Effects of Ridge-Furrow Mulching Rainwater Harvesting Pattern on Maize Rhizosphere Soil Microbial Diversity: Postprint

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Abstract

To clarify the effects of ridge-furrow mulching rainwater harvesting patterns on corn rhizosphere soil microbial community structure and diversity, 'Dengyi 2' was selected as the experimental material. A single-factor completely randomized experimental design was adopted, with conventional flat planting without mulching as the control (CK), and six treatments were established in sequence: ridge mulched with ordinary black plastic film and furrow without mulching (HL), ridge mulched with liquid film and furrow without mulching (YL), ridge without mulching and furrow mulched with straw (NJ), ridge mulched with liquid film and furrow mulched with straw (YJ), and ridge mulched with ordinary black plastic film and furrow mulched with straw (HJ). Illumina high-throughput sequencing technology was used to analyze soil microbial community composition and diversity. The results showed that: (1) The rainwater harvesting patterns with ridge mulching and furrow straw mulching were all beneficial for increasing corn yield and improving water use efficiency. Among these, the HJ treatment exhibited the maximum ear row number, thousand-grain weight, yield, and water use efficiency, which increased by 11.22%, 31.31%, 88.02%, and 79.83% compared with the control CK, respectively, with all differences being significant ($P<0.05$). In contrast, the yield and water use efficiency of the NJ treatment with ridge without mulching and furrow straw mulching were both lower than those of CK. (2) All ridge mulching treatments significantly improved microbial community diversity and altered microbial structure, whereas the NJ treatment with ridge without mulching and furrow straw mulching did not. (3) The microbial community composition of each treatment was influenced by the mulching rainwater harvesting patterns at both phylum and class levels. The dominant bacterial phyla in the soil microbial community were Proteobacteria, Acidobacteria, Gemmatimonadetes, and Bacteroidetes. The dominant bacterial classes were Gammaproteobacteria (accounting for 25.8%), Bacteroidia (accounting for 8.4%), and Alphaproteobacteria (accounting for 7.7%).

Ridge-furrow mulching helped to increase soil microbial richness, diversity, and evenness indices, indicating that the ridge-furrow rainwater harvesting pattern could achieve corn yield increase by altering the structure and composition of soil microorganisms at phylum and class levels.

Full Text

Abstract

To clarify the effects of ridge-furrow mulching and rainwater harvesting patterns on the microbial community structure and diversity of maize rhizosphere soil, this study employed the "Dengyi No. 2" maize variety as experimental material using a single-factor completely randomized design. Conventional flat planting without mulching served as the control (CK). Six treatments were established: ridge covered with ordinary black plastic film and furrow without cover (HL), ridge covered with liquid film and furrow without cover (YL), ridge without cover and furrow mulched with straw (NJ), ridge covered with liquid film and furrow mulched with straw (YJ), ridge covered with ordinary black plastic film and furrow mulched with straw (HJ), and the control. Illumina high-throughput sequencing technology was used to analyze soil microbial community composition and diversity. The results showed that: (1) The ridge-furrow mulching rainwater harvesting mode benefited maize yield increase and improved water use efficiency. Treatment HJ exhibited the maximum values for ear row number, thousand-grain weight, yield, and water use efficiency, which were 11.22%, 31.31%, 88.02%, and 79.83% higher than CK, respectively ($P<0.05$). However, the yield and water use efficiency of treatment NJ were lower than CK. (2) All ridge-mulching treatments significantly increased microbial community diversity and altered microbial structure, whereas the NJ treatment did not. (3) Microbial community composition at the phylum and class levels was affected by the mulching rainwater harvesting mode in all treatments. The dominant bacterial phyla were Proteobacteria, Acidobacteria, Gemmatimonadetes, and Bacteroidetes. The dominant bacterial classes were Gammaproteobacteria (25.8%), Bacteroidia (8.4%), and Alphaproteobacteria (7.7%). Ridge-furrow mulching improved soil microbial richness, diversity, and evenness indices. Thus, the ridge-furrow rainwater harvesting mode can increase maize yield by altering the structure and composition of soil microbial communities at the phylum and class levels.

Keywords: ridge-furrow rainwater harvesting; maize; soil microbial community; mulching

Introduction

The semi-arid Loess Plateau region of northwest China holds significant importance in dryland agricultural production, where rainfed maize represents a major agricultural industry. Deep groundwater levels, uneven seasonal precipitation distribution, water scarcity, and seasonal drought constitute primary constraints on dryland maize development. Research on effective natural precipitation conservation and scientific irrigation is crucial for addressing the severe temporal mismatch between rainfall during the growing period and crop water demand. Ridge-furrow mulching and rainwater harvesting cultivation technology, which employs alternating ridges and furrows in the field with crops planted in furrows and mulch on ridges, effectively reduces and collects surface runoff from ineffective rainfall, decreases surface evaporation, increases soil water content in farmland and the crop root zone, extends the crop water utilization period, and thereby achieves the goals of increasing crop yield and water use efficiency. This technology serves as an effective water-saving agricultural practice in China' s semi-arid rainfed agricultural regions.

Surface mulching (plastic film, straw, etc.) can artificially improve farmland microclimate and provide suitable hydrothermal conditions for crop rhizospheres. Plastic film mulching enhances yield and water use efficiency significantly through its moisture retention and warming effects. However, long-term use of ordinary plastic film causes severe farmland ecosystem pollution due to its non-degradability, which blocks soil water infiltration and affects crop root growth. The emergence of degradable film has solved the problem of farmland residual film pollution. Straw mulching provides sufficient nutrients to stimulate soil microbial activity and increase biodiversity in soil microecosystems. Soil microorganisms play important roles in nutrient cycling and structural maintenance of agricultural ecosystems, and their diversity is involved in internal material and energy cycling in soil. Studies have shown that plastic film mulching can improve soil microbial populations, alter bacterial and fungal structures, and increase the relative abundance of Proteobacteria and Acidobacteria. Currently, research on ridge-furrow rainwater harvesting cultivation technology has focused primarily on soil physicochemical properties, nutrient content, biochemical processes, photosynthetic characteristics, water utilization, and yield, while studies on soil microbial community responses to ridge-furrow mulching are rarely reported. This experiment investigated the effects of ridge-furrow mulching and rainwater harvesting planting systems on maize rhizosphere soil microbial community structure using high-throughput sequencing technology.

1 Materials and Methods

1.1 Study Area Description

The experiment was conducted at the Xichuan Experimental Base of the Gansu Provincial Irrigation Experiment Station (37°52 N, 102°50 E, altitude 1958 m). Located in the hilly region of western Loess Plateau, the base has an average annual temperature of 6.3°C, solar radiation of 592 $\mathrm{kJ \cdot cm^{-1}}$, annual sunshine duration of 2409 h, \$ $10°Caccumulatedtemperatureof2075.1°C, andfrost - freeperiodof141days.Theregionfeaturesatypicalsemi-aridrainfedagriculturalsystemwithasinglecroppery$ $September(accountingfor65.8^{\text{\{-3\}}, organicmattercontentof1.0}\text{\{-1\}}, nitratenitrogenof32.25mg\cdot kg^{\text{\{-1\}}, ammoniumnitrogenof19.56mg\cdot kg}\text{\{-1\}}, availablephosphorusof240.62mg \quad \cdot$

$kg^{\{-1\}, andavailablepotassiumof186.17mg \cdot kg}$ {-1}\$.

1.2 Experimental Design and Sampling

1.2.1 Experimental Design The maize variety "Dengyi No. 2" was used as test material. Ridge-furrow mulching planting technology was adopted with a ridge width of 60 cm and furrow width of 60 cm. Five mulching treatments were established: ridge covered with ordinary black plastic film and furrow without cover (HL), ridge covered with liquid film and furrow without cover (YL), ridge without cover and furrow mulched with straw (NJ), ridge covered with liquid film and furrow mulched with straw (YJ), and ridge covered with ordinary black plastic film and furrow mulched with straw (HJ). Traditional flat planting without mulching served as the control (CK). Each treatment had three replicates, totaling 18 plots arranged randomly. Before sowing, fertilizer was applied once. No irrigation or additional fertilization was applied during the entire growth period, and weeds were removed manually twice to avoid damaging the plastic film.

1.2.2 Soil Sample Collection Three representative maize plants were randomly selected from each treatment. Soil samples were collected at 5-10 cm depth near the root system on the furrow side using a soil auger with 30 cm outer diameter after removing surface impurities. Soil samples from three sampling points were mixed uniformly, then visible roots, weeds, and stones were removed. Each sample was divided into two portions: one was sieved through a 2 mm mesh, placed in a sterile sealed bag, stored in an ice box, transported quickly to the laboratory, and preserved at -80°C for high-throughput sequencing; the other was air-dried after sieving for soil physicochemical property analysis.

1.2.3 Maize Yield and Related Indicators At harvest, the number of effective ears was counted in each plot, and representative plants were selected for yield component analysis. Ear length, ear diameter, ear row number, kernels per row, ear weight, and thousand-grain weight were recorded after air-drying.

1.2.4 Soil Moisture Content Determination Three measurement points were established in each plot at the front, middle, and back positions within the same furrow, 10 cm horizontally from maize roots. Samples were taken at depths of 0-20 cm, 20-40 cm, 40-60 cm, 60-80 cm, and 80-100 cm. Measurements at the same depth were averaged. Additional measurements were taken before and after rainfall and during growth stage transitions. The oven-drying method was used with a DHG-9036A oven at 105°C.

1.2.5 Soil Temperature Measurement A set of geothermometers was installed at the middle position of the furrow side in each plot, 15 cm from maize plants. Measurements were taken at depths of 5 cm, 10 cm, 15 cm, 20 cm, and 25 cm. Readings were taken at 08:00, 10:00, 12:00, 14:00, 16:00, and 18:00.

1.2.6 Water Use Efficiency Water use efficiency (WUE) was calculated as WUE = Y/ET, where Y is maize yield ($\mathrm{kg \cdot hm^{-2}}$) and ET is total water consumption during the growth period (mm), estimated from soil moisture content. According to the Irrigation Experiment Specification (SL13-2004), the simplified calculation formula is:

$$ET_{1-2} = 10\sum_{i=1}^{n} \gamma_i H_i(\theta_{i1} - \theta_{i2}) + I + P + K + C$$

where ET_{1-2} is the water consumption during the calculation period (mm); γ_i is the dry bulk density of the i-th soil layer ($\mathrm{g \cdot cm^{-3}}$); H_i is the thickness of the i-th soil layer (cm); θ_{i1} and θ_{i2} are the mass water contents of the i-th soil layer at the beginning and end of the calculation period, respectively; and I, P, K, and C are irrigation amount, rainfall amount, groundwater recharge, and drainage (surface and subsurface) during the period (mm), respectively. Groundwater depth in the experimental area exceeded 100 m, so K = 0. The experimental area is arid with no irrigation (I = 0) and flat terrain without runoff (C = 0). Rainfall data were obtained from a small meteorological station at the experimental base.

1.3 High-Throughput Sequencing and Data Analysis

1.3.1 Soil High-Throughput Sequencing Soil DNA was extracted using a specific method, and purity and concentration were detected by agarose gel electrophoresis. Appropriate DNA was diluted with sterile water and used as template with Barcode-specific primers for PCR amplification. Primers were 5 -GTGCCAGCMGCCGCGGTAA-3 and 5 -GGACTACHVGGGTWTCTAAT-3 . Amplicon sequencing was completed by Beijing Novogene Bioinformatics Technology Co., Ltd. The Illumina NovaSeq platform generated raw data (Raw Tags), which were filtered for low-quality and short sequences to obtain Clean Tags, then filtered for chimeras to obtain Effective Tags for subsequent analysis. Each soil sample yielded between 40,000-60,000 sequences with average length of 253 bp, and quality data >Q20 accounted for over 88% of effective data. Sequencing data were processed on the Novogene after-sales tool platform (https://magic.novogene.com/): Uparse software (Version 7.0.1001) clustered Effective Tags into Operational Taxonomic Units (OTUs) at 97% similarity. Representative sequences were annotated using the SSUrRNA database SILVA132. Community composition was statistically analyzed at various classification levels. Alpha diversity indices including Chao1, Shannon, Simpson, and PD_{{{whole}}_{{tree}}} were calculated using Mothur software (Version 1.9.1). Beta diversity analysis was performed using principal component analysis (PCA) and non-metric multidimensional scaling (NMDS). SPSS Statistics 19.0 was used for data processing and variance analysis, and Origin 2022 was used for graphing.

2 Results

2.1 Effects of Different Rainwater Harvesting Modes on Maize Yield and Yield Traits

Table 1 shows maize yield and yield traits under different rainwater harvesting modes. No significant differences were observed among treatments for ear length, ear diameter, or kernels per row ($P>0.05$). However, when ridges were covered with the same material, treatments without straw mulching in furrows had no fewer kernels per row than those with straw mulching, possibly because straw-mulched maize entered the grain-filling stage earlier, shortening the booting stage. Treatment HJ had the highest values for ear row number and thousand-grain weight, while treatment NJ had the lowest. No significant differences existed between treatments HJ and YJ, but both were significantly higher than CK ($P<0.05$). All treatments had significantly higher thousand-grain weight than CK, with increases of 31.31%, 30.21%, 28.74%, and 26.33% for HJ, YJ, YL, and HL, respectively ($P<0.05$), though the difference between YL and CK was not significant. Treatment NJ had the lowest thousand-grain weight, though not significantly different from CK, likely due to lower temperatures after sowing without film mulching that delayed emergence, and insufficient grain filling due to temperature drops during the grain-filling stage.

When ridges were covered with the same material, furrow straw mulching treatments showed higher thousand-grain weight than non-mulched furrow treatments, indicating that both ridge mulching and furrow straw mulching benefit maize thousand-grain weight. Yield ranking from high to low was HJ > YJ > YL > HL > CK > NJ. Treatment HJ yield was significantly higher than CK by 88.02% ($P<0.05$). Treatment YJ also showed significantly higher yield than CK by 79.83%, while treatment NJ yield was significantly lower than CK by 9.16% ($P<0.05$). The lower yield in NJ treatment was fundamentally due to lower planting density in ridge-furrow planting compared with flat planting, resulting in fewer ears per plot. This aligns with Hu et al.' s conclusion that the yield-increasing effect of non-mulched ridge-furrow planting cannot compensate for yield reduction caused by sparse planting.

2.2 Effects of Different Rainwater Harvesting Modes on Maize Water Consumption and Water Use Efficiency

Table 2 presents maize water consumption characteristics and water use efficiency under different rainwater harvesting modes. Treatment NJ had the smallest water consumption, significantly different from other treatments, followed by treatment YJ. Among furrow straw-mulched treatments, water consumption ranked NJ > YJ > HJ, indicating that ridge mulching with ordinary black plastic film had stronger water retention than liquid film or no mulching. Water use efficiency ranked HJ > YL > HL > YJ > CK > NJ. Treatment HJ showed significantly higher water use efficiency than CK by 79.83% ($P<0.05$). Except for treatment NJ, all other treatments had significantly higher water use efficiency than CK (P<0.05). Treatment NJ had lower water use efficiency than CK, fundamentally due to lower yield caused by planting density smaller than CK, while water consumption was also too high.

2.3 Sequencing Results and Diversity Analysis

2.3.1 Sequencing Results The rarefaction curve, constructed by randomly extracting sequencing data from samples and plotting the number of species (OTUs) against sequencing depth, is commonly used to describe within-sample diversity. It directly reflects the rationality of sequencing data volume and indirectly reflects species richness in samples. As shown in Figure 2, the rarefaction curves for all treatments became 平缓, indicating that OTU number gradually plateaued with increasing sequencing data, demonstrating that the sequencing data were scientifically sound and reasonable. Each sample yielded 40,000-60,000 sequences with average length of 253 bp, and data efficiency exceeded 88% for all samples.

2.3.2 Alpha Diversity Analysis At 97% similarity threshold, alpha diversity indices were calculated for different samples (Table 3, normalized at cutoff=54179). Sample library coverage rates exceeded 98% for all treatments, indicating high credibility of sequencing results that could reflect actual sample conditions. Treatment HJ showed the highest Observed_{species} index, significantly greater than treatment NJ (P<0.05), indicating that ridge mulching significantly increased soil microbial community richness compared with furrow straw mulching alone. The Chao1 index was also highest in treatment HJ. All ridge-mulching treatments had higher soil microbial Shannon indices than CK, with treatments HJ and YJ showing significant differences (P<0.05), demonstrating that ridge mulching significantly improved soil microbial diversity. No significant differences existed in Simpson indices among treatments, but treatment NJ had the lowest values, indicating that ridge mulching increased soil microbial diversity while furrow straw mulching alone decreased it, though the effects were not significant.

2.3.3 Beta Diversity Analysis Beta diversity analyzes microbial community composition among different samples and reveals community similarity. Non-metric multidimensional scaling (NMDS) is a nonlinear model that reflects species information on a two-dimensional plane, overcoming linear model limitations. NMDS analysis (Figure 3) showed that treatments HJ, YJ, YL, and HL were distributed on the left axis, while treatments CK and NJ were on the right axis, indicating high similarity among the four ridge-mulching treatments and clear differences from CK and NJ. On the vertical axis, differences among treatments were not obvious. Principal component analysis (PCA) extracts principal components from soil factors to analyze microbial diversity functions. The first two principal components explained 18.02% and 12.86% of variance, respectively, with cumulative contribution of 30.88%. PCA showed that treatments HJ, YJ, YL, and HL clustered on the lower axis, indicating similar microbial community structures, while treatments CK and NJ were distributed on the upper axis, showing different microbial community structures.

2.4 Microbial Community Structure and Composition

The relative abundance of top 10 dominant bacterial phyla was statistically analyzed (Figure 4). Under different rainwater harvesting modes, the main bacterial phyla in maize rhizosphere soil were Proteobacteria, Acidobacteria, Gemmatimonadetes, Actinobacteria, Bacteroidetes, Planctomycetes, Chloroflexi, Verrucomicrobia, Firmicutes, and Rokubacteria. Proteobacteria was the dominant phylum across all treatments, accounting for 39.3% of total bacteria, followed by Acidobacteria (16.6%) and Gemmatimonadetes (8.4%). At the class level, the dominant bacterial classes were Gammaproteobacteria (25.8%), unidentified_{Gemmatimonadetes} (8.4%), Alphaproteobacteria (7.7%), unidentified_{Acidobacteria} (7.1%), Bacteroidia (6.3%), and Deltaproteobacteria (5.9%), with a combined proportion of 63.7%. Compared with CK, treatment HJ showed decreased Gammaproteobacteria abundance but increased Alphaproteobacteria and Bacteroidia abundances by 9.42% and 8.63%, respectively.

2.5 Correlation Analysis Between Soil Microorganisms and Moisture/Temperature

Soil microbial communities are highly sensitive to soil environmental factors (temperature, moisture, and nutrient status). Changes in soil environment may cause certain microbial communities to become dominant while suppressing others, leading to changes in community composition and structure. Microbial communities respond differently to climate characteristics, soil properties, and crop types, playing crucial roles in soil quality and nutrient transformation while being influenced by soil hydrothermal factors. Spearman correlation coefficients between different bacterial phyla and soil moisture/temperature are shown in Table 4. Proteobacteria showed significant negative correlation with moisture content, Gemmatimonadetes showed extremely significant negative correlation with moisture, Bacteroidetes showed extremely significant negative correlation with both moisture and temperature, Planctomycetes and Chloroflexi showed significant positive correlation with moisture, Verrucomicrobia showed significant negative correlation with moisture, and Rokubacteria showed extremely significant negative correlation with temperature. Other correlations were not significant.

3 Discussion

Plastic film mulching has been widely adopted in agricultural production due to its effects on increasing temperature and moisture retention, suppressing weed growth, and saving water while increasing yield. Mulching creates suitable microecological environments for crop growth, and its effects on soil environment have been extensively studied. However, few reports exist on the effects of binary ridge-furrow rainwater harvesting planting combining different ridge mulching types with furrow straw mulching on soil microbial composition and function. This study revealed the effects of ridge-furrow mulching on maize rhizosphere soil microbial community diversity and composition through high-throughput sequencing.

The results showed that ridge-furrow mulching did not substantially change the dominant microbial species and their composition but affected their abundance to varying degrees. Proteobacteria was the dominant phylum in all treatments, followed by Acidobacteria, consistent with previous research. Proteobacteria is an important phylum in microbial communities that can utilize unstable carbon sources and has high relative abundance in eutrophic environments. These dominant species are common polycyclic aromatic hydrocarbon-tolerant and pollutant-degrading bacteria. Ridge mulching decreased the relative abundance of Proteobacteria, while furrow straw mulching alone increased it, similar to findings in other studies showing that straw mulching had higher Proteobacteria relative abundance than other mulching treatments. Acidobacteria is less common in moist soil and at high temperatures, but this study showed that all ridge-furrow mulching treatments increased its relative abundance.

Proteobacteria and Actinobacteria had higher abundance in the NJ treatment. Proteobacteria growth generally utilizes unstable carbon sources in soil and can participate in degradation of other microbial cell membranes and walls, using intracellular lipid metabolism to maintain competitive advantages in soils with low soluble organic carbon content. Ridge mulching increased soil alpha diversity compared with bare soil, consistent with reports that mulching improved soil microbial abundance and diversity. Plastic film mulching enhances nutrient availability in surface soil, and abundant active organic carbon and nitrogen provide active substrates for bacterial reproduction. Beta diversity analysis showed that NJ treatment had similar microbial community structure to CK, while other treatments differed significantly from CK. In summary, ridge-furrow mulching changed soil microbial composition at both phylum and class levels, and the ridge-furrow rainwater harvesting mode can influence soil microbial community diversity.

4 Conclusion

(1) The ridge mulching rainwater harvesting mode (HJ and YJ) significantly increased maize yield and water use efficiency. Under the HJ mode (ridge covered with ordinary black plastic film and furrow mulched with straw), yield and water use efficiency increased by 88.02% and 79.83% compared with traditional flat planting without mulching, respectively. The NJ mode (ridge without cover and furrow mulched with straw) decreased maize yield and water use efficiency compared with CK.

(2) Ridge mulching rainwater harvesting modes significantly improved maize

rhizosphere soil microbial community diversity and altered microbial structure, whereas the NJ mode had minimal effects on soil microbial community diversity and structure.

(3) Under different ridge-furrow mulching rainwater harvesting modes, the dominant bacterial phyla were Proteobacteria (39.3%), Acidobacteria (16.6%), Gemmatimonadetes (8.4%), and Bacteroidetes (7.7%). The dominant bacterial classes were Gammaproteobacteria (25.8%), Bacteroidia (8.4%), and Alphaproteobacteria (7.7%). Ridge-furrow mulching improved soil microbial richness, diversity, and evenness indices, thereby increasing maize yield by altering soil microbial structure and composition at phylum and class levels.

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Note: Figure translations are in progress. See original paper for figures.