

Effect of polyaspartic acid on soil water content, soil microbial diversity, cotton yield and fiber quality

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Abstract

Purpose Water-saving is one of the most important problems in agricultural development, especially in arid and semi-arid areas. The effects of polyaspartic acid (PASP) on soil water storage, soil microbial community, soil physiochemical properties, cotton yield and fiber quality were studied to find water-saving material utilized in cotton field.

Materials and methods The experiment was divided into two parts, the first part concerned the direct application of three different amounts of PASP under field conditions. In the second part PASP was mixed with soil in different proportions and the mixtures were put into bottles, which were then buried in the cotton field.

Results and discussion The application of PASP improved the water-holding capacity and thus increased water content available to the cotton root system in the cotton field for a long time, and significantly ($p < 0.05$) increased the content of soil organic matter, available P and ammonium-N. Relative abundances of *Methylophaga*, *Sphingomonas*, *Cupriavidus*, *Pseudeurotium*, *Fusarium* and *Nectria* were significantly affected by applying PASP. Compared to the control group, 15 kg ha⁻¹, 75 kg ha⁻¹ and 150 kg ha⁻¹ of PASP increased seed cotton yield by 3.94%, 8.31% and 7.71%, respectively. The application of PASP also increased reflectance degree, Micronaire and short fiber index of cotton.

Conclusion These results suggested that 75 kg ha⁻¹ of PASP can be appropriate to alleviate drought stress in arid and semi-arid areas.

1 Introduction

Xinjiang is the largest cotton-growing region in China (Han et al. 2020), as an arid and semi-arid region with the typical continental desert climate, the average air temperature ranges from 10°C to 13°C (Wang et al. 2018; Du et al. 2021). The average annual potential evaporation is above 2700 mm while the average annual rainfall is below 80 mm, and this limits the sustainable development of agriculture in this area (Tan et al. 2017).

Water-retaining agent is a polymer composite particle, with a strong water absorption, retention and slow-release properties (Liu et al. 2020; Zhou et al. 2021). Applying water-retaining agent to farmland soil can not only improve soil porosity, change soil physical properties and increase soil water accumulation capacity, but also promote seed germination, increase the emergence rate, and improve soil nutrient structure and microbial environment (Guo et al. 2016). In addition, water-retaining agents can absorb and release nutrients, and promote soil colonization of microorganisms, including bacteria and fungi, to meet the physiological and biochemical requirements of crop (Liu et al. 2014; Sojka et al. 2006). The addition of water-retaining agents, such as polyacrylamide (Ozhan 2021), poly-γ-glutamic acid (Liang et al. 2019), poly-acrylic acid salt and denatured starch (Bai et al. 2011; Hirotama et al. 2008) can alleviate drought stress.

Polyaspartic acid (PASP) is one of the most important water-retention agents with a good chelating ability, scale and corrosion inhibition ability; it is biodegradable, and the degradation products can be absorbed by plants (Migahed et al. 2016). PASP can chelate Ca, Cu, Mg, Fe and other metal ions, avoiding their leaching in soil, thus improving soil nutrient content (Jiao et al. 2020; Hu et al. 2019). PASP, as a controlled-release agent, can improve the uptake of nutrients by some dryland crops, and is an effective material to promote crop growth, and improve N utilization efficiency and crop yield (Deng et al. 2014; Ji et al. 2021). PASP applied to the transplantation of *Xanthoceras sorbifolia* seedlings, increased the survival rate and leaf water content of seedlings by 8–12% and 4–16%, respectively (Wei et al. 2016). The mixed application of polyaspartic acid and N fertilizer increased N accumulation in specific organs of rice, and N recovery efficiency and photosynthetic capacity of leaves, thereby improving growth rate, biomass, and rice yield and quality (Deng et al. 2017).

Microbial communities are very important for plant growth and adapt to various biological and abiotic stresses (Sun et al. 2021), and their composition depends on soil properties (Bååth and Anderson 2003). In the rhizosphere of plants, the interaction between plants and microorganisms is very close, because plants emit a wide range of organic and inorganic compounds (Compant et al. 2010). Applying fertilizer to soil can affect the diversity and function of microbial communities (Wang et al. 2021). The microbial community around plants establishment is not randomly. Plants regulate by manipulating the aboveground-belowground feedbacks between plants and soil microbiota (Bulgarelli et al. 2013; Lau and Lennon 2012). The combination of water-retaining agent and biofertilizer in shrubs increased microbial diversity and soil microbial abundance, and changed soil structure and soil fertility (Zhang et al. 2018). Plant-microorganisms-soil interactions are considered to be the main driver of plant community structure and dynamics. The effect of water-retaining agent on microorganisms, nutrients and plant growth is likely to be determined by water content (Amarasinghe et al. 2021). However, the effects of water-retaining agent (especially PASP) on cotton-soil-microorganisms-water system and their relationships are poorly understood.

Therefore, our study focused on the effects of PASP on the soil water content, soil physiochemical properties, composition of soil microbial community, cotton yield and quality under drought condition. In particular, our study aimed to (1) investigate the effects of PASP on soil water-holding capacity and physiochemical properties during all cotton growth; (2) evaluate the influences of different PASP application rates on the diversity of soil microbial community; (3) explore the effects of PASP on cotton yield and quality. Studying these issues will allow to reveal the mechanism of the effect of PASP on soil microbial physiology and ecology as well as on crop yield and quality in addition to obtain a suitable amount of PASP to be applied to the cotton field in the alkaline sandy loam of Southern Xinjiang, China.

2 Methods And Materials

2.1 Site description and experimental design

The study was carried out in Nankou town, Alar City, Xinjiang province, China (40°38' N, 81°23' E). This region had a typical extreme continental arid desert climate with an average annual rainfall of 40.1-82.5mm and an average temperature of 10.7°C, respectively. There were 180-224d of frost-free period in a year. The annual potential evaporation was approximately 1876.6-2558.9mm. Thus, water loss caused by evaporation was extraordinarily severe. The land was cotton cultivated for more than ten years before the experiment, and the soil type was alkaline sandy loam. The cotton variety selected in this study was Xinluzhong 70, provided by Xinjiang Tarim River Seed Industry Co., Ltd (Alar, China). Cotton field was covered with plastic film to reduce water loss.

The sowing date of cotton was April 18th, 2019; cotton was irrigated 9 times during the whole growth period. Applying basal fertilizer composed of 300 kg ha⁻¹ urea, 375 kg ha⁻¹ diamonium hydrogen phosphate, and 150 kg ha⁻¹ potassium sulfate. During the whole growth period of cotton, 312 kg ha⁻¹ urea was added with drip-irrigation and the nitrogen content of urea was 46%. Application of 525 kg ha⁻¹ drip-irrigation fertilizer containing 50% P₂O₅, 4% K₂O produced by Xinjiang BoShuoSi Ecological Technology Co., Ltd (Xinjiang, China). 4.5% avermectin (0.6kg ha⁻¹) and 20% acetamiprid (0.6kg ha⁻¹) were sprayed on the cotton field to control pests.

The experiment was divided into two parts. In the first part (Scheme 1), PASP was applied at 0 kg ha⁻¹ (CK1), 15 kg ha⁻¹ (PASP1), 75 kg ha⁻¹ (PASP2) or 150 kg ha⁻¹ (PASP3) before ploughing to each 100 m² (10m×10m) plot soil. In the second part (Scheme 2), PASP was mixed with soil at the weight ratio of 1:1 (PASP4), 1:10 (PASP5), 1:100 (PASP6), 1:1000 (PASP7) or 1:10000 (PASP8); the untreated soil was the control (CK2); then 1.2 kg of these mixtures were put into sterilized bottles with holes and buried at 20 cm deep in the cotton field. Soil samples were collected at seedling stage (t1), budding stage (t2), flowering stage (t3) and bolling stage (t4) of cotton, respectively. The arrangement of the

experiment is shown in Fig. 1. The area with bottles was 364m² (26m×14m), the interval between each bottle was 2 m. Twelve repeated samples were set for each soil/PASP mixture, and three replicates were taken for each soil/PASP mixture at each physiological period of cotton.

2.2 Polyaspartic acid

The polyaspartic acid was obtained from Beijing Key Lab of Bioprocess, Beijing University of Chemical Technology. The structure of PASP is shown in Fig.S1. Polysuccinimide (PSI) was ring-opened under alkaline conditions and then cross-linked with epoxy groups to prepare PASP hydrogel (Meng et al. 2015). The PASP hydrogel had a high water absorption rate (500g H₂O / g hydrogel), and its molecular weight was 75 kDa (Wei et al. 2015).

2.3 Measurement of soil water content

Soil moisture analyzers (L99-TS-1, Shanghai Fatai Precision Instrument Co., Ltd., Shanghai, China) were used to measure volumetric soil moisture values around the probe, whose length was 7cm, and the measurement range from 0 to 100%, and the measurement accuracy was around 2–3%. The moisture sensor was FDS100, and the data were recorded every ten min. The probe of soil moisture meter was buried in the soil at the depth of 30cm, a value needed to monitor the water content of cotton roots under field conditions.

2.4 Soil sampling

Soil cores (0–30 cm) were collected from the area of scheme 1 using a cylindrical soil sampler (5 cm inner diameter) at four physiological periods of cotton. In each plot, 5 soil samples were collected at random and mixed. At the same time, three bottles of each PASP/soil mixture were collected from the second part and mixed together. The soil samples were stored in sterilized plastic bags and transported to the laboratory in a portable box containing ice. The soil samples were sieved (2 mm) and then separated into two parts. One portion was air-dried for physiochemical characterization, and the other was stored at -80°C for DNA extraction.

2.5 Measurement of soil properties

Soil organic matter (SOM) content was determined using rapid dichromate Oxidation (Tiessen and Moir 1993). Available P (OP) and available K (AK) were extracted by the Olsen method (Olsen and Sommers 1982) and NH₄OAc extraction method (Richards and Bates 1989), respectively. Soil ammonium-N (AN) was determined using soil ammonium-N kits (Comin Biotechnology Co., Ltd, Suzhou, China) (Liu et al. 2018) according to the manufacturer's instructions, respectively.

2.6 Soil DNA extraction, illumina sequencing, and data analysis

Soil DNA was extracted using a DNeasy Power Soil Kit (Mo Bio / QIAGEN, USA) according to the manufacturer's instruction. The concentration, quality and integrity of the DNA were assessed using a Qubit Fluorometer (Invitrogen, USA) and a NanoDrop Spectrophotometer (Thermo Scientific, USA) before being stored at -20°C in the freezer.

PCR amplification of the bacterial 16S rRNA targeting the V3-V4 variable region was conducted by using 338F (5'-ACTCCTACGGGAGGCAGCA-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3') primers (Xu et al., 2016). The fungal ITS-1 region was amplified by using ITS1R (5'-GCTGCGTTCTTCATCGATGC-3') and ITS5F (5'-GGAAGTAAAAGTCGTAACAAGG-3') primers (Ma et al. 2019). The target fragment in the gel was cut for recovery using a gel recovery kit (AxyPrep DNA Gel Extraction Kit, Axygen, AP-GX-500) and quantified by BioTek Microplate Reader (BioTek Flx800 Microplate Reader; Quant-iT PicoGreen dsDNA Assay Kit, Invitrogen, P7589). Sequencing libraries were generated using the TruSeq DNA Sample Preparation Kit (Illumina, USA) and the Template Prep Kit (Pacific Biosciences, USA). The genome sequencing was conducted on the Illumina Novaseq platform by Personal Biotechnology Co., Ltd (Shanghai, China).

QIIME2 software was used for species taxonomic analysis. Dada2 (Benjamin et al. 2016) was mainly used for primer removal, quality filtering, noise removal, splicing and chimerism removal. It was no longer clustering with similarity, only performed dereplication processing. Each dereplication sequence produced after using Dada2 was called ASVs (amplicon sequence variants). The Silva Database (Release132, <http://www.arb-silva.de>, bacteria) (Quast et al. 2013) was used to determine the 16S rRNA gene sequence, while the UNITE Database (Release 8.0, <https://unite.ut.ee/>, fungi) (Koljalg et al. 2013) was used for the internal transcribed space (ITS) sequence of fungi. The raw sequence reads were submitted to the National Center for Biotechnology Information (NCBI) Sequence Read Archive (SRA) under the accession number PRJNA641758 for bacteria and PRJNA641986 for fungi.

2.7 Yield and fiber quality assessment

Plants from the central four rows of each plot were manually harvested in October. At flowering stage, 30 cotton plants were randomly selected from three areas of each plot to investigate the number of fruit branches and bolls at flowering stage. During each harvest, 30 cotton bolls were picked from lower, middle and upper sections of each plot, respectively, to determine the average single boll weight. After natural air-drying for twenty days, seed cotton was weighed. Seed cotton yield (kg ha^{-1}) was determined for each plot. The fiber quality including reflectance degree, Micronaire, length, short fiber index, uniformity index and strength was measured by the HVI instrument from the Alar Cotton Fiber Testing Institute, Alar City, Xinjiang province, China (Zhang et al. 2016).

2.8 Statistical analysis

The differences between treatments were evaluated using SPSS 16.0 for Windows (SPSS Inc., Chicago, IL, USA) and the Fischer Least Significant Difference (LSD) test. A probability of $p < 0.05$ was considered statistically significant. The bacterial and fungal richness and diversity indices (Chao1, Observed species and Shannon) were estimated using Mothur software (v.1.30.1). Non-metric multidimensional scaling analysis (NMDS) based on the Bray-Curtis distance index calculated was used to illustrate the changes in bacterial and fungal community composition between different samples. The clustering heatmap of abundance data were performed through one-way ANOVA using the R v.3.1.3 program. Redundancy analysis (RDA) (Liu et al. 2019) was employed to show a visual relationship between gene abundance and environmental factors.

3 Results

3.1 Effects of PASP on soil water storage

The soil water storage data measured at the different PASP concentrations in the cotton field are shown in Fig. 2. The soil water content of the field soil treated with PASP was higher than that of CK1, and PASP could keep the cotton root system at a higher water content level for a long time. The average water content for a PASP treatment was maintained between 40.2% and 51.9%. During the whole physiological period of cotton, the average soil water content increased by more than 4% with the application of appropriate PASP. After the cotton field was drop-irrigated, the soil moisture peaked. PASP increased soil water-holding capacity, reduced soil water loss and prolonged soil water holding period after each irrigation. The soil water content decreased a few days after irrigation, and the decrease speed of PASP treatments was slower than that of CK1. The soil water content was related to the concentration of PASP, with the highest values at 75 kg ha^{-1} and 150 kg ha^{-1} . The application of 15 kg ha^{-1} PASP also increased the soil water content, and the effect was much better at the budding stage and flowering stage. Among three concentration, 75 kg ha^{-1} was the best for maintaining available water content around cotton rhizosphere. At different physiological periods of cotton, PASP had different effects on soil moisture retention. At the seedling stage and boll opening stage of cotton, light had a strong effect on soil water content, and this effect depended on the PASP content. At the budding stage and flowering stage, the water content of the cotton field was higher than that of the other two physiological periods.

3.2 Effect of PASP on microbial α and β -diversity

After the cutting and filtering of reads, the number of bacterial sequences varied from 101118 to 189828 per sample (mean = 124603), whereas the number of fungal sequences varied from 101687 to 151372 per sample (mean = 117631). According to the 16S rRNA (bacteria) and ITS (fungi) genes of soil samples, the characteristic sequences (ASVs) were obtained after dereplication (Tables S1, S2). Sequences were annotated by the silva database and unite database.

The comparison of Chao1, Observed species and the Shannon index showed the effects of PASP on bacterial and fungal diversity (Table S3). The richness and diversity of bacterial and fungal communities were significantly increased by applying PASP. At the seedling stage and bolling stage of cotton, the Chao1, Shannon and Observed species index of bacteria were the highest when the PASP concentration was 75kg ha^{-1} . At the flowering period, the Shannon and Observed species index of bacteria were the highest with PASP at 15kg ha^{-1} . At the seedling and bolling stages, of cotton treated with 15kg ha^{-1} and 75kg ha^{-1} , the Chao1, Shannon and Observed species index of fungal communities were higher than those of CK1. At the budding stage, the effect on the fungal communities was the highest when the concentration of PASP was 75kg ha^{-1} . At the higher concentration of the bottled experiment, the richness and diversity of soil bacterial and fungal communities significantly increased at the flowering and bolling stages, when PASP ratio was 1:1000 and 1:10000. However, PASP significantly reduced the richness and diversity of soil microbial communities, when the concentration of PASP in soils exceeded 1:1000.

Changes in the bacterial and fungal community composition among the different soil samples were estimated by nonmetric multidimensional scaling analysis (NMDS) (Fig. 3). Bacterial communities of samples were far away from each other in the same physiological period of cotton based on the Bray-Curtis distance and greatly affected by the PASP concentration (Fig. 3A). Fungal communities at lower PASP concentrations were separated from those at higher concentrations (Fig. 3B). At the seedling stage of cotton, the similarity of microbial communities of soil samples with adjacent concentrations was higher than those of soil samples treated with markedly different PASP concentrations, but the differences of microbial communities gradually increased with time (Fig. 3). The differences in bacterial communities of PASP4, PASP5 and PASP6 samples were small, but they were significantly different from those of other samples. The aggregation degree of soil fungal communities of PASP4.t1, PASP5.t4, PASP6.t4 and PASP7.t1 samples were relatively discrete, and the composition of fungal communities was quite different from each other. Analysis of NMDS revealed that the influence of the PASP concentration on bacterial β -diversity was higher than the influence on fungal β -diversity.

3.3 Bacterial and fungal community composition

Comparisons of the relative abundance of the 20 dominant bacterial and fungal genera are showed in Fig. 4 and Fig.S2. Bacteria were more sensitive to the application of PASP than fungi. Applying PASP to the cotton field, relative abundances of *Pseudomonas*, *Aeromicrobium*, *Rokubacteriales*, and *Nitrospira* did not change too much, while those of *Methylophaga*, *Cupriavidus*, *Panacagrmonas*, *Sphingomonas*, *Bacillus*, *Achromobacter*, and *Candidimonas* changed greatly. The low amount of applied PASP increased the relative abundance of *Methylophaga*, *Sphingopyxis*, *Panacagrmonas*, and *Candidimonas*. *Methylophaga*, *Panacagrmonas*, *Pseudomonas* and *Aeromicrobium* were more abundant at the low PASP concentration and in CK soil samples than at high PASP concentrations, while *Cupriavidus*, *Sphingomonas* and *Acinetobacter* were the most abundant bacterial genera at the higher PASP concentration. Most of the abundant genera in all samples were Gram-negative bacteria, aerobic and facultative anaerobic bacteria. The relative abundance of *Methylophaga* decreased significantly, while the relative abundance of *Aeromicrobium* increased at the flowering stage compared with the other three cotton physiological periods (Fig. 4A, Fig.S2A).

PASP application did not change too much the relative abundances of *Llyonectria* and *Mycosphaerella*, while those of *Pseudeurotium*, *Fusarium*, *Nectria*, *Alternaria*, *Tricharina*, *Cephalotrichum*, and *Mortierella* changed greatly. *Nectria*, *Pseudeurotium*, *Mortierella* and *Cephalotrichum* were more abundant fungal genera at low than at high concentrations of PASP, whereas, *Fusarium*, *Aspergillus* and *Pseudogymnoascus* were more abundant at the high PASP concentrations. Applying PASP increased the relative abundance of *Fusarium* but reduced those of *Pseudeurotium* and *Nectria*. At the bolling stage, the relative abundance of *Alternaria* was significantly higher than at the other three physiological periods (Fig. 4B, Fig.S2B). Therefore, PASP significantly reduced the relative abundance of *Methylophaga*, *Panacagrimonas*, *Pseudeurotium* and *Nectria*, while increased that of *Sphingomonas*, *Cupriavidus* and *Fusarium*. At the bottling test, with the highest concentration of PASP, the soil microbial changed markedly (Fig. 4). In addition, due to the large span of PASP concentrations in the experiment with bottles, the composition of soil microbial community varied greatly among different PASP concentrations.

3.4 Relations between microbial community composition and soil physiochemical properties

The relationships between soil physiochemical properties and microbial community composition were investigated by Redundancy analysis (RDA). The two-dimensional diagrams of RDA explained 39.54% and 15.4% of the whole bacterial and fungal variance, respectively (Fig. 5). SOM, AN and OP significantly increased by increasing the applied PASP rates, while the effect of PASP on AK was non-significant (Fig. 5, Table S4). SOM ($r = 0.474$, $p = 0.015$), AN ($r = 0.868$, $p = 0.001$) and OP ($r = 0.501$, $p = 0.004$) exhibited a significantly effect on the bacterial community composition with AN having the greatest impact on bacterial communities. Relative abundances of *Pseudomonas*, *Aeromicrobium*, and *Sphingomonas* were correlated with soil physiochemical properties strongly. For example, the relative abundances of *Pseudomonas* and *Aeromicrobium* had the strongest negative correlation with AN, SOM, OP and AK, while that of *Sphingomonas* had a strong positive correlation with AN, AK, OP and SOM. Relative abundances of *Panacagrimonas* and *Cupriavidus* had positive correlation with AK and SOM, but had negative correlation with AN, and had a low correlation with OP. The application of PASP changed the composition of microbial communities with greater effects at higher application rates. Samples with similar PASP concentrations had similar composition both PASP4.t1 and PASP5.t1 samples showed the best similarity (Fig. 5A).

AN ($r = 0.700$, $p = 0.001$), OP ($r = 0.743$, $p = 0.001$) and SOM ($r = 0.588$, $p = 0.005$) had a significantly impact on the fungal community composition with OP having the greatest impact on fungal communities. The relative abundances of *Tricharina* and *Cephalotrichum* had strong negative correlation with soil physiochemical properties, while those of *Panacagrimonas*, *Cupriavidus*, *Methylophaga* and *Sphingopyxis* had the weakest correlation with soil physiochemical properties. SOM, OP and AN were positively correlated with the relative abundances of *Fusarium*, but negatively correlated with that of *Pseudeurotium*. *Nectria* and *Llyonectria* had the strongest positive correlation with AK, while *Acremonium* and *Pseudogymnoascus* had the strongest negative correlation with AK. The negative correlation between the relative abundance of *Pseudorotium* and SOM was the strongest one. The higher PASP application rates had greater effect in fungal diversity than lower PASP application rates. The distance of each sample in the fungal community was close with PASP4.t1, PASP4.t3 and PASP5.t3 having low species composition similarity with the other soil samples.

3.5 Effects of PASP on cotton yield and quality

Cotton yield and quality were affected by PASP under field conditions (Table 1). Seed cotton yield and single boll weight were higher in PASP treatments than those of CK1. The application of PASP increased the boll number and fruit branch number of cotton at flowering stage, and the reflectance degree. The most significant ($p < 0.05$) effects by PASP were on Micronaire and short fiber index of cotton, and the effect on the reflectance degree. Compared to CK1, single boll weight increased by 2.1%, 5.48% and 10.14%, and seed cotton yield increased by 3.94%, 8.31% and 7.71% at the PASP

concentrations of 15 kg ha⁻¹, 75 kg ha⁻¹ and 150 kg ha⁻¹, respectively. The maximum seed cotton yield of 6977.38 kg ha⁻¹ was achieved with 75 kg ha⁻¹ of applied PASP.

Table 1
Effects of different PASP application rates on cotton yield and fiber quality

PASP rate (kg ha ⁻¹)	Fiber quality						Cotton yield			
	Rd	MIC	LEN(mm)	SFI (%)	UI (%)	STR(cN/tex)	BN	FBN	SBW(g)	SCY(kg ha ⁻¹)
15	78.37 ± 0.27c	4.81 ± 0.15a	27.37 ± 0.36a	11.67 ± 0.62ab	83.33 ± 0.35ab	27.09 ± 0.66b	10.17 ± 0.39a	6.87 ± 0.28a	6.34 ± 0.08bc	6696.27 ± 218.62a
75	79.10 ± 0.12a	4.97 ± 0.07a	27.66 ± 0.40a	13.07 ± 1.31a	82.97 ± 0.23b	26.51 ± 0.64b	10.37 ± 0.43a	7.03 ± 0.24a	6.55 ± 0.08b	6977.38 ± 155.86a
150	79.07 ± 0.17ab	4.99 ± 0.13a	27.92 ± 0.43a	12.13 ± 0.28ab	83.23 ± 0.48ab	27.19 ± 0.02ab	10.23 ± 0.41a	6.87 ± 0.28a	6.84 ± 0.09a	6939.34 ± 260.12a
CK1	78.53 ± 0.09bc	4.89 ± 0.11a	28.62 ± 0.44a	9.70 ± 0.78b	84.03 ± 0.09a	28.70 ± 0.24a	9.10 ± 0.35b	6.53 ± 0.27a	6.21 ± 0.09c	6442.33 ± 193.80a

Values of cotton yield and quality were mean of three replicates ± SE; n = 30. a, b and c indicate significant differences (p < 0.05; LSD test) in the same column. Rd, Reflectance degree; MIC, Micronaire; LEN, Length; SFI, Short fiber index; UI, Uniformity index; STR, Strength; BN, Boll numbers per plant; FBN, Fruit branches numbers; SBW, Single boll weight; SCY, seed cotton yield.

4 Discussion

4.1 Effect of PASP on soil water content

PASP as a water-retaining agent with amide groups and carboxylic groups, is completely biodegradable (Adelnia et al. 2019), can store irrigation water and improve water availability for vegetables and ornamental species (Montesano et al. 2015). Indeed, PASP maintained water at a higher level in the cotton field than the control after irrigation (Fig. 2). During the budding and flowering stage, the soil water content is higher than that of the other two physiological periods of cotton. The possible reason is that there are more leaves in cotton, and the degree of concealment is higher in these two physiological periods. Wei et al. (2011) studied the effect of polyaspartic acid on maize, showing that water-retaining agents not only increased soil aggregation and soil water content, but also inhibited the water evaporation from soil surface. Here soil moisture content we have showed that applying PASP to cotton fields increased by more than 4%, indicating that PASP can be applied to cotton field, especially in arid and semi-arid areas.

4.2 Effect of PASP on the composition of soil microbial communities

Soil microbial diversity is important in maintaining ecosystem stability (Zhang et al. 2021). The effects of PASP on the soil microbial community was not reported before our study, which showed that moderate PASP application rates increased the richness and diversity of soil microbial community, while excessive PASP application had the opposite effect on bacterial and fungal α-diversity (Table S3). The possible reason is that excessive application of PASP will harden the soil, change the living environment of microorganisms, and then affect the survival of microorganisms.

Fungal community is important in arid soils, and being fungal activity less affected than bacterial activity in these soils (Singh et al. 2021). The composition of bacterial community was more affected by PASP than that of fungal community (Fig. 3), likely due to the better performance of the fungi in arid and semi-arid environment. Fungi can produce more extracellular enzymes under arid and semi-arid conditions, to degrade organic polymer than bacteria (Porrás Alfaro et al. 2008), which makes fungi more adaptable to PASP than bacteria. In the rhizosphere soil of cotton, the relative abundances of *Sphingomonas*, *Streptomyces*, *Gemmatimonas*, *Sphingopyxis*, *Aidotherrmus* and *Jatrophihabitans* are relatively high (Ullah et al. 2018).

4.3 Correlation analyses

It is well established that pH, climate, soil properties and geographic location can affect the composition of soil microbial communities (Shi et al. 2017). PASP can positively affect the absorption and translocation of K^+ by plants (Marschner 2013), but our study, the effect of PASP on AK was not significant (Table S4). AN was the most significant affected soil properties and likely this affected the composition of soil bacterial communities. PASP affected soil N dynamics likely due to the degradation products of PASP, which are low molecular weight N compounds (Deng et al. 2014). OP, AK and AN had a strong correlation with the composition of soil fungal communities, likely because PASP stimulated nutrients-uptake by fungi. However, the relationship between microbial composition and soil properties using DNA through studies based on the detection of genes is biased because of the silence of genes and the presence of DNA from dead microbes (Nannipieri et al. 2019). In addition, the selection of fungal primers also affects fungal diversity (Xue et al. 2019), usually the ITS-1 amplicons provide greater taxonomic and functional resolution as well as coverage of the communities than LSV markers (Ma et al. 2019).

4.4 Effect of PASP on cotton growth

The application of PASP improved soil water-holding capacity and thus cotton yield. Moderate water deficit during fiber elongation and development can significantly reduce fiber length and strength, and increase the Micronaire value (Dağdelen et al. 2009). The application of PASP can reduce the loss of photosynthesis efficiency of plants under strong light to a certain extent, especially for plants planted in drought-stressed areas (Wei et al. 2016). The applied PASP water-retaining agent is degraded into small molecular substances by biological and chemical procedures in the soil. For example, exopeptidase can hydrolyze aspartic acid units from the end of the polyaspartic acid chain, and then be absorbed and utilized by plants to promote the growth of crops (Jalalvandi and Shavandi 2018). The quality of cotton fiber depends on the supply of soil nutrients, especially that of N (Mullins and Burmester 1990; Chen et al. 2010). Adding PASP to cotton fields can not only improve soil porosity and looseness, but also significantly promote seed germination, survival and plant root vitality (Guo et al. 2016; Hu et al. 2019), and increase the boll weight and yield of cotton (Du et al. 2011). In addition, the optimum rate of PASP was 75 kg ha^{-1} in this alkaline sandy loam soil, a similar result observed by Liang et al. (2019) using poly- γ -glutamic acid.

5 Conclusion

These results have demonstrated that applying PASP to cotton field has caused different effects in water content, microbial biomass, diversity, community compositions, nutritional structure, cotton yield and fiber quality. The dynamic changes of soil microbial biomass, community structure and nutrients are closely related to soil water content. The application of appropriate amount of PASP increased soil water content and delayed the decrease of soil water after cotton field irrigation. 16S rRNA and ITS gene sequencing revealed that different microbial community structures of the soil cultivated under different amount of PASP application, and appropriate application of PASP increased the abundance and diversity of soil microbial communities. Applying PASP could alleviate the drought stress effect on the cotton, increased cotton yield and improved fiber quality, which may be caused by the regulation of water content, soil microbial structure, nutrients, and promoting the vitality of cotton roots. In the future, it is worthy of studying the

degradation process of PASP, and the long-term effects of PASP on soil properties and microbial communities for the mechanisms of PASP on soil microbial community diversity.

Declarations

Acknowledgment

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Author contributions

Wenlong Liu planned and conducted the experimental design and all soil samplings, data evaluations, and statistics. Data interpretation and paper writing was done by Wenlong Liu with the support of Prof. Chuanxing Wan, Prof. Hong Zeng and Dr. Jianbo Zhao. Chuanxing Wan contributed to the guidance of experimental operations and the financial support for this work. All authors approved the manuscript.

Disclosure statement

All authors declare no conflict of interest.

Ethical approval

This article does not contain any studies with human participants and/or animals performed by any of the authors. The formal consent is not required in this study.

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Figures

Figure 1

The view of field experiment

ABCDEF represented the row position of the landfill sample in the test field, and the ratio of PASP weight to soil weight was 1:1, 1:10, 1:100, 1:1000, 1:10000, respectively. It is also showed the control soil.

Figure 2

Effect of PASP on soil moisture in the cotton field

Figure 3

The influence of PASP on soil microbial β -diversity based on 16S rRNA and ITS analyses

A, Soil bacterial β -diversity; B, Soil fungal β -diversity. PASP1, PASP2, and PASP3 are of 15 kg ha⁻¹, 75 kg ha⁻¹, and 150 kg ha⁻¹ rates, respectively. PASP4, PASP5, PASP6, PASP7, and PASP8 are weight ratios of PASP and soil at 1:1, 1:10, 1:100, 1:1000 and 1:10000, respectively. CK1 represented the control soil sample of the sprinkled field without PASP, and CK2 represented the control soil sample of bottle experiment. t1, t2, t3 and t4 represent the seedling stage, budding stage, flowering stage and bolting stage of cotton, respectively.

Figure 4

The heat map of genus clustering

A, bacteria ; B, fungi. The red color block indicates that the genus was more abundant in the sample than that of the other samples; the blue color block indicates the abundance of the genus in the sample was lower than that of other samples.

Figure 5

Redundancy Analysis (RDA) about the relationship between soil microbial composition and soil properties

A, The relationships between the soil bacterial taxa and soil properties; B, The relationships between the soil fungal taxa and soil properties. Soil organic matter, SOM; Olsen-P, OP; Avail-K, AK; Ammonium-N, AN.

Supplementary Files

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- [FigureS2AHistogramofhorizontalabundancecompositionofthetop20generabacteria.tif](#)
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