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Effect of Plastic Mulching on Soil Carbon and Nitrogen Cycling-Related Bacterial Community Structure and Function in a Dryland Spring Maize Field

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Abstract: Plastic mulching, given its positive effects on temperature and water retention, has been widely used to solve water shortages and nutrient scarcity in rainfed agricultural soils. This practice affects the physical and chemical processes of soil, including carbon and nitrogen cycling. However, research into microbe-mediated carbon and nitrogen cycling in soil with plastic mulching is still limited. In this study, the structures and functions of the soil bacterial community in non-mulched spring maize, plastic-mulched spring maize, and bareland fallow in a dryland field on the Loess Plateau in China were analyzed to explore the responses of microbe-mediated carbon and nitrogen cycling to plastic mulching. Results showed that the richness of soil bacteria was the highest in bareland fallow. Plastic mulching increased the diversity and richness of soil bacteria to a certain extent ($p > 0.05$), and significantly increased the content of microbial biomass nitrogen (MBN) ($p < 0.05$). Plastic mulching enhanced the total abundances of carbon and nitrogen cycling-related microbes, exhibiting a significant increase in the abundances of *Cellvibrio*, *Bacillus*, *Methylobacterium* and *Nitrospira* ($p < 0.05$). Predicted functional analysis revealed 299 metabolic pathways related to carbon and nitrogen cycling, including methane metabolism, carbon fixation in photosynthetic organisms, and nitrogen metabolism. The number of gene families assigned to carbon and nitrogen cycling-related metabolic pathways was higher in plastic mulched than that in non-mulched spring maize. This study demonstrated that plastic mulching enhances the capacity of carbon and nitrogen cycling, revealing its potential in mediating greenhouse gas emissions in the dryland spring maize fields on the Loess Plateau.

Keywords: plastic mulching; dryland farmland; bacterial community structure; carbon–nitrogen cycling; KEGG

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1. Introduction

Rainfed land, accounting for 55% of the cultivated land in China, is essential to meeting the growing demand for food. However, crop production in rainfed farmland is constrained by limited precipitation, high evaporation, and low levels of soil nutrients [1]. The plastic mulching of crops has recently been widely applied in rainfed farmland regions of China, increasing yield by heightening soil temperature and improving water retention and soil nutrient availability [2–4]. Although studies have reported that plastic mulching promotes soil carbon and nitrogen cycling by altering soil physicochemical properties (i.e., higher soil temperature and moisture conditions) [5–7], concerns have been raised regarding its potential effect on increasing emissions of greenhouse gases, such as carbon dioxide (CO_2), nitrous oxide (N_2O), and methane (CH_4) [8,9]. Therefore, it is necessary to

understand the influencing mechanism of plastic mulching on carbon and nitrogen cycling, especially related to the generation of greenhouse gases.

Microbes are the main drivers of biogeochemical and soil nutrient cycling, especially for soil carbon and nitrogen, material metabolism, and pollutant purification [10]. Functional microbes that mediate soil carbon cycling are dominated by species capable of CO_2 fixation and organic material decomposition, such as cellulose and starch [11], methanogens, and methane oxidizing bacteria [12]. Soil nitrogen cycling is mediated by nitrogen-fixing microbes, ammonia-oxidizing bacteria (AOB), ammonia-oxidizing archaea (AOA) [13–15], and denitrifying microbes. Among these, methanotrophs, organic-decomposing heterotrophic microbes, nitrifying microbes, and denitrifying microbes are the most important mediators of greenhouse gas emissions [16–18]. The influences of plastic mulching on the soil carbon and nitrogen cycling-related microbes deserve further exploration.

Studies have demonstrated the effects of plastic mulching on the structures and diversity of soil microbial community, and soil micro-ecological environment [19–21]. Shen et al. reported that plastic mulching increased carbon metabolic activity and bacterial diversity in semiarid regions of northwestern China [22]. Dong et al. found increases in the richness and functional diversity of a soil microbial community were associated with the early stage of plastic mulching in a rainfed region of northeastern China [23]. However, other research also found that plastic mulching did not significantly affect soil bacterial diversity and community compared to no mulching [24]. Changes in the soil microbial community structures under plastic mulching may affect carbon and nitrogen cycling-related microbes, thereby impacting the soil carbon and nitrogen cycling. However, there is limited understanding of microbe-mediated soil carbon and nitrogen cycling under plastic mulching.

This study aimed to evaluate the effect of plastic mulching on the structures and functions of soil bacterial community in a dryland spring maize field on the Loess Plateau in China. The soil properties, the diversity and community composition of bacteria, and the function of carbon and nitrogen cycling-related bacteria from soils under different treatments were compared, including contrasting non-mulching (NM), plastic mulching (PM), and bareland fallow (BL). We hypothesized that plastic mulching of spring maize could affect carbon and nitrogen cycling-related structures and functions of the bacterial community, thereby playing a major role in carbon and nitrogen cycling, and mediating greenhouse gas emissions. This study provides a reference for the development of agricultural activities and the formulation of carbon emission reduction policies in dryland farmland.

2. Materials and Methods

2.1. Study Area

The experiments were conducted on the Weibei rainfed highland at the Agricultural Ecological Experiment Station of the Loess Plateau (107°40′ E, 35°12′ N) in Changwu County, Shaanxi Province, China. This area is in a warm temperate zone with a semi-arid/semi-humid continental monsoon climate, representative of dryland farming conditions in China. With an average elevation of 1220 m, the area is characterized by an annual average temperature of 9.1 °C and a precipitation of 584 mm. Its annual sunshine hours is 2230 h, with 171 d of frost-free period. The soil comprises a Heilutu silt loam (Calcarid Regosol according to the FAO classification system or an Ultisol according to the U.S. soil taxonomy) [25], with 65% silt, 31% clay, and 4% sand, 8.4 pH.

2.2. Experimental Design and Sampling

Three treatments were involved in the experiment, including non-mulched spring maize (NM), plastic-mulched spring maize (PM), and bareland fallow (BL). In April 2014, three replicates of each treatment were applied to 8 m × 5 m plots in a 54 m × 12 m field with a randomized design. Spring maize (*Zea mays* L., cv. Xianyu 335) was sown at a density of 65,000 plants ha^{-1} in the NM and PM plots. Nitrogen, phosphorous, and

potassium fertilizers were applied at 225 kg N ha^{-1} (as a basal and top dressing at jointing, in a ratio of 6:4), 60 kg P ha^{-1}, and 30 kg K ha^{-1}, respectively. In NM and PM plots, a wide (60 cm) and narrow (40 cm) row spacing pattern was employed, and maize seed was sown with a plant spacing of 30 cm. In the PM plots, maize seeds were sown, and then plastic mulching (polyethylene film 0.008 mm thick) was applied. In the BL plots, no spring maize was sown, and weed growth was controlled.

After two years of planting, soil samples from the maize rhizosphere were collected following harvest in October 2015. In the PM plots, plastic films were immediately removed to prevent the influence of plastic debris on the environment. After removing surface debris, the soil samples were taken from the depth of 0–10 cm in an S-shaped pattern from five points within each plot. Maize roots were gently shaken to remove loose soil, and the remaining soil on the roots was carefully collected using a sterile brush. In addition, soil samples were collected from the same depth (0–10 cm) within the BL plots. All samples were placed in a portable refrigerator, immediately brought to the laboratory and stored at –20 °C before analysis.

2.3. Soil Properties Analysis

Part of the soil samples (n = 3 for NM, PM, and BL treatments, respectively) were air-dried, and sieved to 2 mm to determine the content of ammonium nitrogen (NH_4^+–N), nitrate nitrogen (NO_3^-–N), potential nitrogen mineralizable (PNM), microbial biomass nitrogen (MBN) and microbial biomass carbon (MBC). Afterward, the samples were further sieved to 0.15 mm to determine soil total nitrogen (STN) and soil organic carbon (SOC) contents.

To determine SOC and STN, the samples were denitrified with 10% HCl to remove inorganic carbon, and analyzed by an EA3000 elemental analyzer [26]. PNM was determined by the sealed incubation method [27,28]. Two 10 g soil samples were adjusted to 50% field capacity with water, and cultured in the jar containing beakers at 21 °C for 10 d. After 10 d, one beaker was taken out and leached with 50 mL of 2 mol·L^{-1} KCl solution for 1 h. The contents of NO_3^-–N and NH_4^+–N in the extract were determined by Cleverchem 380 (DeChem-Tech) auto discrete analyzer. The PNM was determined as the difference between NO_3^-–N and NH_4^+–N before and after culture. The other container with moist soil was used for determining MBN and MBC by chloroform fumigation–incubation method [29]. The moist soil was fumigated with ethanol-free chloroform for 24 h, then cultured at 21 °C for 10 d. MBN was determined by the same extraction method as PNM [29]. After fumigation with ethanol-free chloroform for 24 h, the moist soil was placed in a jar with 20 mL water and 2 mL 0.5 mol L^{-1} dilute NaOH for 10 d. After titration with $BaCl_2$ and HCl, the amount of CO_2 released from the soil before and after chloroform fumigation was calculated. The MBC content was the difference between the amount of CO_2 released from the soil before and after fumigation divided by a factor of 0.41 [29].

2.4. DNA Extraction and Sequencing

Within a week after harvest, the total genomic DNA from each soil sample (n = 3 for NM, PM, BL treatments, respectively) was extracted using TIANamp Soil DNA Kit 107 (Tiangen Biotech Inc., Beijing, China), following the manufacturer's instructions. DNA quality and integrity were controlled using 1% agarose gel electrophoresis.

Universal bacterial primers B341F (5′-CCTACGGGNGGCWGCAG-3′) and B785RA (5′-GACTACHVGGGTATCTAATCC-3′) [30] were used to amplify the V3–V4 hypervariable regions of the bacterial 16S rRNA gene. All PCR reactions were carried in 25 μL, consisting of 12.5 μL KAPA HiFi HotStartReadyMix (2×), 0.25 μmol L^{-1} of each primer, 10 ng of DNA template, and PCR-grade water. Amplifications were performed using the following temperature program: 3 min of initial denaturation at 95 °C, followed by 25 cycles of denaturation (95 °C for 30 s), annealing (55 °C for 30 s), extension (72 °C for 30 s), and a final extension at 72 °C for 5 min. PCR amplicon libraries were prepared by combining the PCR products for each sample. After purification, the PCR products from each sample were quantified using the Agilent 2100 Bioanalyzer System (Santa Clara, CA, USA) and

then pooled at equal concentrations. Amplicon sequencing was performed on the Illumina Miseq platform.

2.5. Data Analysis

Taxonomic assignment of the sequences was performed using the RDP classifier [31] with reference to the SILVA database [32]. Sequences were assigned to six taxonomic levels: kingdom, phylum, class, order, family, and genus. The operational taxonomic units (OTUs) at 97% similarity were used to calculate the richness and diversity indices. Rarefaction was used to normalize sample data. Chao1, ACE, Simpson, and Shannon indices were calculated in Mothur [33] as measures of bacterial richness and diversity. The prediction of gene function was performed using the Phylogenetic Investigation of Communities by the reconstruction of the unobserved state (PICRUSt) in the KEGG database (Kyoto Encyclopedia of Genes and Genomes, http://www.genome.jp/kegg/pathway.html, accessed on 25 August 2021). All results were analyzed by analysis of variance (ANOVA) using SPSS 20.0 software. The redundancy analysis (RDA) of species information and environmental factors was carried out using Canoco 5.0.

3. Results and Discussion

3.1. Soil Bacterial Community

A total of 24,258, 28,429, and 40,027 16S rRNA sequences of soil bacteria were obtained from the NM, PM, and BL treatments, respectively, which were assigned to 2434, 2619, and 2726 OTUs, respectively (Table 1). The number of sequences and OTUs was higher in the BL treatment compared with those of PM and NM treatments ($p > 0.05$). There were 2114 OTUs shared across all three treatments. Totals of 2202 OTUs were shared between the NM and PM treatments, 2284 between the NM and BL treatments, and 2386 between the PM and BL treatments. In addition, 62, 145, and 170 OTUs were found unique to the NM, PM, and BL treatments, respectively (Figure 1). The OTUs were classified into 27 phyla, 49 classes, 82 orders, 121 families, and 153 genera. The most abundant phyla were *Proteobacteria*, *Planctomycetes*, and *Acidobacteria*, accounting for more than 50% of the OTUs in each treatment, while *Actinobacteria*, *Gemmatimonadetes*, *Verrucomicrobia*, *Bacteroidetes*, *Chloroflexi*, and *Cyanobacteria* accounted for roughly 30% of the total OTUs in each treatment (Figure 2).

Table 1. The diversity indices of the soil bacterial community in a dryland field under three different treatments.

Sample	NM	PM	BL
Nseqs	24258 ± 3719 a	28429 ± 4287 a	40027 ± 4607 a
OTUs	2434 ± 106 a	2619 ± 202 a	2726 ± 185 a
Chao	2835.333 ± 87.345 a	3016.138 ± 169.537 a	3192.375 ± 140.838 a
ACE	2840.142 ± 82.245 a	2956.100 ± 160.015 a	3088.118 ± 137.283 a
Coverage	0.977 ± 0.005 a	0.981 ± 0.009 a	0.987 ± 0.007 a
Shannon	6.818 ± 0.051 a	6.923 ± 0.159 a	6.866 ± 0.130 a
Simpson	0.00255 ± 0.000 a	0.00249 ± 0.000 a	0.00246 ± 0.000 a

Nseqs: The number of sequences clustered into an operational taxonomic unit (OTU). NM, non-mulched spring maize (control); PM, plastic-mulched spring maize; BL, bareland fallow. Values are mean ± SD (n = 3). Letters followed by different numbers within a row are significantly different at $p < 0.05$.

As a major phylum of Gram-negative bacteria, *Proteobacteria* are free-living (non-parasitic) and responsible for nitrogen fixation [34]. The rank order of the abundance of *Proteobacteria* was 21.536%, 20.560%, and 17.432% in the BL, PM, and NM treatments, respectively, its abundance in PM being 1.179 times that in NM ($p > 0.05$). *Acidobacteria* decompose plant debris or polymers, playing a role in the iron recycling, photosynthesis, and metabolism of single-carbon compounds [35,36]. *Actinobacteria* are known to decompose organic matter and produce diverse enzymes and vitamins, while some members fix atmospheric nitrogen [37]. The rank order of the abundance of *Actinobacteria* and *Acidobac-*

teria was NM > PM > BL ($p > 0.05$). A study showed that the abundance of *Actinobacteria* was lower in non-mulched maize than that in plastic-mulched maize in cinnamon soil [38]. *Cyanobacteria* play a role in CO_2 fixation [38–40]. In this study, the abundance of *Cyanobacteria* was the highest in the BL treatment (0.999%), followed by PM (0.970%) and NM (0.736%) ($p > 0.05$). The peak relative abundance of *Nitrospirae* occurred in PM, and was significantly higher than in NM and BL ($p < 0.05$). These relatively abundant phyla are involved in processes related to carbon and nitrogen metabolism (i.e., organic matter decomposition and nitrogen fixation). The rank order of OTU numbers from the three treatments indicated that plastic mulching could change the metabolic capability of soil microbes for carbon and nitrogen cycling in dryland spring maize field.

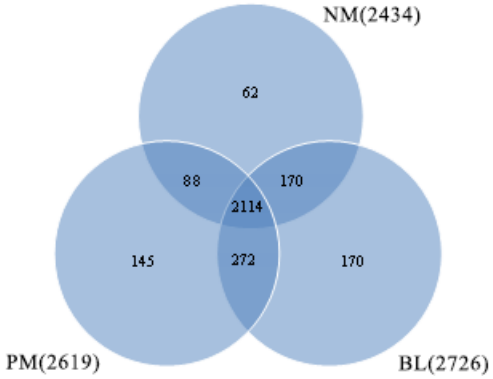

Figure 1. Venn diagram showing the number of unique and common operational taxonomic units for soil microbes in the three treatments. NM: non-mulched spring maize (control); PM: plastic-mulched spring maize; BL: bareland fallow. n = 3 (NM, PM, BL).

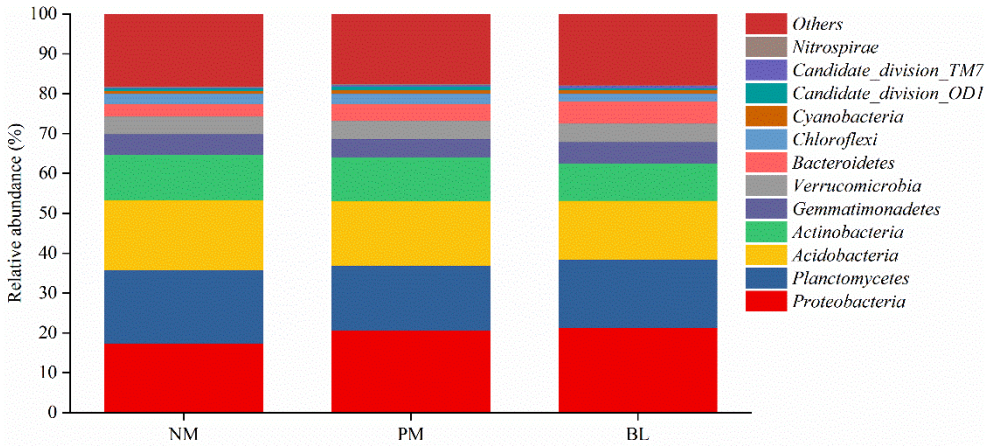

Figure 2. Percent distribution of soil bacterial phyla in the three treatments. NM: non-mulched spring maize (control); PM: plastic-mulched spring maize; BL: bareland fallow. n = 3 (NM, PM, BL).

The predominant genera in the NM, PM, and BL treatments were *Gemmatimonas*, *Sphingomonas*, *Gaiella*, *Haliangium*, and *Solirubrobacter*, accounting for 5.987%, 7.030% and 6.250% of the total genera, respectively (Figure 3). *Gemmatimonas* is a genus in the phylum *Gemmatimonadetes*, predominant in the soil environment, and capable of both aerobic and anaerobic respiration [41]. The abundance of *Gemmatimonas* in PM was lower than that in NM ($p > 0.05$). *Sphingomonas* may utilize various simple molecules, and decompose complex organic compounds [42]. The abundance of *Sphingomonas* in PM was significantly higher than that in NM ($p < 0.05$). *Streptomyces* (PM–BL, $p < 0.05$) and *Nitrospira* (PM–BL, PM–NM, $p < 0.05$) also had the highest relative abundance in PM. While the highest relative abundance of *Lysobacter* (BL–NM, BL–PM, $p < 0.05$), *Ohtaekwangia* (BL–NM, $p < 0.05$), *and*

Blastocatella (BL–NM, BL–PM, $p < 0.05$) were observed in BL. Plastic-mulching and bareland fallow exert a certain effect on the bacterial community.

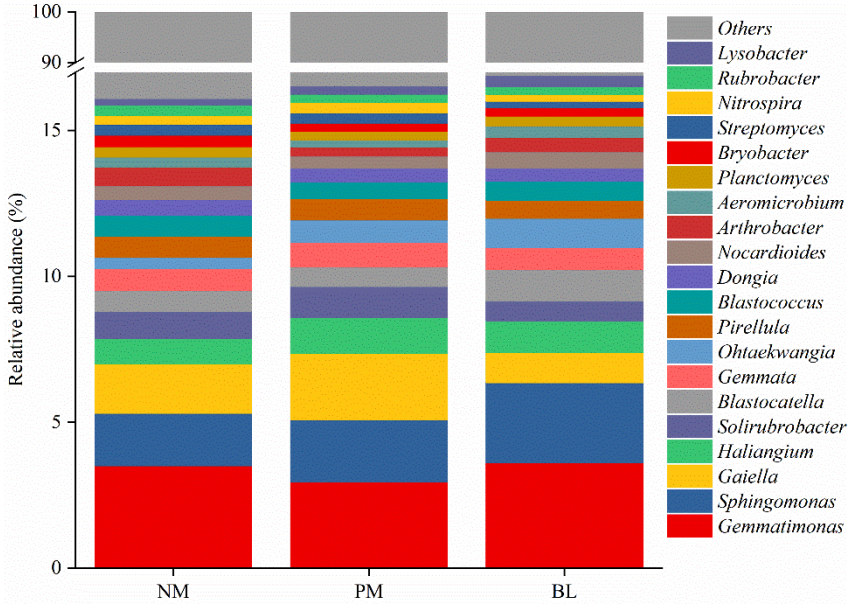

Figure 3. Percent distribution of soil bacterial genera in the three treatments. NM: non-mulched spring maize (control); PM: plastic-mulched spring maize; BL: bareland fallow. n = 3 (NM, PM, BL).

3.2. Diversity of Soil Bacteria

The diversity indices of the soil bacterial communities of the three treatments are shown in Table 1. The sequencing coverage for the NM, PM, and BL treatments was 0.98, 0.99, and 0.98, respectively, indicating that the species' information fully reflected the soil microbes in different treatments. Among the three treatments, the richness (Chao1 and ACE) of the soil bacteria was the highest in BL, and the Chao1 and ACE indices were 6.38% and 4.08% higher in PM than that in NM ($p > 0.05$). The rank order of the Shannon index in the three treatments was PM > BL > NM, and the Simpson index, NM > PM > BL (Table 1, $p > 0.05$). Compared with NM, PM increased the richness and diversity of soil bacteria to a certain extent. This result was consistent with the previous studies which found that plastic mulching increased soil microbial richness and diversity [22,23].

3.3. Functional Group Responses

3.3.1. Soil Carbon Cycling-Related Microbes

Microbial functional communities drive carbon cycling processes, and are vital in response to global climate change and maintaining ecosystem function and stability [34,43]. Respiration in farmland ecosystem soils is derived from plants, animals, and microbes. Approximately 30–50% of the CO_2 released through soil respiration arises from root activity or autotrophic respiration—the remainder arising from the decomposition of organic matter by soil microbes [44]. In addition to the decomposition of organic matter, microbes also mediate carbon fixation, methane metabolism, and other basic processes of carbon cycling [34,43].

In this study, the microbes participating in carbon cycling include methanotrophs and species capable of CO_2 fixation and the decomposition of organic matter (Table 2). Examples of organic matter decomposers were the cellulose-decomposing *Cellvibrio* [11]; starch-decomposing *Bacillus, Nocardia*, and *Pseudomonas*; lignin-degrading *Streptomyces* [45]; lipid-decomposing *Mycobacterium* [46]; and aromatic-decomposing *Arthrobacter* [47]. Most of the carbon cycling-related microbes in PM were higher than that in NM, among which the abundances of *Cellvibrio, Bacillus* and *Methylobacterium* in PM were significantly higher than those in NM ($p < 0.05$). The total abundance of bacteria decomposers of soil organic matter

differed among the three treatments, with a rank of BL > PM > NM ($p > 0.05$). Similarly, a study from our group concluded that soil microbial biomass carbon and nitrogen were lower under spring maize cultivation than under fallow [27]. We suggest that plastic mulching increased the total abundance of soil microbes associated with organic matter decomposition and reduced carbon-fixing bacteria, which may lead to an increase in CO_2 emissions. This hypothesis is supported by a study in the Loess Plateau region which showed that plastic mulching increased CO_2 emissions [3].

Table 2. The abundance of carbon and nitrogen cycling-related functional microbes in the three different treatments.

Function	Microbe	Relative Abundance (%)		
		NM	PM	BL
Carbon cycling	Decomposition of Organics			
	Cellvibrio Nocardia	0 b	0.016 ± 0.008 a	0.034 ± 0.026 a
		0.012 ± 0.003 a	0.012 ± 0.001 a	0.021 ± 0.006 a
	Streptomyces	0.112 ± 0.016 a	0.148 ± 0.019 a	0.071 ± 0.017 b
	Bacillus	0.024 ± 0.005 b	0.079 ± 0.030 a	0.024 ± 0.007 b
	Pseudomonas	0.017 ± 0.005 b	0.022 ± 0.002 b	0.108 ± 0.062 a
	Mycobacterium	0.134 ± 0.038 a	0.180 ± 0.041 a	0.145 ± 0.013 a
	Arthrobacter	0.332 ± 0.054 a	0.192 ± 0.058 a	0.272 ± 0.019 a
	CO_2 fixation			
	Nostoc	0 a	0 a	0.002 ± 0.001 a
	Methanotroph			
	Methylobacterium	0.002 ± 0.001 b	0.013 ± 0.000 a	0.005 ± 0.001 b
Nitrogen cycling	Nitrogen fixation			
	Rhizomicrobium	0.025 ± 0.006 a	0.028 ± 0.009 a	0.022 ± 0.005 a
	Nitrification			
	Nitrosospira	0.019 ± 0.004 a	0.023 ± 0.010 a	0.032 ± 0.018 a
	Nitrospira	0.235 ± 0.017 b	0.298 ± 0.002 a	0.191 ± 0.008 c
	Planctomyces	0.319 ± 0.025 a	0.293 ± 0.107 a	0.296 ± 0.023 a
	Pirellula	0.508 ± 0.030 a	0.551 ± 0.063 a	0.470 ± 0.053 a
	Pseudonocardia	0.040 ± 0.012 a	0.059 ± 0.004 a	0.048 ± 0.004 a
	Denitrification			
	Bacillus	0.024 ± 0.005 b	0.079 ± 0.030 a	0.024 ± 0.007 b
	Pseudomonas	0.017 ± 0.005 a	0.022 ± 0.002 a	0.021 ± 0.002 a
	Flavisolibacter	0.112 ± 0.012 b	0.100 ± 0.033 b	0.246 ± 0.044 a

Nseqs: NM, non-mulched spring maize (control); PM, plastic-mulched spring maize; BL, bareland fallow. Values are the mean ± SD (n = 3). Letters followed by different numbers within a row are significantly different at $p < 0.05$.

Methanogens and *methanotrophs* are two major functional groups that mediate CH_4 production and oxidation in soils, respectively [12]. *Methanogens* belong to the Euryarchaeota and only survive in habitats with very low redox potential [48]. Widely present in soils, swamps, paddy fields, rivers, lakes, forests, and oceans [49], *Methanotrophs* are divided into two types: Type I (*Gammaproteobacteria*) and Type II (*Alphaproteobacteria*) [50]. *Methanotrophs* commonly exist in anaerobic environments, such as swamps and paddy fields [49], whereas this study was conducted in rainfed farmland with aerobic soils. The only group of *methanotrophs* detected in this study was *Methylobacterium*, the abundance of which was significantly higher in PM than in NM ($p < 0.05$). The enhanced water retention with plastic mulching may result in an anaerobic environment in the soil [2–4].

3.3.2. Soil Nitrogen Cycling-Related Microbes

Soil nitrogen cycling includes processes of biological nitrogen fixation, ammonification, nitrification, and denitrification, which are driven by microbes. In this study, bacteria involved in nitrogen cycling were mainly nitrogen-fixing, nitrifying, and denitrifying bacteria. Nitrogen fixation includes symbiotic and non-symbiotic nitrogen fixation. The latter tends to be the main source of nitrogen in the absence of fertilizer application and legumes [51]. In this study, the major nitrogen-fixing bacterium was *Rhizomicrobium*, the

abundance of which was higher in PM than in NM ($p > 0.05$). These results suggest that in general, *Rhizomicrobium* played a major role in nitrogen fixation in the dryland spring maize field.

Nitrification includes ammonia oxidation and nitrite oxidation. The former is catalyzed by AOB [13] and AOA [14]. The AOB detected in this study mainly comprised *Planctomyces*, *Pirellula*, and *Nitrosospira*. The bacteria involved in nitrite oxidation mainly comprised *Nitrospira*, and the abundance was significantly higher in PM than that in NM ($p < 0.05$). Furthermore, *Pseudonocardia* were detected, which are known to directly nitrify ammonia into nitrate [52]. The total abundance of the bacteria involved in nitrification was highest in PM (1.224%), followed by NM (1.120%) and BL (1.037%) ($p < 0.05$). Denitrification mainly refers to dissimilatory denitrification: $NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2$. *Bacillus*, *Pseudomonas*, and *Flavisolibacter* were found to be the major bacteria participating in denitrification in this study. The abundance of *Bacillus* was higher in PM than that in NM ($p < 0.05$)—whereas there was no significant change in the abundance of the other two bacteria involved in denitrification. The highest abundances of these denitrifying bacteria were observed in BL (0.291%) ($p < 0.05$), followed by PM (0.202%) and NM (0.154%) ($p > 0.05$).

Research has shown that 80% of atmospheric N_2O is related to agricultural activities [53], and that soil microbe-mediated N_2O production processes (i.e., nitrification and denitrification) account for roughly 70% of the fluxes in total N_2O emissions [16]. N_2O emission fluxes have been found to positively correlate with the abundance of nitrifying and denitrifying microbes [54]. The major microbial mediators of N_2O emissions in this study were *Planctomyces*, *Pirellula*, *Nitrosospira*, *Nitrospira*, *Pseudonocardia*, *Bacillus*, *Pseudomonas*, and *Flavisolibacter.* The total abundance of bacteria involved in nitrification and denitrification in decreasing rank order in the three treatments was PM (1.425%), BL (1.328%), and NM (1.274%). PM increased the abundance of bacteria involved in nitrification and denitrification ($p > 0.05$), indicating that plastic mulching may enhance N_2O emissions in dryland agricultural fields due to the increase in the total abundance of nitrifying and denitrifying bacteria. These results were consistent with previous studies which found that compared with non-mulching treatment, plastic mulching increased N_2O emissions in temperate upland soil in South Korea and the arid region of China [55,56].

3.4. Relationships between Soil Properties and Bacterial Community Composition

Interactions exist between soil properties and bacterial communities. Soil carbon and nitrogen components (STN, PNM, MBN, NO_3^-–N, NH_4^+–N, SOC and MBC) were analyzed. Compared with NM, MBN in PM increased by 9.71% ($p<0.05$, Table S1), while NO_3^-–N in PM increased by 14.89% ($p > 0.05$). The contents of STN, PNM, SOC, MBC and NH_4^+–N in PM did not significantly change compared to NM ($p > 0.05$). Since the length of the maximum axis (0.69) was less than 3, RDA was used to explore the relationships between soil carbon and nitrogen components, and the top 20 bacteria (*Gemmatimonas, Sphingomonas, Gaiella, Haliangium, Solirubrobacter, Blastocatella, Gemmata, Ohtaekwangia, Pirellula, Blastococcus, Dongia, Nocardioides, Arthrobacter, Aeromicrobium, Planctomyces, Bryobacter, Streptomyces, Nitrospira, Rubrobacter, Lysobacter*) (Figure 4). SOC, NO_3^-–N, PNM and MBC exerted strong effects on bacterial communities. Previous studies also showed that SOC was the predominant factor affecting the bacterial community structures [23]. The top two bacteria (*Gemmatimonas, Sphingomonas*) were positively correlated with MBN, NH_4^+–N and MBC. For the carbon and nitrogen cycling-related microbes, a highly negative correlation was detected among *Arthrobacter, Streptomyces* and SOC, respectively, indicating that *Streptomyces* and *Arthrobacter* participated in the degradation of organic carbon [45,47]. The dominant nitrifying bacteria (*Pirellula* and *Nitrospira*) had a strong positive correlation with STN, PNM, and NO_3^-–N—but had a negative correlation with NH_4^+–N, suggesting their participation in nitrification. These results once again demonstrated the involvement of these bacteria in the carbon and nitrogen cycle.

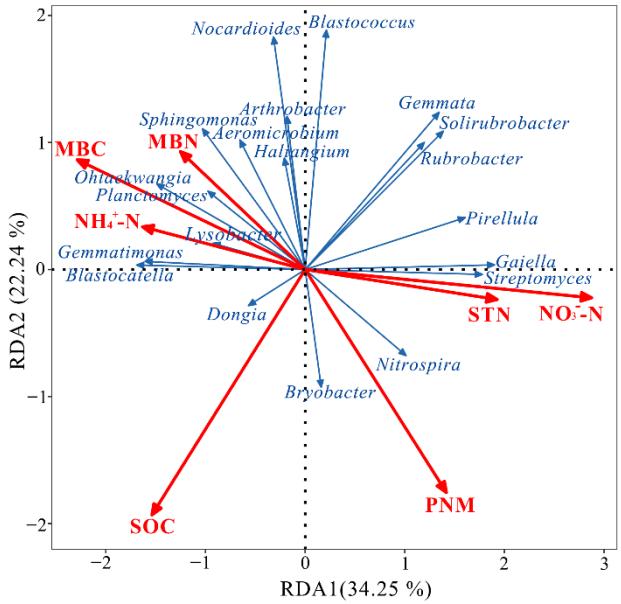

Figure 4. RDA among the higher levels of bacteria (top 20) and soil factors under different mulching treatments.

3.5. Prediction of Functional Genes

There were six major functional gene categories at KO (KEGG Orthology) level 1, including metabolism, genetic information processing, environmental information processing, cellular processes, human diseases, and organismal systems. Among these, metabolism was enriched with the largest number of sequences, accounting for nearly 50% of the total sequences in the three treatments. At KO level 2, 41 functional gene categories were identified, including amino acid metabolism, carbohydrate metabolism, membrane transport, replication and repair, and energy metabolism. The five most abundant categories were consistent with the functional prediction results of the rhizosphere microbial community for healthy and root-rot *Panax notoginseng* in the Wenshan region of Yunnan Province [57]. The classification at KO level 3 corresponded to various metabolic pathways, and for the three treatments, soil microbes were annotated to a total of 299 metabolic pathways (Figure 5).

Except for transporters and general function, the five most enriched gene pathways were ABC transporters, DNA repair and recombination proteins, the two-component system, purine metabolism, and bacterial motility proteins. The extensive expression of ABC transporters has been found in paddy soils under both aerobic and anoxic conditions [58], probably because ABC-type transporters are among the most active transmembrane transport systems in bacteria, archaea, and eukaryotes [59]. Among the 299 metabolic pathways, those associated with carbon and nitrogen cycling included carbon metabolism (glycolysis/gluconeogenesis, carbon fixation in photosynthetic organisms, methane metabolism, etc.) and nitrogen metabolism (nitrogen metabolism) (Figure S1). The relative abundance of these pathways in the treatments in decreasing rank order were BL, PM, and NM. These results indicate that the carbon and nitrogen metabolic capacity of soil microbes is greatest in bareland fallow, and plastic mulching enhances the carbon and nitrogen metabolic capacity of soil microbes compared with non-mulched dryland spring maize.

Figure 5. KEGG annotated metabolic pathways of soil bacteria in the three treatments. NM: non-mulched spring maize; BL: bareland fallow; PM: plastic-mulched spring maize. n = 3 (NM, PM, BL).

4. Conclusions

This study characterized the structure and function of soil bacterial communities under the NM, PM, and BL treatments. Overall, we found that *Proteobacteria* was the most abundant phyla, and *Gemmatimonas* was the most abundant genus. Greater bacterial community richness and diversity were observed in PM compared to NM. Carbon and nitrogen cycling-related bacterial community analyses showed that carbon cycling-related bacteria were mainly composed of organic matter, methanotrophs, and CO_2 fixation, while nitrogen cycling-related bacteria included those capable of nitrogen fixation, nitrification, and denitrification. Compared with NM, PM enhanced the total abundances of carbon and nitrogen cycling-related bacteria, especially *Cellvibrio*, *Bacillus*, *Methylobacterium*, and *Nitrospira* ($p < 0.05$). Furthermore, the predicted functional analysis showed that PM increased carbon and nitrogen metabolism-related metabolic pathways. Based on these results, we speculate that PM enhanced the capacity of soil carbon and nitrogen cycling, and may increase greenhouse gas emissions (CO_2, CH_4, and N_2O). This study revealed the bacterial mechanism of carbon and nitrogen cycling in plastic-mulched dryland spring maize. Our findings provide compelling data for the further evaluation of the effects of different mulching methods on soil carbon and nitrogen cycling and greenhouse gas emissions in dryland farmland.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/agriculture11111040/s1, Table S1: The physical and chemical properties of the maize soil under different treatments, Figure S1: KEGG annotated carbon and nitrogen metabolic pathways of soil bacteria in a dryland field with three different treatments. NM: non-mulched spring maize (control); PM: plastic-mulched spring maize; BL: bareland fallow.

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