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Changes in Soil Properties, Bacterial Communities and Wheat Roots Responding to Subsoiling in South Loess Plateau of China

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Abstract: This study was carried out to investigate effects of subsoiling on the diversity and composition of the bacterial community in a wheat–maize rotation field in the Guanzhong area of Shaanxi Province, China. After the wheat harvest, surface soil samples were collected under two tillage methods (single rotary tillage (RT) and subsoiling + rotary tillage (ST)) to perform high-throughput sequencing and bioinformatics analysis. Soil properties and root length density (RLD) of winter wheat at booting and flowering stages were also studied. Results showed that ST treatment significantly raised the water storage, organic carbon and total nitrogen contents of deep soil (>40 cm), and notably increased the total soil pH, ammonium nitrogen content and RLD in the tillage layer from 0–70 cm at booting stage and 0~100 cm at flowering stage, but the residual nitrate nitrogen significantly decreased by 17.74%. Compared with RT, soil bacterial richness and diversity in the 10~20 cm layer of ST treatment showed a significantly decreased trend. The relative abundances of *GAL15*, *Actinobacteria*, *Nitrospirae*, *Rhizobiales*, *Burkholderiales*, *Pseudomonas* and *Serratia* in the 10–20 cm layer were remarkably increased in ST. Principal Component Analysis (PCA) and Redundancy Analysis (RDA) results showed that surface soil pH, ammonium nitrogen and nitrate nitrogen contents have the strongest effect on the bacterial structure. In addition, there were positive correlations between the RLD and the relative abundances of *Rhizobiales*, *Burkholderiales*, *Pseudomonas* and the ammonium nitrogen content. In conclusion, although subsoiling was not conducive to improving soil bacterial community richness and diversity, it significantly increased soil beneficial bacteria (biological nitrogen-fixing bacteria, ammonifying bacteria, nitrobacteria) abundances, reduced the nitrogen loss caused by denitrifying bacteria, promoted earlier root development and improved the plant utilization ratio of soil nutrients.

Keywords: rotary tillage; soil pH; ammonium nitrogen; nitrate nitrogen; high-throughput sequencing; bacterial diversity; root length density

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1. Introduction

The Guanzhong plain of Shaanxi Province is a typical winter wheat–summer corn rotation area with two crops per year in China, and the soil is mainly loessal soil, black clay soil and Lou soil [1]. Long-term traditional rotary tillage makes the surface layer of soil shallower, the bottom layer of the plow continuously thickens and worsens, and the utilization rate of subsoil water and nutrients decreases year by year [2], which eventually

leads to a series of problems such as poor root development of crops and grain yield reduction [3]. However, farmland subsoil can store nearly 50% of the total nitrogen [4] and 25–70% of the total phosphorus [5], so as to ensure the water supply under extreme drought conditions [6]. In recent years, new tillage practices with large tractors pulling subsoiling plows have disturbed the soil to a depth of 30–50 cm, thus breaking the plow base. This measure improves the soil profile structure, restores the optimal plowing depth [7], promotes the downward extension of crop roots, improves the utilization rate of crops to deep soil resources and finally achieves high and stable yield of crops [8]. The change of tillage methods will inevitably have a significant impact on the physical and chemical properties and microbial environment of the original soil [9]. Remarkably, soil microorganisms play an important role in soil nutrient processing, material circulation and maintaining soil ecological functions [10], and they are sensitive to changes in soil environmental quality [11,12]. Changes in soil microbial population, quantity, community structure and diversity also directly or indirectly affect soil physical and chemical properties and plant root activity [13]. Among them, the change of nitrification bacteria and denitrification bacteria is closely related to the transformation and utilization of soil nitrogen and nitrogen loss [14,15]. The metabolic activities of *Rhizobia* and *Actinomycetes* also indirectly affect the root activity of crops [16,17]. Therefore, in the soil–microbial–plant system, it is of great significance to study the mutual relation and interaction of soil physicochemical properties, microbial activities and crop root development.

Studies have shown that long-term rotary tillage can worsen most soil quality indicators, and such deleterious effects are due to drastic disruption of soil aggregates and consequent exposition of protected soil organic matter to further microbial mineralization [18]. Long-term rotary tillage will also form a relatively hard and thick plow bottom under the shallow plow layer, which affects the development and down planting of crop roots [19]. It has also been pointed out that, compared with the share plow (tillage depth of 30 cm), rotary tillage (tillage depth of 8 cm) can improve soil organic carbon, total nitrogen and phosphorus, as well as microbial carbon, nitrogen and phosphorus storage, but is not beneficial to the turnover of microbial biomass [20]. Meanwhile, the concept of increasing subsoil water and nutrient availability through subsoiling has a long history. However, by the 1970s, the popularity of subsoiling had declined in both academia and agricultural production. This is due to the inconsistent crop yield response of subsoiling, which cannot compensate for the high implementation cost [8], and subsoiling is not conducive to the construction of soil beneficial bio groups and the promotion of no-till technology [21,22]. In recent years, due to the application of large-scale agricultural machinery, more and more attention has been paid to the improved technology of subsoiling and the system of alternate subsoiling, to deal with the problems of soil compaction and plow layer thickening. Studies have shown that using subsoiling and keeping a 5 cm thick plow bottom can obtain the best nitrogen use efficiency and relatively good crop growth environment [23]. Subsoiling and no tillage are more conducive to improving the water storage and conservation capacity of deep soil and increasing the economic benefits of crops [24]. Yang et al. [25] believed that a subsoiling tillage of 30 cm was appropriate in water deficient areas of North China. Yin et al. [26] found that vertical rotary subsoiling (40 cm) could efficiently utilize deep soil moisture and rainfall to promote crop growth under ridge-furrow construction with a plastic film mulching system. Wei et al. [27] believed that the subsoiling technique was suitable for popularization in the southeast and northwest areas of the Loess Plateau, and its effect was better than that of no tillage. In addition, compared with conventional tillage, subsoiling and straw returning treatment could increase soil organic carbon content by 12.4%, increase microbial number and enzyme activity by 33.9% and 25.2%, respectively [28], increase crop root volume by 1.35 times, promote root exudates significantly [29], and are beneficial to the formation and stability of surface soil aggregates [30]. However, there are few studies on the effects of subsoiling on soil microbial community structure and diversity. Zhang et al. [31] found that subsoiling could optimize the rhizosphere microbial community composition, significantly increasing the quantity and diversity of soil microor-

ganisms. Zhang et al. [32] believed that 40 cm subsoiling could promote the growth of soil bacteria and fungi, but the plate counting method they adopted was relatively backward. In recent years, high-throughput sequencing technology has been widely used to study the composition of soil bacterial and fungal communities. This technology can more truly reveal microbial species, community structure and diversity, and it accelerated the study of non-culturable and trace microorganisms in soil [33]. Previous studies using qPCR showed that the average abundance of bacterial 16S rRNA and fungal ITS genes in topsoil under no tillage was higher than that under conventional tillage [34]. The Illumina Hiseq 2500 sequencing platform has recently been used to study the effects of plowed tillage, rotary tillage and no tillage on the microbial community structure in vertical soil profiles; the results showed that the bacterial community quantity attenuated by plowed tillage was less than that by rotary tillage and no tillage from the shallow to deep section [35].

To sum up, domestic and foreign researchers have conducted extensive research on the effects of different soil use and agricultural management methods (mainly no-till systems) on the physical and chemical properties of the surface layer, the number of microorganisms, root development and yield. However, the structure of the soil bacterial community and its relationship with soil physical and chemical properties and crop root growth under subsoiling and rotary tillage has been rarely reported. In this study, based on the localized experimental field with wheat–maize rotation in the south Loess Plateau of China, with the help of the Illumina Hiseq ultra-high-throughput sequencing platform, we took the surface soil (0–20 cm deep) after wheat harvest as the research object to investigate the differences in the number, diversity and community structure of soil bacteria and their relationship with soil physical and chemical properties and root length density under two tillage treatments of single rotary tillage and subsoiling + rotary tillage. This study fills the gap in the study of the effects of subsoiling on soil microecology and provides a scientific basis for optimizing farming practices, revealing the response mechanism of soil bacterial community to tillage system and improving soil water and nutrient utilization.

2. Materials and Methods

2.1. Test Design

This experiment was conducted from October 2016 to July 2017 at the long-term positioning experimental base of winter wheat–summer maize dry farming in the yellow soil area of Fuping County, Weinan City, Guanzhong Region, Shaanxi Province (longitude: 109°17′29″, latitude: 34°76′58″, altitude: 356 m). This area belongs to the continental temperate zone semi-arid, semi-humid climate and lies to the south of the Loess Plateau. The frost-free period lasts from 199–255 d. Annual sunshine in this area is 2200–2500 h, and spring drought often occurs. However, summer temperatures are high, and rainfall is concentrated. According to the statistics of the local meteorological bureau, the annual rainfall in this area ranges from 498 mm to 726 mm in recent years, and there is a significant difference between annual rainfall. The tested soil is loessal soil, loose, soft, with light soil color and the essence of the soil is soil. Before the start of the experiment, pipette particle size analysis [36] was used to measure that the 0–20 cm surface soil contained 49.0% gravel, 42.3% silt and 8.7% clay. Other basic physical and chemical properties are as follows: pH 8.20, organic matter 12.30 g·kg^{-1}, total nitrogen 0.72 g·kg^{-1}, total phosphorus 0.97 g·kg^{-1}, total potassium 12.02 g·kg^{-1}, available nitrogen 116.5 mg·kg^{-1}. The tested winter wheat variety was Xiaoyan 22, which was supplied by Shaanxi Longfeng Seed Industry Co., Ltd. (Weinan, China).

This experiment is based on the long-term positioning experiment of rotary tillage and straw returning management for 5 consecutive years. On this basis, the positioning test of rotary tillage conversion to subsoiling + rotary tillage combined with straw returning was established before winter wheat sowing in 2016. The experimental area (40 m × 18 m) of primary rotary tillage and straw returning was divided into two. Half of the treatments kept the original rotary tillage and straw returning (RT) unchanged as the control (40 m × 9 m). In the other half, the tillage method was changed to subsoiling + rotary tillage combined

with straw returning (ST) (40 m × 9 m). Each set had 3 replicates. The operation process of specific tillage methods is as follows. RT treatment: corn mechanical harvesting → corn straw returning to the field for stubble removal → multi-purpose rotary tillage fertilization and sowing (tillage depth 8–10 cm, Shandong Aolong 2BXFS-270 rotary tillage fertilization seeder) → wheat mechanical harvesting → wheat straw returning to the field for stubble removal → no-tillage direct seeding of corn. ST treatment: corn mechanical harvesting → corn straw returning to the field for stubble removal → shovel subsoiling (35–40 cm subsoiling, Hebei Zhongnongboyuan 1S-264 subsoiling machine) → multi-purpose rotary tiller rotary fertilization and sowing (8–10 cm subsoiling, Shandong Aolong 2BXFS-270 rotary tillage fertilizer seeder) → wheat mechanical harvesting → wheat straw returning to the field for stubble removal → no-tillage direct sowing of corn. The amount of wheat straw returning was about 10.3 t·hm^{-2}. The amount of corn straw returned to the field was about 11.2 t·hm^{-2}. The winter wheat was sown on 17 October with a sowing amount of 187.50 kg·hm^{-2} and row spacing of 20 cm and harvested on 13 June of the following year. Each treatment was managed uniformly by the field. The whole wheat growth period was irrigated twice, 90 mm in winter and 90 mm in booting stage. N 150 kg·hm^{-2}, P_2O_5 150 kg·hm^{-2} and K_2O 37.5 kg·hm^{-2} were applied to winter wheat at the season base, and 163 kg·hm^{-2} urea was applied to each treatment on 5 March of the second year.

2.2. Collection of Wheat Root and Soil Samples

Root samples of winter wheat were excavated by root drilling at booting stage and flowering stage, and one point was taken from each of the three repeating cells in each process. During each sampling, samples were taken at the root of the plant and at 1/2 row spacing in the same growth area of wheat. We drilled 100 cm vertically with a 10 cm diameter root drill, taking a sample every 10 cm. The obtained samples were washed to pick up all the roots for separation and scanning. Vectorization software R2V04 and ArcGIS10.2 were used to statistically calculate root length density (RLD) [37]. Soil compaction (SC) was measured directly in situ in the field using a digital compaction meter under moderate and low soil moisture conditions. Each treatment was repeated three times to investigate the effect of different tillage methods on soil compaction in the 0–45 cm upper layer.

On 14 June 2017, after the winter wheat harvest, the surface soil (0–20 cm) of the field was excavated. Three areas with the same density of wheat root stubble were randomly selected in each plot. The stubble was removed from the soil as a whole, and thin layers of soil (<10 mm) were collected at 0–10 cm and 10–20 cm, respectively, tightly attached to the stubble. In addition, the soil samples obtained from the same depth in 3 areas were crushed and mixed according to the method of 4 sections into zip lock bags. Samples 0–10 cm deep were labeled "A" after the treatment symbol and samples 10–20 cm deep were labeled "B" and stored immediately in the ice box. Each treatment had 3 repeat plots; each plot had a bag of soil samples of different depths, for a total of 6 zip lock bag. They were quickly transported to the laboratory and passed through a 2 mm sieve to remove visible root residues and stones and stored in a refrigerator at −80 °C for high-throughput sequencing analysis of soil bacteria. At the same time, three sampling points with the same stubble density were randomly selected from the RT- and ST-treated experimental areas in the field. From top to bottom, soil samples were collected at depths 0–10, 10–20, 20–30, 30–40, 40–60, 60–80 and 80–100 cm by soil drilling. There were 3 repeat samples for each treatment at different soil depths. The soil sample was screened for 2 mm and divided into two parts after impurity removal. Some fresh soil samples were used for the determination of ammonium nitrogen (NH_4-N), nitrate nitrogen (NO_3-N) and volumetric water content (VWC) in soil. In the other part, soil pH, total nitrogen (TN) and total organic carbon (TOC) were determined after natural air drying.

2.3. Determination of Soil Physical and Chemical Properties

Soil compactness was measured by digital soil compactness meter (SC-900, SPECTRUM, USA). Soil pH was measured by potentiometric method (Metler-Toledo S400-K

multifunctional pH meter), and soil and water ratio was 2.5:1. Soil TN and TOC were determined by continuous flow analyzer (AA3, SEAL, German) and automatic organic carbon analyzer (Primacs ATC100-IC, SKALAR, Holland). Soil NH_4-N and NO_3-N were determined by indophenol blue colorimetry and dual-wavelength ultraviolet spectrophotometry, respectively [38,39]. Soil VWC was determined by drying method.

2.4. Extraction and PCR Amplification of Soil Microbial Total DNA

Soil total DNA was extracted using the E.Z.N.A.® Soil DNA Kit (Omega Bio-Tek, Norcross, GA, USA). Approximately 0.5 g of fresh homogeneous soil was taken from each sample. The kit instructions were followed. DNA concentration and purity were detected by 1% agarose gel electrophoresis. The extracted DNA was diluted with TE buffer (10 mmol·L^{-1} Tris—HCl and 1 mmol·L^{-1} EDTA, PH 8.0) and stored in a −20 °C refrigerator for later use. Primers 338F (5′-ACT CCT ACG GGA GGC AGC A-3′) and 806R (5′-GGA CTA CHV GGG TWT CTA AT-3′) [40,41] were used to amplify the V3-V4 specific recognition region of bacterial 16S rRNA gene. PCR amplification was performed using a 50 μL system, including: 5 μL of 10× Ex Taq buffer (TaKaRa RR001A, Japan), 4 μL of dNTPs (2.5 μmol·L^{-1}), 1 μL each of upstream and upstream primers (10 μmol·L^{-1}), 1 μL of Ex Taq polymerase (5 U·μL^{-1}) and 2 μL of DNA template (1–10 ng), supplemented with ultra-pure water (ddH_2O) to 50 μL. Each reaction was repeated three times. The amplification conditions were as follows: pre-denaturation at 94 °C for 5 min, denaturation at 94 °C for 60 s, annealing at 48 °C for 60 s, stabilization at 72 °C for 60 s, 30 cycles, and extension at 72 °C for 10 min. To distinguish the samples, a specific peptide (barcode) of 8 bp length was added to the 5′ end of the upstream primers of each sample.

2.5. Purifying PCR Product and High-Throughput Sequencing Analysis

DNA amplification was purified and recovered using GeneJET Gel Extraction Kit (Thermo Scientific, Waltham, MA, USA). Quantification was performed using the QuantiFluor™-ST Blue fluorescence quantification system (Promega, Madison, WI, USA). Purified PCR products were mixed at an isogeneity ratio. The high-throughput sequencing was commissioned by Beijing Physicochemical Analysis and Testing Center. Based on Illumina HiSeq 2500 sequencing platform, 400 bp/450 bp primal-end pairing sequence sequencing was performed according to standard operating guidelines (Illumina, San Diego, CA, USA).

2.6. Sequencing Data Processing

The software FLASH (V1.2.7) [42] was first used to incorporate end-pair sequences from the original DNA fragment. According to the minimum overlap, length is 10 bp and the maximum error ratio allowed in overlap area is 0.2 (default), the reads of each sample are spliced. The resulting spliced sequence is the Raw Tags data. Then, Trimmomatic (v0.36) software [43] was used to filter the original Fastq file for quality control (remove joints and low quality bases) by setting sliding window parameters and other parameters as default: for a window of 50 bp, if the average quality value in the window is less than 20, the back-end bases are truncated from the window, and the Tags whose length is less than 75% of the Tags' length after quality control are filtered to obtain high-quality Clean Tags data. Finally, the chimera was removed by software UCHIME (v4.2) [44] to obtain high-quality sequences. The software UCLUST (v1.2.22) [45] was used to cluster the sequences at the 97% similarity level, and 0.005% of all sequence numbers were used as the threshold to filter these Operational Taxonomic Units (OTUs) [46]. The ribosome database RDP classifier (v.2.2) [47] was used in combination with Silva 128 database (www.arb-silva.de/no_cache/download) (accessed on 3 January 2019) to annotate the classification information for each representative sequence. The confidence threshold was 0.8. Soil bacteria α diversity index was analyzed by Mothur (v1.32.1) software (http://www.mothur.org/) (accessed on 4 January 2019) at 97% similarity level [48].

2.7. Statistical Analysis

The software SPSS 20.0 was used to perform multiple comparisons, variance analysis and correlation analysis on soil physicochemical properties, bacteria α diversity index and root length density. The significance level of the difference was $p < 0.05$. Principal Component Analysis (PCA) was used to compare the differences of bacterial community structure among samples. The Mantel test function of PC-ORD 5.0 software was used to screen the environmental factors strongly related to the bacterial community. Then, Redundancy Analysis (RDA) was performed using Canoco 5.0 software to explore the relationship between environmental factors and bacterial communities. The graphics were built using Microsoft Office Excel 2016.

3. Results

3.1. Soil Physical and Chemical Properties of Different Treated Soil Profiles (0–100 cm)

Subsoiling + rotary tillage significantly affected soil compactness of winter wheat topsoil (0–45 cm) (Figure 1). Soil compactness under low water condition was significantly higher than that under moderate soil water condition, and there was a significant difference between soil compactness under RT treatment and ST treatment. Under the condition of moderate soil moisture (Figure 1A), there was no significant difference in compactness in the 0–15 cm soil layer between different treatments. In the 0–12.5 cm soil layer, ST treatment was slightly higher than RT treatment. However, in the 15–45 cm soil layer, the soil compactivity of the RT treatment was significantly higher than that of ST ($p < 0.05$). It increased by an average of 2.30 times. The difference was most significant at 17.5 cm, which increased 4.13 times compared with ST. When the soil was dry (Figure 1B), there was no significant difference in compactness in the 0–7.5 cm soil layer under different treatments. However, when the soil depth was below 7.5 cm, the soil compactivity of RT treatment was gradually higher than that of ST treatment. The maximum value was reached at the soil depth of 15 cm, which was significantly increased by 10.63 times compared with the ST treatment ($p < 0.05$), and the compactness tester was difficult to set down. The compactness tester could be embedded into the soil depth of 35 cm in the ST treatment area. This indicated that the subsoiling + rotary tillage method reduced the compactness of the soil layer of 15–45 cm, which was beneficial to the growth of the root system and the deeper penetration of the crops.

As can be seen from Figure 2A,D, TOC and TN of different treatments have the same variation trend in the soil profile 0–100 cm. TOC and TN of the RT treatment were 23.43% and 20.46% higher than those of the ST treatment in the 0–10 cm topsoil, respectively ($p < 0.05$). There were no significant differences in TOC and TN contents between the two treatments in the 10–40 cm soil layer ($p < 0.05$). However, under 50 cm, ST processing of TOC and TN content was significantly higher than RT ($p < 0.05$), and the average increased by 37.93% and 53.11%, respectively. The content of NH_4-N in soil was the highest in the surface soil and decreased significantly with the increase in soil depth (Figure 2B), while NO_3-N showed an opposite trend (Figure 2C). Compared with RT, ST treatment significantly increased NH_4-N content in 0–100 cm soil layers ($p < 0.01$) by 65.88% on average, while NO_3-N content in each soil layer decreased by 17.74% on average ($p < 0.05$). In the topsoil layer (0–10 cm), the VWC of the ST treatment was slightly higher than that of the RT treatment (8.06% higher on average), but the VWC of the 10–20 cm ST treatment was significantly lower than that of the RT treatment (21.08% lower on average). In the soil layer below 20 cm, the VWC of the ST treatment was increased by 29.35% on average compared with that of RT treatment (Figure 2E). Especially in the deep soil layer below 40 cm, the VWC of the ST treatment was significantly higher than that of RT (47.54% on average), and the difference was significant ($p < 0.05$). With the increase in soil depth, the pH value of the two treatments showed a trend of first increasing and then decreasing (Figure 2F). The pH value of the ST treatment in each soil layer was significantly higher than that of the RT treatment ($p < 0.01$), which was 2.51% higher on average.

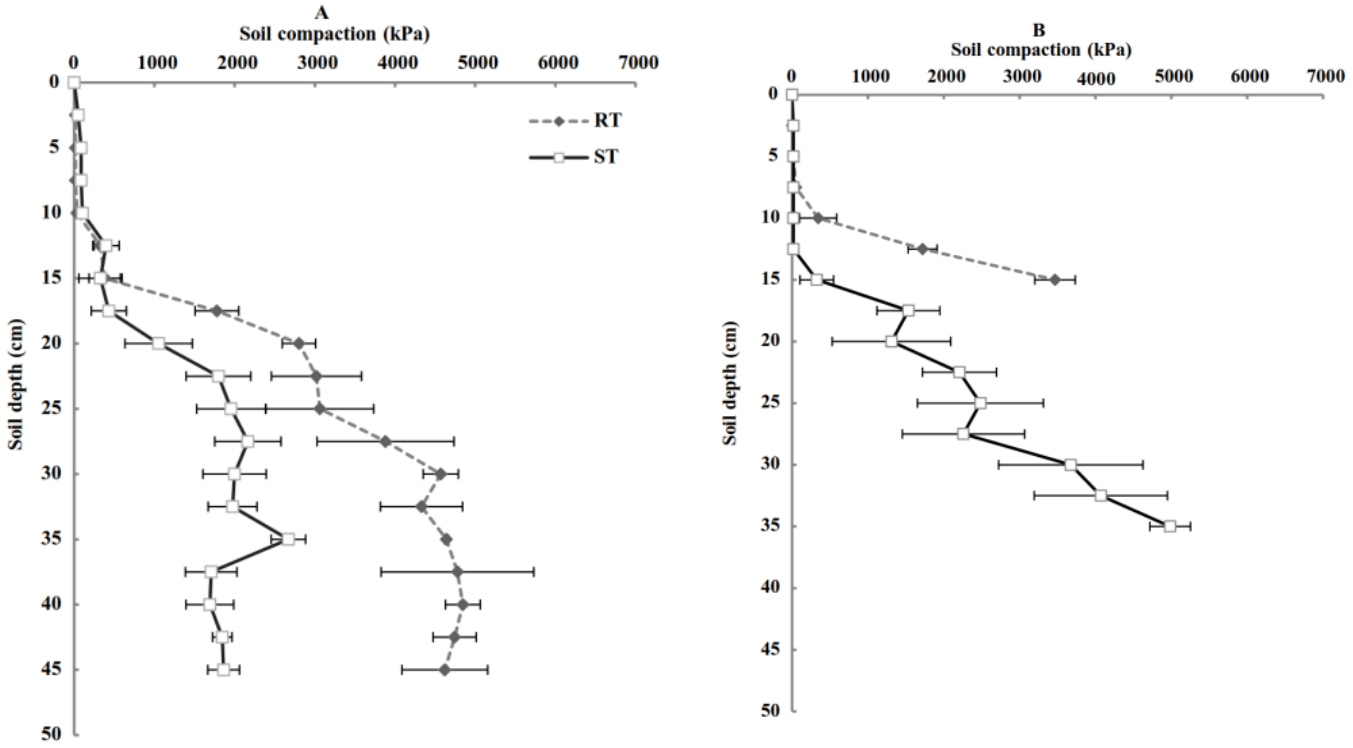

Figure 1. Effect of tillage methods on soil compactness at 0–45 cm ((**A**) moderate moisture; (**B**) low moisture). Data are mean ± standard deviation (n = 3).

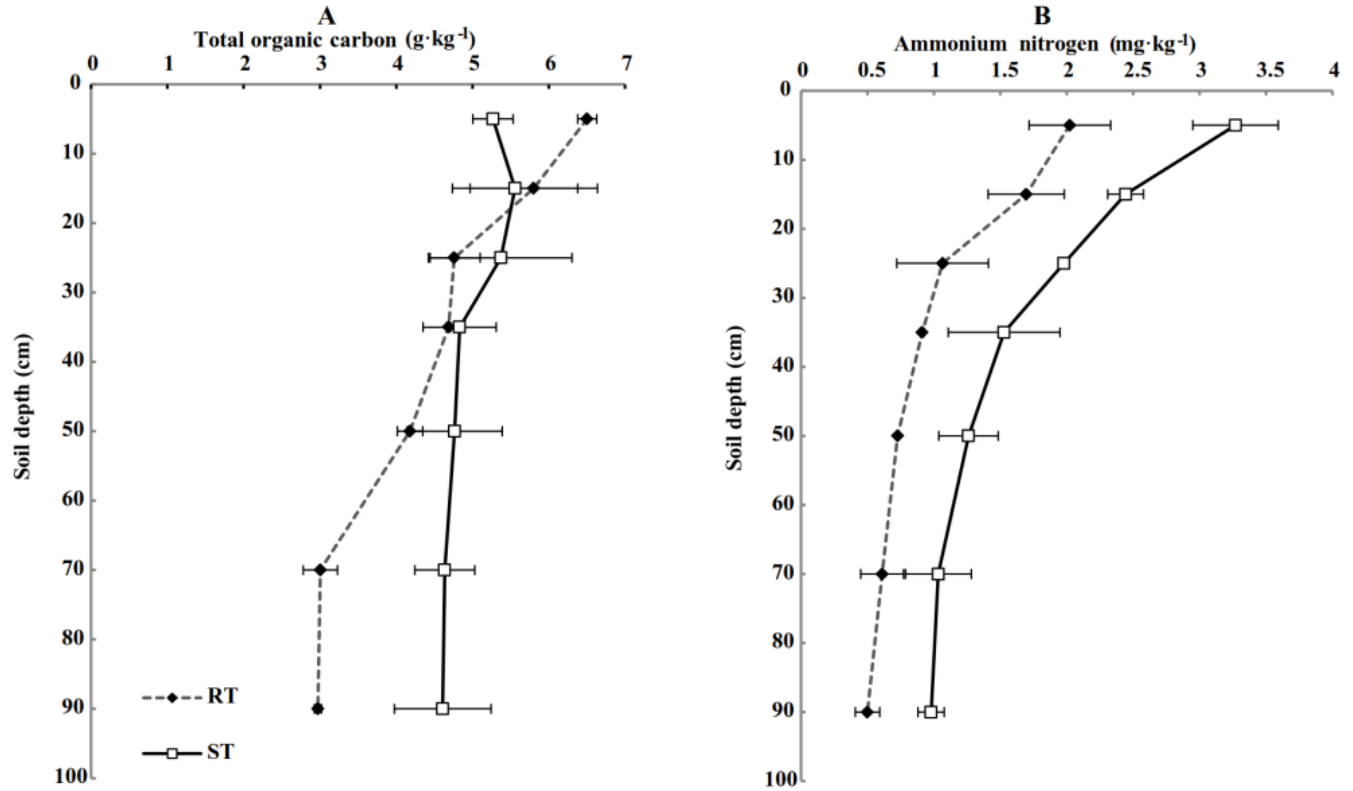

Figure 2. *Cont.*

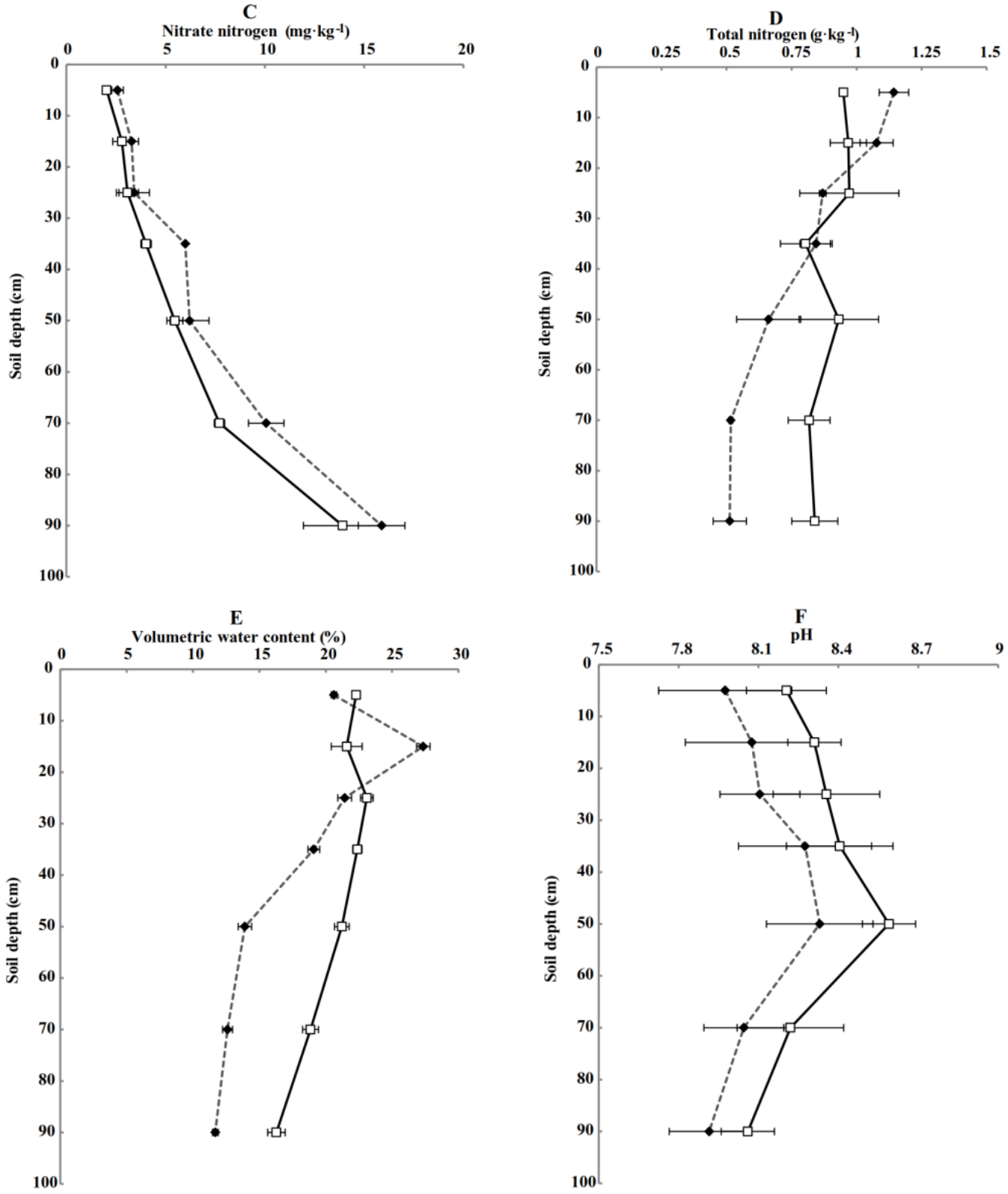

Figure 2. Effect of tillage methods on soil physicochemical property at 0–100 cm after harvest. (**A**) Total organic carbon; (**B**) Ammonium nitrogen; (**C**) Nitrate nitrogen; (**D**) Total nitrogen; (**E**) Volumetric water content; (**F**) pH. Data are means ± standard deviation (n = 3).

3.2. Wheat Root Length Density in Soil Profile (0–100 cm) under Different Treatments

As can be seen from Figure 3, from booting stage to flowering stage, the density of root length at each depth showed a trend of gradual increase, reaching the maximum at flowering stage. The difference in root length density between different treatments reached the maximum at booting stage and began to decrease at flowering stage. The root density decreased logarithmically with the increase in soil depth. At the booting stage of winter wheat (Figure 3A), the root length density of ST treatment was significantly higher than that of RT ($p < 0.05$) at all depths (2.85 times higher on average) when the soil depth was over 70 cm. In the deep soil below 70 cm, the differences between the two treatments in each depth gradually decreased, and the two lines overlapped. After entering the flowering stage (Figure 3B), the root development of winter wheat in each treatment was mature. The root length density of ST treatment was still significantly higher than that of RT. However, the difference in root length density between the two treatments at 0–60 cm was gradually reduced, which was 1.55 times higher on average than that at booting stage. However, in the soil below 60 cm, the difference between the two treatments at each depth increased significantly ($p < 0.05$), and the root length density of ST treatment increased 4.80 times that of RT on average. This indicates that subsoiling + rotary tillage can promote the root system to go down to the depth, accelerate the root growth in both horizontal and vertical directions and is conducive to improving the utilization rate of subsoil resources.

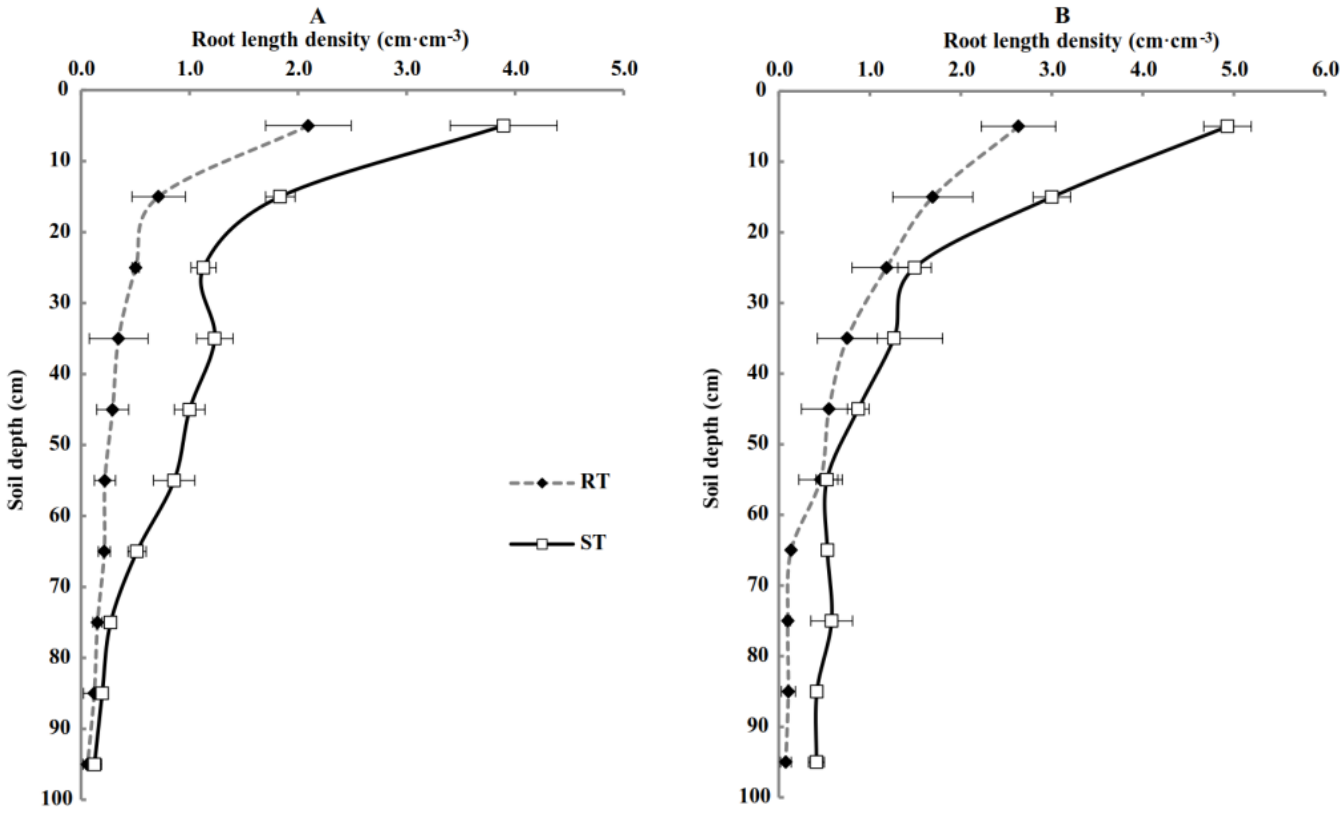

Figure 3. Effect of tillage methods on root length density at 0–100 cm ((**A**) booting stage; (**B**) flowering stage). Data are means ± standard deviation ($n = 3$).

3.3. Bacterial Community Composition in Surface Layer (0–20 cm) of Different Treatments

At the bacterial phylum level, a total of 44 bacterial groups were detected in all the treated soil samples, among which 11 genera were dominant (average relative abundance > 0.85% bacterial communities). The composition of dominant bacteria in each sample was similar, but the abundance varied (Figure 4). In the 0–10 cm soil layer, the relative abundances of *Proteobacteria*, *Bacteroidetes* and *GAL15* in the ST treatment were significantly

higher than that in the RT treatment (1.24, 3.41 and 6.75 times, respectively, $p < 0.001$). The relative abundances of *Acidobacteria*, *Gemmatimonadetes* and *Firmicutes* were significantly lower than that of RT (decreased by 29.94%, 41.36% and 67.80% on average, $p < 0.001$). The relative abundances of *Chloroflexi* and *Planctomycetes* were also significantly lower than that of the RT treatment (19.67% and 29.65%, respectively, $p < 0.01$). In the 10–20 cm soil layer, compared with RT treatment, the relative abundance of *Actinobacteria*, *Nitrospirae* and *GAL15* in ST increased by 1.93, 2.89 and 30.95 times, respectively, with extremely significant differences ($p < 0.001$). However, the relative abundances of *Acidobacteria*, *Gemmatimonadetes*, *Planctomycetes*, *Verrucomicrobia* and *Firmicutes* were significantly lower than that of RT (decreased by 17.31%, 57.78%, 59.39%, 26.19% and 88.62% on average, $p < 0.05$). In addition, with the increase in soil depth, more *Actinomycetes*, *Nitrospirobacteria* and *GAL15* were detected in the ST treatment, with an average increase of 2.29, 2.96 and 12.04 times compared with topsoil (0–10 cm), respectively ($p < 0.001$). The relative abundances of *Gemmatimonadetes* and *Bacteroidetes* were significantly lower than that of topsoil (36.25% and 81.50%, $p < 0.01$, respectively). Compared with topsoil, RT treatment significantly increased the relative abundance of *Firmicutes* in the 10–20 cm soil layer ($p < 0.01$) by an average of 4.17 times.

At the bacterial class level (Figure 5), a total of 128 bacterial groups were detected, among which 12 dominant bacteria were found (the average relative abundance was >3.00%). In the 0–10 cm soil layer, the relative abundances of *Gammaproteobacteria*, *Sphingobacteriia* (belonging to *Bacteroidetes*) and *MB-A2-108* (belonging to *Actinobacteria*) in the ST treatment were significantly increased compared with the RT treatment (average 2.13, 2.03 and 1.85 times higher, respectively, $p < 0.001$), while the relative abundance of *Subgroup_6* (*Acidobacteria*), *Gemmatimonadetes* and *Blastocatellia* (*Acidobacteria*) decreased significantly (26.09%, 46.53% and 41.42%, respectively, $p < 0.001$). The relative abundances of *Thermoleophilia* (*Actinobacteria*), *MB-A2-108* and *Acidimicrobiia* (*Actinobacteria*) were significantly increased by 1.91, 2.70 and 1.49 times in the 10–20 cm soil layer compared with RT ($p < 0.001$), and the relative abundances of *Alphaproteobacteria*, *Subgroup_6* and *Gemmatimonadetes* were significantly decreased (35.25%, 29.49% and 56.62%, respectively, $p < 0.01$). In addition, the ST treatment significantly decreased the relative abundance of *Alphaproteobacteria* and *Gammaproteobacteria* (29.64% and 49.31%, $p < 0.01$, respectively) with increasing soil depth, while the RT treatment increased the relative abundance of *Alphaproteobacteria* and *Gammaproteobacteria* (1.18 and 1.38 times, respectively).

At the level of bacterial order, a total of 194 taxa were found. Nine dominant bacterial orders (average relative abundance > 1.50%) and three typical bacterial genera (typical ammoniating and denitrification bacteria) were selected for comparison (Table 1). In the 0–10 cm soil layer, the relative abundances of *Sphingomonadale* (belonging to *Alphaproteobacteria*), *Xanthomonadales* (belonging to *Gammaproteobacteria*), *Flavobacteriales* (belonging to *Bacteroidetes*) and *Serratia* (typical ammoniating bacteria) treated by ST were significantly higher than that of RT (average of 1.16, 1.91, 36.80 and 7.17 times, respectively, $p < 0.05$). However, the relative abundance of *Nitrosomonadales* (*Betaproteobacteria*) was significantly lower than that of RT (22.17% on average, $p < 0.05$). In the 10–20 cm soil layer, the relative abundance of *Nitrospirales* (*Nitrospirae*) in the ST treatment was significantly higher than that in RT (2.89 times higher on average, $p < 0.05$). The relative abundances of *Sphinomonas* and *Flavobacteria* were significantly lower than that of RT (25.32% and 69.96% on average, $p < 0.05$). In addition, compared with the RT treatment, ST treatment also significantly increased the relative abundance of *Rhizobiales* (*Alphaproteobacteria*), *Burkholderiales* (*Betaproteobacteria*), *Gaiellales* (*Actinobacteria*) and *Pseudomonas* (typical ammoniated bacteria) in the 0–20 cm surface layer (decreased by 1.19–1.32, 2.30–3.17, 1.41–2.15 and 2.68–3.79 times, respectively, $p < 0.05$). This treatment significantly reduced the relative abundance of *Rhodospirillales* (*Alphaproteobacteria*, typical denitrification bacteria) and *Bacillus* (typical denitrification bacteria) in the surface layer (decreased by 27.87–58.15% and 87.19–92.54%, respectively, $p < 0.05$).

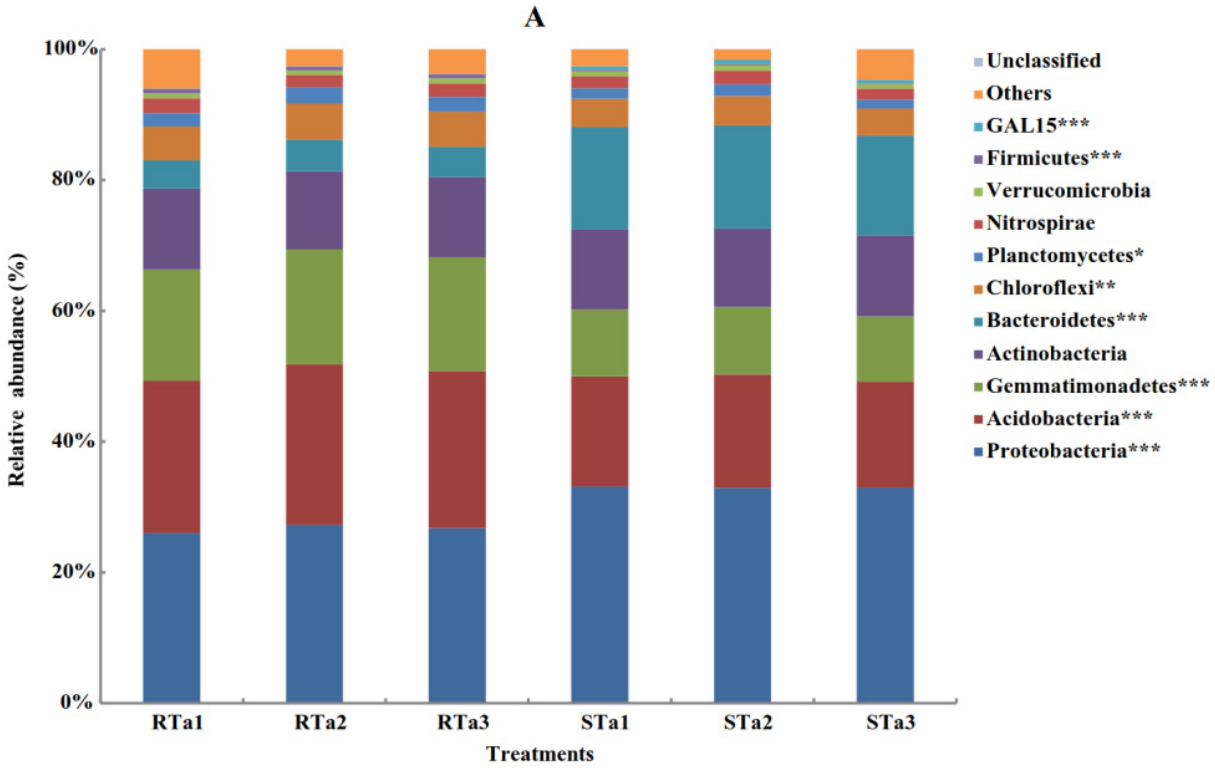

Figure 4. *Cont.*

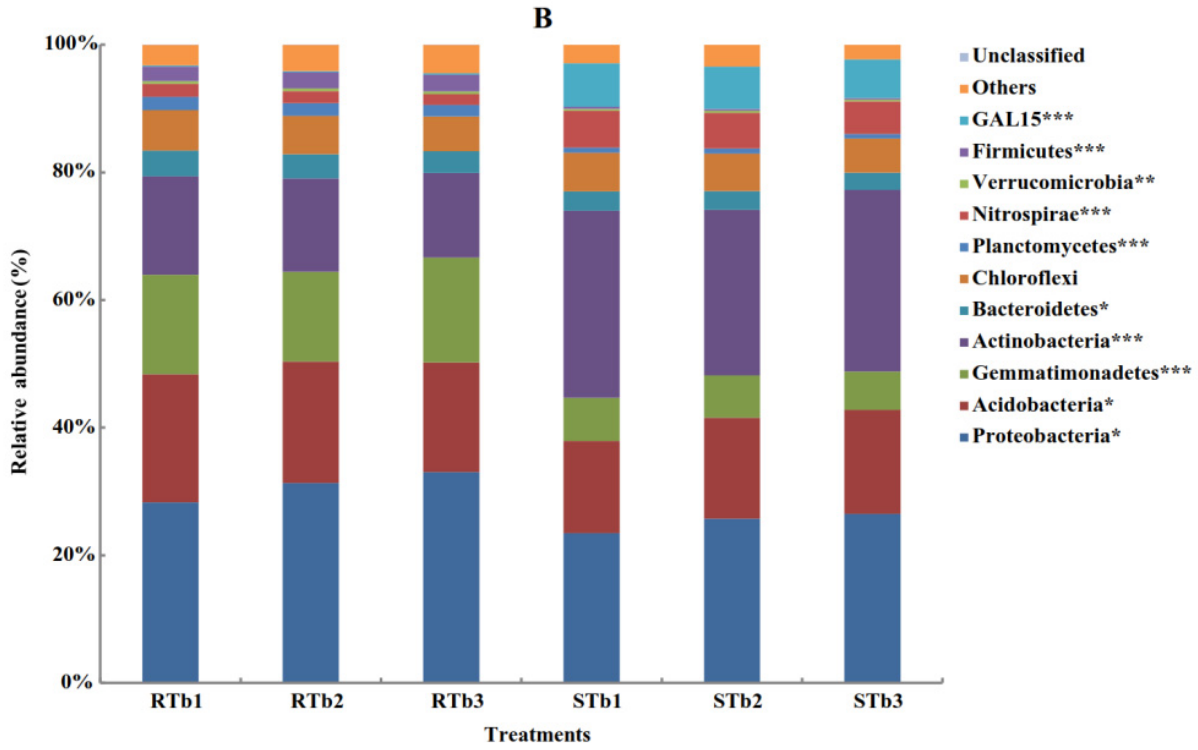

Figure 4. Comparison of the dominant bacterial communities at phylum level (%) in different samples. Number means three replications under the same treatment. (**A**) 0–10 cm; (**B**) 10–20 cm. Significant differences in relative abundance between different treatments are marked with a star: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, the same as below.

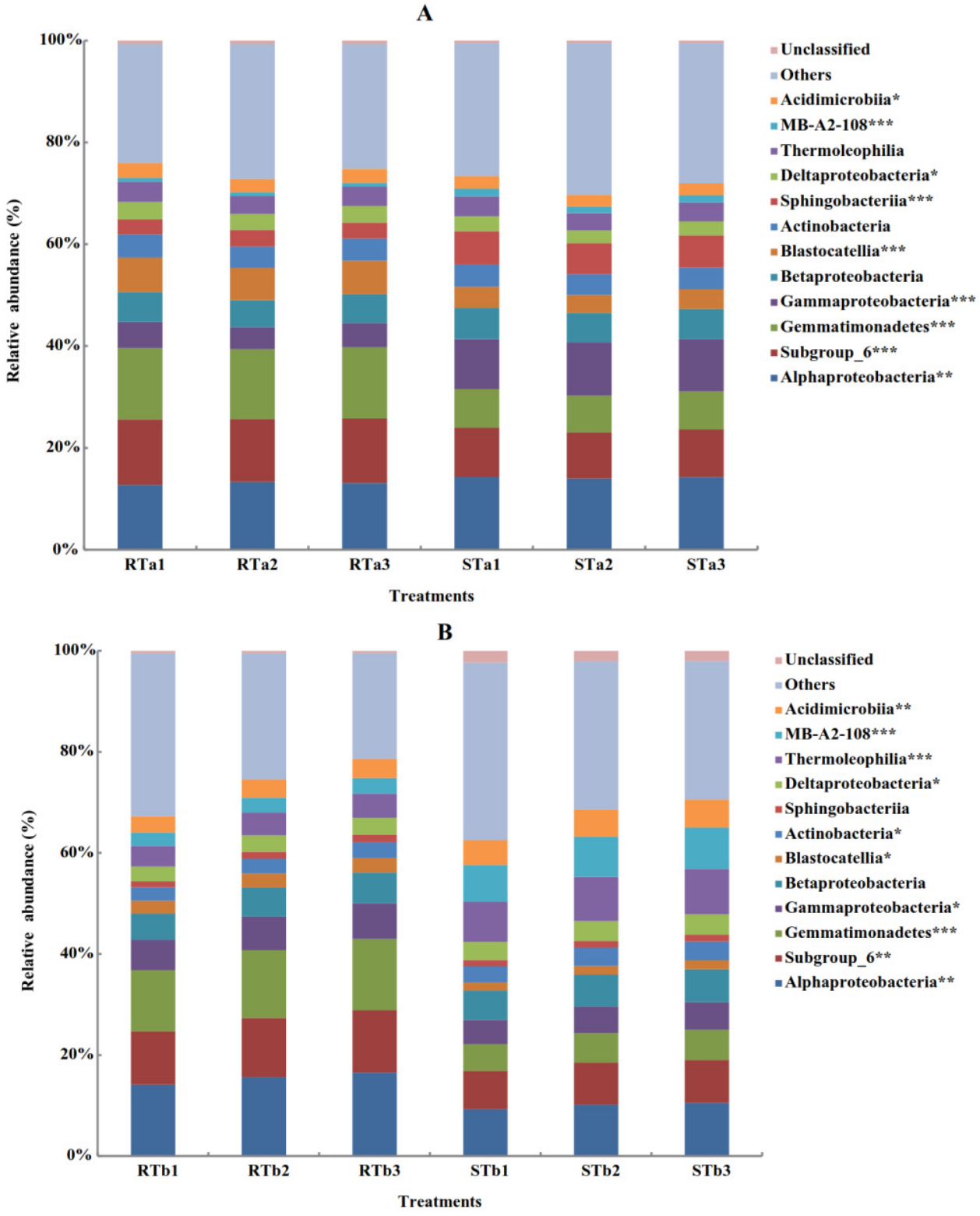

Figure 5. Comparison of the dominant bacterial communities at class level (%) in different samples.

Table 1. Comparison of the dominant bacterial orders and typical bacterial genera and at order and genus levels (%) in different treatments.

Bacterial Types	Rotary Tillage (0–10 cm) RTa	Subsoiling and Rotary Tillage (0–10 cm) STa	Rotary Tillage (10–20 cm) RTb	Subsoiling and Rotary Tillage (10–20 cm) STb
o_Sphingomonadales	5.9130 ± 0.1532 b	6.9066 ± 0.1526 a	3.8256 ± 0.2437 c	2.8571 ± 0.1459 d
o_Xanthomonadales	3.9411 ± 0.3192 b	7.5155 ± 0.1482 a	4.5856 ± 0.2921 b	4.4502 ± 0.2271 b
o_Rhodospirillales	3.6096 ± 0.0760 b	2.6035 ± 0.1552 c	8.9473 ± 0.5699 a	3.7447 ± 0.1912 b
o_Rhizobiales	2.5410 ± 0.1592 b	3.0216 ± 0.1693 a	1.9061 ± 0.1214 c	2.5232 ± 0.1289 b
o_Nitrosomonadales	3.3418 ± 0.2391 a	2.6010 ± 0.1526 b	3.7302 ± 0.2376 a	3.2833 ± 0.1676 a
o_Flavobacteriales	0.2149 ± 0.0109 bc	7.9082 ± 0.1510 a	0.3880 ± 0.0247 b	0.1166 ± 0.0062 c
o_Nitrospirales	2.0583 ± 0.2735 b	1.8522 ± 0.1974 b	1.8945 ± 0.1207 b	5.4689 ± 0.2791 a
o_Burkholderiales	1.0182 ± 0.0135 b	2.3415 ± 0.0295 a	0.7225 ± 0.0460 c	2.2937 ± 0.1172 a
o_Gaiellales	1.4707 ± 0.0541 d	2.0693 ± 0.0516 c	2.8266 ± 0.1801 b	6.0688 ± 0.3097 a
g_Bacillus	0.2384 ± 0.0108 a	0.0305 ± 0.0022 c	0.1953 ± 0.0047 b	0.0146 ± 0.0004 c
g_Pseudomonas	0.0672 ± 0.0021 b	0.2544 ± 0.0091 a	0.0294 ± 0.0038 c	0.0789 ± 0.0021 b
g_Serratia	0.0445 ± 0.0027 b	0.3189 ± 0.0128 a	N	N

Data are means ± standard deviation, $n = 3$. The different lowercase letters in a row indicate significant differences among treatments (Tukey method, $p < 0.05$). RTa, rotary tillage (0–10 cm soil sample); STa, subsoiling + rotary tillage (0–10 cm soil sample); RTb, rotary tillage (10–20 cm soil sample); STb, subsoiling + rotary tillage (10–20 cm soil sample), the same as below.

3.4. Soil Bacterial Diversity Index of Surface Layer (0–20 cm) under Different Treatments

The V3-V4 specific identification regions of 16S rRNA from soil bacteria were sequenced using the Illumina HiSeq 2500 platform. A total of 1,388,877 effective sequences were obtained. The average sequence length was 420.75 bp, the GC content was 56.12–58.53% and the Q20 content was 96.13–96.24%. After screening, >79,598 high-quality sequences were obtained from each sample, including 2126–3664 OTUs, and the sequencing coverage was 0.9951–0.9965 (Table 2). The OTU dilution curve of each sample tended to be flat, indicating that the sequencing depth had basically covered all bacterial species in the sample, and the sequencing results could represent the real situation of the sample.

Table 2. Comparison of α-diversity parameters in different treatments.

Soil Depth (cm)	Sample	Operational Taxonomic Units (OTUs)	Chao1 Index	Shannon Index	Simpson Index	Coverage
0–10	RTa	3632 ± 36 a	4022.20 ± 39.53 a	6.5965 ± 0.0794 a	0.0042 ± 0.0001 c	0.9953 ± 0.0009 a
	STa	3664 ± 38 a	4062.84 ± 42.60 a	6.5772 ± 0.0382 a	0.0044 ± 0.0002 c	0.9951 ± 0.0008 a
10–20	RTb	2934 ± 115 b	3164.35 ± 124.68 b	6.3918 ± 0.0573 b	0.0051 ± 0.0002 b	0.9965 ± 0.0011 a
	STb	2126 ± 54 c	2387.48 ± 61.72 c	6.0779 ± 0.0436 c	0.0059 ± 0.0002 a	0.9958 ± 0.0010 a

Data are means ± standard deviation, $n = 3$. The different lowercase letters in a column indicate significant differences among treatments at $p < 0.05$ level.

Analysis of bacterial α diversity (Table 2) showed that there was no significant difference among all α diversity indices of different treatments in the 0–10 cm topsoil ($p < 0.05$). In the 10–20 cm soil layer, the Chao1 and Shannon indices of ST treatment were significantly lower than RT, while the Simpson index was significantly higher than RT ($p < 0.05$). At the same time, the Chao1 and Shannon indices in the 10–20 cm layer were significantly lower than that in the 0–10 cm layer, while the Simpson index was significantly higher than that in the 0–10 cm layer ($p < 0.05$). PCA was used to compare the β diversity of the soil bacterial community between samples under two tillage methods, as shown in Figure 6. The dimension reduction analysis explained 90.83% of the differences in bacterial structure, among which the first variable axis (PC1) and the second variable axis (PC2) explained 79.14% and 11.69% of the differences in bacterial structure, respectively. Among them, the two treatments (RTa and STa) at 0–10 cm soil depth gathered together, but at 10–20 cm soil depth, the treatment points of STb and RTb were far apart, and the OTU composition of the

two treatments was significantly different, indicating that the effect of tillage method on the bacterial community structure of the 0–10 cm topsoil was not obvious. However, the community structure in the 10–20 cm soil layer was significantly affected. In addition, the two soil layers treated by RT (RTa and RTb) were close to each other, while the different soil layers treated by ST (STa and STb) were far away, indicating that ST treatment had a significant effect on the bacterial community structure in different soil layers.

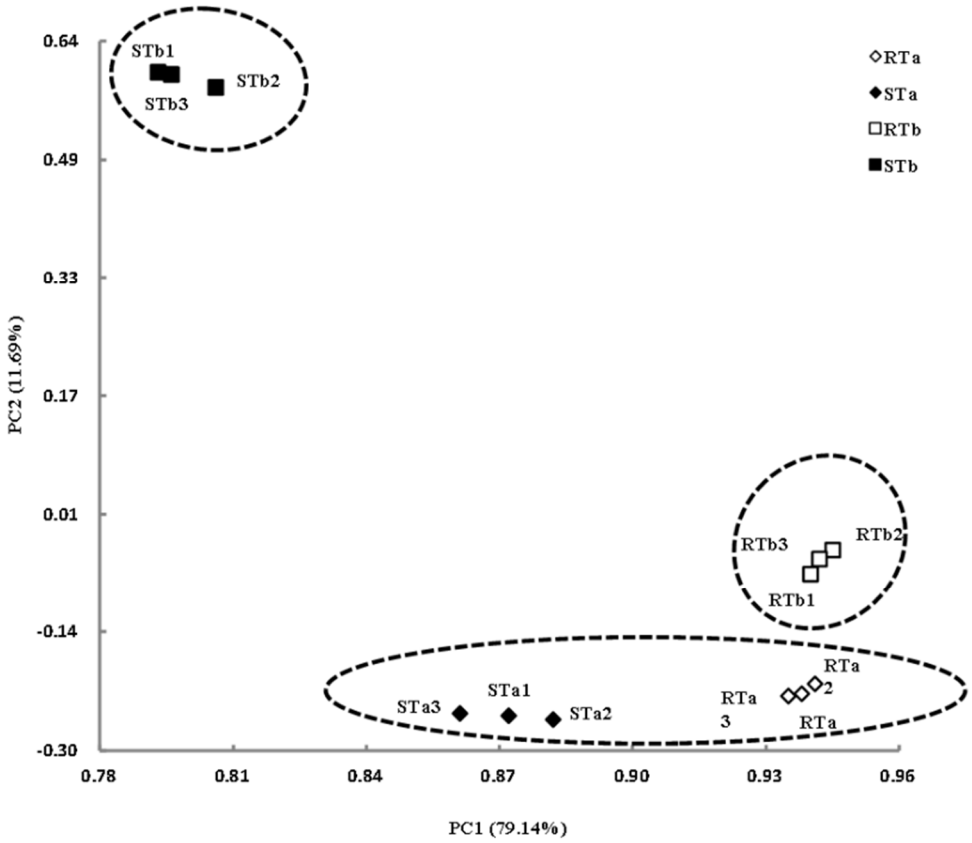

Figure 6. Comparison of bacterial communities in different treatments through Principal Component Analysis (PCA).

3.5. Relationship between Bacterial Community Structure in Surface Layer (0–20 cm) of Different Treatments and Soil Physical and Chemical Properties and Root Length Density

Different tillage methods and sampling depth significantly affected soil physical and chemical properties, root length density and bacteria α diversity index (Table S1). Analysis of variance showed that tillage significantly changed the physical and chemical parameters and diversity index of different treatments, except the C/N ratio (F = 0.359, p = 0.565). Sampling depth also significantly affected other parameters, except total nitrogen (F = 0.549, p = 0.480) and C/N ratio (F = 0.070, p = 0.797). Their interactions significantly changed total nitrogen (F = 1.783, p = 0.218), pH (F = 0.055, p = 0.821), C/N ratio (F = 2.088, p = 0.186), root length density (F = 3.501, p = 0.098) and Simpson index (F = 4.696, p = 0.062), and the significant level of other parameters was $p < 0.05$.

The correlation coefficient R and significant p values of Pearson correlation analysis were used to determine the correlation strength between soil physical and chemical property parameters and root length density. The analysis showed that RLD was significantly positively correlated with NH_4-N content (r = 0.943, $p < 0.01$), negatively correlated with TN content and SC content ($p < 0.05$) and negatively correlated with NO_3-N content (r = −0.898, $p < 0.01$) at flowering time. SC was positively correlated with VWC and NO_3-N content (r = 0.867, 0.869, $p < 0.01$) and negatively correlated with NH_4-N content ($p < 0.05$). There was a significant negative correlation between soil pH and the contents of TN and

TOC (r = −0.805, −0.815, $p < 0.01$). There was a significant positive correlation between TN content and TOC (r = 0.822, $p < 0.01$), and a significant negative correlation between TN content and NH_4-N ($p < 0.05$). The content of NH_4-N was negatively correlated with NO_3-N (r = −0.891, $p < 0.01$) and with TOC ($p < 0.05$).

Further correlation analysis showed (Figure 7) that the Chao1 index showed significant negative correlation with surface nitrate nitrogen content and pH ($p < 0.05$); the Shannon index had a significant negative correlation with soil pH, while the Simpson index had a significant positive correlation with soil pH ($p < 0.05$), indicating that soil pH was the main factor driving the change in soil bacteria α diversity index. The relative abundances of *GAL15*, *MB-A2-108*, *Acidimicrobiia*, *Nitrospirales* and *Gaiellales* were significantly positively correlated with soil pH ($p < 0.05$). The relative abundances of *Xanthomonadales*, *Flavobacteriales*, *Pseudomonas* and *Serratia* had significant positive correlation with NH_4-N content and RLD at flowering stage ($p < 0.01$), and NO_3-N content showed significant negative correlation ($p < 0.01$). The relative abundance of *Rhizobiales* was positively correlated with NH_4-N content and RLD ($p < 0.01$), and negatively correlated with NO_3-N content, VWC and SC ($p < 0.01$). The relative abundance of *Rhodospirillales* showed an opposite trend with these environmental factors. The relative abundances of *Subgroup_6* and *Blastocatellia* were significantly negatively correlated with soil pH ($p < 0.01$) and significantly positively correlated with TOC and TN contents ($p < 0.05$). The relative abundance of *Burkholderiales* was positively correlated with the contents of NH_4-N, pH and RLD ($p < 0.01$), and negatively correlated with the contents of TN and TOC ($p < 0.01$). The correlation between the relative abundance of *Bacillus* and these environmental factors showed the opposite trend. The relative abundance of *Nitrosomonadales* was significantly positively correlated with NO_3-N content ($p < 0.001$) and negatively correlated with NH_4-N content and RLD ($p < 0.001$). The relative abundance of *Gemmatimonadetes* had a significant positive correlation with TN content ($p < 0.001$) and a significant negative correlation with pH and NH_4-N content ($p < 0.01$).

The r value and the significance level p value obtained by the Mantel test were used to exclude the environmental factors with weak correlation with bacterial community distribution in the cultivation layer, which were volumetric water content (r = 0.088, $p = 0.209$) and carbon/nitrogen ratio (r = −0.020, $p = 0.499$), respectively. The remaining seven environmental factors passed the Mantel test (r = 0.468, $p = 0.005$), and the subsequent RDA could be performed. After importing the data, the Canoco software automatically calculated the gradient length, which in this case was 0.8 sd, less than 3, the recommended RDA for linear methods. The results showed that the contents of pH, NH_4-N and NO_3-N in the topsoil had the most significant effect on the bacterial community structure. The cumulative contribution rate of these three variables reached 97%, and the order of influence was: pH > NH_4-N > NO_3-N (all p values < 0.01).

Redundancy Analysis also showed that there was a significant differentiation of soil bacterial communities under different tillage practices (Figure 8). As can be seen from the figure, the NH_4-N content, pH and RLD of the ST treatment are higher, and *GAL15*, *MB-A2-108*, *Xanthomonadales*, *Rhizobiales*, *Flavobacteriales*, *Nitrospirales*, *Burkholderiales*, *Gaiellales*, *Pseudomonas* and *Serratia* were enriched here. The relative abundance of these bacteria was related to higher NH_4-N content, pH and RLD. However, the RT treatment contained higher concentrations of *Subgroup_6*, *Gemmatimonadetes*, *Rhodospirillales*, *Nitrosomonadales* and *Bacillus* and higher contents of TN, TOC and NO_3-N, and the results more directly reflected the differences of bacterial colonies in different treatments and their correlation with soil physical and chemical properties and root length density.

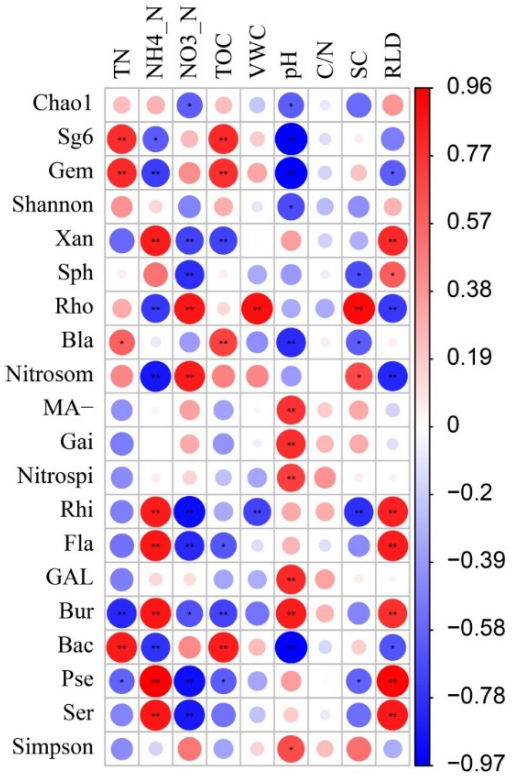

Figure 7. Pearson correlation coefficients between bacterial alpha diversity index, dominant bacterial abundance and soil physicochemical property, root length density in plow layer (0–20 cm). *GAL*: *GAL15*, *Sg6*: *Subgroup_6*, *Gem*: *Gemmatimonadetes*, *Bla*: *Blastocatellia*, *MA-*: *MB-A2-108*, *Aci*: *Acidimicrobiia*, *Sph*: *Sphingomonadales*, *Xan*: *Xanthomonadales*, *Rho*: *Rhodospirillales*, *Rhi*: *Rhizobiales*, *Nitrosom*: *Nitrosomonadales*, *Fla*: *Flavobacteriales*, *Nitrospi*: *Nitrospirales*, *Bur*: *Burkholderiales*, *Gai*: *Gaiellales*, *Bac*: *Bacillus*, *Pse*: *Pseudomonas*, *Ser*: *Serratia*. Significant differences in relative abundance between different treatments are marked with a star: * $p < 0.05$, ** $p < 0.01$. The same as below.

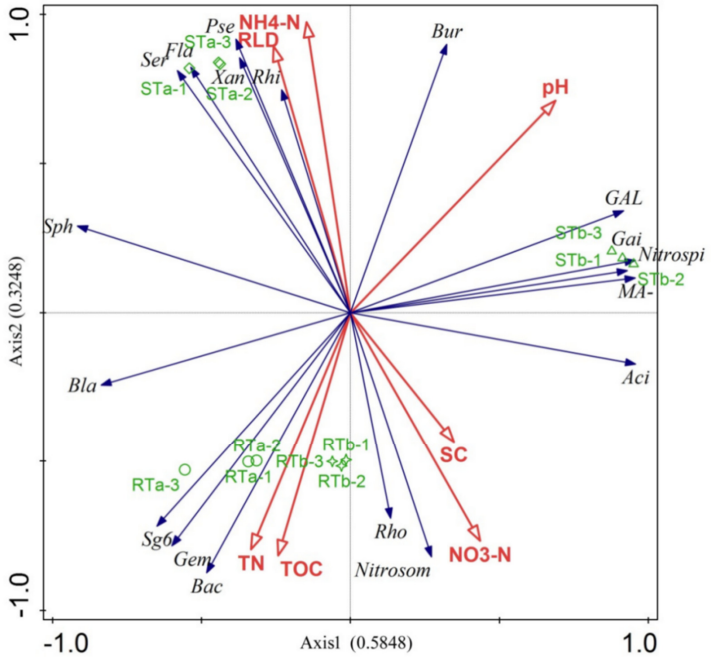

Figure 8. Redundancy Analysis (RDA) of soil properties, root length density and bacterial community structure under different treatments in plow layer.

4. Discussion

4.1. Effects of Subsoiling + Rotary Tillage on Physical and Chemical Properties and Root Length Density of Soil Profile (0–100 cm)

Subsoiling can break the hard plow bottom and improve the plant utilization of subsoil resources [13]. In this study, it was found that subsoiling + rotary tillage significantly reduced the compactedness of the 15–45 cm soil layer (Figure 1), significantly increased the water storage, organic carbon and total nitrogen contents of the deep soil (>40 cm), significantly increased the pH and ammonium nitrogen contents of the whole soil layer and reduced the nitrate nitrogen residue and leaching loss (Figure 2). Moreover, the root length density of the 0–70 cm deep soil at booting stage and 0–100 cm deep soil at flowering stage of winter wheat were significantly increased (Figure 3), especially the root length density of the 60–100 cm deep soil at flowering stage. These findings indicate that subsoiling + rotary tillage can accelerate root growth in both horizontal and vertical directions, promote the early development of roots and deeper roots, improve the utilization rate of subsoil water and nitrate nitrogen, improve the quality of subsoiling and nutrient cycling of organic fertilizer and alleviate the water and nutrient stress of crops.

4.2. Effects of Subsoiling + Rotary Tillage on Bacterial Diversity in Topsoil (0–20 cm)

Diversity index is an important index to describe the characteristics of community structure. It is used to judge the stability of the community system. The higher the Chao1 and Shannon values are, the smaller the Simpson values are, indicating the higher species richness and diversity [49]. A large number of studies have shown that excessive human disturbance during agricultural production can significantly reduce the diversity of soil microbial community structure and biological activity [50]. In addition, the number and diversity of bacterial colonies decreased with soil depth, and the relative abundance of *Proteobacteria* decreased most significantly [51]. The diversity analysis in this paper also showed that the abundance and diversity of topsoil flora were significantly affected by tillage methods and sampling depth (Table S1). Subsoiling + rotary tillage significantly decreased the bacterial abundance and diversity in the 10–20 cm soil layer ($p < 0.05$), and the bacterial colony abundance and diversity showed a downward trend with the increase in soil depth (Table 2). In addition, soil pH is the key factor driving the change in soil microbial activity and community diversity [52]. In this study, the Chao1 and Shannon indices of cultivator colonies were negatively correlated with pH, while the Simpson index was positively correlated with pH (Figure 7), indicating that soil pH was an important factor affecting the abundance and diversity of cultivator bacterial communities. As mentioned above, subsoiling + rotary tillage significantly increased soil pH (Figure 2F). The increase in pH led to the decrease in the Chao1 and Shannon indices and the increase in the Simpson index, and it was concluded that the increase in pH was the main reason for the decrease in the abundance and diversity of the bacterial community in the tillage layer after subsoiling.

4.3. Differences in Bacterial Community Composition and Structure in Surface Layer under Different Tillage Treatments and Their Relationship with Physical and Chemical Properties and Root Length Density

Subsoiling technology can break the plow bottom, improve soil permeability, improve surface layer structure and soil environment and inevitably cause changes in soil microbial community structure, which means that some microbial communities can adapt to the changed environment, while others cannot [53]. The relative abundance of *GAL15*, *Actinobacteria* and *Nitrospirae* increased significantly by subsoiling + rotary tillage in the topsoil layer, especially in the 10–20 cm soil layer ($p < 0.001$) (Figure 4). Among them, the relative abundances of *Acidimicrobiia*, *MB-A2-108*, *Thermoleophilia* (the dominant order is *Gaiellales*) (all belong to *Actinobacteria*), *Nitrospirales*, *Rhizobiales* (*Alphaproteobacteria*), *Burkholderiales* (*Betaproteobacteria*) and some typical ammoniating bacteria (*Pseudomonas* and *Serratia*) were significantly increased, while the relative abundance of *Nitrosomonadales* (*Betaproteobacteria*, ammonia-oxidizing bacteria) was not significantly different in

the 10–20 cm soil layer under different tillage treatments (Figure 5, Table 1). Most of these bacteria play a certain role in soil nitrogen cycling and maintain positive and beneficial interactions with plant root growth [54]. Among them, *Rhizobiales* and *Burkholderiales* are involved in biological nitrogen fixation, and most of them are concentrated in plant roots and surrounding areas [55]; *Pseudomonas* and *Serratia* are typical aerobic ammoniators, which are involved in the decomposition of organic nitrogen to produce ammonia available to plants [56,57]; as the main groups of aerobic nitrification bacteria, *Nitrospirales* and *Nitrosomonadales* can oxidize ammonia or ammonium salts in soil to nitrite and nitrite to nitrate [58]; *Acidimicrobiia*, *MB-A2-108*, and *Thermoleophilia* belong to *Actinobacteria*, which prefer neutral or slightly alkaline soils and are involved in the decomposition of organic nitrogen to produce ammonia and antibiotics. They have been considered to have outstanding effects on biological control, promoting the interaction between crop growth and root system, etc. [59]. The correlation analysis (Figure 7) and RDA (Figure 8) in this paper also showed that the relative abundances of these flora were positively correlated with soil pH, NH_4-N content and RLD ($p < 0.01$), and negatively correlated with NO_3-N content ($p < 0.01$). The preliminary understanding is that subsoiling + rotary tillage increased the pH and permeability of the surface soil, promoted the decomposition of organic nitrogen by aerobic ammoniating bacteria and *Actinobacteria*, accelerated the mineralization of organic matter and increased the content of ammonium nitrogen. At the same time, subsoil tillage broke the plow bottom to accelerate the root growth, promoted the biological nitrogen fixation of *Rhizobiales* and *Burkholderiales* and nitro oxidation of *Nitrospirales*, reduced the residual nitrite in the soil and improved the soil ammonium nitrogen content and nitrate nitrogen utilization rate. However, with the increase in ammonium nitrogen content, the relative abundance of *Nitrosomonadales*, which uses ammonia or ammonium salt as energy source, did not change significantly. This may be due to the reduction in CO_2 (reduction in carbon source) due to the exposure of organic matter due to subsoil tillage and the competition between bacteria. In addition, studies have shown that *Nitrosomonadales* is adapted to the growth of low-concentration ammonium nitrogen environment, and there is no significant correlation with ammonium nitrogen content [60]. The specific reasons need further research and verification.

In addition, subsoiling + rotary tillage significantly decreased the relative abundance of *Acidobacteria*, *Gemmatimonadetes* and *Firmicutes* in the surface layer ($p < 0.05$), among which the relative abundance of *Subgroup_6*, *Blastocatellia* (all belong to *Acidobacteria*), *Rhodospirillales* (belong to *Alphaproteobacteria* and typical denitrification bacteria) and *Bacillus* (belong to *Firmicutes* and typical denitrification bacteria) showed a trend of significant decrease (Figures 4 and 5, Table 1). *Acidobacteria* are widely distributed in the soil environment, accounting for about 20% of the total bacteria [61]. It played a very important role in the process of soil material circulation and ecological environment construction [62]. A large number of studies have shown that the relative abundance of *Acidobacteria* is significantly negatively correlated with soil pH, and significantly positively correlated with average precipitation and organic carbon content [63]. Some studies have found that *Gemmatimonadetes* and *Bacillus* are also significantly correlated with soil pH, organic carbon and water content [64]. *Rhodospirillum* and *Bacillus* can use nitrite and nitrate nitrogen as nitrogen sources to perform denitrification under anoxic conditions [65]. In this study, correlation analysis (Figure 7) and RDA (Figure 8) also showed that the relative abundances of *Subgroup_6*, *Blastocatellia*, *Gemmatimonadetes* and *Bacillus* were significantly negatively correlated with soil pH, and significantly positively correlated with organic carbon and total nitrogen contents. The relative abundance of *Rhodospirillales* was positively correlated with soil compaction and nitrate nitrogen content, but negatively correlated with ammonium nitrogen content and RLD ($p < 0.01$). Therefore, subsoiling + rotary tillage increased soil pH, decreased soil compactness and reduced the number of acidophilic bacteria and denitrification bacteria, thus slowing down soil denitrification and inhibiting the loss of soil available nitrogen.

At the level of microbial classification, existing studies have shown that pH, organic matter composition, soil types and plants have a great influence on soil microbial community structure [63]. Microbial metabolic processes may also alter the biogeochemical cycle of soil nutrient elements, including decomposition of organic matter, nitrogen conversion and storage. These microbial-mediated processes will affect soil physical and chemical properties, and then affect soil microbial diversity, bacterial community composition, function and soil ecosystem processes, and promote or inhibit root nutrient uptake and growth. Plant root growth and the production of root exudates also provide carbon sources and energy for microorganisms. The composition and quantity of exudates affect the species, reproduction and metabolic activities of microorganisms [66], resulting in the formation of a special microecological environment around the root system that is different from other parts of the soil. Therefore, there is a complex trilateral relationship between the soil, microorganism and plant. The changes in soil structure and environment caused by different tillage systems and agronomic management will inevitably affect the growth of crops and the distribution of microorganisms in the soil. In this study, it was concluded that subsoiling + rotary tillage broke the plow bottom, increased soil pH and ammonium nitrogen content, reduced nitrate nitrogen residue and promoted root growth. In addition, subsoiling was not conducive to the abundance and diversity of the bacterial community in the plow layer, but it significantly increased the soil bacteria (biological nitrogen-fixing bacteria, ammonifying bacteria, denitrifying bacteria), reduced the nitrogen loss caused by denitrifying bacteria and improved the soil nutrient utilization of plants.

Therefore, how should we correctly apply subsoiling technology, long-term application or short-term application? It is believed that long-term subsoiling tillage has proven to be an effective measure for remediating the plow layer and improving the structure and physical properties of the soil [67]. However, the subsoiling needs to be equipped with high-power tractors, which ordinary farmers cannot afford. Meanwhile, the reasonable plow layer takes time to rebuild after subsoiling, because it also positively affects soil physical and chemical properties, crop yield increase, etc. [68]. In my opinion, a subsoiling operation should be carried out after 2–3 years of topsoil cultivation. We should pay attention to the remarkable effect of combining subsoiling and minimal tillage with building a reasonable arable layer. In addition, this study only analyzed the effects of subsoiling + rotary tillage on some soil physical and chemical properties, bacterial community structure and wheat root length density, and did not investigate the overall soil microbial community (including archaea, fungi, etc.) and crop root exudates, in which the complex interaction mechanism between soil, microorganisms and plants needs further study and proof.

5. Conclusions

Subsoiling has a certain effect on the physical and chemical properties of the soil profile (0–100 cm). Subsoiling significantly reduced soil compactness from 15 to 45 cm, significantly increased soil water content, organic carbon and total nitrogen content in deep soil (>40 cm), significantly increased pH, ammonium nitrogen content and wheat root length density in the whole soil layer and decreased nitrate nitrogen residue and leaching loss. In addition, compared with single rotary tillage, subsoiling was not conducive to improving soil bacterial community richness and diversity. Subsoiling indeed changed the composition and structure of the bacterial community in the topsoil layer, significantly increasing the abundance of *GAL15*, *Actinobacteria*, *Nitrospirae*, *Rhizobiales*, *Burkholderiales*, *Pseudomonas* and *Serratia*, and significantly decreasing the abundance of *Acidobacteria*, *Gemmatimonadetes*, *Rhodospirillales* and *Bacillus* in the topsoil. This reveals that subsoiling could significantly increase soil beneficial bacteria (biological nitrogen-fixing bacteria, ammonifying bacteria and nitrobacteria) abundances and reduce the nitrogen loss caused by denitrifying bacteria. In addition, the interaction of soil, microorganism and plant had a three-sided relationship. There was a significant positive correlation between root length density and the contents of *Rhizobiales*, *Burkholderiales*, *Pseudomonas*, *Serratia* and

ammonium nitrogen, and a significant negative correlation between root length density and the contents of *Rhodospirillales*, *Nitrosomonadales* and nitrate nitrogen.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy12102288/s1, Table S1: Effects of tillage, depth and their interaction on soil physicochemical property, root length density and bacterial alpha diversity index.

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