JSEE Journal of Sustainable Energy & Environment

Journal of Sustainable Energy & Environment

13 (2022) 39-46

Nitrous oxide and carbon dioxide production in soils from the 46-year long term fertilization treatments in response to temperature and moisture changes

Chuleeporn Sriyam[1], Amnat Chidthaisong[1,*], Kazuyuki Yagi[2], Sirintornthep Towprayoon[1] and Sudarut Tripetchkul[3]

[1]The Joint Graduate School of Energy and Environment, King Mongkut's University of Technology Thonburi, 126 Prachauthit Rd., Bangmod, Tungkru, Bangkok, Thailand and Center of Excellence on Energy Technology and Environment (CEE), PERDO, Ministry of Higher Education, Science, Research and Innovation, Bangkok, Thailand
[2]Adaptation & Water Unit, Institute for Global Environmental Strategies (IGES), Kanagawa, Japan
[3]School of Bioresources and Technology, King Mongkut's University of Technology Thonburi, Thailand
*Corresponding author: amnat.chi@kmutt.ac.th

Abstract: This study aimed to determine the rates of N_2O and CO_2 production under different soil temperature and moisture conditions. Soil samples were collected from the 46-years long-term fertilizer experiment plots in Lopburi province. Three treatments were included in the present study; control or no fertilizer (NF), chemical fertilizer application (CF) and organic fertilizer application (OM). The soils were incubated at 25°C, 30°C, and 35°C under moisture content of 60 and 80% water-filled pore space (%WFPS) for 14 days. Long term fertilizer experiment (46 years) revealed that the application of cow or chicken manure has increased soil carbon stock (SOC) by 5-7 MgC ha^{-1} compared to that of no fertilizer and chemical fertilizer application. The N_2O production rate from all treatments was increased 2-9 times under 60% WFPS and 5-18 times under 80% WFPS when the temperature was increased from 25° to 30°C, but was decreased when the temperature was increased from 30° to 35°C, except the CF which was increased. The OM had highest N_2O and CO_2 at any temperatures investigated. Only the temperature above 30°C that the effects of moisture on N_2O production was observed. The mean value of N_2O production in OM under 60% WFPS and 80% WFPS differed only under 25°C. On the other hand, CO_2 production rates was enhanced 1-2 times along with increasing temperature and moisture. These results indicate that the long-term fertilizer application has significantly changed the production potential of N_2O and CO_2, and so their sensitivities to temperature and moisture changes.

Keywords: Long-term experiment, N_2O and CO_2 production, soil temperature, soil moisture.

1. Introduction

Evidences of adverse impacts of global climate change have called for the actions to reduce the emissions of greenhouse gases (GHGs) worldwide [1]. Agricultural management practices, such as crop cultivation and chemical fertilizer usage, contribute significantly to the emission of greenhouse gases (GHGs), including methane (CH_4), and nitrous oxide (N_2O). In Thailand, GHGs emissions from agricultural sector were 58,486 GgCO_{2eq} or 15.7% of national total GHG emissions in 2018 [2]. The main agricultural sources consist of rice cultivation (51.28% of total agricultural emissions), enteric fermentation (17.19%), and direct N_2O emission from managed soils (14.90%).

While lowland crop (rice cultivation) is the major emission source of CH_4, upland crop cultivation under well-aerated conditions is the major source of N_2O [3]. However, large spatial and temporal variations in the emission of N_2O were usually found in the past studies. Soil moisture content, oxygen availability, pH, inorganic nitrogen (ammonium nitrogen ($NH_4^+ - N$) and nitrate nitrogen ($NO_3^- - N$)), soil organic carbon (SOC), and soil temperature are the factors that control the rate of N_2O emission [4-6]. To effectively apply mitigation options, it is important to understand the mechanisms that these factors influence N_2O production and emission that lead to such high spatial and temporal variations.

The mineralization and decomposition of dead plants or animals by microbes can increase N availability to nitrification and denitrification [7]. Understanding of N mineralization is therefore important to improve the reliability of N_2O emission estimates across landscape or regions. Key factors that control the N mineralization are soil temperature and water content that are related to climate conditions at the site [8- 11]. Previous study in a long-term field experiment in Thailand showed that organic matter application and N fertilization impact microbial biomass, their activity and CO_2 emissions [12]. No information on the emissions of N_2O has been available yet.

Laboratory incubation experiments can be used to determine N dynamics in soils since the direct N transformation study is difficult under the field conditions [13-14]. The rates of N mineralization can be determined by measuring the substrates, such as organic N and products such as NH_4^+-N, NO_3^--N. Laboratory incubation experiments can also assess the mechanisms of N mineralization in relation to production of N_2O because the main process of mineralized N production in soils are nitrification and denitrification that release N_2O as the by-product.

Accordingly, this study aims to determine the rates of N_2O and CO_2 production of soil collected from different field management plots of a long-term experiment sites, and to understand the responses of soils to different temperatures and moisture. The comparison among the treatments of no fertilizer (NF), organic fertilizer application (OM), and chemical fertilizer (CF) was performed. The potential of agricultural management practices (applications of chemical fertilizer and organic matter) for mitigating GHGs emissions were also discussed.

2. Materials and Methods

2.1 Study sites for soil collection

Soil samples were collected from a long-term experiment site in Lopburi Province, central Thailand. The site is located within the Lopburi Agricultural Research and Development Center, Department of Agriculture (14°48'03.6"N and 100°47'58.3"E). The annual rainfall in 2020 at this site was 1286 mm and the mean annual air temperature was 28.0°C. Maize and other crops (mungbean, rice bean, cowpea, and soybean) have been rotated since 1976. During the period of this study, the cropping systems was maize rotated with mungbean. The texture of the soil was loam (%sand, % silt, and % clay of 51, 35.2, and 13.8%, respectively) [15]. Maize cultivation at this site was treated with chemical and organic N fertilizers as shown in Table 1. In September 2020, soil samples (0-20 cm) were collected from three different treatments (3 replicates from each treatment) after harvest. These were no fertilizer (NF), organic fertilizer application (OM), and chemical fertilizer application (CF). The rates of N input were 78.13 kg N ha^{-1} as manure, and 93.75 kg N ha^{-1} as 15-5-5 composite fertilizer for OM and CF, respectively.

2.2 Soil sampling and analysis

Soil samples were collected from the plots of three selected treatments (e.g., no fertilizer (NF), organic matter application (OM), and chemical fertilizer (CF)). The samples collected from three replicated plots of the same treatment were mixed as a composite soil sample and stored at 4°C in sterile plastic bags until being further processed. Large pieces of organic material, visible plant debris, and rocks were removed by hand and then the composite soil samples were sieved through a 2-mm mesh. The samples were then air-dried in a controlled temperature room (~28°C) for approximately two days to lower the moisture content to 20-25% WFPS and then the air-dried samples were stored at 4°C until further use.

Soil pH were measured in water suspension (soil:H_2O ratio of 1:1) [16]. The wet oxidation method of Walkey and Black was used for the analysis of soil organic matter (OM) and soil organic carbon (SOC) [17]. Total nitrogen was measured by the Kjeldahl method as described by Bremner [18]. The phenoldisulphonic acid method was used for determining the amount of nitrate ($NO_3^- - N$) in the soil samples [19-20]. The amount of ammonium is determined using the indophenol blue method [21]. Soil organic carbon (SOC) stock was calculated as indicated in the equation 1:

$$SOC\ [\mathrm{Mg\ ha^{-1}}] = \mathrm{OC} \times \mathrm{LT} \times \mathrm{BD} \times (1 - \mathrm{RF}) \times 10^4 \qquad (1)$$

Where OC is the percent of organic carbon concentration. LT is the layer thickness (m). BD is the bulk density (Mg m^{-3}). And RF is the rock fragment content fraction [22].

2.3 Laboratory incubation experiment and GHG analysis

Thirty (30) grams of sieved and air-dried soil samples were weighed and placed in a 100mL narrow bottle. The bottles were closed with a rubber septum. Two sets of the soils were prepared, one with the moisture content at 60% and another with 80% WFPS. The moisture was adjusted by adding distilled water until reaching the designated moisture level, and maintained at these levels throughout the incubation. The bottles then were incubated at 25°C, 30°C, and 35°C for 14 days. The headspace gas samples were collected for N_2O and CO_2 analyses after 1, 5, 7, 9, 12, and 14 days of incubation.

The concentrations of N_2O and CO_2 were then determined by a gas chromatograph (GC) equipped with electron capture detector (ECD) for N_2O analysis and with flame ionization detector (FID) for CO_2 analysis [23]. Before and after the gas sample analysis, a set of standard concentrations of N_2O and CO_2 were determined.

Table 1. The fertilization and management history at the Lopburi site for each treatment No-fertilizer (NF), Organic matter/fertilizer application (OM), and Chemical fertilizer treatment (CF).

Year	Treatment	Management practices				
		Crop rotation		Mulch application	Chemical fertilizer	Organic fertilizer
		1st crop	2nd crop			
1976-1979	NF	Maize	Mungbean	-	-	-
	OM	Maize	-	Vinyl sheet	-	-
	CF	Maize	Mungbean	-	✓*	-
1980-1989	NF	Maize	Mungbean	-	-	-
	OM	Maize	Rice bean	-	-	-
	CF	Maize	Mungbean	-	✓**	-
1990-1995	NF	Maize	Mungbean	-	-	-
	OM	Maize	Rice bean	-	-	-
	CF	Maize	Mungbean	-	✓**	-
2006-2008	NF	Maize	Mungbean	-	-	-
	OM	Maize	Soybean	-	✓***	-
	CF	Maize	Mungbean	-	✓**	-
2009-2010	NF	Maize	Mungbean	-	-	-
	OM	Maize	Cowpea	-	✓****	CKM
	CF	Maize	Mungbean	-	✓*****	-
2011-2015	NF	Maize	Mungbean	-	-	-
	OM	Maize	Mungbean	-	-	RHC
	CF	Maize	Mungbean	-	✓*****	-
2016-2020	NF	Maize	Mungbean	-	-	-
	OM	Maize	Mungbean	-	-	CWM
	CF	Maize	Mungbean	-	✓*****	-

*At a rate of 100-100-50 kg of N-P_2O_5-K_2O ha^{-1}, ** 62.5-62.5-0 kg of N-P_2O_5-K_2O ha^{-1}, *** 41.5-41.5-41.5 kg of N-P_2O_5-K_2O ha^{-1}, **** 9-3-3 (56.26-18.75-18.75 kg of N-P_2O_5-K_2O ha^{-1}), and ***** 15-5-5 (93.75-31.25-31.25 kg of N-P_2O_5-K_2O ha^{-1}), CKM: Chicken manure, CWM: Cow manure (6250 kg ha^{-1}), and RHC: Rice husk charcoal.

2.4 Soil extraction and measurement of dissolved N

The soil samples collected at 1, 5, 7, 9, 12, and 14 days of incubation, were immediately extracted with 2 M (mol L^{-1}) KCl (1:10, soil: KCl solution ratio), shaken for 60 min, and the soil-KCl extractions were filtered with Whatman No.42 filter paper. This was used for the analysis of dissolved N by Kjeldahl method [21, 24]. Soil N transformation rate (i.e.production or assimilation) was estimated by adapting the following equation [25-27]:

$$\mathrm{N_{transformation}} = -\mathrm{N_i} + \mathrm{N_e} \tag{2}$$

Where $\mathrm{N_i}$, $\mathrm{N_e}$ represent the amount dissolved N at the initial and the end of incubation, respectively. The negative value therefore indicates net consumption/assimilation while the positives ones indicate the net production of dissolved N in the incubated soil solution.

2.5 Statistical analysis

Statistical analyses were carried out by using the SPSS Statistics 26 package (SPSS Inc., USA). One-way analysis of variance (ANOVA) was conducted to see the effects of treatment, and temperature on GHGs emission rates, transformation rate of dissolved N and difference of soil characteristics at 95% level of confidence (p-value < 0.05). The mean values were also compared by the Duncan's Multiple Range test (DMRT). Independent t test (two-way) was conducted to see the effect of soil moisture on GHGs emission rates and net N mineralization (p-value < 0.05).

3. Results and Discussion

3.1 Soil characteristics affected by different long term fertilization

Results of soil analysis reveals that various key characteristics of soils have been changed after 46 years of long-term fertilization. Soil pH, total C, total N and inorganic N content were significantly changed by fertilizer treatment (Table 2). Soil amendment with organic material (OM treatment) has significantly increased total C, N and inorganic N content ($\mathrm{NH_4^+}$ and $\mathrm{NO_3^-} - \mathrm{N}$). Long-term application of chemical fertilization (CF) was resulted in enhanced soil acidity as compared to other treatments. The content of nitrate (NO_3-N) was also enhanced under chemical fertilization. Changes in C and N contents were resulted in changing C/N ratio of soil, such that the NF soil had highest mean of C:N ratio of 13.32, and followed by OM (12.66) and CF (9.96), respectively. However, the long-term fertilization practice has not changed the soil texture, as texture of soils from all treatments remained the same as it was at the initial stage, which was classified as loam with an average of 51% sand, 35.2% silt and 13.8% clay content.

The apparent effect of long-term fertilization practices was the enhanced soil carbon stock. The application of cow or chicken manure has increased 5-7 MgC ha^{-1} compared to that of no fertilizer and chemical fertilizer application. Singh et al. also revealed that the application of farmyard manure and fertilizer increased the SOC stock to 22.36 Mg C ha^{-1} from the long term for 44 years in fertilization application under jute-rice-wheat cultivation [28]. Moreover, Anandakumar et al. reported that the carbon sequestration rates in the integrated application of farmyard manure and fertilizer were higher than the soils that were applied with only chemical fertilization [29]. The enhanced carbon sequestration in the integrated fertilizer could increase plant growth, root biomass, and CO_2 fixation, which balances the NPK fertilizer and input organic carbon [30]. However, as described below that organic amendment also increased the production (and probably the emission) of N_2O. Further study on the entire ecosystem balance between increasing greenhouse gas emissions and enhancing soil carbon sequestration is needed to reach the conclusion whether the system help reduce or enhance the emission of greenhouse gases in the absolute term.

Enhancing SOC stock in OM plot was also possibly caused by rice husk charcoal application during 2011-2015 (Table 1). Several studies have shown that rice husk biochar increased SOC in various cropping systems. In paddy soil, for example, Koyama and Hisayoshi reported the soil C sequestration rate of 1.65 kg m^{-2} in the top soil after the field was applied with rice husk charcoal for three years [31]. Zhang et al. applied biochar in maize cultivation and found that biochar increased 4.9-6.3 g kg^{-1} in SOC. Biochar application therefore could contribute to the observed enhanced SOC stock in these plots [32].

It is noted that SOC stock under NF was greater than CF. The reason behind this difference is not known but may be related to the transformation of nitrate as concentration of nitrate under CF was much higher than in other plots (Table 1). Utilization of nitrate and soil carbon by microbes especially during anaerobic conditions releases gaseous products such as N_2O, N_2 and CO_2 could result in reducing soil carbon. This issue warrants further detailed study.

3.2 Production rates of N_2O

The N_2O production rates from different three temperature conditions and statistical analysis are shown in Figure 1 (a-c) and Table 3. The N_2O production rates in all treatments at 25°C and under both moisture contents were quite stable below 40 ng N_2O g^{-1} soil d^{-1} during incubation period (14 days). The maximal production rate of N_2O during incubation at 25°C was found in OM treatment at 80%WFPS on day 7th of incubation (38.92 ng N_2O g^{-1} soil d^{-1}), while the minimum was found in NF treatment at 60%WFPS on 14th day (0.13 ng N_2O g^{-1} soil d^{-1}). The means value of N_2O in OM treatment was significantly higher than (p-value < 0.05) in NF and CF under the same conditions. Production of N_2O in NF and CF was not significantly different. Moreover, the mean value of N_2O production in OM between 60%WFPS and 80% WFPS differed only at 25°C, where N_2O production under 60% WFPS was significantly higher than at 80% WFPS.

Similar to that of under 25°C, N_2O production in the OM treatment at 30° and 35°C for both 60%WFPS and 80%WFPS was significantly higher than that of NF and CF. However, when increasing temperature from 30°C to 35°C the production rate was declined. On the other hand, N_2O production rate at 80% WFPS was always greater than at 60% WFPS in all treatments.

The means values of N_2O production rate under the same soil moisture content and same treatment were shown in Table 3. The N_2O production rates of NF, OM and CF soils at 60% WFPS increased significantly at 30°C, while the rates among all other treatments were not significant different at 25° and 35°C.

Table 2. Physical and chemical properties of the soil used in the current study after 46 years of cultivation under different fertilizer treatment. The different lowercase letters (a, b, c) indicate the significant differences among treatments by ANOVA.

Treatment	pH (1:1 H_2O)	Total C ($g\ kg^{-1}$)	Soil C stock ($Mg\ ha^{-1}$)	Total N ($g\ kg^{-1}$)	$\mathrm{NH_4^+} - \mathrm{N}$ ($mg\ kg^{-1}$)	$\mathrm{NO_3^-} - \mathrm{N}$ ($mg\ kg^{-1}$)	Mean C/N ratio
NF	6.59^b	8.40±0.40^b	29.92±1.53^b	0.63±0.07^a	7.89±4.89^a	38.93±6.40^a	13.32^b
OM	7.13^c	9.69±0.29^c	34.57±0.96^c	0.77±0.02^a	57.56±2.14^b	47.33±3.67^a	12.66^b
CF	5.79^a	7.51±0.04^a	26.66±0.22^a	0.76±0.08^a	5.58±3.70^a	86.03±11.31^b	9.96^a
P-value	<0.001	0.001	0.001	0.080	<0.001	0.001	0.013

Table 3. N_2O production rate (ng N_2O g^{-1} soil d^{-1}) in soil from different treatments under different moisture and temperature conditions. The capital letters are used to indicate significant differences among treatments under same temperature, the symbols (*, #, $) are used to indicate significant different among temperatures within the same treatment, and the lowercase letters are used to indicate significant difference between 60% and 80% WFPS within each treatment and temperatures.

Moisture (%WFPS)	Treatment	Temperature (°C)		
		25	30	35
60	NF	$1.54\pm1.59^{A*a}$	$3.65\pm3.78^{A\#a}$	$1.96\pm1.12^{A*a}$
	OM	$10.50\pm2.80^{B*a}$	$83.66\pm87.83^{B\#a}$	$17.24\pm12.05^{B*a}$
	CF	$1.54\pm0.83^{A*a}$	$13.62\pm7.99^{A\#a}$	$4.59\pm4.65^{A*a}$
80	NF	$2.12\pm2.44^{A*a}$	$19.42\pm8.62^{A\#b}$	$9.57\pm8.76^{A\$b}$
	OM	$30.19\pm6.24^{B*b}$	$171.12\pm56.10^{B\#b}$	$46.26\pm25.66^{B*b}$
	CF	$1.76\pm1.26^{A*a}$	$8.29\pm4.95^{A\#b}$	$13.00\pm9.08^{A\$b}$

Table 4. CO_2 production rate (µg CO_2 g^{-1} soil d^{-1}) in soil from different treatments under different moisture and temperature conditions. The capital letters are used to indicate significant differences among treatments under same temperature, the symbols (*, #) are used to indicate significant different among temperatures within the same treatment, and the lowercase letters are used to indicate significant difference between 60% and 80% WFPS within each treatment and temperatures.

Moisture (%WFPS)	Treatment	Temperature (°C)		
		25	30	35
60	NF	$16.58\pm6.24^{A*a}$	$19.17\pm10.36^{A*a}$	$33.50\pm18.15^{A\#a}$
	OM	$33.76\pm24.35^{B*a}$	$43.80\pm22.14^{B*a}$	$66.38\pm55.89^{B\#a}$
	CF	$14.13\pm7.34^{A*a}$	$20.93\pm7.68^{A\#a}$	$25.05\pm16.06^{A\#a}$
80	NF	$18.49\pm5.24^{A*a}$	$18.33\pm9.35^{A*a}$	$37.02\pm17.64^{A\#a}$
	OM	$38.63\pm28.44^{B*a}$	$48.48\pm27.89^{B*a}$	$70.84\pm63.03^{B\#a}$
	CF	$15.87\pm8.73^{A*a}$	$16.71\pm7.28^{A*a}$	$19.35\pm10.04^{A*a}$

It is well documented that most of N_2O in soil is produced under aerobic condition through nitrification and anaerobic condition through denitrification [8-9]. Sensitivity of N_2O production has also been widely studied [6, 33-34]. In this study we showed that temperature sensitivity is altered according to cultivation practices. Using a long term soil enabled us to compare the effects among treatment with confidence that these were attributed to different fertilization treatment.

Firstly, it was revealed that long-term application of organic fertilizer (cow or chicken manure) enhances the N_2O production significantly compared to no fertilizer or chemical fertilizer. Highest production of N_2O was always observed in the soil from OM treatment (Table 3). Secondly, for each soil N_2O production was increased when the incubation temperature was increased from 25° to 30°C. Further increasing temperature to 35°C was resulted in declined N_2O production, except in CF treatment at 80% WFPS. For the immediate effects of changing soil moisture, it was found that there was no significant difference in response among treatments at 25°C. However, at 30 and 35°C N_2O production was generally stimulated when increasing moisture from 60 to 80% WFPS. The stimulation of N_2O production by moisture was also reported in serval studies [4, 10, 35-37]. These observations thus indicate that application with OM has resulted increasing N_2O production as well as its sensitivity to temperature and moisture changes. Under 60%WFPS, for example, N_2O production was increased 2.4, 8.0 and 8.8 folds when temperature was increased from 25 to 30°C in NF, OM and CF treatments, respectively. These were more obvious under 80%WFPS, which were 9.2, 17.9 and 4.7 folds, respectively. Thus, there was a combined moisture and temperature effects such that under high moisture content the effects of temperatures were greater than at the lower soil moisture content. These results indicate that one possible way to lower N_2O production and thus emission may be done through controlling soil moisture level. Yet, the appropriate moisture level for plant growth and reduced N_2O emission in the field still needs further investigation.

The increase N_2O production in response to temperature increase may indicate the stimulation of nitrification and denitrification, since temperature indirectly affects on enzymatic process on C and N cycling [6]. Beside, an increase temperature may also result in the depletion of soil oxygen to lead to enhanced soil anaerobiosis. The different responses among treatments, accordingly, may reflect the differences in activity of nitrifiers and denitrifiers. It is probably can be said that both groups of microbes may be more active and abundance in OM soil than other treatments. Keeney et al. and Castaldi described that the ratio of N_2O/N_2 decreases with temperature above 37°C, and the temperature greater than 50°C induces nitrite (NO_2^-) chemodenitrification that produces N_2 gas by thermophilic nitrate consumer [33-34]. Castaldi also revealed the significant correlation between temperature and N_2O emission rate in couple with an increase respiration and O_2 consumption [34]. Several studies have reported

that soil moisture content more than 60%WFPS is necessary to influence the denitrification because under high soil moisture oxygen supply to soil is reduced [4, 10]. Davidson et al. revealed that the optimum soil moisture for N_2O emission was between 70 and 80% WFPS [36]. These facts (the sensitivity of denitrification) may be the reason that the effects of soil temperature were more apparent under 80% WFPS than under 60%. Moreover, some fungi and archea are capable of denitrification [38-40]. This may be another reason for high N_2O production rate in OM treatment under 80% WFPS. The N_2O production rate could be stimulated by the readily degradable C in OM soil that might be possible to increase denitrification under high moisture content [41]. The denitrification in the incubated soil under high WFPS were mostly stimulated by heterotrophic denitrification [42].

It was also observed that the production rate under NF and CF treatment at 25° and 35°C was decreased during incubation, while under OM treatment it was increased. Previous studies reported that the production of N_2O depends on soil moisture and soil pH. When nitrification is the main process of N_2O production, the production rate was increased with an increase in soil pH [43]. With relative higher substrates for microbial activities, N_2O production in OM soil therefore continued to increase along the incubation time. On the other hand, depletion of available substates may result in decreasing N_2O production in NF and CF soils. These may partly explain the increased N_2O production along the incubation time that was found in OM soil, as this soil had higher pH than other treatment. In addition, N_2O was also decreased in acidic soil [44].

3.3 Production rates of CO_2

The CO_2 production rates from different temperature and soil moisture conditions are shown in Figure 1 (d-f). Similar to case of N_2O production, application of manure significantly enhanced CO_2 production. Increasing incubation temperature and moisture also stimulated CO_2 production. However, the increase rates were in the ranges of 1.05-2 times compared to the control plot. Thus, the responses of CO_2 production were relatively benign when compared to the cases of N_2O that temperature increases the production more than 2 folds as described above.

The different CO_2 production is related to the carbon content of soils, 7.45±0.28 g kg^{-1} of carbon content for OM, follow by 6.46±0.37 g kg^{-1} and 5.77±0.04 g kg^{-1} for NF and CF, respectively. Moreover, it is generally known that increasing temperature usually leads to increase microbial respiration and thus the release of CO_2 [45]. It was observed that the CO_2 production rates from all treatment as shown in Figure 1 (d-f) after day 5th decreased until the end of incubation. Such phenomenon was described by Reichstain et al. who pointed out that the carbon mineralization rates of soil incubated at 5, 15, and 25°C was decreased with incubation period, as a results of acclimatization and depleted substrate availability [46].

Figure 1. Production rates of N_2O (a-c), and CO_2 (d-f) under different temperature and moisture conditions during 14 days of incubation.

3.4 Production and consumption of dissolved N

Changes of soil N (dissolved organic + inorganic N) during incubation period (14 day) are shown in Figure 2. Under a given incubation temperature or moisture, dissolved N was increased along with the incubation time. The maximal value during incubation at 25°C was found in NF treatment at 60% WFPS on the 14th day of the incubation (106.75 µg $NH_4^+ - N$ g^{-1} soil), whereas the minimum was found in NF treatment at 60% WFPS on the first of incubation (22.17 µg NH_4 g^{-1} soil). The amount of dissolved N was declined when temperature was increased from 25° to 30°C and increased from at 30° to 35°C. This was consistent with the results of N_2O production. As described above, N_2O production increased when temperature was increased from 25° to 30°C. This N_2O production therefore was a result of mineralization of dissolved orgnic N and subsequently inorganic N consumption. OM treatment also showed the higher dissolved N than other treatments. For the effects of soil moisture, it was observed that during the first 5-7 days of incubation, dissolved N under 60% WFPS was usally higher than under 80% WFPS.

However, when the incubation period was prolonged beyond about 9th days, the opposite where dissolved N under 60%WPFS was lower than under 80%WFPS. Increase in the amount of dissolved N during the first 7 days may result from the active mineralization of organic N under lower moisture condition (and thus aerobic conditions under 60% WFPS). Later on consumption and production N_2O and CO_2 may result in the depletion of dissolved N as observed. On th other hand, under higher moisture condition (i.e. 80% WFPS), mineralization may be slow so that total dissolved N was low.

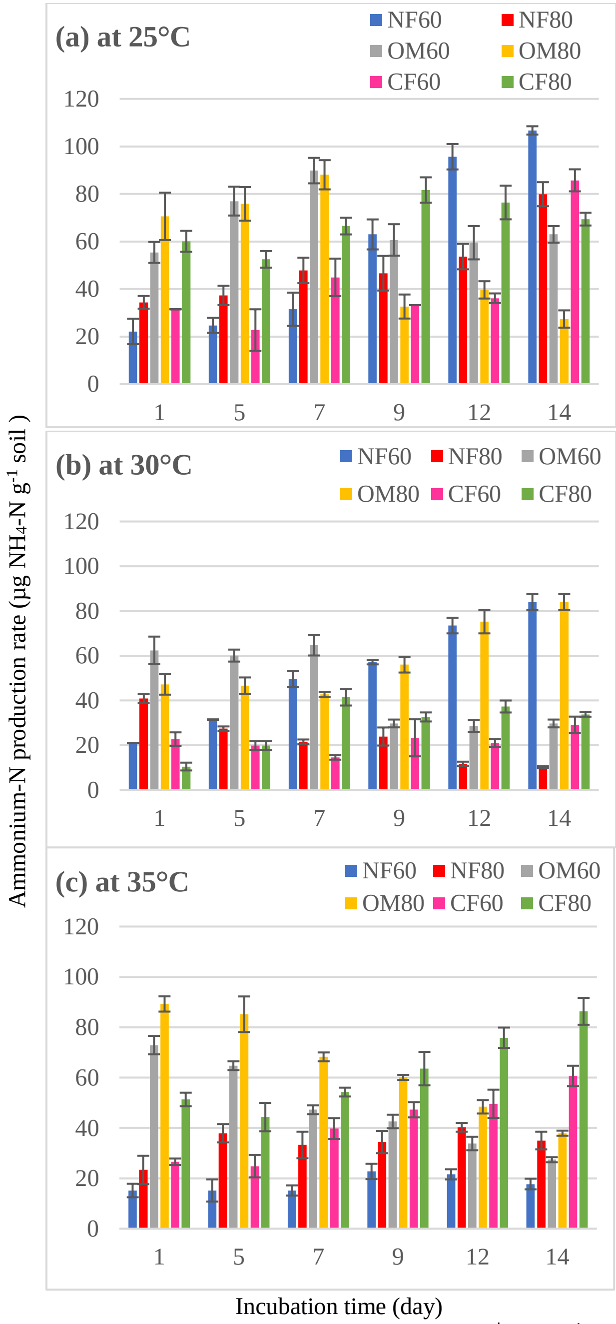

Figure 2. The dissolved N production rate (µg $NH_4^+ - N$ g^{-1} soil) from diferent treatment and temperature during incubation period.

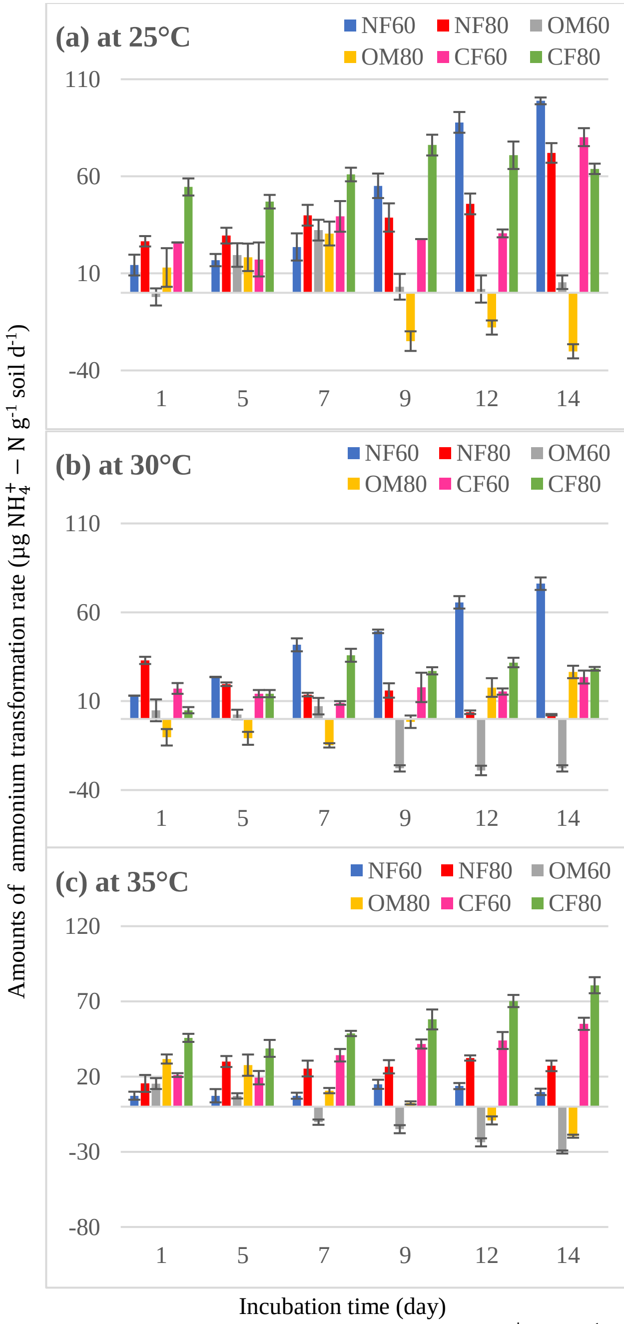

Figure 3. The dissolved N transformation rate (µg $NH_4^+ - N$ g^{-1} soil d^{-1}) from diferent treatment and temperature during incubation period.

Table 5. Statistical analysis of dissolved N transformation rate (μg $NH_4^+ - N$ g^{-1} soil d^{-1}) in from different treatments under different moisture and temperature conditions. The capital letters are used to indicate significant differences among treatments under same temperature, the symbols (*, #) are used to indicate significant different among temperatures within the same treatment, and the lowercase letters are used to indicate significant difference between 60% and 80% WFPS within each treatment and temperatures.

Moisture (%WFPS)	Treatment	Temperature (°C)		
		25	30	35
60	NF	49.41±35.19^{A*a}	44.90±22.71^{A*a}	10.04±4.02$^{A\#a}$
	OM	10.01±13.23^{B*a}	-11.67±17.34$^{B\#a}$	-9.44±16.69$^{B\#a}$
	CF	36.81±21.51^{A*a}	16.20±5.64$^{C\#a}$	35.88±13.49^{C*a}
80	NF	42.08±15.91^{A*a}	14.72±10.68$^{A\#b}$	26.14±6.53$^{A\$b}$
	OM	-1.85±24.58^{B*a}	1.08±16.33^{B*b}	7.29±19.18^{B*b}
	CF	62.18±10.72^{C*b}	23.68±11.18$^{C\#b}$	57.03±15.47^{C*b}

The mean net transformation rate of dissolved N at the same soil moisture content and same treatment are showed in Table 5 and Figure 3. The means of dissolved N transformation rate of NF, OM, and CF for both 60% and 80% WFPS at all temperature were significantly different, except at 25°C under 60% WFPS.

The negative dissolved N transformation rate was found in OM treatment indicates N lost from soil and this was in line with the enhanced N_2O production. The decrease in inorganic N may be also related to N immobilization by microorganism [47-49].

4. Conclusions

In this study, the effects of temperature and moisture on soil N_2O and CO_2 production in soils taken from long-term fertilizer experiment study were investigated. The results reveal that various soil characteristics were altered after 46 years of cultivation. The production of N_2O and CO_2 was also affected by such fertilizer treatment. The application of organic materials has increased soil organic carbon, stimulated the production of N_2O and CO_2. N_2O production was enhanced when the soil incubation temperature was increased from 25 to 30°C, but declined when the temperature was further increased to 35°C. Changing the moisture from 60% to 80% WFPS stimulated the production of N_2O. On the other hand, CO_2 production was found to increase along with temperature. These results imply the different sensitivity of N and C mineralization to temperature. The findings also imply that managing soil moisture level through irrigation such that soil moisture is kept sufficiently low for plant growth and to avoid high moisture level (e.g. 80%WPFS) that promote N_2O production and emission.

Acknowledgement

This study was supported by the National Research Council of Thailand (NRCT) through the International Research Network (IRN) under the project of climate change mitigation by sustainable agriculture soil management in Thailand under climate change and tropical soil carbon research network, and by the Joint Graduate School of Energy and Environment (JGSEE), King Mongkut's University of Technology Thonburi (KMUTT), Thailand. Also, we would like to thank the Lopburi Agricultural Research and Development Center, Department of Agriculture, for their assistance with soil sampling and other related background information.

References

[1] IPCC (The Intergovernmental Panel on Climate Change). 2022. Climate Change 2022: Mitigation of Climate Change. In Shukla, P.S. *Contribution of Working Group III to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change*. Cambridge, UK and New York, NY, USA: Cambridge University Press.

[2] ONEP (Office of Natural Resources and Environmental Policy and Planning). 2022. *Thailand's Fourth National Comunication*. Bangkok, Thailand: Office of Natural Resources and Environmental Policy and Planning Press.

[3] Cerri, C.C., Maia, S., Galdos, M.V., Cerri, C.E.P., Feigl, B.J. and Bernoux, M. 2009. Brazilian greenhouse gas emission: The important of agriculture and livestock, *Scientia Agricola*, 66(6), 831-843.

[4] Dobbie, K.E. and Smith, K.A. 2003. Nitrous oxide emission factors for agricultural soils in Great Britain: The impact of soil water-filled pore space and other controlling variables, *Global Change Biology*, 9(2), 204-218.

[5] Dalal, R.C., Wang, W., Robertson, G.P. and Parton, W.J. 2003. Nitrous oxide emission from Australian agricultural lands and mitigation option: A review, *Australian Journal of Soil Research*, 41, 165-195.

[6] Butterbach-Bahl, K., Baggs, E.M., Dannenmann, M., Kiese, R. and Zechmeister-Boltenstern, S. 2013. Nitrous oxide emissions from soil: How well do we understand the processes and their controls?, *Philosophical Transactions of the Royal Society*, 368, 1-13.

[7] Knapp, J.S. and Bromley-Challoner, K.C.A. 2003. Recalcitrant organic compounds. In: Mara, D. and l Horan, N. (eds): *Handbook of Water and Wastewater Microbiology*, (pp. 559-595).

[8] Bremner, J.M. 1997. Sources of nitrous oxide in soil, *Nutrient Cycling in Agroecosystems*, 49, 7-16.

[9] Firestone, M.K. and Davidson, E.A. 1989. Microbiological basis of NO and nitrous oxide production and consumption in soil. In Andreae, M.O.: *Exchange of Trace Gases between Terrestrial Ecosystems and the Atmosphere* (pp. 7-21). New York, USA: John Wiley and Sons.

[10] Drury, C.F., Zhang, T.Q. and Kay, B.D. 2003. The non-limiting and least limiting water ranges for soil nitrogen mineralization, *Soil Science Society of America Journal*, 67, 1388-1404.

[11] Keiluweit, M., Bougoure, J.J., Nico, P.S., Pett-Ridge, J., Weber, P.K. and Kleber, M. 2015. Mineral protection of soil carbon counteracted by root exudates, *Nature Climate Change*, 5(6), 588-595.

[12] Matsumoto, N., Paisancharoen, K. and Hakamata, T. 2008. Carbon balance in maize field under cattle manure appliction and no-tillagr cultivation in Northeast Thailand, *Soil Science and Plant Nutrition*, 54, 277-288.

[13] Nakadai, T., Koizumi, H., Usami Y., Satoh, M. and Oikawa, T. 1993. Examination of the method for measuring soil respiration in cultivated land: Effect of carbon dioxide concentration on soil respiration, *Ecological Research*, 8(1), 65-71.

[14] Mueller, T., Jensen, L S., Nielsen, N. E. and Magid, J. 1998. Turnover of carbon and nitrogen in a sandy loam soil following incorporation of chopped maize plants, barley straw and blue grass in the field, *Soil Biology and Biochemistry*, 30(5), 561-571.

[15] Sugino, T., Nobuntou, W., Srisombut, N., Rujikun, P., Luanmanee, S. and Punlai, N. 2013. Effects of long-term organic material applications and green manure crop cultivation on soil organic carbon in rainfed areas of Thailand, *International Soil and Water Conservation Research*, 1(3), 29-36.

[16] Schofield, R.K. and Taylor, A.W. 1955. The measurement of soil pH, Soil Science Society of America, 19, 164-167.

[17] Walkley, A. and Black, I.A. 1934. An Examination of the Degtjareff method for determining soil organic matter, and a proposed modification of the chromic acid titration method, *Soil Science*, 37(1), 29-38.

[18] Bremner, J.M. 1960. Determination of nitrogen in soil by the Kjeldahl method, *The Journal of Agricultural Science*, 55(1), 11-33.

[19] Taras, M.J. 1950. Phenoldisulfonic acid method of determining nitrate in water. Photometric study, *Analytical Chemistry*, 22(8), 1020-1022.

[20] Bremner, J.M. 1965. Organic forms of soil nitrogen. In Norman, A.G. (eds): *Method of Soil Analysis Part 2 Chemical and Microbiological Properties, 9.2* (pp. 1238-1255). Madison: American Society of Agronomy and Soil Science Society of America.

[21] Keeney, D.R. and Nelson, D.W. 1982. Nitrogen-Inorganic form. In Page, A.L.: *Method of Soil Analysis* (pp. 643-693). Madison, WI, USA: American Society of Agronomy Inc. - American Society of Soil Science Inc.

[22] Tadiello, T., Perego, A., Valkama, E., Schillaci, C. and Acutis, M. 2022. Computation of total soil organic carbon stock and its standard deviation from layered soils, *MethodsX*, 9, 101662, https://doi.org/10.1016/j.mex.2022.101662.

[23] Cha-un, N., Chidthaisong, A., Yagi, K., Sudo, S. and Towprayoon, S. 2017. Greenhouse gas emissions, soil carbon sequestration and crop yields in a rain-fed rice field with crop rotation management, *Agriculture, Ecosystems & Environment*, 237, 109-120.

[24] Bremner, J.M. and Keeney, D.R. 1966. Determination and isotope-ratio analysis of different forms of nitrogen: 3. Exchangeable ammonium, nitrate, and nitrite by extraction-distillation methods, *Soil Science Society of America Journal*, 30, 577-582.

[25] Delphin, J.E. 2000. Estimation of nitrogen mineralization in the field from an incubation test and from soil analysis, *Agronomie*, 20, 349-361.

[26] Pihlatie, M., Pumpanen, J., Rinne, J., Ilvesniemi, H., Simojoki, A., Hari, P. and Vesala, T. 2007. Gas concentration driven fluxes of nitrous oxide and carbon dioxide in boreal forest soil, *Chemical and Physical Meteorology*, 59(3), 458-469.

[27] Mohanty, M., Reddy, K.S., Probert, M.E., Dalal, R.C., Subba Rao, A. and Menzies, N.W. 2011. Modeling N mineralization from green manure and farmyard manure from a laboratory incubation study, *Ecological Modelling*, 222, 719-726.

[28] Singh, A.K., Behera, M.S., Mazumdar, S.P. and Kundu, D.K. 2019. Soil carbon sequestration in long-term fertilization under jute-rice-wheat agro-ecosystem, *Communications in Soil Science and Plant Analysis*, 50(6), 739-748, DOI: 10.1080/00103624.2019.1589483.

[29] Anandakumar, S., Bakhoum, N., Chinnadurai, C., Malarkodi, M., Arulmozhiselvan, K., Karthikeyan, S. and Balachandar, D. 2022. Impact of long-term nutrient management on sequestration and dynamics of soil organic carbon in a semi-arid tropical Alfisol of India, *Applied Soil Ecology*, 177, 104549, https://doi.org/10.1016/j.apsoil.2022.104549.

[30] He, Y.T., He, X.H., Xu, M.G., Zhang, W.J., Yang, X.Y. and Huang, S.M. 2018. Long-term fertilization increases soil organic carbon and alters its chemical composition in three wheamaize cropping sites across central and south China, *Soil and Tillage Research*, 177, 79-87.

[31] Koyama, S. and Hisayoshi, H. 2017. Rice yield and soil carbon dynamics over three years of applying rice husk charcoal to an Andosol paddy field, *Plant Production Science*, 20(2), 176-182.

[32] Zhang, A., Liu, Y., Pan, G., Hussain, Q., Li, L., Zheng, J. and Zhang, X. 2011. Effect of biochar amendment on maize yield and greenhouse gas emissions from a soil organic carbon poor calcareous loamy soil from Central China plain, *Plant Soil*, 351, 263-275.

[33] Keeney, D.R., Fillery, I.R. and Marx, G.P. 1979. Effect of temperature on the gaseous nitrogen products of denitrification in a silt loam soil, *Soil Science Society of America Journal*, 43(6), 1124-1128.

[34] Castaldi, S. 2000. Responses of nitrous oxide, dinitrogen and carbon dioxide production and oxygen consumption to temperature in forest and agricultural light-textured soils determined by model experiment, *Biology and Fertility of Soil*, 32, 67-72.

[35] Linn, D.M. and Doran, J.W. 1984. Effect of water-filled pore space on carbon dioxide and nitrous oxide production in tilled and nontilled soils, *Soil Science Society of America Journal*, 48(6), 1267-1272.

[36] Davidson, E.A., Keller, M., Erickson, H.E., Verchot, L.V. and Veldkamp, E. 2000. Testing a conceptual model of soil emissions of nitrous and nitric oxides, *Bioscience*, 50, 667-680.

[37] Ruser, R., Flessa, H, Russow, R., Schmidt, G., Buegger, F. and Munch, J.C. 2006. Emission of N_2O, N_2 and CO_2 from soil fertilized with nitrate: effect of compaction, soil moisture and rewetting, *Soil Biology & Biochemistry*, 38, 263-274.

[38] Stein, L.Y. and Klotz, M. 2016. The nitrogen cycle, *Current Biology*, 26(3), 94-98.

[39] Li, Y., Chapman, S., Nicol, G.W. and Yao, H. 2018. Nitrification and nitrifiers in acidic soils, *Soil Biology and Biochemistry*, 116, 290-301.

[40] Rajta, A., Bhatia, R., Setia, H. and Pathania, P. 2019. Role of heterotrophic aerobic denitrifying bacteria in nitrate removal from wastewater, *Journal of Applied Microbiology*, 128(5), 1261-1278.

[41] Wang, L., Cai, Z., Yang, L. and Meng, L. 2005. Effects of disturbance and glucose addition on nitrous oxide and carbon dioxide emissions from a paddy soil, *Soil and Tillage Research*, 82, 185-194.

[42] Ameloot, N., Meanhout, P., De Neve, S. and Sleutel, S. 2016. Biochar-induced N_2O emission reductions after field incorporation in a loam soil, *Geoderma*, 267, 10-16.

[43] Granli, T. and Bockman, O.C. 1994. Nitrous oxide from agriculture, *Norwegian Journal of Agricultural Sciences*, 12, 1-128.

[44] Yamulki, S., Harrison, R.M., Goulding, K.W.T. and Webster, C.P. 1997. N_2O, NO and NO_2 fluxes from a grassland: Effect of soil pH, *Soil Biology and Biochemistry*, 29, 1199-1208.

[45] Fang, C. and Moncrieff, J.B. 2001. The dependence of soil CO_2 efflux on temperature, *Soil Biology and Biochemistry*, 33(2), 155-165.

[46] Reichstein, M., Bednorz, F., Broll, G. and Katterer, T. 2000. Temperature dependence of carbon mineralization: conclusions from a long-term incubation of subalpine soil samples, *Soil Biology and Biochemistry*, 32, 947-958.

[47] Flowers, T.H. and Arnold, P.W. 1983. Immobilization and mineralization of nitrogen in soils incubated with pig slurry or ammonium sulphate, *Soil Biology and Biochemistry*, 15(3), 329-335.

[48] Kirchmann, H. and Anders, L. 1993. Relationship between N immobilization and volatile fatty acids in soil after application of pig and cattle slurry, *Biology and Fertility of Soils*, 15, 161-164.

[49] Sorensen, P. and Jensen, E.S. 1995. Mineralization-immobilization and plant uptake of nitrogen as influenced by the spatial distribution of cattle slurry in soils of different texture, *Plant Soil*, 173, 283-291.