Greenhouse Gas Emissions and Bacterial Community Structure as Influenced by Biodegradable Film Mulching in Eastern China

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Abstract: Machine transplanting technology of biodegradable films has solved the problems of the higher cost of artificial film and the serious environmental pollution of polyethylene film residue. Previous studies have shown the positive impact of mulching on mitigating global warming potential. However, the mechanisms underlying the association between greenhouse gas emissions and the bacterial community structure in paddy field soil with biodegradable film mulching (BM) still remain limited. In this study, greenhouse gas emissions and the associated bacterial community in non-mulching, biodegradable mulching in a paddy field in Eastern China were analyzed over the 2019 and 2020 rice growing seasons. Rice mulching cultivation significantly inhibited CH4 emissions from a rice paddy, mainly due to the significant reduction in methane emission peaks. Film mulching significantly increased the diversity of the bacterial community as revealed by 16S rRNA gene sequencing. The relative abundance of methanogens was decreased, while the relative abundance of methanotrophs was increased in the paddy soil due to the BM treatment, with the change pattern basically consistent with CH4 emissions. The N2O emissions during the growth period showed a pronounced downward trend. However, the total abundance of bacteria involved in nitrification and denitrification was higher under BM. Mulching cultivation improved the soil nutrient availability and significantly increased the yield by 5.0%. BM inhibited the greenhouse gas emission intensity (GHGI) of the paddy field by 46.9%. Film mechanical transplanting could promote yield increases and significantly mediate the warming potential (GWP) of greenhouse gases in the paddy fields of the Middle-Lower Yangtze Area. The rational use of film mechanical transplanting would play a role in carbon neutrality in paddy fields. This study provided a theoretical basis for paddy field emission reduction and sustainable agricultural development.

Keywords: bacterial community structure; biodegradable film; greenhouse gas; rice; yield

1. Introduction

Global warming due to rising atmospheric greenhouse gas concentrations has become a major environmental issue. Global atmospheric greenhouse gas levels have increased significantly due to human activities, such as the burning of fossil fuels, livestock and various agricultural activities, and deforestation [1]. Farmland ecosystems are the main source of greenhouse gas emissions, producing nearly 8% and 32% of the global anthropogenic emissions of CH4 and N2O [2,3]. However, nearly 50% of cropland methane emissions and 10% of cropland nitrous oxide emissions come from rice paddies [4,5]. Therefore, it is critical to reduce greenhouse gas emissions from paddies while ensuring rice yields.
Greenhouse gas (GHG) emissions are affected by a wide range of factors, including fertilization, crop types, tillage systems, irrigation, climate change, and soil physicochemical properties, each of which plays major roles in GHG emissions [6–9]. For example, rice varieties are important factors influencing GHG emissions. The differences in CH₄ and N₂O emissions between rice varieties were 6 and 14 times, respectively [10]. It was estimated that CH₄ and N₂O emissions in paddy fields are approximately four times those of dryland systems [11]. Various agronomic practices can also directly affect GHG emissions of CO₂, CH₄, and N₂O [12]. The total methane (CH₄) emissions from rice fields account for approximately 7–17% of total global CH₄ emissions [13]. Factors, such as the tillage patterns, microbial activity, fertilization, and soil microbial carbon (SMC) stocks, determine whether the soil is a source or sink of methane emissions [12,14]. The soil moisture, temperature, and volumetric capacity alter the activities of methanogenic bacteria and methanotrophs, in turn affecting the direction of CH₄ emission or absorption [15]. Methane emission is closely related to microbial methanogens and methanotrophs that are affected by the soil oxygen content [16]. The flooding conditions in continuously flooded rice production systems create an anoxic environment that favors methane production by anaerobic methanogenic archaea, leading to methane emissions [17]. The nitric oxide from agricultural soils is a major source of atmospheric N₂O and is expected to account for about 60% of global emissions by 2030 [18]. Paddy fields are an important source of increased atmospheric N₂O concentrations, and how to address N₂O emissions from rice paddies is a key issue [4,5,19]. Various studies have shown that the emission of nitrous oxide from soil is largely controlled by the nitrification and denitrification processes, where nitrogenous compounds are converted to nitrous oxide by nitrification and denitrification-related microorganisms [20–22]. The degree of soil anaerobiosis and the nitrate content influence the soil nitrification and denitrification processes [23]. Under anaerobic conditions, nitric oxide is released as an intermediate compound when nitrite is reduced to dinitrogen gas in the presence of denitrifying microorganisms. Under aerobic conditions, denitrifying microorganisms release N₂O when they reduce nitrite to dinitrogen gas. Under aerobic conditions, nitric oxide is released when autotrophic nitrifying bacteria convert nitrite to nitrate [12].

The mechanized planting technology of a biodegradable plastic film for rice has been continuously developing, as it has advantages, such as water saving and drought resistance, increasing temperature and soil moisture, inhibiting weeds and controlling pests and diseases, and improving the nitrogen utilization efficiency [24–27]. However, the effect of film mulching on GHG emissions from rice fields has varied among trials; for example, mulching reduced methane emissions by 45–85%, but it increased N₂O emissions compared with the controls [28]. Yao et al. (2017) showed that mulching reduced GHG emissions compared with conventional treatments, with a 54% reduction in total CH₄ and N₂O emissions [29]. Plastic film mulching (PFM) may have the potential to increase GHG emissions because it increases the microbial activity of the soil [30]. A meta-analysis showed that mulching significantly increased crop yields by 48.6%, reduced CH₄ emission in 64.2% of paddy fields, and reduced CH₄ uptake in 16.1% of drylands, but increased soil N₂O emissions by 23.9% [31].

The results of previous studies on greenhouse gas emissions under plastic film mulching have been divergent, and the effects of biodegradable film mulching on greenhouse gas emissions from paddy fields remain poorly understood. Therefore, a 2-year field experiment was performed in a rice paddy field in eastern China to investigate the influencing mechanisms associated with biodegradable film mulching on soil greenhouse gas emissions and the bacterial community structure. The expectations were as follows: (1) to clarify the effects of biodegradable film cultivation on greenhouse gas emissions from rice fields; (2) to explore the changes of soil-related microorganisms under mulching conditions; and (3) to reveal the influential relationships between the soil microorganisms, soil nutrients, and greenhouse gas emissions.
2. Materials and Methods

2.1. Experimental Site and Materials

Field experiments were conducted in 2019–2020 at the Fuyang Experimental Base of the China Rice Research Institute (30°5′ N, 119°55′ E) located in the Middle-Lower Yangtze Area, a mid-latitude subtropical monsoonal climate zone. The experimental site has clayey rice soils, and the organic matter content of the 0–20 cm soil layer was 52.0 g·kg⁻¹, total N 2.66 g·kg⁻¹, total P 0.82 g·kg⁻¹, available P 19.7 mg·kg⁻¹, and available K 138.5 mg·kg⁻¹. The biodegradable film was supplied by BASF. The film had a width of 1.8 m and a thickness of 0.01 mm. The degradation started at around 45 days after mulching and was completed during the rice growing season. The performance of this type of biodegradable film complies with the national standard for Biodegradable Agricultural Mulch Film (GB/T 35795-2017). The biodegradable film used in this study was provided by BASF SE, with a weight of approximately 12 kg per roll and the ability to cover an area of roughly 1000 square meters.

2.2. Experimental Design and Treatments

Rice seeds (Oryza sativa L., cv. Yongyou 538) were obtained from Zhejiang Ningbo Seeds Co., LTD., Hangzhou, China. The experiment consisted of two treatments: biodegradable film mulching (BM) and no film mulching (CK). The plots are designed in randomized blocks with three replicates per treatment. The area of each plot is 70.2 m² (18 m × 3.9 m). The experimental design ensured that the same fertilizers for the two treatments were equal during a single rice growth period. The fertilizer, applied once, contained 240 kg ha⁻¹ of pure N, 450 kg ha⁻¹ of calcium superphosphate (containing P₂O₅ ≥ 12.5%), and 225 kg ha⁻¹ of potassium chloride (containing K₂O ≥ 57%). The seeds were screened before sowing to sift out unsaturated seeds. The seeds were dried after 48 h of chemical immersion and kept at a 30–35% seed humidity. After sowing, the seeds were dark germinated in a stacked tray for 48 h (dark room constant temperature 32 °C, air relative humidity 100%), and the seeds were placed in the field for seedling cultivation. At 20 days, the seedlings were planted by machine using a Yanmar rice transplanter (Yanmar Holdings Co., Ltd., Suzhou, China) with a hanging mulching machine. The application of the film involved an initial laying onto the rice field using a specialized film-mulching machine, followed by perforation using a punching machine. The seedlings were then inserted into the punched holes using a transplanting machine to complete the mulching and planting process. The row and plant spacing was 30 cm × 16 cm for a density of 208,000 clusters per hectare.

2.3. Sample Collection and Analysis

2.3.1. CH₄ and N₂O Sampling and Analysis

Methane and nitric oxide were collected and measured in the rice fields using a static box-gas chromatography method. The gas sampling chamber consisted of a stainless-steel base and a sampling box consisting of two parts, a middle box and a top box, both 50 cm high with a 50 cm × 50 cm bottom area, made of stainless steel, and insulated with a sponge and tin foil wrapping as a means of maintaining a constant temperature inside the chamber. The base and the top of the middle chamber were equipped with a water tank for sealing. The static box was covered with a stainless-steel base that had been buried approximately 15 cm deep in the field during sampling. The greenhouse gases were collected every 5–10 days according to the actual weather conditions, with each sampling time fixed at 09:00–11:00 a.m. The sampling time points were 0, 10, 20, and 30 min after the placement of the cover box, with each automatic sampling volume being approximately 500 mL, and the temperature inside the closed box was recorded. The gas samples were analyzed by a GC-2030 gas chromatograph. The detection conditions were FID for CH₄ at 200 °C and 60 °C and ECD for N₂O at 300 °C and 60 °C. The flow rates of nitrogen, hydrogen, and air were 30 mL·min⁻¹, 40 mL·min⁻¹, and 400 mL·min⁻¹, respectively.
2.3.2. Soil Sample Collection and Analysis

The soil samples were taken from the cultivated layer (0–20 cm) at the panicle initiation stage and full heading stage, according to the five-point sampling method, and then mixed after removing impurities. The mixed soil was filtered through a 2 mm sieve and packed into sterile 2.0 mL centrifuge tubes. Then, the soil sample (about the size of peanut rice, no more than 1/3 of the volume) was taken from each tube into sterile 2.0 mL centrifuge tubes, and 3–5 tubes of each sample were obtained as backup, and were immediately transferred to –80 °C for storage. The physical and chemical properties of the soil in the experiments were determined as shown in Figure S1 and Table S1 of the Supplementary data.

For the genomic DNA extraction, microbial community DNA was extracted using the NucleoSpin Soil Kit (Macherey-Nagel, Düren, Germany) in accordance with the manufacturer’s instructions. The extracted DNA was then quantified using a Qubit Fluometer and a Qubit dsDNA BR Assay kit (Invitrogen, Carlsbad, USA). The PCR amplification was performed using degenerate primers, 341F (5’-ACTCCTACGGGAGGCACAG-3’) and 806R (5’-GGACTACHVGGGTWTCTAAT-3’), to amplify the V3-V4 variable regions of the bacterial 16S rRNA gene. Both the forward and reverse primers were labeled with linker sequences, padding, and Illumina adapters. After amplification, the libraries were qualified using an Agilent 2100 bioanalyzer (Agilent, Palo Alto, USA) before sequencing. Validated libraries were used for sequencing according to the Illumina standard pipelines to generating 2 × 300 bp paired-end reads on the Illumina MiSeq platform (BGI, Shenzhen, China). The original reads were filtered to remove low-quality bases and adaptors. Paired-end reads were added to tags by the Fast Length Adjustment of Short reads program (FLASH, v1.2.11) to obtain the tags. The tags were clustered into OTUs with a cutoff value of 97% using UPARSE software (v7.0.1090) and the chimera sequences were detected by comparison with the Gold database using UCHIME (v4.2.40). The OTU representative sequences were classified using a Ribosomal Database Project (RDP) Classifier v2.2 with a minimum confidence threshold of 0.6, and trained on the Greengenes database v201305 by QiIME v1.8.0. All tags were back-tracked to OTUs using USEARCH_global to obtain the statistical table of OTU abundance for each sample. The alpha diversity was estimated by MOthur (v1.31.2) at the OTU level. The Venn plots were plotted with R package “VennDiagram” version 3.1.1. The redundancy analysis was plotted with R 4.1.1. The heatmap was plotted with R package v3.4.1 and R package “gplots”.

2.3.3. Plant Sample Collection and Analysis

Each plot was randomly assigned 6.67 m² for yield measurement at the maturing stage. After single beating and sun drying, the quality and moisture content of the rice were determined, and then converted to a standard moisture content of 14.5% to be recorded as the yield at harvest.

2.4. Methane and Nitric Oxide Data Calculations

The emission flux of greenhouse gases is determined by calculating the rate of change in the gas concentration over time within an enclosed container. The formula for this calculation is presented as follows:

\[
F = \eta \times H \times \frac{\Delta c}{\Delta t} \times \frac{273}{273+T}
\]

In this formula, \(F\) is the emission flux; \(\eta\) is the density of the gas at standard conditions (1.25 kg·m⁻³ for N₂O and 0.714 kg·m⁻³ for CH₄); \(H\) is the height of the sampling chamber; \(\Delta c/\Delta t\) is the rate of change of the gas concentration in the chamber over time during sampling (mL·m⁻³·h⁻¹); \(T\) is the average temperature in the chamber at the time of sampling (°C); and 273 is the gas equation constant.

Global warming potential (GWP) is an index that quantifies the potential of a substance to contribute to the greenhouse effect, expressed as the mass of CO₂ that would
produce an equivalent effect to the greenhouse gas in question over a period of 100 years. The formula for this calculation is presented as follows:

\[ \text{GWP} = F_{\text{CH}_4} \times 25 + F_{\text{N}_2\text{O}} \times 298 \]

In this formula, \( F \) is the total emission of greenhouse gases on a time scale of 100 years; the global warming potential (GWP) per unit mass of \( \text{CH}_4 \) and \( \text{N}_2\text{O} \) is 25 and 298 times that of \( \text{CO}_2 \) [32].

The greenhouse gas emission intensity (GHGI) is an indicator that assesses the overall greenhouse effect of different treatments by comparing the warming potential of \( \text{CH}_4 \) and \( \text{N}_2\text{O} \) to the rice yield. The formula for this calculation is presented as follows:

\[ \text{GHGI} = \frac{\text{GWP}}{Y} \]

In this formula, \( \text{GWP} \) is the global warming potential (t ha\(^{-1}\), calculated as \( \text{CO}_2 \)), and \( Y \) is the crop yield (t ha\(^{-1}\)) [33].

2.5. Statistical Analyses

The data were analyzed using SAS version 9.0 (SAS Institute, Cary, NC, USA). One-way analysis of variance (ANOVA) was used to compare the means of each treatment using a 5% probability level, according to standard procedures. A least significant difference (Duncan’s) test was used to identify significant differences among the treatment means. All figures were prepared using SigmaPlot 10.0 (Systat Software, Inc., London, UK).

3. Results

3.1. The Dynamics of \( \text{CH}_4 \) Emission Fluxes

This graph showed the change of \( \text{CH}_4 \) emission flux in the rice field after transplanting. During the entire growth period of rice, the \( \text{CH}_4 \) emission flux from the paddy field had significant periodic variation, primarily in the early flooding period of rice after transplanting (Figure 1). However, there was a seasonal difference in the \( \text{CH}_4 \) emissions from two rice fields. In 2019, the \( \text{CH}_4 \) emission fluxes showed emission peaks at 28 and 56 d after transplanting in the CK and BM treatments. The \( \text{CH}_4 \) emission fluxes showed the highest values of 39.5 and 10.1 mg m\(^{-2}\)h\(^{-1}\) in the CK treatment. However, the peak \( \text{CH}_4 \) emission of BM treatment was significantly lower than that of the CK treatment, and were 17.1 and 5.65 mg m\(^{-2}\)h\(^{-1}\), respectively. The \( \text{CH}_4 \) emission fluxes showed clear emission peaks at 27 and 77 d after transplanting in 2020, with the highest values of 33.7 and 6.72 mg m\(^{-2}\)h\(^{-1}\) in the CK treatment, respectively. The \( \text{CH}_4 \) emission peaks of rice fields in the BM treatment appeared at 27 and 57 d after transplanting, and the peaks were significantly lower than those in the CK treatment; they were 19.3 and 15.3 mg m\(^{-2}\)h\(^{-1}\), respectively. The peaks of the BM treatment were significantly reduced by 42.7% at 27 d, while the peaks of the BM treatment were increased by 56.1% at 57 d after transplanting.
3.2. The Dynamics of N₂O Emission Fluxes

This graph shows the change of N₂O emission flux in the rice field after transplanting. The BM mulching altered the characteristics of N₂O emission in the growth period, with emissions concentrated in the middle and late growth stages. There were three and two emission peaks in the rice growing seasons of 2019 and 2020, respectively (Figure 2). The emission peak of the BM treatment was 56.3 mg·m⁻²·h⁻¹ on the 35th day after transplanting, and the emission peak of the CK treatment was 171.2 mg·m⁻²·h⁻¹ on the 63rd day after transplanting. The peak N₂O emission from the rice field decreased by 67.1% in the BM treatment compared with CK. On the 27th day after transplanting in 2020, the BM and CK treatments showed emission peaks with values of 31.5 and 34.4 mg·m⁻²·h⁻¹, while there was no significant difference between the BM and CK treatments. However, the peak N₂O emission of the BM treatment was 91.3 mg·m⁻²·h⁻¹ at 77 d after transplanting, significantly lower than that of CK treatment, and the peak was decreased by 42.7% compared with CK.

3.3. Yield and Greenhouse Gas Intensity

As shown in Table 1, the BM treatment could effectively reduce the CH₄ emissions from the paddy fields. Compared with CK, the BM treatment significantly reduced CH₄ emissions.
emissions by 44.9% and 41.8% in 2019 and 2020, respectively. The effect of BM treatment on N₂O emissions was similar to that of CH₄ emissions in paddy fields. Compared with CK, the N₂O emissions were significantly reduced by 58.1% in 2019. During the 2-year rice growing season, the GWP and GHGI were lower than in the conventional paddies under the BM treatments, with decreases of 44.8% and 46.9%, respectively. Compared with conventional paddy cultivation, averaged across both of the rice seasons, the shoot biomass and grain yield were significantly increased due to the biodegradable film mulching by 11.9% and 5.0%, respectively (Table 1).

Table 1. The average values of seasonal CH₄ emissions, N₂O emissions, shoot biomass, grain yield, GWP, and GHGI showing their responses to the mulching cultivation.

<table>
<thead>
<tr>
<th>Year</th>
<th>Treatment</th>
<th>CH₄ Emissions</th>
<th>N₂O Emissions</th>
<th>Shoot Biomass</th>
<th>Grain Yield</th>
<th>GWP</th>
<th>GHGI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>(kg ha⁻¹)</td>
<td>(kg ha⁻¹)</td>
<td>(t ha⁻¹)</td>
<td>(t ha⁻¹)</td>
<td>(kg ha⁻¹)</td>
<td>(kg·ha⁻¹)</td>
</tr>
<tr>
<td>2019</td>
<td>CK</td>
<td>204.68 ± 13.03 a</td>
<td>0.74 ± 0.04 a</td>
<td>28.51 ± 2.45 a</td>
<td>10.59 ± 0.04 b</td>
<td>7331.81 ± 220.53 a</td>
<td>0.69 ± 0.02 a</td>
</tr>
<tr>
<td></td>
<td>BM</td>
<td>112.86 ± 10.74 b</td>
<td>0.31 ± 0.01 b</td>
<td>30.26 ± 0.83 a</td>
<td>11.02 ± 0.07 a</td>
<td>3753.44 ± 228.41 b</td>
<td>0.34 ± 0.02 b</td>
</tr>
<tr>
<td>2020</td>
<td>CK</td>
<td>249.87 ± 2.18 a</td>
<td>0.75 ± 0.08 a</td>
<td>24.01 ± 0.10 b</td>
<td>11.44 ± 0.15 b</td>
<td>8503.57 ± 199.38 a</td>
<td>0.74 ± 0.01 a</td>
</tr>
<tr>
<td></td>
<td>BM</td>
<td>145.37 ± 4.11 b</td>
<td>0.47 ± 0.05 a</td>
<td>28.23 ± 0.2 a</td>
<td>12.12 ± 0.07 a</td>
<td>5041.98 ± 175.66 b</td>
<td>0.42 ± 0.02 b</td>
</tr>
</tbody>
</table>

Note: CK: no mulching; BM: biodegradable film mulching. Values are mean ± SE (n = 3). The letters followed by different numbers within a column are significantly different at p < 0.05.

3.4. Abundance and Diversity of Soil Bacteria

Each OTU was usually considered as a microbial species. Totals of 40,762 and 40,990 soil bacterial 16S rRNA sequences were obtained from the CK treatment at the panicle differentiation (PI) and heading date (HD), respectively, and 36,476 and 38,145 analogous sequences were obtained from the BM treatment. The sequences were assigned to 3709, 3814, and 3732, 3978 OTUs, respectively (Figure 3). The chao1 index was an index to calculate the community richness. A larger chao index indicated a higher number of species in the sample. The Chao1 index estimator was used to indicate the species richness, and the Chao1 index of BM was 5.5% higher than that of CK at each growth stage. The Shannon index was an index to calculate the community diversity. Higher Shannon values indicated a higher community diversity. The Shannon index of the BM treatment also showed a significant increase, indicating that BM mulching could increase the bacterial richness in paddy soil to a certain extent. A Venn diagram was used to represent the relationships between multiple data sets. It was important to understand what two or more groups of things have in common, which elements were unique to one or more of these groups, and what characteristics none of these groups exhibited. The Venn diagram of OTUs showed that there were 2114 OTUs shared in the two soil model samples, while 465 and 456 OTUs were found unique to BM and CK at the PI stage, and 434 and 315 OTUs were found unique to BM and CK at the HD stages, respectively (Figure 4).
Figure 3. The diversity indices of the soil bacterial community in a paddy field influenced by the mulching cultivation. Chao1 (a), Shannon (b), Coverage (c), Otu number (d); CK: no mulching; BM: biodegradable film mulching; PI: panicle initiation stage; HD: full heading stage. Values and error bars are the mean ± SE (n = 5), the bars superscripted by different lowercase letters are significantly different at 0.05 level among treatments.

Figure 4. Venn diagram showing the number of unique and common operational taxonomic units for the soil microbes influenced by the mulching cultivation. CK: no mulching; BM: biodegradable film mulching; PI: panicle initiation stage; HD: full heading stage.
3.5. Structure and Composition of the Soil Bacterial Community

The number of OTUs was roughly broken up into 32 phyla, 69 classes, 81 orders, 174 families, and 440 genera. The common dominant bacterial phyla in the paddy soil under the two treatments were: Proteobacteria, Acidobacteria, Chloroflexi, Nitrospirae, Planctomycetes, Actinobacteria, Bacteroidetes, Firmicutes, Gemmatimonadetes, and Verrucomicrobia (Figure 5). The abundance of Proteobacteria was 34.37% and 35.37% in the CK and BM treatments, and the abundance of Nitrospirae was 4.97% and 5.61% in the CK and BM treatments during the PI stage, respectively. BM mulching significantly increased the relative abundance of Cyanobacteria, its abundance in BM being 9.0 times that in CK during the PI stage. However, the abundances of Proteobacteria, Nitrospirae, and Cyanobacteria were the opposite during the HD stage. The relative abundance of Planctomycetes was decreased by 43.4%, while the relative abundance of Bacteroidetes was increased by 54.9% in the BM treatment during the PI stage.

At the genus level, the predominant bacteria (top 10 in relative abundance) in the CK and BM treatments were: Acidobacteria, Thermodesulfovibrio, Bradyrhizobium, Aquisphaera, Geobacter, Rhodoplanes, Sphingomonas, Anaeromysobacter, Gemmatimonas, and Reyranella (Figure 6). Compared with CK, the relative abundance of Gemmatimonas was decreased by 22.9%, and the relative abundance of Sphingomonas was decreased by 39.7% in BM during the PI stage. Thermodesulfovibrio, Anaeromysobacter, and Syntrophorhabdus had the highest relative abundances in BM during the PI stage, while each was lower during the HD stage.
Figure 6. The relative abundances (%) of soil bacterial genera influenced by the mulching cultivation. CK: no mulching; BM: biodegradable film mulching; PI: panicle initiation stage; HD: full heading stage.

Heatmaps showed the variability of data, especially in the face of large data. The heatmap visualization can visualize the distribution or variation of the data. A heatmap showed that the abundances of methanogens and methanotrophs differed significantly among the treatments and different growth stages (Figure 7). CH₄ emission occurred mainly in the early and middle terms of rice growth, and the abundance of methanogens in the soil during the PI stage was higher than that during the HD stage. There were only two groups of methanogens detected in this study, and their abundances in CK were higher than in the BM treatment at the PI stage. The nine groups of methanotrophs detected in paddy soil comprised Type I, Type II, and unclassified methanotrophs. Type I methanotrophs were composed of *Methylocaldum*, *Methylococcus*, *Methylomonas*, *Methylosarcina*, and *Methylobacter*. Type II methanotrophs were composed of *Methylocystis* and the unclassified methanotrophs included *Methyloparacoccus*, *Methyloversatilis*, and *Methylogae*. Compared with CK, the BM treatment had much higher relative abundance of Type I methanotrophs and a correspondingly higher relative abundance of Type II methanotrophs at the PI stage, while the same treatment had lower relative abundance of Type II methanotrophs at the HD stage.
The abundance of most soil organic matter related microorganisms in BM was higher than that in CK, and the abundances of Nocardia, Streptomyces, Syntherohorhabdus, Dehalogenimonas, and Georgiuchia in BM was significantly higher than those in CK. The major nitrogen-fixing bacteria were Kledonobacter, Sideroxydans, and Rhodomicrobium, and their total abundance was higher in BM than in CK. The heatmap shows that the main microbial mediators responsible for regulating the N2O emissions in this study were Flavisolibacter, Thiobacillus, Hyphomicrobium, Nitrosospira, and Nitrosospiro. The abundances of Nitrosospiro and Nitrosospira were lower in BM than that in CK at the PI stage, and the abundance of Nitrosospira was higher in BM than in CK at the HD stage ($p < 0.05$). The abundances of iron-sulfate-reducing bacteria were higher in BM than in CK at the PI stage, while being lower in BM than in CK at the HD stage. The abundance of bacteria involved in iron and sulfur metabolism was increased due to BM mulching, such as Desulfatiglans, Desulfovibrio, Thermodesulfovibrio, and Geobacter. Compared with CK, the abundance of nitrifying bacteria in the BM treatment decreased by 21.5%, while the abundance of denitrifying bacteria increased by 56.8%. At the heading stage, the abundance of soil nitrifying bacteria in the BM treatment increased by 20.3%, while the abundance of denitrifying bacteria decreased by 14.8%.
3.6. Relationships between the Soil Properties and Bacterial Community Composition

Redundancy analysis (RDA) is a ranking method of regression analysis combined with principal component analysis and an extension of multiresponse variable regression analysis. The effects of soil environmental factors on the bacterial community structure after BM mulching were analyzed (Figure 8). The soil variables explained 64.87% of total variation, with the first axis accounting for 36.3% and the second axis accounting for 28.57% at PI stage. The soil variables explained 76.09% of total variation, with the first axis accounting for 56.03% and the second axis accounting for 20.06% at the HD period. The soil bacterial community in the PI stage was influenced by the soil AN and TN, while in the HD stage, the soil bacterial community was influenced by the soil AN and SOM (Table 2). The soil bacterial community structure of CK and BM was primarily related to TP, AN, and TN at the PI stage, while being related to TP, AN, and SOM at the HD stage, according to the carrier analysis. Compared with CK, AN in the BM treatment was increased by 30.4%. The contents of AP, AK, and pH in BM were not significantly different compared with CK.

![Figure 8](image)

Figure 8. Redundancy analyses of the soil bacterial community structures influenced by the mulching cultivation during the PI (a) and HD (b) stages. CK: no mulching; BM: biodegradable film mulching; PI: panicle initiation stage; HD: full heading stage.

<table>
<thead>
<tr>
<th></th>
<th>TN (g kg⁻¹)</th>
<th>TP (g kg⁻¹)</th>
<th>AP (mg kg⁻¹)</th>
<th>AN (mg kg⁻¹)</th>
<th>AK (mg kg⁻¹)</th>
<th>SOM (g kg⁻¹)</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>CK-PI</td>
<td>2.40 ± 0.01</td>
<td>0.75 ± 0.01</td>
<td>23.25 ± 2.14</td>
<td>58.80 ± 1.21</td>
<td>151.04 ± 1.42</td>
<td>52.58 ± 0.49</td>
<td>4.98 ± 0.02</td>
</tr>
<tr>
<td>BM-PI</td>
<td>2.80 ± 0.04</td>
<td>0.76 ± 0.01</td>
<td>23.61 ± 0.86</td>
<td>99.98 ± 1.19</td>
<td>149.23 ± 1.73</td>
<td>53.33 ± 0.46</td>
<td>5.04 ± 0.02</td>
</tr>
<tr>
<td>CK-HD</td>
<td>2.87 ± 0.02</td>
<td>0.84 ± 0.01</td>
<td>18.53 ± 0.49</td>
<td>106.54 ± 2.42</td>
<td>152.72 ± 1.84</td>
<td>53.84 ± 0.40</td>
<td>4.98 ± 0.02</td>
</tr>
<tr>
<td>BM-HD</td>
<td>2.85 ± 0.02</td>
<td>0.86 ± 0.01</td>
<td>18.62 ± 0.44</td>
<td>132.68 ± 2.50</td>
<td>149.14 ± 1.44</td>
<td>54.90 ± 0.21</td>
<td>5.03 ± 0.02</td>
</tr>
</tbody>
</table>

Note: CK: no mulching; BM: biodegradable film mulching. Values are mean ± SE (n = 5). Letters followed by different numbers within a column are significantly different at p < 0.05. SOM, soil organic matter (sulfuric acid–potassium dichromate oxidation method); TN, total nitrogen (Kjeldahl method); AN, available nitrogen (alkali hydrolysis diffusion method); AP, available phosphorus (molybdenum-antimony resistance colorimetric method); AK, available potassium (flame photometry). Soil pH (electrode method).

4. Discussion

The CH₄ fluxes from the soil to the atmosphere were the results of the combined action of three processes, namely, production, transport, and consumption. These processes were closely related to the soil and environmental factors, and affecting any of these factors would alter the CH₄ emissions from the paddy fields [34]. In this study, the mechanical transplanting of BM altered the CH₄ emission characteristics, with significantly reduced CH₄ fluxes from the rice fields in 2019 and 2020, with decreases of 44.9% and 41.8%,
respectively (Figure 1). The main function of the film mulching was to reduce the peak value of CH₄ emissions in the paddy field. To investigate the causes of the greenhouse gas emission reduction, most of the previous studies have started with an abundance of greenhouse gas-related genes. A few studies have further analyzed the relationship between the soil microorganisms and greenhouse gas emissions, but they have also focused on these microorganisms with greenhouse gas-related genes and have not comprehensively analyzed the changes in the soil bacterial community structure.

To investigate the response of microorganisms related to greenhouse gas emissions to mulching measures in paddy fields, the relative abundances of the soil bacterial communities were determined during the growth season (Table 2). The film mulching significantly decreased the relative abundance of methanogens, and increased the relative abundance of methanotrophs compared with the control. Tang et al. (2014) showed that the CH₄ emissions from paddy fields were closely related to the variation in methanogens and methanotrophs [35]. Watanabe et al. (2007) showed that soil methanogens were the key microorganisms for methane production in paddy fields [36]. Methanogens belong to the Euryarchaeota and need to act on soil organic matter to produce CH₄ under extremely anaerobic conditions [37]. Biofilm mulching in paddy fields improved soil oxygen content, and the O₂ exposure increased sulfate and Fe⁢⁺ concentrations in soil. Sulfate-reducing bacteria and iron-reducing bacteria were able to use stronger microbial reduction of inorganic compounds as electron acceptors, thus surpassing the competition of methanogens for substrates H₂ and acetic acid [38,39].

Methanotrophs are obligate aerobic bacteria that use CH₄ as the sole energy and main carbon source [40]. The quantitative changes of the two functional microorganisms affected the production and consumption of CH₄. The soil aeration was a vital factor in changes in the methanogen and methanotroph communities. If the paddy soil had been flooded, it will reduce the soil redox potential, causing the rapid reproduction of methanogens and resulting in large CH₄ emissions. Film mulching does not require water layer in the field, and this improved O₂ availability and promoted the proliferation of a methanotrophic community [38]. The BM treatment significantly increased the relative abundance of type I methanotrophs (Methylocaldum, Methylococcus, Methylomonas, Methylosarcina) in the PI stage. Type I methanotrophs could promote methane oxidation at both low and high concentrations of methane [41]. Type I methanotrophs were more active than type II methanotrophs in the paddy soils, although their overall abundance was lower compared with type II methanotrophs [42]. BM treatment could increase the soil temperature in the early stages of rice growth and improve the activity of soil microorganisms, especially methanotrophs. Flessa et al. (1995) found that the oxidation of CH₄ was significantly positively correlated with temperature [43]. Additionally, the film would directly block or delay the diffusion of CH₄ to the outside during the transport of CH₄, so that the methane-oxidizing bacteria used the undischarged CH₄ as a carbon source to oxidize; this may be one reason why BM reduced the peak CH₄ emission [44].

Further analysis of soil nutrients showed that BM mulching significantly improved the availability of soil nitrogen during the PI and HD growth stages (Table 2). CH₄ fluxes showed a negative correlation with the total nitrogen content [45], and nitrate may stimulate methanotrophs to assimilate methane carbon, as demonstrated using the DNA stable isotope probe method [46]. The available nitrogen in the paddy soil exists largely in the form of ammonium nitrogen, while the content of nitrate nitrogen is less. Film mulching reduced the nutrient loss and promoted soil mineralization, and the higher concentration of NH₄⁺ ions would limit the oxidation of CH₄ due to the oxidation of ammonium to produce toxic hydroxylamine and nitrite substances [47]. Moreover, CH₄ fluxes were significantly and positively correlated with the soil’s organic carbon content as carbon and energy sources of methanogens [48]. About 60–90% of the CH₄ produced in a paddy field is discharged into the atmosphere through plant aerenchyma. As the link between soil and plants, the morphological and physiological characteristics of rice roots also affect CH₄ emission.
Farmland soil is one of the most important N₂O sources, accounting for about 70% of global atmospheric emissions [44]. Different farmland management practices varied greatly in soil N₂O emissions [49]. The total seasonal N₂O emissions from paddy fields under plastic film mulching were significantly higher than those under conventional cultivation [30]. However, biodegradable film mulching significantly decreased N₂O emission by 47.7% during the growth period compared with CK in the study (Table 1). Soil moisture is an important factor affecting the N₂O emissions from paddy fields [50], and its emission flux depends on the variation of soil moisture [51]. The alternation of soil drying and wetting will cause severe nitrification and denitrification, leading to a large amount of N₂O emissions from paddy fields, and long-term flooding of rice fields can significantly reduce N₂O emissions [52]. The amount of ammonium and oxygen in the soil also affected the nitrous oxide emissions, which was an intermediate product of ammonium oxidation, and when NO₃⁻ was not further oxidized to NO₂⁻, the nitrous oxide emissions were higher. These processes were mainly driven by the microorganisms in the soil.

N₂O emission fluxes were positively related to the abundances of nitrifying and denitrifying microorganisms [53]. In the study, the main microbial media of N₂O emission were Nitrosospira, Nitrospira, Thiobacillus, Flavisolibacter, and Hyphomicrobiurn. The total abundance of nitrifying and denitrifying bacteria related to soil N mineralization in the BM treatment was higher than that in CK, indicating that film mulching may promote the N₂O emission from paddy soils. However, N₂O emissions during the entire growth period showed a pronounced downward trend. The results were completely consistent with early research demonstrating that mulching reduced N₂O emissions in rice paddy soils compared to non-mulching treatments [54]. This may be related to the growth of rice plants and the differences in soil nitrogen absorption and utilization, and may also be the result of film mulching hindering its outward release. Therefore, it is necessary to further study the effect of plastic film mulching on N₂O emissions from paddy fields regarding rice root and soil nitrogen uptake and utilization.

The GWP is a general index to measure the warming capacity of greenhouse gases, which could effectively assess the impact of greenhouse gases on global climate change [29]. The warming potential of CH₄ in rice fields was much higher than that of N₂O on a century time scale. Although the BM treatment increased N₂O emissions to some extent, it significantly reduced CH₄ emissions from paddy fields and reduced the overall warming potential. Chen et al. (2021) showed that reducing CH₄ emissions could effectively reduce the intensity of greenhouse gas emissions from paddy fields [55]. Compared with CK, the 2-year film mulching treatment significantly reduced the greenhouse gas emission intensity from the paddy fields (Table 1). Therefore, biodegradable film mulching planting could reduce greenhouse gas emissions and increase the yield of rice, conclusions that are consistent with the balance of environmental and economic benefits of sustainable agricultural development. However, our study needs to be further improved. In future studies, we also need to strengthen the influence of the soil biology, fungi, and enzyme indicators on greenhouse gas emissions. In this study, the soil microbial communities were analyzed, but the potential contribution of other soil fauna to GHG emissions was not specifically explored. The fungi and soil enzyme activities, which play key roles in soil nutrient cycling and greenhouse gas production, were also not investigated in this study, which limits our understanding of the relationship between the soil enzyme activities and greenhouse gas emissions. Therefore, for a more comprehensive understanding of the effects of soil ecosystems on GHG emissions, future studies could further consider these factors to provide more in-depth and comprehensive findings.

5. Conclusions

The biodegradable film mulching machine planting mode had a significant impact on the CH₄ and N₂O emissions from rice fields. In this study, film-covering planting significantly reduced the CH₄ emissions and led to changes in soil bacterial abundance and the community structure, such as increased Proteobacteria abundance. Furthermore, BM
decreased the relative abundance of methanogens while increasing the relative abundance of type I methanotrophs in the rice paddy soil. The N2O emissions during the growth period showed a pronounced downward trend. However, the total abundance of nitrifying and denitrifying bacteria was higher in the BM treatment. The soil bacterial communities were primarily affected by the improvement of soil nitrogen nutrient availability. BM significantly improved the yield by 5.0% and significantly inhibited the greenhouse gas emission intensity (GHGI) of the paddy field by 46.9%. Film mechanical transplanting could significantly mediate the warming potential (GWP) of greenhouse gases in paddy fields, increase the rice yield, and provide a new way for the green and efficient production of rice. The paddy field emission reduction is an important direction for sustainable agriculture development. This helps mitigate global climate change and slow down global warming. This research also provides the basis for the formulation of carbon emission reduction policies and the development of agricultural activities in paddy fields.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy13061535/s1. Figure S1: Weather data in the experimental location Hangzhou in 2019 and 2020; Table S1: Heatmap data of soil bacterial community at genus level to be involved in greenhouse gas emission influenced by the mulching cultivation.

Author Contributions: Y.Z. (Yikai Zhang) and Y.Z. (Yuping Zhang) conceived the project and designed the experiments; J.X. (Jiahuan Xiong) and T.Y. performed the experiments; Y.Z. (Yuping Zhang) and Y.Z. (Yikai Zhang) provided funding; K.S., Y.G., and Z.W. analyzed the data; Y.Z. (Yuping Zhang), H.C., Y.W., and J.X. (Jing Xiang) contributed the reagents/materials/analysis tools; J.X. (Jiahuan Xiong) and Y.Z. (Yuping Zhang) drew the figures and wrote the manuscript. All authors have read and agreed to the published version of the manuscript.

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References


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