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Effects of Biodegradable Film Mulching on Bacterial Diversity in Soils

Yinhao Xue
Shenyang Agricultural University; Rural Energy and Environment Agency, Ministry of Agriculture and Rural Affairs
Tuo Jin
Rural Energy and Environment Agency, Ministry of Agriculture and Rural Affairs
Chengyu Gao
Lanzhou University
Chongxiao Li
Agricultural ecology and resource protection technology extension station of Gansu province Lanzhou
Tao Zhou
Agricultural ecology and resource protection technology extension station of Gansu province Lanzhou
Dongshi Wan
Lanzhou University
Mengran Yang (1504526812@qq.com)
State Key Laboratory of Grassland Agro-Ecosystem, School of Life Sciences, Lanzhou University, Lanzhou, 730000, Gansu, P. R. China https://orcid.org/0000-0003-3771-8748

Research Article

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Abstract

The spread of biodegradable plastic film (BDFs) not only increase grain yield but also reduce the environmental pollution from plastic film to a large extent. Soil microbes have been considered to be involved in biodegradation processes. However, the study of microbes diversity in soil mulched with biodegradable plastic film remains limited. Here, we compared the diversity of microbes between soils with biodegradable film and non-biodegradable film (NBDF) mulch. The results showed that BDFs affected on the soil quality parameters, such as total C, P and NH_4^+-N, but also on the microbes species richness (ACE; Chao1) and diversity (Simpson index; Shannon index). In terms of dominant phyla and genera, BDFs and NBDF can influence the abundance of disparate species. Furthermore, BDFs could also contribute to improve the richness of the important functional bacterial groups in soil, e.g. *Pedomicrobium* and *Comamonas*, both of which have been found to be involved in the degradation of plastic residues in soil. Finally, we found that BDFs improved the transformation of nitrogen through increased significantly the abundances of Nitrobacter and Nitrospira. Our results highlight the impact of BDFs mulch on the abundance of functional bacteria in the soil.

1. Introduction

With the increasing of human population, the yield and quality of grain have become the most serious problems. The emergence of plastic film mulching (PFM) has solved a problem, as precipitation and other water resources are used more efficiently to attain the required food production. PFM is primarily used to protect shoots and seedlings and maintain or increase soil humidity and temperature through insulation and evaporation prevention (Steinmetz et al., 2016). Furthermore, PFM may increase yields; extend the growing season; conserve soil moisture and water-use efficiency; increase fertilizer use efficiency; prevent soil erosion with weed growth; and reduce weed pressure, as well as consequently reduce herbicide and fertilizer use. In recent years, the usage of PFM has been increasing in worldwide (Cuello et al., 2015). For example, in China, about 14.7×10^5 tons PFM have been used on crops (Gao et al., 2019), most of which is distributed in 19.8 million hectares of agricultural land, while in Europe, areas of PMF cover approximately 162,000 ha (Briassoulis and Dejean, 2010), and in the U.S.A, only in vegetable production, more than 130,000 metric tons of plastics are used (LI et al., 2014). However, PFM have improved crop yield significantly and solved greatly the food crisis, but also results in a series of issues as well. For example, residues of plastics in the soil have become one of the major environmental pollution sources. The residues include microplastics, phthalates, and agrochemicals, all of which have been found to lead to substantial amounts of plastic waste residue accumulation and may possibly liberate toxic additives into the soil (Steinmetz et al., 2016) and promote soil water repellency and soil degradation. At present, the residues from PFM in the soil are also a serious problem, and burning and disposing of PFM in open fields or landfills are common. Although PFM is legal, it has been repeatedly banned due to its simplicity and low operating cost, which has a huge negative impact on product quality, human health and the environment. Therefore, whether BDFs can be used as an alternative to NBDF has been a hot topic.

Biodegradable plastic mulch (BPM), synthesized first in the mid-1970s (Briassoulis and Dejean, 2010; LI et al., 2014), consists of either BDFs plastics (i.e., plastics produced from fossil materials) or biobased plastics (i.e., plastics synthesized from biomass or renewable resources) (Tokiwa et al., 2009) and mainly indicate the instead of traditional geoponic PFM, which can't be recovered from the soil for financial or technical reasons. BPM primarily consists of biobased plastics that will biodegrade after the end of their effective process without leaving toxic and polluting remains in the soil (Li et al. 2014). The advantages of BDFs are as follows: improved tensile strength, flexibility, and controllability of rupture and degradation; pollution free; enhanced ability to increase the soil temperature and preserve the soil moisture and satisfy the demand of crop production with mulching (Yan et al., 2016). However, some shortcomings of BDFs are also exposed as the service time increases. For instance, there is perceived uncertainty about the weak market transparency, product properties, a lack of uniformity in the product, and a discouraging cost of biodegradable plastic mulch compared to conventional mulch in certain applications (Briassoulis and Dejean 2010). For some reason,

bewilderment still existence regarding the capability of these materials under real soil conditions. Studies show the incomplete breakdown of BDFs could lead to an accumulation of plastic fragments and particulates in soils (Sintim and Flury, 2017). Therefore, whether BDFs may become a desirable alternative to traditional PFM with solve agricultural plastic problems remains a question.

Microbes are an important part of soil, and their diversity and function, i.e., their abundance and activity, as well as the community structure (Puglisi et al., 2012), may act as a prime indicator of soil fertility and quality. Microbes are sensitive to soil contaminants, their activity and composition are the primary biological indicators of alters in the soil environment, as they play a key role in carbon, phosphorus, nitrogen, and potassium cycling in the soil (Qian et al., 2018). Recent reports have found that PFM can also influence the activities and composition of soil microbes (Cook et al., 2006; Subrahmaniyan et al., 2006). Some bacterial strains in the soil have been found to be involved in microplastic degradation. However, biodegradable plastic mulches are now widely used and can be amalgamated into the soil at the end of the crop season and decomposed by microbes, such as bacteria, fungi and algae, eventually released into the soil in the form of CO_2 and H_2O (Ashley and Jo, 2012). Thus, the abundance and activity of soil microbes may be change along with the disposal of BDFs.

In this study, we evaluated the effects on microbes diversity, abundance in soils mulched by three potentially biodegradable films (BDFs: BDF1, BDF2, BDF3) and one nonbiodegradable films (NBDF, also named polyethylene film), respectively. We also quantified the abundance of some functional microbes that can improve soil fertility as well as soil properties, or accelerate degradation of the BDFs. Our results highlight the effect of the BDFs mulch on soil microbes diversity that might be involved in the degradation of BDFs.

2. Materials And Methods

2.1 Sampling location and collection

The sampling site is located in the potato planting demonstration area of Shandan County (38°47'1.90"N, 101°05'11.33E, Gansu Province, China), where potatoes are cultivated in double ridges-furrow with different mulches. Five soil samples (CK, NBDF, BDFs, including BDF1, BDF2, BDF3) were collected using a soil columnar sampler. CK refers to soil without plastic mulches, NBDF samples were covered with nonbiodegradable mulches (polyethylene), and three BDFs samples present the three potentially biodegradable mulches which are different in thickness, color and manufacturer. Furthermore, among the five sampling locations, there was no significant difference in field management measures such as watering and fertilization. In this study, the PE film is white and 0.008 mm thickness. Three biodegradable plastic films used in this study are BDF1 (Black, 0.008 mm thickness), BDF2 (White, 0.008 mm thickness) and BDF3 (Black, 0.008 mm thickness, manufactured by Xinjiang), respectively. Starch is the main synthetic raw material of these degradable membranes, which have the characteristics of large tensile strength, could be degraded into H_2O and CO_2 by microbes in soil, and absolutely friendly to the environment.

The soil samples were evenly collected from the 15-20cm above the planting ridge, where the BDFs has cracked and some of them began to degrade, while BNDF is intact. Each sample was collected from at least 5 points and three biological replicates for the data statistics. The collected samples were packed and transported back to the laboratory at 4°C using for soil DNA extraction and other analysis. All of the samples were stored at -20°C.

2.2 Soil property measurements

The soil samples using for soil property determination were dried in air. Conspicuous plants, stones and other substances were removed from the soil samples, and transported back to a clean and ventilated indoor environment with dehumidified air after the soil samples were collected. For the soil property analyses, the soil samples were sieved (to five

mm and two mm) and stored at 4°C until analyses were performed within the next two to three weeks (Acosta-Martínez et al., 2011). Two copies of 5 mm screened soil samples, each 200g, were ground fine, and 1 part was thoroughly processed at 2 mm for soil pH determination (DELTA320 pH). The rapid determination of total N in the soil was performed by an automatic Kjeldahl apparatus (DDY9820). The NH_4^+-N (indophenol blue colorimetry), NO_3^--N (colorimetry of phenoldisulfonic acid) and total P (digestion colorimetry) were determined from air-dried soil by spectrophotometry. The organic C was determined by TOC-V (series SSM-5000A) solid sample measurement. The conductivity was determined by a conductometer.

2.3 DNA extraction from the soil samples and polymerase chain reaction (PCR) amplification

DNA was isolated from at least 2 g of mixed soil using a MoBio High-throughput PowerSoil DNA Isolation Kit (Young et al., 2015) and purified on agarose gels. The 16S rRNA gene fragment (V3-V4) of the appropriate size and sequence was amplified using universal primers 1492R (5'-GGTTACCTTGTTACGACTT-3') and 27F (5'-AGAGTTTGATCCTGGCTCAG-3') (Krumins et al., 2009). The PCR conditions were as follows: 94°C for 5 min; then 25 cycles of 45 s of denaturation at 94°C, 60 s of annealing at 55°C, and 60 s of extension at 72°C; followed by 10 min at 72°C (Sharma et al., 2009; Sabine and Ralf, 1997).

2.4 16S rRNA gene fragment analysis

The purified PCR products were sequenced by the Illumina HiSeq2500 platform at Novogene Technologies Corporation (Beijing, China). The high-quality reads within 97% similarity were clustered into operational taxonomic units (OTUs) using UCLUST (Edgar, 2010) in QIIME (version 1.8.0) (Caporaso et al., 2010). The bioinformatics analysis, including the creation of Venn diagrams (Chen and Boutros, 2011) and calculation of alpha diversity indexes (Grice et al., 2009), were performed on the biocloud platform (www.biocloud.org). Species richness was prognosis by utilize the Chao 1 and ACE methods, while quantitative species richness or evenness was prognosis by nonparametric Simpson and Shannon's indexes in Mothur (Version v.1.30). We created UPGMA dendrogram (an unweighted pair group method with arithmetic mean) using Fast UniFrac analysis (Amin et al., 2017). The UPGMA was used to analyze the correlations between the five samples.

2.5 Determining the abundance of soil Nitrobacter and Nitrospira

The total DNA was extracted from the soil samples using a DNeasy Power Soil kit (Qiagen GmbH, Germany). The nitrifier communities from all the soil samples were examined by qRT-PCR-related analyses using specific primers (S4). The number of nitrifying bacteria in the samples was analyzed based on the data from the different fragments obtained from specific amplifications (Li et al., 2017).

2.6 Data statistics

A two-sided p value < 0.05 was considered statistically significant; the confidence intervals were set at 95%. Statistical analyses were performed using SPSS 19.0 software. Normally distributed continuous variables are expressed as the means ± standard errors of the mean (SEM), and non-normally distributed continuous variables are expressed as the median (interquartile range). One-way analysis of variance was used to compare multiple groups.

3. Results

3.1 Soil properties

In order to evaluate the effects of BDFs and NBDF on soil environmental factors, the relevant parameters were determined. Organic C value was significantly different (P = 0.005 < 0.05) between BDFs and NBDF. Total P value was

significantly different (P = 0.045 < 0.05) between BDFs with NBDF. As well as, pH, total N, NH_4^+-N, NO_3^--N and conductivity were no significantly different among CK, BDFs, NBDF (P = 0.294 > 0.05, P = 0.344 > 0.05, P = 0.294 > 0.05, P = 0.339 > 0.05, and P = 0.119 > 0.05, respectively). Results suggesting that the presence or absence of mulch does not affect these factors in the soil, nevertheless, some variation of soil factors was obviously between BDFs and NBDF. Conductivity was significantly different between BDF3 and NBDF (Duncan's test). The pH of all samples was slightly alkaline; the quantitative of total N was higher in NBDF (1.94 ± 0.81) than BDFs. However, the maximum values of NH_4^+-N and NO_3^--N were found in BDFs (BDF2 83.87 ± 51.69; BDF3, 4.06 ± 1.28) (Table 1). The BDFs samples showed significant differences in NO_3^--N and conductivity. In addition, different changes exist in BDFs for soil physical and chemical factors. Total P and organic C were higher in BDFs than CK, and the NBDF has the maximum value of total P and conductivity and the minimum value of organic C. In other words, PFM (NBDF and BDFs) can change the value (rise or fall) of physical and chemical factors in the soil. There is no obvious rule in two types mulch, so it is impossible to distinguish which type of mulch is more suitable for soil planting.

Table1 Selected soil properties under different the PFM and CK

Samples	Soil pH	Total N (g/kg±SD)	NH_4^+-N (mg/kg±SD)	NO_3^--N (mg/kg±SD)	Total P (g/kg±SD)	Organic C (g/kg±SD)	Conductivity (us/cm±SD)
CK	8.34±0.32	1.05±0.39	3.23±0.60	59.09±5.30	2.02±0.35*	8.98±0.57	37.87±5.46
NBDF	8.15±0.10	1.94±0.81	3.41±0.41	70.93±11.60	2.60±0.43	8.28±0.68	49.93±3.33
BDF1	8.27±0.07	0.80±0.57	3.47±0.22	47.68±10.01	2.09±0.23*	9.77±0.63*	34.50±12.41
BDF2	8.32±0.02	0.55±0.24	4.06±1.28	46.61±2.93	2.27±0.24	8.77±0.41	31.63±3.92*
BDF3	8.19±0.10	1.22±0.74	2.73±0.44	83.87±51.69	2.15±0.37*	9.33±0.86*	45.00±12.10

Table 1 Selected soil properties under the different PFM treatments and the CK.

The means (±1 standard deviation) of the different soil properties (including soil pH, total N, NH_4^+-N, NO_3^--N, total P, organic C and conductivity) in the soil of the PFM treatments and the CK.

3.2 Diversity of bacterial

A total of 782586 clean tags were obtained for all samples after removing the low-quality and chimeric tags. Approximately 50% of the effective tags are paired-end reads (Table S1). At the 97% OTU level, the quality tags can be distributed into 1655 OTUs, consisting of 437 microorganism genera in 28 phyla (Table S2; Fig. 1) (Schneider et al., 2015). CK lacks one phylum compared with those in other samples, with the least number of genera. There were 1555 OTUs shared as a core group of all samples, and, NBDF had no unique OTUs (Fig. 2). The data suggesting that high numbers of microbes species were found in all samples. For the five samples bacterial communities, the CK had the lowest ACE (1609) and Chao 1 (1617) indexes (Table S3), and BDFs (BDF3) had the highest species richness (ACE 1637 and Chao1 1644). BDFs (except BDF2) had higher ACE and Chao 1 indexes than NBDF. The BDF3 showed the richest species diversity (Simpson index: 0.0034 and Shannon index: 6.44), CK had the lowest species diversity (Simpson index: 0.0045 and Shannon index: 6.23). Each index of the NBDF sample was slightly different than BDFs, with no significant

differences. The results suggested that PFM is beneficial for increasing the richness of the soil microbial community and improving the diversity and richness of soil microbial species. In addition, the values of microbial species diversity and richness from high to low are as follows: BDFs, NBDF and CK.

3.3 Correlations of the bacterial taxa

The unweighted pair group method with arithmetic mean (UPGMA) (Sui et al., 2013) was used to conduct hierarchical clustering of the samples in R to judge the similarity of the species compositions among all samples. When the samples from the different sites were compared at the 95% confidence level, the meaningful values ranged between 0.021 and 0.038 (Fig. 4). The UPGMA dendrogram created based on the UniFrac distance matrix exhibited three major clades (Fig. 3). The sample CK formed one clade relatively independent from the other samples, and it also has the farthest genetic distance from the other four samples. The BDFs clade was basically identical to the NBDF clade, and there was little genetic distance between BDF2 and NBDF. NBDF and BDFs samples were different with CK. In three clades, BDFs (BDF1 and BDF3) formed a single closed, independent cluster; among all samples, the highest similarity was found between BDF1 and BDF3.

As a whole, the reciprocal similarity of the three BDFs samples was the highest; while that of the CK sample was the lowest. There were obvious effects of mulching on soil microbial species composition and the similarity distances of the CK, NBDF and BDFs ranged from far to near.

3.4 Bacterial community composition

3.4.1 Abundance of phyla

Amplicon sequencing of bacterial 16S rRNA genes (V3-V4 region) was used to assess the influence of applying four different films on the soil bacterial community (Dawson et al., 2017). The five soil samples were dominated by bacteria belonging to the phyla Proteobacteria (Batzke et al., 2007), Acidobacteria, Bacteroidetes, Gemmatimonadetes, Chloroflexi, Verrucomicrobia, Nitrospirae, Armatimonadetes and Fusobacteria, with each of these phyla constituting more than 0.1% of the relative abundance of the detected 16S rRNA genes (Fig. 5a).

The dominant phylum Proteobacteria was the most abundant in BDFs (BDF2: 36.25%, BDF3: 36.96%), followed by the NBDF sample (32.13%) and CK (31.05%). The abundance of Acidobacteria in four film mulching (NBDF: 20.85%, BDF1: 22.30%, BDF2: 20.57%, and BDF3: 17.44%) were lower than CK (24.10%). The species abundance of most dominant bacterial phyla Verrucomicrobia and Armatimonadetes were slightly higher in BDFs than CK. There was no significant difference in species abundance between the NBDF and BDFs, for example, the Gemmatimonadetes (NBDF: 11.66%, BDF1: 10.90%, BDF2: 9.62%, and BDF3: 9.52%) and Chloroflexi (NBDF: 6.47%, BDF1: 6.57%, BDF2: 6.15%, and BDF3: 5.71%).

However, there was a slight difference of species abundance among the three BDFs treatments, the highest species abundance of Proteobacteria (36.96%) and Bacteroidetes (20.33%) in BDF3, as well as, the Chloroflexi (6.57%) in BDF1. The abundances of species in the following phyla increased in NBDF relative to the CK: Gemmatimonadetes (from 10.06% in CK to 11.66% in NBDF), Chloroflexi (from 6.09–6.47%), Actinobacteria (from 3.82–3.90%), Verrucomicrobia (from 1.22–1.62%), and Nitrospirae (from 0.55–0.91%). The exception was Bacteroidetes, which decreased from 20.12% in the CK sample to 18.76% in the NBDF sample. Mulching (BDFs, NBDF) changed the abundance of the different species in the CK soil, especially the dominant phyla.

In addition, Proteobacteria, Acidobacteria and Bacteroidetes are the most common dominant bacterial phyla in all samples. It is interesting to note that these three kinds of bacteria showed different states in CK compared with the samples from the four mulching treatments. The lowest difference abundances of different species between the samples

was found in the Proteobacteria, the highest difference was found in the Acidobacteria, and almost no difference was found in the Bacteroidetes. In NBDF, the species abundances of the three phyla showed an increasing trend compared with BDF1 and BDF2. The species abundances of the Proteobacteria and Bacteroidetes was higher in BDF3 than the other two BDFs treatments. There was no significant difference in species abundances of the three dominant bacteria in all samples, and the species abundance value was between the lowest and the highest. Gemmatimonadetes and Nitrospirae were two phyla that can play an important role in soils. The species abundances of Gemmatimonadetes and Nitrospirae were higher in NBDF than other samples, and their abundances in CK were between the NBDF and BDFs. In addition, the abundances of species from these two important phyla in BDF2 and BDF3 were lower than that in BDF1.

3.4.2 Abundance of genera

In this study, the top twenty different taxonomic genera were examined from the five samples. The five dominant genera were *uncultured_bacterium_c_Subgroup_6* (from 5.58–7.95%), *uncultured_bacterium_f_Gemmatimonadaceae* (from 4.43–6.01%), *uncultured_bacterium_f_Saprospiraceae* (from 3.89–4.84%), *uncultured_bacterium_o_Subgroup_7* (from 2.27–4.18%) and *Sphingomonas* (from 2.53–3.62%), which were common in all the samples (Fig. 5b).

Dominant bacterial genera were generally more abundant in CK than NBDF and BDFs, while the functional genera (*Sphingomonas*, *Haliangium, Lysobacter, Arenimonas*) were generally lower in CK than in NBDF and BDFs. There was no significant difference in species abundances between the NBDF and BDFs. The abundances of *uncultured_bacterium_f_Gemmatimonadaceae*, *Haliangium* and *Lysobacter* were the highest in BDF1, BDF2 and BDF3 had the highest species abundances of *Sphingomonas* and *Arenimonas*, respectively. It is worth noting that in NBDF, there are two functional genera, *Uncultured_bacterium_f_Gemmatimonadaceae* and *Arenimonas*, with maximum values, and one group that reached the lowest value (*Sphingomonas*).

In addition, the species abundances of the other four important functional bacterial genera in CK was lower than that in all plastic film treatments (except for *Lysobacter*). Interestingly, the increases or decreases in relative abundance were driven no only by the normalized sequence counts of abundant OTUs (≥3% of the relative abundance per OTU) (Qian et al., 2018) but also by the normalized sequence counts of the low-abundance OTUs (<3% relative abundance per OTU). For example, this pattern is seen in the relative abundances of the low-abundance OTUs in the genera *uncultured_bacterium_c_S0134_terrestrial_group* (1.41% - 2.06%), *uncultured_bacterium_f_Blastocatellaceae* (1.21% - 2.53%), *Arenimonas* (1.25% - 2.51%), *Bryobacter* (1.01% - 1.83%) and so on. The low-abundance genera account for approximately 45.28% of all the OTU sequences in the five samples. The results indicate that the abundance of species does not determine the importance of species in the population completely, and in most cases, it plays a role in regulating the population function.

3.5 Functional genera

The bacterial genera *Pedomicrobium* and *Comamonas*, which can participate in the degradation of soil plastics (Shah et al., 2008), were found in this study. Compared to CK and NBDF, the abundance of species from the *Pedomicrobium* in the BDFs have increased significantly, while between BDFs and NBDF, the abundance of *Pedomicrobium* in all the BDFs is higher than that in the NBDF (except BDF1 sample). In additional, there was no significant difference in the abundance of *Comamonas* species between the CK and NBDF samples; the abundance levels were almost uniform, although there were significant differences among BDFs, CK and NBDF. The abundances of *Comamonas* species were higher in all BDFs treatments (1, 2, 3) than CK and NBDF (Fig. 6). Both functional genera were found in the three kinds of samples (BDFs, NBDF, and CK), and the general trend was that species abundances were higher in the BDFs samples than CK and NBDF samples, and the abundances in NBDF were similar to CK samples. In general, the mulching of BDFs can change the abundance of species in the dominant genera relative to that in the CK and increase the abundance of species in the functional genera. In particular, this functional strain that can degrade microplastics in soil is one of the most important indicators for biodegradable membrane. *Pedomicrobium* and *Comamonas* existence in BDFs, it expresses the availability

and functionality of three kinds of biodegradable plastic films in this paper, and it is also an important basis for distinguishing non-degradable films.

3.6 The abundance of Nitrobacter and Nitrospira in soils

To investigate whether mulching with BDFs or NBDFs affected the functional microbial population in soils, we detected the number of nitrifying bacteria in the soil samples using qRT-PCR methods. The number of nitrifying bacteria is determined by the number of two important functional genes, which have been amplified by three pairs of specific primers. The results showed that the number of *nxrA* in the PFM (NBDF and BDFs) was significantly higher than that in the CK sample, and there were significant differences between the BDF1 and CK. The number of *nxrB* in the NBDF and BDFs samples (except BDF1) was higher than CK, and both the BDF2 and BDF3 were significantly different from CK. Therefore, compared with the CK sample, the number of *Nitrobacter* communities (*Nitrobacter nxrA* and *Nitrobacter nxrB*) was increased in the BDFs and NBDF samples. The content of *Nitrobacter* in the BDFs were higher than that in the NBDF sample. It can be found from the data that the number of *Nitrobacter* communities in the three treatment groups is gradually increased in the order of CK, NBDF and BDFs. This is an effective illustration of the advantages of mulching and the best use of biodegradable mulching

In addition, the number of *Nitrobacter nxrB* in the BDF1 sample was decreased compared with that in the CK and NBDF samples, which proved that there are some differences in *Nitrobacter* between the different BDFs. The number of *Nitrospira* communities was reduced almost to zero in the BDFs and NBDF samples (Fig. 7). Both *Nitrobacter* and *Nitrospira* were present in the CK sample, and the mulching of plastic films contributes to the rise of *Nitrobacter* and reduces the content of *Nitrospira*. In addition, the increase of *Nitrobacter* in response to the biodegradable film is greater than that in response to NBDF.

4. Discussion

4.1 BDFs mulch could improve the soil quality

Soil quality indicators can reflect changes in soil intuitively and effectively (Li et al., 2008). Soil pH affects the availability of nutrients, plant growth and microbial activity (Domagała-Świątkiewicz and Siwek, 2013). Here, in the soils mulched with different films, the pH was stable at 8.20 ± 0.14 (no significant difference), similar results have also found in term of the total P, NH_4^+-N, or NO_3^- -N. However, the content of total N, NO_3^- -N and total P in NBDF and BDF2 were higher than in that of CK. It is suggested that film mulch could improve the soil quality (Arutchelvi et al., 2008). PFM increases soil nutrients; for example, PFM maximizes soil moisture savings, protects soil nutrients from loss (Kader et al., 2017). The difference in organic C in each sample may be due to the activities of soil organisms, which influence C retention and, consequently, aggregation and soil structure (Domagała-Świątkiewicz and Siwek, 2013), or improve the ability of organic matter degradation by soil microorganism (Liu et al., 2012). Meanwhile, BDFs have a higher retention effect on soil organic C than NBDF, which had the maximum conductivity value. A possible explanation is the NBDF leaches toxic substances into the soil for a long time, leading to an increase in soil conductivity (Carteau et al., 2014). In addition, the differences among BDFs in thickness, color and composition, that all together could affect soil structure, quality and activity (Alkayssi and Alkaraghouli, 1991; Sun et al., 2015)

4.2 BDFs could increase the diversity and richness of functional microbes species in the soil

The plastic film mulch can lead to the change of soil properties, such as solar radiant intensity, temperature, humidity, and microbes (Fan and Liu, 2003; Yadav and Yadav, 2013). In term of microbes, which species diversity and richness in NBDF and BDFs soil samples were all higher than in CK. The more reasonable explanation is the NBDF residue period

increases phthalate concentrations, which leads to significant decreases in soil microbial carbon and nitrogen, enzyme activities and microbial diversity (Wang et al., 2016), while the biodegradable film can degrade in one year and its residue does not remain in the soil for a long time (Barragán et al., 2016).

Furthermore, the dominant bacterial phyla, such as Proteobacteria, Acidobacteria, Bacteroidetes and Gemmatimonadetes), and common bacterial phyla, including Actinobacteria, Chloroflexi, Verrucomicrobia and Nitrospirae (Farmer et al., 2016), were all found in our study. Among them, the abundance of the Proteobacteria was no significantly different among CK, BDFs and NBDF. Gemmatimonas has the beneficial effect of fixing atmospheric nitrogen under low oxygen pressure conditions (DeBruyn et al., 2013). With the exception of the BDF3, the PFM systems showed a higher abundance of *uncultured_bacterium_f_Gemmatimonadaceae* species, suggesting that PFM can enhance *Gemmatimonas* activity and promote nitrogen fixation. Meanwhile, PFM could also enhance the abundance of Actinobacteria, Nitrospirae and Alphaproteobacteria, in which Actinobacteria can secrete antibiotics over long periods to inhibit disease (Landwehr et al., 2016), while Nitrospirae (Zhang et al., 2015) and Alphaproteobacteria (Trujillo et al., 2015) play an important role in nitrogen fixation and soil nitrification. Collectively, mulch with film could be contribute to resistance to disease and soil nitrification.

4.3 BDFs mulch could increase the abundance of microbes involved in plastic degradation.

Microbes, such as bacteria and fungi in the soil, are involved in the degradation of BDFs and plastic film residue (Shah et al., 2008). The NBDF, for example plastic film, phthalate esters take up 20–60% of it, which contaminate the environment and affect the soil microbial communities and enzymatic activities (Qian et al., 2018). However, soil microbes prefer polymers, especially plastics, as substrates. For example, the genus *Sphingomonas* can degrade the toxic substance bromine acid and participate in the nitrogen cycle in soil (Vasilyeva and Strijakova, 2007). Samples CK and BDF2 have higher *Sphingomonas* abundance than NBDF and BDF 1 and BDF 3). Since a possible competition between *Gemmatimonas* and *Sphingomonas*, PFM increased *Gemmatimonas* abundance and inhibited *Sphingomonas* development. *Haliangium* is a common saprophyte in soil, and it plays an important role in the nutrient cycle of soil (Tayyab et al., 2018). *Haliangium* abundance in BDFs and NBDF samples was higher than CK in this study. It is obvious that NBDF and BDFs could improve soil microbial activities by enhancing the abilities of saprophytic bacteria and soil nutrient recycling. Moreover, *Pedomicrobium* and *Comamonas* are bacteria that have been reported to have the ability to degrade plastics residues (Shah et al., 2008). The number of *Pedomicrobium* and *Comamonas* were significantly higher in BDFs than CK and NBDF. The presence of both functional bacteria is likely to be a key distinction between biodegradable and nonbiodegradable membranes and their highest abundances in BDFs also suggested that both microbes have been involved in the degradation of BDFs.

The BDFs and NBDF form a bottleneck on the soil surface that impacts soil temperature, moisture and soil-air gas exchange, indirectly altering the microorganism communities (Bandopadhyay et al., 2018). Nitrobacteria involve in regulating the balance of inorganic nitrogen (N forms) in soils (Attard et al., 2010) through facilitating nitrification, which is a vital step of the nitrogen cycle (Poly et al., 2008). In this study, the abundance of three functional nitrobacteria were determined by Quantitative Real-time PCR. Both oxidizing bacteria (NOB), *Nitrobacteria* and *Nitrospira*, have involved in soil nitrification from ammonia to nitrate and play a crucial role in nitrogen biogeochemical cycling and plant nutrition. The results showed that the number of nitrobacteria in BDFs was higher than CK and NBDF. BDFs change the species abundance of the nitrobacteria community in soil, and selectively increased two of the species (*Nitrobacter nxrA* and *nxrB*). The decrease in *Nitrospira* quantity in BDFs and NBDF may have less of an effect than CK on the degradation process of plastics. The increase in the number of these two nitrobacteria also show that the cover of biodegradable film used in this study has an effect on the nitrobacteria community in the soil.

5 Conclusions And Perspectives

PFM (plastic film mulch) could increase significantly the diversity and richness of the soil microbial species. Especially, BDFs could improve the soil quality and the species abundance of functional genera (e.g. *Pedomicrobium*, *Comamonas* and nitrifying microbes), which would be involved in the degradation of BDFs or nitrification in soil.

The degradation of BDFs need soil microbial community that could be influenced by various environmental factors, including sunlight, rainfall, soil moisture and temperature, and film type. Up to now, there are still some problems that need to be solved in the use of biodegradable film, e.g. long degradation times, incomplete degradation, and the possibility of residual particles in the soil. Therefore, in the future, biodegradable film should be particularly improved in many ways, such as material innovation, degradation process optimization and agronomic technology in sustainable agriculture.

Declarations

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Conflicts of interest We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the position presented in, or the review of, the manuscript entitled.

Availability of data and material Complete original data.

Code availability: Not applicable.

Authors' contributions: Yinghao Xue, Providing the site for the experiment and providing the right to sample, and plays an important role in writing articles.

Tuo Jin, Chengyu Gao, Chongxiao Li and Tao Zhou, Sample collection and processing, PFM collection, soil screening, experimental data statistics.

Dongshi Wan, Microbial sample processing and sequencing analysis.

Mengran Yang, Overall experimental design including chart drawing review

Ethics approval: Not applicable.

Consent to participate: We the undersigned declare that this manuscript entitled “Effects of biodegradable film mulching on bacterial diversity in soils” is original, has not been published before and is not currently being considered for publication elsewhere.

We confirm that the manuscript has been read and approved by all named authors and that there are no other persons who satisfied the criteria for authorship but are not listed. We further confirm that the order of authors listed in the manuscript has been approved by all of us.

We understand that the Corresponding Author is the sole contact for the Editorial process. He/she is responsible for communicating with the other authors about progress, submissions of revisions and final approval of proofs.

Consent for publication: All authors agree to publish this article in Plant Ecology

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Figures

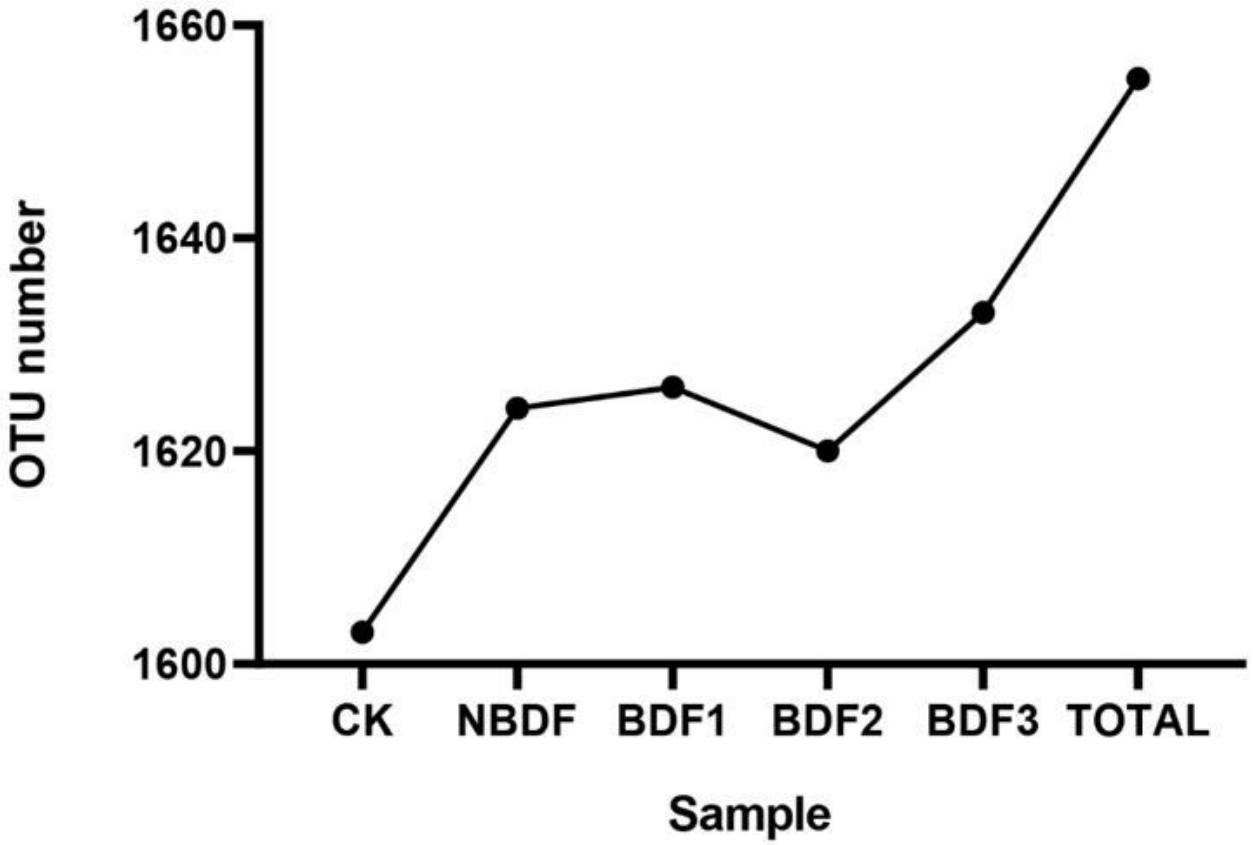

Figure 1

OTU distribution of all sample. UCLUST in QIIME (version 1.8.0) software was used to cluster tags and obtain OTUs at a 97% similarity level. The taxonomic annotation of the OTUs was carried out based on the taxonomic databases of Silva (bacteria) and UNITE (fungi).

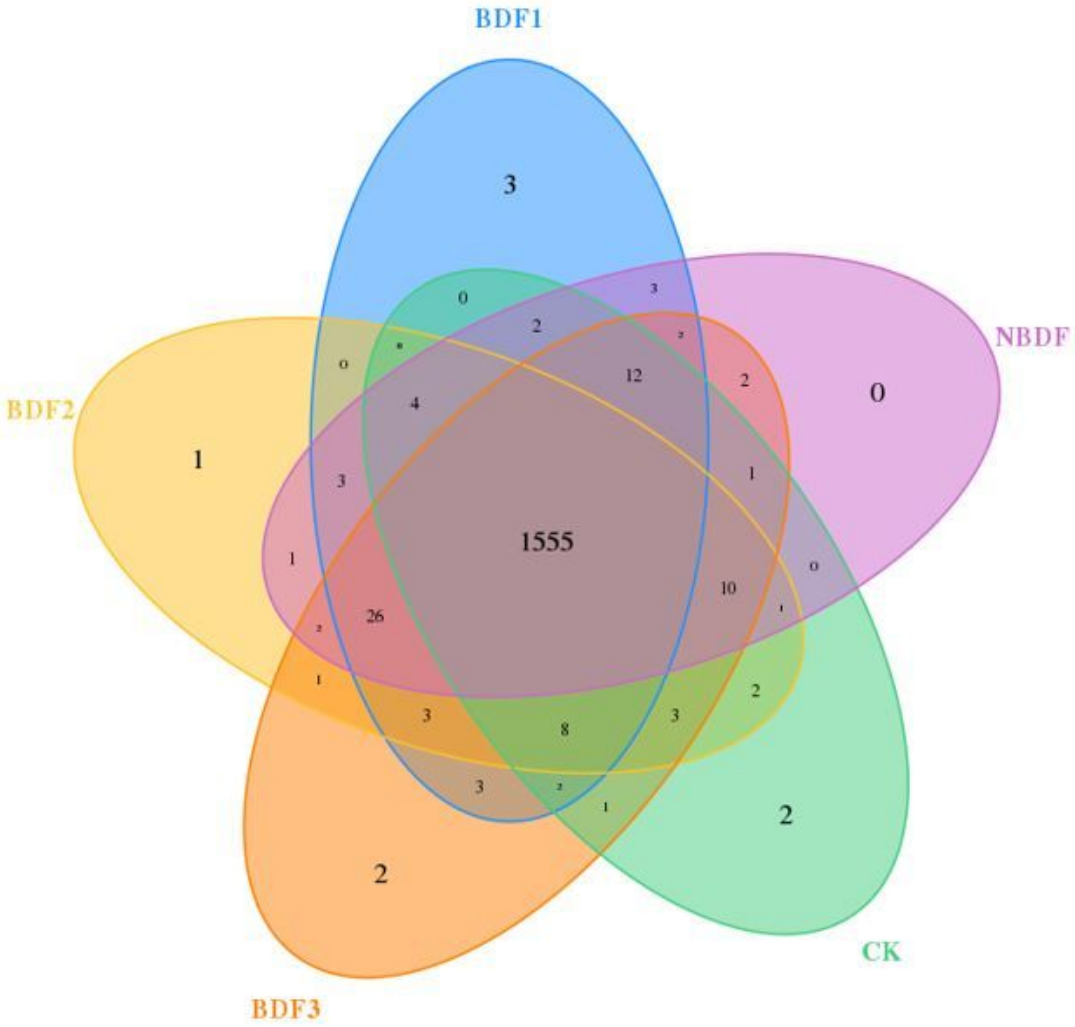

Figure 2

Venn diagram of all samples At the 97% similarity level, the number of OTUs in each sample was obtained. A Venn diagram was used to display the number of common and unique OTUs in the samples. Combined with the species represented by the OTUs, the common microorganisms in the different environments could be found. The number of overlapping parts of the graphs is the total number of common OTUs between multiple samples, while the number of nonoverlapping parts is the number of unique OTUs in each sample.

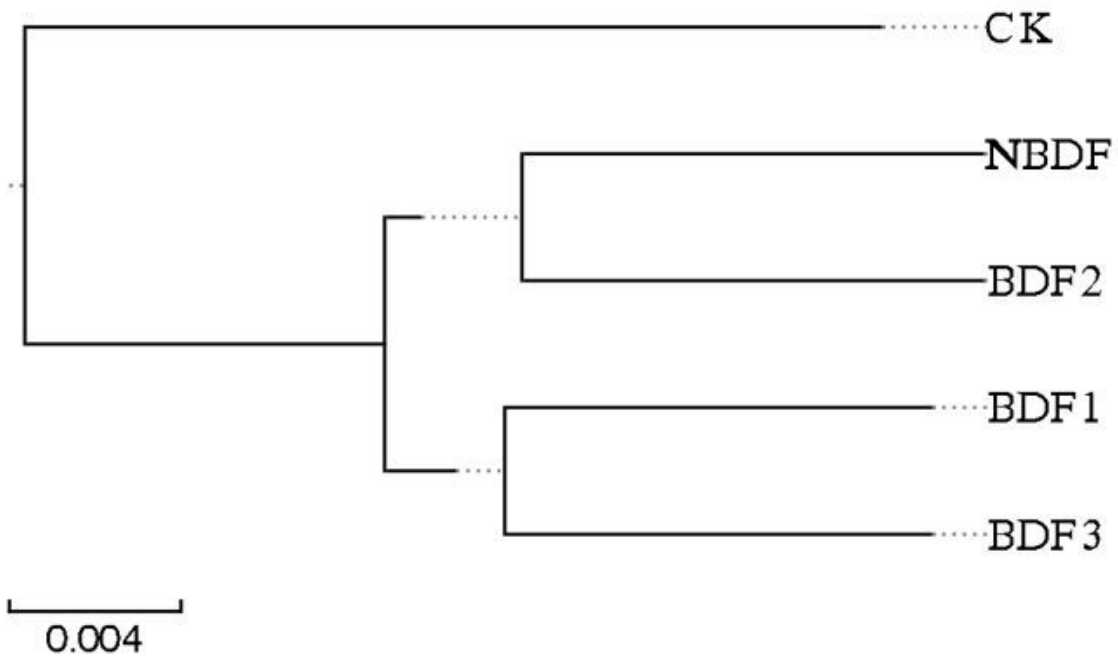

Figure 3

UPGMA dendrogram based on the UniFrac data distance matrix for the comparison of all the samples The phylogenetic tree generated by the UPGMA (unweighted pair group method with arithmetic mean) is a simple embodiment of the species tree. After each divergence, the length of the branches from the common ancestor node to the two OTUs is the same. The closer the samples were, the shorter the branches, indicating that the species composition of the two samples was more similar.

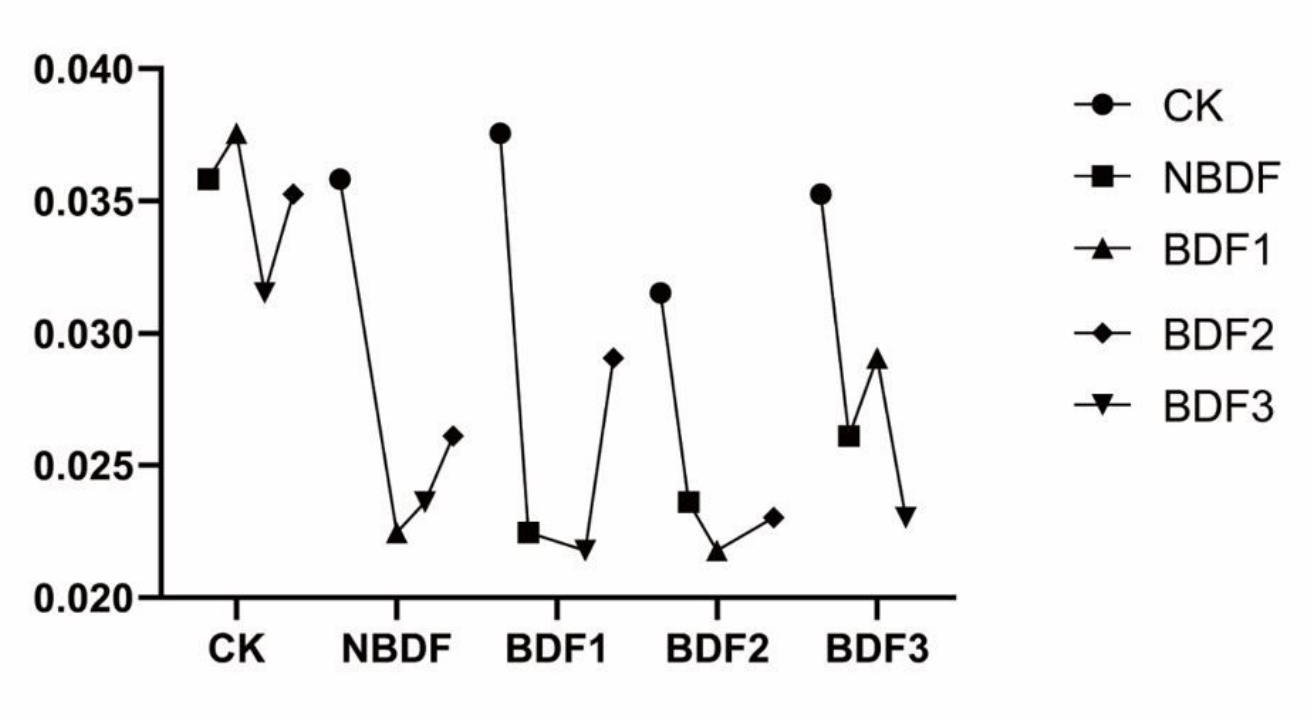

Figure 4

Graph based on the UPGMA dendrogram The genetic distance between each sample was used to draw a line graph, which directly reflected the similarities and differences among the five samples.

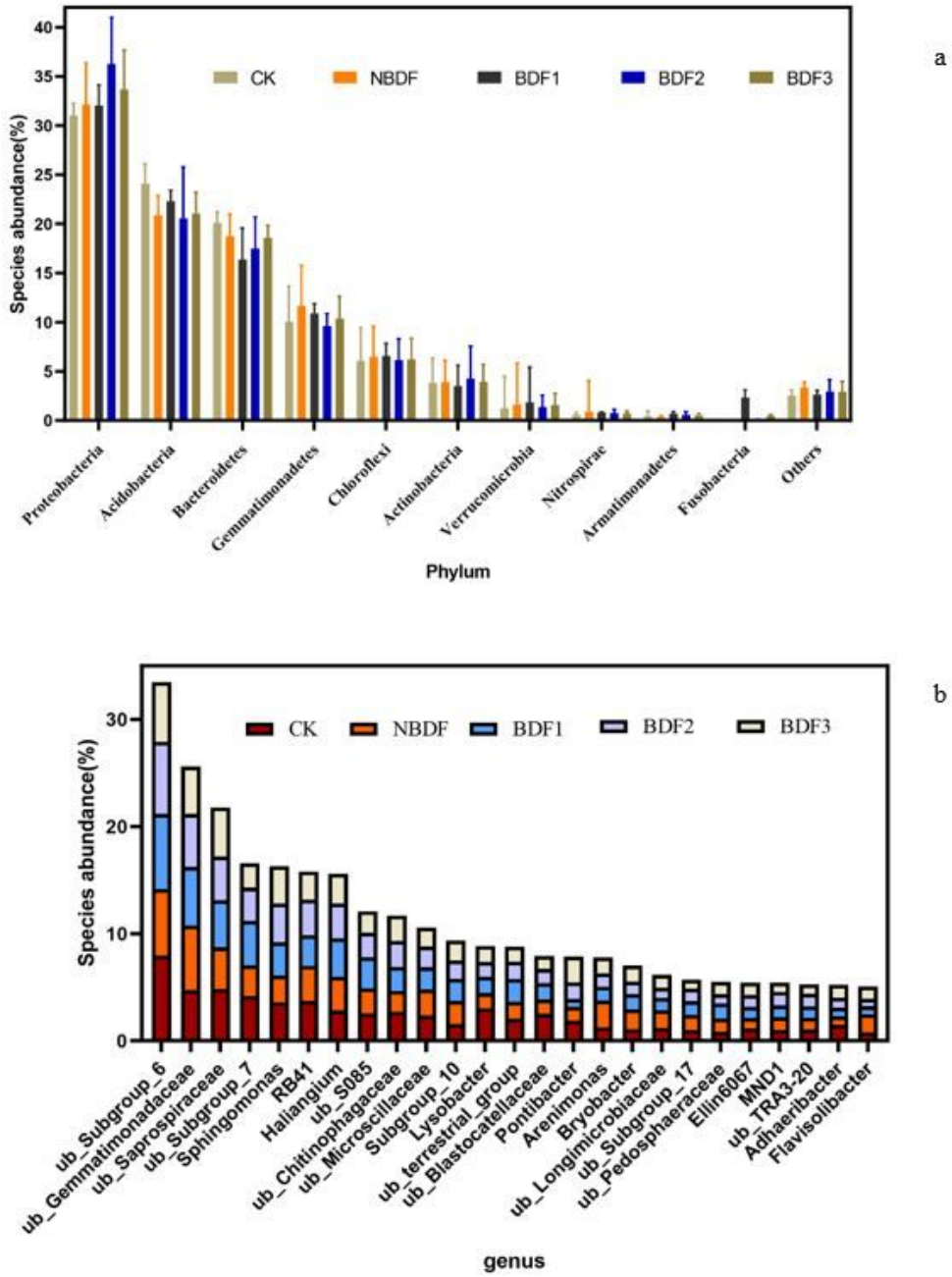

Figure 5

Soil bacterial community composition of all the samples at the (a) phylum level and (b) genus level. Each color represents a species, and the length of the color block (histogram) indicates the relative abundance of the species. We show only the top 10 species at the phylum level; the remaining species were combined and displayed as others (a). The top 20 different taxonomic genera were selected from the five samples (b). As shown in the figure, unclassified represents the species that have not been taxonomically annotated.

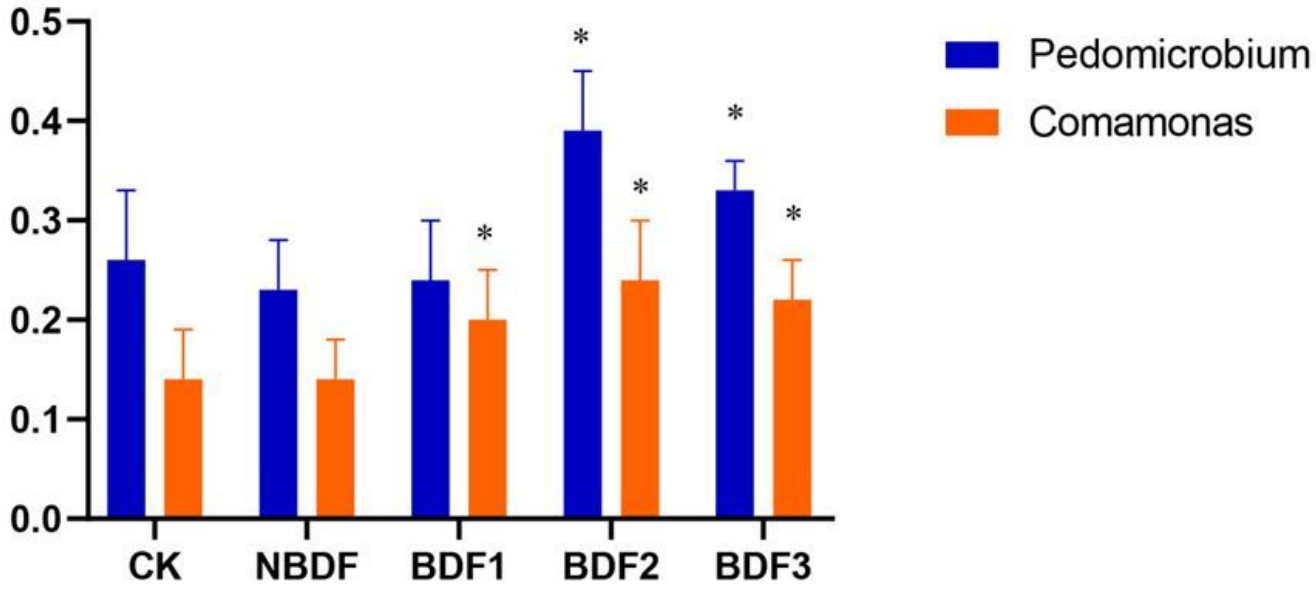

Figure 6

Functional genera samples The two functional genera have been shown to be involved in the degradation of plastics in the soil. The two colors bars represent the two genera, Pedomicrobium and Comamonas, and the height of the bars represents the abundance of species in both genera in the five samples.

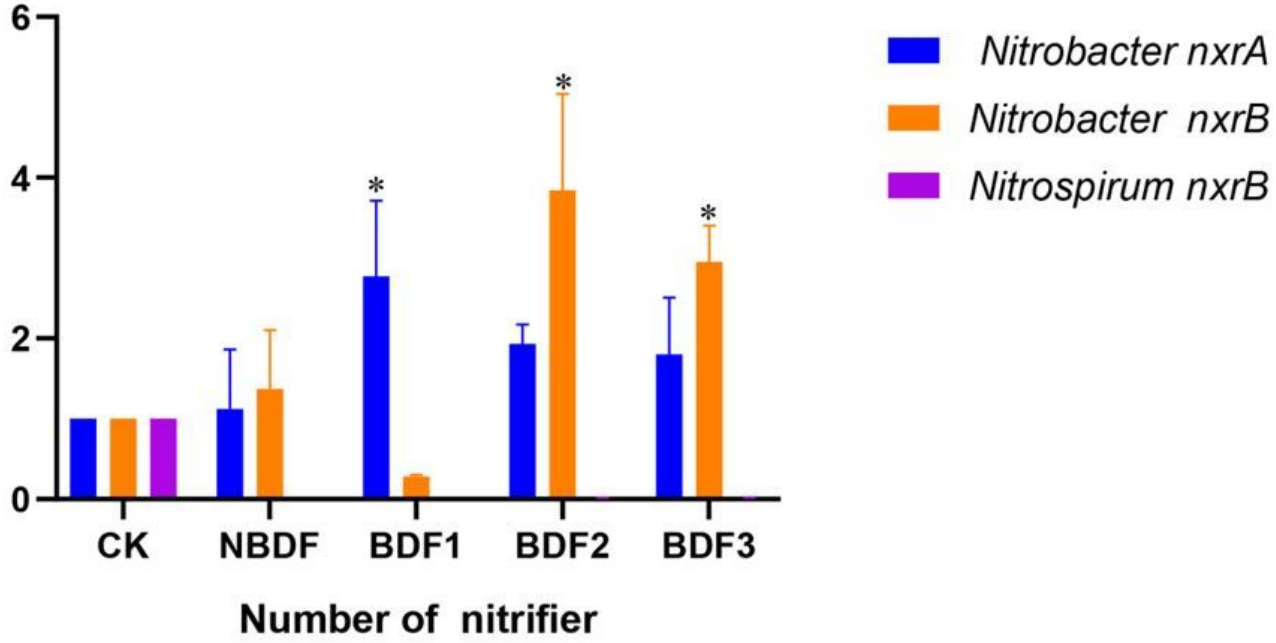

Figure 7

Number of nitrifying microorganisms The number of nitrifying microorganisms in the different samples is shown in the figure, and the three different colors represent Nitrobacter nxrA, Nitrobacter nxrB, and Nitrospira nxrB.

Supplementary Files

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- SupplementalTable.docx