




## Article

# Effects of Combination of Water-Retaining Agent and Nitrogen Fertilizer on Soil Characteristics and Growth of Winter Wheat under Subsoiling Tillage in South Loess Plateau of China

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**Abstract:** This study was carried out to evaluate the effects of the combined application of water-retaining agents and nitrogen fertilizers on soil physicochemical properties, bacterial communities, and root growth under winter wheat planting mode in the Guanzhong area of Shaanxi Province. Based on the positioning experiment of dry farming in the loessal soil area of Shaanxi Province, four treatments were set up by using the tillage method of subsoiling + rotary tillage and straw returning: only fertilization (U), only water retention agent sodium polyacrylate (C<sub>3</sub>H<sub>3</sub>NaO<sub>2</sub>)<sub>n</sub> (S), combined use of water-retaining agent sodium polyacrylate (C<sub>3</sub>H<sub>3</sub>NaO<sub>2</sub>)<sub>n</sub> and fertilizer (US), and control group CK (no treatment). The ultra-high-throughput sequencing of 16S rRNA genes of soil bacteria was performed by the Illumina Hiseq platform, and the effects of different tillage measures on soil bacterial diversity and community structure were analyzed. In addition, the effects of these tillage measures on soil physicochemical properties and winter wheat root length density at booting and flowering stages were evaluated. The results indicated that the combination of the water-retaining agent and fertilizer markedly enhanced the contents of ammonium nitrogen, available phosphorus, and available potassium in the 0–20 cm soil layer, significantly increased the soil moisture content, and promoted the deep growth of roots. The root length density was 4.70 times higher than that of the control group at the booting stage. In addition, the combined application alleviated the decrease in soil microbial diversity caused by individual fertilization, especially significantly increasing the abundance of Gemmatimonadetes, Acidobacteria, and Planctomycetes in the 0–10 cm soil layer. This study reveals the potential of the combined use of water retention agents and fertilizers to optimize the soil environment and enhance winter wheat yield, which provides a scientific basis for improving local agricultural practices.

**Keywords:** subsoiling; water-retaining agent; nitrogen fertilizer; soil physicochemical properties; bacterial community structure



**Citation:** Wang, H.; Huo, Q.; Wang, T.; Lv, G.; Li, W.; Ren, J.; Zhang, S.; Li, J. Effects of Combination of Water-Retaining Agent and Nitrogen Fertilizer on Soil Characteristics and Growth of Winter Wheat under Subsoiling Tillage in South Loess Plateau of China. *Agronomy* **2024**, *14*, 1287. <https://doi.org/10.3390/agronomy14061287>

Academic Editors: Cezary A. Kwiatkowski and Elzbieta Harasim

Received: 16 May 2024

Revised: 10 June 2024

Accepted: 12 June 2024

Published: 14 June 2024



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## 1. Introduction

As a representative winter wheat-to-summer corn crop rotation region in China, the main soil types in Guanzhong Plain of Shaanxi Province include loess soil, black loessial soil, and tier soil [1,2]. Over the past three decades, agricultural production in this region

has mainly relied on small-scale agricultural machinery for shallow cultivation. Long-term repeated cultivation and inadequate nutrient management have led to shallower soil layers, thicker plow bottoms, and weakened soil buffering capacity. These problems have aggravated soil erosion and nutrient imbalance, seriously restricting the high and stable yield of crops and the efficient use of resources. Although subsoiling integrated with straw returning is used to improve soil structure, the problems of soil nutrient loss and insufficient water management are still prominent. Water-retaining agents are structurally cross-linked hydrophilic polymers capable of absorbing large quantities of water or aqueous fluids (up to 1000 times their original weight or volume) within a relatively short period of time [3]. In addition, the use of water-retaining agents has improved soil water-retaining capacity, but its effective combination with soil physical conditioning and biological activity improvement has not been fully resolved [4–8]. The soil bottom layer stores a large amount of nutrients such as nitrogen [9] and phosphorus [10], which are crucial for ensuring water and nutrient supply under extreme drought conditions [11]. However, the utilization efficiency of these resources in traditional agricultural practices is usually low, which leads to the urgent need for more effective farming methods. In the Guanzhong Plain, subsoiling combined with straw returning is a common tillage method [12]. By breaking the hard plow pan, improving the soil profile structure, and prompting the crop roots to take root more deeply, the utilization rate of deep soil resources by crops is effectively improved [13]. In addition, the addition of water-retaining agents markedly improved the soil water and fertilizer environment and soil structure [14]. It maximizes the absorption of water and nutrients by crop roots by preventing the deep infiltration of water and soil nutrient loss, thereby helping to achieve high and stable crop yields [15]. However, the use of subsoiling and water-retaining agents inevitably changes the soil physicochemical properties, which in turn affects the soil microbial environment. For example, subsoiling optimizes soil structure by reducing soil salinity and pH, which is beneficial to fungal growth [16]. Existing research indicates that the addition of nitrogen fertilizer directly affects soil microorganisms by changing the inorganic nitrogen content in the soil or indirectly affects these microorganisms by changing the carbon availability, carbon–nitrogen ratio, and pH value of the soil [17]. When the soil pH value is lower than 5, the availability of soil nutrients is greatly reduced, which inhibits the decomposition of crop residues. As a result, the mineralization process and basal respiration of carbon, nitrogen, and phosphorus in the soil are slowed down, which reduces the biomass and enzyme activity of microorganisms and has a negative impact on the metabolic function of bacteria and fungi [18–20]. In addition, soil salinization and excessive use of inorganic fertilizers can significantly weaken the adsorption capacity of water-retaining agents [21,22] and inhibit the activity of soil microorganisms and the mineralization of organic matter, thereby reducing the effectiveness of soil nutrients and hindering the healthy thriver of crops [23]. The appropriate application of water-retaining agents has been proven to significantly enhance the water-holding capacity of the soil and improve nutrient availability and soil conditioning, thereby raising crop yield and enhancing soil microbial diversity [24]. The use of water-retaining agents markedly changes the structure of soil microbial communities, resulting in an increase in the abundance of bacteria and actinomycetes, while the abundance of fungi decreases [25].

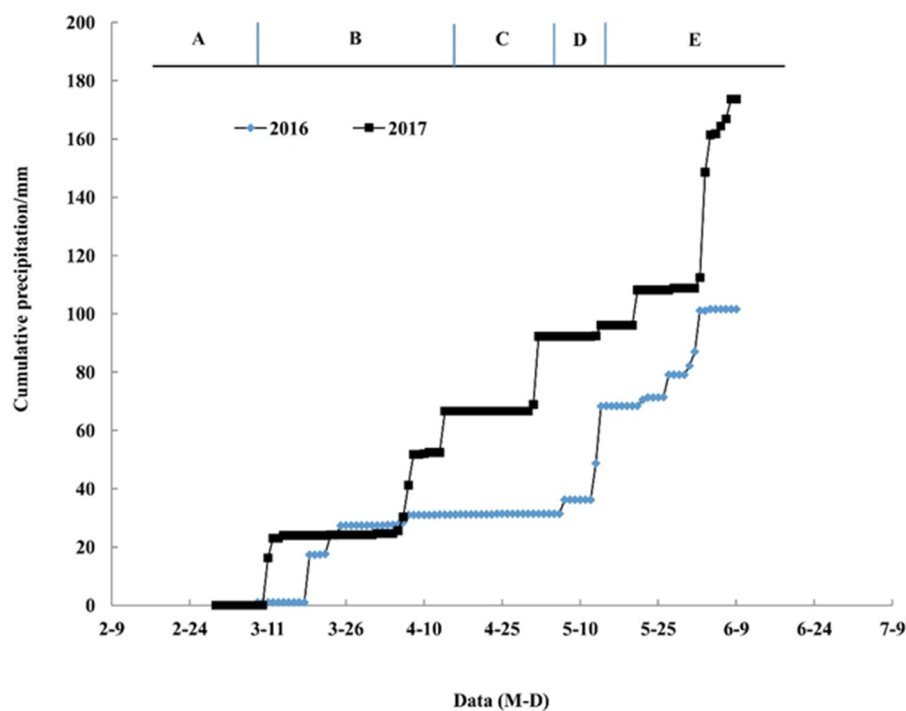
Although scholars at home and abroad have extensively studied the effects of different soil types and agricultural management methods on root development, crop production, physical and chemical properties of the soil, and microbial communities in the plow layer, comprehensive knowledge about winter wheat root thriver, root soil physicochemical properties, and bacterial community diversity and richness based on subsoiling tillage systems in semi-humid yellow loess soil is still deficient. This study utilized a positioning experimental platform for the dryland winter wheat summer maize planting model in the loessal soil area of Guanzhong Plain, Shaanxi Province, focusing on the topsoil of winter wheat fields. The effects of subsoiling tillage, fertilization, and water-retaining agent treatment on winter wheat yield, rhizosphere soil physicochemical properties, and bacterial communities were systematically evaluated. Combined with ecological data-processing

software, this study further explored the correlation between these three variables, aiming to reveal the reaction mechanism of the soil bacterial community to fertilization and water-retaining agent treatment and provide a scientific basis for ameliorating soil moisture and nutrient utilization.

## 2. Materials and Methods

### 2.1. Test Design

This trial was performed from October 2015 to July 2017 at a long-term positioning experimental base for winter wheat summer maize dryland agriculture in the Guanzhong region, Shaanxi Province (longitude: 109°17'9", latitude: 34°76'58", altitude: 356 m). The region has a continental temperate semi-arid and semi-humid climate, with a frost-free period of 199 to 255 days and an average annual temperature of 12 to 14 °C. The annual sunshine duration is 2200 to 2500 h, and the annual precipitation is between 498 and 726 mm. The interannual variation of precipitation is significant. The soil in the test region is loess, loose in texture and light in color, which belongs to the primary soil. The analysis of the basic physicochemical properties of the soil before the test showed that [26] the 0~20 cm plow layer soil contained 49.0% gravel, 42.3% silt, and 8.7% clay. Other basic physicochemical properties are as follows: bulk density is 1.32 g·cm<sup>-3</sup>, pH is 8.20, the organic matter content is 12.30 g·kg<sup>-1</sup>, total nitrogen is 0.72 g·kg<sup>-1</sup>, total phosphorus is 0.97 g·kg<sup>-1</sup>, total potassium is 12.02 g·kg<sup>-1</sup>, and available nitrogen is 116.50 mg·kg<sup>-1</sup>. The tested winter wheat variety is Xiaoyan 22. As shown in Figure 1, the cumulative precipitation of winter wheat from the turning green period to the harvest period in 2016 and 2017 was 101.60 mm and 173.30 mm, respectively, indicating a significant difference in precipitation between the two years. In addition, the middle jointing stage to the flowering stage (27 March to 11 May) of wheat in 2016 experienced a long period of drought, which was a critical period for water demand of wheat and had a negative impact on wheat growth.



**Figure 1.** Accumulated precipitation of winter wheat from the turning green period to the mature period (A, turn-green period; B, jointing period; C, heading period; D, flowering period; E, grain-filling period).

This study was based on the long-term positioning test of subsoiling and straw returning management for 5 successive years [27]. On this foundation, a water-retaining agent and fertilizer application positioning test was established before winter wheat sowing in 2016. The fertilizer used in this experiment is urea, and the water-retaining agent used is sodium polyacrylate ( $C_3H_3NaO_2$ ) $_n$ . It is a cationic polyacrylate/high water absorbent polymer with a particle size range of 0.8~1.0 mm, milky white, provided by Nippon Shokubai Co., Ltd. (Tokyo, Japan).

The experiment was carried out in a 40 m × 20 m subsoiling and straw returning test area, and four treatments were set up: (1) only subsoiling and straw returning (CK); (2) application of urea (U) after subsoiling and returning straw to the field; (3) application of the water-retaining agent (S) after subsoiling and returning straw to the field; and (4) application of urea and the water-retaining agent (US) simultaneously after subsoiling and returning straw to the field. The tillage process includes returning to the field after the mechanical harvesting of maize → performing subsoiling (chisel plowing with a depth of 35 to 40 cm) → wheat sowing → wheat mechanical harvesting → wheat straw returning → maize no-tillage direct seeding. After deep loosening tillage measures, we first sprayed the fertilizer with a sprayer to wet the fertilizer, mixed the water-retaining agent and fertilizer at a ratio of 1:10, and mixed them thoroughly so that they adhered to each other evenly. We spread the mixed water-retaining agent and fertilizer in the sowing furrow first, with a soil depth of 8~10 cm, and then covered the top with another layer of soil before sowing. The water-retaining rate was 45 kg·hm<sup>-2</sup>, all treatments were sown on 17 October every year, the seed dosage was 187.50 kg·hm<sup>-2</sup>, the row spacing was 20 cm, and the harvest was on June 13 of the next year. Throughout the entire wheat growing season, each treatment was subjected to unified field management, including two rounds of irrigation: once in winter and another during the booting stage, in the form of border irrigation, with a watering rate of 90 mm. Basal fertilization was applied to winter wheat before sowing each year in fertilization treatments. Basal fertilization included N 150 kg·hm<sup>-2</sup>, P<sub>2</sub>O<sub>5</sub> 150 kg·hm<sup>-2</sup>, and K<sub>2</sub>O 37.5 kg·hm<sup>-2</sup>. Fertilization treatments (U and US) were uniformly treated with urea (163 kg·hm<sup>-2</sup>) on 5 March of the following year. The topdressing urea was broadcast to the soil surface just before irrigation.

## 2.2. Test Methods

### 2.2.1. Winter Wheat Growth Indicators and Yield Determination

All wheat plants were harvested, and we calculated the total number of plant spikes in a marked 1 m<sup>2</sup> area from each community. Subsequently, 18 representative plants were selected for further analysis, including the determination of spike length, the number of grains per spike, the fresh weight of grains per spike, the dry weight of grains per spike, and the total weight of grains.

### 2.2.2. Bacterial Analysis of Wheat Roots and Rhizosphere Soil Sample Collection

Wheat root samples were collected by a root drilling method in the jointing period, booting period, and flowering period of winter wheat.

An area with consistent wheat growth in each community was selected, and soil samples were vertically drilled up to 100 cm deep at the 1/2 row spacing of the wheat roots by using a root drill with a diameter of 10 cm. Samples were taken at 10 cm intervals. After washing and root separation of the samples, the root length density (RLD) was measured using R2V04 vectorization software and ArcGIS 10.2 [28].

In June 2016 and June 2017, after harvesting winter wheat, four topsoils (0~20 cm) with similar stubble density of winter wheat roots were stochastically collected in each community using the excavation method, and thin layers of soil attached to the residual roots were gathered from depths of 0~10 cm and 10~20 cm, respectively. The samples were mixed and sampled according to the quartering method. After passing through a 2 mm mesh sieve, they were refrigerated at -80 °C for subsequent high-throughput sequencing analysis. Meanwhile, three sampling points with consistent residue density

were randomly selected in each community, and soil samples with depths of 0~10, 10~20, 20~30, 30~40, 40~60, 60~80, and 80~100 cm were collected by the soil drilling method from top to bottom. After sieving the soil sample through a 2 mm mesh sieve, it was separated into two sections. One section of the fresh soil sample was used for the measurement of soil ammonium nitrogen, nitrate nitrogen, and water content, while the other section was used for the measurement of soil available phosphorus and available potassium after natural air drying [12].

### 2.2.3. Determination of Soil Physicochemical Properties

The determination of soil physicochemical properties involved the following: measurement of soil water content using the drying method, measurement of soil ammonium nitrogen using the indophenol blue colorimetric method, measurement of nitrate nitrogen using dual-wavelength ultraviolet spectrophotometry, measurement of available phosphorus using the leaching-molybdenum antimony colorimetric method, and measurement of available potassium using the ammonium acetate leaching-flame photometer method [29,30].

### 2.2.4. Soil Microbial DNA Extraction and PCR Amplification and Sequencing

Microbial genomic DNA was extracted from soil samples using the E.Z.N.A.<sup>®</sup> Soil DNA Kit (Omega Bio-tek, Norcross, GA, USA) kit. DNA quality and concentration were measured using Nanodrop2000 (Thermo Fisher Scientific, Inc., Waltham, MA, USA). DNA samples were stored at  $-20^{\circ}\text{C}$  for subsequent experiments. The V3-V4 region of the bacterial 16S rRNA gene was amplified with primers 338F (5'-ACTCCTACGGGAGGCAGCA-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3') [31,32]. 8bp barcode sequences were added at the 5' end of the upstream and downstream primers to distinguish different samples. Finally, a universal primer with a barcode sequence was synthesized and amplified on an ABI9700 PCR machine (Applied Biosystems, Inc., Woburn, MA, USA). The size of the amplified target band was detected by 1% agarose gel electrophoresis, 170 V and 30 min. The PCR products were automatically purified using the Agencourt AMPure XP (Beckman Coulter, Inc., Brea, CA, USA) nucleic acid purification kit. We used Nanodrop2000 (Thermo Fisher Scientific, Inc., Waltham, MA, USA) to preliminarily test the library concentration, used Agilent 2100 Bioanalyzer (Agilent Technologies, Inc., Santa Clara, CA, USA) to detect the library fragment size, and used the ABI Step One Plus Real-Time PCR System (Applied Biosystems, Inc., Woburn, MA, USA) to accurately quantify library concentration. The final library was sequenced on the Illumina Miseq/Nextseq2000/Novaseq6000 (Illumina, Inc., San Diego, CA, USA) platform with a sequencing strategy of PE250/PE300. The sequencing raw data have been submitted to the SRA database of NCBI.

### 2.3. Sequencing Data Processing

FLASH (v1.2.7) [33] software was used to merge the end-paired sequences from the original DNA fragments, and the reads of each sample were spliced to obtain the spliced sequence, which was the original Tags data (Raw Tags). Then, Trimmomatic (v0.36) software [34] was used to perform quality control filtering (remove joints and low-quality bases) on the original Fastq file to obtain high-quality Tags data (Clean Tags). Finally, the chimeras were removed using software UCHIME (v4.2) [35] to obtain high-quality sequences, and the sequences were clustered at a similarity level of 97% using software UCLUST (v1.2.22) [36]. These operational taxonomic units (OTUs) were filtered using a threshold of 0.005% of all sequencing sequences [37]. We used a ribosomal database project classifier (RDP classifier) (v2.2) [38] combined with the Silva128 database ([www.arb-silva.de/no\\_cache/download](http://www.arb-silva.de/no_cache/download) (accessed on 5 March 2020)) to annotate classification information for each representative sequence, with a confidence threshold of 0.8. Soil bacteria  $\alpha$  diversity index analysis using Mothur (v1.32.1) software (<http://www.mothur.org/> (accessed on 5 March 2020)) at a 97% similarity level [39].



## 2.4. Statistical Analysis

One-way analysis of variance (ANOVA), LSD multiple comparisons, and Pearson's correlation analysis were performed using SPSS.25 software to analyze the data on soil physicochemical properties, growth indexes of winter wheat, root length density, and the bacterial diversity index of different treatments. The difference in bacterial community structure between samples was compared by principal coordinates analysis (PCoA) using the *vegan* and *stats* packages in R language, and the graph was constructed by Prism10.

## 3. Results

### 3.1. Effects of Different Treatments of Fertilization and Water-Retaining Agents on the Growth and Yield of Winter Wheat

From Table 1, it can be observed that there were marked differences in the effects of different treatments on the emergence rate, stem and tiller growth, and yield of winter wheat. In terms of emergence rate, "US (combined fertilization and water-retaining agent) > U (fertilization only) > S (water-retaining agent only) > CK (control)", among which the emergence rate of the US treatment was 37.45% and 10.10% higher than the average values of the control group and the water-retaining agent only treatment group over two years, respectively. In addition, compared with U treatment and S treatment, the maximum tiller number of US treatment increased by 24.75% and 12.33%, respectively. In terms of yield, data from 2016 and 2017 showed that US, U, and S treatments all increased the number of spikes, thousand-grain weight, and total yield of winter wheat. The thousand-grain weight and number of spikes of wheat treated with US increased by an average of 10.64% and 7.82%, respectively, compared to S treatment, and the total yield increased by 21.02%. The difference in the number of grains per spike was not significant. Compared to the unfertilized treatment, the fertilization treatment significantly increased yield, thousand-grain weight, and the number of spikes, with an average increase of 68.48%, 24.76%, and 76.15%, respectively.

**Table 1.** Effects of different fertilization and water-retaining agent treatments on the growth and yield of winter wheat.

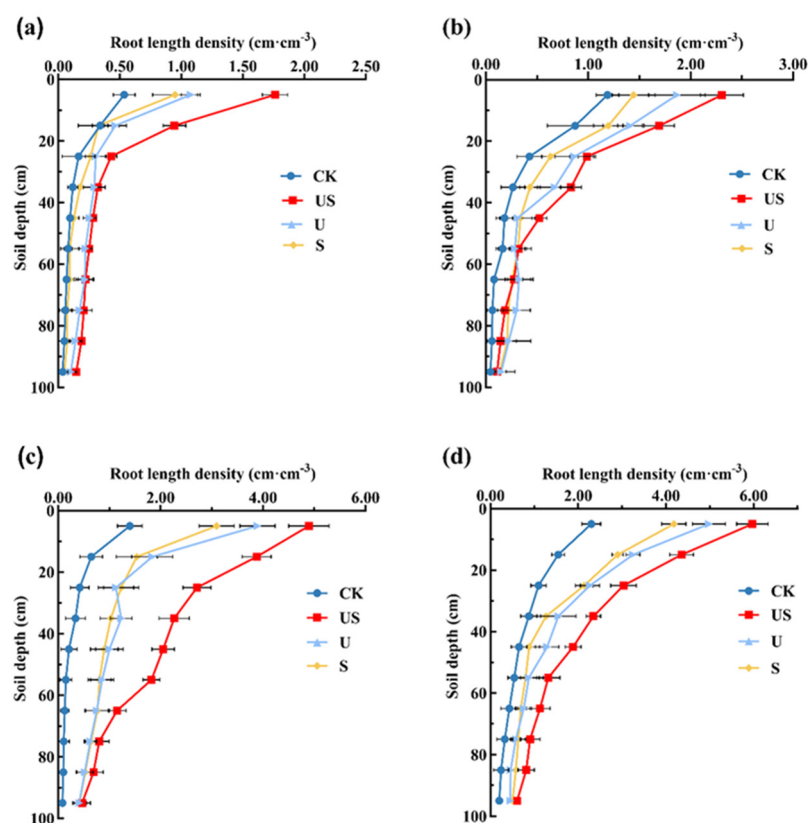
Year	Treatment	Emergence Rate (%)	Maximum Tiller Number ( $10^4 \text{ hm}^{-2}$ )	Panicle ( $\times 10^4 \text{ hm}^{-2}$ )	1000-Grain Weight (g)	Yield ( $\text{kg}\cdot\text{hm}^{-2}$ )
2016	CK	62.8 ± 2.3 c	635.3 ± 52.7 c	387.3 ± 22.1 c	34.2 ± 1.3 c	3390.7 ± 110.5 d
	U	80.4 ± 2.9 ab	1344.3 ± 83.5 a	628.7 ± 35.2 b	44.9 ± 1.6 a	5790.4 ± 225.8 b
	S	76.1 ± 2.7 b	1143.2 ± 71.7 b	630.0 ± 32.6 b	40.5 ± 2.2 b	5107.5 ± 192.5 c
	US	83.3 ± 3.3 a	1455.6 ± 62.8 a	694.5 ± 26.1 a	45.7 ± 2.1 a	6619.2 ± 311.3 a
2017	CK	59.4 ± 3.6 c	462.2 ± 41.7 d	347.0 ± 20.3 c	37.3 ± 1.8 b	4683.9 ± 171.7 c
	U	80.3 ± 3.4 ab	1248.1 ± 71.5 b	659.2 ± 22.6 ab	44.1 ± 1.4 a	7783.9 ± 251.3 a
	S	76.3 ± 2.2 b	1023.4 ± 55.3 c	638.7 ± 10.2 b	41.5 ± 1.6 a	7117.7 ± 156.8 b
	US	84.5 ± 2.8 a	1425.3 ± 82.6 a	673.1 ± 13.5 a	45.0 ± 1.3 a	8002.4 ± 336.8 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.

### 3.2. Effects of Different Treatments of Fertilization and Water-Retaining Agents on Root Length Density

Figure 2 shows the root length density of winter wheat during the jointing and flowering periods in 2016, as well as the booting and flowering periods in 2017. In the two-year data, with the passage of the wheat growth cycle, the root length density of each depth gradually increased and reduced logarithmically with the increase in soil depth. In 2016, the difference in root length density among different treatments was small, but in the booting period in 2017, the difference between the treatments reached the maximum. The difference started to reduce after the flowering period. During the 2016 jointing period, at soil depths above 30 cm, the root length density of U and S was almost the same, which was 35.72% lower than that of US. However, at soil depths below 30 cm, the root length

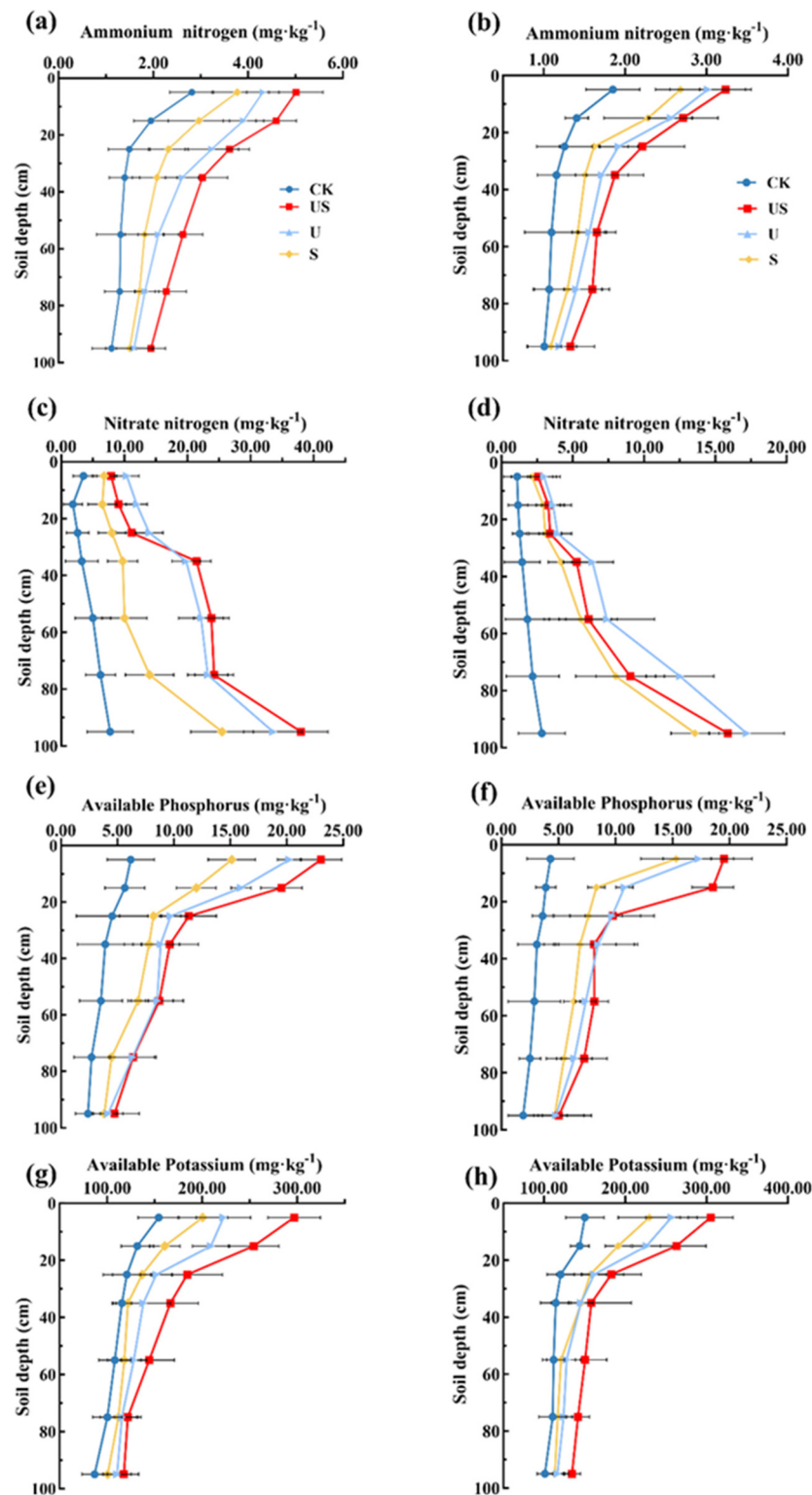
density of U increased markedly, which was 153.67% and 78.45% more than that of CK and S, respectively. Entering the flowering period, the root length density of winter wheat treated with US in soil depths above 40 cm was significantly higher than that of S by 57.11% and U by 20.81% in 2016; in the deep soil below 70 cm, the differences among the three treatments decreased, and the curves tended to overlap. In the booting period in 2017, the root length density of US at a soil depth above 70 cm was markedly more than that of CK and S, which was 4.70 times and 1.01 times that of CK and S, respectively. The root length density of US was also higher than that of U by an average of 75.38%. Entering the flowering period, the root length density of wheat treated with US in soil depths above 40 cm was 49.88% higher than that of S and 30.41% higher than that of U. However, as the soil depth increased to below 50 cm, the difference in root length density among the treatments began to decrease.



**Figure 2.** Effects of different treatments of fertilization and water-retaining agents on root length density of 0~100 cm ((a): jointing period in 2016; (b): flowering period in 2016; (c): booting period in 2017; (d): flowering period in 2017).

### 3.3. Effects of Different Treatments of Fertilization and Water-Retaining Agents on Soil Physicochemical Properties

The study found that the content of ammonium nitrogen ( $\text{NH}_4^+\text{-N}$ ) was the largest in the surface soil and reduced markedly with the increase in soil depth (Figure 3a,b). In the surface soil of 0~20 cm depth, the  $\text{NH}_4^+\text{-N}$  content caused by different treatments varied greatly, and the difference was significant. Compared with the control group (CK), the treatment of fertilization (U), water-retaining agents (S), and combined fertilization and water-retaining agents (US) in 2016 increased the soil  $\text{NH}_4^+\text{-N}$  content by 72.39%, 41.36%, and 101.59%, respectively. In 2017, these increases were 71.33%, 52.38%, and 82.56%, respectively.



**Figure 3.** Effects of different treatments of fertilization and water-retaining agents on soil nutrient availability from 2016 to 2017 ((a): ammonium nitrogen content in 2016; (b): ammonium nitrogen content in 2017; (c): nitrate nitrogen content in 2016; (d): nitrate nitrogen content in 2017; (e): available phosphorus content in 2016; (f): available phosphorus content in 2017; (g): available potassium content in 2016; (h): available potassium content in 2017).

For the content of nitrate nitrogen ( $\text{NO}_3^-$ -N), the data in 2016 showed that the content of  $\text{NO}_3^-$ -N in each layer of soil during the growth period of winter wheat generally showed



a downward tendency. The content of  $\text{NO}_3^-$ -N in the upper and middle layers of 0~60 cm depth was lower, while the content of  $\text{NO}_3^-$ -N in the deep soil below 60 cm gradually increased. The trend of change in 2017 was similar to this. In the 40~100 cm soil layer in 2016, in comparison to CK, the  $\text{NO}_3^-$ -N content of U, S, and US treatments increased significantly by 3.16 times, 3.53 times, and 1.61 times, respectively. In 2017, the difference between the three treatments and CK increased significantly from the soil layer below 60 cm, with an increase of 4.29 times, 2.97 times, and 3.53 times, respectively.

As for the content of phosphate (AP), two years of research data indicate that the difference in AP content in the rhizosphere soil of winter wheat was not significant ( $p < 0.05$ ) among different soil depths, and overall, it gradually reduced the rise in soil depth. In 2016, in comparison to CK, the AP content of U, S, and US treatments in the 0~20 cm surface soil was markedly enhanced by 2.05 times, 1.29 times, and 2.59 times, respectively. In 2017, the US treatment had the highest AP content in the same soil layer, with significant increases of 303.91%, 17.83%, and 39.88% compared to CK, U, and S treatments, respectively.

Regarding the content of available potassium (AK), compared with the deep soil below 20 cm, the AK content of each treatment was significantly different in the shallow soil above 20 cm ( $p < 0.01$ ). The AK content of US treatment was the highest at a depth of 10~20 cm in 2016, which increased by 108.56%, 21.45%, and 58.24%, respectively, compared with CK, U, and S treatments. In 2017, the AK content of U, S, and US treatments significantly increased by 64.28%, 30.12%, and 93.16% contrasted with CK in the 0~20 cm soil layer.

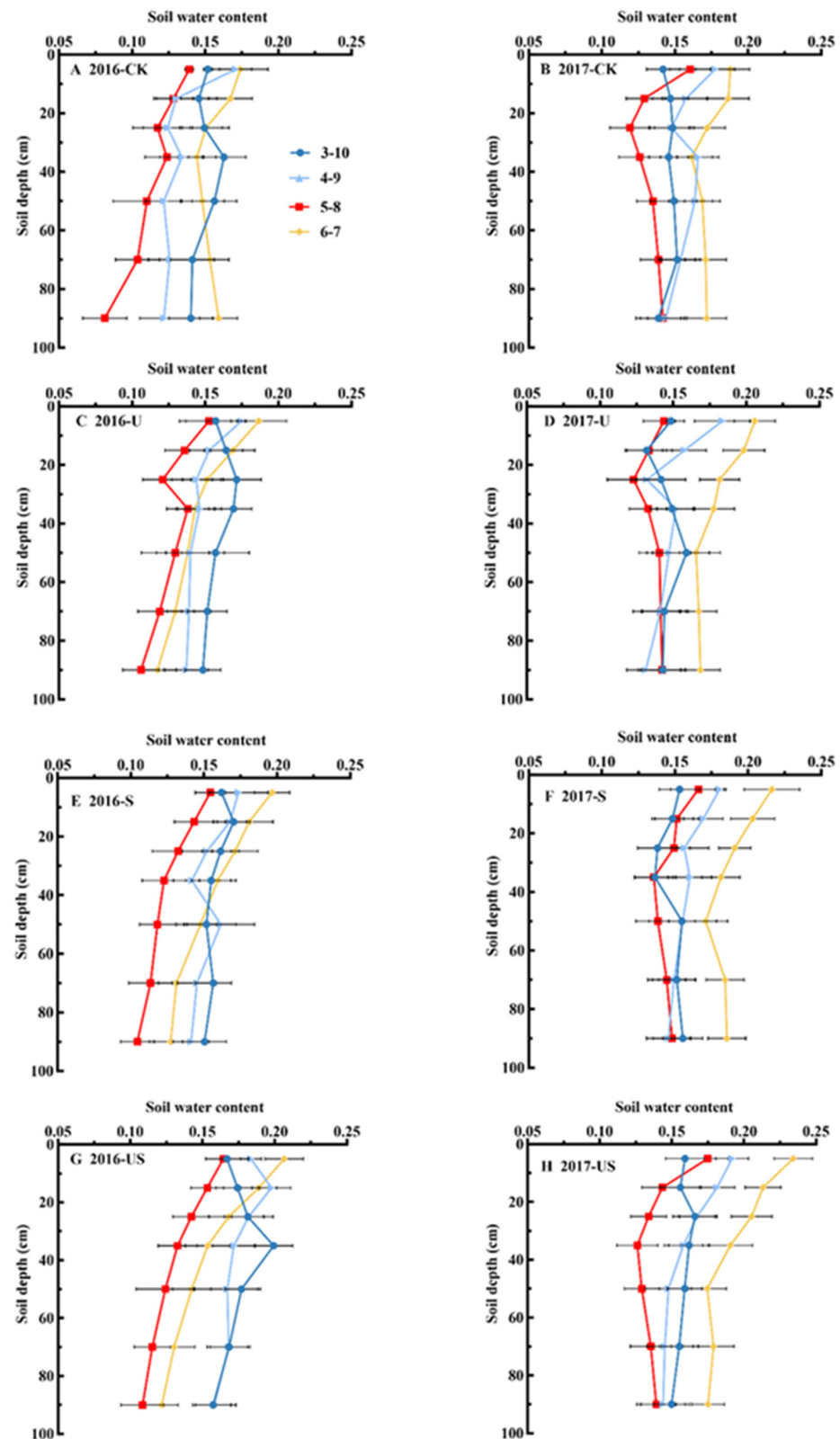
Figure 4 shows that in the 2016 harvest, soil water content was higher in US and S treatments and markedly higher in 0~20 cm topsoil than CK treatments by an average of 28.89% and 23.01%, respectively; in the 2017 harvest, soil water content was higher in US and S treatments and markedly higher in 0~20 cm topsoil than CK treatments by an average of 19.52% and 12.07%, respectively. Due to higher precipitation in 2017, the water uptake of deep soil was limited, resulting in the water content of deep soil (>40 cm) treated with CK and S being higher than that in 2016, and the average water content during the whole growth period was 16.97% and 14.18% higher than before, respectively.

### 3.4. Effects of Different Treatments of Fertilization and Water-Retaining Agents on Soil Microbial Communities

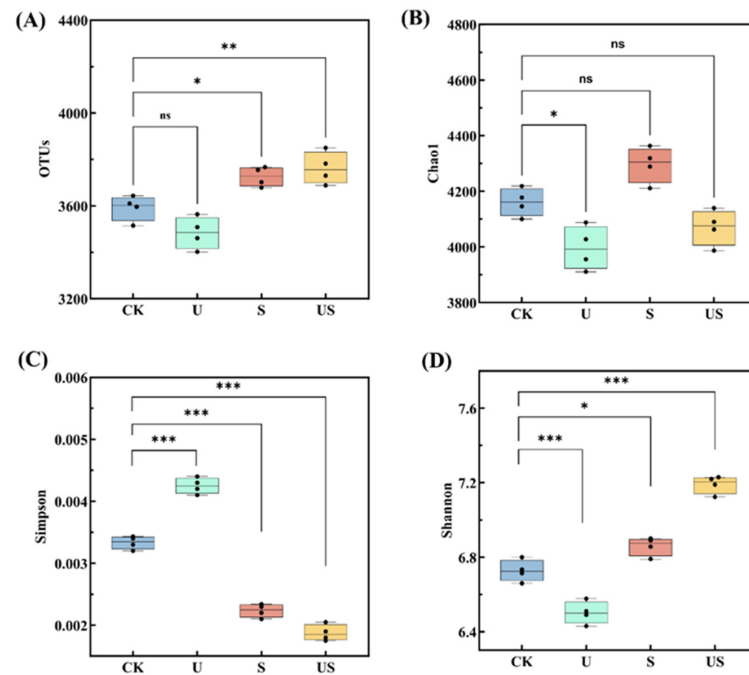
#### 3.4.1. Soil Bacterial Diversity Index in the Cultivated Layer (0~20 cm) under Different Treatments

In this study, the Illumina HiSeq 2500 platform (San Diego, CA, USA) was used to sequence the 16S rRNA V3-V4 region of soil bacteria. A total of 1,388,877 valid sequences were obtained, with an average sequence length of 420.33 bp. GC content refers to the fraction or percentage of GC base pairs in a genome. The GC content ranged from 56.12% to 58.53%, and the Q20 mass value was between 96.12% and 96.33%. After flattening and screening, each sample obtained over 79,598 high-quality sequences, including 2126 to 3742 operational taxonomic units (OTUs), with sequencing coverage ranging from 0.9942 to 0.9958. The OTUs dilution curves of all samples tended to be flat, indicating that the sequencing depth was sufficient to cover all bacterial species in the samples, ensuring that the sequencing results could truly reflect the microbial status of the samples.

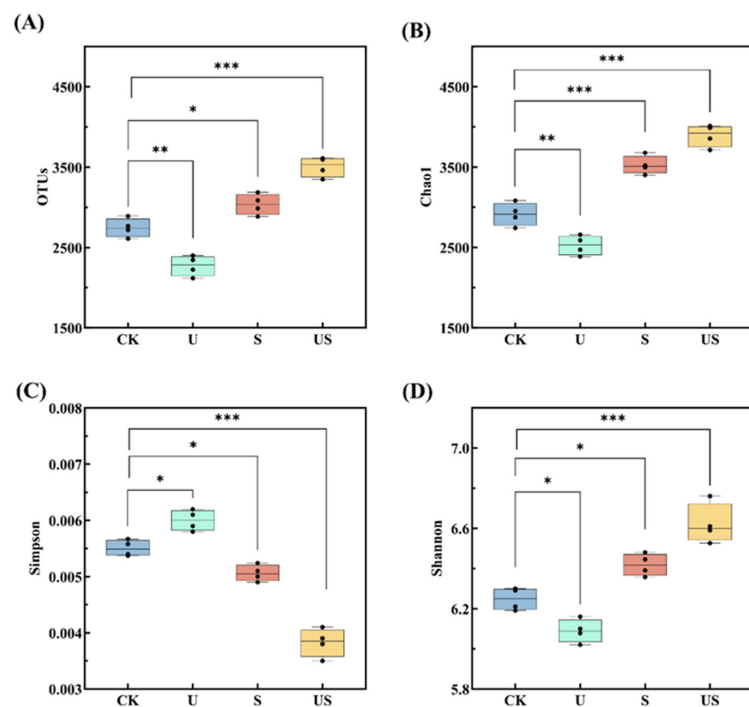
According to Figures 5 and 6, it has been observed that there were marked differences in the microbial diversity index in different cultivation layers among different treatments. In the 0~10 cm surface soil, in comparison to the control group (CK), US and S treatments significantly increased the number of OTUs and Shannon index, while the Simpson index decreased significantly, and the Chao1 index did not change significantly. In the 10~20 cm soil layer, the OTUs, Chao1, and Shannon indexes of S and US treatments were markedly higher than those of CK, while the Simpson index was significantly less than that of CK. In the whole 0~20 cm soil layer, in comparison to other treatments, OTUs, Chao1, and Shannon indexes of U treatment were the lowest, and its Simpson index was the highest.



**Figure 4.** Distribution of soil moisture profiles at different growth stages under different fertilization and water-retaining agent treatments in 2016 and 2017 (date, Month-Day. 3-9, turn green; 4-10, joining; 5-11, grain filling; 6-8, harvest).



**Figure 5.** Soil bacterial diversity index of 0~10 cm cultivation layer under different treatments. (A) represents the OTUs diversity index under different treatments, (B) represents the Chao1 diversity index under different treatments, (C) represents the Simpson diversity index under different treatments, (D) represents the Shannon diversity index under different treatments. Significant differences in diversity index between different treatments were marked with star. \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$ , ns means no significant differences at  $p > 0.05$ .

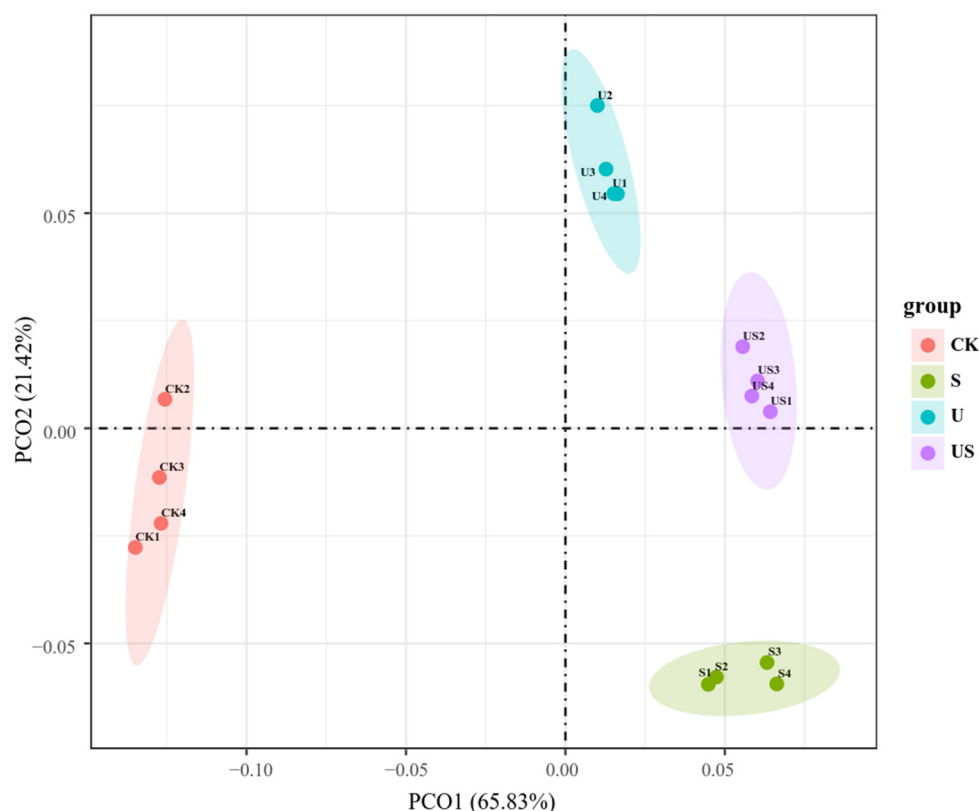


**Figure 6.** Soil bacterial diversity index of 10~20 cm cultivation layer under different treatments. (A) represents the OTUs diversity index under different treatments, (B) represents the Chao1 diversity index under different treatments, (C) represents the Simpson diversity index under different treatments, (D) represents the Shannon diversity index under different treatments. Significant differences in diversity index between different treatments were marked with star. \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$ .

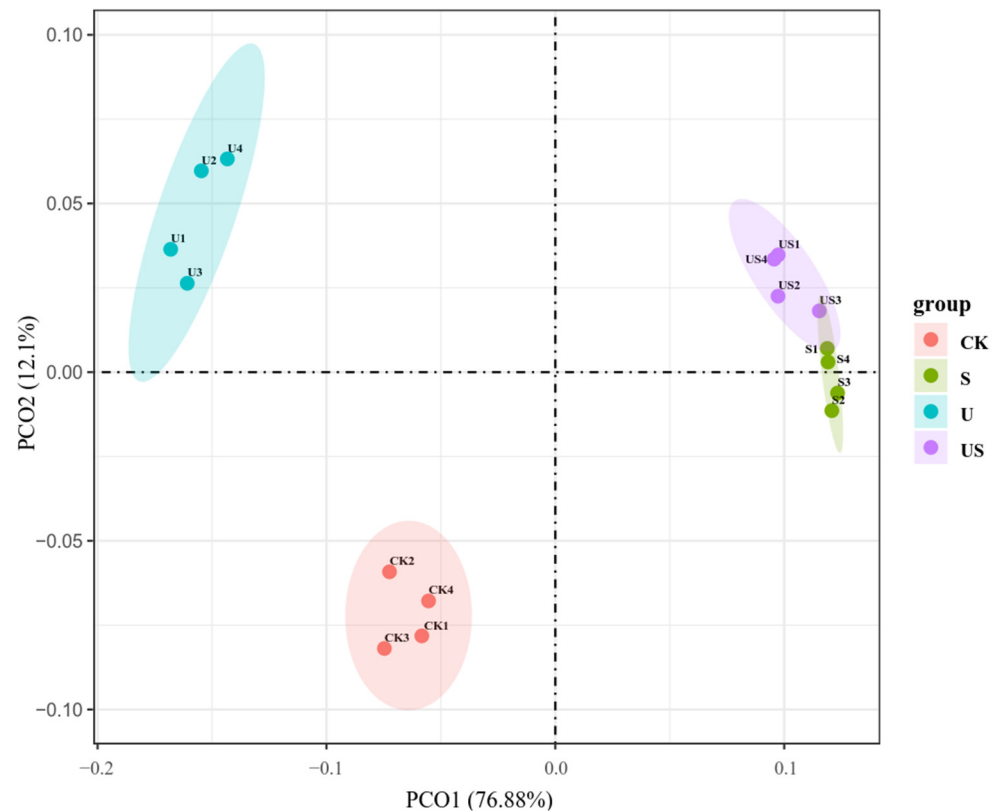
For the sake of further analyzing the effects of different treatments on the Beta diversity of soil bacterial communities, principal component analysis (PCA) at the OTU level was performed on 16 samples of four treatments. According to Figures 7 and 8, the results indicated that in the 0~20 cm soil layer, the distribution of samples between different treatments was more dispersed, indicating that fertilization and application of the water-retaining agent had a marked effect on the bacterial community composition of the soil tillage layer. We explained 87.25% of the differences in microbial community structure in the 0~10 cm soil layer through dimensionality reduction analysis, with the first principal axis (PCO1) and second principal axis (PCO2) explaining 65.83% and 21.42% of the differences in microbial community structure, respectively. In the 10~20 cm soil layer, 88.98% of the differences in bacterial community structure were explained. The first principal axis (PCO1) and the second principal axis (PCO2) explained 76.88% and 12.1% of the differences in bacterial community structure, respectively. In the soil layer of 10~20 cm depth, the distribution distance of samples after S and US treatments was relatively close, indicating that these two treatments have similar effects on the bacterial community in the soil layer.

### 3.4.2. Composition of Bacterial Communities in Different Treatment Layers (0~20 cm)

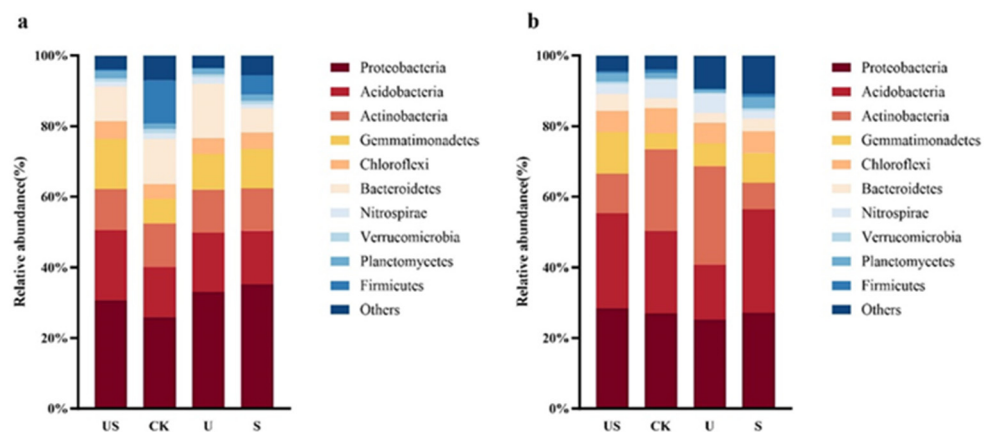
At the phylum level, 47 and 45 bacterial phyla were detected in the samples of 0~10 cm and 10~20 cm soil layers, showing that the bacterial composition of different soil layers was generally similar, but the relative abundance of each strain was markedly different. As can be seen in Figure 9, the top ten bacterial phyla in terms of relative abundance in the two soil horizons showed significant differences, indicating that the use of both fertilizers and water-retaining agents affects the relative abundance of bacterial taxa in the soil.



**Figure 7.** Principal component analysis of bacterial community differences between different treatment groups in the 0~10 cm cultivation layer.



**Figure 8.** Principal component analysis of bacterial community differences between different treatment groups in the 10~20 cm cultivation layer.



**Figure 9.** Comparison of the relative abundance of dominant bacterial communities between different treatments at the phylum taxonomic level ((a): 0~10 cm; (b): 10~20 cm).

In the 0~10 cm soil layer, compared with the control group (CK), the US treatment resulted in an increase of 50.95%, 28.59%, and 28.71% in the relative abundance of Gemmatimonadetes, Acidobacteria, and Planctomycetes, while the relative abundance of Firmicutes, Bacteroidetes, and Verrucomicrobia decreased by 96.65%, 22.84%, and 23.02%, respectively. S treatment significantly enhanced the relative abundance of Proteobacteria (26.56%) and Gemmatimonadetes (27.87%) and reduced the relative abundance of Firmicutes (128.54%) and Bacteroidetes (47.09%). The U treatment resulted in an increase of 17.34% and 12.97% in the relative abundance of Bacteroidetes and Nitrospirae, while the relative abundance of Firmicutes and Verrucomicrobia reduced by 98.45% and 35.71%, respectively. The rela-



tive abundance of Actinobacteria and Chloroflexi did not show significant changes in the four treatments.

In the soil layer of 10~20 cm depth, in comparison to the control group (CK), US treatment significantly boosted the relative abundance of several bacterial phyla: Gemmatimonadetes increased by 61.95%, Actinobacteria increased by 51.75%, Bacteroidetes increased by 43.27%, and Planctomycetes increased by 41.94%. In addition, US treatment significantly reduced the relative abundance of Firmicutes and Nitrospirae by 52.94% and 45.09%, respectively. In U treatment, the relative abundance of Gemmatimonadetes increased by 30.82%, while the relative abundance of Firmicutes, Verrucomicrobia, Planctomycetes, and Acidobacteria decreased by 72.55%, 45.61%, 44.44%, and 33.59%, respectively. S markedly boosted the relative abundance of Gemmatimonadetes and Planctomycetes by 46.86% and 55.41% and significantly declined the relative abundance of Actinobacteria and Nitrospira by 67.37% and 59.34%, respectively. The relative abundance changes of Proteobacteria and Chloroflexi were not significant among the four treatments.

3.5. Relationship between Bacterial Community Structure and Soil Physicochemical Properties, Root Length Density, and Yield in Different Treatments (0~20 cm)

Pearson correlation analysis indicated that there was a marked positive correlation between root length density (RLD) and soil water content (SWC), nitrate nitrogen ( $\text{NO}_3^-$ -N), ammonium nitrogen ( $\text{NH}_4^+$ -N), available phosphorus (AP), and available potassium (AK) at the flowering stage (Figure 10). Meanwhile, there was a highly significant negative correlation between root length density and soil bulk density (SBD) and the Shannon index. In addition, soil water content (SWC) was extremely markedly and positively linked to  $\text{NH}_4^+$ -N, AP, AK, and RLD, markedly positively correlated with  $\text{NO}_3^-$ -N, and negatively correlated with the Shannon index. The number of operational taxonomic units (OTUs) was significantly negatively correlated with SBD. The Simpson index was markedly positively correlated with  $\text{NH}_4^+$ -N and  $\text{NO}_3^-$ -N and was significantly negatively correlated with the Chao1 index. Finally, the yield showed an extremely significantly positive correlation with AN, NN, AP, AK, SWC, and RLD and a significantly negative correlation with SBD.

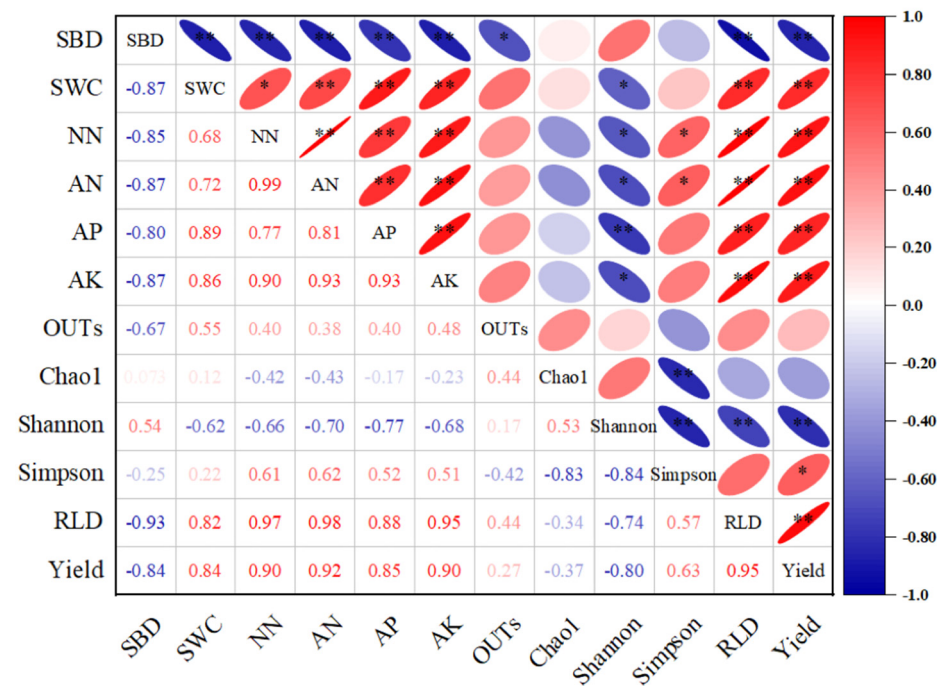


Figure 10. Pearson correlation analysis of bacterial alpha diversity index with soil physicochemical properties and root length density in plow layer (0~20 cm). \* means significant correlation ( $p < 0.05$ ); \*\* means extremely significant correlation ( $p < 0.01$ ).

## 4. Discussion

### 4.1. Effects of Fertilization and Water-Retaining Agent on Growth and Yield of Winter Wheat under Different Treatments

In 2015~2016, the economic yield of winter wheat in all treatment groups was less than the average level in 2016~2017. This phenomenon is mainly attributed to the severe drought stress throughout the growth period of wheat from 2015 to 2016 and the occurrence of different degrees of wheat root rot from the flowering to the filling stage, which led to a decrease in yield during that year. Compared to other years, winter wheat from 2016 to 2017 maintained good growth conditions throughout the entire growth period, resulting in a higher yield per unit of wheat. In addition, we found that fertilization (U) and water-retaining agents (S) could dramatically raise the yield of winter wheat by comparing the yield data of two consecutive years, especially the synergistic effect of combined fertilization and water-retaining agents (US). The results of this study clearly demonstrate the significant effect of the combined application of fertilizer and water-retaining agents (US) on improving soil water-retaining capacity and structure, which significantly enhances soil water and fertilizer utilization efficiency. In addition, the US treatment effectively alleviated the negative effects of drought and nutrient deficiency on winter wheat growth by increasing soil temperature and maintaining appropriate water and nutrient levels, thereby creating more favorable growth conditions [14]. Compared with the existing literature, this study not only verified the positive role of water-retaining agents in improving crop productivity but also specifically explored their synergistic effects when combined with fertilizers. Previous studies usually focused on the effect of a single soil amendment or fertilizer [40], while this study explained in detail the overall improvement effect of the combination of fertilizer and water-retaining agents on soil and crop characteristics, providing conclusive data support for the effectiveness of the combined strategy. However, this study also found that although the combined use of water-retaining agents and fertilizers can significantly increase production and improve soil quality in most cases, the effect is not obvious in certain soil layers. This difference may be associated with the unique physical and chemical properties of the soil layer, which may affect the treatment effect. Therefore, this finding emphasizes that when implementing soil management strategies, it is necessary to customize the application plan according to the specificity of the soil to ensure the optimal management of water and fertilizer.

### 4.2. Effects of Different Treatments of Fertilization and Water-Retaining Agents on Root Length Density of Winter Wheat

The root system is an essential part of crops for water and nutrient uptake, and it is extremely sensitive to changes in soil moisture. These changes directly affect the physiological characteristics and growth of the root system, thereby determining the morphology of the aboveground parts of the plant [41,42]. In this experiment, the application of fertilizers and water-retaining agents markedly promoted the growth of winter wheat roots, and the combination of the two showed the best effect. By enhancing the water content of the rhizosphere soil and slowly releasing water, the water-retaining agents form an effective water 'reserve', optimize the physiological activity of the root system and the growth state of the entire plant, and effectively reduce the adverse effects of drought stress on crops [43]. Zhang et al. [44] showed that water-retaining agents can significantly enhance the growth rate and quality of crop roots. The results of this study were not only consistent with this but also further revealed that the response of winter wheat roots to fertilization and water-retaining agents was different at different growth stages. Especially at the booting stage, the change in root length density was more significant with the increase in water and nutrient demand. Although the root growth during the jointing stage was relatively slow in 2016, the growth rate of the root system significantly accelerated during the booting stage. This change may be closely related to the effects of fertilization and water-retaining agents at different growth stages, especially during peak demand periods. The increased use of water-retaining agents and fertilizers enables the root system to more effectively

absorb nutrients and water, promoting root morphogenesis and aboveground reproductive growth. Although this study provides preliminary insights, there are several limitations. First of all, this study did not distinguish the effects of different types of water-retaining agents in detail. Future work should evaluate in detail the effects of various chemical water-retaining agents on the growth of winter wheat. In addition, it is recommended to extend the research to field experiments under different climatic conditions to optimize the ratio of water-retaining agent to fertilizer so as to improve the wide applicability and practical application value of the research results.

#### *4.3. Effects of Different Treatments of Fertilization and Water-Retaining Agents on Soil Physicochemical Properties*

This study conducted an in-depth analysis of the effects of fertilizer (U), water-retaining agents (S), and their combined use (US) on available nutrients in soil. The results indicated that the use of fertilizer (U) and water-retaining agents (S), as well as their combination (US), could markedly enhance the content of available nutrients in soil. Among them, the effect of using water-retaining agents alone was greater than that of applying fertilizer alone. The molecular structure characteristics of water-retaining agents enable them to adsorb nutrients [45], reducing the leaching loss of nutrients, especially nitrogen, manifested in an increase in  $\text{NO}_3^-$ -N and  $\text{NH}_4^+$ -N content. This demonstrates the dual role of water-retaining agents in improving soil fertility and water-retaining capacity, providing better soil conditions for the growth of crops such as winter wheat [46]. Wei Ningning [47] and Zhou Jiangtao [48] found that water-retaining agents can improve soil aeration and enhance soil fertility. This study further demonstrates the significant effect of water-retaining agents in controlling nitrogen leaching and increasing the content of available nutrients, especially available potassium in the soil. In addition, this study also pointed out the changes in nutrient content in different soil layers, especially the significant increase in  $\text{NH}_4^+$ -N content in shallow soil, which is in contrast to the high  $\text{NO}_3^-$ -N content in deep soil, reflecting the hierarchical effect of applying water-retaining agents. It was found that the content of  $\text{NO}_3^-$ -N in deep soil was higher, which may be due to the fact that nitrate nitrogen in deep soil is difficult for crops to directly absorb and utilize, resulting in its accumulation. This phenomenon suggests that when applying water-retaining agents and fertilizers, we need to consider the differences in their effects in different soil layers, as well as how to optimize application strategies to promote the utilization of deep nutrients by crops. Although this study provides strong evidence on the effects of fertilization and water-retaining agents on soil physicochemical properties, there is insufficient research on the diversity of water-retaining agent types and their interactions with different soil types. Future research can consider exploring the effects of different types of water-retaining agents under different soil conditions, as well as adjusting fertilization and water-retaining agent usage strategies based on soil characteristics to achieve optimal water and nutrient management.

#### *4.4. Effects of Different Treatments of Fertilization and Water-Retaining Agents on Soil Microbial Communities*

The results of the experiment indicated that the microbial diversity indexes (OTUs, Chao1 index, and Shannon index) of separate fertilization treatments were drastically lower than those of CK in the 0~20 cm soil layer, indicating that fertilization may inhibit the activity or abundance of some microorganisms. In contrast, the application of water-retaining agents and the combined use of water-retaining agents and fertilizers increased soil bacterial diversity. This may be due to the fact that water-retaining agents improve the soil moisture status, alleviate drought stress, and create a more favorable environment for microbial activity [49]. Water-retaining agents, as polymeric organic matter, can provide carbon and energy sources for certain bacteria, so increased application of water-retaining agents will promote the development of certain microorganisms and increase microbial abundance in the soil [50]. In addition, water-retaining agents indirectly promote microbial respiration and growth by changing the physical and chemical properties of soil, such as

raising soil pH and cation availability [51]. Wang et al. [52] showed that water-retaining agents can markedly enhance the microbial biomass of soil around rubber trees. This study further revealed that water-retaining agents can also improve microbial diversity in farmland soil. Moreover, we found that water-retaining agents were particularly beneficial to the abundance of specific bacteria such as Acidobacteria and Nitrospirae, which showed high metabolic activity in humid and low-nutritional environments [53]. In this study, fertilization treatment reduced the growth of Actinobacteria, which may be related to the form and concentration of nitrogen in the fertilizer because excessive nitrogen levels can inhibit the activity of certain microbial populations [54]. The use of water-retaining agents reduced this effect. Although this study provided important insights into the effects of fertilization and water-retaining agents on soil microbial communities, the specific effects of different types and concentrations of fertilizers and water-retaining agents on microbial diversity were not explored in detail. Future studies should consider different fertilization and water-retaining agent formulations and their application effects under diverse soil types and climatic conditions to better understand how these treatments affect the structure and function of microbial communities, thereby optimizing soil ecology and improving its fertility.

In conclusion, this study comprehensively assessed the impact of fertilizer (U), water-retaining agents (S), and their combined use (US) on the growth, yield, and soil characteristics of winter wheat. The experimental data revealed that the combined treatment significantly increased the yield and soil fertility of winter wheat by optimizing the soil water-retaining capacity and improving the structure under drought stress. This synergistic effect is reflected in not only increasing the utilization rate of soil water and fertilizer but also providing a more favorable growth environment for winter wheat by regulating soil temperature and nutritional status. In addition, the rational application of the water-retaining agent and fertilizer significantly improved root health and plant growth vigor, especially in key growth stages such as the booting stage, which effectively promoted root development and nutrient absorption. However, although this study provided empirical support for soil management practices and revealed the benefits of applying water-retaining agents and fertilizers to increase crop yield and soil health, the heterogeneity of treatment effects in different soil layers was also found in the study. This difference highlights the need for future research to explore in depth the interaction of different types and concentrations of fertilizers with water-retaining agents, as well as their performance under diverse soil and climate conditions. In order to further optimize soil management strategies and improve crop production efficiency, future work should focus on customized soil improvement plans, taking into account the physical, chemical, and biological characteristics of the soil.

## 5. Conclusions

- (1) Improvement of soil nutrients and physical properties: water-retaining agents significantly enhanced the content of ammonium nitrogen and facilitated nitrate nitrogen uptake and utilization in shallow soil, improved soil physical structure, increased soil water content, and created good conditions for the accumulation of available phosphorus and potassium.
- (2) Improvement of winter wheat productivity: water-retaining agents significantly enhanced the total production of winter wheat by increasing the seedling emergence rate, spike rate, grain number per spike, and thousand-grain weight, showing its key role in optimizing crop growth environment and improving productivity.
- (3) Promotion of root development: the combined use of water-retaining agents and fertilizer significantly increased root length density under subsoiling tillage conditions, particularly in the soil layer below 30 cm at the jointing stage and above 70 cm at the booting stage, which enhanced the utilization ability of roots to deep soil resources.
- (4) The impact of microbial diversity: the application of water-retaining agents significantly enhanced the abundance and diversity of bacteria in the 10~20 cm soil layer, revealing its important potential in improving microbial habitats. In contrast, fertiliza-



tion alone resulted in a marked decline in bacterial abundance and diversity in the 0~10 cm surface soil.

**Author Contributions:** Conceptualization, T.W. and G.L.; formal analysis, Q.H.; investigation, H.W., S.Z. and J.L.; methodology, W.L. and J.R.; resources, H.W.; data curation, Q.H.; writing—original draft preparation, H.W. and Q.H.; writing—review and editing, H.W., Q.H., T.W., G.L., W.L., J.R., S.Z. and J.L.; visualization, Q.H. and H.W.; project administration, W.L., J.L. and S.Z.; funding acquisition, T.W. and G.L. All authors have read and agreed to the published version of the manuscript.

**Funding:** Funding was provided by the National Key R&D Program of China [grant 2021YFB3900602], the Special Fund for Agro-Scientific Research in the Public Interest [grant 201503116-12], and Hebei Provincial Water Resources Science and Technology Plan Project [2020-07; 2022-20; 2024-63].

**Data Availability Statement:** Data are contained within the article.

**Conflicts of Interest:** The authors declare no conflicts of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

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