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Impact of Long-Term Gravel Mulching on Soil Bacterial and Fungal Communities in the Semi-Arid Loess Plateau of Northwestern China

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Gravel mulching is a traditional method of water conservation in the semi-arid regions of China. In this study, we investigated the soil microbial community in a field in China's Gaolan County which has been gravel mulching for over a period of 18 yr. Compared with the non-mulch control, total organic nitrogen (TON), microbial biomass carbon (MBC), and microbial biomass nitrogen (MBN) were all significantly increased in the field with gravel mulching for over 13 yr. Moreover, after 18 yr, the soil bulk density and sand content increased significantly, thus degrading the soil microenvironment. Gravel mulch significantly altered the bacterial community structure and composition, increased the abundance of Acidobacteria, Gemmatimonadetes, Bacteroidetes, and Firmicutes, and decreased the abundance of Actinobacteria compared with the control. Gravel mulch also significantly changed the fungal community structure and composition; the soils were found to have a greater abundance of Basidiomycota and Zygomycota and reduced abundance of Ascomycota and Glomeromycota compared with the control soils after long-term gravel mulching. Redundancy analysis (RDA) revealed that the bacterial genera after 18 yr of mulching were dominated by Incertae_Sedis, Blastocatella, Desulfovibrio, Bacteroides, Gemmatimonas, Parabacteroides and Alloprevotella, and that the composition of the bacterial community was related to soil pH, bulk density, MBC and MBN. However, significant decreases in the diversity indices of Chao1, abundance-based coverage estimator (Ace) and Shannon after 18 yr of mulching demonstrated negative effects on the complexity of the soil microbial community.

Key Words: bacterial genera, fungal community, gravel mulching, soil bulk density, soil microbial community

Abbreviations: ACE – abundance-based coverage estimator, BD – bulk density, MBC – microbial biomass carbon, MBN – microbial biomass nitrogen, OTU – operational taxonomic unit, PCoA – principal coordinate analysis, QIIME – Quantitative Insights Into Microbial Ecology, RDA – redundancy analysis, TOC – total organic carbon, TON – total organic nitrogen

INTRODUCTION

Gravel mulch is a traditional method of water conservation that has been used for hundreds of years in the Loess region of northwestern China, which lies in the transitional zone between the arid and semi-arid regions. In the Loess region, the mean annual precipitation is between 250 and 350 mm, of which nearly 70% occurs between June and September (Li and Gong 2002). The annual pan evaporation ranges from 1500 to 2000 mm. The gravel mulch used in the Loess region is a porous layer of gravel that is approximately 10 cm thick that lies on the soil surface. Mulch reduces the risk of crop failure, which frequently occurs due to a combination of low precipitation and high evaporation that creates severe deficits in soil moisture. This mulching technique has been promoted and widely adopted due to the lack of sufficient water and high cost of irrigation in the Gansu province. In the 1990s, 118 000 ha of mulched watermelon fields were established in Gansu Province (Li 2003).

Gravel mulch effectively reduces water evaporation and runoff, increases the soil temperature in crop fields, retains soil moisture and affects the microenvironment (Li 2003; Wang et al. 2008; Ma and Li 2011). The efficiency of mulching varies widely, depending on the characteristics (position, thickness, particle size, coverage degree and roughness) of the gravel mulch (Yuan et al. 2009; Xie et al. 2006, 2010). Yuan et al. (2009) reported that gravel mulches reduced evaporation by 49.1–83.6% compared with the bare soil. In a study conducted in the semi-arid Loess region of northwestern China, the soil temperature at a depth of 10 cm was 0.5–4.5 °C warmer than that at a comparable depth below bare topsoil when the topsoil was covered with gravel mulch (Li 2003). In addition, Ma

and Li (2011) found that gravel-sand mulches conserved soil water compared with the bare soil and that the soil water content increased with increasing mulch thickness.

Most of the previous studies focused on the positive effects of mulching on soil water conservation in crop fields. However, we found that gravel mulches increased the organic carbon fractions and enzymatic activities (peroxidase, dehydrogenase, invertase, β-glucosidase, alkaline phosphomonoesterase, and urease) in the soil over 11 yr of mulching, but almost all of the indicators declined considerably after 16 yr of mulching (Qiu et al. 2014). Our findings suggest that although the use of gravel mulch is a promising management technique to conserve water, its capability to improve soil quality declines after 16 yr of mulching. The microbial composition of a soil has often been proposed as an early and sensitive indicator of soil ecological stress and the success of restoration processes in both natural and agricultural ecosystems (Dick 1994). Soil microorganisms play important roles in organic carbon mineralization, nutrient cycling, and disease transmission and resistance, all of which are associated with soil health (Hollister et al. 2013).

To date, little is known about the effects of gravel mulch on communities of soil microorganisms. In this study, therefore, field experiments were conducted to investigate the long-term effects of gravel mulch on the soil physicochemical properties and soil microbial communities. The study objectives were (1) to determine the impact of long-term gravel mulching on soil microbial communities using the MiSeq platform of next-generation sequencing and (2) to study the relationship between microbial communities and soil properties.

MATERIALS AND METHODS

Site Description and Sampling

A long-term field experiment was conducted at the Gaolan Research Station of Ecology and Agriculture, Northwest Institute of Eco-Environment and Resources, Chinese Academy of Sciences. The station is located in the northwestern Loess Plateau (Lanzhou City, Gaolan County, Gansu Province; 36°13'N, 103°47'E) at an altitude of approximately 1800 m above sea level. Based on 30 yr of records, the mean annual rainfall is 263 mm, of which nearly 70% occurs between June and September. The region's mean annual temperature is 8.4°C, with a monthly mean temperature reaching a maximum of 20.7°C in July and a minimum of −9.1°C in January. The soil is a silt loam (sand = 123 g kg^{-1}; silt = 669 g kg^{-1}; clay = 208 g kg^{-1}) of Loess origin and belongs to the Haplic Orthic Aridisols.

The field study was conducted in 1996 at the station to investigate the long-term effects of gravel mulching. The mulch used was composed of 50% big grain size gravel (2–6 cm) and 50% small grain size gravel (2–5 mm) by volume and placed on the surface of the soil in a 10-cm thick layer. The study compared three mulching durations (9, 13, and 18 yr) with a non-mulched control. Watermelon (*Citrullus lanatus*) was seeded at an in-row spacing of 1.0 m in rows that were 0.6 m apart. All of the treatments received 150 kg N ha^{-1} (as nitrate), 90 kg P_2O_5-equivalent ha^{-1}, 99 kg of K_2O-equivalent ha^{-1}, and 30 000 kg of manure (sheep) ha^{-1}. No irrigation was provided. Three replicates were used for each treatment, and the individual plot size was 10 × 10 m. We used the same tillage cultivation in the control plots as that in the mulched plots throughout the study. Only the rows of plants were tilled between crops; the mulch was not disturbed in the between-row spaces throughout the study, and most of the aboveground biomass was removed during harvest.

The soil in all of the treatments (below the mulch layer in the mulched plots) was sampled to a depth of 15 cm at 10 randomly established locations per plot in September 2014. The subsamples were combined to provide a single sample for each plot. After sifting the samples through a 2-mm sieve and thorough homogenization, one portion of each sample was air-dried for the determination of chemical properties, and the other was stored at −70°C for subsequent DNA extraction.

Determination of Soil Physicochemical Properties and DNA Extraction

Samples were analyzed following the procedures in AFNOR (2004). AFNOR 31–107 was used to assess the particle size distribution. Total organic carbon (TOC) was determined by potassium dichromate ($K_2Cr_2O_7$) oxidation at 170–180°C, followed by titration with 0.1 mol L^{-1} ferrous sulphate (Walkley and Black 1934). Total organic nitrogen (TON) was measured using the Kjeldahl digestion method. Total P was extracted in aqua regia following ISO 11466 (ISO 1995). The soil pH was determined according to ISO 10390 (ISO 1994): 5 g of soil was shaken in 25 mL of 1 M KCl for 5 min and allowed to stand for 2 h, and then, the pH was recorded using a glass electrode. The carbon concentration in the soil microbial biomass (MBC) was determined using the modified chloroform fumigation-extraction method developed by Gregorich et al. (1994). Soil microbial biomass N (MBN) was analyzed using chloroform fumigation and K_2SO_4 extraction based on the method of Brookes et al. (1985). Fumigated samples were flushed for 24 h with chloroform and extracted with 0.5 M K_2SO_4 immediately afterwards to prevent microbial regrowth. MBN was calculated as

the difference between fumigated and non-fumigated samples. Total soil genomic DNA was extracted using the PowerSoil DNA Isolation Kit (MoBio Laboratories Inc., USA) following the manufacturer's instructions and then stored at −70 °C for subsequent analysis.

PCR Amplification, Sequencing and Statistical Analysis

The Miseq sequencing strategies for bacteria and fungi used in this study followed the respective protocols described by Kozich et al. (2013). Briefly, the gene-specific primers F515 and R806 were constructed based on the V4 region of the bacterial 16S rRNA gene, while primers ITS1F and ITS2 were constructed based on the ITS1 region of the fungal internal transcribed spacer (ITS). 16S rRNA tag-encoded high-throughput sequencing was conducted using the Illumina MiSeq platform at the Biomarker Technologies Co., Ltd., Beijing, China. Pairs of reads from the original DNA fragments were aligned based on the method described previously (Magoč and Salzberg 2011). Sequencing reads were assigned to each sample according to their individual unique barcode. Sequences were analyzed with the Quantitative Insights Into Microbial Ecology (QIIME) software package and UPARSE pipeline (Caporaso et al. 2010). The reads were first filtered for quality by the method available in QIIME. Default settings were used for Illumina processing in QIIME. The UPARSE pipeline was then used to identify operational taxonomic units (OTUs) at 97% similarity. For each OTU, a representative sequence was selected for an assignment of the taxonomic composition using the Ribosomal Database Project (RDP) classifier (Wang et al. 2007). Then, the estimated species richness was identified by rarefaction analysis; the Chao1, abundance-based coverage estimator (ACE) and Shannon indices of diversity were determined for five libraries using methods described previously (Schloss et al. 2009).

The similarity between microbial communities of different samples was determined using UniFrac analysis. QIIME calculates both weighted and unweighted UniFrac. Principal coordinate analysis (PCoA) based on weighted UniFrac metric matrices was performed to explore the differences in bacterial and fungal community structures among all soil samples. Redundancy analysis (RDA) was performed using CANOCO4.5 for Windows to examine the relationships between the frequencies of abundant phyla, samples and selected soil variables (Etten 2005).

Data Analysis

Data were analyzed by analysis of variance (ANOVA), and when the ANOVA results were significant, the means for the control and for each mulch duration were compared using Fisher's least significant difference test, with significance at $p < 0.05$ ($LSD_{0.05}$).

RESULTS

Effects of Long-term Gravel Mulching on the Soil Physicochemical Properties

Table 1 summarizes the physicochemical parameters of the soils assessed in this study in 2014. Compared with the control, bulk density increased significantly in the A treatment. For all mulching durations, TOC, TON, and total P ranged from 4.94 to 6.19, 0.64 to 0.81, and 0.62 to 0.75 g kg^{-1} dry soil, respectively (Table 1). TOC in the C treatment was higher than in the control, but not significantly, and TOC was significantly lower in the A treatment. TON increased with the duration of mulching, and the difference became significant after 13 yr of mulching; however, TON decreased significantly after 18 yr of mulching.

Total P decreased with increasing duration of mulching, although the total P in the C treatment was significantly higher than in the control. MBC was lowest in the control and reached its maximum value in the B treatment, and MBC was markedly higher in the B and C

Table 1. Soil physicochemical properties in the field study conducted in Gaolan County.

Treat-ments	BD(g cm^{-3})	pH	Sand(%)	Clay(%)	Silt(%)	TO(g kg^{-1})	TON(g kg^{-1})	C: N	Total P (g kg^{-1})	MBC	MBN
A	1.52a	8.14a	78.31c	17.97c	3.72b	4.94b	0.64c	7.72c	0.62b	42.92c	9.62bc
B	1.44b	8.12a	75.79b	20.57b	3.64b	6.08a	0.81a	7.51a	0.66b	52.25a	13.39a
C	1.41b	8.11a	73.98a	22.61a	3.41b	6.19a	0.76ab	8.14b	0.75a	45.29b	11.33ab
Control	1.37b	8.21b	72.93a	22.48a	4.59a	6.15a	0.72b	8.54b	0.63b	41.45c	7.88c

BD, bulk density; TOC, total organic carbon; TON, total organic nitrogen; MBC, microbial biomass carbon; MBN, microbial biomass nitrogen

Values of a parameter followed by different letters differ significantly between treatments (ANOVA followed by Fisher's least significant difference test, $p < 0.05$).

Control = cropland without mulch; A = cropland with 18 yr of gravel-sand mulch; B = cropland with 13 yr of gravel-sand mulch; C = cropland with 9 yr of gravel-sand mulch

treatments than in the control. MBN was dramatically increased in the B and C treatments than in the control, but decreased greatly (to a level not significantly different from that in the control) in the A treatment. The C:N ratio increased significantly in the A and B treatments; however, the ratios were all significantly lower than in the control.

Microbial Community Analysis

There were 635,924 valid reads from 12 samples after filtering low-quality reads and chimeras and trimming the adapters, barcodes, and primers. All of the valid reads were classified from phylum to genus according to QIIME using default settings. Table 2 shows the number of operational taxonomic units (OTUs) and diversity indices in the different treatments and that the OTUs in the gravel mulch treatments (A, B and C treatment) were higher than those in the control. The non-parametric richness indices of Chao1 and Ace, evaluated at 3% dissimilarity, showed similar comparative values for each treatment. The following trend was found in terms of the Shannon index: C > B > Control > A.

The taxonomic distribution at the phylum level is summarized in Fig. 1. *Proteobacteria* was the most abundant phylum in all samples, accounting for 26.69–32.57% of the total valid reads, with an average relative abundance of 29.72%. *Acidobacteria* was the second most abundant phylum in all samples, with an average relative abundance of 17.43%. The other dominant phyla were *Gemmatimonadetes* (12.11–17.39%, averaging at 15.60%), *Actinobacteria* (6.73–29.26%, averaging at 13.43%), *Bacteroidetes* (5.64–11.19%, averaging at 7.69%), *Firmicutes* (3.78–5.29%, averaging at 4.42%), and *Chloroflexi* (2.75–4.91 %, averaging at 3.48%).

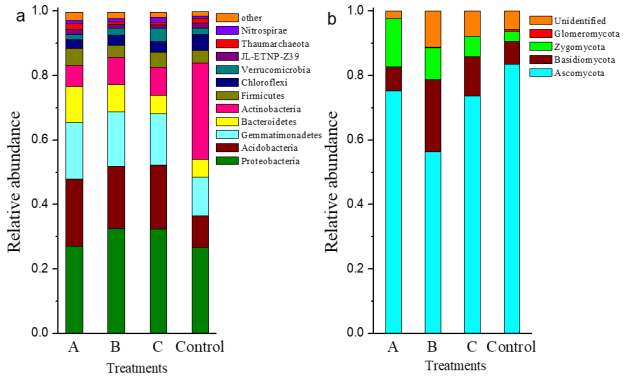

Fig. 1. Relative abundance of bacterial phyla (**a**) and fungal phyla (**b**) in the different treatments. "Other" indicates phyla with an extremely low abundance, including *Armatimonadetes*, *BHI80-139*, *Chlamydiae*, *Chlorobi*, *Cyanobacteria*, *Deferribacteres*, *Deinococcus-Thermus*, *Elusimicrobia*, *Euryarchaeota*, *Fibrobacteres*, *Fusobacteria*, *OD1*, *Planctomycetes*, *SM2F11*, *TM6*, *Thermotogae*, *TM7*, *WCHB1-60* and *WS3*.

Table 2. Number of operational taxonomic units (OTUs) and diversity indices.

Treatments	OTUs (97%)	ACE	Chao1	Shannon
A	1889	1909	1918	9.42
B	1922	1962	1972	9.50
C	1915	1941	1966	9.54
Control	1871	1903	1916	9.43

ACE: abundance-based coverage estimator

The most abundant genera within different samples were determined as shown in Fig. 2. The most abundant genera included *Sphingomonas*, *Haliangium* and *Bryobacter*, which were dominant in all four treatments, with average relative abundances greater than 1%. Other genera, including *Bacillus*, *Arthrobacter*, *Nitrospira*, *Gemmatimonas*, *Desulfovibrio*, *Escherichia-Shigella*, *Nordella*, *Skermanella*, *Rhodobium*, *Solirubrobacter*, *Blastocatella*, *Bacteroides*, *Gaiella*, *Woodsholea*, and *Flavisolibacter*, were detected in all samples and were dominant in several samples. Furthermore, the duration of gravel mulching enriched the genera *Blastocatella*, *Alloprevotella*, *Desulfovibrio*, *Bacteroides*, *Incertae_Sedis*, *Parabacteroides*,

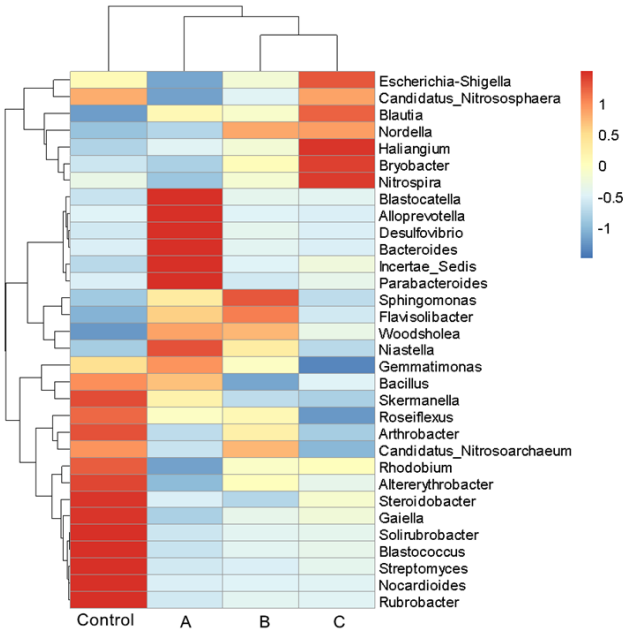

Fig. 2. Heatmap analysis of the distribution of dominant genera in the four samples. Relative values (0–1) for the microbial genera are depicted by color intensity. Abundance is expressed as the value of the target sequences relative to the total high-quality sequences from each soil sample.

Niastella, *Gemmatimonas*, *Bacillus*, *Woodsholea* and *Skermanella* compared with the control.

Microbial Community Structure and RDA Analysis

The similarities among the microbial communities in the four treatments were evaluated using cluster analysis. As shown in Fig. 2, cluster analysis revealed that the bacterial communities could be clustered into three groups: Group I, which contains samples C and B, and Groups II and III,

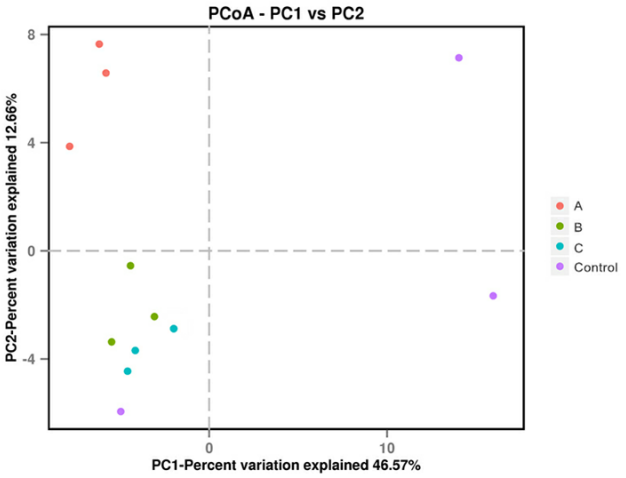

Fig. 3. Principal coordinate analysis (PCoA) plot based on the 16S rRNA sequencing genes from four treatments. The scatter plot shows principal coordinate 1 (PC1) vs. principal coordinate 2 (PC2). The percentages are the percentage of variation explained by the components.

which contain only one sample each, designated as treatment A and the control, respectively. Principal coordinate analysis (PCoA) clearly demonstrated that the soil microbial community varied between the different treatments (Fig. 3). The control was separated distinctly from the A, B and C treatments along with the first component (PCoA1) and second component (PCoA2). This pattern indicates that the microbial community structure is correlated with the duration of gravel mulching.

A correlation between the microbial community and selected soil physicochemical properties was discerned by RDA analysis (Fig. 4). As such, the MBC, MBN, TOC, and bulk density (BD) concentrations appear to be the most important parameters. As shown in Fig. 4, the bacterial genera in the control were dominated by *Bacillus*, *Patulibacter*, *Streptomyces*, *Solirubrobacter*, *Nocardioides*, *Rubrobacter*, *Gaiella*, and *Euzebya*, and were related to the soil C/N ratio and TOC content. The bacterial genera in the A treatment were dominated by *Incertae_Sedis*, *Blastocatella*, *Desulfovibrio*, *Bacteroides*, *Gemmatimonas*, *Parabacteroides* and *Alloprevotella* and were related to the soil pH and BD. The bacterial genera in the B treatment were dominated by *Flavisolibacter*, *Sphingomonas* and *Haliangium* and were related to the soil pH, BD, MBC and MBN, while the bacterial genera in the C treatment were dominated by *Arthrobacter*, *Nitrospira* and *Bryobacter* and were related to TOC, TON and the total P content.

DISCUSSION

The microbiological and biochemical status of a soil has often been proposed as an early and sensitive indicator of soil ecological stress and the success of restoration processes in both natural and agricultural ecosystems. This study is the first work providing information on the soil microbial community as affected by gravel mulch using the MiSeq platform of next-generation sequencing.

Based on the results of our pilot experiment, the soil bulk density increased significantly after 18 yr of mulching and TOC decreased with increasing duration of mulching. However, the total P, MBC, and MBN decreased significantly after 18 yr of mulching. The high bulk density of the soil caused by long-term gravel mulching would lead to a negative effect on microbiological properties by degrading microbial habitats as a result of poor aggregate formation of coarser particles (Udawatta et al. 2008). This deterioration in soil quality after long-term gravel mulching may also be due to lack of input of organic matter, because most of the

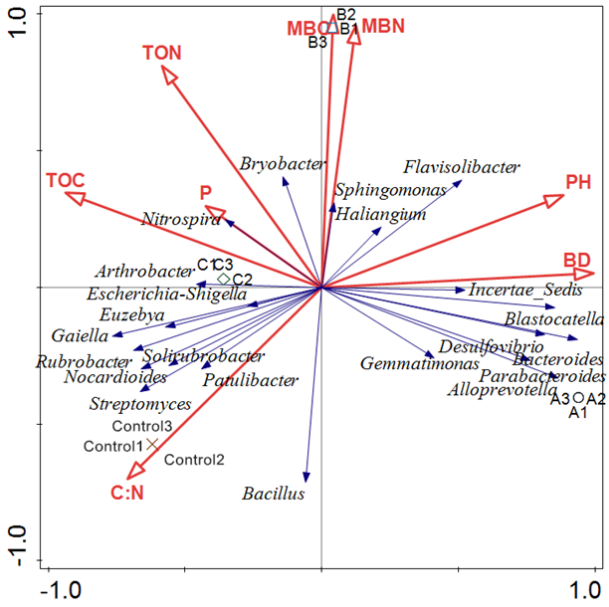

Fig. 4. Redundancy analysis (RDA) based on the 16S rRNA gene data and selected soil physicochemical properties in the different treatments.

aboveground biomass was removed during harvest and the gravel mulch layer would prevent leaves or stem material from reaching the surface soil. The positive effects of the gravel mulch on MBC and MBN could be due to the capability of mulching to increase soil temperature, which has been reported in previous investigations at our study site (Wang et al. 2008). However, after 18 yr of mulching, soil MBC and MBN decreased significantly to the same level as in the control. This result agrees with that of Wang et al. (2010), who reported that the soil temperature began to decrease over time due to mixing of the gravel mulch layer with the topsoil after long-term tillage. The increased MBC and MBN in the mulched treatments may also have resulted from increased soil moisture in the mulched soils (Ma and Li 2011). Soil water content is an important factor limiting microbial activity. Since lots of soil microorganisms require an aquatic environment to perform their life processes and are largely dependent on water availability, microbial biomass and activity declined as a consequence of water deficiency (Drenovsky et al. 2004; Uhlírová et al. 2005).

In the present study, the results of PCoA showed that the soil bacterial and fungal community structures observed under treatment with gravel mulch differed from those found in the control. The microbial community structure observed in the A treatment differed from that in the B or C treatment, indicating that the duration of gravel mulching also correlates with changes in community structure (A, B and C were cropland with 18, 13 and 9 yr of gravel-sand mulch, respectively. The control was cropland without mulch). The number of OTUs in the gravel mulch treatments (A, B and C treatments) was higher than that in the control, and the richness (diversity) indices Ace, Chao1 and Shannon showed a similar trend with respect to mulching: C > B > Control > A. The higher OTUs and diversity indices obtained in the B and C treatments demonstrated that gravel mulch has a positive effect on the growth of soil microorganisms, which could be due to the capability of mulching to increase the soil temperature (Wang et al. 2008). However, after 18 yr of mulching (A treatment), the OTUs and diversity indices decreased significantly, to a level lower than that in the control treatment, which indicates a negative effect on the growth of soil microorganisms that could be due to the significant decrease in temperature observed after long-term mulching (Wang et al. 2010). This change in soil microorganisms may be an important reason for the decrease in watermelon yields after long-term gravel mulching. The watermelon yields at the study site were highest after 5 yr of mulching (26 267.33 kg ha^{-1}), and decreased gradually with increasing mulching duration; the yield after 15 yr of mulching was 19.9% lower than the yield after 5 yr of mulching (Liang et al. 2011).

Phyla abundance analysis revealed that *Proteobacteria, Acidobacteria, Gemmatimonadetes*, *Actinobacteria, Bacteroidetes*, and *Firmicutes* were the most abundant bacterial phyla in all of the soil samples, with some variation in their relative abundance. This finding roughly corresponds to the results of previous studies which investigated agricultural or other types of soils in which these phyla accounted for most of the sequences examined using deep 16S rRNA pyrosequencing or Illumina MiSeq sequencing (Caporaso et al. 2011; Shen et al. 2015). The observed greater abundance of *Proteobacteria*, *Gemmatimonadetes*, and *Bacteroidetes* may have resulted from an enhanced cycling of essential elements, which may improve soil fertility and plant growth efficiency (Lesaulnier et al. 2008). The greater abundance of *Acidobacteria* could be explained by the recent results of Jones et al. (2009), which demonstrated that pH is strongly correlated with the abundance of *Acidobacteria*. In concordance with the conclusion of Jones et al. we found that *Acidobacteria* was less in the control compared with the gravel mulch treatments. This result may be correlated with higher soil pH in the control compared with the other treatments. *Bacteriodetes* in agricultural systems can rapidly exploit bioavailable organic matter and colonize aggregates, which are beneficial for crops (Abell and Bowman 2005). Moreover, *Ascomycota* and *Basidiomycota* were the most abundant fungal phyla in all of the soil samples, indicating agreement with previous studies that utilized the Illumina sequencing platform to show that *Ascomycota* and *Basidiomycota* are the most abundant phyla, accounting for more than 60% of the total sequences in soil samples (McGuire et al. 2013; Schmidt et al. 2013).

After long-term gravel mulching, the soils were found to have a greater abundance of *Acidobacteria, Gemmatimonadetes*, *Bacteroidetes*, and *Firmicutes* and reduced abundance of *Actinobacteria* compared with the control soils. The greater abundance of *Firmicutes* in gravel mulch treatments was significantly correlated with mineralizing the organic matter, which promotes nutrient absorption and plant growth (Goldfarb et al. 2011). Genera abundant analysis showed that gravel mulch enriched the quantities of genera *Haliangium, Blastocatella, Woodsholea, Flavisolibacter* and reduced the quantities of genera *Bacillus, Arthrobacter, Skermanella, Solirubrobacter, Rhodobium* and *Gaiella* compared with the control. Moreover, the duration of gravel mulch enriched the quantities of genera *Blastocatella, Alloprevotella, Desulfovibrio, Bacteroides, Incertae_Sedis, Parabacteroides, Niastella, Gemmatimonas, Bacillus, Woodsholea* and *Skermanella*. Besides, *Ascomycota* and *Basidiomycota* were the

most abundant fungal phyla in all of the soil samples, accounting for more than 70% of the total sequences in soil samples. Phyla abundant analysis (Fig. 1) showed that long-term gravel mulching enriched the quantities of phyla *Basidiomycota and Zygomycota* and reduced the quantities of phyla *Ascomycota* and *Glomeromycota* compared with the control.

Soil bacterial communities respond quickly to environmental changes (Rinnan et al. 2007). Our RDA analysis showed that the diversity of soil bacteria is significantly correlated with some soil physiochemical properties. The MBC, MBN, TOC, and BD concentrations appear to be the most important parameters. The gravel mulch treatments were all related to soil pH, BD, MBC and MBN. Therefore, the variation of BD, MBC, and TOC were the main causes of the changes in soil microorganism composition. Dick et al. (1988) reported that the immediate causes of decreased nutrient availability in soils with high bulk density were changes in the microbial biomass caused by deterioration of the microenvironment in the compacted soil. In addition, Udawatta et al. (2008) reported that soil compaction negatively affected soil microbiological properties by degrading microbial habitats as a result of poor aggregate formation by coarser particles. The variation of BD caused by long-term gravel mulching may therefore change soil microorganism composition by decreasing soil permeability to air and water and as a result of poor aggregation. Organic carbon availability is a major determinant of soil microbial community composition (Drenovsky et al. 2004). The organic matter would provide a substrate for soil microorganisms, and would therefore contribute to MBC accumulation in the soil. The data of Kautz et al. (2004) suggest that the change in soil microorganism composition after long-term mulching probably depends on a lack of available substrates and nutrients for soil microbes.

CONCLUSION

Our results demonstrate that the application of gravel mulch can significantly alter soil properties and soil microbial communities: (1) mulching generally increased TON, MBC, and MBN during the first 13 yr, but all of these indicators declined considerably after 18 yr of mulching; (2) 18 yr of mulching increased the soil bulk density and sand content, which led to degradation of the soil microenvironment; (3) gravel mulch significantly altered the microbial structure and composition, i.e., after long-term gravel mulching, the soils were found to result in increasing the abundance of *Acidobacteria*, *Gemmatimonadetes*, *Bacteroidetes*, and *Firmicutes* and reducing the abundance of *Actinobacteria* compared with the soils from the control treatment; and (4) the richness indices of Chao1, ACE and Shannon, evaluated at 3% dissimilarity, showed a similar trend of C > B > Control > A, which demonstrated the negative effects on soil microorganisms after 18 yr of gravel mulching.

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