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RESEARCH ARTICLE

Effects of inorganic and organic fertilizers on CO_2 and CH_4 fluxes from tea plantation soil

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Agricultural practices such as fertilization considerably influence soil greenhouse gas fluxes. However, the effects of fertilization on greenhouse gases fluxes remain unclear in tea soil when soil nitrogen is low. In the present study, soil CO_2 and CH_4 fluxes under various fertilization treatments in tea soil were investigated during a 50-day period. The experiment consisted of five treatments: no fertilizer (CK), single nitrogen (urea, N), single oilseed rape cake fertilizer (R), nitrogen + cake fertilizer (2:1, NR1), and nitrogen + cake fertilizer (1:2, NR2). The fertilization proportion of NR1 and NR2 was determined by the nitrogen content of nitrogen fertilizer and cake fertilizer. The results revealed that the single application of nitrogen had no significant effect on soil CO_2 flux. However, the addition of cake fertilizer significantly increased CO_2 emissions through enhanced soil microbial biomass carbon (MBC). Additionally, CO_2 emissions were directly proportional to the amount of carbon (C) in the fertilizer. All treatments were minor sinks for CH_4 except for the treatment NR1. Specifically, the cumulative CH_4 fluxes of NR1 and NR2 were significantly higher than rest of the three treatments, which implies that application of urea and oilseed rape cake reduced the capability of CH_4 oxidation in tea soil. Structural equation models indicated that soil CO_2 flux is significantly and positively correlated with soil dissolved organic carbon, MBC and soil pH, while mineral nitrogen content was the main factor affecting CH_4 flux. Overall, the application of oilseed rape cake increased the oxidation of CH_4 and promoted soil C sequestration but inevitably increased the soil CO_2 emissions.

Keywords: Nitrogenous fertilizer, Oilseed rape cake fertilizer, Greenhouse gases, Microbial metabolisms, Structural equation models

1. Introduction

The tea plant (Camellia sinensis), a perennial evergreen woody plant, acts as an important cash crop in tropical and subtropical areas (Yang et al., 2018). It is grown in multiple developing countries such as China, India, Kenya (Wang et al., 2020). The high-quality tea in China is most commonly cultivated in the mountainous regions due to the favorable climate and soil conditions (Yan et al., 2018). The addition of chemical and organic fertilizers can significantly stimulate the tea yield. Chemical fertilizers provide essential nutrients to tea plants and thus improve the bush tea shoot mass and the production of compounds (Mudau et al., 2005; Yang et al., 2014). The addition of organic materials not only maintains soil fertility but also improves soil structure and soil porosity (Kallenbach et al., 2010; Wu et al., 2019). However, fertilization changes community structure of microbes and affects the decomposition of organic matter (Bao et al., 2016; Wei et al., 2019). Furthermore, the addition of fertilizer affects the greenhouse gas (GHG) fluxes from soil (Shimizu et al., 2013; Chen et al., 2017; Liu et al., 2018; Wu et al., 2018).

Carbon dioxide (CO_2) and methane (CH_4) contribute significantly to global warming, and they are greatly influenced by anthropogenic and agricultural activities (Kallenbach et al., 2010; Shah et al., 2016). The application of organic and inorganic fertilizers highly influence soil CO_2 and CH_4 emissions (Nyamadzawo et al., 2014; Khan et al., 2017). Many studies have shown that application of chemical fertilizers increases soil CO_2 emissions (Shao et al., 2014; Zamanian et al., 2018; Zhang et al., 2019), whereas the effect of nitrogen (N) fertilizer on soil CH_4 emissions is not consistent. Kong et al. (2019) observed that the application of N fertilizer improved the soil carbon (C) substrate and benefited the methanogens

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proliferation to improve methanogenic activities and thus promote CH_4 emissions. In addition, methane monooxygenase participates in the oxidation process of ammonia (NH_3) after using ammonium nitrogen (NH_4^+-N), influencing the catalytic oxidation of CH_4 (Willison et al., 1995). However, Bodelier and Laanbroek (2004) reported that the application of N fertilizer directly promote the oxidation of CH_4 by nitrifying bacteria. Adding straw and green manure to soil are important measures to improve soil organic matter (Wu et al., 2019; Yu et al., 2020). The application of organic fertilizers can promote microbial decomposition activities and root respiration, leading to the increase of the CO_2 emission of soil (Qiu et al., 2015; Li et al., 2019). The addition of straw and green manure can improve soil methane production potentials and the abundances of methanogens (Zhou et al., 2020). However, Hoang et al. (2019) found that returning burned straw to the field could reduce seasonal cumulative CH_4 emission. Therefore, the results of fertilization types on soil GHG emissions are still controversial, especially the impact of fertilization on CH_4 fluxes.

In addition to the single application of chemical or organic fertilizer, the combined application of both is the main measure of agricultural fertilization (Zhou et al., 2019; Qaswar et al., 2020). Different proportions of fertilizers with differences in C/N ratio may affect soil physicochemical properties, and soil CO_2 and CH_4 emission (Finn et al., 2016; Zhou et al., 2017; Gwon et al., 2019). Fertilizers with low C/N ratio can substantially increase the decomposition of residues and contribute to GHG emissions (Abera et al., 2014; Zhou et al., 2019). But studies have found that adding fertilizers with low C/N ratio can reduce CH_4 emissions (Kim et al., 2012). Oilseed rape cake is a low C/N ratio residue, and GHG emission characteristics from soil following combined application of oilseed rape cake and chemical fertilizer is not clear (Raheem et al., 2019). Soil properties had direct or indirect effects on CO_2 and CH_4 emission. Soil dissolved organic carbon (DOC) is an indicator of the C available to soil microbes, and has close relationship with the respiration and denitrification of heterotrophic microbes (Boyer and Groffman, 1996). Iqbal et al. (2008) observed a positive correlation between DOC and soil CO_2 flux, and DOC provided energy to methanogens to promote CH_4 production (Zhou et al., 2020). In addition, some studies have shown that soil respiration was positively correlated with soil DOC and soil microbial biomass carbon (MBC) (Ge et al., 2020; Wu et al., 2020). Soil nitrate nitrogen (NO_3^--N) and NH_4^+-N not only provides essential nutrients for microbes but also indirectly affects soil microbes and gas emissions. The process of nitrification and denitrification was affected by the soil pH and affected the activity of soil microbes (Sauze et al. 2017; Li et al., 2020). Furthermore, denitrifying intermediates can inhibit methanogenic microorganisms during nitrate reduction (Clarens et al., 1998; Bao et al., 2016). Moreover, NH_4^+-N inhibited the oxidation of CH_4 by competitive action in upland soils (Schimel, 2000), but Bodelier et al. (2000) found ammonium actually stimulates CH_4 oxidation and methanotroph growth in rice-paddy soils. Therefore, clarifying the relationship between soil physicochemical properties and gas flux under different fertilization methods plays an important role in controlling GHG emissions of tea plantations.

Soil respiration is the main flux of C between the atmosphere and soil, and soil C loss can be quantified by soil CO_2 and CH_4 emission (Khan et al., 2017; Liu et al., 2019). Studies have shown that fertilization can increase soil C sequestration and reduce GHG emissions (Wu et al., 2019). By exploring the emission characteristics of soil CO_2 and CH_4 in tea plantations, we can understand the soil C sequestration and provide theoretical basis for improving the productivity of tea. In addition, N content of fertilizers is an important factor affecting CO_2 and CH_4 fluxes. Studies have shown that using low levels of N fertilizers produces low magnitudes of CO_2 and CH_4 (Shao et al., 2014; Li et al., 2019). In the present, the N concentrations of fertilizers were controlled at a low level, and then explored the CO_2 and CH_4 emission characteristics of different fertilization methods. We selected the soil of tea plantations in central China for incubation experiment to measure soil CO_2 and CH_4 fluxes. Organic and inorganic fertilizers were applied in the present study. The specific objectives of this study were to (1) compare the emission pattern and characteristics of CO_2 and CH_4 in tea plantations soil under different fertilization types and proportions, (2) identify the relationships between soil properties and CO_2 and CH_4 fluxes, and (3) choose minimal carbon emission fertilization types and proportions and to provide a scientific basis for reducing agricultural pollution in tea plantations. We hypothesized that mixed application of nitrogen and oilseed rape cake fertilizer will increase CO_2 and CH_4 emissions under the condition of lower nitrogen addition, and soil C content and mineral N content are the main factors affecting CO_2 and CH_4 emissions.

2. Materials and methods

2.1. Experimental soil

The surface soil (0 ~ 20 cm) in this study was collected from the tea garden of Heshengqiao town (29°02′ ~ 30°18′N, 133°31′ ~ 144°58′E), Xianning city, Hubei province, China. The region has the characteristics of typical plain-hills area and belongs to the typical subtropical monsoon climate. The annual average temperatures and precipitation are 16.8 °C and 1577 mm, respectively. The red soil in this area can be classified as Ultisols, as well as some Alfisols and Oxisols, on the basis of US soil taxonomy. The field had been under gone tea plantation for more than 10 years. The average elevation of the sampling site is about 35 m, and the surface soil of tea garden was collected from 0 ~ 20 cm by the diagonal multipoint mixing. A composite soil sample was made after removing visible organic residues and the small stones. The soil was passed through a 2-mm sieve after air-drying and used for incubation experiments. The properties of the soil were as follows: 45.3% sand, 22.6% silt, 15.7% clay, pH 4.57, bulk density 1.41 g cm^{-3}, 17.2 g kg^{-1} total C (TC), 1.04 g kg^{-1} total N (TN), 64.2 mg kg^{-1} NH_4^+-N, and 19.1 mg kg^{-1} NO_3^--N.

2.2. Experiment design

The cake fertilizer used in the experiment was oilseed rape cake, which was dried, crushed, and set aside after 100 mesh sieving. The total C content of rapeseed cake was 446 g kg^{-1}, the total N content was 51.2 g kg^{-1}, and C/N ratio was 8.71. Urea aqueous solution was used as N fertilizer, instead of granule form. Five treatments were designed in this study: no fertilizer (CK), single nitrogen (urea, N), single cake fertilizer (R), nitrogen + cake fertilizer (2:1, NR1), and nitrogen + cake fertilizer (1:2, NR2), respectively. The N concentrations of all fertilization treatments were limited to 100 mg kg^{-1}. The fertilization proportion of NR1 and NR2 was determined by the nitrogen content of nitrogen fertilizer and cake fertilizer. The C contents of R, NR1, and NR2 were 870 mg kg^{-1}, 290 mg kg^{-1}, and 580 mg kg^{-1}, respectively.

A weight of 200 g air-dried soil was placed in a 1000 mL glass bottle. Distilled water was added to moisten the soil, and the soil was preincubated for 10 days in the dark to activate soil microbes. At the end of preincubation, different types and proportions of fertilizer were added to the glass bottles. Three replicates were set for each treatment. At the same time, water content of distilled water was adjusted as 60% for the entire experiment, and the water lost by evaporation was replenished by weighing every 2 days to keep the soil water content constant. Top of the glass bottle was closed by a butyl rubber stopper having two holes. The glass tubes with three valve hose were inserted into holes, which was used to exchange and sample gas. All treatment bottles were incubated aerobically in a biochemical incubator at 25 °C ± 1.0 °C for 50 days. The incubation bottles consisted of two sets, one set was used to collect gas, and the other one was used to collect soil samples to determine soil DOC, MBC, pH, NH_4^+-N, and NO_3^--N.

Gases were sampled from glass bottles each day for the first 7 days, each 2 or 3 days till day 22, and each 5 days till the last day 50. In addition, soil samples of each treatment were destructively sampled at days 1, 3, 7, 13, 22, 41, and 50. Before sampling, the butyl rubber stopper was removed to ensure thorough gas exchange between the ambient air and atmosphere inside each glass bottle. The upper headspace gas sample of the glass bottle was collected as the initial gas concentration and recorded the sampling time. After 2 hours of closure, the gas samples were collected again and stored in a prevacuumed bottle.

2.3. Methods for the calculation and determination of gases

The calculation formula of CO_2 and CH_4 fluxes of soil greenhouse gases was as follows (Zheng et al., 1998):

$$F = \rho \times \frac{V}{m} \times \frac{dc}{dt} \times \frac{273}{T}$$

Where F is the fluxes of CO_2 and CH_4 (mg kg^{-1} h^{-1}), a positive value represents the emission of this gas from soil to the atmosphere, and a negative value represents the absorption of this gas by the soil. ρ is the density of gas under standard conditions, the density of CO_2 and CH_4 are 1.98 kg m^{-3}, 0.714 kg m^{-3}, respectively; V is the glass bottle upper effective space volume (m^3); m is the dry weight of soil sample, and the unit is (g); dc/dt is within the unit time gas concentration change; T is the absolute temperature. The concentration of CO_2 and CH_4 was determined by gas chromatograph (Agilent 7890A, California, USA). Cumulative emissions of CO_2 and CH_4 were calculated by multiplying the average emission rate of two adjacent gases by the emission time.

2.4. Soil analysis

Soil pH was measured in a 1:2.5 soil: water (w/v) mixture using a pH meter connected with a glass electrode (InsMark™ IS126, Shanghai, China). Soil bulk density was determined using ring sampler weighing method. The particle composition was measured with a laser particle size analyzer (Master-sizer 2000, Malvern, UK). Total C (TC) and total N (TN) were analyzed by elemental analyzer (Vario MACRO Cube, Elementar, Germany). Dissolved organic carbon was extracted with 0.5 mol L^{-1} K_2SO_4 and the extracts were measured using a Liqui TOCII analyzer (Elementar, Germany; Jones and Willett, 2006; Wang et al., 2020). Soil microbial biomass carbon was determined by chloroform fumigation-K_2SO_4 extraction method(Brookes et al., 1985). The difference between the total C in nonfumigated and fumigated samples was considered as MBC, and the conversion factors was 0.45. Soil was extracted and filtered by 1 mol L^{-1} KCl, and the contents of NH_4^+-N and NO_3^--N in the extract were measured by Seal Analytical chemistry AA3 flow analyzer.

2.5. Statistical analysis

Statistical analysis of the data was conducted using SPSS 21.0 (SPSS Inc., Chicago, IL, USA). A one-way analysis of variance and a Duncan multiple comparisons ($P < 0.05$) test were used to assess the significance of differences among different treatments (Liu et al., 2020). All bar graphs and broken line graphs were drawn using Origin 2018. Using the corrplot package in R (v.3.5.2) for pairwise comparisons, and the vegan package in R was used for Mantel tests (Sunagawa et al., 2015; Liu et al., 2019). To understand the relationship between CO_2, CH_4, and soil properties, we correlated CO_2 flux and CH_4 flux with soil properties by partial (geographic distance-corrected) Mantel tests. The structural equation modeling (SEM) framework was used to investigate direct and indirect effects of environmental variables on CO_2 flux and CH_4 flux. The χ^2 values, P values, RMSEA, and AIC were adopted to evaluate the overall goodness of structural equation model fit. SEM was performed by Amos 17.0 software package (Smallwaters Corporation, Chicago, IL, USA).

3. Results

3.1. Soil CO_2 emission flux and cumulative emission

In all treatments, soil CO_2 fluxes showed a gradual decrease in the first 27 days of incubation and finally stay stable (**Figure 1a**). The CO_2 fluxes of the CK, NR1, NR2 treatments peaked after a small increase in the first 4 days, and the fluxes of CO_2 under four fertilization treatments was higher than those in CK. At the later stage of incubation, CO_2 fluxes of each treatment were relatively stable

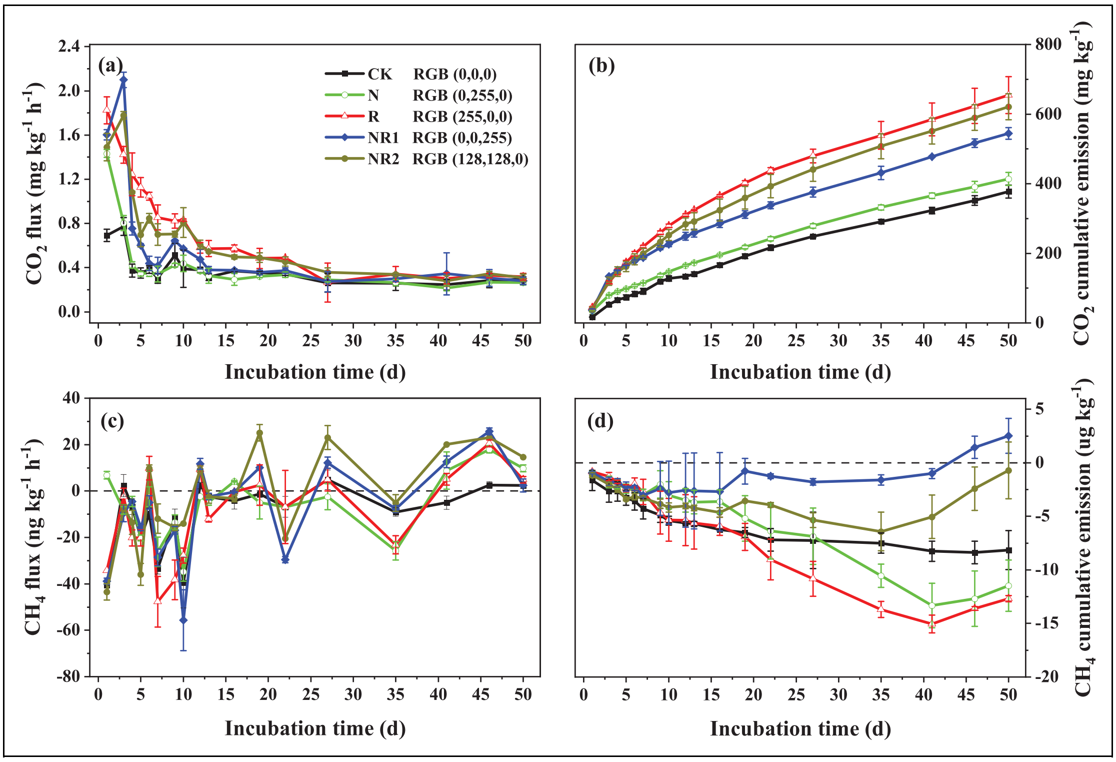

Figure 1. CO_2 (A) and CH_4 (C) fluxes, and cumulative CO_2 (B) and CH_4 (D) emissions in five different treatments. CK (soil), N (soil + urea), R (soil + rape cake fertilizer), NR1 (soil + urea, rape cake fertilizer (2:1)), NR2 (soil + urea, rape cake fertilizer (1:2)). Error bars represent standard error of the mean ($n = 3$). DOI: https://doi.org/10.1525/elementa.2021.090.f1

Table 1. CO_2 and CH_4 fluxes under N fertilizer and oilseed rape cake fertilizer. DOI: https://doi.org/10.1525/elementa.2021.090.t1

	CO_2			CH_4		
Treatment	**Variation ($mg\ kg^{-1}\ h^{-1}$)**	**Average ($mg\ kg^{-1}\ h^{-1}$)**	**Cumulative Emission ($mg\ kg^{-1}$)**	**Variation ($ng\ kg^{-1}\ h^{-1}$)**	**Average ($ng\ kg^{-1}\ h^{-1}$)**	**Cumulative Emission ($\mu g\ kg^{-1}$)**
CK	0.25 ~ 0.77	0.39 ± 0.04 d	377 ± 18.3 c	–40.5 ~ 4.86	–11.2 ± 1.40 a	–8.16 ± 1.83 b
N	0.22 ~ 1.43	0.42 ± 0.01 d	414 ± 18.7 c	–34.8 ~ 17.9	–4.47 ± 9.37 a	–11.5 ± 2.40 b
R	0.26 ~ 1.82	0.75 ± 0.03 a	654 ± 52.9 a	–47.7 ~ 20.5	–9.09 ± 7.18 a	–12.7 ± 0.29 b
NR1	0.27 ~ 2.10	0.58 ± 0.01 c	544 ± 16.9 b	–55.7 ~ 25.7	–1.49 ± 2.01 a	2.51 ± 1.63 a
NR2	0.28 ~ 1.78	0.68 ± 0.05 b	621 ± 37.7 a	–43.5 ~ 25.1	–2.51 ± 4.48 a	–0.70 ± 2.66 a

CK (soil), N (soil + urea), R (soil + rape cake fertilizer), NR1 (soil + urea, rape cake fertilizer (2:1)), NR2 (soil + urea, rape cake fertilizer (1:2)). Different letters within a column mean significant difference ($P < 0.05$). Data are means ± *SD* of three independent replicates.

between 0.27 and 0.32 mg kg^{-1} h^{-1}. During the incubation, the CO_2 fluxes of the N treatment were significantly lower than those in the three treatments with cake fertilizer ($P < 0.05$; **Table 1**). Compared with the control, the treatment with single nitrogen fertilizer had no significant effect on the CO_2 emission. The average CO_2 fluxes from high to low were, in turn, R > NR2 > NR1 > N > CK. The values were 0.75 ± 0.03, 0.68 ± 0.05, 0.58 ± 0.01, 0.42 ± 0.01, and 0.39 ± 0.04 mg kg^{-1} h^{-1}, respectively (**Table 1**).

The cumulative CO_2 emission increased gradually in the process of incubation, and it sharply increased in the early stages (**Figure 1b**). In the study period, cumulative CO_2 emissions in R, NR2, and NR1 were significantly higher than those in CK ($P < 0.05$; **Table 1**). The R treatment had the highest cumulative emissions of 654 ± 52.9 mg kg^{-1}, which is 1.73 times higher than that of the CK treatment (**Table 1**). Cumulative CO_2 emission of the CK and N treatment were 377 ± 18.3 mg kg^{-1}, 414 ± 18.7 mg kg^{-1} with no significant difference ($P > 0.05$).

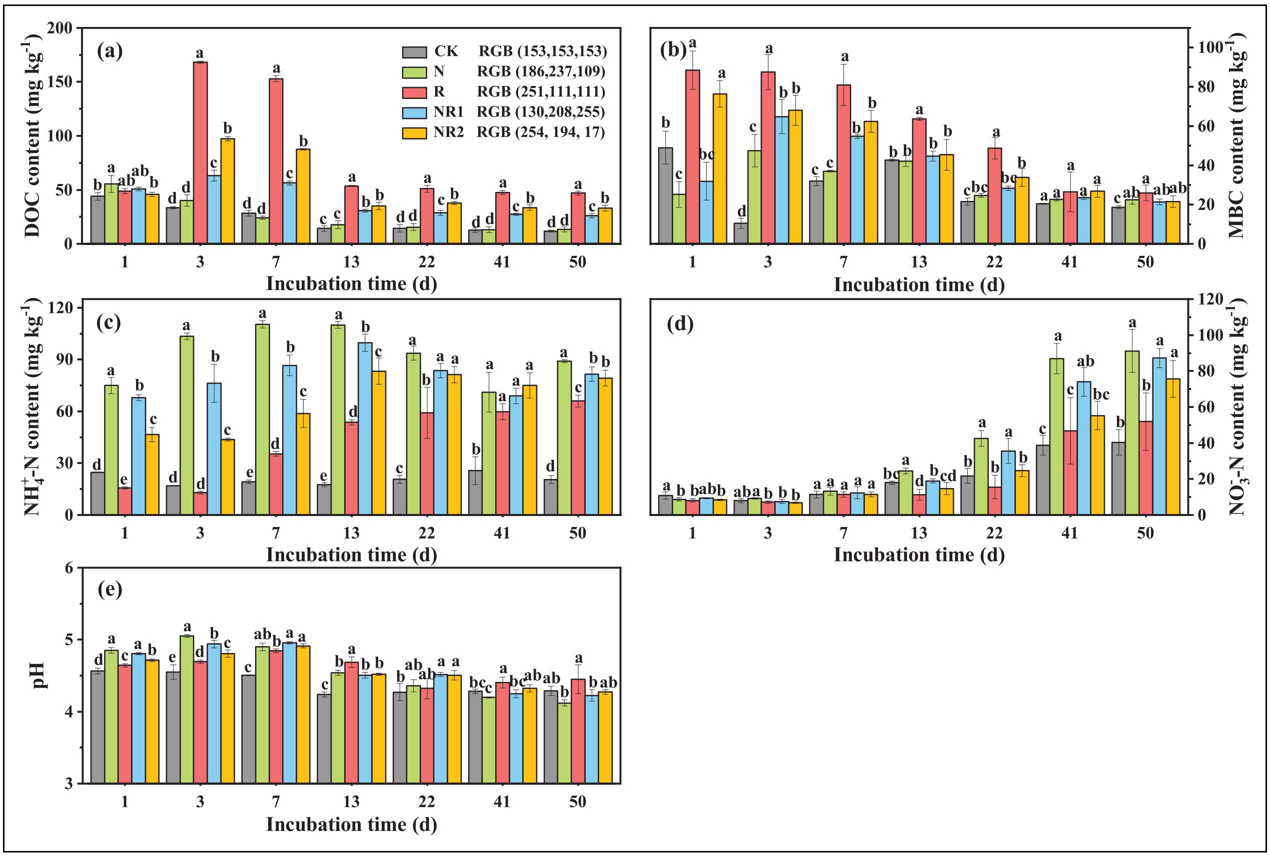

Figure 2. Changes in soil DOC contents (A), MBC contents (B), NH_4^+-N contents (C), NO_3^--N contents (D) and pH (E) after five different treatments. CK (soil), N (soil + urea), R (soil + rape cake fertilizer), NR1 (soil + urea, rape cake fertilizer (2:1)), NR2 (soil + urea, rape cake fertilizer (1:2)). Data were analyzed by one-way analysis of variance and means were compared by Duncan test. Different letters indicate significant differences ($P < 0.05$) among the different treatments. DOI: https://doi.org/10.1525/elementa.2021.090.f2

3.2. Soil CH_4 emission flux and cumulative emission

There were some fluctuations of CH_4 fluxes during the whole incubation process. However, the overall trend of CH_4 fluxes changed from negative value to positive value, that is, the soil gradually changed from absorbing CH_4 to discharging throughout the study stage (**Figure 1c**). At the early stage of incubation, CH_4 showed absorption trend in treatments NR2, CK, NR1 and R with absorption values of 43.5 ± 3.49, 40.5 ± 0.32, 38.9 ± 1.32, and 34.3 ± 1.21 ng kg^{-1} h^{-1}, respectively. At the end of the incubation, all treatments showed CH_4 emissions, and the emissions of NR2, N, R, NR1, and CK were 14.7 ± 2.78, 9.77 ± 1.41, 4.88 ± 1.39, 2.46 ± 2.82, and 2.44 ± 1.44 ng kg^{-1} h^{-1}, respectively. During the incubation, there was no significant difference between treatments (**Table 1**).

The soil cumulative CH_4 emission of NR2 CK, N and R showed net absorption, and its cumulative emissions at the end of the incubation were –0.70 ± 2.66, –8.16 ± 1.83, −11.5 ± 2.40, and −12.7 ± 0.29 μg kg^{-1}, respectively (**Figure 1d**). Among them, the absorption of CH_4 by the three treatments (N, R, and NR2) increased first and then decreased. The NR1 treatment switched to net emissions after 41 days, and the final cumulative emissions were 2.51 ± 1.63 μg kg^{-1}. The cumulative CH_4 emissions in the NR1 and NR2 treatments were significantly higher than that in the CK, N, and R treatments ($P < 0.05$; **Table 1**).

3.3. Changes in soil properties

Throughout the study stage, the contents of DOC and MBC in the R treatment were significantly higher than other treatments (**Figure 2a** and b). At the end of the incubation, DOC content in the R, NR2, NR1, and N treatments was 2.91, 1.66, 1.21, and 0.12 times higher than the CK treatment, respectively. Compared with the treatment with single N fertilizer, the average content of DOC in R, NR2, NR1 treatment was significantly increased ($P < 0.05$; **Table 2**). The content of MBC treated by fertilization showed a trend of decreasing gradually with the development of incubation time (**Figure 2b**). In the whole incubation, the average MBC content of each treatment was R > NR2 > NR1 > N > CK from high to low (**Table 2**).

The NH_4^+-N content of the N treatment was the highest in the incubation process (**Figure 2c**). Moreover, the average contents of NH_4^+-N in each treatment (N, NR1, NR2, R, CK) were 94.4 ± 4.28, 81.1 ± 0.42, 67.9 ± 1.21, 41.5 ± 3.74, and 20.7 ± 1.87 mg kg^{-1}, respectively and there were significant differences among the treatments ($P < 0.05$; **Table 2**). The trend of NO_3^--N content in soil gradually increased with time, especially in N and NR1 treatments(**Figure 2d**). The average contents of NO_3^--N in the treatment of N and NR1 were significantly higher than the treatment of R ($P < 0.05$; **Table 2**). In addition,

Table 2. The changes of the pH value and the content of DOC, MBC, NH_4^+-N, and NO_3^--N in five different treatments. DOI: https://doi.org/10.1525/elementa.2021.090.t2

Treatment	DOC (mg kg^{-1})		MBC (mg kg^{-1})		NH_4^+-N (mg kg^{-1})		NO_3^--N (mg kg^{-1})		pH	
	Variation	Average	Variation	Average	Variation	Average	Variation	Average	Variation	Average
CK	11.9 ~ 43.9	20.3 ± 3.23^e	10.5 ~ 48.9	26.2 ± 1.42^d	16.8 ~ 25.6	20.7 ± 1.87^e	7.94 ~ 40.4	21.3 ± 2.20^{bc}	4.24 ~ 4.57	4.37 ± 0.04^b
N	12.9 ~ 55.5	25.6 ± 0.76^d	22.4 ~ 47.5	30.8 ± 4.74^{cd}	71.1 ~ 110.4	94.4 ± 4.28^a	8.72 ~ 91.2	40.8 ± 0.74^a	4.12 ~ 5.05	4.62 ± 0.08^a
R	47.0 ~ 168	83.2 ± 3.61^a	25.9 ~ 88.5	57.9 ± 3.01^a	12.9 ~ 65.9	41.5 ± 3.74^d	7.15 ~ 51.9	18.1 ± 7.54^c	4.32 ~ 4.85	4.58 ± 0.03^a
NR1	26.2 ~ 63.2	40.5 ± 1.00^c	21.4 ~ 64.8	35.8 ± 3.35^c	67.9 ~ 99.7	81.1 ± 0.42^b	7.49 ~ 87.2	34.9 ± 1.69^a	4.22 ~ 4.96	4.60 ± 0.01^a
NR2	33.1 ~ 97.3	52.9 ± 1.39^b	21.5 ~ 76.4	45.1 ± 1.70^b	43.7 ~ 83.2	67.9 ± 1.21^c	6.86 ~ 75.6	26.6 ± 3.99^b	4.27 ~ 4.91	4.59 ± 0.03^a

CK (soil), N (soil + urea), R (soil + rape cake fertilizer), NR1 (soil + urea, rape cake fertilizer (2:1)), NR2 (soil + urea, rape cake fertilizer (1:2)). Different letters within a column mean significant difference ($P < 0.05$). Data are means ± *SD* of three independent replicates.

the average NO_3^--N content of the R treatment was the lowest, which was 18.1 ± 7.54 mg kg^{-1}.

The change of soil pH value with time under different treatments was shown in **Figure 2e**. Soil pH value in each treatment varied within the range of 4.12–5.05. The average pH value of the four fertilization treatments was significantly higher than the CK treatment (**Table 2**). At the end of incubation, compared with CK, the pH value of R treatment increased by 0.01 units, while the pH value of N, NR1, and NR2 treatment decreased by 0.21, 0.06, and 0.04 units, respectively.

3.4. The relationship between soil CO_2, CH_4 flux and soil properties

According to the correlation results between soil properties, pH showed a correlation with NO_3^--N ($r = -0.8$), DOC ($r = 0.73$), and MBC ($r = 0.67$), while NO_3^--N had a negative correlation with DOC ($r = -0.66$) and MBC ($r = -0.68$), and DOC had a positive correlation with MBC ($r = 0.73$; **Figure 3**). Correlation results can be used to determine collinear problems among factors and construct structural equation models. To understand the relationship between CO_2, CH_4, and soil properties, we correlated CO_2 flux and CH_4 flux with soil properties by partial (geographic distance-corrected) Mantel tests. Overall, DOC and MBC had the strongest correlations with CO_2 flux, followed by NO_3^--N and pH. The correlation between CH_4 flux and NO_3^--N was the strongest, while DOC was not significantly correlated (**Figure 3**).

The effects of different fertilization treatments showed a reasonable fit to our hypothesized causal relationships, as shown by the properties of the SEM ($\chi^2 = 7.04$, $P = 0.42$, RMSEA = 0.01, AIC = 35.0, **Figure 4a**; $\chi^2 = 3.80$, $P = 0.70$, RMSEA = 0.00, AIC = 33.8, **Figure 4c**). NH_4^+-N exerted an indirect negative effect on CO_2 flux and CH_4 flux, as shown by CO_2 (–0.14) and CH_4 (–0.16) release, and NO_3^--N showed a negative control on CO_2 (–0.15) and CH_4 (–0.32), respectively. The emission for both CO_2 and CH_4 was positively affected by DOC. The MBC showed a direct effect on CO_2 (+0.43) and CH_4 (+0.21) emission, respectively. And pH mainly exerted a positive control on CO_2 release (+0.63) and CH_4 formation (+0.32) (**Figure 4b** and d).

4. Discussion

The trend of CO_2 emissions under different fertilization types was consistent, but there were significant differences among treatments. The CO_2 fluxes showed a gradual decline during the whole incubation period (**Figure 1a**), which was consistent with the content of MBC (**Figure 2b**). Previous studies have reported that soil CO_2 emission was affected by multiple factors, including organic matter content (Iqbal et al., 2008), microbial activities (Sauze et al., 2017), and different fertilization treatments (Nyamadzawo et al., 2014). However, for indoor cultivated soil without vegetation cover, microbial respiration in soil was the main way of CO_2 emission (Zhang et al., 2016). Therefore, with the available C and N sources of microbes gradually reduced, the CO_2 emission rate slowed down and finally stabilized, which was coincident with the study

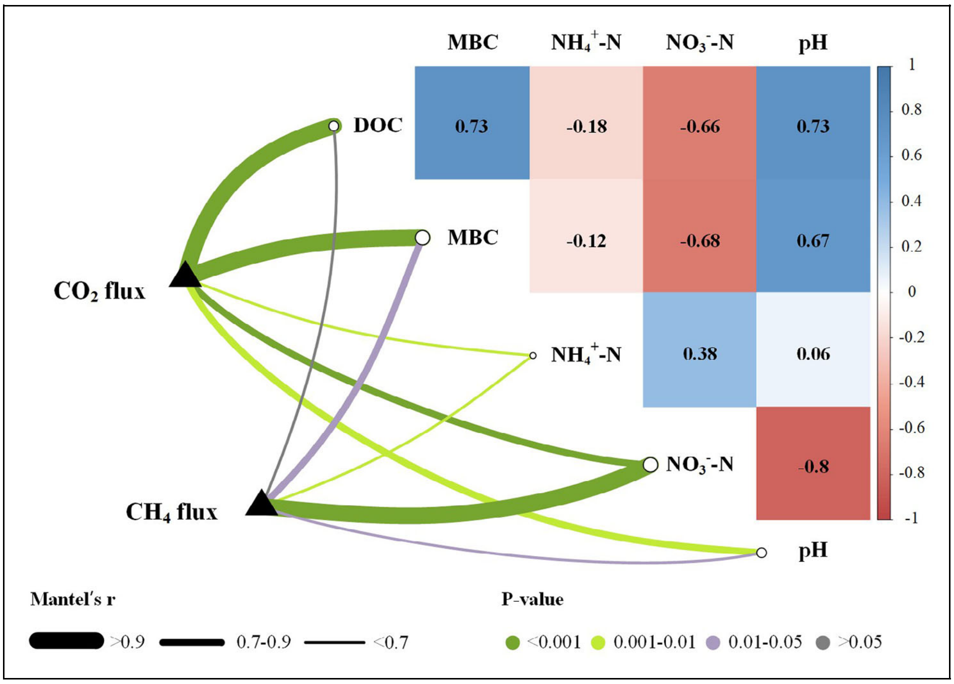

Figure 3. Environmental drivers of CO_2 and CH_4 fluxes. Pairwise comparisons of physicochemical property are shown, with a color gradient denoting Spearman's correlation coefficients. CO_2 flux and CH_4 flux were related to each environmental factor by partial (geographic distance-corrected) Mantel tests. Edge width corresponds to the Mantel's *r* statistic for the corresponding distance correlations, and edge color denotes the statistical significance based on 9,999 permutations. DOI: https://doi.org/10.1525/elementa.2021.090.f3

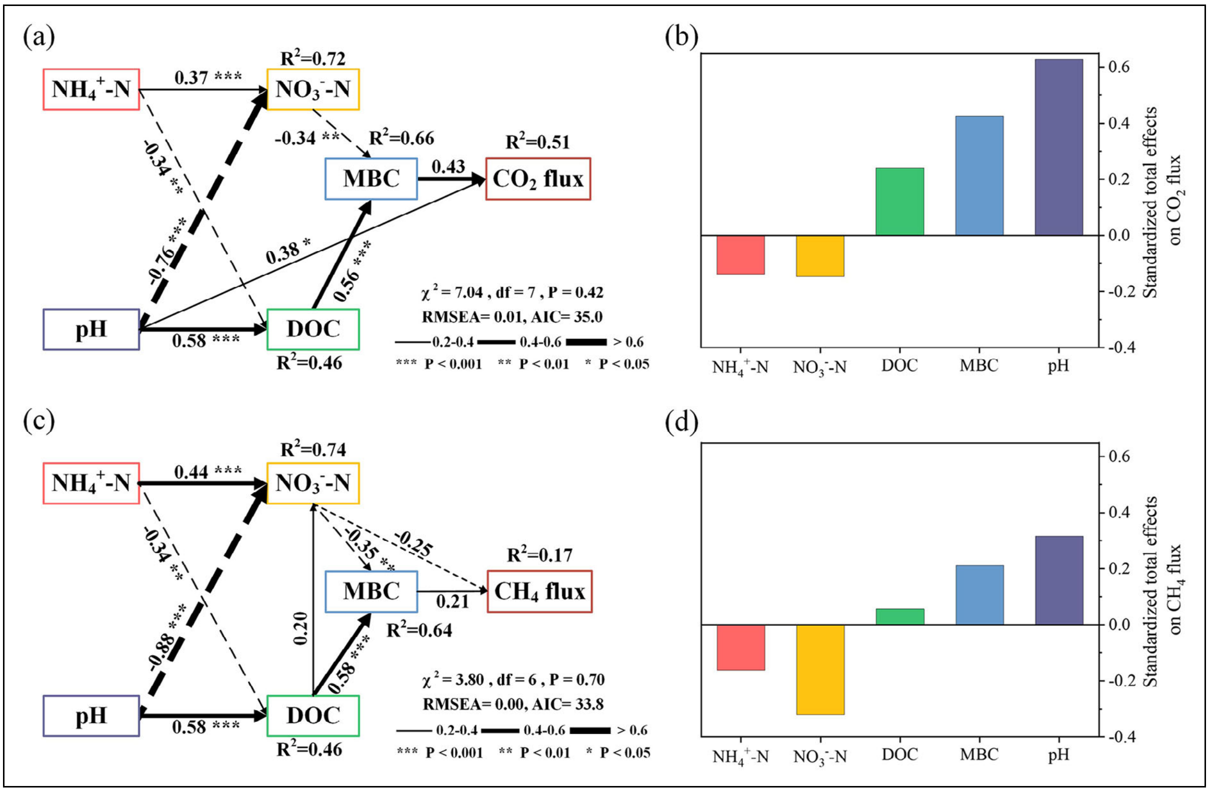

Figure 4. The structural equation model (SEM) showing the effects of NH_4^+-N, NO_3^--N, microbial biomass C (MBC), dissolved organic C (DOC), and pH on CO_2 (A) and CH_4 (C) fluxes. Standardized total effects of soil NH_4^+-N, NO_3^--N, MBC, DOC, and pH on CO_2 (B) and CH_4 (D) fluxes as revealed by SEM. The width of the arrows indicates the strength of the standardized path coefficient. Solid lines represent positive path coefficients and dashed lines represent negative path coefficients. R^2 values represent the proportion of the variance explained for each endogenous variable. DOI: https://doi.org/10.1525/elementa.2021.090.f4

result of Senbayram et al. (2019). Compared to the CK treatment, single application of N fertilizer had no significant effect on soil CO_2 flux (**Table 1**). This result indicated that the addition of organic matter was the main source of CO_2 production (Singh et al., 1999). In addition, organic carbon as the substrate of soil respiration could provide available C and N for microbial decomposition (Witt et al., 2000; Qiu et al., 2015; Shah et al., 2016), and organic materials such as straw and cake fertilizer applied into the soil are conducive to the formation of soil aggregates and increase the soil porosity and promote the diffusion of CO_2 (Kallenbach et al., 2010). This was also the reason why CO_2 emission of three cake fertilizer treatments was higher than the N treatment (**Table 1**).

Among them, the CO_2 emission of single cake fertilizer treatment was higher than that of nitrogen fertilizer and cake fertilizer mixed treatment (**Figure 1b**). This could be due to the lower C/N ratio of oilseed rape cake (8.71) which is conducive for high respiration and ultimately CO_2 emissions (Kim et al., 2012; Raheem et al., 2019). In addition, specific C/N ratio is required for microbial activities to meet their nutritional requirements, and the addition of the residue with a low C/N ratio in this study made the N content a factor not limiting microbial activity (Finn et al., 2016; Wei et al., 2019). Therefore, the C content of fertilizer was an important factor affecting CO_2 emission in this study. The C content in R treatment was the highest, which provided more C sources for soil microbial activities. This was also supported by the result of CO_2 emission that was positively affected by DOC and MBC, as revealed by SEM (**Figure 4b**). As an indicator of microbial available C, DOC can indirectly affect soil respiration by influencing microbial activity and MBC (Boyer and Groffman, 1996; Wu et al., 2020). A previous study has shown that the abundance of soil phototrophs increased most at higher soil pH, promoting the production of CO_2 (Sauze et al., 2017). This is consistent with the results of the structural equation model that CO_2 was positively affected by pH (**Figure 4b**). In addition, CO_2-C is considered to be the sole C source promoting the autotrophic growth of nitrifiers (Xia et al., 2011; Zhang et al., 2019). Soil pH can indirectly affect soil CO_2 emissions by affecting soil nitrification rate (Li et al., 2020).

CH_4 fluxes mainly remained negative and low during the study period(**Figure 1c**). Our finding was similar to other research results from cultivated land (Li et al., 2019) and grassland (Shimizu et al., 2013). The main reason is that the experiment was carried out under the condition of aerobic, and CH_4 can be oxidized by methanotroph, so it is not conducive to the production of CH_4 (Zhou et al., 2020). However, CH_4 emissions do exist during the whole incubation process (**Figure 1c**). This could be due to uneven distribution of soil particles and water leading to the formation of anaerobic microzone, resulting in CH_4 emissions (Kong et al., 2019). Moreover, Hurkuck et al. (2012) observed that in addition to microbial activity, organic compounds such as lignin and pectin showed substantial release of CH_4. The cumulative emissions of CH_4 from each treatment in a short period of 50 days were mainly concentrated in negative values (**Figure 1d**), which can be considered that the soil of the tea garden is the net absorption sink of CH_4 in a short time and be similar to farmland (Jacinthe and Lal, 2005).

There was no significant difference between the CH_4 fluxes among treatments. However, the cumulative CH_4 fluxes in two treatments that mixed application of N fertilizer and cake fertilizer (NR1, NR2) were significantly higher than the other three (CK, N, R; **Table 1**). The R treatment showed the highest CH_4 absorption; it is possible that more abundant C sources were provided to methanotroph than other treatments (Wang et al., 2019; Zhou et al., 2020). However, compared with the N treatment, NR1 and NR2 reduced CH_4 absorption (**Figure 1d**), and previous studies have reported that NH_4^+-N has a similar molecular shape and size to CH_4, and NH_4^+-N can be used as a substrate for methanotroph to inhibit the absorption of CH_4 (Bedard and Knowles, 1989; Schimel, 2000). This is consistent with the results that the NH_4^+-N content of NR1 and NR2 was higher in incubation (**Figure 2c**). In addition, NH_4^+-N produces NO_2^- in the oxidation process, which may poison methanotroph in a short time and inhibit the oxidation of CH_4 (Dunfield and Knowles, 1995). Although the N treatment had the highest NH_4^+-N content, there is no C source for microorganisms to carry out oxidation activities. This explains why NR1 absorbed the least CH_4 in this result (**Figure 1d**). The input of oilseed rape cake provided available C sources for methanogen and methanotroph (Seghers et al., 2005), but the input of nitrogen affected microbial activities and indirectly affected CH_4 emission (**Figure 4c**). The N treatment had the highest NO_3^--N content, and its cumulative CH_4 emissions were lower than the control treatment (**Table 2**; **Figure 1d**). Some studies have found that nitrate has an inhibitory effect on CH_4 production (Lu et al., 2000). On the one hand, denitrification intermediates (NO_2^-, NO, N_2O) from nitrate reduction can inhibit methanogens (Clarens et al. 1998; Liu et al., 2017). On the other hand, CH_4 emission is reduced when nitrate reducers are more competitive than methanogens for common substrates (Bao et al., 2016). This was shown by the NO_3^--N, which indicated a negative effect for CH_4 as revealed by structural equation models (**Figure 4d**).

It is worth noting that the results of CO_2 and CH_4 emissions in this experiment were obtained by using tea plantation soil under indoor incubation conditions rather in the presence of tea plants. The uptake of soil nutrients by tea plants, root exudates, water and climatic factors can affect the soil CO_2 and CH_4 emissions under planting conditions (Xiang et al., 2008; Kechavarzi et al., 2010; Du et al., 2020). Besides, the soil sampled from tea plant field was disturbed in terms of grinding and adding treatments, and thus soil organic matter become more vulnerable to the decomposition increasing the amount of substrate for microorganisms and affecting greenhouse gas emission (Six et al., 2000). Therefore, filed studies are necessary to deeply and systematically exploration of influencing rules and mechanisms pertinent to GHGs from tea plant soils.

5. Conclusions

The application of urea and oilseed rape cake fertilizer showed a significant difference for the CO_2 and CH_4 fluxes from tea plantation soil. The single application of N fertilizer had no significant impact on soil CO_2 emissions. However, the addition of cake fertilizer significantly increased soil MBC content and CO_2 emissions, especially the treatment of single cake fertilizer showed the highest emissions. The C content of fertilizer was positively proportional to the CO_2 emissions of soil. The cumulative emissions of CH_4 from each treatment were mainly concentrated in negative values, which can be considered that the soil of the tea garden is the net absorption and sink of CH_4 in a short time. Mixed application of N fertilizer and cake fertilizer reduced the soil absorption of CH_4. It is likely that NH_4^+-N was involved in microbial oxidation activity and inhibited the absorption of CH_4. In addition, soil pH, MBC, and DOC were the main factors affecting CO_2 fluxes, while CH_4 fluxes were mainly affected by mineral nitrogen contents. In conclusion, under the condition of low nitrogen addition, the single application of cake fertilizer increased the CO_2 emission but increased the oxidation of CH_4 and promoted soil C sequestration. The mixed application of urea and oilseed rape cake fertilizer reduced the emission of CO_2 but also increased CH_4 emissions. These results are helpful to further understand the CO_2 and CH_4 emission processes in tea plantation soil and provide potential strategies to reduce CO_2 and CH_4 emissions and improve soil C sequestration through improved fertilization management.

Data accessibility statement

All data generated or analyzed during this study are included in this article, and the underlying data are presented in the supplemental material.

Supplemental files

The supplemental files for this article can be found as follows:

Data 1-CO_2 emission.xlsx
Data 2-CH_4 emission.xlsx
Data 3-Soil property.xlsx

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Competing interests

The authors have no competing interests to declare.

Author contributions

SL and SZ contributed equally to this work.

Contributed to conception and design: SL, SZ, LF.

Contributed to acquisition of data: SL, SZ, GS, MS, WJ, YC, CD.

Contributed to analysis and interpretation of data: SL, SZ, GS, LF.

Drafted and/or revised the article: SZ, GS, MS, WJ, YC, CD.

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