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Effects of compound water retention agent on soil nutrients and soil microbial diversity of winter wheat in saline-alkali land

Yunshuo Xu, Yu Gao, Wubo Li, Shuang Chen, Yajun Li and Yan Shi*

Abstract

Water retention agents have been applied to agricultural fields to improve the growing conditions of crops, but the potential of these agents in saline soils is poorly understood. The effects of compound water retention agents on nutrient content and soil microbial diversity of saline winter wheat soils were investigated in a field experiment with no water retention agent (CK) and 30 kg hm² of commercial attapulgite water retention agent (T4) as control and different amounts of compound water retention agents as treatments (15 kg hm²-T1, 30 kg hm²-T2, 45 kg hm²-T3). The study showed that the application of water retention agents increased the soil water content. From anthesis to harvest stage, the decreases in soil alkali-hydrolyzed nitrogen, available phosphorus, available potassium and organic matter content were greater in T2 and T3 than in the other treatments. At harvest stage, the alkali-hydrolyzed nitrogen content of T2 was significantly lower than that of CK and T4 6.19–8.83% and 4.62–5.39%, respectively. The soil available phosphorus content of T2 was significantly lower than that of CK 8.14–8.83%. The relative abundance of Actinobacteria, Proteobacteria and Acidobacteria as well as the Shannon and Simpson indices of T2 reached their maximum at harvest stage. T2 showed the best performance in terms of overall number of OTUs. The compound water retention agent may regulate soil nutrient content and accelerate plant nutrient accumulation by regulating soil water content and soil microbial abundance composition.

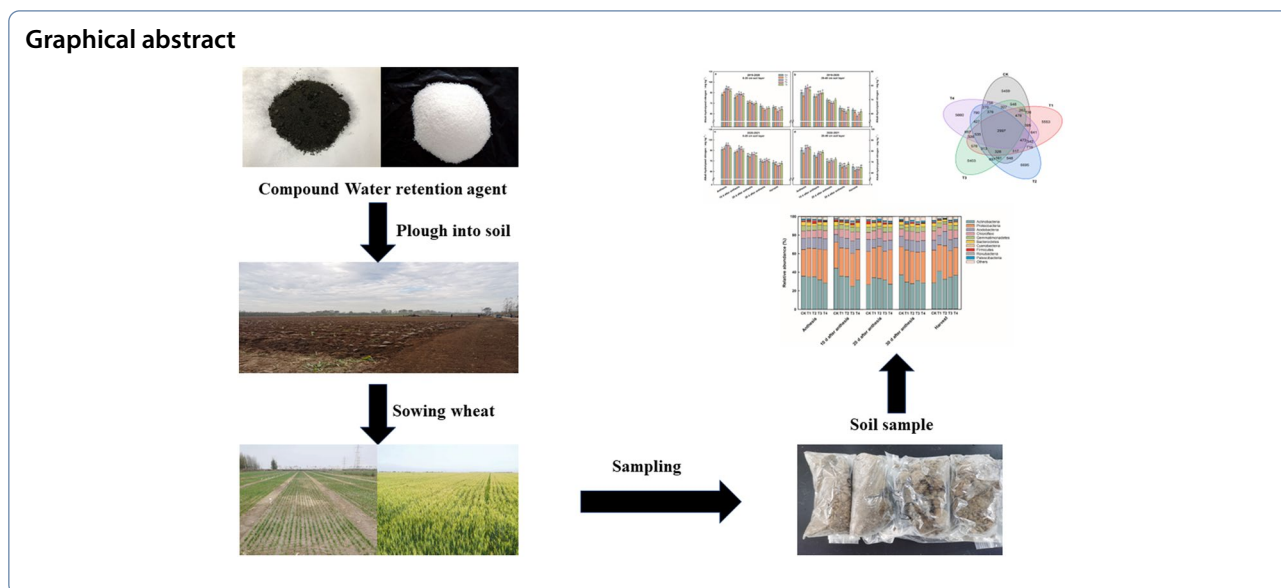
Keywords: Winter wheat, Saline-alkali land, Compound water retention agent, Soil nutrients, Soil microbial diversity

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Introduction

Wheat is one of the most important food crops in the world [1, 2], the nutritional value and economic value of which are very considerable as it provides vital quantities of high-quality starch, protein, vitamins, and other nutrients [3, 4]. As the world's largest producer and consumer of wheat, the development of wheat industry in China is of great significance to guarantee not only social stability, but also world food security [5, 6]. However, there are still many typical abiotic stresses affecting the sustainability of wheat production worldwide, soil salinization is one of the most challenging problems that restricts the normal growth and production of wheat. In China, saline-alkali land accounts for more than 20% of the total arable land area, and problems such as soil compaction, poor permeability, slow nutrient release, and changes in soil physical and chemical properties are prevalent [7], as well as plant osmotic stress, nutrient deficiency due to salt-related imbalance and oxidative stress on plants [8]. Therefore, there are many problems in planting wheat on saline-alkali land, such as low yield and poor quality [9].

Water retention agent, also called soil amendment, and super absorbent resin, is a kind of super water absorption, water retention performance of the new polymer resin, its principle is mainly the use of natural polymer materials, adding monomer, initiator, crosslinking agent polymerization [10]. Water retention agent can quickly absorb and maintain a lot of water, and can repeatedly lose water and absorb water, applied to the soil can play the role of water storage and moisture retention, can effectively improve the soil structure, promote the formation of soil aggregates [11]. It is a novel product widely accepted by farmers after chemical fertilizer, pesticide and plastic

film, which can effectively improve the drought resistance of crops and save water resources [12]. Water retention agent used in field production can fully absorb and maintain a large amount of water under the condition of sufficient rain or irrigation, while when the soil is dry and short of water, it can slowly release water for crop utilization and improve water use efficiency [13, 14]. Therefore, water retention agent is a new kind of polymer chemical water saving material with broad application prospect [15].

Water retention agent has been used in saline-alkali land improvement and saline-alkali land cultivation due to its excellent functions of water storage and soil structure improvement, but there are few relevant types of research and the mechanism is not completely clear [16–18]. Therefore, this study explored the effects of compound water retention agent on soil nutrients and soil microbial diversity of winter wheat in saline-alkali land, providing theoretical and practical basis for the improvement of saline-alkali land by water retention agent and the application of water retention agent in winter wheat cultivation in saline-alkali land.

Materials and methods

Experimental location

The field experiments were conducted in the experimental base of Qingdao Agricultural University (N37°0'27.24", E119°22'15.79") from October 2019 to June 2020 and from October 2020 to June 2021. The soil was moderately saline-alkali soil (salt content 0.32%), pH value 8.4, alkali-hydrolyzed nitrogen 81.2 mg kg⁻¹, available phosphorus 22.5 mg kg⁻¹, available potassium 171.4 mg kg⁻¹, organic matter 12.0 g kg⁻¹. Precipitation

during wheat growth period is shown in Fig. 1. Precipitation from April to June of 2021 is higher than that from April to June of 2020.

Materials

Jimai 44 (*Triticum aestivum* L.) was used as experimental material. The compound fertilizer (N–P₂O₅–K₂O:18–20–7) and attapulgite water retention agent were provided by Sinochem Shandong fertilizer Co., Ltd. and Dongying Huaye New Materials Co., Ltd. Attapulgite (chemically pure), bentonite (chemically pure) and rare earth (chemically pure) were purchased from Fujian youqiyi Pharmaceutical Trading Co., Ltd. Biochar (superior pure, Zhengzhou Niute Agricultural Technology Co., Ltd) was sieved through 300 meshes and then dried for standby. Acrylic acid and acrylamide (analytical pure, Macklin Biochemical Co., Ltd, Shanghai, China), span 60 (chemically pure, Sinopharm Chemical Reagent Co., Ltd, Shanghai, China), potassium persulfate (analytical pure, Kermel Chemical Co., Ltd., Tianjin, China), and N, N'-methylene bisacrylamide (analytical pure, BASF Chemical Co., Ltd, Tianjin, China) were used as purchased.

Preparation of the hydrogel

The compound water retention agent was made using the method of Dong with minor modifications [19]. Mix the right amount of acrylic acid and acrylamide, add the right amount of attapulgite (58%), rare earth (14%), biochar (14%), bentonite (14%), and span 60 in turn, stir and mix, pass nitrogen gas for 30 min, then add cross-linker and initiator in a nitrogen atmosphere, dry to constant weight at 75 °C after the completion of

the polymerization reaction, mechanical crushing and spare.

Experimental design

A randomized block design was used. A total of 5 treatments were set, with 3 repetitions. Each plot was 15 m² (3 × 5 m). All treatments were fertilized with 750 kg hm² of compound fertilizer as the base. Treatments were: Control (CK): no application of water retention agents; Treatment 1 (T1): application of 15 kg hm² of compound water retention agent; Treatment 2 (T2): application of 30 kg hm² of compound water retention agent; Treatment 3 (T3): application of 45 kg hm² of compound water retention agent; Treatment 4 (T4): application of 30 kg hm² of commercialized attapulgite water retention agent.

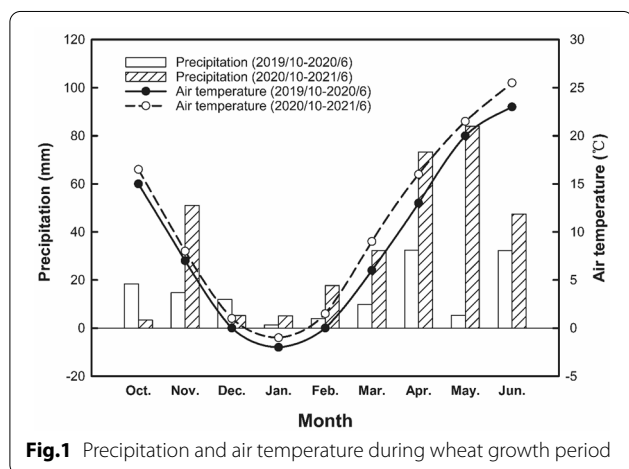
The application rate of the water retention agent was developed based on the results of previous studies [20]. Before sowing, the compound fertilizer and water retention agent will be fully mixed and evenly, and then evenly spread in each district, the way of tillage it into the soil. The sowing rate was 150 kg hm⁻². All fertilizers were used as base fertilizer for one time fertilization, and no topdressing operation was carried out later.

Determination items and methods

Samples were taken from anthesis stage to harvest stage at an interval of 10 days. Two soil layers of 0–20 cm and 20–40 cm between wheat rows were drilled with soil. After natural air drying, it will be broken, respectively, through 0.25 and 1 mm screen for reserve. It was used to determine the content of alkali-hydrolyzed nitrogen, available phosphorus, available potassium and organic matter in soil. Another 20 g was quickly put into the aluminum box and closed, and brought back immediately to measure the soil moisture content. Soil samples of 0–20 cm layer were taken into a 5-ml centrifuge tube, placed in liquid nitrogen immediately, and then transferred to a refrigerator at –80 °C for storage.

The aluminum box method was used for soil moisture content. The aluminum box containing fresh soil samples was dried at 105 °C for 12 h and weighed. The alkali-hydrolyzed nitrogen (a general term for ammonium nitrogen, nitrate nitrogen, and organic nitrogen that is readily hydrolyzed) content in soil was determined by alkali diffusion method. The content of available phosphorus in soil was determined by molybdenum–antimony resistance colorimetry. The content of soil available potassium was determined by flame photometer with ammonium acetate extraction.

Soil microbial diversity was measured, sequenced and analyzed using Illumina Miseq high-throughput sequencing platform by Shanghai Personalbio Biotechnology Co.,



LTD. A highly variable V3V4 region of about 468 bp in length of 16S rRNA gene was used. NEB Q5 DNA high-fidelity polymerase was used for PCR. Sequencing primers were: 338F (5'-barcode + ACTCCTACGGGAGGCAGCA-3'), 806R (5'-GGACTACHVGGGTWTCTAA T-3'). PCR system is shown in Table 1.

TruSeq Nano DNA LT Library Prep Kit of Illumina was used to build the Library. Two-terminal sequencing of 2 × 250 bp was performed using MiSeq Reagent Kit V3 (600cycles) on a MiSeq machine. First, the library (Index not repeatable) needed to be used on the computer was diluted to 2 nM by gradient, and then the sample was mixed in proportion to the required amount of data. The mixed library was denatured into a single chain by 0.1 N NaOH for computer sequencing.

Data processing

Microsoft Excel 2019 and SPSS 26 software were adopted for data processing and one-way ANOVA. Duncan's new multiple range method was applied for multiple comparisons ($P \leq 0.05$). SigmaPlot 14.0 and Origin 2022 software were used for graphing.

Results and analysis

Soil moisture

As shown in Fig. 2a, the soil moisture content of the 0–20 cm soil layer in the different treatments differed relatively significantly in each period from 2019 to 2020. Overall, treatments with water retention agent application (T1, T2, T3, and T4) were higher than CK, with T3 reaching the maximum soil moisture content in all periods and T2 the second highest. The soil moisture content of T2 and T3 was significantly higher than that of CK from anthesis to maturity of wheat. At anthesis, the soil moisture content of T2 was significantly higher than that of T4. The changes in different treatments in soil moisture content in the 0–20 cm soil layer from 2020–2021 were basically the same as those in 2019–2020 (Fig. 2c). As illustrated in Fig. 2b, d, soil moisture content in the

20–40 cm soil layer of different treatments did not differ significantly in all periods and performed basically the same in both years.

Soil nutrient contents

Soil alkali-hydrolyzed nitrogen

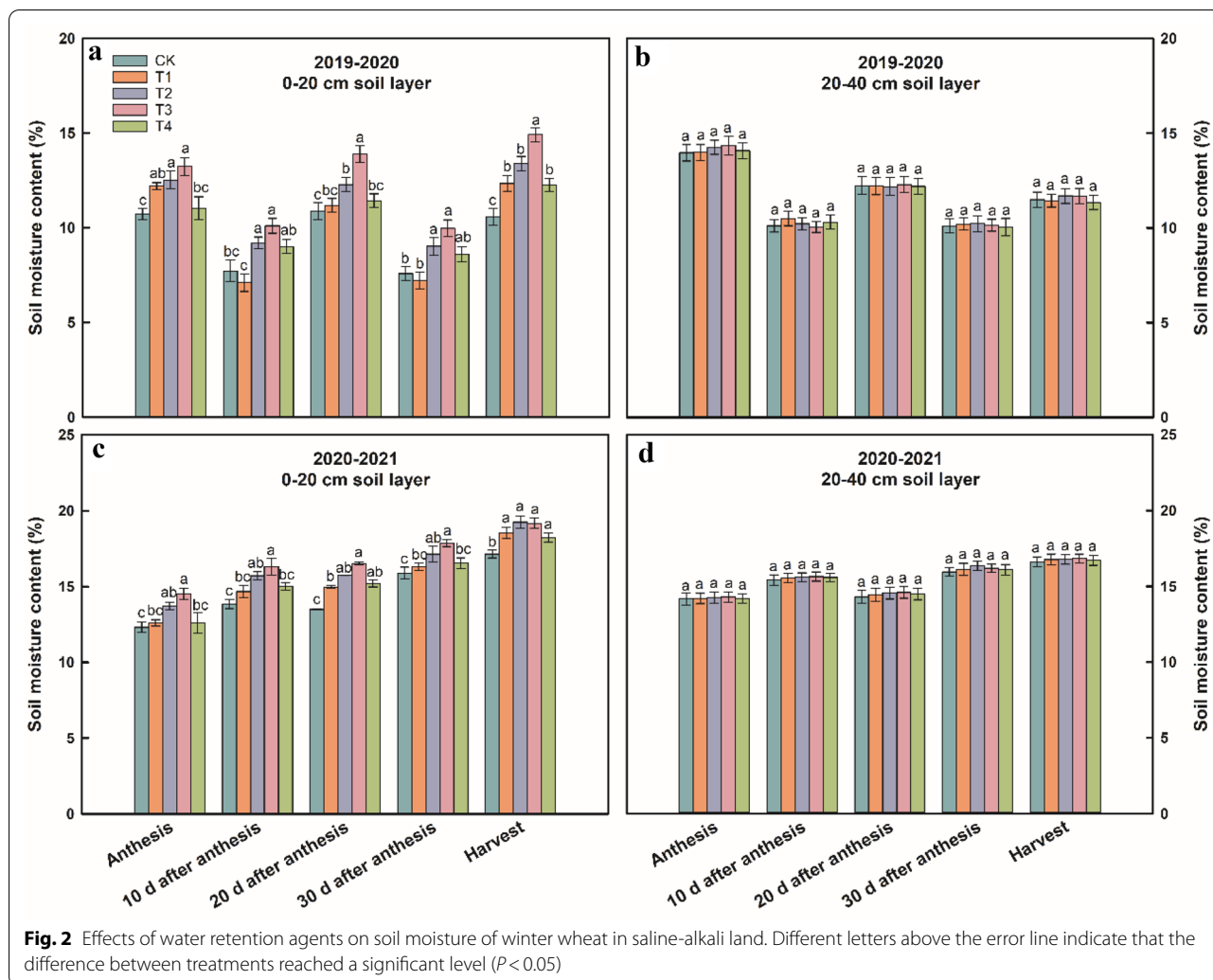
As indicated in Fig. 3, the soil alkali-hydrolyzed nitrogen content in different treatments showed a decreasing trend with the increase of days after anthesis in both 0–20 cm and 20–40 cm soil layers, and the content and the reduction of soil alkali-hydrolyzed nitrogen in 0–20 cm soil layer were greater than that in 20–40 cm soil layer. The two soil layers, 0–20 cm and 20–40 cm, showed the greatest decrease from 10 days after anthesis to 30 days after anthesis, and the decrease in soil alkali-hydrolyzed nitrogen was significantly greater in all treatments (T1, T2, T3, and T4) with water retention agent application than in CK. As demonstrated in Fig. 3a, c, the decreases in alkali-hydrolyzed nitrogen for each treatment in the 0–20 cm soil layer in 2019–2020 were T2 (34.4%) > T3 (31.3%) > T4 (27.9%) > T1 (26.0%) > CK (20.3%), and the decreases in alkali-hydrolyzed nitrogen for each treatment in the 0–20 cm soil layer in 2020–2021 were T2 (30.4%) > T3 (29.3%) > T1 (23.8%) > T4 (23.6%) > CK (20.5%). In 2019–2020, the alkali-hydrolyzed nitrogen content of T2 and T3 was significantly higher than that of CK, and that of T2 was significantly higher than that of T4 in the 0–20 cm soil layer at anthesis. At 20 days after anthesis, the alkali-hydrolyzed nitrogen content of T2 was not significantly different from that of CK. At harvest, the alkali-hydrolyzed nitrogen content of T2 was significantly lower than that of CK and T4 by 8.83% and 5.39%, respectively, and the difference between T2 and T3 was not significant. In the 20–40 cm soil layer, soil alkali-hydrolyzed nitrogen content was significantly lower in T2 and T3 than in CK and T4 at 20 days after anthesis and at harvest. The trend of alkali-hydrolyzed nitrogen content in different treatments in 2020–2021 was similar to that in 2019–2020. At harvest, the alkali-hydrolyzed nitrogen content of T2 in the 0–20 cm soil layer was significantly lower than that of CK and T4 by 6.19% and 4.62%, respectively.

Soil available phosphorus

From the beginning of the anthesis of wheat, the soil available phosphorus content in 0–20 cm and 20–40 cm soil layers showed a decreasing trend with the increase in the number of days after anthesis, with a greater decrease from 10 days after anthesis to 30 days after anthesis. 0–20 cm soil layer had higher available phosphorus content than 20–40 cm soil layer (Fig. 4). In 2019–2020, from the anthesis stage to 10 days after anthesis, soil available phosphorus content was significantly higher in the

Table 1 PCR system

Components	Quantity (μl)
Q5 high-fidelity DNA polymerase	0.25
5* Reaction buffer	5
5* High GC buffer	5
dNTP (10 mM)	2
Template DNA	2
Former primer (10uM)	1
Reverse primer (10uM)	1
ddH ₂ O	8.75



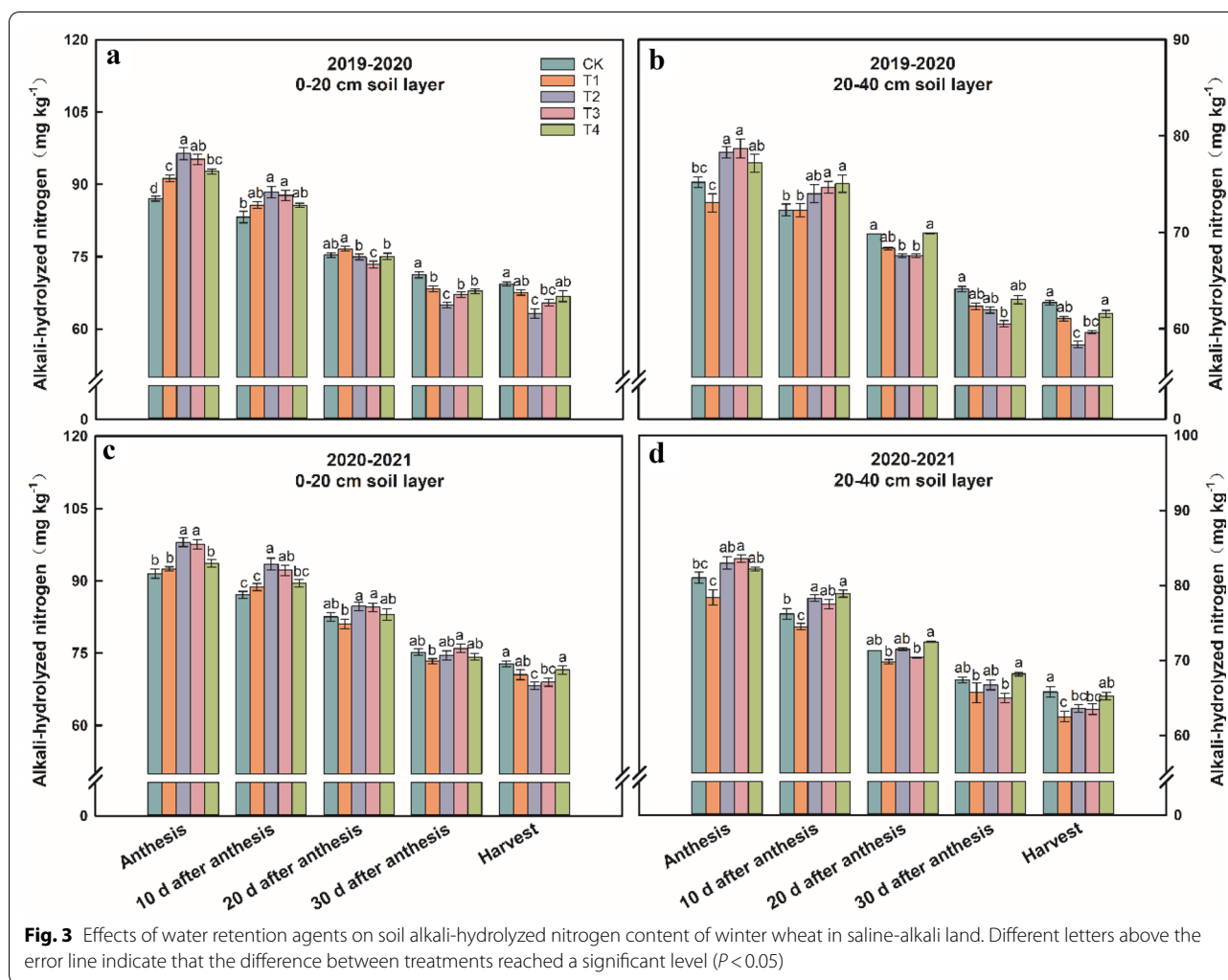
0–20 cm soil layer in T2 and T3 than in CK, and higher in T2 than in T4, but the differences were not significant. From 20 days after anthesis to the harvest stage, the soil available phosphorus content of T2 was lower than that of the other treatments. At the harvest stage, the soil available phosphorus content of T2 was significantly lower than that of CK by 8.14% (Fig. 4a). In the 20–40 cm soil layer, the available phosphorus content of T3 was significantly higher than that of CK from the anthesis to 10 days after anthesis, and the difference between T2 and T4 was not significant. At the harvest stage, the soil available phosphorus content of T2 was lower than that of CK and T4 by 8.5% and 4.39% (Fig. 4b). The trend of soil available phosphorus content in 2020–2021 was basically the same as that in 2019–2020 (Fig. 4c, d).

The changes of soil available phosphorus content in the 0–20 cm soil layer are shown in Fig. 4a, b, and the specific reduction of soil available phosphorus content in the 0–20 cm soil layer from 2019 to 2020 was

T2 (40.4%) > T3 (36.1%) > T1 (34.4%) > T4 (31.5%) > CK (25.5%). And the reduction of soil available phosphorus content for each treatment in 2020–2021 was specifically shown as T2 (34.6%) > T3 (32.7%) > T4 (31.3%) > T1 (29.3%) > CK (25.6%). Furthermore, the changes of soil available phosphorus content in the 20–40 cm soil layer are presented in Fig. 4c, d. The decrease of soil available phosphorus content in each treatment from 2019 to 2020 was specifically shown as T2 (37.8%) > T3 (35.5%) > T4 (34.0%) > T1 (33.8%) > CK (28.1%), and the decrease of each treatment from 2020 to 2021 was specifically shown as T2 (36.9%) > T3 (33.1%) > T1 (28.2%) > T4 (26.3%) > CK (23.4%).

Soil available potassium

Soil available potassium content of different treatments showed a gradual decrease with the increase of days after anthesis in wheat, with a greater decrease from 10 days after anthesis to 30 days after anthesis (Fig. 5).



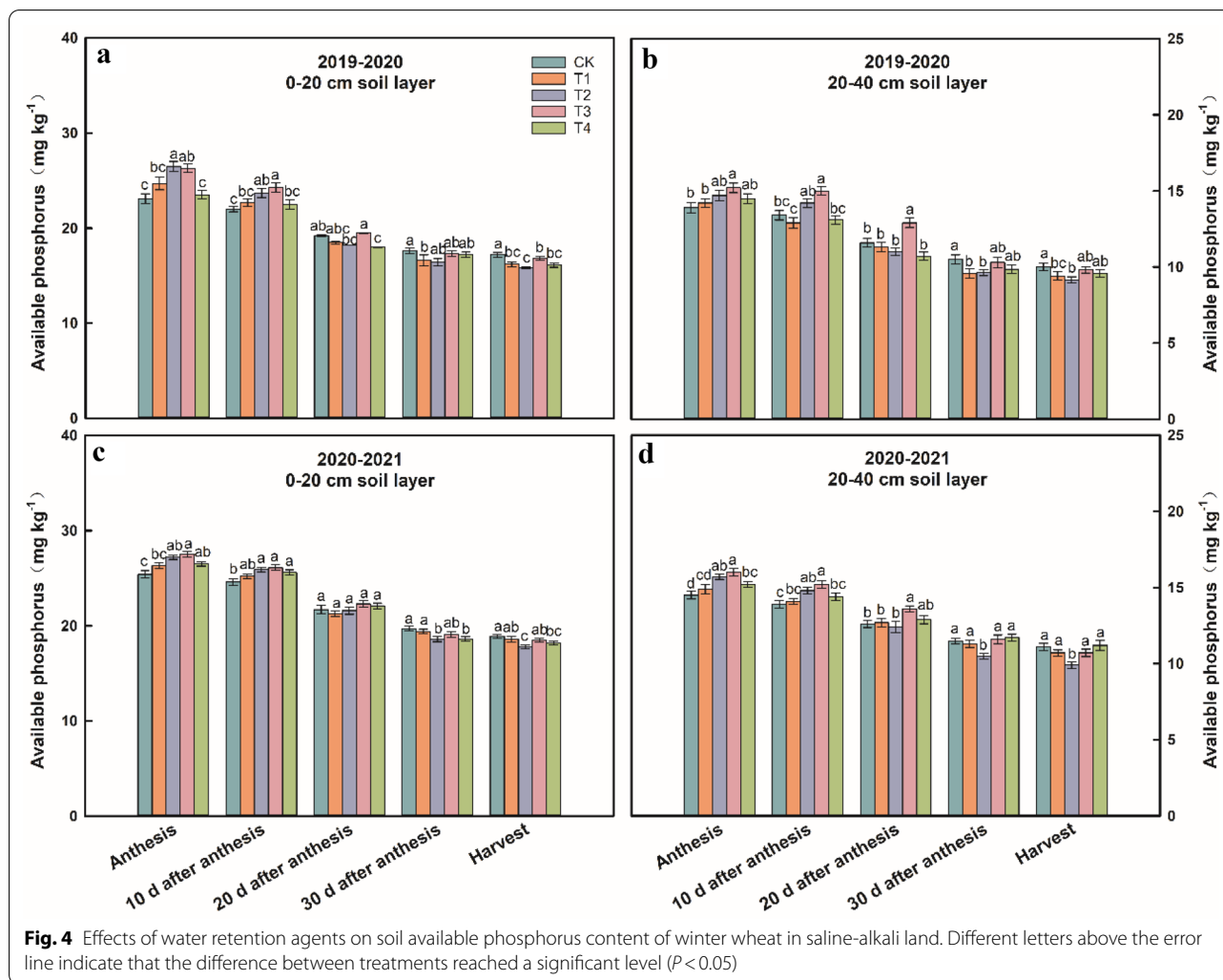
In 2019–2020, the soil available potassium content of T2 and T3 in the 0–20 cm soil layer was significantly higher than that of CK and T4 from the anthesis stage to 10 days after anthesis. At 20 days after anthesis and 30 days after anthesis, the differences in available potassium content among CK, T1, T2, and T3 were not significant, but all were lower than that of T4 (Fig. 5a). In the 20–40 cm soil layer, from 10 days after anthesis to harvest, the differences in the available potassium content between T2 and CK and T4 were not significant (Fig. 5b). The trend of soil available potassium content in 2020–2021 was basically the same as that in 2019–2020 (Fig. 5c, d).

As shown in Fig. 5a, b, the decreases in soil available potassium content were significantly greater in all treatments from 2019 to 2020 than in CK, with larger decreases of 21.8% and 19.5% in T3 and T2, respectively. The decreases in soil available potassium content were greater in T3 (22.9%) and T2 (20.9%) than in CK (12.8%) and T4 (14.3%) from 2020 to 2021. Figure 5c, d

demonstrates the trend of changes in soil available potassium content in the 20–40 cm soil layer with increasing days after anthesis. And the decrease in soil available potassium content for each treatment from 2019 to 2020 was shown as T3 (24.1%) > T2 (21.7%) > T4 (17.0%) > T1 (16.9%) > CK (14.5%). The decrease of available potassium content in each treatment from 2020 to 2021 was shown as T3 (25.2%) > T2 (22.1%) > T4 (18.2%) > T1 (17.3%) > CK (14.8%).

Soil organic matter

The organic matter content of the different treatments in the 0–20 cm and 20–40 cm soil layers trended downward as the number of days after anthesis increased in both years. The soil organic matter content of the 0–20 cm soil layer was higher than that of the 20–40 cm soil layer in all stages (Fig. 6). The decrease in soil organic matter content was significantly greater in all treatments than in CK in the 0–20 cm soil layer. As depicted



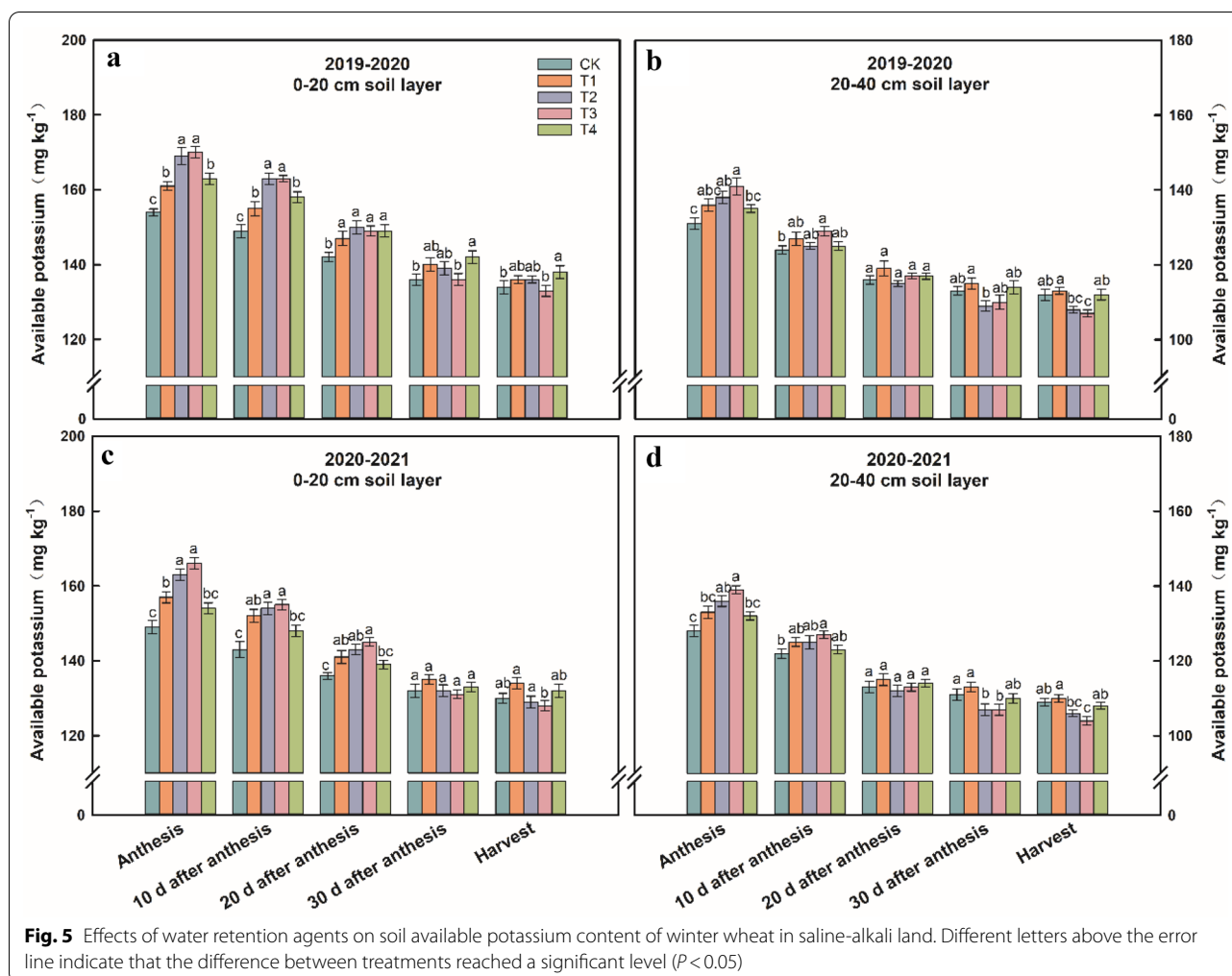
in Fig. 6a, the decrease in soil organic matter content was greater in both T3 (11.8%) and T2 (10.8%) than in CK (8.8%) in 2019–2020. In 2020–2021, the greatest decrease in soil organic matter content was observed in T3 with a decrease of 12.1% (Fig. 6c). The treatments in the 20–40 cm soil layer showed inconsistent decreases in both years. The decrease in soil organic matter content with increasing number of days after anthesis was $T3 > T2 > CK > T4 > T1$ in 2019–2020 (Fig. 6b), and $T2 > T3 > T4 > T1 > CK$ in 2020–2021 (Fig. 6d).

Soil microbial diversity

Relative abundance of soil colonies

As shown in Fig. 7, the relative abundance of soil bacteria in each treatment with water retention agent was different from the anthesis stage to the harvest stage of wheat. The relative abundance of Actinobacteria and Proteobacteria was higher in each treatment. The relative abundance of Acidobacteria, Chloroflexi and

Gemmatimonadetes decreased successively. The relative abundance of Bacteroidetes, Cyanobacteria, Firmicutes, Rokubacteria and Patescibacteria was relatively small. The relative abundance of Actinobacteria in each treatment was lower than CK at anthesis stage, 10 days after anthesis and 30 days after anthesis, and higher than CK at 20 days after anthesis and harvest stage. The relative abundance of Proteobacteria in each treatment was significantly higher than CK from anthesis stage to 10 days after anthesis, and $T4 > CK > T2 > T1 > T3$ in each treatment on 20 days after anthesis. The relative abundance of Proteobacteria in T2 and T3 was higher than that in other treatments T4 at 30 days after anthesis. The relative abundance of Proteobacteria was highest in T2 during harvest period. The relative abundance of Acidobacteria in each treatment was significantly higher than CK at 10 and 20 days after anthesis stage. The relative abundance of Acidobacteria in T2 and T3 was lower than that in other treatments at anthesis stage, and T2 was the

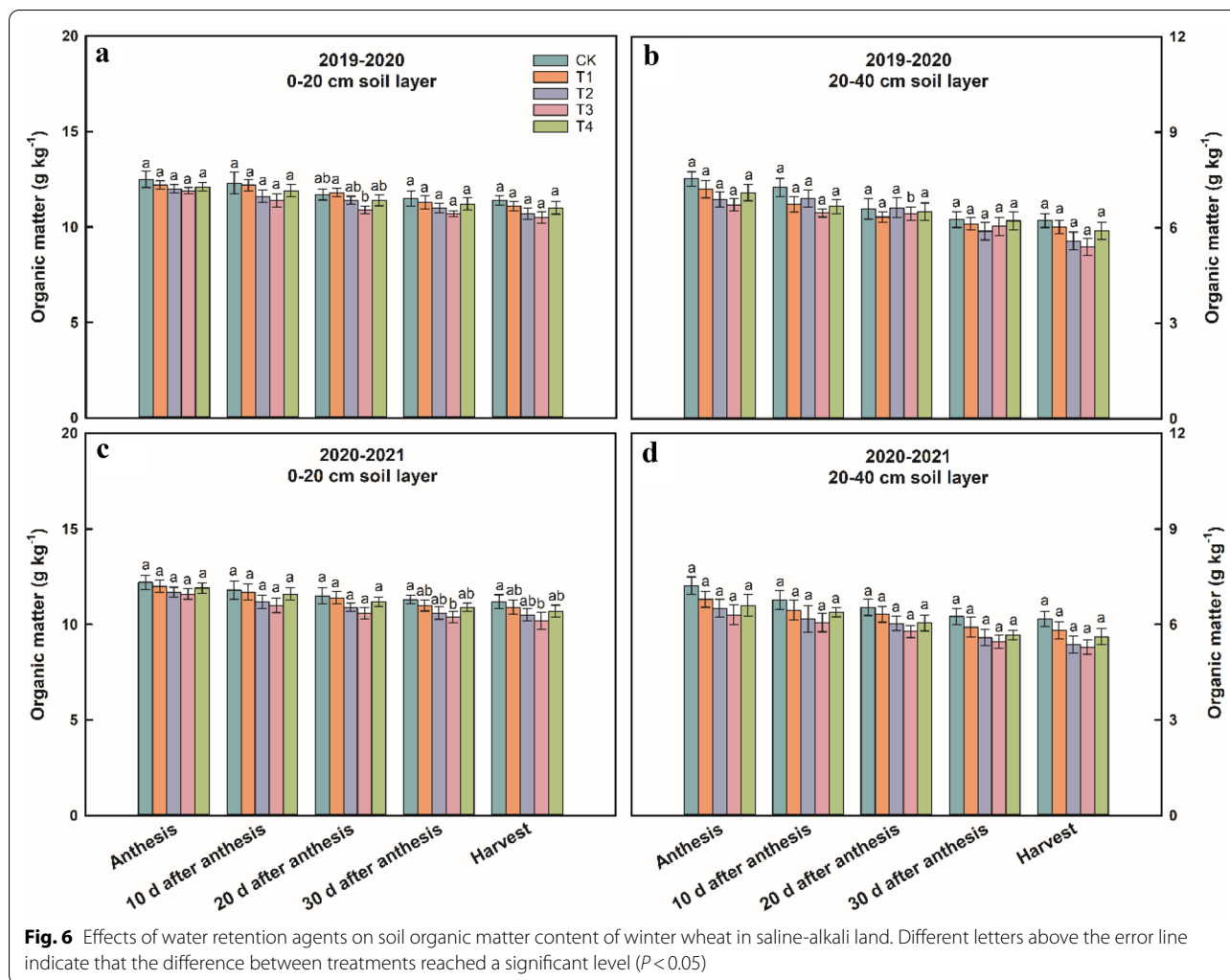


highest at harvest stage. The relative abundance of Chloroflexi in all treatments treated with water retention agent was significantly higher than that in the control group from anthesis stage to 30 days after anthesis stage, and the relative abundance of T1, T2 and T4 was lower than that in CK at harvest stage. The relative abundance of Gemmatimonadetes in each treatment treated with water retention agent was significantly higher than that in the control group at 10 and 30 days after anthesis stage.

Soil microbial taxa at each level

As shown in Fig. 8, there was no significant difference in the number of units at the classification level of domain and phylum between different treatments during the period from anthesis stage to harvest stage. At the level of class classification, there was no significant difference in the number of microbial units at anthesis stage and 20 days after anthesis. At 10 days after anthesis and harvest stage, the number of microbial units applied with water retention agent was significantly higher than

that of the control group, among which T2 was the largest, and T1 and T3 were significantly higher than other treatments at 30 d after anthesis stage. At the level of order classification, the number of microbial units in each treatment was significantly higher than that in the control group in each stage. At the level of order classification, T3 had the largest number of microbial units, and there was no significant difference between T2, T3, and T4 at anthesis stage. At the level of order classification at 10 days after anthesis, the number of microbial units was $T3 > T2 > T4 > T1 > CK$. At the level of order classification, the number of T3 and T4 microbial units was the highest 20 days after anthesis, but there was no significant difference between them, and there was no significant difference between T1 and T2. At the level of order classification, T1 had the largest number of microbial units at 30 days after anthesis, while there was no significant difference between T2, T3, and T4. At harvest stage, the number of T2 microbial units was the largest at order level, followed by T3 and T4. The



number of microbial units at the level of family classification in each treatment treated with water retention agent was significantly higher than that in the control group at anthesis stage, 10 days and 30 days after anthesis. At anthesis stage and 10 days after anthesis, the number of microbial units at the family taxonomic level of T2 and T3 was the largest, and T1 was the largest at 30 days after anthesis stage. There was no significant difference between T1 and CK in the number of microbial units at the level of family classification at 20 days after anthesis and at harvest stage, while T2, T3 and T4 were significantly higher than CK. The number of microbial units in each treatment was significantly higher than that in the control group at the level of genus classification. The number of microbial units at the genus level of T3 was the largest at anthesis stage, T2 was the largest at 10 and 20 days after anthesis, T1 was the largest at 30 days after anthesis stage, and T2 was the largest at harvest stage. The number

of microbial units at species classification level in all treatments was significantly higher than CK in all treatments except T1 at anthesis stage, and significantly higher than control group at 10 days after anthesis. As shown in Fig. 8, there was no significant difference in the number of units at the classification level of domain and phylum between different treatments during the period from anthesis stage to harvest stage. At the level of class classification, there was no significant difference in the number of microbial units at anthesis stage and 20 days after anthesis stage. At 10 days after anthesis stage and harvest stage, the number of microbial units applied with water retention agent was significantly higher than that of the control group, among which T2 was the largest, and T1 and T3 were significantly higher than other treatments at 30 days after anthesis stage. At the level of order classification, the number of microbial units in each treatment was significantly higher than that in the control group in each

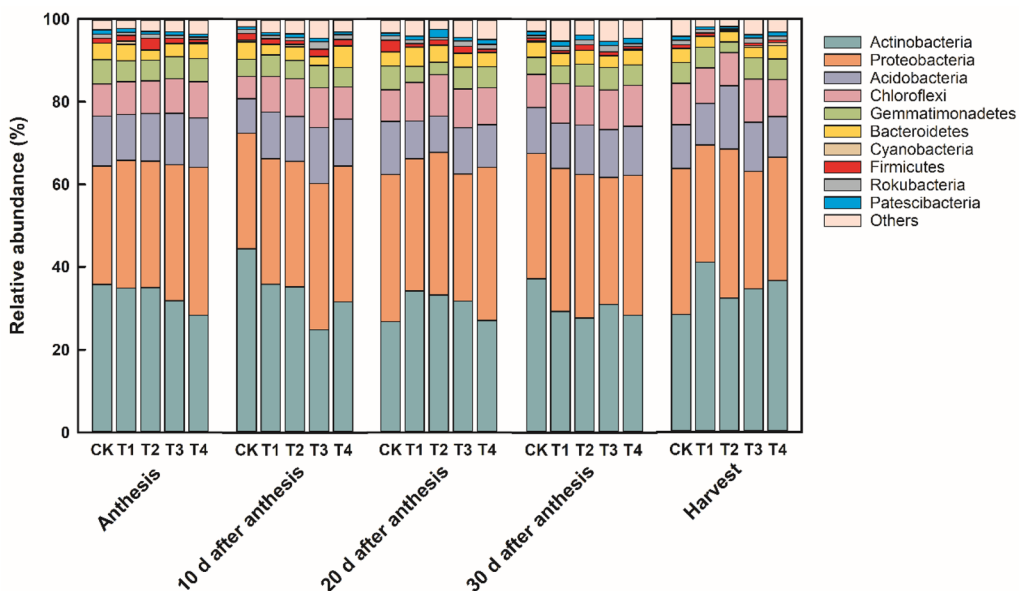


Fig. 7 Effects of water retention agents on relative abundance of winter wheat soil colonies in saline-alkali land. Annotation: every 5 columns from left to right is a period, anthesis stage, 10 days after anthesis, 20 days after anthesis, 30 days after anthesis, and harvest stage

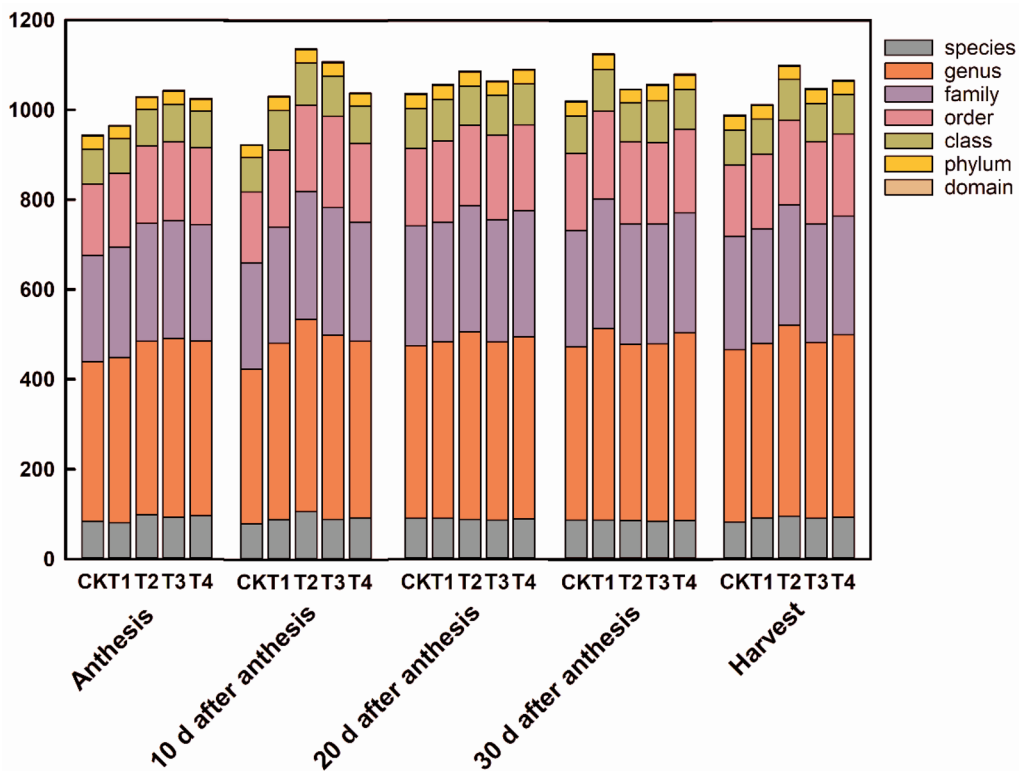


Fig. 8 Effects of water retention agents on the number of soil microbial taxa at each level of winter wheat in saline-alkali land. Annotation: every 5 columns from left to right is a period, anthesis stage, 10 days after anthesis, 20 days after anthesis, 30 days after anthesis, and harvest stage

period. At the level of order classification at anthesis stage, T3 had the largest number of microbial units, and there was no significant difference between T2, T3 and T4. At the level of order classification at 10 days after anthesis stage, the number of microbial units was T3 > T2 > T4 > T1 > CK. At the level of order classification, the number of T3 and T4 microbial units was the highest 20 days after anthesis stage, but there was no significant difference between them, and there was no significant difference between T1 and T2. At the level of order classification, T1 had the largest number of microbial units at 30 days after anthesis stage, while there was no significant difference between T2, T3 and T4. At harvest stage, the number of T2 microbial units was the largest at order level, followed by T3 and T4. The number of microbial units at the level of family classification in each treatment treated with water retention agent was significantly higher than that in the control group at anthesis stage, 10 days and 30 days after anthesis stage. At anthesis stage and 10 days after anthesis stage, the number of microbial units at the family taxonomic level of T2 and T3 was the largest,

and T1 was the largest at 30 days after anthesis. There was no significant difference between T1 and CK in the number of microbial units at the level of family classification at 20 days after anthesis stage and at harvest stage, while T2, T3, and T4 were significantly higher than CK. The number of microbial units in each treatment was significantly higher than that in the control group at the level of genus classification. The number of microbial units at the genus level of T3 was the largest at anthesis stage, T2 was the largest at 10 and 20 days after anthesis, T1 was the largest at 30 days after anthesis, and T2 was the largest at harvest stage. The number of microbial units at species classification level in all treatments was significantly higher than CK in all treatments except T1 at anthesis stage, and significantly higher than control group at 10 days after anthesis and at harvest stage, among which T2 was the largest, and there was no significant difference in each treatment at 20 days after anthesis and 30 days after anthesis. And at harvest stage, among which T2 was the largest, and there was no significant difference in each treatment at 20 days and 30 days after anthesis.

Table 2 Soil microbial Alpha diversity index under different treatments

Treatment		Chao1	Observed species	Shannon	Simpson	Pielou's evenness	Faith's PD	Good's coverage
Anthesis stage	CK	4004.09e	3828.1e	10.253d	0.9877c	0.8783ab	271.27d	0.9922ab
	T1	4457.49d	4254.5d	10.531b	0.9933b	0.8794a	288.21c	0.9906a
	T2	4635.11c	4317.7c	10.655a	0.9974a	0.8792a	297.60b	0.9888a
	T3	4883.03a	4616.2a	10.657a	0.9947ab	0.8755c	314.97a	0.9887a
	T4	4738.11b	4437.0b	10.452bc	0.9976a	0.8792a	303.98b	0.9890a
10 days after anthesis stage	CK	3897.35e	3731.5d	9.7380d	0.9901c	0.8207d	266.67d	0.9919a
	T1	5177.51c	4596.5b	10.469c	0.9940b	0.8605c	291.96c	0.9853b
	T2	6177.44a	5409.5a	10.812a	0.9962ab	0.8719a	345.09a	0.9819c
	T3	4968.27d	4496.7c	10.592b	0.9977a	0.8729a	316.20b	0.9868b
	T4	5273.61b	4598.4b	10.527bc	0.9971a	0.8652b	299.65c	0.9842b
20 days after anthesis stage	CK	4629.93d	4246.8c	10.727d	0.9885c	0.8925a	285.54d	0.9895a
	T1	5565.50b	5102.6b	10.787bc	0.9936b	0.8709d	336.42a	0.9830d
	T2	5716.78a	5158.2a	10.906a	0.9978a	0.8843b	339.30a	0.9861b
	T3	5434.63c	4828.8d	10.750c	0.9977a	0.8768c	319.39bc	0.9848c
	T4	5409.12c	4862.9c	10.844b	0.9943b	0.8854b	324.49b	0.9853bc
30 days after anthesis stage	CK	5095.99d	4615.9d	10.791c	0.9946c	0.8858c	303.99d	0.9851a
	T1	5320.62c	4733.9c	10.796c	0.9972ab	0.8843c	326.05b	0.9859a
	T2	5274.11c	4753.3c	10.844b	0.9987a	0.8878b	317.70bc	0.9855a
	T3	5547.55b	5028.8b	10.659d	0.9982a	0.8757d	327.20b	0.9860a
	T4	6113.21a	5417.2a	11.096a	0.9988a	0.8946a	357.43a	0.9819b
Harvest stage	CK	4820.73d	4416.0d	9.6090e	0.9845c	0.8523d	281.42b	0.9870a
	T1	5365.94c	4727.3c	10.596d	0.9956b	0.8681c	286.30b	0.9844c
	T2	5578.76a	4966.9a	10.874a	0.9986a	0.8878a	320.69a	0.9843c
	T3	5496.28b	4869.7b	10.812b	0.9981a	0.8822b	318.27a	0.9842c
	T4	5497.94b	4999.2a	10.711c	0.9945b	0.8880a	321.30a	0.9857b

Annotation: different letters after the same column of data in each stage indicate a significant difference of 5%

Soil microbial Alpha diversity index

As shown in Table 2, various indexes of soil microbial Alpha diversity varied with different treatments from anthesis stage to harvest stage. Chao1 index and Observed Species index are used to represent the richness of each sample Species. Chao1 index and Observed Species index of the treatment with water retention agent were significantly higher than those of the control group in all periods, indicating that the application of water retention agent can significantly improve the microbial richness of winter wheat soil in saline-alkali land. The richness of T3 was the highest at anthesis stage, and that of T2 was significantly higher than that of other treatments at 10 days, 20 days after anthesis and harvest stage. The richness of T4 was the highest at 30 days after anthesis stage. Shannon index and Simpson index were used to indicate the diversity of sample species. The Shannon index of T3 was significantly lower than that of CK 30 days after anthesis stage, and there was no significant difference between T1 and CK. The Shannon index and Simpson index of the treatments applied with water retention agent were significantly higher than those of CK in the other stages, indicating that the application of water retention agent could improve the soil microbial diversity of winter wheat in saline-alkali land, and the diversity of T2 in each treatment was the best from anthesis stage to harvest stage. Pielou's Evenness index was used to represent the evenness of each sample species. At the anthesis stage, the evenness of T3 decreased, while the evenness of other treatments remained basically the same. At the 10 days after anthesis and the harvest stage, the evenness of each treatment with water retention agent was significantly higher than that of the control group, among which T2 showed the best performance. At 20 days after anthesis, the evenness of all treatments was lower than CK, and the decrease of T2 and T4 was the least. At 30 days after anthesis, there was no significant difference in evenness between T1 and CK, while other treatments were significantly higher than CK. Faith's PD Index represents the evolutionary diversity of sample species. The application of water retention agent can significantly improve the evolutionary diversity of soil microbial species of winter wheat in saline-alkali land, and the overall performance of T2 and T3 is better. Good's Coverage index was used to represent the coverage of the sample library, and the values of each treatment were around 0.98 to 0.99, indicating that the sequencing results could represent the real situation of microbes in the sample.

Soil microbial OTU quantity

According to the number of OTUs in different treatments, a Venn diagram was made. The number of

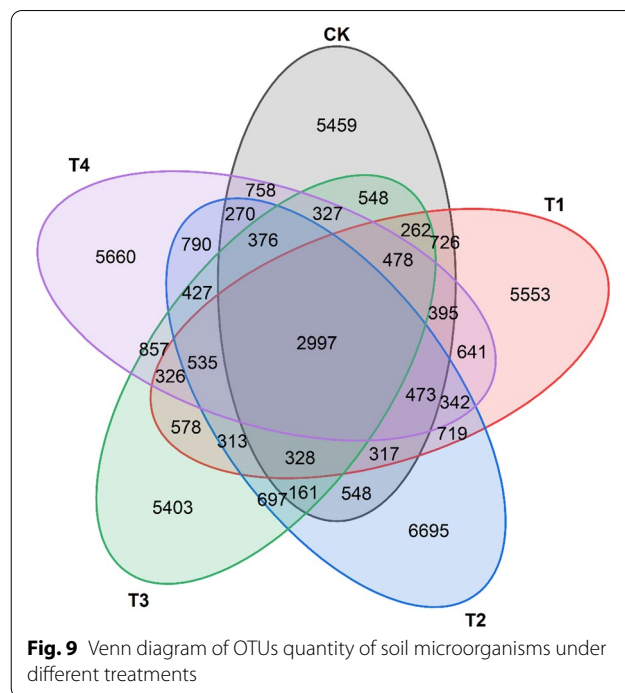


Fig. 9 Venn diagram of OTUs quantity of soil microorganisms under different treatments

common OTUs and the number of specific OTUs among different treatments are shown in Fig. 9. The total amount of OTU in each treatment showed as T2>T4>T1>T3>CK. The results showed that applying water retention agent could significantly increase OTU quantity of winter wheat soil microorganisms in saline-alkali soil. The total number of OTUs specific to each treatment was T2>T4>T1>CK>T3. The results showed that proper application of water retention agent could significantly increase the number of microbial specific OTUs in winter wheat soil in saline-alkali land, but excessive application would lead to the decrease of OTUs. In general, T2 has the best comprehensive performance. Both the total number of OTUs and the number of specific OTUs in T2 are significantly higher than other treatments.

Discussion

Soil moisture

This study showed that the application of a compound water retention agent could significantly increase the soil water content of saline winter wheat in saline-alkali land, which increased with the amount of compound water retention agent within a certain range. Other studies found that the application of water retention agent could increase photosynthetic rate and yield of spring millet by increasing soil water content [22]. Water retention agents, due to their strong water absorption capacity and volume change during the wet and dry cycle, can

improve the basic physical properties of soil after mixing with soil, such as increase water absorption and desorption capacity, reduce bulk weight, increase total porosity, significantly improve permeability and air permeability, inhibit soil water evaporation and prevent soil leakage, thus increasing the water content of soil [21].

Soil nutrients

The stage of anthesis to harvest is an important time for wheat growth and development. Studies have demonstrated that water retention agents can retain soil nutrients and promote nutrient uptake by plants [23, 24]. In this experiment, the contents of soil alkali-hydrolyzed nitrogen, available phosphorus and available potassium of each treatment with water retention agent were significantly higher than those of the control at the anthesis stage of wheat, indicating that the application of water retention agent can preserve soil nutrients and reduce nutrient loss before the anthesis stage of wheat [25]. The compound water retention agent applied in this study contains bentonite, biochar, rare earths and albite. Bentonite is a natural layered silica-aluminate mineral clay with properties of water molecule adsorption, ion exchange and good adhesion, which can enhance the salt resistance of water retention agent [26]. Biochar has favorable physicochemical properties and nutrient regulating effects, which can improve soil bulk structure, enhance soil nutrient adsorption and retention, and meet the water demand of plant growth [27]. The water retention agent adsorbs nutrients from the soil while retaining water, reducing nutrient loss in the early stages of wheat growth, improving the microenvironment of the soil, reducing the stressing effect of salinity on wheat, and ensuring the nutrient uptake capacity of wheat and its nutrient requirements in the later stages of growth.

The soil organic matter content in different treatments was lower than that of the control from anthesis to harvest stage, and the decrease rate was higher than that of the control, which might be because the application of water retention agent increased soil water content and promoted the decomposition of organic matter into inorganic nutrients [28].

Soil microbial diversity

Soil microorganisms play an important role in the transformation and supply of soil nutrients, which can regulate organic and inorganic nutrients, decompose organic matter, and affect the availability of nutrients, thus affecting crop yield [29]. The dominant phyla in this study were Actinobacteria, Proteobacteria, Acidobacteria, Chloroflexi, and Gemmatimonadetes. Actinobacteria are active in dry environments [30], which may be the reason why the abundance of Actinobacteria in this study has

decreased in most periods. Proteobacteria are related to the absorption of nitrogen and phosphorus by plants and the transformation of organic matter [31]. In this study, the treatment with water retention agent consumes more available nutrients and decomposition of organic matter. Therefore, this may be the reason why the abundance of Proteobacteria in the treatment with water retention agent in this study is significantly higher than that in other treatments. Acidobacteria is a kind of oligotrophic taxon, which can maintain metabolic activity under low nutrient conditions [32]. This suggests that the higher relative abundance of Acidobacteria in the treatment with water retention agent in this study may be due to the better utilization of available nutrients.

The species richness, diversity and evenness of soil microorganism were increased by applying water retention agent from anthesis to harvest stage. Studies have shown that soil microbes have a strong dependence on soil water, and bacteria's acquisition of nutrients mainly depends on the flow of water film in soil. Within a certain range, soil microbial richness and diversity are positively correlated with soil water content [33]. Enhanced soil nutrient cycling driven by bacterial communities is usually associated with higher microbial diversity [34, 35]. The results of this experiment are similar to those of previous studies. In this experiment, soil microbial diversity may be improved by increasing soil water content and improving soil physical and chemical properties by applying water retention agent.

Conclusions

In this study, a compound water retention agent was manufactured from acrylic acid, acrylamide, bentonite, biochar, attapulgite, rare earth, N-N methylene bisacrylamide, and potassium persulfate. (1) The application of water retention agent was beneficial in improving soil water content, soil nutrient status and soil microbial diversity compared to the treatment without water retention agent. (2) The application of the same amount of the compound water retention agent (T2) was more effective compared to the treatment with the attapulgite water retention agent (T4). In this experiment, a variety of natural and environmentally friendly materials were used as active ingredients of water retention agents, which help to reduce the stress of wheat in the harsh soil environment and avoid their own toxicity. However, since the use of chemical materials such as acrylic acid and acrylamide is still unavoidable, the current problem is to select natural and biodegradable materials as their alternatives and to maintain the reproducibility and consistency of water retention agent performance in complex field environments for mass production and agricultural applications.

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Author contributions

YS and YX designed the experiment. YX, YG, WL, SC, and YL completed the experiment. YX and YG carried out data analysis and graphic production. YX participated in the writing of the manuscript. YS performed the final editing of the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets used or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations**Ethics approval and consent to participate**

Meets ethical standards applicable to the research discipline.

Consent for publication

All authors agree to the publication of the work.

Competing interests

The authors declare that there is no conflict of interest.

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