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Effects of Soil Residual Plastic Film on Soil Microbial Community Structure and Fertility

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Abstract Plastic films have previously displayed tremendous potential to increase water use efficiency in farmland and the yield of cash crops; however, long-term use of plastic film in soils can influence soil physiological and biochemical characteristics and change its biota. The present study aimed to investigate the effects of residual plastic film pollution on soil microbe community structure and fertility in Xinjiang province, China. Residual plastic film-contaminated soil and non-contaminated soil in Xinjiang farmland were selected for this study. The results indicated that residual plastic film pollution changed the structure of the soil biological community by significantly decreasing and increasing the abundance of *Actinomycetes* and *Proteobacteria*, respectively; further, the pollution decreased soil organic matter and inorganic nitrogen content by downregulating microbial genes related to soil carbon and nitrogen cycles and decreasing related enzymatic activities. The present results indicated that long-term residual plastic film exposure (more than 10 years) in farmland significantly decreases soil fertility and alters the microbial community structure.

Keywords Plastic film · Microbial community structure · 16S rDNA sequencing · Soil fertility

1 Introduction

Application of plastic films on soil is a global agricultural practice for increasing crop production, achieving earlier harvests, improving fruit quality, and increasing water-use efficiency (Wu 2002; Wei et al. 2015; Steinmetz et al. 2016). The surface area of agricultural land covered with plastic films has increased worldwide by approximately 7% in the last 10 years (Wang et al. 2016). Previous reports indicated that with the use of plastic film technology, grain and cash crop production increased by 20–35% and 20–60%, respectively, and water-use efficiency was improved by 20–60% (Liu et al. 2014). Plastic film for soil was introduced in China in the late 1970s, and the proportion of land covered by the films increased tremendously at a rate of 30% per year between 1991 and 2004, especially in the northern regions of China (Espí et al. 2006). In Xinjiang, the northwest province of China, the use of plastic films increased from 7.0 to 34.8 kg ha^{-1} from 1991 to 2011; however, it was accompanied with a series of pollution hazards from residual pollution films (RPFs) (Jambeck et al. 2015; Wang et al. 2016).

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Soil is closely related to human survival and development (Doran et al. 2003). The composition and diversity of microbial communities in soils play an important role in maintaining soil quality (Kennedy and Smith 1995; Rong et al. 2017). Microbes are sensitive to soil contaminants, and their composition and activity are the primary biological indicators of changes in the soil environment, as they play a key role in carbon, nitrogen, phosphorus, and potassium cycling in the soil (Avidano et al. 2005; Bergkemper et al. 2016; Delgado-Baquerizo et al. 2016). Plastic film contains approximately 20–60% phthalate esters (PEs), which ubiquitously contaminate the environment and affect microbial communities and soil enzymatic activities (Chen et al. 2013; Fu and Du 2011; Jambeck et al. 2015; Zhou et al. 2005). PE content in all non-cultivated soils was reported to be the lowest (Xu et al. 2008), while residual PE concentration in RPF soil was reported to vary from 124 to 1232 μg g^{-1} in Xinjiang; this concentration increased with the use of the plastic film (Kong et al. 2012; Wang et al. 2007). PEs were reported to inhibit fluorescein diacetate hydrolysis and dehydrogenase activity (Wang et al. 2016) or enhance phosphatase activity (Xie et al. 2010; Zhou et al. 2005), which might be attributed to different environmental conditions or experimental conditions to manage RPF contamination.

Most studies on the effects of RPF on soil microbial populations and enzymatic activity were conducted under laboratory conditions, not in outdoor farmland (Chen et al. 2013; Wang et al. 2016), and few studies have attempted to elucidate the effects of long-term use of plastic films on soil fertility and microbial community composition. Hence, the present study analyzed the soil microbial abundance and community composition and enzymatic activity in soils of the field environment, where plastic films have been used for more than 10 years, through high-throughput sequencing.

2 Materials and Methods

2.1 Soil Sample Collection

The upper 20-cm layer of the soil (with surface soil removed) from two different sites in Urumqi (44° 7′ N, 87° 31′ E, Xinjiang, China) was collected on the basis of the level of plastic film contamination; the first site comprised fields containing plastic film mulch used during crop production, and the second site contained no plastic film mulch. Three soil cores at different depths (0–20 cm) sampled across a horizon distance 200 cm away from each site were combined as a single sample with four replicates. Subsamples for soil physicochemical analysis were stored at − 20 °C and analyzed within 1 week. Subsamples for molecular analysis were stored at − 80 °C.

2.2 Soil Physicochemical Analysis

The soil was initially naturally dried at approximately 25 °C and then filtered through a sieve (< 2 mm) to eliminate the plant debris. Next, the soil was homogenized for chemical analysis. The following chemical properties of the soil were determined: soil pH, electrical conductivity (EC), soil organic matter (SOM) and total nitrogen (TN) content, ammonium nitrogen (NH_4^+-N), and nitrate nitrogen (NO_3^--N). Soil pH and EC were measured in a 1:2.5 (*w*/*w* soil: water) suspension using a pH meter and conductivity meter, respectively. TN content in the soil was determined using Kjeldahl's method (Raya-Moreno et al. 2017; Sen et al. 2017). NH_4^+-N and NO_3^--N content was determined in accordance with the method of Wu et al. (2017). SOM content was determined using the potassium dichromate oxidation method (González-Pelayo et al. 2006).

2.3 Estimation of Enzymatic Activities

Soil enzymatic activities were assayed and considered an indicator of soil microbial activity (Qian et al. 2007b). Soil dehydrogenase (sDHA) induces 2,3,5-triphenyltetrazolium chloride to generate red triphenyl formazance. Phosphatase (S-ACP) catalyzes the conversion of disodium phenyl phosphate to phenol. Urease (S-UE) hydrolyzes urea to produce ammonia. Solid-β-glucosidase (S-β-GC) catalyzes the conversion of glucoside of *p*-nitroben-beta-d pyrana to generate *p*-nitrophenol. Soil chitinase (S-Chi) hydrolyzes chitin and produces a red compound with dimethylaminobenzaldehyde. Levels of sDHA, S-ACP, S-UE, S-NR, S-β-GC, and S-Chi were measured spectrophotometrically at 485, 660, 578, 540, 400, and 585 nm using the corresponding reagent kit (Suzhou Comin Biotechnology Co., Ltd., Suzhou, Jiangsu, China), as per the manufacturer's guidelines.

2.4 DNA Extraction and 16S rDNA Sequencing

The PowerSoil® DNA Isolation Kit (Mo Bio Laboratories Inc., Carlsbad, CA, USA) was used to extract genomic DNA from (0.25 ± 0.02 g) soil in accordance with the manufacturer's instructions. The quantity and concentration of DNA were measured and evaluated, as previously reported (Qian et al. 2007a). The extracted DNA was amplified using polymerase chain reaction (PCR) with forward primer 314F (5′-CCTA YGGGRBGCASCAG-3′) and reverse primer 805R (5′-GGACTACNNGGGTATCTAAT-3′) of V3-V4, followed by 16S rDNA sequencing. The 16S rDNA amplicon sequencing was conducted on the Illumina HiSeq 2500 platform (Zhejiang Tian Ke Hi-Tech Development Co., Ltd. Hangzhou, China). When the sequence similarity ≥ 97%, the gene sequences were assigned into one operational taxonomic unit (OTU). The soil microorganisms were classified into different taxa by aligning the OTU with the SILVA database and using the RDP Classifier software (Version 11.4) for further analysis. Alpha diversity indices including Chao1, Shannon, Simpson, and observed species were calculated with QIIME (Version 1.9.1). Beta diversity of both weighted and unweighted unifrac was calculated by QIIME (Version 1.9.1). Principal component analysis (PCA) was performed by R software (Version 3.2.2).

2.5 Quantitative Polymerase Chain Reaction Assays

The effects of RPF on expression of soil carbon and nitrogen cycle-related genes in soil microorganisms were investigated using quantitative polymerase chain reaction (qPCR). The reaction contained the following: 1 μL template DNA (10 ng μL^{-1}), 5 μL SYBR Green Realtime PCR Master Mix (Toyobo, Osaka, Japan), 0.4 μL each of the forward and reverse primers, and 3.2 μL double-distilled water (Li et al. 2018). All operations were carried out on ice. The cycling conditions were as follows: initial denaturation at 95 °C for 10 min, followed by 40 cycles of denaturation at 95 °C for 15 s, annealing at 56 °C for 30 s, and 72 °C for 1 min, and final extension at 72 °C for 10 min. Real-time quantitative PCR (qRT-PCR) was performed using an Eppendorf Mastercycler® ep RealPlex4 system (Wesseling-Berzdorf, Germany), as described previously (Lu et al. 2018). A total of seven genes were detected, including three carbon cycle-related genes (*cbb*L, *chi*A, and β-*glu*) and four nitrogen cycle-related genes (*nif*H, *amo*A, *nos*Z, and *nir*K) (Song et al. 2017). Primer sequences are listed in Table S1.

2.6 Statistical Analysis

All data are presented as means ± standard error (SE) and were compared using one-way analysis of variance (ANOVA). All statistical analyses were performed using StatView 6.0 software (Statistical Analysis Systems Institute, Cary, NC, USA). The differences were considered significant when the probability (p) was less than 0.05 or 0.01. Multivariate data were imported into the SIMCA-P+ 14.0 software package (Umetrics, Umea, Sweden) to assess differences in soil community structure through PCA.

3 Results

3.1 Effects of Plastic Film on Physicochemical Properties of Soil

Soil chemical properties of each group are provided in Table 1. The content of TN, NH_4^+-N, and NO_3^--N significantly decreased by 42.10%, 17.7%, 50.53%, respectively, compared with that in control soil. SOM content was 3.15×10^4 mg kg^{-1} in control soil but decreased to 48.57% (1.53×10^4 mg kg^{-1}) in the plastic film-covered soil. Conversely, compared with that in control soil, EC and phosphorus content increased from 4.38 ms cm^{-1} and 34.56 mg kg^{-1} to 9.83 ms cm^{-1} and 45.87 mg kg^{-1}, respectively. Compared to that in control soil, soil pH and sulfur content did not change significantly in the RPF-exposed soil; the pH was maintained at about 7.3 in both soils.

3.2 Effects of Plastic Film on Soil Enzymatic Activity

Enzymatic activity in the different soils is shown in Fig. 1. Compared with that in control soil, sDHA activity was slightly reduced, and S-ACP activity was slightly increased in the RPF soil, while S-UE in the plastic film-covered soil decreased significantly from 922.38 mg day^{-1} g^{-1} in the control to 747.01 mg day^{-1} g^{-1}. Simultaneously, both S-β-GC and S-Chi decreased by 24.94% and 24.87%, respectively, compared with that in control soil. Conversely, S-NR significantly increased by 25.42% in plastic film-covered soil, compared with that in control soil.

Table 1 The primary physical and chemical properties of the soils in control (Con) and plastic film-covered soil (Treat)

Group	NH_4-N ($\mu g\ g^{-1}$)	NO_3-N ($\mu g\ g^{-1}$)	TN (%)	SOM (%)	P ($\mu g\ g^{-1}$)	S (%)	EC ($ms\ cm^{-1}$)	pH
Con	2.77 ± 0.097	24.42 ± 2.72	0.19 ± 0.0006	3.15 ± 0.05	34.56 ± 3.58	0.048 ± 0.01	4.38 ± 0.06	7.41 ± 0.02
Treatment	2.28 ± 0.07*	12.08 ± 1.45*	0.11 ± 0.004*	1.53 ± 0.05*	45.87 ± 1.19*	0.06 ± 0.004	9.81 ± 0.71*	7.22 ± 0.09

*Significant differences at the $p < 0.05$ level between treatments

3.3 Effects of Plastic Film on the Diversity and Richness of Soil Microorganism Communities

To determine the effect of plastic film pollution on soil microbial community structure, we obtained approximately 539,963 reads from Illumina sequencing, with 60,635–79,731 reads per sample. The average numbers of OTUs significantly increased by 9.72% in the plastic film-covered soil, compared with those in control soil. As shown in Table 2, the number of Chao1 and observed species in the plastic film-exposed soil was 4.34% and 7.83% higher than that in control soil, respectively, while the average Shannon diversity indices in the control soil were not significantly different from those in the plastic film-covered soil. The Simpson values of all groups were close to 1, indicating high coverage rates of sequencing in this study. PCA revealed that the bacterial community structure changed and indicated more than 50% species variance by two axes. As shown in Fig. 2, the bacterial community in plastic film-covered soil was different from that in control soil.

To further investigate the differences in taxonomic distribution between plastic film-covered soil and control soil ecosystems, metagenomic biomarkers were identified using linear discriminate analysis (LDA) effect size (LEfSe), and differentially abundant taxa were determined from the metagenome data with default parameters. Ninety-five distinguishing taxa in the two different soils had LDA scores greater than 1.2. As shown by the circular cladogram, Phylum *Actinobacteria* was the most abundant in the control soil. In plastic film-covered soil, the abundance of *Proteobacteria*, *Bacteroidetes*, and *Cyanobacteria* increased significantly, and Phylum *Proteobacteria* was the most abundant (Fig. 3).

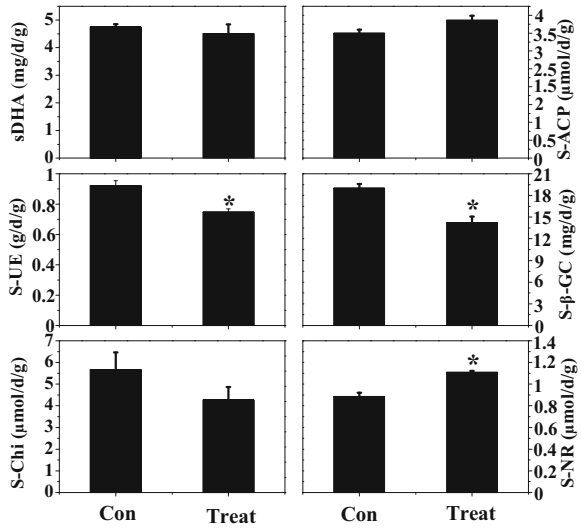

Fig. 1 Enzyme activities in control soil (Con) and plastic film-covered soil (Treat). Asterisk indicates significant differences at the $p < 0.05$ level between treatments. Error bars indicate the standard error of the mean ($n = 4$)

3.4 Composition and Changes in Bacterial Communities

The bacterial community taxonomic distribution between plastic film-covered and control soil was further investigated, and the 10 most abundant phyla are shown in Fig. 4. Compared with that in the control, the abundance of *Actinobacteria* decreased from 32.15 to 14.41%; *Acidobacteria*, *Firmicutes*, and *Thaumarchaeota* displayed the same trend, and their abundance decreased from 9.61 to 8.00%, 4.03 to 3.74%, and 0.76 to 0.43%, respectively. The abundance of *Chloroflexi* remained at approximately 5% in the plastic film-covered soil and control soil. The control soil contained approximately 27.18% *Proteobacteria*; however, the abundance increased to 33.47% in the plastic film-covered soil. The abundance of *Gemmatimonadetes* increased from 13.33 to 20.69%, and *Bacteroidetes*, *JL-ETNP-Z39*, and *Nitrospirae* displayed the same trend and increased from 4.16 to 7.52%, 0.80 to 2.44%, and 0.86 to 1.43% abundance, respectively.

Table 2 Operational taxonomic unit (OTU) sequences and the alpha diversity indices of control group (Con) and the plastic film-covered soil (Treat)

Simple	OTUs	Chao1	Observed species	Shannon	Simpson
Con	2227	2549.20	2042.5	8.751	0.9928
Treatment	2443.5	2659.85	2202.5	8.755	0.9921

At the genus level (Fig. 5), the abundance of *Sphingomonas*, *Bacillus*, *Defluviicoccus*, *Xanthomonas*, *Nitrospira*, and *Gemmatimonas* in plastic film-covered soil increased by 58.36%, 37.35%, 21.76%, 313.42%, 102.72%, and 30.81%, respectively, compared with that in control soil. In contrast, the abundance of *Skermanella*, *Arthrobacter*, *Nocardioides*, *Enterobacter*, *Solirubrobacter*, and *Pseudomonas* decreased in plastic film-covered soil by 40.43%, 67.02%, 71.81%, 58.76%, 76.72%, and 70.75%, respectively, compared with that in the control.

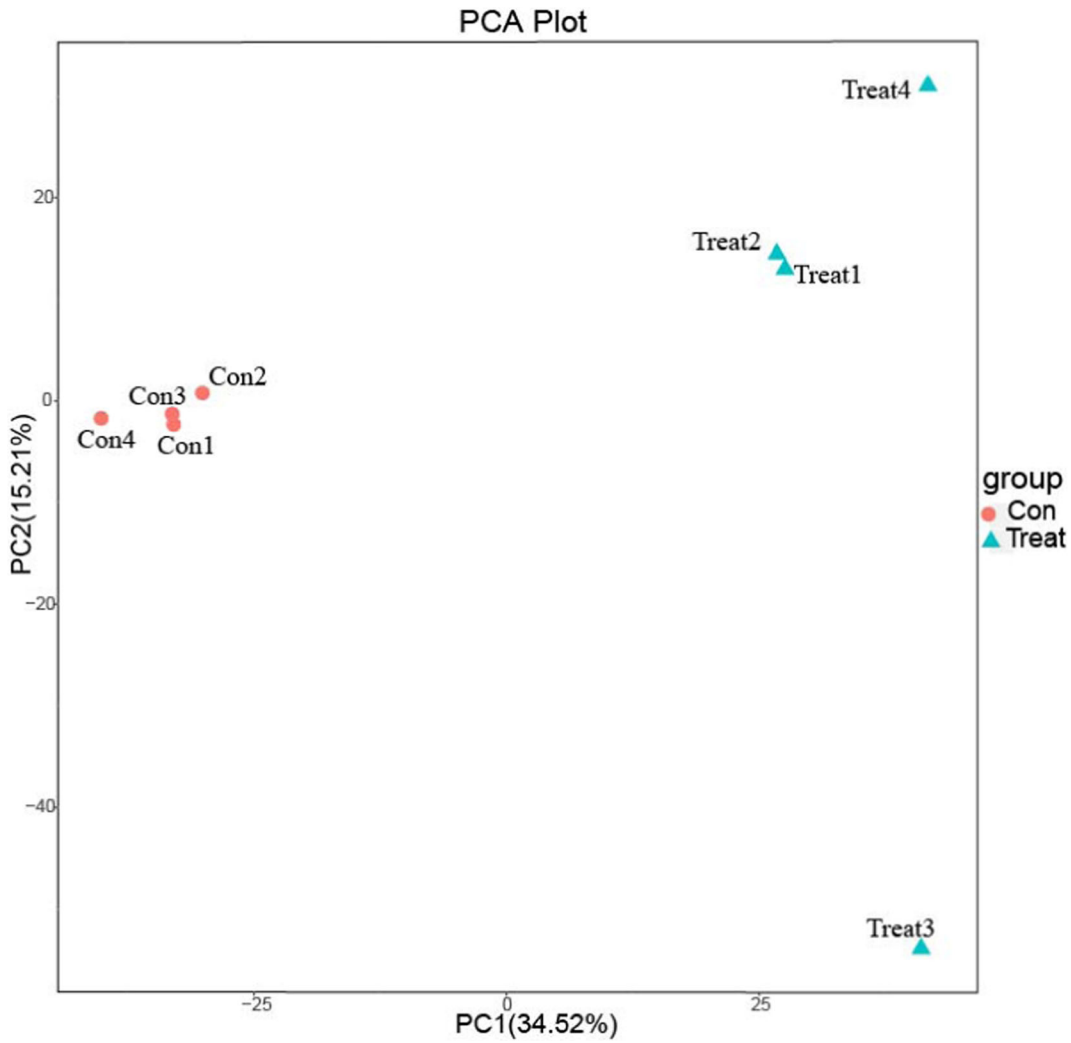

Fig. 2 Multivariate classification of the soil bacterial communities under different treatments. Eigenvalues and eigenvectors were sorted by principal component analysis (PCA) of average well color development in two different soils

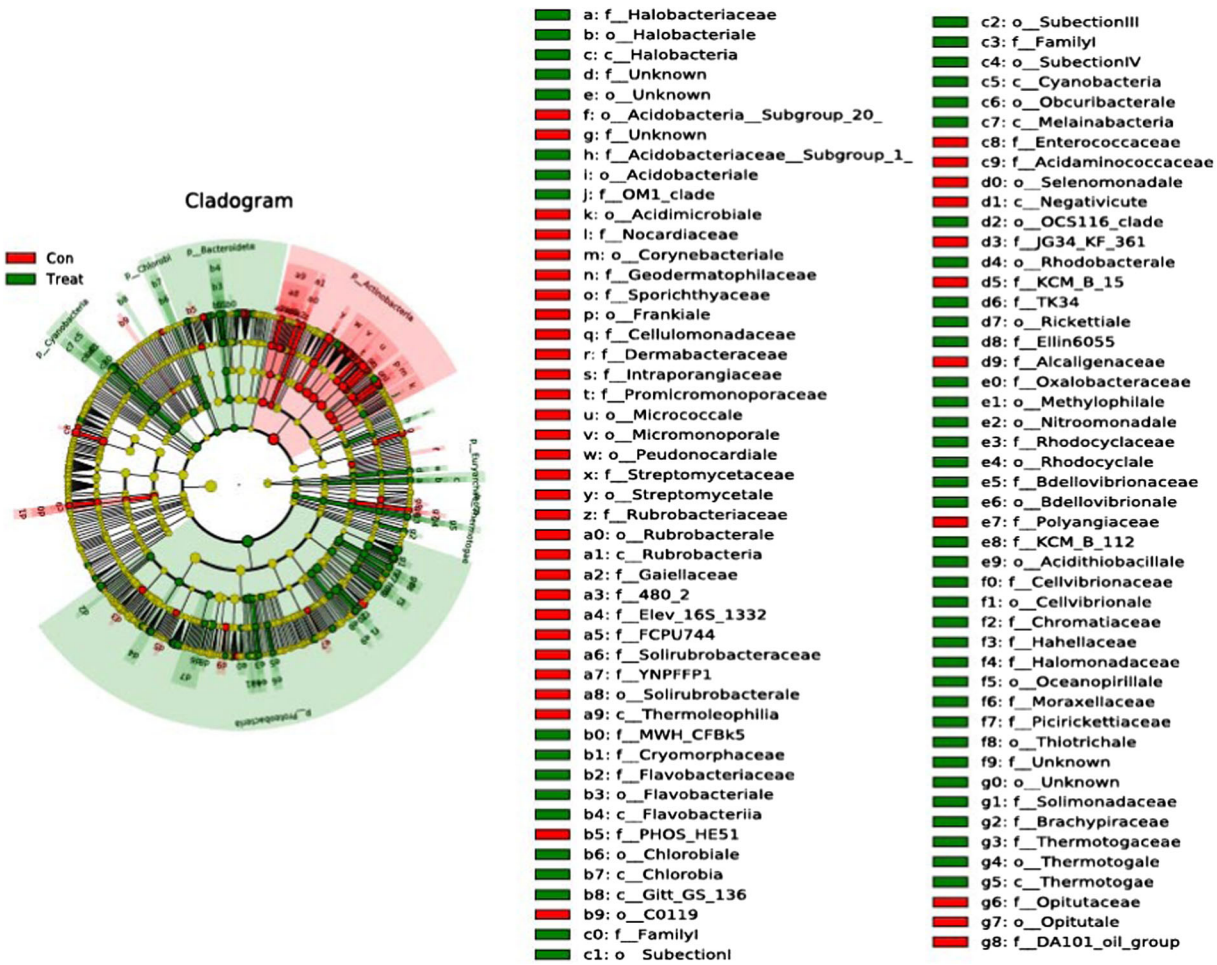

Fig. 3 LEfSe cladograms showing taxa with different abundance values. Small circles and shading with different colors in the diagram represent abundance of those taxa in the respective treatment group. Yellow circles represent non-significant differences in abundance between treatment groups for corresponding taxa. LEfSe detected 95 biomarkers at different taxonomic levels with differential abundance among different treatment groups

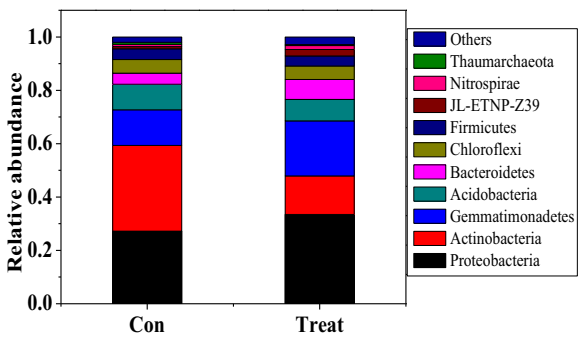

Fig. 4 Relative abundance of top 10 bacterial communities at phylum level in control soil (Con) and plastic film soil (Treat) groups

3.5 Changes in Expression of Soil Carbon and Nitrogen Cycle-Related Genes

There was a significant difference in the abundance of genes involved in carbon cycling (Fig. 6a). The abundance of *cbb*L, a key gene in soil microbial carbon fixation, decreased to 53.1% in the plastic film-covered soil. The abundance of *chi*-A and β-*glu*, both related to the carbon cycle, decreased to 51.2% and 79.2%, respectively, in plastic film-covered soil. Interestingly, the abundance of genes was consistent with the activities of the relevant enzymes; the activities of S-β-GC and S-Chi decreased by 24.94% and 24.87% in

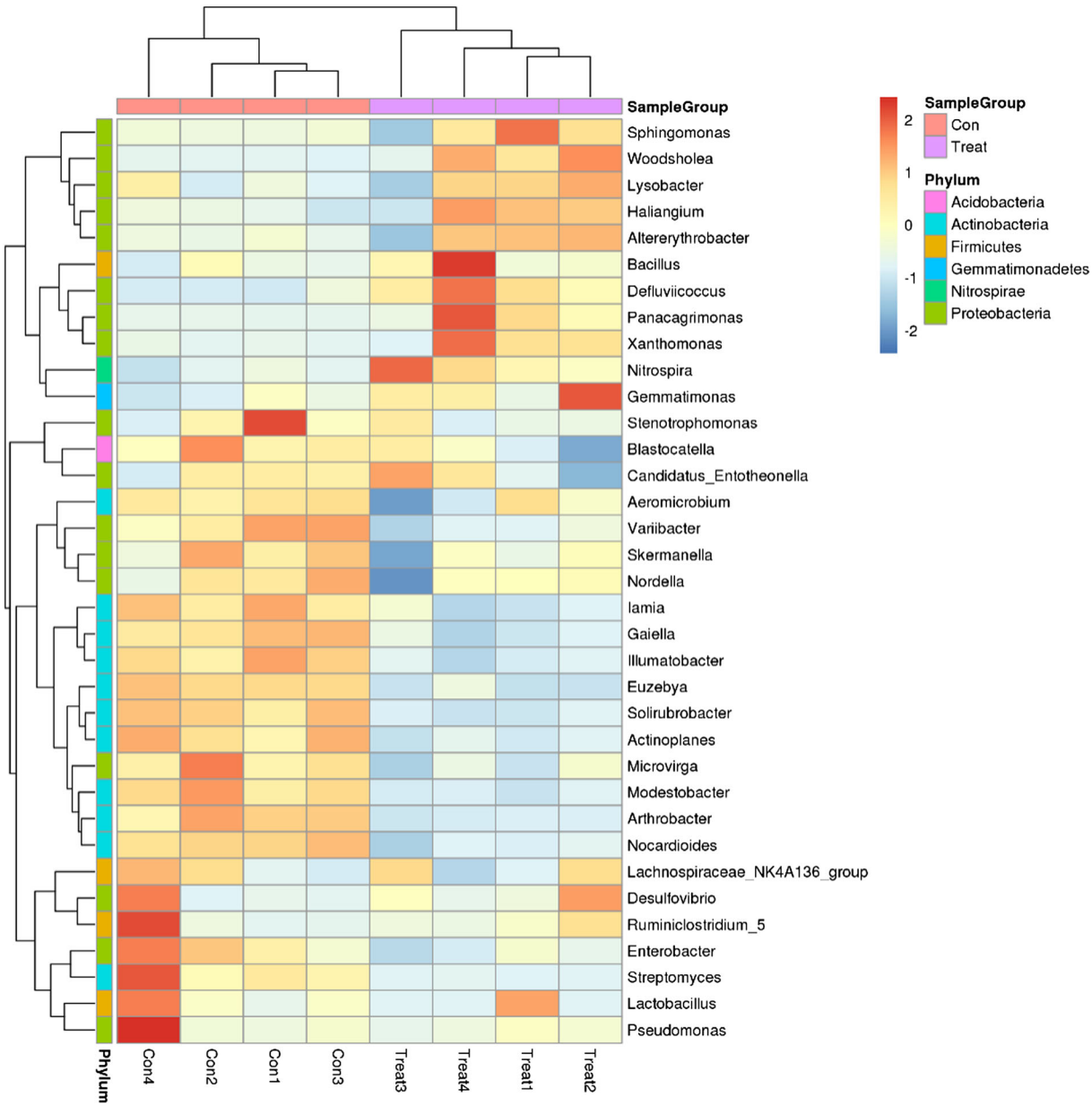

Fig. 5 Species abundance clustering heat map of genera. (Vertical for the sample information, horizontal for the species annotation information, the figure on the left side of the tree as a cluster tree; the top of the cluster tree for the cluster between clusters. The corresponding value of the intermediate heat map is the Z value obtained by normalizing the relative abundance of each row. The Z value of a sample on a certain category is the relative abundance of the sample on the classification and the average relative abundance difference is divided by the standard deviation of all samples on the classification.)

plastic film-covered soil, respectively, compared with those in control soil.

The abundance of the nitrogen fixation-related gene *nif*H increased by about 48.34%, while that of *nos*Z and *nir*S increased by 80.45% and 83.09%, respectively, in the plastic film-covered soil; however, the abundance of *nir*K, also related to denitrification, decreased by 37.22%. The abundance of *amo*A, an ammonia oxidation-related gene, decreased by 9.77% in the plastic film-covered soil (Fig. 6b).

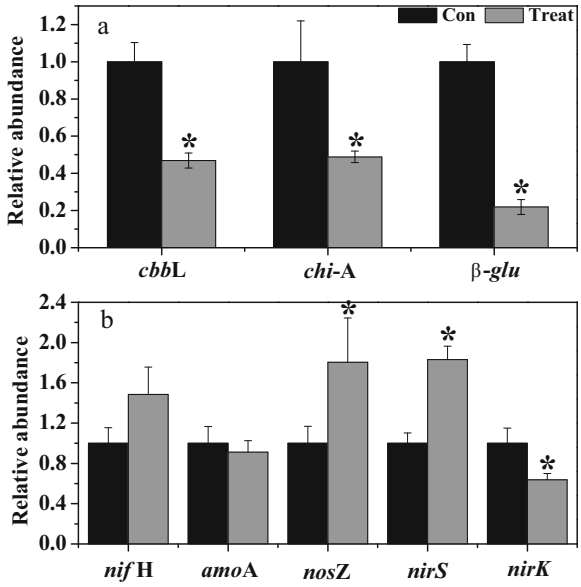

Fig. 6 The effects of the plastic film on the abundance of soil carbon cycle-related genes (a) and nitrogen cycle-related genes (b). Soil in control soil (Con) and film soil (Treat). Asterisk indicates significant differences at the $p < 0.05$ level between treatments. Error bars indicate the standard error of the mean (n = 4)

4 Discussion

4.1 Soil RPF Affects Soil Physicochemical Properties, Enzymatic Activity, and Abundance of Soil Carbon and Nitrogen Cycle-Related Genes

We evaluated the effects of soil RPF on soil physicochemical properties, enzymatic activities, and the abundance of soil carbon and nitrogen cycle-related genes. A plastic film is very effective in controlling the growth of weeds, limiting soil water evaporation, and increasing crop production (Wu 2002; Steinmetz et al. 2016); however, the long-term use of plastic film in soil decreased soil TN and SOM, while EC content significantly increased in plastic film-exposed soil, indicating a high degree of soil salinization (Rhoades et al. 1990). These results indicated that the RPF in soil can be considered a pollutant during long-term exposure in fields.

In the present study, RPF also significantly reduced S-β-GC activity, owing to the inhibitory effect of the contaminants of RPF and the reduction of the organic substrate (Chen et al. 2009; Xie et al. 2010). S-UE, a key component in the soil nitrogen cycle found in bacteria, fungi, and higher plants (Samborska et al. 2004), was also reduced significantly in plastic film-covered soil. Reduction in S-β-GC and S-UE levels has been reported to exert a negative effect on soil fertility and the nitrogen–carbon cycle (Qian et al. 2007b; Zhou et al. 2005). sDHA and S-ACP were considered indicators of soil microbial activity and functional diversity. The effects of plastic film on S-ACP and sDHA activities were not remarkable, which may be attributed to the adaptation of microbes to the long-term exposure to plastic film residue; however, plastic film can affect S-ACP and S-UE activity during short-term exposure (Xie et al. 2010).

Soil carbon is mainly divided into two parts: one part is derived from carbon fixation by microorganisms, and the other is the organic matter decomposed by soil microorganisms. In the present study, a carbon-fixation gene (*cbb*L) and two carbon source hydrolase-coding genes (β-*glu* and *chi*-A) in plastic film-covered soil decreased to different degrees in accordance with changes in corresponding enzyme activities of β-glucosidase and S-Chi. These results indicated that soil carbon cycle-related genes and enzymatic activity were negatively correlated with the presence of plastic film residue in soil, which may have contributed to reductions in SOM content and soil fertility.

Urease is a key component in the nitrogen cycle in soil and is commonly expressed in bacteria (Zhou et al. 2005). Figure 1 shows that urease activity was significantly inhibited. The inhibition of urease might be attributed to disruption by pollutants in RPF. Similarly, some previous studies reported that PEs in plastic film can inhibit urease and β-glucose activities in the soil (Wang et al. 2009; Xie et al. 2010). Upadhyay et al. (2009) reported that *Bacillus* also has nitrogen fixation capacity. A nitrogen fixation gene (*nif*H) was more abundant in plastic film-covered soil than in control soil, probably owing to the increase in *Azotobacter*. Furthermore, RPF significantly increased the abundance of denitrification genes, which may be because of the increase in the abundance of denitrifying microorganisms in the plastic film-covered soil. In this study, the gene copy numbers of *nir*S and *nos*Z, but not those of *nir*K, were positively correlated with S-NR activity, indicating that *nir*S-type and *nos*Z-type denitrifiers contribute more to nitrate reduction. This finding indicated that *nir*S-type and *nos*Z-type denitrification was more active than the *nir*K-type. Overall, these findings indicated that the functional communities involved in denitrification respond differently to plastic film-covered soils, which may affect soil fertility (Tao et al. 2018).

4.2 Bacterial Community, Diversity, and Richness in Two Different Residual Plastic Film-Exposed Soils

Illumina sequencing results for 16S rDNA revealed that the bacterial community in plastic film-covered soil was notably different from that in control soil. The change in bacterial composition between plastic film-exposed soil and control soil showed that the effects of the plastic film can alter the soil microbiome to contain more abundant *Proteobacteria*, *Bacteroidetes*, *Gemmatimonadetes*, and *Nitrospirae* than in control soil. A high abundance of denitrification genus was observed for *Proteobacteria* and *Bacteroidetes* (Heylen et al. 2006). In our study, *nir*S and *nos*Z abundance increased significantly in plastic film-covered soil. *Gemmatimonadetes* are well adapted to not only arid but also oligotrophic conditions (Hanada and Sekiguchi 2014), which indicates that RPF changed soil fertility and resulted in the generation of tolerant microorganisms in an unhealthy environment. *Nitrospirae* are nitrite-oxidizing bacteria ubiquitous in terrestrial environments that play major roles in biological nitrogen cycling and soil nitrification in agricultural ecosystems (Xia et al. 2011). In the present study, abundances of some bacterial phyla in plastic film-covered soils differed significantly from those in control soil, indicating a gradual adaptation to the plastic film contamination.

PCA revealed that most of the altered soil microorganisms were significantly affected by RPF. *Bacillus*, which has high anti-fungal ability, also has nitrogen fixation capacity (Son et al. 2009). *Xanthomonas* are bacterial plant pathogen (Da et al. 2002). *Sphingomonas* strains are often isolated from contaminated soils as degraders of polycyclic aromatic hydrocarbons and can be considered important biocatalysts for soil bioremediation (Leys et al. 2004). The application of harmful substances will inevitably cause the emergence of certain species of bacteria (Cai et al. 2015). The abundance of these genera in plastic film-covered soil was significantly higher than that in control soil; these results indicated that RPF increased the number of degrading bacteria and may increase the risk of pest infestation in crops. *Sphingomonas* and *Pseudomonas* can promote nitrate reduction to produce nitrogen (Chen et al. 2017b; Tao et al. 2018). *Pseudomonas* is also a microbial surfactant that promotes denitrification in the nitrogen cycle (Chen et al. 2017a, b; Wang et al. 2017). These genera were more abundant in plastic film-covered soil, indicating that RPF increases the abundance of denitrifying microorganisms, thereby accelerating the soil denitrification process. The application of harmful substances will inevitably cause the emergence of certain species of bacteria (Cai et al. 2015). Nitrogen-fixing bacteria, degrading bacteria, and some plant pathogen bacteria are also increased in our study, and with the prolongation of the plastic film pollution time, the decrease of the pollutant concentration, and the cross of the soil under the natural environment, some microorganisms in polluted soil may be gradually restored. This maybe the reason that microorganism diversity was increased after RPF exposure.

5 Conclusion

Using two types of soils with different levels of plastic film residue, soil physicochemical properties, organic matter content, nitrogen content, microbial community structure, enzymatic activities, carbon and nitrogen cycle-related genes, and fertility were determined to be associated with the presence of RPF. Specifically, RPF pollution decreased the SOM and soil TN content, regulated the activity of carbon and nitrogen cycle-related genes in soil, and hence reduced soil fertility. Long-term RPF pollution caused some bacteria to adapt to the environment and increase in abundance, and the distribution and relative abundance of these microorganisms also changed significantly.

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References

Avidano, L., Gamalero, E., Cossa, G. P., & Carraro, E. (2005). Characterization of soil health in an Italian polluted site by using microorganisms as bioindicators. *Applied Soil Ecology, 30*, 21–33.

Bergkemper, F., Scholer, A., Engel, M., Lang, F., Kruger, J., Schloter, M., & Schulz, S. (2016). Phosphorus depletion in forest soils shapes bacterial communities towards phosphorus recycling systems. *Environmental Microbiology, 18*, 1988–2000.

Cai, Z., Wang, J., Ma, J., Zhu, X., Cai, J., & Yang, G. (2015). Anaerobic degradation pathway of the novel chiral insecticide paichongding and its impact on bacterial communities in

soils. *Journal of Agricultural and Food Chemistry, 63*, 7151–7160.

Chen, H., Yao, J., Wang, F., Choi, M. M., Bramanti, E., & Zaray, G. (2009). Study on the toxic effects of diphenol compounds on soil microbial activity by a combination of methods. *Journal of Hazardous Materials, 167*, 846–851.

Chen, H., Zhuang, R., Yao, J., Wang, F., & Qian, Y. (2013). A comparative study on the impact of phthalate esters on soil microbial activity. *Bulletin of Environmental Contamination and Toxicology, 91*, 217–223.

Chen, Q., Li, T., Gui, M., Liu, S., Zheng, M., & Ni, J. (2017a). Effects of ZnO nanoparticles on aerobic denitrification by strain *Pseudomonas* stutzeri PCN-1. *Bioresource Technology, 239*, 21–27.

Chen, S., Li, X., Lavoie, M., Jin, Y., Xu, J., Fu, Z., & Qian, H. (2017b). Diclofop-methyl affects microbial rhizosphere community and induces systemic acquired resistance in rice. *Journal of Environmental Sciences (China), 51*, 352–360.

Da, S. A., Ferro, J. A., Reinach, F. C., Farah, C. S., Furlan, L. R., & Quaggio, R. B. (2002). Comparison of the genomes of two xanthomonas pathogens with differing host specificities. *Nature, 417*, 459–463.

Delgado-Baquerizo, M., Grinyer, J., Reich, P. B., Singh, B. K., & Allen, E. (2016). Relative importance of soil properties and microbial community for soil functionality: insights from a microbial swap experiment. *Functional Ecology, 30*, 1862–1873.

Doran, J. W., Karlen, D. L., & Weinhold, B. J. (2003). Soil quality: humankind's foundation for survival. *Journal of Soil & Water Conservation, 58*, 171–179.

Espí, E., Salmerón, A., Fontecha, A., García, Y., & Real, A. I. (2006). Plastic films for agricultural applications journal of plastic. *Journal of Plastic Film & Sheeting, 22*, 85–102.

Fu, X., & Du, Q. (2011). Uptake of Di-(2-ethylhexyl) phthalate of vegetables from plastic film greenhouses. *Journal of Agricultural and Food Chemistry, 59*, 11585–11588.

González-Pelayo, O., Andreu, V., Campo, J., Gimeno-García, E., & Rubio, J. L. (2006). Hydrological properties of a mediterranean soil burned with different fire intensities. *Catena, 68*, 186–193.

Hanada, S., & Sekiguchi, Y. (2014). *The phylum gemmatimonadetes* (pp. 677–681). Berlin: Springer.

Heylen, K., Gevers, D., Vanparys, B., Wittebolle, L., Geets, J., Boon, N., & De Vos, P. (2006). The incidence of *nir*S and *nir*K and their genetic heterogeneity in cultivated denitrifiers. *Environmental Microbiology, 8*, 2012–2021.

Jambeck, J. R., Geyer, R., Wilcox, C., Siegler, T. R., Perryman, M., & Andrady, A. (2015). Marine pollution. Plastic waste inputs from land into the ocean. *Science, 347*, 768–771.

Kennedy, A. C., & Smith, K. L. (1995). Soil microbial diversity and the sustainability of agricultural soils. *Plant & Soil, 170*, 75–86.

Kong, S., Ji, Y., Liu, L., Chen, L., Zhao, X., & Wang, J. (2012). Diversities of phthalate esters in suburban agricultural soils and wasteland soil appeared with urbanization in China. *Environmental Pollution, 170*, 161–168.

Leys, N. M., Ryngaert, A., Bastiaens, L., Verstraete, W., Top, E. M., & Springael, D. (2004). Occurrence and phylogenetic diversity of sphingomonas strains in soils contaminated with polycyclic aromatic hydrocarbons. *Applied & Environmental Microbiology, 70*, 1944–1955.

Li, X., Ke, M., Zhang, M., Peijnenburg, W. J. G. M., Fan, X., & Xu, J. (2018). The interactive effects of diclofop-methyl and silver nanoparticles on Arabidopsis thaliana: growth, photosynthesis and antioxidant system. *Environmental Pollution, 232*, 212–219.

Liu, E. K., He, W. Q., & Yan, C. R. (2014). 'White revolution' to 'white pollution'—agricultural plastic film mulch in China. *Environmental Research Letters, 9*(9), 091001.

Lu, T., Zhu, Y., Xu, J., Ke, M., Zhang, M., Tan, C., et al. (2018). Evaluation of the toxic response induced by azoxystrobin in the non-target green alga chlorella pyrenoidosa. *Environmental Pollution, 234*, 379–388.

Qian, H., Hu, B., Cao, D., Chen, W., Xu, X., & Lu, Y. (2007a). Bio-safety assessment of validamycin formulation on bacterial and fungal biomass in soil monitored by real-time PCR. *Bulletin of Environmental Contamination & Toxicology, 78*, 239–244.

Qian, H., Hu, B., Wang, Z., Xu, X., & Hong, T. (2007b). Effects of validamycin on some enzymatic activities in soil. *Environmental Monitoring and Assessment, 125*, 1–8.

Raya-Moreno, I., Canizares, R., Domene, X., Carabassa, V., & Alcaniz, J. M. (2017). Comparing current chemical methods to assess biochar organic carbon in a Mediterranean agricultural soil amended with two different biochars. *Science of the Total Environment, 598*, 604–618.

Rhoades, J. D., Shouse, P. J., Alves, W. J., Manteghi, N. A., & Lesch, S. M. (1990). Determining soil salinity from soil electrical conductivity using different models and estimates. *Soil Science Society of America Journal, 54*, 46–54.

Rong, Y., Wang, Y., Guan, Y., Ma, J., Cai, Z., & Yang, G. (2017). Pyrosequencing reveals soil enzyme activities and bacterial communities impacted by graphene and its oxides. *Journal of Agricultural and Food Chemistry, 65*, 9191–9199.

Samborska, A., Stepniewska, Z., & Stepniewski, W. (2004). Influence of different oxidation states of chromium (VI, III) on soil urease activity. *Geoderma, 122*, 317–322.

Sen, F., Uzunsoy, I., Basturk, E., & Kahraman, M. V. (2017). Antimicrobial agent-free hybrid cationic starch/sodium alginate polyelectrolyte films for food packaging materials. *Carbohydrate Polymers, 170*, 264–270.

Son, S. H., Khan, Z., Kim, S. G., & Kim, Y. H. (2009). Plant growth-promoting rhizobacteria, *Paenibacillus polymyxa* and *Paenibacillus lentimorbus* suppress disease complex caused by root-knot nematode and fusarium wilt fungus. *Journal of Applied Microbiology, 107*, 524–532.

Song, H., Xu, J., Lavoie, M., Fan, X., Liu, G., & Sun, L. (2017). Biological and chemical factors driving the temporal distribution of cyanobacteria and heterotrophic bacteria in a eutrophic lake (west lake, China). *Applied Microbiology and Biotechnology, 101*, 1–12.

Steinmetz, Z., Wollmann, C., Schaefer, M., Buchmann, C., David, J., & Tröger, J. (2016). Plastic mulching in agriculture. Trading short-term agronomic benefits for long-term soil degradation? *Science of the Total Environment, 550*, 690–705.

Tao, R., Wakelin, S. A., Liang, Y., Hu, B., & Chu, G. (2018). Nitrous oxide emission and denitrifier communities in drip-irrigated calcareous soil as affected by chemical and organic fertilizers. *Science of the Total Environment, 612*, 739–749.

Upadhyay, S. K., Singh, D. P., & Saikia, R. (2009). Genetic diversity of plant growth promoting rhizobacteria isolated

from rhizospheric soil of wheat under saline condition. *Current Microbiology, 59*, 489–496.

Wang, B., Wang, Q., Liu, W., Liu, X., Hou, J., & Teng, Y. (2017). Biosurfactant-producing microorganism *Pseudomonas sp*. SB assists the phytoremediation of DDT-contaminated soil by two grass species. *Chemosphere, 182*, 137–142.

Wang, J., Lv, S., Zhang, M., Chen, G., Zhu, T., & Zhang, S. (2016). Effects of plastic film residues on occurrence of phthalates and microbial activity in soils. *Chemosphere, 151*, 171–177.

Wang, L., Jiang, G. B., Cai, Y. Q., He, B., Wang, Y. W., & Shen, D. Z. (2007). Cloud point extraction coupled with HPLC-UV for the determination of phthalate esters in environmental water samples. *Journal of Environmental Sciences, 19*, 874–878.

Wang, X., Yuan, X., Hou, Z., Miao, J., Zhu, H., & Song, C. (2009). Effect of di-(2-ethylhexyl) phthalate (DEHP) on microbial biomass C and enzymatic activities in soil European. *Journal of Soil Biology, 45*, 370–376.

Wei, Q., Hu, C., & Oenema, O. (2015). Soil mulching significantly enhances yields and water and nitrogen use efficiencies of maize and wheat: a meta-analysis. *Scientific Reports, 5*, 2045–2322.

Wu, D., Senbayram, M., Well, R., Brüggemann, N., Pfeiffer, B., & Loick, N. (2017). Nitrification inhibitors mitigate N_2O emissions more effectively under straw-induced conditions favoring denitrification. *Soil Biology and Biochemistry, 104*, 197–207.

Wu, P. (2002). Water and nitrogen use efficiency of lowland rice in ground covering rice production system in south China. *Journal of Plant Nutrition, 25*, 1855–1862.

Xia, W., Zhang, C., Zeng, X., Feng, Y., Weng, J., & Lin, X. (2011). Autotrophic growth of nitrifying community in an agricultural soil. *The ISME Journal, 5*, 1226–1236.

Xie, H. J., Shi, Y. J., Zhang, J., Cui, Y., Teng, S. X., & Wang, S. G. (2010). Degradation of phthalate esters (PAEs) in soil and the effects of PAEs on soil microcosm activity. *Journal of Chemical Technology & Biotechnology, 85*, 1108–1116.

Xu, G., Li, F., & Wang, Q. (2008). Occurrence and degradation characteristics of dibutyl phthalate (DBP) and di-(2-ethylhexyl) phthalate (DEHP) in typical agricultural soils of China. *Science of the Total Environment, 393*, 333–340.

Zhou, Q. H., Wu, Z. B., Cheng, S. P., He, F., & Fu, G. P. (2005). Enzymatic activities in constructed wetlands and di-n-butyl phthalate (DBP) biodegradation. *Soil Biology and Biochemistry, 37*, 1454–1459.