing, Formal analysis. R.M.: Writing—review and editing, Formal analysis, Software. Y.L.: Writing—review and editing, Data curation. Z.X.: Writing—review and editing, Visualization, Supervision, Funding acquisition, Conceptualization. M.W.: Writing—review and editing, Supervision. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by the National Key Research and Development Program Young Scientist Project (2023YFD1901900), the Agricultural Science and Technology Innovation Program (ASTIP-TRIC06-2026), the Taishan Scholars Program (Xiaobin Li), and the Shandong Provincial Natural Science Foundation (ZR2024MC118). The funders had no role in designing the study, data collection and analysis, publication decision, or manuscript preparation.

Data Availability Statement: The original contributions presented in this study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding authors.

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

References

1. Hopmans, J.W.; Qureshi, A.S.; Kisekka, I.; Munns, R.; Grattan, S.R.; Rengasamy, P.; Ben-Gal, A.; Assouline, S.; Javaux, M.; Minhas, P.S.; et al. Critical knowledge gaps and research priorities in global soil salinity. In *Advances in Agronomy*; Sparks, D.L., Ed.; Elsevier: Amsterdam, The Netherlands, 2021; Volume 169, pp. 1–191. [\[CrossRef\]](#)
2. Bernstein, N.; Kafkafi, U. Root growth under salinity stress. In *Plant Roots: The Hidden Half*, 3rd ed.; Waisel, Y., Eshel, A., Kafkafi, U., Eds.; Marcel Dekker: New York, NY, USA, 2002; pp. 787–805. [\[CrossRef\]](#)
3. Song, C.-F. Deterioration and governance: Ecological environmental problems in contemporary China. *Soc. Stud.* **1990**, *2*, 24–29. (In Chinese)
4. Wang, J.; Tian, D.; Knapp, A.K.; Chen, H.Y.H.; Luo, Y.; Li, Z.; Hou, E.; Huang, X.; Jiang, L.; Niu, S. Precipitation manipulation and terrestrial carbon cycling: The roles of treatment magnitude, experimental duration and local climate. *Glob. Ecol. Biogeogr.* **2021**, *30*, 1909–1921. [\[CrossRef\]](#)
5. Xiao, C.; Wu, P.; Zhang, L.; Song, L. Robust increase in extreme summer rainfall intensity during the past four decades observed in China. *Sci. Rep.* **2016**, *6*, 38506. [\[CrossRef\]](#) [\[PubMed\]](#)
6. Tang, Y.; Huang, A.; Wu, P.; Huang, D.; Xue, D.; Wu, Y. Drivers of summer extreme precipitation events over East China. *Geophys. Res. Lett.* **2021**, *48*, e2021GL093670. [\[CrossRef\]](#)
7. Liu, B.; Wang, S.; Liu, X.; Sun, H. Evaluating soil water and salt transport in response to varied rainfall events and hydrological years under brackish water irrigation in the North China Plain. *Geoderma* **2022**, *422*, 115954. [\[CrossRef\]](#)
8. Bertsch, P.M.; Thomas, G.W. Potassium status of temperate region soils. In *Potassium in Agriculture*; Munson, R.D., Ed.; ASA, CSSA, SSSA: Madison, WI, USA, 1985; pp. 129–162. [\[CrossRef\]](#)
9. Zhang, H.; Jiang, N.; Zhang, S.; Zhu, X.; Wang, H.; Xiu, W.; Zhao, J.; Liu, H.; Zhang, H.; Yang, D. Soil bacterial community composition is altered more by soil nutrient availability than pH following long-term nutrient addition in a temperate steppe. *Front. Microbiol.* **2024**, *15*, 1455891. [\[CrossRef\]](#)
10. Lv, M.; Zhu, S.; Shi, Y.; Shu, S.; Li, A.; Fan, B. Study on soil leaching risk of reuse of reclaimed fertilizer from micro-flush sanitary wastewater. *Water* **2022**, *14*, 2823. [\[CrossRef\]](#)
11. de León-Lorenzana, A.S.; Delgado-Balbuena, L.; Domínguez-Mendoza, C.; Navarro-Noya, Y.E.; Luna-Guido, M.; Dendooven, L. Reducing salinity by flooding an extremely alkaline and saline soil changes the bacterial community but its effect on the archaeal community is limited. *Front. Microbiol.* **2017**, *8*, 466. [\[CrossRef\]](#)
12. Kissel, D.E.; Sonon, L.; Vendrell, P.F.; Isaac, R.A. Salt concentration and measurement of soil pH. *Commun. Soil Sci. Plant Anal.* **2009**, *40*, 179–187. [\[CrossRef\]](#)

13. Yang, C.; Li, K.; Lv, D.; Jiang, S.; Sun, J.; Lin, H.; Sun, J. Inconsistent response of bacterial phyla diversity and abundance to soil salinity in a Chinese delta. *Sci. Rep.* **2021**, *11*, 12870. [\[CrossRef\]](#)
14. Gunde-Cimerman, N.; Plemenitaš, A.; Oren, A. Strategies of adaptation of microorganisms of the three domains of life to high salt concentrations. *FEMS Microbiol. Rev.* **2018**, *42*, 353–375. [\[CrossRef\]](#)
15. Sun, F.; Fan, L.; Deng, G.; Kuzyakov, Y.; Zhang, Y.; Wang, J.; Li, Y.; Wang, F.; Li, Z.; Tariq, A.; et al. Responses of tropical forest soil organic matter pools to shifts in precipitation patterns. *Soil Biol. Biochem.* **2024**, *197*, 109530. [\[CrossRef\]](#)
16. Li, X.; Zhang, Q.; Ma, J.; Yang, Y.; Wang, Y.; Fu, C. Flooding irrigation weakens the molecular ecological network complexity of soil microbes during the process of dryland-to-paddy conversion. *Int. J. Environ. Res. Public Health* **2020**, *17*, 561. [\[CrossRef\]](#)
17. Rath, K.M.; Fierer, N.; Murphy, D.V.; Rousk, J. Linking bacterial community composition to soil salinity along environmental gradients. *ISME J.* **2019**, *13*, 836–846. [\[CrossRef\]](#)
18. Sun, H.; Qu, J.; Wang, X.; Zheng, W.; Li, C.; Liu, Y. The response of soil organic nitrogen fractions and nitrogen availability to salinity in saline soils of the Yellow River Delta. *Chin. J. Eco-Agric.* **2021**, *29*, 1397–1404. (In Chinese) [\[CrossRef\]](#)
19. Lyu, H.; Zhao, Y.; Gong, X.; Wan, J.; Guo, H.; Xu, S. Review of techniques and case studies for saline-alkali land amelioration in the coastal regions of China. *Hydrogeol. Eng. Geol.* **2025**, *52*, 25–43. (In Chinese) [\[CrossRef\]](#)
20. Guan, B.; Xie, B.H.; Yang, S.S.; Hou, A.X.; Chen, M.; Han, G.X. Effects of five years' nitrogen deposition on soil properties and plant growth in a salinized reed wetland of the Yellow River Delta. *Ecol. Eng.* **2019**, *136*, 160–166. [\[CrossRef\]](#)
21. Khan, I.; Tariq, M.; Nadeem, F.; Khan, M.S.; Alharbi, S.A.; Alfarraj, S.; Ercisli, S.; Bozhuyuk, A.U.; Zhuang, P. Understanding the influence of precipitation and nitrogen depositions on the soil health and growth indices of invasive (*Solidago canadensis* L.) and native (*Wedelia chinensis*) association. *BMC Plant Biol.* **2025**, *25*, 845. [\[CrossRef\]](#) [\[PubMed\]](#)
22. Lu, R.K. *Analytical Methods of Soil Agrochemistry*; China Agricultural Science and Technology Press: Beijing, China, 1999. (In Chinese)
23. Sun, M.; Li, M.; Zhou, Y.; Liu, J.; Shi, W.; Wu, X.; Xie, B.; Deng, Y.; Gao, Z. Nitrogen deposition enhances the deterministic process of the prokaryotic community and increases the complexity of the microbial co-network in coastal wetlands. *Sci. Total Environ.* **2023**, *856*, 158939. [\[CrossRef\]](#)
24. Bao, S.D. *Analysis for Soil and Agro-Chemistry*, 3rd ed.; China Agriculture Press: Beijing, China, 2000. (In Chinese)
25. Sun, J.; Song, W.; Sun, X.; Cui, J.; Ma, H. Analysis of soil salinization characteristics in coastal area of Panjin City, Liaoning Province. *Water* **2025**, *17*, 2666. [\[CrossRef\]](#)
26. Liu, C.; Zhao, D.; Ma, W.; Guo, Y.; Wang, A.; Wang, Q.; Lee, D.-J. Denitrifying sulfide removal process on high-salinity wastewaters in the presence of *Halomonas* sp. *Appl. Microbiol. Biotechnol.* **2016**, *100*, 1421–1426. [\[CrossRef\]](#) [\[PubMed\]](#)
27. Chen, S.; Zhou, Y.; Chen, Y.; Gu, J. fastp: An ultra-fast all-in-one FASTQ preprocessor. *Bioinformatics* **2018**, *34*, i884–i890. [\[CrossRef\]](#)
28. Magoč, T.; Salzberg, S.L. FLASH: Fast length adjustment of short reads to improve genome assemblies. *Bioinformatics* **2011**, *27*, 2957–2963. [\[CrossRef\]](#)
29. Callahan, B.J.; McMurdie, P.J.; Rosen, M.J.; Han, A.W.; Johnson, A.J.A.; Holmes, S.P. DADA2: High-resolution sample inference from Illumina amplicon data. *Nat. Methods* **2016**, *13*, 581–583. [\[CrossRef\]](#)
30. Bolyen, E.; Rideout, J.R.; Dillon, M.R.; Bokulich, N.A.; Abnet, C.C.; Al-Ghalith, G.A.; Alexander, H.; Alm, E.J.; Arumugam, M.; Asnicar, F.; et al. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. *Nat. Biotechnol.* **2019**, *37*, 852–857. [\[CrossRef\]](#)
31. Wang, X.; Riaz, M.; Babar, S.; Eldesouki, Z.; Liu, B.; Xia, H.; Li, Y.; Wang, J.; Xia, X.; Jiang, C. Alterations in the composition and metabolite profiles of the saline-alkali soil microbial community through biochar application. *J. Environ. Manag.* **2024**, *352*, 120033. [\[CrossRef\]](#)
32. Wang, Z.; Zhu, S.; Yu, R. *Saline-Alkali Soils in China*; Nanjing Institute of Soil Science, Chinese Academy of Sciences: Nanjing, China, 1998; pp. 153–154. (In Chinese)
33. Sun, X.; Wang, Z.; Zhao, C.; Xu, L.; Dong, Y. Impact assessment of saline-sodic agricultural drainage on water quality in vented area of Chagan Lake. *Trans. Chin. Soc. Agric. Eng.* **2011**, *27*, 214–219. (In Chinese)
34. Ringham, M.C.; Hoke, G.D.; Huntington, K.W.; Aranibar, J.N. Influence of vegetation type and site-to-site variability on soil carbonate clumped isotope records, Andean piedmont of Central Argentina (32–34°S). *Earth Planet. Sci. Lett.* **2016**, *440*, 1–11. [\[CrossRef\]](#)
35. Al-Silevany, B.; Mehmedany, L. The effect of wetting and drying cycles on potassium release in three soil orders. *J. Ecol. Eng.* **2023**, *24*, 218–226. [\[CrossRef\]](#)
36. Li, J.; Gao, Y.; Zhang, X.; Tian, P.; Li, J.; Tian, Y. Comprehensive comparison of different saline water irrigation strategies for tomato production: Soil properties, plant growth, fruit yield and fruit quality. *Agric. Water Manag.* **2019**, *213*, 521–533. [\[CrossRef\]](#)
37. Richards, L.A. (Ed.) *Diagnosis and Improvement of Saline and Alkali Soils*; USDA Agriculture Handbook No. 60; United States Department of Agriculture: Washington, DC, USA, 1954.
38. Sparks, D.L. *Environmental Soil Chemistry*, 2nd ed.; Academic Press: San Diego, CA, USA, 2003.

39. Bargrizan, S.; Smernik, R.J.; Mosley, L.M. Constraining the carbonate system in soils via testing the internal consistency of pH, pCO₂ and alkalinity measurements. *Geochem. Trans.* **2020**, *21*, 4. [\[CrossRef\]](#)
40. Li, T.; Wang, S.; Liu, S.; Zhang, X.; Dong, H.; Dai, S.; Chai, L.; Li, H.; Lv, Y.; Li, T.; et al. Trade-offs of organic amendment input on soil quality and crop productivity in saline-alkali land globally: A meta-analysis. *Eur. J. Agron.* **2025**, *164*, 127471. [\[CrossRef\]](#)
41. Hartikainen, H.; Yli-Halla, M. Chloride and sulphate solutions as extractants for soil P: I. Effect of ionic species and ionic strength on P desorption. *Agric. Food Sci.* **1982**, *54*, 287–296. [\[CrossRef\]](#)
42. Nigon, L.L.; Kaiser, D.E. Factors affecting potassium retention in sandy soils. *Soil Sci. Soc. Am. J.* **2025**, *89*, e70094. [\[CrossRef\]](#)
43. Tang, S.; Liu, Y.; Jian, C.; Xu, Y.; Zhang, W.; Wang, Z.; Yang, J. Research progress on effects of alternate wetting and drying irrigation on water and nitrogen use efficiency, yield and quality of rice. *J. Huazhong Agric. Univ.* **2022**, *41*, 184–192. (In Chinese)
44. Filipović, L.; Krevh, V.; Chen, R.; Defterdarović, J.; Kovač, Z.; Mustač, I.; Bogunović, I.; He, H.; Baumgartl, T.; Filipović, V. Quantification of intra- vs. inter-row leaching of major plant nutrients in sloping vineyard soils. *Water* **2023**, *15*, 759. [\[CrossRef\]](#)
45. Haynes, R.J.; Swift, R.S. Effects of air-drying on the adsorption and desorption of phosphate and levels of extractable phosphate in a group of acid soils, New Zealand. *Geoderma* **1985**, *35*, 145–157. [\[CrossRef\]](#)
46. Dodd, I.C.; Puértolas, J.; Huber, K.; Pérez-Pérez, J.G.; Wright, H.R.; Blackwell, M.S.A. The importance of soil drying and re-wetting in crop phytohormonal and nutritional responses to deficit irrigation. *J. Exp. Bot.* **2015**, *66*, 2239–2252. [\[CrossRef\]](#) [\[PubMed\]](#)
47. Setia, R.; Gottschalk, P.; Smith, P.; Marschner, P.; Baldock, J.; Setia, D.; Smith, J. Soil salinity decreases global soil organic carbon stocks. *Sci. Total Environ.* **2013**, *465*, 267–272. [\[CrossRef\]](#) [\[PubMed\]](#)
48. Kang, S.; Rodrigues, J.L.M.; Ng, J.P.; Gentry, T.J. Hill number as a bacterial diversity measure framework with high-throughput sequence data. *Sci. Rep.* **2016**, *6*, 38263. [\[CrossRef\]](#)
49. Das, A.K.; Lee, D.-S.; Woo, Y.-J.; Sultana, S.; Mahmud, A.; Yun, B.-W. The Impact of Flooding on Soil Microbial Communities and Their Functions: A Review. *Stresses* **2025**, *5*, 30. [\[CrossRef\]](#)
50. 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. [\[CrossRef\]](#) [\[PubMed\]](#)
51. Joshi, M.N.; Sharma, A.C.; Pandya, R.V.; Patel, R.P.; Saiyed, Z.M.; Saxena, A.K.; Bagatharia, S.B. Draft genome sequence of *Pontibacter* sp. nov. BAB1700, a halotolerant, industrially important bacterium. *J. Bacteriol.* **2012**, *194*, 6329–6330. [\[CrossRef\]](#) [\[PubMed\]](#)
52. Bovio-Winkler, P.; Guerrero, L.D.; Erijman, L.; Oyarzúa, P.; Suárez-Ojeda, M.E.; Cabezas, A.; Etchebehere, C. Genome-centric metagenomic insights into the role of Chloroflexi in anammox, activated sludge and methanogenic reactors. *BMC Microbiol.* **2023**, *23*, 45. [\[CrossRef\]](#)
53. Gschwend, F.; Aregger, K.; Gramlich, A.; Walter, T.; Widmer, F. Periodic waterlogging consistently shapes agricultural soil microbiomes by promoting specific taxa. *Appl. Soil Ecol.* **2020**, *155*, 103623. [\[CrossRef\]](#)
54. de Vries, F.T.; Griffiths, R.I.; Bailey, M.; Craig, H.; Girlanda, M.; Gweon, H.S.; Hallin, S.; Kaisermann, A.; Keith, A.M.; Kretschmar, M.; et al. Soil bacterial networks are less stable under drought than fungal networks. *Nat. Commun.* **2018**, *9*, 3033. [\[CrossRef\]](#)
55. Li, Y.; Wang, J.; Li, E.; Yang, X.; Yang, J. Shifts in microbial community structure and co-occurrence network along a wide soil salinity gradient. *Microorganisms* **2024**, *12*, 1268. [\[CrossRef\]](#)
56. Wang, Y.; Xie, Y.; Ma, H.; Zhang, Y.; Zhang, J.; Zhang, H.; Luo, X.; Li, J. Responses of soil microbial communities and networks to precipitation change in a typical steppe ecosystem of the Loess Plateau. *Microorganisms* **2022**, *10*, 817. [\[CrossRef\]](#)
57. Röttgers, L.; Faust, K. From hairballs to hypotheses—biological insights from microbial networks. *FEMS Microbiol. Rev.* **2018**, *42*, 761–780. [\[CrossRef\]](#)
58. Yan, N.; Marschner, P.; Cao, W.; Zuo, C.; Qin, W. Influence of salinity and water content on soil microorganisms. *Int. Soil Water Conserv. Res.* **2015**, *3*, 316–323. [\[CrossRef\]](#)
59. Haj-Amor, Z.; Araya, T.; Kim, D.-G.; Bouri, S.; Lee, J.; Ghilou, W.; Yang, Y.; Kang, H.; Jhariya, M.K.; Banerjee, A.; et al. Soil salinity and its associated effects on soil microorganisms, greenhouse gas emissions, crop yield, biodiversity and desertification: A review. *Sci. Total Environ.* **2022**, *843*, 156946. [\[CrossRef\]](#) [\[PubMed\]](#)
60. Fierer, N.; Jackson, R.B. The diversity and biogeography of soil bacterial communities. *Proc. Natl. Acad. Sci. USA* **2006**, *103*, 626–631. [\[CrossRef\]](#)
61. Wu, Y.; Zeng, J.; Zhu, Q.; Zhang, Z.; Lin, X. pH is the primary determinant of the bacterial community structure in agricultural soils impacted by polycyclic aromatic hydrocarbon pollution. *Sci. Rep.* **2017**, *7*, 40093. [\[CrossRef\]](#) [\[PubMed\]](#)
62. Cai, S.; Xu, S.; Zhang, D.; Liang, Y.; Zhu, H. Vegetation-driven changes in soil salinity ions and microbial communities across tidal flat reclamation. *Microorganisms* **2025**, *13*, 1184. [\[CrossRef\]](#)
63. Liu, B.; Wang, S.; Kong, X.; Liu, X. Soil matric potential and salt transport in response to different irrigated lands and soil heterogeneity in the North China Plain. *J. Soils Sediments* **2019**, *19*, 3982–3993. [\[CrossRef\]](#)
64. Linkemer, G.; Board, J.E.; Musgrave, M.E. Waterlogging effects on growth and yield components in late-planted soybean. *Crop Sci.* **1998**, *38*, 1576–1584. [\[CrossRef\]](#) [\[PubMed\]](#)

65. Roy, R.N.; Finck, A.; Blair, G.J.; Tandon, H.L.S. Nutrient management guidelines for some major field crops. In *Plant Nutrition for Food Security: A Guide for Integrated Nutrient Management*; FAO Fertilizer and Plant Nutrition Bulletin No. 16; Food and Agriculture Organization of the United Nations: Rome, Italy, 2006; pp. 235–262.
66. Stavi, I.; Thevs, N.; Priori, S. Soil salinity and sodicity in drylands: A review of causes, effects, monitoring, and restoration measures. *Front. Environ. Sci.* **2021**, *9*, 712831. [[CrossRef](#)]
67. Eriksen, J.; Olesen, J.E.; Askegaard, M. Sulphate leaching and sulphur balances of an organic cereal crop rotation on three Danish soils. *Eur. J. Agron.* **2002**, *17*, 1–9. [[CrossRef](#)]
68. Kissel, D.E.; Saha, U.K.; Sonon, L.S. *Soil Salinity Testing, Data Interpretation, and Recommendations*; Circular 1019; University of Georgia Cooperative Extension: Athens, GA, USA, 2022.

Disclaimer/Publisher’s Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.