



Article Mitigated Greenhouse Gas Emissions in Cropping Systems by Organic Fertilizer and Tillage Management

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Abstract: Cultivating ecological benefits in agricultural systems through greenhouse gas emission reduction will offer extra economic benefits for farmers. The reported studies confirmed that organic fertilizer application could promote soil carbon sequestration and mitigate greenhouse gas emissions under suitable tillage practices in a short period of time. Here, a field experiment was conducted using a two-factor randomized block design (organic fertilizers and tillage practices) with five treatments. The results showed that the application of microbial fertilizers conserved soil heat and moisture, thereby significantly reducing CO₂ emissions (6.9–18.9%) and those of N₂O and CH₄ fluxes during corn seasons, compared with chemical fertilizer application. Although deep tillage increased total CO₂ emissions by 4.9–37.7%, it had no significant effect on N₂O and CH₄ emissions. Application of microbial organic fertilizer increased corn yield by 21.5%, but it had little effect on the yield of wheat. Overall, application of microbial fertilizers significantly reduced soil GHG emission and concurrently increased yield under various tillage practices in a short space of time. With this, it was critical that microbial fertilizer be carefully studied for application in wheat–corn cropping systems.

Keywords: microbial fertilizer; tillage practice; wheat-corn cropping system; greenhouse gas; crop yield

1. Introduction

The contribution of agricultural production to greenhouse gas (GHG) emission is estimated at 20% [1–3]. Especially, though synthetic fertilizers are important for crop output, they not only emit the large quantities of GHG emissions, but also destroy soil quality and fertility. China is the largest nitrogen fertilization-consumption country in the world, accounting for ~30% of global nitrogen (N) use. In addition, continuous nitrogen input (>400 kg·ha⁻¹·year⁻¹) has become another driving factor of increasing carbon C and N emissions [4–6]. Meanwhile, the combination of returning straw with tillage practices also have obviously contributed to the large quantities of GHG emissions in the North China Plain (NCP), a key grain production area in China. There is therefore the need for effective measures to improve farm management (e.g., fertilization, tillage practices, irrigation, etc.) to increasing soil C and N reserves, to reduce GHG emissions, and to enhance crop yield [7–9]. The fertilizer system is one of the most direct ways of emitting large amounts of GHGs. Inorganic fertilizers impact N₂O emissions by directly or indirectly influencing the processes of nitrification and denitrification [10,11]. Additionally, organic fertilizers significantly affect soil CO₂ and CH₄ emissions [12], as soil C can be produced from soil



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Copyright: © 2022 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). organic matter degradation. However, the combined application of organic and inorganic fertilizers has been shown to reduce soil GHG emissions [13,14]. This is because organic fertilizers promote the formation of macroaggregates and soil carbon sequestration [15]. Microbial fertilizer, an organic fertilizer with an active microbial community, quickly changes the soil microbial community, rapidly improves soil fertility, and increases crop yield. Singh and Strong [16] recommended that microbial organic fertilizers that improve the activity of methane oxidizing bacteria can also increase crop yield and the potential for methane sink. Liu et al. [17] introduced microbial inoculants (micro-organism agents) to accelerate straw decomposition and found that treatments with microbial inoculants increased seasonal CH₄ emissions, but reduced N₂O emissions. Meanwhile, previous studies [18,19] have also noted that tillage can differently affect soil environment and soil fertility. This is because of the disturbance of soil's physical structure by different tillage practices [20]. Additionally, the change in the soil temperature and soil moisture from the disturbance of soil's physical structure directly influences GHG emissions from soil by influencing the processes of microbial decomposition and root respiration [21,22]. Changing soil water content and soil temperature have the potential to greatly influence soil GHG emissions, which are key driving factors for optimal microbial activity [23,24].

Here, we hypothesize that tillage practices could increase crop yield and mitigate GHG emissions with microbial fertilizer application. Understanding this point requires the investigation of the suitability of tillage for microbial fertilizer application. Moreover, uncertainty remains in the short-term effects of the use of various microbial fertilizers application under different tillage practices on GHG emissions. The relationship between GHG emissions, soil moisture, and soil temperature is also not entirely clear. Therefore, the objective of this study was to investigate the short-term effects of different microbial fertilizers and tillage practices on GHG emissions, soil moisture, and soil temperature in wheat–corn cropping systems in NCP. Additionally, based on the characteristics of GHG emissions and crop yield under different microbial fertilizer applications and tillage practices, a suitable cultivation system was discussed for a wheat–corn cropping system in NCP.

2. Materials and Methods

2.1. Field Site

The field experiment was conducted from wheat sowing (October) to corn harvest (Next October) at Yucheng Comprehensive Experiment Station of Chinese Academy of Sciences (116°36′ E, 36°57′ N, 21.2 m above sea level), which is in Dezhou County of Shandong Province, North China. The site is representative of the agriculturally intensive areas in NCP with an annual mean temperature of 13.1 °C and precipitation of 593.2 mm, of which summer precipitation is 68% of the annual total. The experimental site was established in a winter wheat/summer maize cultivation field. The soil type in this area is fluvo-aquic and the soil texture is silt loam (sand = 12%, silt = 66%, and clay = 22%). The initial soil physical and chemical properties are: bulk density = 1.46 g·m⁻³, pH = 8.0, soil organic C = 1.5%, total N = 0.64 g·kg⁻¹, total phosphorus = 0.84 g·kg⁻¹, and total potassium = 19.99 g·kg⁻¹.

2.2. Experimental Design

The field experiment was conducted with a wheat–corn cropping system, a typical cultivation practice in NCP. Wheat (Jimai 22) was planted in October 2016 and harvested in June 2017, while corn (Zhengdan 958) was planted in June 2017 and harvested in October 2017.

The study was conducted using a randomized block design (field plot size of $5 \text{ m} \times 5 \text{ m}$) with three replicates. The main factors were fertilizer applications, including normal chemical fertilizer and reduced chemical fertilizer plus two microbial agents, one of which was a microbial organic fertilizer and another was a microbial decomposition agent (used individually). The secondary factors were tillage practices, including conventional rotary

tillage (RT) and deep plowing (DT). Five treatments were designed: TF (RT with chemical fertilizer), TE (RT with microbial organic fertilizer plus 70% chemical fertilizer), TJ (RT with microbial decomposition agent plus 70% chemical fertilizer), DTE (DT with microbial organic fertilizer plus 70% chemical fertilizer), and DTJ (DT with microbial decomposition agent plus 70% chemical fertilizer). Further details of the field operations conducted in the plot experiments throughout the wheat–corn cropping system are presented in Table 1.

Treatment	Tillage before Sowing		Basal Fertilization Rate						Topdressing Rate	
meannent	Wheat	Corn	Wheat		Corn			Wheat	Corn	
			CCF	ETS	JS	CCF	ETS	JS	Ure	ea
			kg∙N∙ha ⁻¹	kg∙ha−1	L∙ha ⁻¹	kg·N∙ha ^{−1}	kg∙ha−1	L∙ha ⁻¹	kg·N·	ha ⁻¹
TF	RT		112.5	-	-	112.5	-	-	112.5	112.5
TE	RT		78.75	3000	-	78.75	3000	-	78.75	78.75
TJ	RT	NT	78.75	-	30	78.75	-	30	78.75	78.75
DTE	DT		78.75	3000	-	78.75	3000	-	78.75	78.75
DTJ	DT		78.75	-	30	78.75	-	30	78.75	78.75

Table 1. Field operations applied to plots throughout the experimental periods.

TF: rotary tillage with chemical fertilizer; TE: rotary tillage with ETS plus 70% chemical fertilizer; TJ: rotary tillage with JS plus 70% chemical fertilizer; DTE: deep plowing with ETS plus 70% chemical fertilizer; DTJ: deep plowing with JS plus 70% chemical fertilizer; RT: rotary tillage; DT: deep plowing; NT: no-tillage; CCF: combined chemical fertilizer; ETS: microbial organic fertilizer, remarked as "ETS"; JS: microbial decomposition agent, remarked as "JS".

RT was performed at the depth of 10-15 cm and DT performed at the depth of 30 cm. The operations of RT and DT treatments were performed following maize harvest. It started with the sowing of winter wheat with all the corn crop straws and residues ploughed into the soil. Combined chemical fertilizer (CCF of N 26%, P 12%, and K 10%) was used as a base fertilizer and urea was applied as a topdressing at jointing stages of wheat and flare opening of maize, respectively. The tested microbial organic fertilizers (denoted as "ETS") were taken from barnyard manure and plant straw of ETS microflora were taken to the fermentation process, of which total C concentration was 13.25% and N concentration 1.78%. ETS was applied at 3000 kg \cdot ha⁻¹, containing the same N concentration at ~30% the normal amount in N fertilizer. The tested microbial decomposition agent (denoted as "JS") was a farm-oriented liquid microbial decomposition agent containing ~ 2.0×10^9 CFU·mL⁻¹ bacteria [25]. Specifically, *Firmicutes*, *Proteobacteria*, and Bacteroidetes were the dominant phyla among the microflora in the "JS", which efficiently decompose fiber and other macromolecular organic matters with synergistic effects. JS was applied at 30 L·ha⁻¹ and sprayed on the soil surface in accordance with the 1:150 ratio of JS:water before wheat sowing. The two microbial fertilizers have been registered in the Department of Plantation Management, Ministry of Agriculture and Rural Affairs of the People's Republic of China with assigned access numbers of 2011–0781 and 2011–0801, respectively. ETS and JS were both applied before fertilization and irrigation, with tillage occurring at the same time to guarantee uniform use of the fertilizer and microbial agent.

2.3. Soil GHG Flux Calculation

Fluxes of GHG were measured in each plot using the active–passive closed chamber system [26]. The system consists of a circular stainless-steel base (19 cm inner diameter and 22 cm external diameter), surrounded by a trough (3 cm width and 5 cm height) and a cylindrical, opaque chamber made of polyvinyl chloride (20 cm inner diameter and 15 cm height).

Small, living plants were removed inside the base at least 1 d before measurements were taken to avoid any relation to the gas sampling. The gas samples were collected between 9:00–11:00 a.m. once every month for laboratory analysis, using a 50 mL syringe at 0–, 10–, 20–, and 30–min intervals. Samples were transferred to a sealed aluminum foil air bag, taken to the lab, and analyzed within 24 h using a gas chromatograph (Agilent

7890 A, Agilent Technologies, Santa Clara, CA, USA). Additionally, the chamber and air temperatures were recorded using portable thermometers, and the soil temperature and moisture measured using a soil sensor were recorded.

The rates of GHG flux were calculated from the measured slope of linear increase or decrease from the change in gas concentration within the chamber over time. After chamber closure, chamber headspace height, air pressure, and air temperature within the chambers were calculated as follows [27]:

$$F = K \times (273 \times (273 + T_a)^{-1}) \times (M \times V^{-1}) \times H \times (dc \times dt^{-1})$$

$$\tag{1}$$

where *F* is GHG flux rate (μ g N₂O·m⁻²·h⁻¹, μ g CH₄·m⁻²·h⁻¹, and mg CO₂·m⁻²·h⁻¹); *K* is the conversion coefficient (0.001 for N₂O and CH₄, 1 for CO₂); *T_a* (°C) is the air temperature within the chamber; *M* is molecular weight (44 g N₂O·mol⁻¹, 16 g CH₄·mol⁻¹, and 44 CO₂·mol⁻¹); *V* is mole volume under standard atmospheric condition (22.4 L·mol⁻¹); *H* (m) is chamber headspace height; and $dc \times dt^{-1}$ (μ L·L⁻¹·h⁻¹) is change in concentration of N₂O, CH₄, and CO₂.

Cumulative GHG flux was calculated using the following equation [28]:

$$S = \frac{\sum_{i=1}^{n} (F_i \times 24 \times T_i)}{100}$$
(2)

where *S* is cumulative GHG fluxes (kg N₂O·ha⁻¹, kg CH₄·ha⁻¹, and kg CO₂·ha⁻¹) during the experimental season; *i* is *i*-th measurement; T_i is days between two consecutive dates of measurement; *n* is total number of times measurement is done; and 100 is the conversion factor.

The global warming potential (GWP) in CO_2 -equivalent for N_2O and CH_4 calculated using the following equation [28]:

$$GWP_{N_2O} = N_2O \times 298 \tag{3}$$

$$GWP_{CH_4} = CH_4 \times 25 \tag{4}$$

Regression analysis was used to relate GHG flux data with soil temperature (exponential or polynomial function) and soil moisture (polynomial or linear function) [29].

2.4. Soil Measurements

Soil temperature and moisture were taken using a portable sensor probe (ECH₂O, Decagon, Pullman, WA, USA) at the 0–20 cm and 20–40 cm soil depths in each plot at the same time of gas samples collection. Air temperature and precipitation data were provided by Yucheng Comprehensive Experiment Station.

2.5. Crop Yield

Crop grain yield $(kg \cdot ha^{-1})$ and straw yield $(kg \cdot ha^{-1})$ of winter wheat and summer corn were determined for samples collected in each plot on 16 June 2017 and on 4 October 2017, respectively. The harvested area agreed with the whole experimental plot.

2.6. Statistical Analysis

SPSS (version 19.0, SPSS Inc., Chicago, IL, USA) was used to test significant differences among the different samples in one-way ANOVA. Treatments were considered to be fixed effects and replications as random effects at a significance level of 0.05. The least significant difference among treatments was determined using Duncan Multiple Range Test. Pearson's correlation coefficients were calculated to examine the relationships of the soil factors and the GHG emissions. Data were presented as means \pm standard errors of the three replicates.

3. Results

3.1. Soil Moisture

The precipitation and soil moisture contents of the different treatments during the experimental period are shown in Figure 1. Soil moisture content was consistently highest in the TE treatment for the 0–20 cm soil depth and lowest in the DTE treatment for the study period (Figure 1A, Table S1). Significant differences were exhibited between the treatments from sowing to elongation of winter wheat. Compared with the DTE treatment, soil moisture content was higher (p < 0.05) by 17.0–70.4% under TE treatment and by 7.9–44.3% under the TJ treatment. In the 20–40 cm depth (Figure 1B, Table S1), soil moisture content under the different treatments for the winter wheat period ranked as follows: TF > TJ > TE > DTJ > DTE. During the corn growth period, soil moisture was highest under TF and lowest under DTE treatment (p < 0.05).



Figure 1. Soil moisture in different soil layers (0–20 cm depth (**A**), 20–40 cm depth (**B**)) for different treatments, and precipitation in study area (**C**). TF: rotary tillage with chemical fertilizer; TE: rotary tillage with ETS plus 70% chemical fertilizer; TJ: rotary tillage with JS plus 70% chemical fertilizer; DTE: deep plowing with ETS plus 70% chemical fertilizer; DTJ: deep plowing with JS plus 70% chemical fertilizer.

3.2. Soil Temperature

Differences in soil and atmospheric temperature varied significantly among the different treatments (Figure 2A,B, Table S2). In the 0–20 cm soil layer, the difference between soil and atmospheric temperature was significantly higher (p < 0.05) in TE than in the other treatments from sowing to overwintering and from elongation to grain-filling of wheat. The temperature difference was lowest in the TJ treatment at the corn period. The temperature difference under different treatments in the 20–40 cm soil layer was higher than those in the 0–20 cm soil layer across the treatments. In the 20–40 cm soil layer, temperature difference in the DTE and the DTJ treatments was significantly higher (p < 0.05) than in other treatments, and those in the TF treatment were lowest for the period from the sowing to the greening stage of wheat. Temperature difference in the TE and the TJ treatments were significantly higher (p < 0.05) than in the other treatments for the corn period.



Figure 2. The differences in soil temperature and air temperature at different soil layers (0–20 cm depth (**A**), 20–40 cm depth (**B**)) under different treatments, and air temperature in study area (**C**). TF: rotary tillage with chemical fertilizer; TE: rotary tillage with ETS plus 70% chemical fertilizer; TJ: rotary tillage with JS plus 70% chemical fertilizer; DTE: deep plowing with ETS plus 70% chemical fertilizer; DTJ: deep plowing with JS plus 70% chemical fertilizer.

3.3. CO₂ Flux Characteristics

The dynamics of GHG flux for the experimental period is plotted in Figure 3A and Table S3. There were similar seasonal variations in CO₂ flux for all the treatments, with peak CO₂ fluxes occurring at the elongation stage of wheat and at the heading stage of maize. From the sowing to the greening stage of wheat, the order of CO₂ flux was: DTJ > DTE > TF > TE > TJ; where CO₂ flux under the DTJ treatment was significantly higher (p < 0.05) than that under any the other treatments. The main CO₂ flux peak was observed under the TF treatment (538.1 g·m⁻²·h⁻¹) and it was at the elongation stage of wheat, but with no significant difference among the different treatments. In the corn period,

the range of mean CO₂ flux for the DTJ and the DTE treatments was 316.8–816.8 g·m⁻²·h⁻¹, which was higher than that for the TF treatment and significantly higher (p < 0.05) than those for the TE and the TJ treatments.



Figure 3. GHG fluxes (CO₂ (**A**), N₂O (**B**), CH₄ (**C**)) in the experimental periods under different treatments. The vertical bars represent standard errors (n = 3). B and T: the dates of base fertilizer applications and top dressings, respectively. TF: rotary tillage with chemical fertilizer; TE: rotary tillage with ETS plus 70% chemical fertilizer; TJ: rotary tillage with JS plus 70% chemical fertilizer; DTE: deep plowing with ETS plus 70% chemical fertilizer; DTJ: deep plowing with JS plus 70% chemical fertilizer.

The correlations between CO₂ flux and soil moisture and temperature in the different treatments are shown in Figure 4A,B and Figure 5A,B. The plots suggest that CO₂ flux can be described better in terms of soil temperature than soil moisture, especially for the 20–40 cm soil layer (Table 2). It has to be noted that R^2 for CO₂ flux plotted against the two soil factors at the 0–20 cm soil layer under the DTE and DTJ treatments were much stronger than that under TF. Additionally, in the 20–40 cm soil layer, R^2 for flux with soil temperature under TE and TJ dropped, compared to those under the TF treatment. However, the reverse was the case under the DTE and DTJ treatments.

3.4. N₂O Flux Characteristics

The peak N₂O flux was observed at the elongation to the heading stage of wheat and at the elongation stage of corn (Figure 3B, Table S3). The two peak values for the winter and summer seasons were observed, respectively, under the TJ (76.6 μ g·m⁻²·h⁻¹) and TF (165.0 μ g·m⁻²·h⁻¹) treatments. From the sowing to the elongation stage of wheat, N₂O flux under the DTE and DTJ treatments was highest across the treatments. N₂O flux under the TF treatment was significantly higher (p < 0.05) than that under other treatments at the elongation stage of corn, but not significantly different for the other treatments for all maize growth stages.



Figure 4. Scatterplots between CO₂ emission and soil moisture at 0–20 cm (**A**) and 20–40 cm (**B**) depth; between N₂O emission and soil moisture at 0–20 cm (**C**) and 20–40 cm (**D**) depth; and between CH₄ emission and soil moisture at 0–20 cm (**E**) and 20–40 cm (**F**) depth under different treatments. TF: rotary tillage with chemical fertilizer; TE: rotary tillage with ETS plus 70% chemical fertilizer; JI: rotary tillage with JS plus 70% chemical fertilizer; DTE: deep plowing with ETS plus 70% chemical fertilizer.

Overall, N₂O flux can be described better by soil temperature than by soil moisture (Figure 4C,D and Figure 5C,D and Table 2). N₂O flux under rotary tillage treatment (TF, TE, and TJ) was not significantly correlated with soil moisture. However, N₂O flux under DTE and DTJ was positively correlated (linear function, Table 2) with soil moisture at the 0–20 cm soil layer (R^2 0.32–0.35). In terms of N₂O flux and soil temperature for all the soil layers, R^2 under TF was highest across all the treatments. Additionally, the correlation between TE and TJ was much stronger than that under the DTE and DTJ treatments, separately.

3.5. CH₄ Flux Characteristics

CH₄ flux occurred as a source of gas sequestration in winter wheat and a source of gas emissions in summer corn, with the main CH₄ peak flux occurring at the heading stage during the wheat and corn growth period (Figure 3C, Table S3). From overwintering to the greening of wheat, CH₄ peak flux occurred under the DTJ treatment, with a range of -0.13 to $-0.24 \ \mu g \cdot m^{-2} \cdot h^{-1}$. At the heading stage of wheat, CH₄ flux was highest under the TJ treatment (0.63 $\ \mu g \cdot m^{-2} \cdot h^{-1}$, p < 0.05). At the heading stage of corn, the amount of CH₄ flux under the various treatments ranked as: TF > TJ > DTJ > DTE > TE. Therefore, CH₄ flux under the TF treatment was significantly higher (p < 0.05) than the other treatments.

CH₄ flux did not depend on soil moisture and temperature for microbial fertilizer treatment under rotary tillage (Table 2). It has to be noted that the CH₄ flux under the DTE and DTJ treatments was negatively correlated (polynomial function) with soil temperature (Figure 5E,F), and R^2 was less than it was in the TF treatment.



Figure 5. Scatterplots between CO_2 emission and soil temperature at 0–20 cm (**A**) and 20–40 cm (**B**) depth; between N_2O emission and soil temperature at 0–20 cm (**C**) and 20–40 cm (**D**) depth; and between CH_4 emission and soil temperature at 0–20 cm (**E**) and 20–40 cm (**F**) depth under different treatments. TF: rotary tillage with chemical fertilizer; TE: rotary tillage with ETS plus 70% chemical fertilizer; DTE: deep plowing with ETS plus 70% chemical fertilizer.

3.6. Total GHG Emission

The total CO₂ emissions during the experimental period was highest under the DTJ treatment, with those under the other treatments ranked as follows: DTJ > TF > DTE > TE > TJ for the wheat season and DTJ > DTE > TF > TJ > TE for the corn season (Figure 6A). The GWP in CO₂-equivalent for N₂O during the wheat period under the TJ and DTJ treatments was significantly higher (p < 0.05) than under other treatments. The GWP in CO₂-equivalent for N₂O during the corn period in the TF treatment increased by 37.2–109.6% (p < 0.05) over that in the other treatments. While the GWP in CO₂-equivalent for CH₄ under the TF and DTJ treatments for wheat was negative, that under the TE (125.1 CO₂-equivalent kg·ha⁻¹, p < 0.05) and DTE (99.9 CO₂-equivalent kg·ha⁻¹, p < 0.05) treatments was highest. The GWP in CO₂-equivalent for CH₄ in the corn field was highest (p < 0.05) under the TF treatment, with an increase of 131.5–273.9% over that under the other treatments.

Factor	Treatments	Layer	Туре	$CO_2 (mg \cdot m^{-2} \cdot h^{-1})$		N_2O (µg·m ⁻² ·h ⁻¹)		CH ₄ (μg·m ⁻² ·h ⁻¹)	
Tuctor				<i>R</i> ²	р	R^2	р	R^2	р
	TF		Linear	0.317	**	0.071	ns	0.203	**
	TE		Quadratic	0.112	ns	0.072	ns	0.681	***
		0–20 cm	Linear	0.003	ns	0.005	ns	0.004	ns
	TJ		Quadratic	0.080	ns	0.007	ns	0.003	ns
			Linear	0.015	ns	0.038	ns	0.028	ns
			Quadratic	0.072	ns	0.046	ns	0.041	ns
	DTE		Linear	0.498	***	0.345	***	0.001	ns
	DTJ		Quadratic	0.697	***	0.302	**	0.338	**
			Linear	0.382	**	0.312	**	0.341	**
Moisture			Quadratic	0.583	***	0.318	**	0.521	***
	TF	20–40 cm	Linear	0.025	ns	0.042	ns	0.183	*
	TE		Quadratic	0.270	**	0.158	*	0.557	***
			Linear	0.056	ns	0.020	ns	0.017	ns
	TJ DTE		Quadratic	0.327	**	0.044	ns	0.025	ns
			Linear	0.057	ns	0.052	ns	0.032	ns
			Quadratic	0.396	**	0.059	ns	0.102	ns
			Linear	0.060	ns	0.076	ns	0.044	ns
	DTJ		Quadratic	0.240	*	0.083	ns	0.303	**
			Linear	0.251	**	0.143	*	0.327	**
			Quadratic	0.482	***	0.158	*	0.483	***
	TF		Linear	0.597	***	0.375	**	0.307	**
	TE		Quadratic	0.610	***	0.498	***	0.408	***
		0–20 cm	Linear	0.642	***	0.403	***	0.016	ns
	TJ		Quadratic	0.667	***	0.424	***	0.171	ns
			Linear	0.656	***	0.319	**	0.158	*
			Quadratic	0.684	***	0.323	**	0.160	ns
	DTE		Linear	0.668	***	0.408	***	0.200	*
	DTJ		Quadratic	0.726	***	0.602	***	0.374	**
			Linear	0.635	***	0.406	***	0.334	**
Temperature	-		Quadratic	0.682	***	0.459	***	0.368	**
	TF		Linear	0.547	***	0.361	**	0.294	**
	TE	20–40 cm	Quadratic	0.570	***	0.455	***	0.419	***
			Linear	0.596	***	0.385	**	0.029	ns
	TJ		Quadratic	0.635	***	0.403	**	0.198	*
			Linear	0.611	***	0.295	**	0.129	*
			Quadratic	0.653	***	0.296	**	0.131	ns
	DTE		Linear	0.605	***	0.411	***	0.165	*
	DTJ		Quadratic	0.684	***	0.585	***	0.268	**
			Linear	0.581	***	0.402	***	0.333	**
			Quadratic	0.652	***	0.450	***	0.357	**

Table 2. Determination coefficient (R^2), significance level (p), for the plot of GHG flux and soil moisture and then temperature under the different treatments.

Linear function (Y = dx + e), quadratic function ($Y = ax^2 + bx + c$). *, **, and *** indicate statistically significant at p < 0.05, p < 0.01, and p < 0.001, respectively. ns: not significant. TF: rotary tillage with chemical fertilizer; TE: rotary tillage with ETS plus 70% chemical fertilizer; TJ: rotary tillage with JS plus 70% chemical fertilizer; DTE: deep plowing with ETS plus 70% chemical fertilizer; DTJ: deep plowing with JS plus 70% chemical fertilizer.

3.7. Crop Yield

Crop yield and production efficiency under the different treatments are listed in Table 3. For wheat, straw yield under TF (12,364 kg·ha⁻¹, p < 0.05) was significantly greater than that under other treatments. However, the difference was not significant among the different treatments for wheat grain yield. The rank order of corn grain yield for the different treatments was: TE > DTJ > DTE > TJ >TF. Straw yield was highest under the TJ treatment for corn, but not significantly different between the TJ, TE, or DTE treatments.



Figure 6. The GWP in CO₂-equivalent for N₂O and CH₄ (**A**) and CO₂ emissions (**B**) under different treatments for wheat–corn periods. The vertical bars represent standard errors (n = 3). TF: rotary tillage with chemical fertilizer; TE: rotary tillage with ETS plus 70% chemical fertilizer; TJ: rotary tillage with JS plus 70% chemical fertilizer; DTE: deep plowing with ETS plus 70% chemical fertilizer; DTJ: deep plowing with JS plus 70% chemical fertilizer.

Table 3. The crop yield under different treatments.

Treatment	W	heat	Maize			
	Grain Yield (kg∙ha ⁻¹)	Straw Yield (kg∙ha ^{−1})	Grain Yield (kg∙ha ^{−1})	Straw Yield (kg∙ha ^{−1})		
TF	8591.4 ± 1376.4 a	$12364.2 \pm 1300.3 \text{ a}$	$11364.7 \pm 2012.2 b$	11515.4 ± 596.1 a		
TE	8194.5 ± 1296.7 a	$10001.0 \pm 977.2 \mathrm{b}$	13810.0 ± 851 a	12570.0 ± 1351 a		
TJ	$9095.7 \pm 212.8 \text{ a}$	$10326.5 \pm 366.1 \mathrm{b}$	$11497.3 \pm 659.3 \mathrm{b}$	13170.0 ± 991.4 a		
DTE	8647.3 ± 884.9 a	$10161.6 \pm 553.1 \ {\rm b}$	$12455.9 \pm 760.7 \text{ ab}$	12300.0 ± 2177.4 a		
DTJ	8808.2 ± 985.5 a	$9489.6 \pm 1513.7 \mathrm{b}$	12637.7 \pm 785.4 ab	$11580.0 \pm 1549.3 \text{ a}$		

Values are means (n = 3); different letters in the same column indicate significant difference between treatment and control for the same salt content at p < 0.05. TF: rotary tillage with chemical fertilizer; TE: rotary tillage with ETS plus 70% chemical fertilizer; TJ: rotary tillage with JS plus 70% chemical fertilizer; DTE: deep plowing with ETS plus 70% chemical fertilizer; DTJ: deep plowing with JS plus 70% chemical fertilizer.

4. Discussion

4.1. Effect of Tillage and Fertilizer on Soil Moisture/Temperature

Soil moisture and soil temperature were directly influenced by fertilizer systems and tillage practices. Studies showed that organic fertilizers (e.g., microbial organic fertilizer and manure) promoted soil aggregation, increased soil porosity, reduced soil bulk density, and enhanced stability of mechanical aggregates [30,31]. This effectively improves the initial infiltration rate of soils and enhances the water retraining capacity of soils [32]. Consistent with this conclusion, it was noted that the application of microbial organic fertilizer, or "ETS", was most effective in terms of water retention at the 0-20 cm soil layer under the investigated cultivation treatments (Figure 1). Lu et al. [33] tested dry farming wheat and showed that relative to chemical fertilizer, organic amendment improved soil water storage by 5.1–6.5%. Soil water retention capacity was significantly improved, suggesting that the results of this study were consistent with those of other studies. Meanwhile, the application of microbial organic fertilizers improved subsoil temperature in this study (Figure 2). Zhang et al. [34] reported that exogenous organic carbon can reduce soil temperature fluctuation, which is an effect of atmospheric environment on soil temperature. In this study, treatment application of ETS had the most obvious heat conservation in the 0-20 cm soil layer under low soil temperature conditions in winter. Both treatments also showed obvious cooling effects under high soil temperature conditions in summer. For the experimental period, the difference between soil and air temperature in treatments with

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chemical fertilizer was the smallest (Figure 2). It suggested that under chemical fertilizer application, there was an obvious effect of air temperature on soil temperature. It was also noted that the effects of soil moisture conservation and heat preservation under microbial decomposition agent "JS" treatment were less compared with ETS treatment (Figures 1 and 2). On the one hand, JS enhanced straw degradation to provide available nutrients to crops. The process of microbiological straw degradation increased moisture consumption and decreased soil water content [35]. Relative to JS, ETS contained more organic carbon and nitrogen. Input of C and N were also among the primary factors affecting soil water and heat retention in the study area [36,37].

Improving tillage practices can improve soil properties and enhance water use efficiency [38]. Buragiene et al. [39] reported that deep tillage increased the proportion of soil macropores and thereby promoted gas exchange between soil and the atmosphere. Thus, the difference between soil temperature and air temperature under deep tillage was lower than that under conventional rotary tillage. It was further found that soil moisture in the 0–20 cm and the 20–40 cm soil layers under deep tillage was lower than that under rotary tillage (Figure 1). Moreover, soil temperature under deep tillage was more susceptible to change than under rotary tillage (Figure 2). Compared with rotary tillage, deep tillage improved soil structure, promoted energy flow and substance exchange between soil and air, and decreased soil heat preservation and water retention capacity [40,41].

4.2. Effect of Tillage and Fertilizer on GHG Flux and the Relationship with Moisture/Temperature

CO₂ **Flux.** In this study, the application of ETS and JS reduced CO₂ emissions under rotary tillage (Figure 3). This was consistent with the findings in other studies [42,43], where it was noted that the sole application of organic fertilizer promoted soil respiration while the combined application of organic and inorganic fertilizer promoted soil carbon sequestration and reduced GHG emissions. The combined application of organic and inorganic fertilizers reduced C emissions compared with the sole application of organic fertilizer. This was because it improved the immobilization of active C (e.g., microbial biomass C, readily oxidizable C) [43–45]. Thus, the results of this study showed that CO₂ emissions under microbial fertilizers application are reduced significantly (Figure 6).

For the significant effect of deep tillage on soil CO_2 emissions, soil CO_2 emissions under deep tillage were significantly higher than that under rotary tillage (Figure 6). Compared with rotary tillage, deep tillage exacerbated soil disturbance, changed soil structure, and enhanced soil organic matter degeneration; all of which increased CO_2 emissions [46,47].

In agreement with other studies [48,49], a strong exponential increase was noted for CO₂ emissions with increasing temperature for all the treatments. In addition, while microbial fertilizer weakened the correlation between CO₂ flux and soil temperature under rotary tillage, microbial fertilizer with deep tillage strengthened that correlation (Table 2). First, soil microbial activity increased with increasing soil temperature under the application of microbial fertilizer, causing soil C sequestration by micro-organisms to also be enhanced [50,51]. Second, changes in the correlation between soil temperature and respiration with microbial fertilizer were the result of change in soil microbial community structure and not microbial fertilizer [52]. Then, there were still uncertainties associated with the modeling of the strong dependence of soil respiration on soil temperature [53]. Soil disturbance due to deep tillage resulted in more CO2 release with microbial fertilizer, which in turn partly exacerbated the influence of soil temperature on soil respiration [21,48]. In addition, microbial fertilizer with deep tillage increased the correlation between CO_2 flux and soil moisture (Table 2), which could have originated from the improvement of soil aeration. It was therefore suggested that tillage practices may affect soil CO_2 flux by modifying the interaction between soil moisture and temperature.

 N_2O Flux. Synthetic N fertilizers exacerbate soil N_2O emissions. For instance, urea and nitrate fertilizers cause more N_2O emissions than organic fertilizers, especially microbial fertilizers [54,55]. As also noted in this study, N_2O emissions under chemical fertilizer treatment were significantly higher than those under ETS or JS. In addition, N_2O emissions under JS were higher than those under ETS in wheat fields (Figure 6). To a large extent, this was because JS promoted the decomposition of straw without nitrogen fixation. Previous studies [44,45] reported that the combined application of organic and inorganic fertilizers enhanced the activity of organic C and N and the immobilization of ammonium N. This equally explained why N₂O emissions under ETS were lower than those under the JS treatment, as organic matter in ETS enhanced N fixation. Additionally, microbial fertilizer can also alter soil denitrification by changing the functional gene of denitrifying agents [56,57], which could limit N₂O flux with the application of microbial fertilizer.

In this study, no significant difference in total N_2O emissions existed between the two tillage practices (Figure 6), but N_2O emissions were significantly affected by tillage practices in previous studies [58,59]. Although deep plowing changed soil aeration [22], microbial fertilizer application changed denitrification by influencing the N-cycling gene. Thus, the change in micro-organisms with N-cycling may compensate for the effect of deep tillage.

Although soil N₂O production showed similar Gaussian functions with soil waterfilled pore space [60,61], N₂O emissions were better described by soil temperature than by soil moisture in this study (Table 2). Meanwhile, N₂O emissions showed an increase with soil temperature (Figure 5). First, a nonlinear (exponential) increase in N₂O flux with increasing temperature was observed by other authors [62]. Then, the influence from soil temperature was stronger than that under soil water content, which has been attributed to limitation of soil moisture under dry farming. In a previous study, the optimal water-filled pore space for N₂O flux was 60–80% field capacity [29]. Thus, anaerobic conditions in topsoil hardly occur in upland fields in North China. It was therefore likely that microbial fertilizers influence N₂O emissions in upland fields by modifying soil temperature and not soil moisture.

CH₄ Flux. Treatments with microbial fertilizers showed the increase in methane emissions, but a net sink of CH₄ under the treatment with chemical fertilizer in winter wheat periods was found in this study (Figure 6). Methane sinks are common in agricultural soils in temperate zones and in well-drained soils [63,64]. Meanwhile, exogenous organic C (e.g., manure, organic fertilizer, and microbial fertilizer) can increase CH₄ emissions [65]. Chemical fertilizers promoted CH₄ flux more than microbial fertilizers in the summer corn fields (Figure 6). This can be explained by the fact that soil water content was higher in summer (Figure 1) and also that N fertilizer application enhanced CH₄ emission under higher water-filled pore spaces [66].

No significant difference existed in CH_4 emissions between the two tillage practices, except for treatment with JS application in the wheat field (Figure 6). Bista et al. [67] observed that soil CH_4 emissions increase for tillage practices, but Zhang et al. [68] suggested that deep tillage reduces CH_4 emissions because of reduced abundance of methanogens, which remains inconclusive. Moreover, deep plowing did not affect ETS treatment, which could be traceable in substrates of methanogens of microbial organic fertilizers.

CH₄ flux did not depend on soil moisture and temperature under microbial fertilizer with rotary tillage (Table 2). Although soil moisture was identified as the main factor affecting CH₄ flux, microbial fertilizer application directly influenced CH₄ oxidation by adjusting the methanogenic bacteria community [17]. Additionally, this direct influence may be stronger than that from the change in soil moisture and temperature by microbial fertilizer application. The correlation between CH₄ absorption/emissions with soil temperature was significant and positive [69], although our results did not confirm such a relationship. CH₄ flux under deep tillage was negatively correlated (polynomial function) with soil temperature, especially when the optimal soil moisture for CH₄ flux was reached at 10–20% (Figure 5). Deep tillage has probably exacerbated soil water consumption as soil temperature increases in temperate zones [70].

4.3. Effect of Tillage and Fertilizer on Crop Yield

The application of microbial fertilizer combined with chemical fertilizer can significantly increase crop yield, compared with sole application of chemical fertilizer (Table 3). This is in agreement with the finding that combined microbial organic fertilizer and chemical fertilizer support better maize yield than wheat yield [71]. ETS and JS application increased grain and straw yields of maize, but had no significant effect on grain yield of wheat (Table 3). Indeed, it takes time for microbial fertilizers to increase crop yield [65,72]. Compared with organic fertilizer, Liu et al. [17] reported that microbial agents could maintain grain yield. Thus, the application of microbial fertilizer with cutting 30% chemical fertilizer can stabilize crop productivity.

Subsoils below normal tillage store large stocks of nutrients. Deep tillage could improve the availability of stored nutrients in the subsoil for use by plants and thereby increase crop yield [40]. However, the deep tillage treatment did not result in higher crop yields than the rotary tillage treatments (Table 3). Chen et al. [73] and Zhai et al. [74] observed that the effect of tillage on crop yield was not significant in the short-term, indicating that there was a gradual and potential effect of the tillage model. In summary, tillage practices did not significantly influence crop yield in the short-term in this experimental study.

4.4. Limitations

Overall, controversial issues exist on the effects of microbial fertilizer applications and tillage practices on GHG flux characteristics. It was difficult to be conclusive in terms of the effect of microbial fertilizer on GHG emissions due to the lack of detailed soil biological indicators responsible for microbially driven GHG emissions [75,76]. While the results of this study confirmed that microbial fertilizer application can reduce GHG emissions, total GHG emissions were likely underestimated. This is because of the low measuring frequency for soil GHG flux. The correlation between GHG flux, soil moisture, and temperature are also fully clear. This was also due to the lack of field data at each cultivation—e.g., before and after irrigation or precipitation. However, it was clear that microbial fertilizer application under different tillage practices drove the response of GHG flux to soil moisture and temperature. In addition, microbial fertilizer application and tillage practices had an immediate effect on GHG characteristics [25,29]. Nevertheless, this study lacked long-term observations to analyze the long-term effect of microbial fertilizer application and tillage practices on GHG emissions, which required further studies.

5. Conclusions

This study showed that application of microbial fertilizers not only conserved soil heat, but also soil moisture. Compared with chemical fertilizers, microbial fertilizers reduced CO_2 emissions by 6.9–18.9% and N_2O and CH_4 fluxes during the corn cultivation season. Deep tillage increased total CO_2 emission by 4.9–37.7%, but had no significant effect on total N_2O and CH_4 emissions, which did not support soil heat and moisture conservation. Microbial fertilizer application and tillage practices influenced soil CO_2 flux by modifying soil moisture and temperature. However, N_2O and CH_4 fluxes were better described by soil temperature than soil moisture under microbial fertilizer application. Microbial fertilizer application increased corn yield. Specifically, "ETS" microbial organic fertilizer application increased corn yield by 21.5% but had no significant effect on wheat yield. Overall, the application of microbial fertilizer significantly reduced soil GHG emissions and concurrently increased yield when combined with tillage. Thus, microbial fertilizers need further consideration for research and application in wheat-corn cropping systems.

Supplementary Materials: The following supporting information can be downloaded at: https: //www.mdpi.com/article/10.3390/land11071026/s1. Table S1: Soil moisture in different soil layers for each treatment. Table S2: Soil temperature in different soil layers for each treatment. Table S3: GHG fluxes in the experimental periods under different treatments. **Author Contributions:** Conceptualization, H.G., J.L. and Z.O.; methodology, H.G. and Z.O.; writing—original draft, H.G., data curation, H.G.; writing—review and editing, J.L.; validation, Y.Z., Z.L. and R.H.; formal analysis, Y.Z., Z.L. and R.H.; supervision, J.L. and Z.O. All authors have read and agreed to the published version of the manuscript.

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