

**NITROUS OXIDE EMISSIONS FROM VIETNAMESE
AGRICULTURAL SOIL WITH HIGH AMMONIUM INPUT
UNDER AEROBIC CONDITIONS**

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ABSTRACT

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Increasing use of excess nitrogen (N) fertilizers and compost at low N use efficiency to maximize yields in farming systems and croplands resulted in increasing N loss through nitrous oxide (N₂O) emissions. Nitrous oxide in agricultural soil from nitrification and denitrification were reported to be dependent on N input and temperature. Most of the previous studies have been conducted at temperatures between 5–25°C, at anaerobic conditions and low N application rates. Agriculture production in tropical countries may be associated with high N₂O and CO₂ because these two gases are temperature dependent. Although effects of high N application rates and temperature on N₂O emissions have not been studied well. Their interactions in tropical areas are very important, which will provide useful information on N management for tropical agriculture. The studies in the present thesis were designed to:

- 1) determine interactive effects of NH₄⁺ application rates and temperature on N₂O emissions;
- 2) determine interactive effects of different compost types, commercial compost and chicken compost, on N₂O and CO₂ emissions at high temperature; and
- 3) mitigate N₂O and CO₂ emissions from agricultural soil with different types of coffee waste biochar, normal biochar and functional biochar, at different temperatures.

We found that: 1) cumulative N₂O emissions increased with increasing NH₄⁺ application rates from 0 to 800 mg N kg⁻¹ but decreased from 800 to 1200 mg N kg⁻¹ and their emissions increased in the order of 35°C, 20°C, 30°C and 25°C. Autotrophic nitrification was the only process producing N₂O at 35°C while other processes e.g., nitrifier denitrification and coupled nitrification-denitrification occurred at the other temperatures; 2) greater N₂O and CO₂ emissions were seen in chicken compost with less NH₄⁺ and higher available carbon. More N₂O and CO₂ emissions occurred at higher chicken compost application rates. The temperature dependency of these two gases was not clear; 3) mitigation effects of N₂O and CO₂ emissions with biochar amendment were observed at the highest temperature. The addition of normal biochar produced less N₂O emissions, but more CO₂ emissions compared to that of functional biochar. In conclusion, cumulative N₂O emissions were significantly affected by NH₄⁺ application rates and temperature, and their interactions. The highest N₂O emissions was found at 800 mg N kg⁻¹ and 25°C. The lowest application rate of chicken compost and amendment of coffee waste biochar can be good options in terms of soil improvement and environmental benefits, especially at the highest temperature.

Keywords: Agricultural soil, Biochar, Carbon dioxide, Coffee waste, Chicken compost, Commercial compost, Interactive effects, High ammonium, Nitrogen, Nitrous oxide, Temperature.

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CHAPTER 1. INTRODUCTION

1.1. Background

Nitrous oxide is an important anthropogenic greenhouse gas (GHG) and ozone depleting gas, constituting 7% of the anthropogenic greenhouse effect. On a molecular basis, N_2O has 310 and 16 times higher global warming potential than that of carbon dioxide (CO_2) and methane (CH_4), respectively over a 100-year period (Smith *et al.*, 2007). Agriculture is a source of N_2O , CO_2 and CH_4 , and accounted for 10–12% CO_2 -equivalent to total global GHG emissions (Smith *et al.*, 2007). According to Food and Agriculture Organization (2015), Vietnam fertilizer application rate is great, 439 kg ha^{-1} compared with average level of middle income countries, 154 kg ha^{-1} (FAOSTAT, 2018a). On the other hand, utilization efficiency of N fertilizers was 46–86% (Khai *et al.*, 2007) and resulted in N substantial annual surpluses, greater N_2O emissions and NO_3^- loss to surface and ground waters (Khai *et al.*, 2007; Wang *et al.*, 2008; Zhang *et al.*, 2016b). In addition, N_2O emissions from Vietnamese agricultural soils have been projected to increase with time (Fig. 1.1).

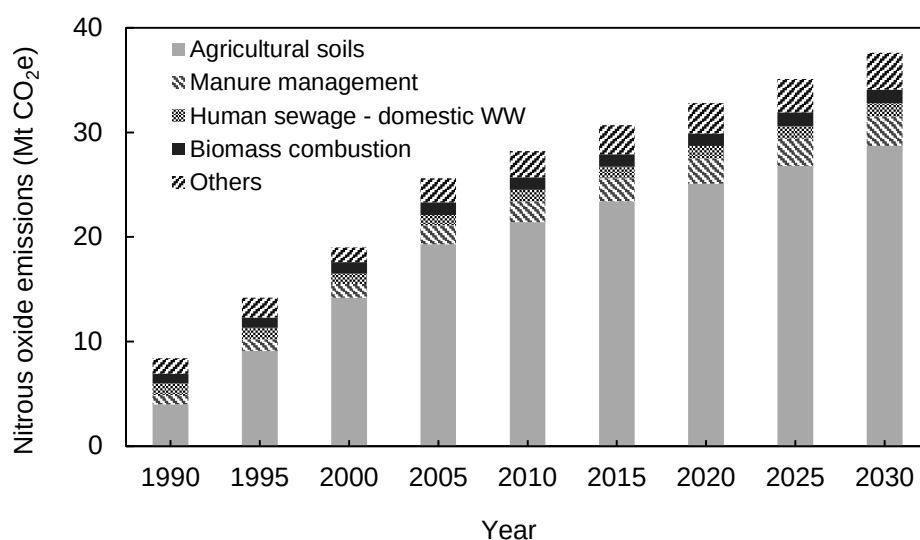


Figure 1.1. Vietnamese N_2O emissions from different sectors between 1990 and 2030 (EPA, 2012). ‘Others’ here includes energy and industrial processes sectors. MtCO_2e is million metric tons of carbon dioxide equivalents.

1.2. Aim and outline of the thesis

The main objective of this research is to examine the effects of nitrogen (N) input and temperature on N_2O emissions from tropical agricultural soil and how to mitigate the emissions with agricultural residues (Fig. 1.2). The primary source of N input which Vietnamese farmers often used excess was a combination of chemical fertilizers, manure (poultry, pig and cattle) and compost to maximize yields in their vegetable farming systems and croplands. However, utilization efficiency of N fertilizer was 46–86%, leading to N substantial surpluses, and N loss through N_2O emission and NO_3^- . Understanding the effects of N input in different sources and application rates under temperature change plays an important role in fertilizer management and improves agricultural practices in vegetable crop systems in tropical countries like Vietnam.

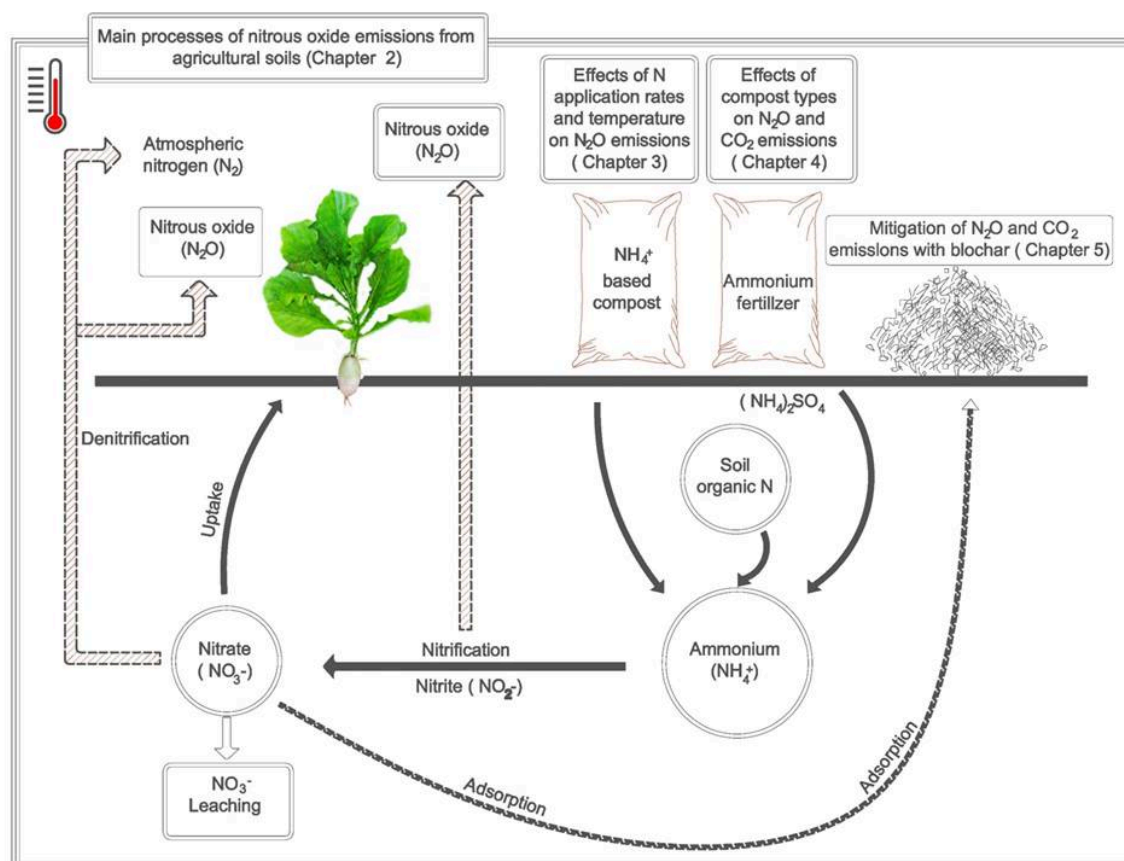


Figure 1.2. Organization of the thesis

The specific objectives of each chapter are to:

- (i) outline the main processes of N₂O emissions from agricultural soils (chapter 2);
- (ii) determine effects of N application rates and temperatures on N₂O emissions from agricultural soil (chapter 3);
- (iii) determine effects of compost types on N₂O and CO₂ emissions from agricultural soil at high temperature (chapter 4);
- (iv) mitigate N₂O and CO₂ emissions from agricultural soil amended with different types of biochar at different temperatures (Chapter 5).

Chapter 2 presented the main processes of N₂O emissions from agricultural soil and factors affecting N₂O emissions under aerobic conditions. Nitrous oxide emissions from agricultural soil and factors controlling N₂O emissions under anoxic and anaerobic conditions (absence of oxygen) have been well studied while those under aerobic conditions have not been frequently reported. This is because N₂O emissions from anaerobic denitrification were significantly higher than from aerobic nitrification. In this thesis, main N₂O-derived processes and factors controlling its emissions in the aerobic microcosms study were summarized and reported. Importantly, summary and critical knowledge gaps were introduced to explain why the thesis was conducted.

Chapter 3 showed effects of high NH₄⁺ application rates (400–1200 mg N kg⁻¹) at 5°C intervals between 20–35°C on N₂O emissions from Vietnamese agricultural soil used for intensive vegetable crops under aerobic conditions for 28 days. About 85% of rural households in Vietnam grew fruit and vegetables and their production and area harvested increased steadily from 1980–2005. Increasing use of excess N fertilizers to maximize yields at low nutrient use efficiency is the primary driver for increasing N loss via NO₃⁻ leaching and N₂O emissions. However, N₂O emissions from Vietnamese agricultural soil used for intensive vegetable cultivation have not been studied well. Most of previous studies about nitrification and nitrification-derived N₂O have been

carried out at low NH_4^+ less than 400 mg N kg^{-1} and temperature under 25°C . Our experiments were designed to determine effects of high NH_4^+ inputs and temperatures on N_2O emissions from a laboratory microcosms study. Our study showed that autotrophic nitrification, nitrifier denitrification and coupled nitrification-denitrification were considered as the predominant processes producing N_2O . The cumulative N_2O emissions were significantly affected by NH_4^+ application rates and temperatures, and their interactions. This chapter showed a specific high amount of NH_4^+ inhibiting N_2O emissions from acidic agricultural soil. In addition, the interactive effects of N application rates and temperatures on N_2O emissions explained a different trend in temperature dependence of N_2O emissions at high N application rates.

Chapter 4 was designed to determine N_2O and CO_2 emissions from Vietnamese agricultural soil collected at the same field site described in the Chapter 3 and amended with compost. Two NH_4^+ -based compost types, commercial compost and chicken compost, with the field application rates of 1%, 2% and 4% were selected to examine N_2O and CO_2 emissions through ammonia oxidation pathway. The microcosm was incubated at $25\text{--}35^\circ\text{C}$ for 28 days to ensure complete mineralization of compost and ammonia oxidation. This chapter reported that N_2O and CO_2 emissions were dependent on compost types and application rates and showed different responses to increasing temperature. In a word, 1% application rate of chicken compost was recommended for vegetable crops and environmental aspects.

Chapter 5 introduced the application of biochar produced from waste as an amendment for soil improvement and mitigation of N loss through N_2O emissions and NO_3^- leaching. Two types of coffee waste biochar, normal biochar and functional biochar with a high NO_3^- adsorption capacity of 20 g N kg^{-1} were added to the same agricultural soil used in the Chapter 4. The biochar-added soil microcosms were conditioned with $100 \text{ mg NO}_3^- \text{ kg}^{-1}$ and aerobically incubated at $25\text{--}35^\circ\text{C}$ for 21 days. The study reported that coffee waste biochar could reduce N_2O and CO_2 emissions at the highest temperature.

Chapter 6 discussed key findings about the effects of N input (with different sources and application rates) and temperatures, and their interactions on N₂O and CO₂ emissions from agricultural soil used for intensive vegetable cultivation. The role of coffee waste biochar in the mitigation of N₂O and CO₂ emissions and its limitations were further discussed in this chapter.

Lastly, the author drew some conclusions and suggested for further studies.

CHAPTER 2. LITERATURE REVIEW

2.1. Main processes of N₂O emissions from agricultural soils under aerobic conditions

Nitrous oxide emissions from soil occur through multiple biological pathways, autotrophic and heterotrophic nitrification, denitrification, chemo-denitrification, nitrifier denitrification, coupled nitrification-denitrification (Butterbach-Bahl et al., 2013; Zhang et al., 2015). Nitrous oxide production and consumption could occur simultaneously in different micro-sites in the same soil. Nitrification took place in the surface layer, whereas denitrification dominated in anaerobic zones (deep layers, waterlogged areas or the interior of soil aggregates) (Leffelaar, 1986). Nitrous oxide was mainly produced at aerobic-anaerobic interfaces from which it could diffuse to the soil surface.

2.1.1. Autotrophic nitrification

Nitrification is the oxidation of NH₄⁺ or ammonia (NH₃) to NO₃⁻ via nitrite (NO₂⁻). These reactions are carried out by two groups of microorganisms (Bock et al., 1986): ammonia-oxidizers with a autotrophic representative *Nitrosomonas europaea* (Klemedtsson et al., 1999) and nitrite-oxidizers with a representative *Nitrobacter winogradskyi*. Some intermediates are produced during nitrification, hydroxylamine (NH₂OH) and NO₂⁻. Apart from autotrophic nitrifiers using nitrification as an energy source for fixing CO₂, heterotrophic nitrifiers use organic carbon (C) as a source of C and energy (Robertson and Kuenen, 1990). Intermediates and products of autotrophic and heterotrophic nitrification are the same, and the enzymes of the two processes have been shown to differ from each other. The NH₃ monooxygenase from heterotrophic nitrifiers as studied in *Pseudomonas denitrificans* is not inhibited by acetylene (C₂H₂) (Richardson et al., 1998). Heterotrophic nitrifiers can oxidize urea and NH₃ (Papen et al., 1989). Under aerobic conditions, heterotrophic can produce higher N₂O per cell than autotrophic nitrifiers and heterotrophic nitrification might produce significant amounts of N₂O under favorable conditions, e.g., low pH, high oxygen (O₂) amounts and availability of organic material (Papen et al., 1989; Anderson et al., 1993).

2.1.2. Chemodenitrification

Chemodenitrification is the process that N_2O is formed during NH_3 oxidation through chemical decomposition of intermediates between NH_4^+ and NO_2^- such as NH_2OH or NO_2^- itself (Chalk and Smith, 1983).

2.1.3. Nitrifier denitrification

Nitrifier denitrification is a pathway of nitrification, which occurs when the incomplete oxidation of NH_2OH (oxidation of NH_3 to NO_2^-) and then followed by the reduction of NO_2^- and NO_3^- to N_2O and N_2 (denitrification) (Ritchie and Nicholas, 1972; Hooper and Terry, 1979). The organisms involved in nitrifier denitrification are probably mostly autotrophic NH_3 -oxidizers (Kuai and Verstraete, 1998). Nitrifier denitrification has been suggested to be an important source of N_2O under low organic carbon contents, low O_2 levels and low pH (Wrage *et al.*, 2001), while the ammonia oxidation pathway for N_2O is favored with high NH_3 , low NO_2^- contents and high nitrification rates (Wunderlin *et al.*, 2012).

2.1.4. Coupled nitrification-denitrification

Coupled nitrification-denitrification is not a distinct process. This coupling between nitrification and denitrification can take place in soil. Nitrification takes place in the aerobic surface layer, whereas denitrification dominates in the anaerobic zones. This means that the production of N_2O is highest under conditions that are optimal for both nitrifiers and denitrifiers.

Generally, under aerobic conditions, N_2O can be generated from different pathways, e.g., nitrification, chemodenitrification, nitrifier denitrification or coupled nitrification-denitrification. Fig. 1.3 shows the potential N_2O pathways in soils (heterotrophic nitrification has not been considered here) and associated genes adapted from Wrage *et al.* (2001), and Glass and Orphan (2012). The overlapping boxes symbolize the possibility of a coupling between nitrification and denitrification. As nitrifier denitrification is a pathway of nitrification, the boxes for nitrification

and denitrification overlap, but the sequential reactions separate into the different branches from NO_2^- onwards. In summary, only nitrifiers carry out nitrifier denitrification, whereas nitrifiers and denitrifiers are involved in coupled nitrification - denitrification. Furthermore, NO_2^- is reduced in nitrifier denitrification and NO_3^- is not formed whereas it may be formed as an intermediate in coupled nitrification-denitrification.

Autotrophic nitrification and denitrification are the most important pathways for N_2O emissions. Nitrifier denitrification, chemo-denitrification and heterotrophic nitrification are also known to contribute to N_2O emissions under some conditions. It has been suggested that heterotrophic nitrification is not significant in agricultural soil nor important as a source of NO or N_2O . Similarly, chemo-denitrification is thought to be less important than denitrification or autotrophic nitrification as a source of N_2O from agricultural soil.

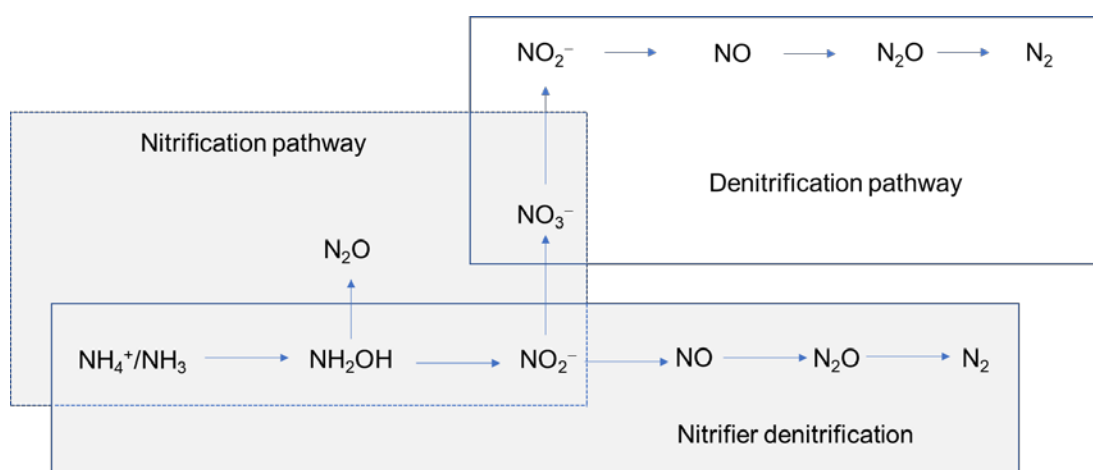


Figure 1.3. Potential pathways of N_2O production in soil. *Adapted from Wrage et al. (2001).*

2.2. Factors affecting N_2O emissions from agricultural soils

2.2.1. Soil N input

Nitrous oxide production generally increased with addition of N fertilizer (Tenuta and Beauchamp, 2003) and was dependent on fertilizer N sources (Eichner, 1990; Bouwman and Boumans, 2001). Under aerobic conditions, cumulative N_2O emissions were normally in the order

of urea > $(\text{NH}_4)_2\text{SO}_4 = (\text{NH}_4)_2\text{HPO}_4 = \text{NH}_4\text{H}_2\text{PO}_4 > \text{NH}_4\text{NO}_3 > \text{Ca}(\text{NO}_3)_2$ (Tenuta and Beauchamp, 2003). Nitrifier denitrification was the main source of N_2O from ammoniacal fertilizer at low O_2 concentrations with urea producing more N_2O than $(\text{NH}_4)_2\text{SO}_4$ (Zhu *et al.*, 2013). A greater loss of N_2O was found from urea than the other N fertilizers (Bouwman and Boumans, 2001; Tenuta and Beauchamp, 2003; Zhu *et al.*, 2013). During ammonia oxidation with high NH_4^+ contents (> 80 mg N kg^{-1}) the soil matrix will actively consume oxygen and accumulate high NO_2^- and leading to suboxic conditions inducing nitrifier denitrification. Nitrification and nitrifier denitrification accounted for 35–53% and 4–58% of total N_2O emissions, respectively (Huang *et al.*, 2014). However, the relative difference in N_2O emissions from different N fertilizer sources should be considered with caution because it was related to the combined effects of factors such as: sampling periods, types and amounts of fertilizer. (Eichner, 1990).

2.2.2. Temperature

Soil temperature is a key factor controlling microbial activities. Since both nitrification and denitrification processes have been demonstrated as being driven by nitrification and denitrification bacteria, soil temperature could impact both transformation processes via its effects on bacterial activities. Nitrous oxide emissions increased with rising incubation temperature (Smith *et al.*, 1998) due to the fact that rates of enzymatic processes generally increase with temperature as long as other factors (e.g., substrate or moisture) have no limiting effect. The optimum production of N_2O was 42°C from nitrification and 54.5°C for denitrification (Benoit *et al.*, 2015).

2.2.3. Soil aeration and water status

The primary effect of water on N_2O production in aerobic and partially aerobic soils is to restrict O_2 levels by reducing the air-water interfacial area within air-filled pores, and producing an anaerobic condition (Davidson, 1992). Under low oxygen availability (0.5% and 3%) following urea or $(\text{NH}_4)_2\text{SO}_4$ application to loam, clay loam and sandy loam soils, nitrifier

denitrification was the main source of N₂O production (Zhu *et al.*, 2013).

A soil water content equivalent to 60% of a water holding capacity (WHC) is the point of maximum aerobic microbial activity and the optimum moisture for nitrification (Linn and Doran, 1984). Nitrous oxide emission has been found to be highly correlated with water filled pore space (WFPS), with the highest emission under 70% WFPS coming from both nitrification (35–53%) and denitrification (44–58%) pathways in an intensively managed calcareous Fluvo-aquic soil (Huang *et al.*, 2014). The favorable conditions for N₂O production from nitrification are between 30% and 70% WFPS (Hu *et al.*, 2015).

The N₂O production primarily occurred by nitrification at air-filled porosity levels of around 65% (Tenuta and Beauchamp, 2003). At higher O₂ partial pressure (> 0.5 vol. %), nitrification is expected to occur, provided there is sufficient water for optimum activity nitrifiers (Bollmann and Conrad, 1998); as the soil WFPS increases (and pO₂ decreases), the rate of N₂O production and the proportion of N₂O to NO₃⁻ produced also increases (Smith *et al.*, 2003).

2.2.4. Available soil organic carbon

Increasing the soil organic C content can increase N₂O emissions (Brentrup *et al.*, 2000). Available C influences nitrification and denitrification reactions because it can stimulate microbial growth and activity, and provide the organic carbon needed by soil denitrifiers (Cameron *et al.*, 2013). In addition, microbial growth increases oxygen consumption and creates favorable conditions for denitrification.

2.2.5. Soil pH

Microbial activities are influenced by soil pH, which is the most important factor in determining the kinetics of soil nitrification (Cheng *et al.*, 2004). Nitrification activity is generally higher at higher soil pH (> 6) (Bremmer and Blackmer, 1981). Nitrous oxide emissions tend to increase with increasing pH, at least in the range of pH 6 to 8. However, the rate of N₂O production from autotrophic nitrification decreases with increasing pH in acid soils up to 5 (Granli and

Bockman, 1994). Denitrification occurs over a wide range of soil pH (5 to 8).

2.2.6. Soil texture and land-use

Nitrous oxide production was greater in clay loam than in loam and sandy loam soils (Zhu *et al.*, 2013), which likely relates to soil nitrifier populations and nitrification rates (Fortuna *et al.*, 2012). Nitrifier populations inhabiting clay surfaces have been shown to be protected from the effects of H^+ produced from NH_3 oxidation (Powell and Prosser, 1991).

Effects of land-use types on N_2O emissions from fertilized soils are related to cultivation practices including N application rates and crop types. Nitrification contributed to 97% of the N_2O emissions in sugarcane soil followed by 72% in the cereal cropping soil while only around 20% of N_2O was produced from nitrification in vegetable soil. The proportion of nitrified nitrogen as N_2O (P_{N_2O} -value) varied across different soils, with the highest P_{N_2O} -value (0.26‰) found in the cereal cropping soil, which was around 10 times higher than that in other three systems (Liu *et al.*, 2016).

2.3. Summary and critical knowledge gaps

There are many studies about N_2O emissions from agricultural soil. However, most of them were carried out to determine specific effects of temperature at a fixed N application rate on N_2O emissions from agricultural soil (Smith *et al.*, 1998; Benoit *et al.*, 2015) or to examine those of different N application rates on N_2O emissions from agricultural soil at specific temperature (Gödde and Conrad, 1999; Acton and Baggs, 2011; Deppe *et al.*, 2017). Other studies examined the relationships between different N sources and N_2O emissions (Tenuta and Beauchamp, 2003) or the inhibition of nitrification and N_2O -derived nitrification at high NH_4^+ contents (Deppe *et al.*, 2017). Studies about temperature dependence of N_2O emissions from agricultural soil were conducted using a short-term laboratory incubation during only 4 hours by Benoit *et al.* (2015) or in a long-term field study over 10 months by Smith *et al.* (1998).

The contribution of nitrification and denitrification to N_2O emissions from agricultural soils

were affected by different types of land uses e.g., vegetable soil, sugarcane, dairy pasture or cereal cropping systems (Liu *et al.*, 2016). In addition, N₂O emissions may be greater in the tropical countries because N₂O and N₂O-derived processes are of temperature dependence when soil mineral N is not limiting (Smith *et al.*, 1998; Benoit *et al.*, 2015). Thua Thien Hue province, Vietnam is in the monsoon tropical area, with average temperatures of 20°C in winter and 29°C in summer, especially occasional extreme daily temperature up to 40–41°C in the summer (Nguyen *et al.*, 2005). About 85% of rural households in Vietnam grew fruit and vegetable. In addition, vegetable production area increased steadily from 1998–2005 (Johnson *et al.*, 2008). Till now, there are no studies about N₂O emissions from this soil type in Vietnam. Therefore, this study provided background information about N₂O emissions from Vietnamese croplands.

CHAPTER 3. EFFECTS OF AMMONIUM APPLICATION RATES AND TEMPERATURES ON NITROUS OXIDE EMISSIONS

3.1. Introduction

Nitrous oxide (N_2O), a potent greenhouse gas with a global warming potential 310 times that of CO_2 for a 100-year timescale (Smith *et al.*, 2007), in agricultural soil mainly comes from microbial processes, nitrification and denitrification. Many studies reported that N_2O emissions from soil were dependent on temperature (McKenney *et al.*, 1980; Smith *et al.*, 1998; Bagherzadeh *et al.*, 2008; Benoit *et al.*, 2015; Nag *et al.*, 2016). Net N_2O production from denitrification increased with increasing temperature in the range of 4–25°C (McKenney *et al.*, 1980). An exponential relationship between N_2O flux during denitrification from a cut grassland site and soil temperatures was observed at 12–32°C (Smith *et al.*, 1998). A study conducted to determine temperature dependence of N_2O production of a luvisolic soil in batch experiments at seven temperatures from 5°C to 45°C showed that N_2O emissions increased with increasing temperatures at 20–45°C from nitrification and at 5–45°C from denitrification (Benoit *et al.*, 2015). However, their experiments were carried out at a lower content of ammonium (NH_4^+) (30 mg N kg^{-1}) in a relatively short incubation time (4 h) with a studied soil belonging to temperate zones (daily average temperature between –2°C and 16.5°C). On the other hand, Zhang *et al.* (2016) showed N_2O emissions slightly fluctuated between 8.0–10.6, 8.8–11.0 and 8.3–10.6 $\mu\text{g kg}^{-1} \text{d}^{-1}$ from a pine forest, an oak forest and a subalpine meadow, respectively at three different incubation temperatures (8°C, 18°C and 28°C). It may be because the N_2O emission rates were not significantly correlated with the soil temperature in the mountain forest-meadow ecosystem (Zhang *et al.*, 2016a).

Under aerobic conditions, N_2O emissions were generally greater with urea than other nitrogen (N) fertilizers, including ammonium nitrate, calcium nitrate, ammonium sulfate, mono-ammonium and di-ammonium phosphates and were associated with nitrite (NO_2^-) accumulation (Tenuta and

Beauchamp, 2003). Previous studies indicated that increasing NH_4^+ application rates from 0 to 400 mg N kg^{-1} soil have been shown to increase nitrification and N_2O emissions (Well *et al.*, 2008; Huang *et al.*, 2014). However, a high NH_4^+ content ($\geq 1000 \text{ mg N kg}^{-1}$ soil) could inhibit nitrification and reduce N_2O emissions (Deppe *et al.*, 2017). Most of the previous studies were carried out to determine specific effects of temperature at a fixed N application rate on N_2O emissions from agricultural soils (Smith *et al.*, 1998; Benoit *et al.*, 2015) and different ecosystems (Zhang *et al.*, 2016a) or to examine those of different N application rates at a specific temperature (Gödde and Conrad, 1999; Acton and Baggs, 2011; Deppe *et al.*, 2017). Other studies were to examine the relationships between different N sources and N_2O emissions (Tenuta and Beauchamp, 2003) or the inhibition of nitrification and N_2O derived from nitrification at NH_4^+ contents higher than 1000 mg N kg^{-1} (Deppe *et al.*, 2017). Studies about temperature dependence of N_2O emissions from agricultural soils were conducted in a short-term laboratory incubation for only 4 hours by Benoit *et al.* (2015) or in a long-term field study over 10 months by Smith *et al.* (1998). Our study was designed to examine interactive effects of NH_4^+ application rates and temperatures on N_2O emissions from tropical agricultural soil over 28 days. Their interactions in tropical areas are very important, which will provide useful information on N management for tropical agriculture.

Thua Thien-Hue province, Vietnam is in the monsoon tropical area, with daily-average ambient temperatures of 20°C in winter and 29°C, occasionally up to 40–41°C, in summer (Nguyen *et al.*, 2005). The primary source of nutrient input in general was a combination of chemical fertilizer, manure (poultry, pig and cattle) and NH_4^+ added compost (ex. Song Huong compost) (Van and Maeda, 2018). Most rural households in Vietnam grow vegetables, and the total production and cultivation area have increased for the past five years (2011–2016) (FAOSTAT, 2018b). Farmers often applied excess N to achieve economic benefits in their vegetable farming systems and croplands. The average fertilizer application rate in Vietnam is 439 kg ha^{-1} , higher than an average of 154 kg ha^{-1} for middle income countries (Gross National

Income per capita between \$1,006 and \$12,235) (FAOSTAT, 2018a). On the other hand, utilization efficiency of N fertilizer was 46–86% in Hanoi, leading to N substantial surpluses (Khai *et al.*, 2007). This surplus N in soil may increase N₂O emissions and NO₃⁻ losses to surface and ground waters (Wang *et al.*, 2008; Davidson, 2009; Pang *et al.*, 2009; Zhang *et al.*, 2016b; Yi *et al.*, 2017).

The contribution of nitrification and denitrification to N₂O emissions from agricultural soils was affected by land-use types at a specific temperature. Based on ¹⁵N-tracing incubation experiments at 50% water-filled pore space (WFPS), Liu *et al.* (2016) reported that nitrification was the main contributor to N₂O emissions in soils from sugarcane, dairy pasture and cereal cropping systems, while denitrification played a major role in N₂O emissions in the vegetable soil under the same experimental conditions. Nitrogen application rates and temperature were considered to affect N₂O production processes and their contributions to the total N₂O emissions. Our hypotheses were that interactions between NH₄⁺ application rates and temperatures had significant effects on cumulative N₂O emissions from a sandy loam soil in Vietnam. Nitrous oxide emissions would be inhibited at a specific NH₄⁺ application rate or temperature because of their interactions.

3.2. Materials and methods

3.2.1. Soil

Surface soil (0–10 cm) was collected from a field used for intensive vegetable cultivation in Phu Mau Commune, Phu Vang District, Thua Thien Hue Province, Vietnam (16°29'53 N, 107°34'47 E) in June 2017. The soil was classified as Fluvisol (FAO, 1938), with the texture of sandy loam (63% sand, 23% silt and 14% clay). This field had been planted in rotation with mustard (*Brassica juncea*) and amaranth (*Amaranthus mangostanus*) prior to the sampling in 2017. Air-dried soil was passed through a 2-mm sieve and characterized: pH (H₂O, 1:5) 6.3 ± 0.0; EC (1:5) 0.073 ± 0.0 dS m⁻¹; NH₄⁺ 2.2 ± 0.7 mg N kg⁻¹; NO₃⁻ 30.8 ± 1.0 mg N kg⁻¹; TC 9 g C kg⁻¹; TN 0.7 g N kg⁻¹ and C/N

ratio 13. Nitrite (NO_2^-) was not found. Analytical methods were the same as in Section 2.4.

3.2.2. Soil incubation experiments

Aerobic incubations were performed in 125-mL glass bottles that contained 5 g air-dried soil and 0, 0.39, 0.79, and 1.18 mL of $(\text{NH}_4)_2\text{SO}_4$ 5000 mg N L^{-1} solution mixed with 1.42, 1.03, 0.63, and 0.24 mL of deionized water to achieve 0, 400, 800 and 1200 mg N kg^{-1} soil, coded 0 N, 400 N, 800 N and 1200 N, respectively at 60% water holding capacity (WHC), equivalent to 40% WFPS or a gravimetric water content of 30 g g^{-1} dry soil. These bottles were covered with a piece of polyethylene plastic film that allowed gaseous exchange and prevented evaporation, then kept in incubators at 20°C, 25°C, 30°C or 35°C for 28 days. All incubation experiments were performed in triplicates (336 bottles in total).

3.2.3. Measurement of nitrous oxide concentration

Gas samples for N_2O measurements were collected 3 h after starting incubation (day 1) and on days 3, 5, 7, 14, 21 and 28. The background N_2O concentration in the laboratory air was measured. The water lost by evaporation was compensated weekly by adding deionized water using a micro pipette to the pre-specific weight.

On days 1, 3, 5, 7, 14, 21 and 28, the incubation bottles were flushed with the laboratory air for 30 seconds by using a mini pump (MP-2N, Shibata, Japan) and closed by a butyl rubber septum for 3 h. After that, air pressure of these bottle samples was measured by an air pressure gauge (Handy manometer, NIDEC Copal Electronics, Japan). Nitrous oxide concentration was determined by injecting 0.5 mL of headspace gas samples to a gas chromatograph (GC-8A, Shimadzu, Japan) (coefficient variation, CV was 1.3% at 1.5 ppm) equipped with an isothermal (ambient to 350°C) ^{63}Ni -containing electron capture detector (ECD).

A N_2O emission rate was calculated by using the following equation:

$$F_i = \rho \times C \times (V_g + V_L \times \alpha) \times 273 / (W \times (273 + T)) / t \quad (1)$$

where F_i is the N_2O emission rate ($mg\ kg^{-1}\ h^{-1}$), t is the closing time before collecting the gas (h), ρ is the density of N_2O ($1.25\ kg\ m^{-3}$), C is the N_2O concentration (ppm_v), V_g (m^3) is the head volume, V_L (m^3) is the volume of liquid phase, α is the Bunsen absorption coefficient for N_2O at 20°C (0.63), 25°C (0.54), 30°C (0.46) or 35°C (0.40), W (kg) is the oven-dry weight of soil, and T is the temperature (°C) at measurement. The background N_2O concentration was subtracted from the measured concentration prior to data analysis. Cumulative N_2O emission was calculated from the integrated flux over the incubation period (by integration of N_2O fluxes over period (days) from one measurement to the next).

After the completion of gas measurement on days 1, 3, 5, 7, 14, 21 and 28, these soil samples were extracted to determine pH, and mineral N contents (NO_3^- , NO_2^- and NH_4^+).

3.2.4. Soil analyses

All soil samples were subjected to measurement of pH at a soil: water ratio of 1:5 after shaking at 175 rpm for 1 h, using a digital pH (F-23, Horiba, Japan).

Total carbon (TC) and nitrogen (TN) contents of soil were determined by the dry combustion method using a NC analyzer (NC-22F, Sumika-Bunseki Center, Japan). Soil mineral N (NH_4^+ , NO_3^- , and NO_2^-) was extracted with $2\ mol\ L^{-1}$ KCl (soil: solution ratio of 1:10) after shaking at 175 rpm for 1 h and measured by spectrophotometry using a continuous flow analyzer (QuAatro 2-HR, Bltec, Japan) according to APHA et al. (2005).

3.2.5. Data analysis

One-way analysis of variance (ANOVA) with Tukey's HSD posthoc test was used to examine effects of pH, NO_3^- , NO_2^- or NH_4^+ on N_2O emissions ($p < 0.05$). Pearson's correlation coefficient was performed to test relationships between physico-chemical properties of soil samples and cumulative N_2O emissions over 28 days at different temperatures. A two-way ANOVA was used to determine effects of N application rates and temperatures on cumulative N_2O emissions over 28

days. All statistical calculations were performed using the SPSS software (SPSS 22.0, IBM, US).

3.3. Results

3.3.1. Soil pH and mineral N contents

Ammonium addition lowered soil pH by up to 1.5 units for 28 days, and pH value was generally the lowest in the 1200 N treatment (Fig. 1, $p < 0.05$). In addition, pH values of the N-added treatments at 35°C and 1200 N at 30°C significantly declined to less than 5 after 7 days while those at 20–25°C did after 14 incubation days.

Soil mineral N contents in the N-added treatments were greater than in the 0 N treatment over the entire period, except for NO_3^- contents at 20°C and NO_2^- on day 1 (Fig. 1). Soil NH_4^+ contents in 400 N and 800 N treatments slightly decreased in the first week after NH_4^+ application. The NH_4^+ contents in the 1200 N treatment were the lowest on day 21 at 20–25°C compared to other days while they were not significantly different at 30°C and 35°C after 7 incubation days ($p < 0.05$). Soil NO_3^- contents sharply increased in the 1200 N treatment at 30°C and in the N-added treatments at 35°C on day 7, and their contents were higher than those at 20°C and 25°C ($p < 0.05$).

Table 3.1. Pearson's correlation coefficients of cumulative N_2O emissions with soil pH, and NH_4^+ , NO_3^- , and NO_2^- on day 28 at different temperatures

Variable	Temperature	pH	NO_3^-	NH_4^+	NO_2^-
N_2O	20°C	0.276	-0.624	0.914 **	0.476 0.276
	25°C	0.137	-0.347	0.522	0.236 0.137
	30°C	0.306	0.037	0.126	0.529 0.306
	35°C	-0.152	0.229	-0.754 *	0.456 -0.152

** : Correlation is significant at the 0.01 level.

* : Correlation is significant at the 0.05 level.

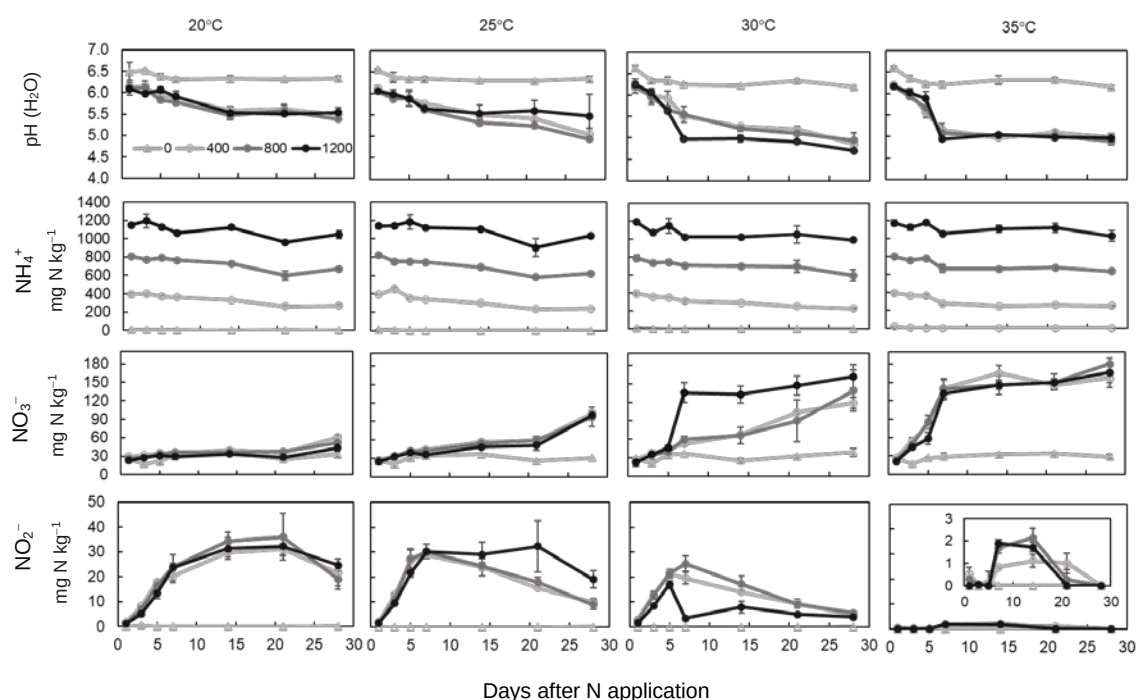


Figure 3.1. pH and mineral soil N contents following NH₄⁺ application of 0, 400, 800 and 1200 mg N kg⁻¹ soil at 20°C, 25°C, 30°C and 35°C. Errors bars represent standard errors of means ($n = 3$). Differences between NH₄⁺ reduction and NO₂⁻ or NO₃⁻ production in N-added treatments were 65–83, 55–87, 39–82, and 6–8 mg N kg⁻¹ at 20 °C, 25 °C, 30 °C and 35 °C, respectively on day 28.

With a decrease in NH₄⁺ contents, soil NO₂⁻ contents in the N-added treatments increased and achieved the highest values on day 7 at 25–35°C, and then decreased towards 28 incubation days, except for 1200 N at 30°C and 400 N treatments at 35°C (Fig. 1). Overall, the results showed that increasing temperature resulted in a decline in soil NO₂⁻ contents and an increase in NO₃⁻ in 400 N, 800 N and 1200 N treatments on day 28.

3.3.2. Nitrous oxide emissions

Nitrous oxide emission rates were higher in the N-added treatments than in the 0 N treatment, increased from day 1 to day 14 or 21 and then decreased on day 28, except for the 1200 N treatment at 20°C (Fig. 2). Peak N₂O emissions in the N-added treatments occurred after the second week of NH₄⁺ applications at 20°C and 25°C, while those were achieved on day 7 or day 21 at 35°C. At

20–30°C, 400 N and 800 N treatments had the same pattern in N₂O emission rates, the peaks appeared on day 14 and then decreased towards the end of the incubation period while in the 1200 N treatment, N₂O emissions peaked on day 21 or even increased towards day 28 at 20°C. Significant differences were observed in cumulative N₂O emissions among 0 N and N-added treatments (Table 2, $p < 0.001$).

3.3.3. N₂O emissions vs. NH₄⁺ application rates and temperatures

Results from a two-way ANOVA showed that NH₄⁺ application rates, temperatures and their interactions had significant effects on cumulative N₂O emissions ($p < 0.001$). Regardless of temperature, cumulative N₂O emissions were found to increase from 0 to 800 mg N kg⁻¹ but decreased from 800 to 1200 mg N kg⁻¹ (Table 2 and Fig. 3). Nitrous oxide emissions in terms of temperature dependence increased in the order of 35°C, 20°C, 30°C and 25°C (Fig. 3, $p < 0.001$).

On the other hand, results showed that cumulative N₂O emissions were the highest at 800 N and 25°C while the lowest emissions occurred at the highest NH₄⁺ application rate and temperature, 1200 N and 35°C (Table 2 and Fig. 3).

Table 3.2. Cumulative N₂O emissions for 28 days at different temperatures (mg N kg⁻¹)

N rate	20°C	25°C	30°C	35°C
0 N	0.1 ± 0.0 ^{cA}	0.1 ± 0.0 ^{cA}	0.2 ± 0.1 ^{cA}	0.1 ± 0.1 ^{cA}
400 N	11.9 ± 0.6 ^{bB}	17.1 ± 0.3 ^{bA}	15.1 ± 1.5 ^{bA}	6.0 ± 0.7 ^{aC}
800 N	13.9 ± 0.1 ^{aB}	20.1 ± 1.6 ^{aA}	18.3 ± 1.4 ^{aA}	6.2 ± 0.3 ^{aC}
1200 N	15.6 ± 1.3 ^{aB}	19.1 ± 1.0 ^{abA}	15.6 ± 1.0 ^{abB}	4.1 ± 0.3 ^{bC}

Values are means ± standard deviation ($n = 3$). Cumulative N₂O emissions are not significantly different ($p > 0.05$) if they share the same letter. The lowercase letters (a, b, and c) show differences among different treatments in a specific temperature. The uppercase letters (A, B, and C) show differences among different temperatures.

3.4. Discussions

3.4.1. Effects of NH_4^+ application rates on N_2O emissions

The autotrophic nitrification should be a dominant N transformation process under conditions that soil was at 30–60% WFPS (Bateman and Baggs, 2005), soil pH was above 5 (optimum 7 to 9) and NH_4^+ was abundant (after application of NH_4^+ based fertilizer) at optimum temperature 25–35°C (Zaman *et al.*, 2007; Zaman and Nguyen, 2010; Zaman *et al.*, 2012). The present experimental conditions (40% WFPS, pH > 5 and abundant NH_4^+) were favorable for the autotrophic nitrification process. The decrease in NH_4^+ accompanied with the increase in NO_3^- in N-added treatments (Fig. 1) indicated that autotrophic nitrification was the predominant N_2O -producing process. Acton and Baggs (2011) also reported that nitrification was the largest contribution to N_2O production over 23 days in 360–1100 mg N kg^{-1} treatments, accounting for 57–83% of the total N_2O emissions.

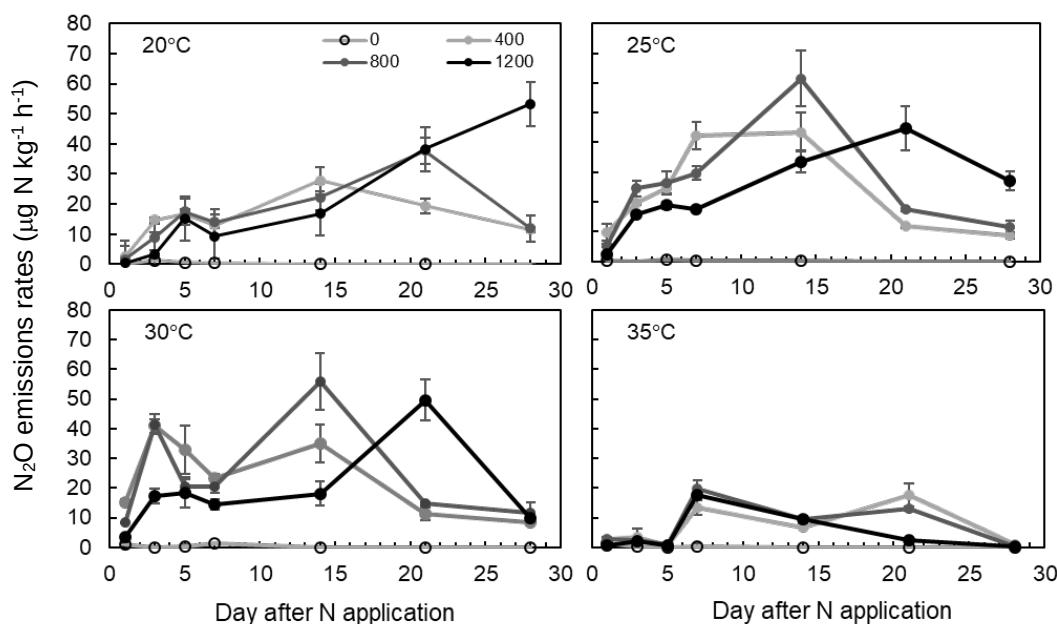


Figure 3.2. Nitrous oxide emissions for 28 incubation days at 20°C, 25°C, 30°C and 35°C. 0, 400, 800 and 1200 are the NH_4^+ application rates, mg N kg^{-1} soil ($n = 3$, $p < 0.05$).

On the other hand, the difference between decreased NH_4^+ and increased NO_3^- on day 14 (Fig. 1) suggests that other processes occurred, e.g., denitrification, coupled nitrification-denitrification, except at 35°C. The exception related to N_2O -producing process at 35°C will be discussed later. Zhu et al. (2013) reported that nitrifier denitrification was an important source of N_2O emissions following urea or ammonium sulfate application to loam, clay and sandy loam soils. At a NH_4^+ content higher than 80 mg N kg⁻¹, ammonia oxidation under aerobic conditions could consume oxygen and accumulated high NO_2^- in soil, leading to suboxic conditions that induced nitrifier denitrification in soil (Zhu *et al.*, 2013; Huang *et al.*, 2014). Therefore, the reduction of NO_2^- on day 7 or 14 in the present study should be due to nitrifier denitrification and/or coupled nitrification-denitrification.

In the present study, cumulative N_2O emissions did not increase exponentially with increasing NH_4^+ application rates. Cumulative N_2O emissions were inhibited at NH_4^+ content higher than 800 mg N kg⁻¹ at 25°C, 30°C and 35°C (Fig. 3). This critical NH_4^+ content was similar to Acton and Baggs (2011) but not to Deppe et al. (2017). Acton and Baggs (2011) noted that cumulative N_2O emissions were found to increase from 360 to 710 mg N kg⁻¹ but decreased from 710 to 1400 mg N kg⁻¹ when inputted as $^{14}\text{NH}_4^{15}\text{NO}_3$ or $^{15}\text{NH}_4^{14}\text{NO}_3$. On the other hand, Deppe et al. (2017) showed that nitrification-derived N_2O emissions were inhibited at NH_4^+ contents more than 450 mg N kg⁻¹ during the 21-day incubation period at 16°C. The difference between the present study and Deppe et al. (2017) would be the contribution of nitrification or denitrification to the total N_2O production and interactive effects of NH_4^+ application rates and temperature. However, mechanisms related to the impacts of NH_4^+ application rates on N_2O production processes were not determined in this study.

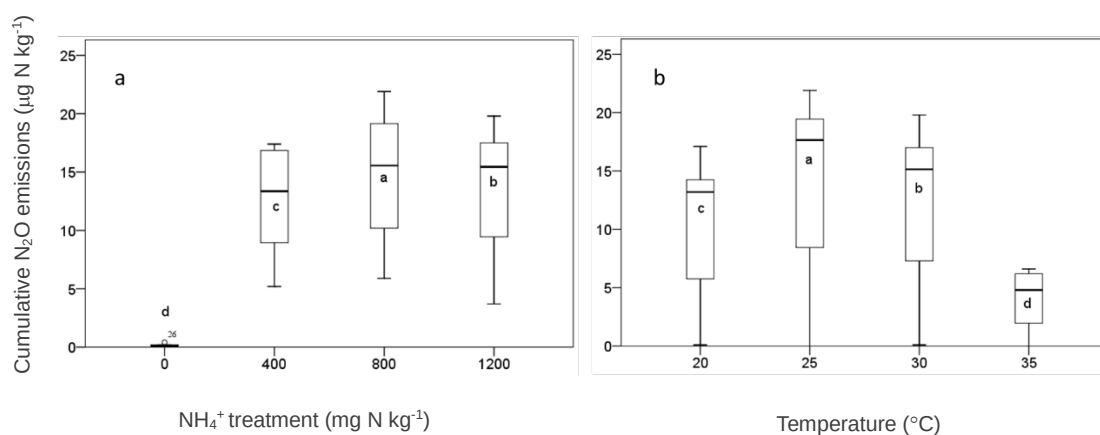


Figure 3.3. Interactive effects of cumulative N₂O emissions and NH₄⁺ application rates and temperatures. Different lowercases (a, b, c and d) show differences among temperatures for each N treatment. Different uppercases (A, B, C and D) show differences among treatments at each temperature ($p < 0.001$, $n = 12$) from a two-way ANOVA.

Table 3.3. N balance for 28 days in different N treatments at four temperatures

N rate	Variables	20°C	25°C	30°C	35°C
0 N	N input ¹⁾	37.2	37.2	37.2	37.2
	N ₂ O ²⁾	0.0 (0.4)	0.1 (0.3)	0.2 (0.6)	0.1 (0.3)
	Mineral N ³⁾	33.0	31.0	39.3	28.9
400 N	N input	420.3	422.9	421.9	416.3
	N ₂ O	11.9 (2.8)	17.1 (4.0)	15.1 (3.6)	6.0 (1.4)
	Mineral N	352.2	353.5	351.7	405.5
800 N	N input	815.2	819.5	827.7	820.5
	N ₂ O	13.9 (1.7)	20.1 (2.4)	18.3 (2.2)	6.2 (0.8)
	Mineral N	741.7	729.5	742.4	814.4
1200 N	N input	1201.9	1208.8	1199.6	1201.2
	N ₂ O	15.6 (1.3)	19.1 (1.6)	15.6 (1.3)	4.1 (0.3)
	Mineral N	1115.5	1150.8	1157.6	1191.7

Note:

¹⁾ The contents of NH₄⁺, NO₃⁻, and NO₂⁻ of the soil prior to incubation, mg N kg⁻¹ soil.

²⁾ The cumulative N₂O-N emissions for 28 days, mg N kg⁻¹ soil.

³⁾ Contents of NH₄⁺, NO₃⁻, and NO₂⁻ on day 28, mg N kg⁻¹ soil.

Number in *brackets* () is the percentage of N₂O over the N input.

Interestingly, there was a different trend in N₂O emission rates with the highest NH₄⁺ application, 1200 N at 20°C, 25°C and 30°C (Fig. 2). Rates of N₂O emission still increased on day 28 at 20°C and those peaks appeared later than at other NH₄⁺ application rates. Those late peaks of N₂O flux at 1200 N were similar to Acton and Baggs (2011) at the highest application rate, 1400 mg N kg⁻¹. They suggested that the late peak of N₂O flux likely reflected a slower growth rate of ammonia oxidizing bacteria. In our study, peaks of NO₂⁻ content were also broader in the 1200N treatments than other N-added treatments (Fig. 1), which was presumably a result of slower growth rates of ammonia oxidizing or denitrifying bacteria.

In the present study, NH₃ toxicity for nitrification could be neglected as shown in Fig.1 because soil pH in all N-added treatments was less than 6.1 and NH₃ fractions were in the range of 0.04–0.14% at 20–35°C.

Microcosms in our study were immediately incubated after adding water to air-dried soil. The priming effect of the dry-wet condition on microorganisms in soil may affect nitrification and N₂O emission in soil (Rudaz *et al.*, 1991). However, this kind of drastic changes in soil moisture sometimes happens in farmland of Vietnam and therefore our experimental procedure simulated tropical soil conditions.

3.4.2. Interactive effects of NH₄⁺ application rates and temperature on N₂O emissions

Temperature dependence of cumulative N₂O emissions in our study was different from Benoit *et al.* (2015) who reported an exponential relationship between N₂O emissions and temperature at 5–45°C. In the present study, N₂O emissions peaked at 25°C and decreased to the lowest at 35°C (Fig. 3). The different trend was presumably due to the different contribution of nitrification and denitrification to the N₂O emissions. More distinct rises in NO₃⁻ contents with increasing temperature (Fig. 1) indicated that the conversion of NH₄⁺ to NO₃⁻ were greater at higher temperatures. The NO₂⁻ contents in the late period (after 7 days) were lower with increasing temperatures (Fig. 1). This is because more NO₂⁻ was consumed at higher temperatures by the

consecutive nitrification to NO_3^- , nitrifier denitrification, and coupled nitrification-denitrification. The second nitrification step from NO_2^- to NO_3^- did not produce N_2O unlike nitrifier denitrification or coupled nitrification-denitrification processes (Wrage *et al.*, 2001). Differences between NH_4^+ reduction and NO_2^- or NO_3^- production in N-added treatments were 65–83, 55–87, 39–82, and 6–8 mg N kg^{-1} at 20°C, 25°C, 30°C and 35°C, respectively on day 28 (Fig. 1). Consequently, the second step of nitrification was the predominant process to reduce NO_2^- at 35°C while the three processes concurrently occurred at lower temperatures. This could be the main reason why N_2O emissions were the lowest at 35°C. In addition, cumulative N_2O emissions for 28 days and mineral N contents in total on day 28 were nearly equal to the N input, which suggests that atmospheric N_2 and other forms were not produced at 35°C (Table 3).

Peaks of N_2O emission in N-added treatments appeared later at 20°C than at 25°C and 30°C (Fig. 2). This trend was consistent with soil NO_2^- content, which peaked at 20°C one week later than at 25°C and 30°C (Fig. 1). This is because N_2O production was enhanced by higher NO_2^- content (Castro-Barros *et al.*, 2016).

Cumulative N_2O emissions declined clearly from 800 mg N kg^{-1} to 1200 mg N kg^{-1} at 35°C while remaining unchanged at other temperatures (Table 2 and Fig. 3). In other words, the greatest NH_4^+ application rates reduced N_2O emissions at the highest temperature of 35°C. On the other hand, there were no differences in NO_2^- or NO_3^- contents among N-added treatments (Fig. 1), indicating that nitrification rates were not different between N-added treatments at 35°C. Deppe *et al.* (2017) noted that both nitrification and N_2O emission declined with increasing NH_4^+ application rates. Our results showed that only N_2O emission was suppressed by high NH_4^+ input. It is possible that N_2O emission ratio through NH_4^+ to NO_2^- was lower at 35°C. The response of nitrifiers and denitrifiers to temperature is conditioned by climate. For example, the optimum temperature for nitrification in a tropical Australian soil was 35°C (Myers, 1975) whereas that was 20°C and nitrification was ceased at 30°C in a temperate Canadian soil (Malhi and McGill,

1982). In the present study, nitrification was not suppressed at 35°C.

3.5. Conclusions

This study aimed at identifying the effects of N application rates, temperatures and their interactions on N₂O emissions from Vietnamese agricultural soil treated with high NH₄⁺ contents under aerobic conditions from 20°C to 35°C. Nitrous oxide emissions in the present study were from autotrophic nitrification, nitrifier denitrification and coupled nitrification-denitrification at 20–30°C. At 35°C, the lowest N₂O emissions occurred because they were derived only from autotrophic nitrification.

Cumulative N₂O emissions increased with increasing NH₄⁺ application rates from 0 to 800 mg N kg⁻¹ and then declined to 1200 mg N kg⁻¹ while emissions increased in the order of 35°C, 20°C, 30°C and 25°C. The interactive effects of N application rates and temperature on cumulative N₂O emissions were also observed. Cumulative N₂O emissions were suppressed at 1200 N which might be due to slower growth rates of ammonia oxidizing or denitrifying bacteria at 20–30°C, and lower N₂O emission ratio through NH₄⁺ to NO₂⁻ at 35°C. In conclusion, N₂O emissions were not exponentially correlated with NH₄⁺ application rates and temperatures. Higher NH₄⁺ application rates at higher temperatures suppressed N₂O emissions.

CHAPTER 4. EFFECTS OF COMPOST TYPES ON NITROUS OXIDE AND CARBON DIOXIDE EMISSIONS AT HIGH TEMPERATURES

4.1. Introduction

Intensification of crop production accompanied by high nitrogen (N) input has been found to increase nitrous oxide (N₂O) emissions (Raut *et al.*, 2015) and resulted in substantial annual N surpluses, causing high nitrate (NO₃⁻) concentration in surface and groundwater (Khai *et al.*, 2007). Utilization of manure compost to supplement plant nutrients and soil quality improvement is common in Vietnam because of its availability and low cost. However, farmers often used excess N input for their crops to achieve economic benefits and consequently caused some problems related to nitrogen loss by NO₃⁻ leaching and N₂O emissions. Organic carbon (C) and N in compost, when added to soil, often increased N₂O and carbon dioxide (CO₂) emissions compared with the control in a 105-day aerobic incubation study at 22°C (Ping *et al.*, 2016). Emissions of N₂O from wetland soil amended with cattle manure increased considerably with increasing N content in the applied manure. The N₂O fluxes following high N manure applications were significantly higher ($P < 0.05$) when compared with fluxes after application of low N manure (Masaka *et al.*, 2016). In Vietnam, no study was conducted to investigate the effect of compost and temperature on emissions of N₂O and CO₂ emissions from an agriculture soil. Here, we conducted a laboratory study on an agriculture soil collected from a tropical area of Vietnam that compost from chicken manure and commercial compost were used for their crops.

In addition, total cumulative N₂O emissions were enhanced by N application (Li *et al.*, 2016) and an increasing temperature interval from 25°C to 35°C (García-Marco *et al.*, 2014; Liang *et al.*, 2016). Cumulative N₂O and CO₂ were increased by 2.8 times and 1.6 times, respectively with an increase of 10°C (15–25°C) (García-Marco *et al.*, 2014). Most of these studies on N₂O and CO₂ emissions have been conducted at temperatures between 5°C and 25°C (García-Marco *et al.*, 2014; Farquharson, 2016; Ping *et al.*, 2016). Little was known about N₂O and CO₂ emissions at

tropical regions with increases in temperature intervals of 25–30°C and 30–35°C. Agriculture production in tropical countries may be associated with higher emissions of N₂O and CO₂, both because of exponential temperature dependence when soil mineral N is not limiting (Smith *et al.*, 1998). Knowledge of high temperature effects on N₂O and CO₂ emissions with different mineral N contents is to provide background data in agricultural management through fertilizer management and prevent nitrate contamination of surface and ground water.

More specifically, the objectives of the study were (i) to investigate changes in N₂O and CO₂ emissions from Vietnamese agricultural soil amended with commercial compost (SH) and chicken compost (CC) with different application rates and (ii) to evaluate temperature responses of N₂O and CO₂ emissions from these compost-treated soils.

4.2. Materials and methods

4.2.1. Soil

Surface soil (0–10 cm) was collected from a vegetable field in Phu Mau commune, Phu Vang district, Thua Thien Hue province, Vietnam (16°29'53 N and 107°34'47 E) in November 2016. The soil was classified as Fluvisols, texture with 63.5% sand, 22.6% silt and 13.9% clay. This soil had been cropped with mustard (*Brassica juncea*) and amaranth (*Amaranthus mangostanus*). Air-dried soil was passed through a 2-mm sieve and characterized (Table 4.1). The soil is alkaline soil (pH > 7) with low nutrient contents, and C/N ratios are in the range of most of the field soils (Weinfurtner, 2008).

4.2.2. Commercial compost and chicken compost

Commercial compost (Song Huong Compost, SH hereafter) was collected from its factory in Phong Dien district, Thua Thien Hue province. The compost was produced from peat (an accumulation of partially decayed vegetation or organic matter under anaerobic condition) and was air-dried for a year. The peat was grilled, mixed with lime and fermented with the addition of microbe mixture for 2-3 months. Then the fermented peat was mixed with ammonium sulfate ((NH₄)₂SO₄, 3%N).

Table 4.1. Basic characteristics of soil and compost types.

Materials	pH	EC (dS m ⁻¹)	NO ₃ ⁻ (mg kg ⁻¹)	NH ₄ ⁺ (mg kg ⁻¹)	TC (g kg ⁻¹)	TN (g kg ⁻¹)	C/N ratio
Soil	7.8	0.052	6.5	1.3	7.5	0.7	10.7
Commercial Compost (SH)	4.8	15	312.4	35979	241.5	47.8	5.1
Chicken Compost (CC)	9.1	9	37.8	1825	290.6	29.6	9.8

pH and EC were determined ($n = 3$) at a 1:5 ratio of material and deionized water (weight basis).

Chicken compost (CC, hereafter) was made by local farmers from chicken manure mixed with bedding material (rice straw). Chicken manure was added microbe mixture (1 kg powder of microbe mixture to 1m³ chicken manure) and covered by blue polyethylene sheet for 6 months. Then, watering the solid pile of fermented manure to make it become loose, and being kept in the air for 10–15 days before use.

Both compost materials were air-dried and ground to pass a 2-mm sieve. The main characteristics of commercial compost and chicken compost were shown in Table 4.1.

4.2.3. Soil incubation

Each 125-mL bottle received 5 g of air-dried soil or soil-compost mixture. Soil treated with 1%, 2% or 4% (w/w) commercial (SH1, SH2 and SH4, respectively) or chicken compost (CC1, CC2 and CC4, respectively) and the control (without compost, CT) were prepared in triplicate. Deionized water was added evenly to the soil surface with a mini-pipette to set the moisture content to 60% water holding capacity (WHC). These soil bottles were covered with a piece of polyethylene plastic film to reduce evaporation while ensuring gaseous exchange, and then incubated at 25°C, 30°C and 35°C by laboratory incubators. The water lost by evaporation was compensated for weekly by adding deionized water using the mini-pipette to the pre-specific weight.

Before collecting gas samples for N₂O and CO₂ measurement, the air in the incubation bottles was exchanged for 30 seconds by using a mini pump (MP-2N, Sibata) and closed for 3 h on days 1, 3, 5, 7, 14, 21 and 28. Air pressure of these samples was measured by an air pressure gauge (handy manometer, Copal Electronics) just before collecting the gas.

Emission rates of N₂O and CO₂ were measured by gas chromatograph (GC-8A, Shimadzu, Japan) equipped with an electron capture detector (ECD) and a thermal conductivity detector (TCD), respectively. Gas emission rates (mg kg⁻¹ dry soil h⁻¹) were calculated by using the following equation:

$$V_i = \rho \times C \times (V_g + V_L \times \alpha) \times 273 / (W \times (273 + T)) / t \quad (4.1)$$

where V_i is the N₂O or CO₂ emission rate (mg kg⁻¹ h), t is the closing time before collecting the gas (h), ρ is the density of N₂O (1.25 kg m⁻³) or CO₂ (0.5357 kg m⁻³) at 25°C, C is the N₂O or CO₂ concentration (ppm_v), V_g (m³) is the head volume, V_L (m³) is the volume of liquid phase, α is the Bunsen absorption coefficients for N₂O and CO₂ at 25°C (0.539 and 0.614), 30°C (0.464 and 0.541) and 35°C (0.404 and 0.481), respectively, W (kg) is the oven-dry weight of soil, T is the temperature at determination. Background N₂O and CO₂ concentrations were subtracted from the measured concentration prior to data analysis. Cumulative N₂O and CO₂ emissions were calculated from the integrated flux over the incubation period.

Additional 15 bottles (3 replicates × 5 days) for each treatment were incubated in the same manner to determine pH, EC, NO₃⁻ and NH₄⁺ of the soil extracts on days 1, 7, 14, 21 and 28.

4.2.4. Soil and compost analyses

All soil samples were subjected to measurement of pH and EC at a soil: water ratio of 1:5 after shaking at 175 rpm for 1 h, using a digital pH meter (F-23, Horiba, Japan) and EC meter (DS-14, Horiba, Japan), respectively.

Total carbon (TC) and nitrogen (TN) contents of soil or soil treated with compost were determined by the dry combustion method using a CN coder (MT-700, Yanaco, Japan). Soil

mineral N was extracted with 2 M KCl solution (soil/water of 1:10) and measured with a continuous flow analyzer (QuAAtro 2-HR, Bltec, Japan) by colorimetric methods.

4.2.5. Data analysis

The temperature sensitivity coefficient (Liang *et al.*, 2016), Q_{10} , was calculated using the following equation:

$$Q_{10} = (R_2/R_1)^{10/(T_2-T_1)} \quad (4.2)$$

where R_1 and R_2 are the emission rates of N_2O and CO_2 at T_1 and T_2 , respectively; T_2 and T_1 are incubation temperatures ($^{\circ}C$).

Two-way ANOVA was used to evaluate the responses of N_2O and CO_2 emissions to temperature and compost additions. Correlation analysis by Pearson's correlation coefficient was performed to test relationships between physico-chemical properties of soil samples prior to incubation and cumulative emissions of N_2O and CO_2 day 28 in the SH and CC treatments. All statistical calculations were performed using SPSS software (SPSS 22.0).

4.3. Results and discussions

4.3.1. Properties of amendments

Commercial compost was acidic while chicken compost was alkaline (Table 4.1). The high NH_4^+ content of SH could be a reason for the low pH compared to CC because NH_4^+ is in equilibrium with ammonia (NH_3) and hydrogen ions (H^+) (Masters, 1998). Electrical conductivity (EC), and NO_3^- and NH_4^+ contents in SH were approximately 1.7, 8.3 and 19.7 times higher than in CC, respectively. The C/N ratio of CC was twice as high as that of SH (Table 4.1) because the SH included $(NH_4)_2SO_4$. The soil C/N ratio is in the range of common values (Weinfurtner, 2008) while that of CC was in the range of good quality compost, 10–20 (Campbell, 1988). When adding compost to soil, soil pH was reduced in the SH treatments but increased in the CC treatments (Table 4.2, $p < 0.05$). The pH value was in the order of SH4 < SH2 < SH1 < CT < CC1, CC2 and CC4. Both types of compost enhanced EC values of the soil. The EC was generally in the order

of CT < CC1 < CC2 < CC4, SH1 < SH2 < SH4 (Table 4.2, $p < 0.05$).

Table 4.2. Main chemical properties of soil and soil mixtures used for the incubation experiment.

Treatment	% Compost	ID	pH	EC (dS m ⁻¹)	NO ₃ ⁻ (mg kg ⁻¹)	NH ₄ ⁺ (mg kg ⁻¹)	DOC (mg kg ⁻¹)	C/N ratio
Soil		CT	7.8±0.1 ^b	0.052±0.0 ^f	6.5±0.3 ^b	1.3±0.4 ^g	62.5±2.7 ^d	12.5
Commercial compost (SH)	1%	SH1	7.3±0.1 ^c	0.674±0.0 ^c	7.6±0.7 ^{ab}	329.4±9.5 ^c	60.1±8.6 ^d	9.7
	2%	SH2	7.1±0.1 ^d	1.259±0.1 ^b	7.6±0.9 ^{ab}	693.9±5.6 ^b	70.8±1.6 ^d	8.8
	4%	SH4	6.8±0.1 ^e	2.325±0.1 ^a	8.7±0.5 ^a	1386.2±19.0 ^a	76.1±0.8 ^d	7.4
Chicken compost (CC)	1%	CC1	8.0±0.1 ^a	0.228±0.0 ^e	6.4±0.4 ^b	30.0±1.0 ^f	324.8±7.0 ^c	11.7
	2%	CC2	8.0±0.0 ^a	0.431±0.0 ^d	6.4±0.1 ^b	61.8±1.7 ^e	611.4±16.7 ^b	11.2
	4%	CC4	8.0±0.1 ^a	0.694±0.0 ^c	7.0±0.2 ^b	118.9±3.5 ^d	1108.3±104.7 ^a	10.5

Note: pH and EC were determined ($n = 3$) at a 1:5 ratio of material and deionized water (weight basis). Data are means ± standard deviation. Different letters indicate difference among treatments ($p < 0.05$, $n = 3$). CT is treatment without compost, SH1, SH2 and SH4 are treatments that receive SH at rates of 1%, 2% and 4% (w/w) to the soil, and CC1, CC2 and CC4 are treatments that receive CC at rates of 1%, 2% and 4% (w/w) to the soil.

The NO₃⁻ content of the soil was only raised with SH4 treatment and did not change in the other applications. NH₄⁺ contents in both SH and CC treatments were much higher than those of the soil, with the order of SH4 > SH2 > SH1 > CC4 > CC2 > CC1 > CT (Table 4.2, $p < 0.05$).

4.3.2. Soil pH, EC and mineral nitrogen contents at the end of incubation

Soil pH in SH treatments decreased with time and was lower at 30°C and 35°C than at 25°C. In the CC treatments, there was no significant difference in pH values (Fig. 4.1a, b and c). Electrical conductivity in the SH treatments slightly increased but EC was insignificant difference in the CC treatments during the incubation period (Fig. 4.1d, e and f).

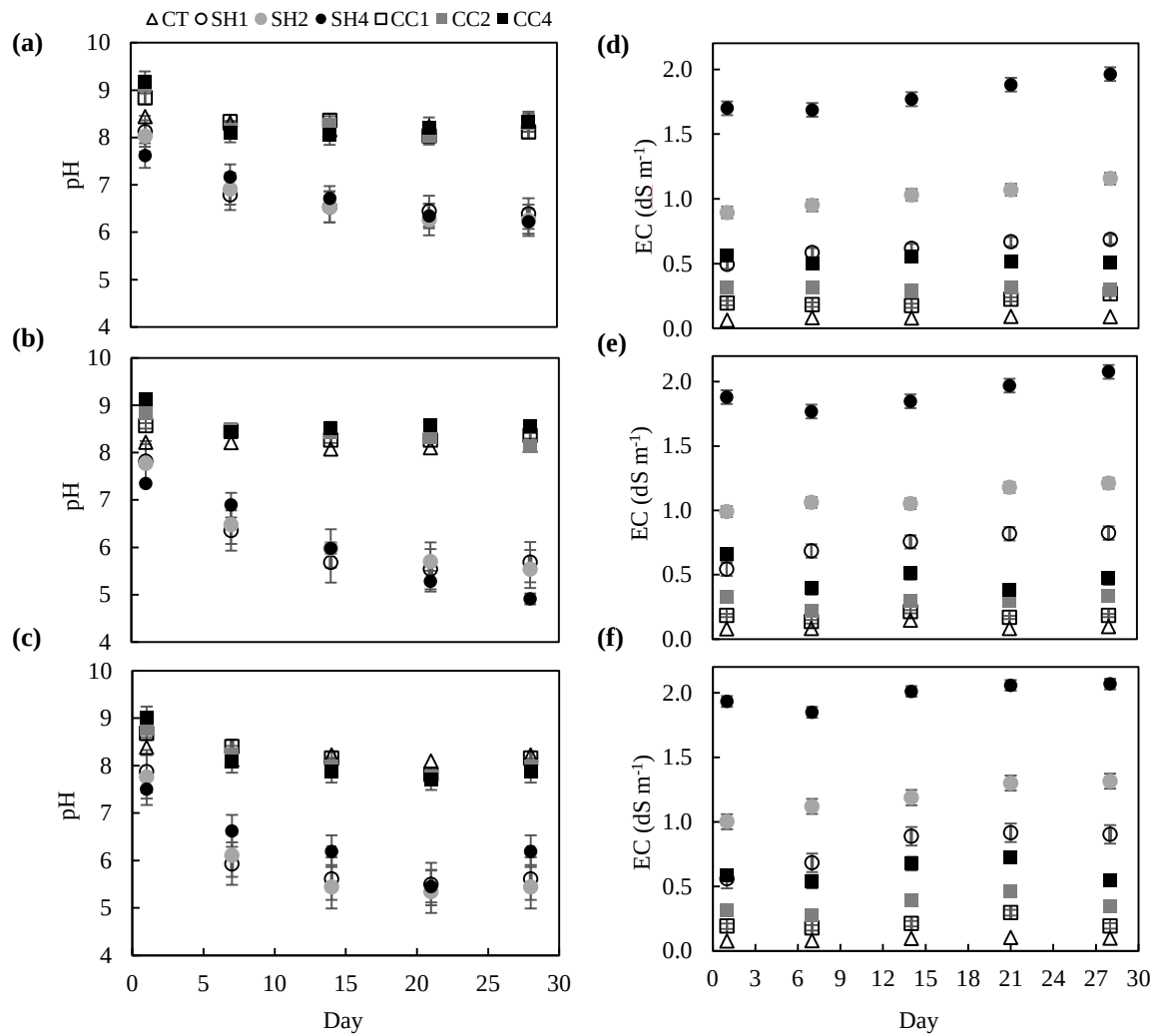


Figure 4.1. pH and EC of different treatments at 25°C (a & d), 30°C (b & e) and 35°C (c & f).

The bars indicate the standard error of the mean ($n = 3$, $p < 0.05$).

As shown in Figure 4.2, soil NH_4^+ and NO_3^- contents ranged from 0.4 to 1553.0 mg N kg⁻¹ and 0.1 to 680.6 mg N kg⁻¹, respectively. The increase of NO_3^- contents in the SH and CC treatments accompanied the decline of NH_4^+ contents (Fig. 4.2 and 4.3). The NH_4^+ and NO_3^- contents in the SH treatments were much higher than in the CC treatments on day 28 at the same application rates. The NH_4^+ in the SH treatments declined with time and was higher (> 10 mg N kg⁻¹, except SH1) at the end of the incubation period (Fig. 4.2d, e and f) while it decreased to less than 1 mg N kg⁻¹ after the first week of incubation in the CC treatments (Fig. 4.3d, e and f). The net N nitrification of the

SH and CC treatments were in the range of 8.6–24.0 mg N kg⁻¹ d⁻¹ (SH4), 10.3–14.5 mg N kg⁻¹ d⁻¹ (SH2), 8.5–15.0 mg N kg⁻¹ d⁻¹ (SH1), 4.5–7.0 mg N kg⁻¹ d⁻¹ (CC4), 3.4–3.9 mg N kg⁻¹ d⁻¹ (CC2), 1.5–3.2 mg N kg⁻¹ d⁻¹ (CC1).

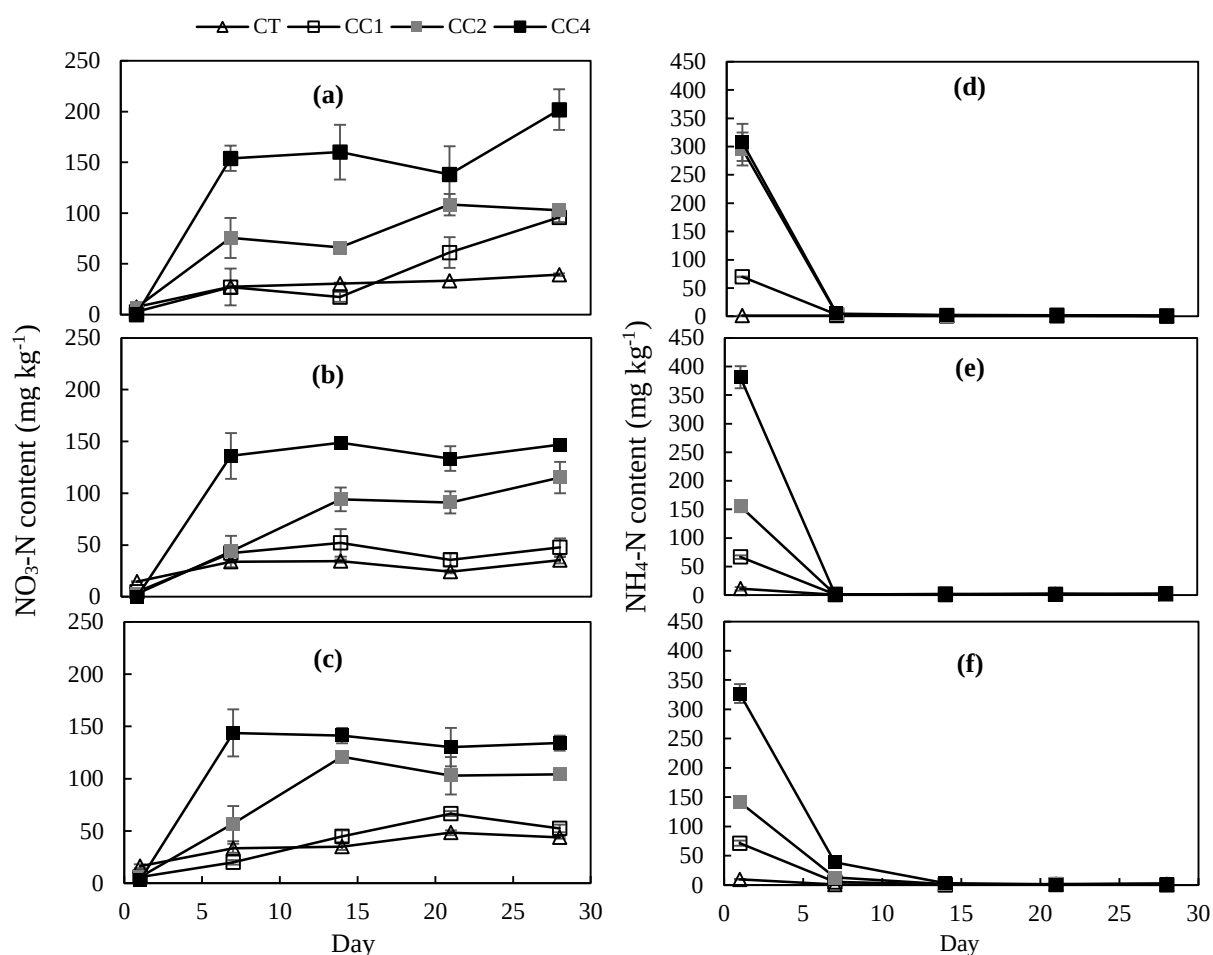


Figure 4.2. NO₃⁻ and NH₄⁺ contents of SH treatments at 25°C (a & d), 30°C (b & e) and 35°C (c & f). The bars indicate the standard error of the mean ($n = 3$, $p < 0.05$).

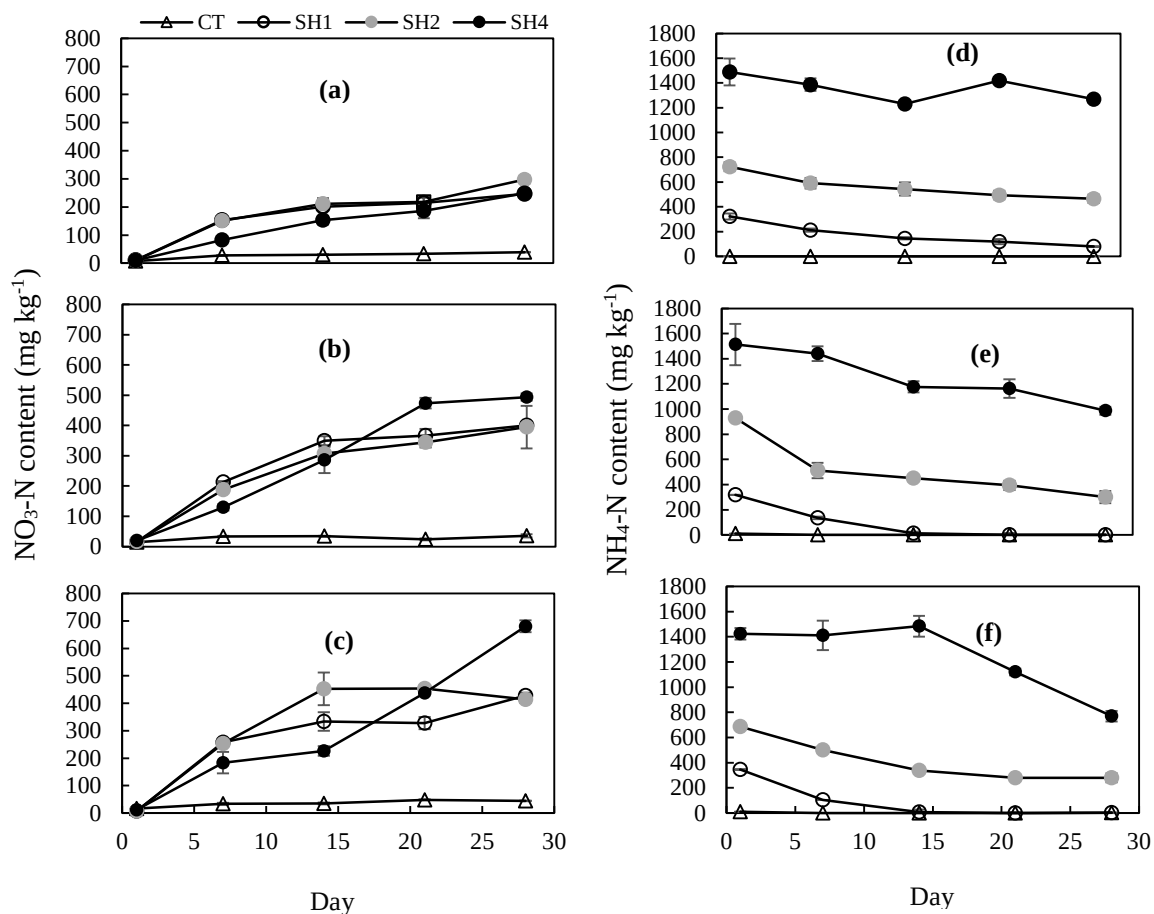


Figure 4.3. NO_3^- and NH_4^+ contents of CC treatments at 25°C (a & d), 30°C (b & e) and 35°C (c & f). The bars indicate the standard error of the mean ($n = 3, p < 0.05$).

4.3.3. Nitrous oxide emissions and effects of compost application rates

This study was aerobically conducted at 60% WHC with high NH_4^+ content and therefore N_2O production was related to nitrification. The N_2O emission in the CC treatments was positively correlated with NH_4^+ and NO_3^- contents, and DOC at each temperature (Table 4.4). This agrees with previous articles (Sánchez-Martín *et al.*, 2008; Cavalli *et al.*, 2015; Farquharson, 2016; Ping *et al.*, 2016), reporting that more N_2O production under aerobic conditions was related to NH_4^+ levels in soil. Our study showed that N_2O fluxes in the compost treatments were greater than in CT in the first two-week incubation (Fig. 4.4). The peak N_2O fluxes in CC treatments was observed in the first week of incubation and decreased towards 28 incubation days (Fig. 4.4d, e and f), which was proven by the significant reduction of NH_4^+ content and increase of NO_3^- content due to the

nitrification process (Fig. 4.3). While peak N_2O fluxes in SH treatments were less distinct, occurred later and were more extended, especially SH2 (Fig. 4.4a, b and c). The N_2O emission in the SH treatments still occurred after the first week incubation accompanied the decreased NH_4^+ and increased NO_3^- contents till the end of incubation (Fig. 4.2). On day 28, the higher availability of NH_4^+ content ($> 10 \text{ mg N kg}^{-1}$) in the SH treatments than that of the CC treatments ($< 1 \text{ mg N kg}^{-1}$) was observed, especially in the SH2 and SH4 treatments (Fig. 4.2). For a higher application rate, higher N_2O emissions was observed in the CC treatments (Fig. 4.7b & d, $p < 0.05$). In the SH treatments, medium application level (SH2) resulted in the greatest N_2O emission, and the highest application emitted the lowest N_2O concentration (Fig. 4.7a, $p < 0.05$), regardless of temperature.

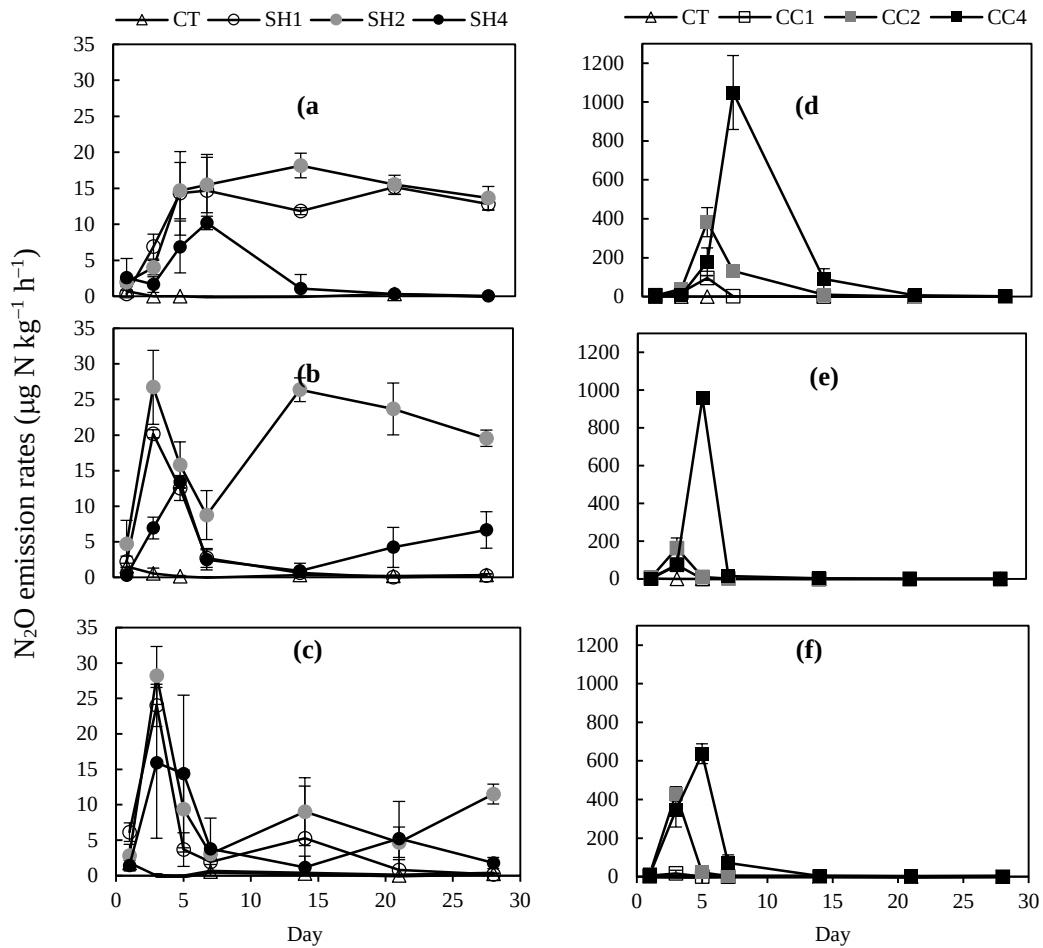


Figure 4.4. N_2O emissions in SH and CC treatments at 25°C (a & d), 30°C (b & e) and 35°C (c & f). The bars indicate the standard error of the mean ($n = 3$, $p < 0.05$).

Table 4.3. Cumulative N₂O (mg N kg⁻¹) and CO₂ (g C kg⁻¹) emissions from different treatments for 28 days at three temperatures.

Gas	Temp.	CT	SH1	SH2	SH4	CC1	CC2	CC4
N ₂ O	25°C	0.1±0.0 ^d	8.2±0.6 ^{cd}	9.4±1.2 ^{cd}	1.8±0.5 ^d	6.4±1.0 ^{cd}	36.5±4.0 ^b	117.0±29.4 ^a
	30°C	0.2±0.1 ^d	2.1±0.3 ^d	13.2±1.0 ^b	2.7±0.9 ^d	3.9±0.7 ^{cd}	8.9±2.7 ^{bc}	51.8±4.1 ^a
	35°C	0.2±0.1 ^c	2.8±0.9 ^c	5.5±0.5 ^c	3.2±1.9 ^c	1.1±0.9 ^c	22.6±2.2 ^b	56.1±4.7 ^a
CO ₂	25°C	1.0±0.0 ^d	1.0±0.0 ^d	1.0±0.0 ^d	1.0±0.0 ^d	3.3±0.6 ^c	5.0±0.0 ^b	8.0±0.0 ^a
	30°C	1.0±0.0 ^d	1.0±0.0 ^d	1.0±0.0 ^d	1.0±0.0 ^d	4.3±0.6 ^c	6.7±0.6 ^b	11.3±0.6 ^a
	35°C	1.0±0.0 ^d	1.0±0.0 ^d	1.0±0.0 ^d	1.0±0.0 ^d	3.0±0.1 ^c	4.3±0.6 ^b	6.3±0.6 ^a

Note: Data are means ± standard deviation. Different letters indicate difference among treatments at each temperature ($p < 0.05$, $n = 3$). CT is treatment without compost, SH1, SH2 and SH4 are treatments that receive SH at rates of 1%, 2% and 4% (w/w) to the soil, and CC1, CC2 and CC4 are treatments that receive CC at rates of 1%, 2% and 4% (w/w) to the soil.

In general, N₂O fluxes in the CC treatments were greater than those in the SH treatments. It is because nitrogen is present in the form of stable organic N in the SH compost, and thus mineralization was slow and occurred over time, and low soil exchangeable NH₄⁺.

In addition, high availability of NH₄⁺ in the SH treatments could limit the nitrification and N₂O production from nitrification, as well as denitrification derived N₂O emission (Deppe *et al.*, 2017). When the NH₄⁺ content ranged from 400 to 5000 mg NH₄⁺ kg⁻¹, nitrification declined with increasing NH₄⁺ level and no nitrification occurred in the 5000 mg NH₄⁺ kg⁻¹ soil treatment as ammonium sulfate (Deppe *et al.*, 2017). With increasing NH₄NO₃ content (from 355 to 720 mg NH₄⁺ kg⁻¹), net nitrification and N₂O production from nitrification decreased by one and two thirds, respectively (Acton and Baggs, 2011). However, nitrification and N₂O emission have been showed to increase with NH₄⁺ contents from 0 to 400 mg NH₄⁺ kg⁻¹ (Huang *et al.*, 2014; Farquharson, 2016; Ping *et al.*, 2016). These findings explained why the SH treatments produced lower N₂O emissions

than the CC treatments did, and the highest NH_4^+ content resulted in the least N_2O production in the SH4 treatment. It was also confirmed by the lowest net N nitrification of SH4 and the lowest % N_2O emission over the mineral N input among the treatments.

When denitrification is a main source of N_2O emission, increasing pH results in decreasing N_2O emissions (Čuhel *et al.*, 2010; Signor and Cerri, 2013; Cheng *et al.*, 2015), and when nitrification is the main source of N_2O emissions, increasing pH results in increasing N_2O emissions (Bremmer and Blackmer, 1981; Signor and Cerri, 2013). Decreasing pH in the SH and CC treatments (Fig. 4.1) decreased N_2O emissions in the SH treatments but increased N_2O emissions in the CC treatments. This is the reason why N_2O emission in the CC treatments was much higher than in the SH treatments despite the higher initial NH_4^+ content in the SH treatments than in the CC.

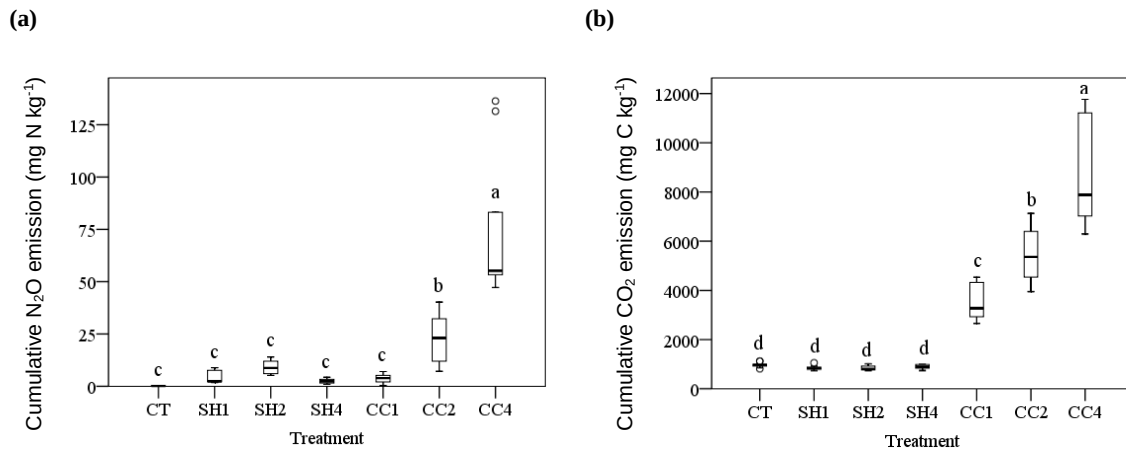


Figure 4.5. Responses of cumulative N_2O (a) and CO_2 (b) over three means of temperatures. Tukey box plot shows 25% (bottom of box) and 75% (top of box), the median (the horizontal line in the box). The whiskers represent the highest (top) and lowest (bottom). Outliers and extreme values are represented by circles beyond the whiskers.

Significant differences were observed in cumulative N_2O emissions among CC treatments, and SH treatments and non-amended soils (Fig. 4.5, $p < 0.05$). With the CC treatments, cumulative

N₂O emissions increased with application rates. The N₂O cumulative emissions from CC1 (1.1–6.4 mg N kg⁻¹), CC2 (8.9–36.5 mg N kg⁻¹), CC4 (51.8–117.0 mg N kg⁻¹) were ten to thousand times higher than the CT (0.1–0.2 mg N kg⁻¹) (Table 4.3, $p < 0.01$). The highest cumulative N₂O emission was from CC4 (Fig. 4.5a, $p < 0.05$).

Cumulative N₂O emissions of SH1, SH2, SH4, CC1, CC2 and CC4 for 28 days were 0.6–2.1%, 0.8–1.9%, 0.0–0.2%, 2.6–13.8%, 12.7–51.9% and 41.0–92.0% of mineral N input (NH₄⁺ and NO₃⁻), respectively (Table 4.3, Fig. 4.5a, $p < 0.05$). Our results showed that net nitrification in the SH treatments was higher than that in the CC treatments whereas %N₂O over mineral N input was much higher in the CC treatments than in SH2 and SH4 treatments. The N₂O emission from the CC treatments did not occur by day 28, different from day 49 reported by Ping et al. (2016) and day 42 reported by Chiyoka et al. (2011) for laboratory incubation at 22°C. However, N₂O emissions from the SH treatments still occurred after 28 incubation days. It means that compost from the CC derived C and N substrates was more degraded and mineralized than the SH.

4.3.4. Carbon dioxide emissions and effects of compost application rates

Carbon dioxide fluxes in CC treatments were much higher than those of SH treatments and CT. The CO₂ fluxes in the SH and CC treatments peaked on day 1 or day 3 and then decreased till the end of the incubation period (Fig. 4.6), due to the consumption of labile and readily mineralizable C pools. This result agrees with Chiyoka et al. (2011), who reported that CO₂ emission was the greatest after the set-up and decreased to constant levels after the first week (Chiyoka *et al.*, 2011). The emission of CO₂ in the CC treatments was much higher than that in the SH treatments due to the greater DOC in the CC treatments than in the SH treatments (Table 4.2, $p < 0.05$). Higher application rates resulting in higher CO₂ emission in the CC treatments (Fig. 4.5b, $p < 0.05$) were explained by the positive correlation between CO₂ concentration and DOC contents (Table 4.4).

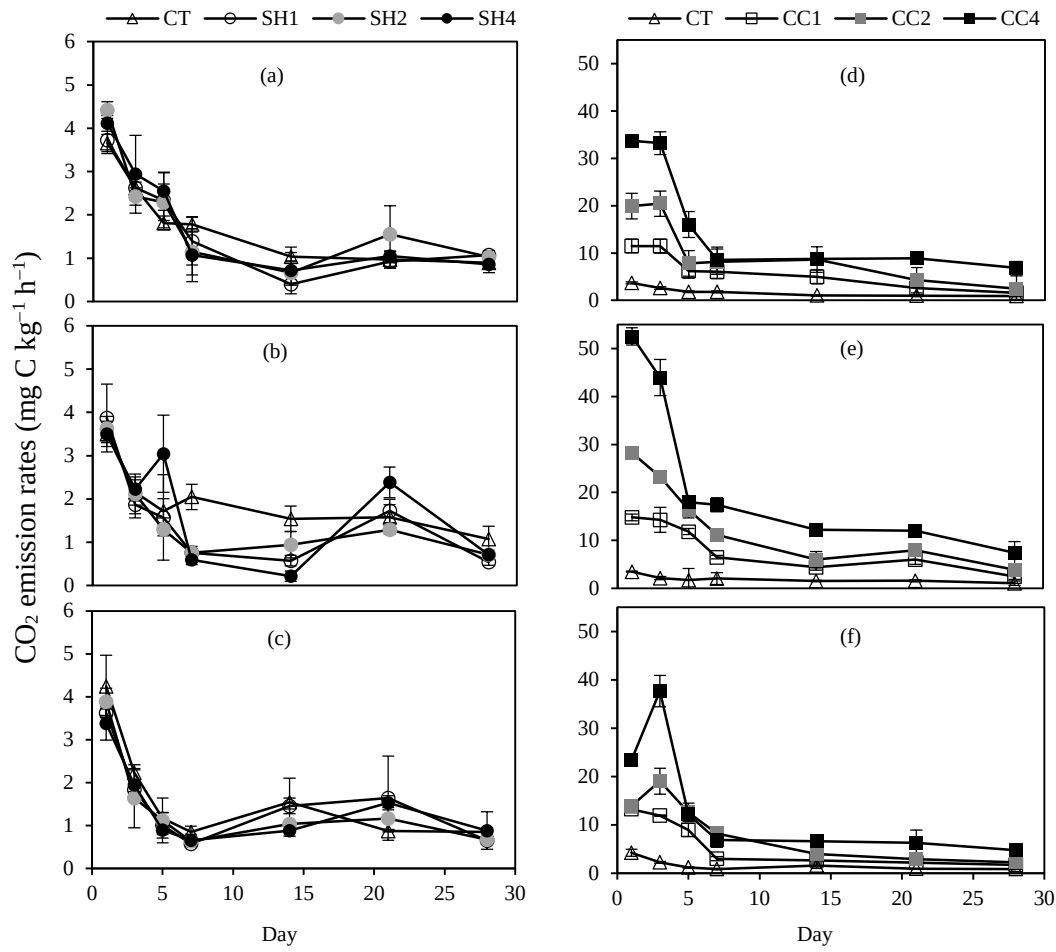


Figure 4.6. CO₂ emissions in SH and CC treatments at 25°C (a & d), 30°C (b & e) and 35°C (c & f). The bars indicate the standard error of the mean ($n = 3$, $p < 0.05$).

On average, less than 1% of the applied C was emitted as CO₂ from the SH treatments, while the proportion of C lost was 33% in the CC treatments. This means that more C supplied with the SH treatments was sequestered by the soil while the larger part of C supplied with the CC treatments was lost.

Cumulative CO₂ emissions were dependent mainly on compost type. The CO₂ emissions from SH treatments were not different from the CT. However, CC additions significantly increased the cumulative CO₂ emissions (Fig. 4.5b, $p < 0.05$). Compared with the CT (1.0 g C kg⁻¹), CO₂ cumulative emissions from CC1 (3.0–4.3 g C kg⁻¹), CC2 (4.3–6.7 g C kg⁻¹), CC4 (6.3–11.3 g C kg⁻¹) were 3 to 11 times higher (Table 4.3, Fig. 4.5b, $p < 0.05$).

Table 4.4. Pearson correlation coefficients of cumulative N₂O and CO₂ emissions from different treatments for 28 days to the treated-soil properties prior to the incubation at different temperatures ($n = 9$).

Treatment	Variable	Temperature	pH	EC	NO ₃ ⁻	NH ₄ ⁺	DOC
Commercial compost (SH)	N ₂ O	25°C	0.782*	-0.845**	-0.704*	-0.859**	-0.564
		30°C	0.025	-0.121	-0.458	-0.129	0.225
		35°C	-0.001	-0.016	-0.442	-0.045	0.084
	CO ₂	25°C	-0.332	0.203	-0.251	0.181	0.566
		30°C	-0.529	0.612	-0.267	0.654	0.270
		35°C	0.257	-0.235	-0.154	-0.240	-0.465
Chicken compost (CC)	N ₂ O	25°C	0.272	0.919**	0.829**	0.939**	0.899**
		30°C	-0.060	0.933**	0.783*	0.964**	0.953**
		35°C	0.140	0.984**	0.922**	0.990**	0.974**
	CO ₂	25°C	0.049	0.992**	0.707*	0.995**	0.993**
		30°C	0.086	0.976**	0.915**	0.995**	0.983**
		35°C	0.144	0.983**	0.921**	0.983**	0.964**

** . Correlation is significant at 0.01 (2-tailed).

* . Correlation is significant at 0.05 (2-tailed).

The cumulative CO₂ emissions of CC1, CC2 and CC4 for 28 days represented 17.9–32.3%, 25.1–42.9% and 29.6–54.1% of initial input TC, respectively. However, the cumulative CO₂ emissions of SH treatments were very low, from 0 to 0.2% of the initial TC input (Table 4.1 & 4.3).

When considering the cumulative N₂O and CO₂ emissions during the whole incubation period, among the treatments, CC1 was the best choice because of the lowest conversion of N₂O (Fig. 4.5a), lower CO₂ emission (Fig. 4.5b), and least NO₃⁻ release and very low NH₄⁺ (Fig. 4.3a, b and c) leading to mitigate atmosphere and soil contamination. However, in this study, effects of compost application rates on soil quality and crop growth have not been considered and might be further studied.

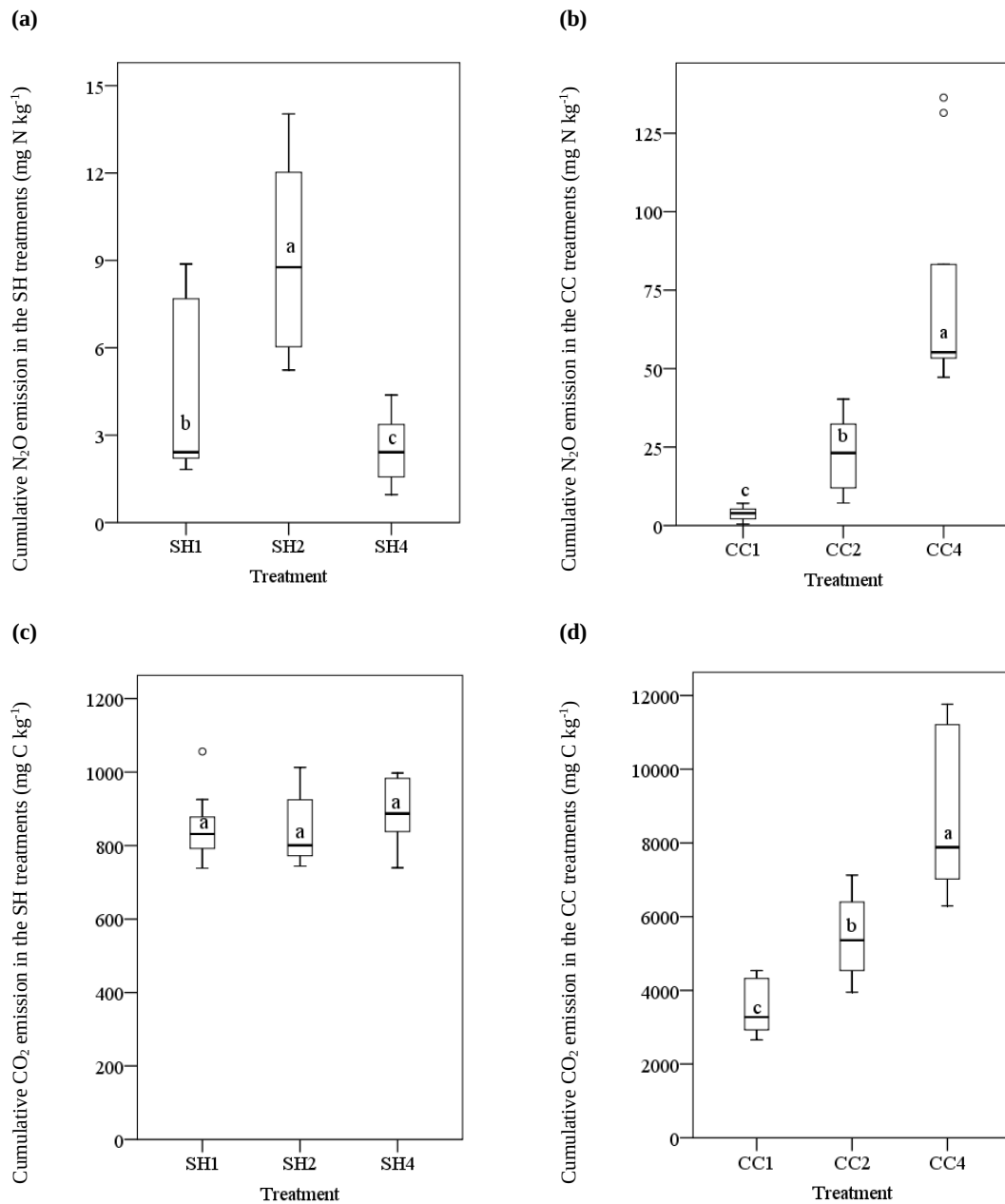


Figure 4.7. Response of N₂O to application rates of SH treatment (a), CC treatment (b), Response of CO₂ to application rates of SH treatment (c), to CC treatment (d). Tukey box plot shows 25% (bottom of box) and 75% (top of box), the median (the horizontal line in the box). The whiskers represent the highest (top) and lowest (bottom). Outliers and extreme values are represented by circles beyond the whiskers.

4.3.5. Temperature responses of N₂O and CO₂ emissions

Increasing temperature from 25°C to 30°C, no significant difference in N₂O emissions was observed in the SH treatments (Fig. 4.8a, $p < 0.05$) but CC treatments resulted in lowering N₂O

emissions (Fig. 4.8b, $p < 0.05$). In the interval of 30–35°C, lower N₂O emissions for higher temperature occurred in the SH treatments while higher N₂O emissions for higher temperature was observed in the CC treatments.

The CO₂ emission was not significantly different in the SH treatments when increasing temperature (Fig. 4.8c, $p < 0.05$). However, in the CC treatments, it was enhanced from 25°C to 30°C and then declined from 30°C to 35°C (Fig. 4.8d, $p < 0.05$).

Table 4.5. Responses of cumulative N₂O and CO₂ for 28 days to temperature and their application rates in a two-way ANOVA.

Factors	N ₂ O (SH)			N ₂ O (CC)		
	df	f	<i>p</i>	df	f	<i>p</i>
Temperature (Temp)	2	19.2	< 0.0001	2	25.3	< 0.0001
Application rate (AR)	2	117.9	< 0.0001	2	118.1	< 0.0001
Temp × AR	4	32.1	< 0.0001	4	9.4	< 0.0001
Factors	CO ₂ (SH)			CO ₂ (CC)		
	df	f	<i>p</i>	df	f	<i>p</i>
Temperature (Temp)	2	1.3	> 0.05	2	295.7	< 0.0001
Application rate (AR)	2	0.6	> 0.05	2	837.5	< 0.0001
Temp × AR	4	2.7	> 0.05	4	30.5	< 0.0001

Table 4.6. Q_{10} values for CO₂ and N₂O emissions from different treatments at each temperature interval.

	Temp	CT	SH1	SH2	SH4	CC1	CC2	CC4
N ₂ O	25–30°C	3.4	0.1	2.0	2.1	0.4	0.1	0.2
	30–35°C	0.9	1.8	0.2	1.4	0.1	6.5	1.2
CO ₂	25–30°C	1.3	1.0	0.7	1.1	1.8	1.6	2.0
	30–35°C	0.7	1.2	0.9	0.8	0.4	0.4	0.3

CT is treatment without compost, SH1, SH2 and SH4 are treatments that receive SH at rates of 1%, 2% and 4% (w/w) to the soil, and CC1, CC2 and CC4 are treatments that receive CC at rates of 1%, 2% and 4% (w/w) to the soil.

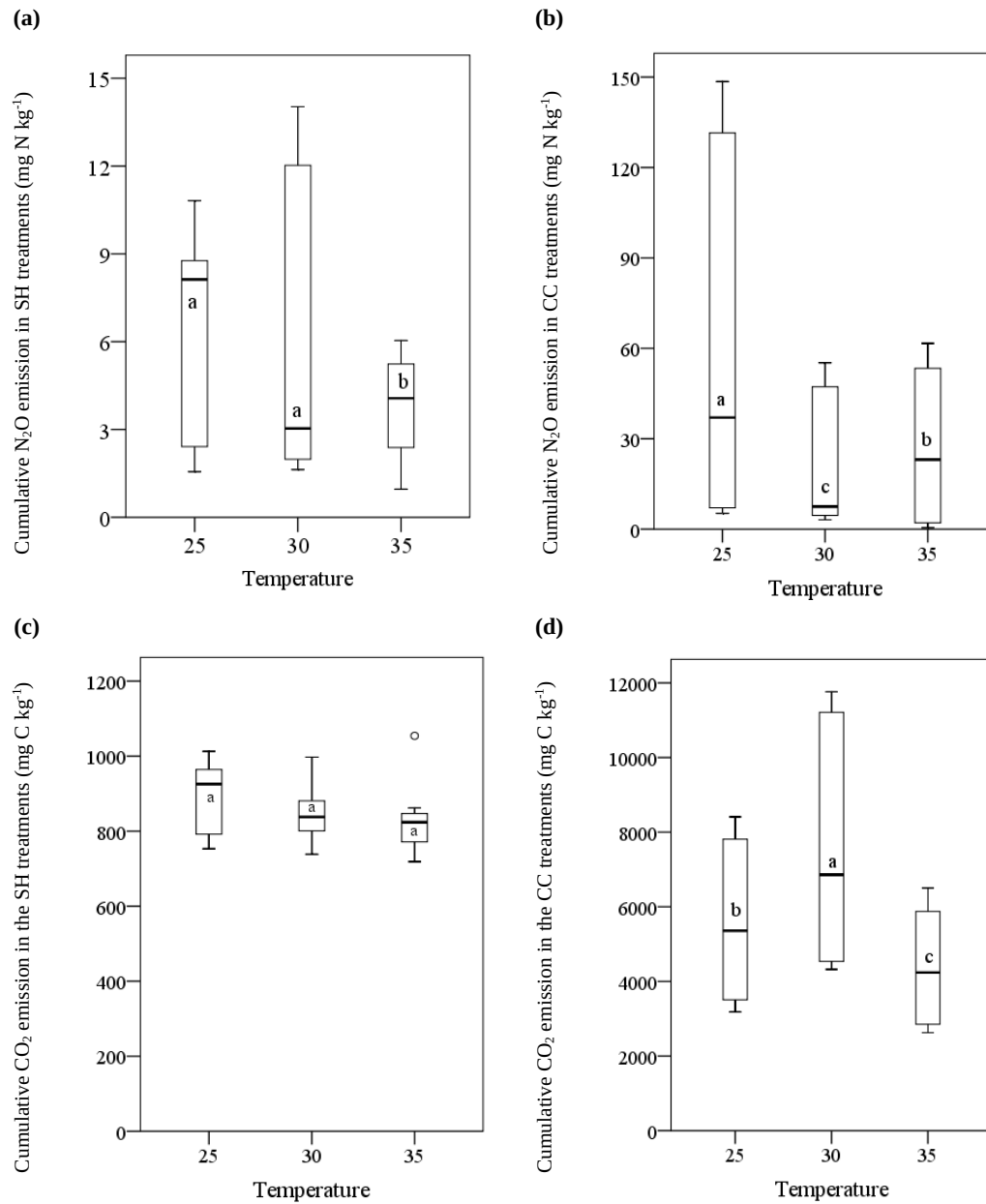


Figure 4.8. Temperature response of N_2O in the SH treatments (a), in the CC treatments (b), Response of CO_2 in the SH treatments (c), in the CC treatments (d). Tukey box plot shows 25% (bottom of box) and 75% (top of box), the median (the horizontal line in the box). The whiskers represent the highest (top) and lowest (bottom). Outliers and extreme values are represented by circles beyond the whiskers.

More specifically, when considering Q_{10} values, our results showed that SH4 and CC1 had unique trends in the two temperature intervals (25–30°C and 30–35°C) (Table 4.6). Higher temperature produced higher N_2O emission in SH4 ($Q_{10} > 1$) but higher temperature resulted in lower N_2O emission in CC1 ($Q_{10} < 1$) (Table 4.6). SH1, CC2 and CC4 had the same fluctuation trends as N_2O emission at the two temperature intervals (Table 4.6). In addition, all the CC treatments and SH4 resulted in higher CO_2 emission at higher temperature ($Q_{10} > 1$) between 25–30°C but less CO_2 emission at higher temperature ($Q_{10} < 1$) between 30–35°C (Table 4.6).

The N_2O and CO_2 emissions from compost-treated agriculture soil in Vietnam were strongly affected by temperature (Table 4.5). The optimal temperatures for nitrification and denitrification were different. Optimal temperature for denitrification rates was from 25°C to 35°C (Braker *et al.*, 2010). In addition, for nitrification, N_2O was essentially produced starting at 30°C (Benoit *et al.*, 2015). Optimal temperatures for N_2O production from nitrification was 42°C and from denitrification was 54.5°C (Benoit *et al.*, 2015). Our study showed that the maximum N_2O emissions occurred at 25°C in both SH and CC treatments and decreased from 25°C or 30°C to 35°C for the SH treatments (Fig. 4.8a), decreased from 25°C to 30°C and then increased from 30°C to 35°C for the CC treatments (Fig. 4.8b). It seems to differ from the previous (Signor and Cerri, 2013; Benoit *et al.*, 2015), reporting that an increase of temperature could enhance N_2O and CO_2 emissions. The above study was conducted in batch experiments at different intervals of 5, 10, 16, 20, 30, 37 and 45°C with optimal conditions for nitrification and denitrification (Benoit *et al.*, 2015). In addition, the evidence suggests that respiratory Q_{10} was dependent on range of measurement temperatures and declined with increasing measurement-temperature intervals (Tjoelker *et al.*, 2001).

The Q_{10} values of N_2O (25–30°C) in the CC treatments and SH1 ranged from 0.1 to 0.4, indicating that higher temperature lowered N_2O emission, which was different from those reported by other researchers (Smith *et al.*, 1998; García-Marco *et al.*, 2014). The Q_{10} values of N_2O

emissions from SH1, SH4 and CC4 treatments in the interval of 30–35°C were in accordance with the previous study, reporting Q_{10} values ranged from 1.6 to 4.1 (Smith *et al.*, 1998).

The Q_{10} values of the present study showed that CO₂ emissions increased with increasing temperature from 25°C to 30°C in the SH and CC treatments, except for SH2. However, from 30°C to 35°C, higher temperature resulting in lower CO₂ emissions was observed, except for SH1 (Table 4.6). Our results agreed with the previous study reporting an declined Q_{10} with increases in temperature intervals of 12–18°C, 18–24°C and 24–30°C (Tjoelker *et al.*, 2001), but disagreed with the study in the intervals of 8–18°C and 18–28°C (Zhang *et al.*, 2016a).

4.4. Conclusions

The study was designed to determine the effects of compost additions and temperature on N₂O and CO₂ emissions from a vegetable field in Vietnam at the temperature range from 25°C to 35°C. Our results showed that N₂O and CO₂ emissions were significantly affected by temperature and compost additions, and by their interactions. Higher N₂O and CO₂ emissions were seen in the CC treatments than those in the SH treatments because high NH₄⁺ contents in the SH treatments inhibited nitrification and N₂O emissions. In response to temperature, cumulative N₂O emissions decreased but CO₂ emissions showed no significant difference in the SH treatments while N₂O and CO₂ emissions in the CC treatments did not show constant trends. With increasing NH₄⁺ application rates, N₂O emissions were greater in the CC treatments while those did not differ in the SH treatments. Our study results suggested that 1% application rate of CC was the best choice when considering N₂O and CO₂ emissions and release of NO₃⁻ and NH₄⁺ at the end of incubation period.

CHAPTER 5. MITIGATION OF NITROUS OXIDE AND CARBON DIOXIDE EMISSIONS WITH BIOCHAR AT THREE TEMPERATURES

5.1. Introduction

Nitrous oxide is a long-lived gas in the atmosphere with global warming potential 298 times that of CO₂ for a time horizon of 100 years (Metz *et al.*, 2007). Agriculture is a source of N₂O, CO₂ and CH₄, and accounted for 10–12% CO₂-equivalent to total global greenhouse gas (GHG) emissions (Smith *et al.*, 2007). Intensive vegetable cropping systems often receive excess nitrogen (N) application and result in greater N₂O emissions and water quality deterioration (Khai *et al.*, 2007; Wang *et al.*, 2008; Zhang *et al.*, 2016b).

Biochar is considered a useful soil amendment to improve soil properties, increase carbon sequestration and reduce GHG emissions from soil (Xie *et al.*, 2016). Amendment of soil with biochar reduced N₂O emissions by 54% (Cayuela *et al.*, 2014) and 76% (Sánchez-García *et al.*, 2014). However, there have been studies reporting no difference or even an increase in N₂O emissions after biochar application (Spokas and Reicosky, 2009; Yoo and Kang, 2012; Sánchez-García *et al.*, 2014; Zhang *et al.*, 2014). Spokas *et al.* (2009) reported that two chars (Biosource™ and Macadamia shell) increased the observed N₂O production by 295% and 1627%, respectively. On the other hand, the addition of 16 different biochars to agricultural soil (10% w/w) resulted in different responses in CO₂ emission: five chars increased it, two chars reduced it and nine caused no significant change (Spokas and Reicosky, 2009).

The major factors controlling N₂O emission from agricultural soil are soil N availability, dissolved organic C (DOC), temperature, moisture and pH. Total cumulative N₂O emission was enhanced by N application (Li *et al.*, 2016) and high temperature (García-Marco *et al.*, 2014; Liang *et al.*, 2016). Increasing temperature could enhance N₂O emission from soils by enhancing nitrification and denitrification rates. The optimal temperature for the nitrification process was 32.5°C, whereas that for denitrification was 45°C (Benoit *et al.*, 2015). Soil respiration is also

stimulated by a temperature increase. Panosso et al. (2011) showed that CO₂ emission in tropical areas was often higher than in temperate regions. Because most of the studies were conducted at 0–25°C, the effects of high temperature on CO₂ (Signor *et al.*, 2013; Liang *et al.*, 2016) and N₂O (Liang *et al.*, 2016) have not been well documented. Little is known about high temperature effects on N₂O and CO₂ emissions from biochar- amended soil in tropical vegetable fields. In this study, we selected a field used for intensive vegetable cultivation in central Vietnam where the groundwater was contaminated with NO₃⁻, probably due to excess use of N fertilizer. Normal biochar and a biochar with high NO₃⁻ adsorption capacity, a functional biochar produced from coffee waste, were used as soil amendments to evaluate the ability to mitigate N₂O emissions from the Vietnamese agricultural soil. The functional biochar was reported to adsorb about 20 g N-NO₃⁻ kg⁻¹ (Yokoyama J. T *et al.*, 2007) and we hypothesized that functional biochar produces less N₂O when added to soil compared to the normal biochar due to its adsorption ability.

The objectives of this study were to 1) determine the effects of different coffee waste biochar materials on N₂O and CO₂ emissions and 2) investigate the response of N₂O and CO₂ emissions from agricultural soil to different temperatures.

5.2. Materials and methods

5.2.1. Soil characterization

Soil was collected from 0 to 10 cm depth in a vegetable field in Phu Mau Commune, Phu Vang district, Thua Thien Hue province, Vietnam (16°29'53 N, 107°34'47 E). This soil is a sandy loam (Fluvisol) with 71% sand, 18% silt and 11% clay. The study field had been planted with mustard (*Brassica juncea*) and amaranth (*Amaranthus mangostanus*). The air-dried soil sample was passed through a 2-mm sieve and characterized as follows: pH (1:5), 7.8; EC (1:5), 0.116 dS m⁻¹; NO₃⁻, 41 mg N kg⁻¹; NH₄⁺, 0.3 mg N kg⁻¹; TN, 0.7 g kg⁻¹; TC, 7.5 g kg⁻¹; C/N ratio, 11 and cation exchange capacity (CEC), 6.63 cmol_c kg⁻¹.

Table 5.1. Properties of coffee waste materials.

Materials	pH	EC ($\mu\text{S cm}^{-1}$)	CEC ($\text{cmol}_c \text{ kg}^{-1}$)	TC (g kg^{-1})	TN (g kg^{-1})	C/N ratio
Raw material	6	721	40	521	25	21
Normal biochar	10	580	28	772	42	18
Functional biochar	5	474	12	634	28	23

pH and EC were measured ($n = 3$) at a 1:10 ratio of material and deionized water (weight basis).

5.2.2. Coffee waste biochar

Normal (NB) and functional coffee waste biochar materials (FB) were incinerated at 600°C for 1 h. The raw material of the biochar (waste coffee grounds) was collected from a coffee canning factory in Okayama, Japan. The functional biochar was manufactured by placing the used coffee grounds in 1 M CaCl_2 solution and stirring for 24 h at room temperature, then dried at 110°C for 5 h, followed by carbonizing at 600°C for 1 h and washing with 6 M HCl (Yokoyama J. T *et al.*, 2007). The functional biochar was reported to have a NO_3^- adsorption capacity of 20 g kg^{-1} (Yokoyama J. T *et al.*, 2007). The mean particle diameter and specific surface area of the functional biochar were 938 μm and 94.7 $\text{m}^2 \text{ g}^{-1}$ on a dry-matter basis, respectively. The normal biochar was produced by drying the coffee grounds at 110°C and carbonizing at 600°C for 1 h (Yokoyama J. T *et al.*, 2007). Characteristics of the raw and biochar materials are presented in Table 5.1.

5.2.3. Incubation tests

Soil microcosms were constructed in 125 mL glass bottles. Each bottle received 5 g of air-dried soil or soil-biochar mixture. Treatments consisted of the addition of normal coffee waste biochar (NB) or functional coffee waste biochar (FB). The control, which received soil without biochar, was compared with the other treatments. All treatments were performed in triplicate. The application rate of biochar was 2% by weight (24 t ha^{-1} , by assuming 0.1 g biochar over 5 g dried soil in a 125 mL glass bottle) which represents a common field application rate (Shackley *et al.*,

2013). After adding biochar, soil microcosms were adjusted to 60% water holding capacity (WHC) and conditioned with 100 mg NO₃⁻ kg⁻¹ dry soil (adding 0.44 mL of KNO₃ 1000 mg N L⁻¹ and 0.98 mL distilled-deionized water (NB-treated soil), and 1.13 mL distilled-deionized water (FB-treated soil)). These bottles were covered with a polyethylene plastic film to reduce evaporation while allowing air exchange, then incubated at 25°C, 30°C or 35°C for 21 days.

Before collecting gas samples, the incubation bottles were flushed by air for 30 seconds at a rate of 30 mL s⁻¹ and then closed by a butyl rubber septum for 3 h on days 1, 3, 5, 7, 14 and 21. Air pressure of these samples was measured by an air pressure gauge (Handy manometer, Copal Electronics) just before the gas collection. After each sampling, deionized water was added to maintain a constant soil water content by weight. Concentrations of N₂O and CO₂ were measured by gas chromatographs (GC-8A, Shimadzu, Japan) equipped with an electron capture detector (ECD) and a thermal conductivity detector (TCD), respectively, and gas emission rates (mg kg⁻¹ soil h⁻¹) were calculated by using the following equation:

$$V_i = \rho \times C \times (V_g + V_L \times \alpha) \times 273 / (W \times (273 + T)) / t \quad (1)$$

where V_i is N₂O or CO₂ emission (mg kg⁻¹ h⁻¹), t is closing time before collecting gas, ρ is the density of N₂O-N (1.25 kg m⁻³) or CO₂-C (0.5357 kg m⁻³), C is the N₂O or CO₂ concentration (ppm_v), V_g (m³) is the head volume, V_L (m³) is the volume of the liquid phase, α is the Bunsen absorption coefficient, W (kg) is the oven-dry weight of soil, and T is the temperature at determination.

The same soil microcosms were incubated in parallel to determine soil characteristics (pH, EC, DOC, DTN) and mineral N (NO₃⁻, NH₄⁺) on days 0, 7 and 21 of incubation.

The temperature sensitivity coefficient, Q_{10} , was calculated using this equation (Liang *et al.*, 2016):

$$Q_{10} = (R_2/R_1)^{10/(T_2-T_1)} \quad (2)$$

where T_2 and T_1 are the incubation temperatures (°C); R_1 and R_2 are the emission rates of T_1 and T_2 , respectively.

5.2.4. Soil and biochar analyses

All soil samples were subjected to measurement of pH and electricity conductivity (EC) at a soil: water ratio of 1:5 after shaking for 1 h at 175 rpm, using digital pH (F-23, Horiba, Japan) and EC meters (DS-14, Horiba, Japan), respectively. To measure pH and EC of NB and FB, a 1:10 ratio of a material to deionized water (weight/basis) was used. Total carbon (TC) and total nitrogen (TN) contents of soil were determined by the dry combustion method using a CN coder (MT-700, Yanaco, Japan). Dissolved organic carbon (DOC) and total extractable nitrogen (DTN) in the extracts (soil: water ratio of 1:10) filtered through 0.2 μm filter were measured with a TOC analyzer (TOC-L_{CSN}, Shimadzu, Japan). The soil mineral N was extracted with 2 M KCl (soil/water ratio of 1:10) and measured with a continuous flow analyzer (AutoAnalyzer QuAAtro 2-HR, Bltec, Japan) by colorimetric methods. Cation exchange capacity (CEC) of soil, NB and FB were determined by using the 1 M ammonium acetate extraction at pH 7.0.

5.2.5. Statistical analysis

Analysis of variance (ANOVA) with Tukey's HSD posthoc test was used to examine the significant differences ($p < 0.05$) in N_2O and CO_2 emissions, pH, EC, NO_3^- , NH_4^+ , DTN and DOC among treatments. A two-way ANOVA was used to analyze the effects of biochar, temperature and their interactions on soil properties, soil mineral N and N_2O and CO_2 emissions. Pearson's correlation was performed for cumulative N_2O as the main factor with dependences of CO_2 , pH, EC, NO_3^- , NH_4^+ , DTN and DOC among treatments after 21 incubation days. All statistical calculations were performed using SPSS software (SPSS 16.0).

5.3. Results

5.3.1. Soil properties

Initial pH values of CT, NB and FB were 7.8, 7.9 and 7.5, respectively. Addition of normal biochar did not significantly change pH or EC while addition of functional biochar reduced pH and increased EC (Table 5.2, $p < 0.05$) compared to the control. Initial NO_3^- and NH_4^+ were not significantly different in the FB while they were lower in the NB than the CT (Table 5.2, $p < 0.05$).

Table 5.2. Properties of initial soil amended with/without biochar.

Treatments	pH	EC ($\mu\text{S cm}^{-1}$)	NO_3^- (mg N kg^{-1})	NH_4^+ (mg N kg^{-1})
Soil (CT)	7.8 $\pm$ 0.1 ^a	116 $\pm$ 4 ^b	12.0 $\pm$ 0.4 ^a	2.6 $\pm$ 0.2 ^a
Soil mixed with 2% normal biochar (NB)	7.9 $\pm$ 0 ^a	131 $\pm$ 3 ^b	10.8 $\pm$ 0.4 ^a	2.4 $\pm$ 0.1 ^b
Soil mixed with 2% functional biochar (FB)	7.5 $\pm$ 0 ^b	504 $\pm$ 18 ^a	11.4 $\pm$ 0.3 ^{ab}	2.5 $\pm$ 0 ^{ab}

Data are means $\pm$ standard deviation. Different letters in each column denote significant differences ($p < 0.05$, $n = 3$) according to Tukey's HSD test.

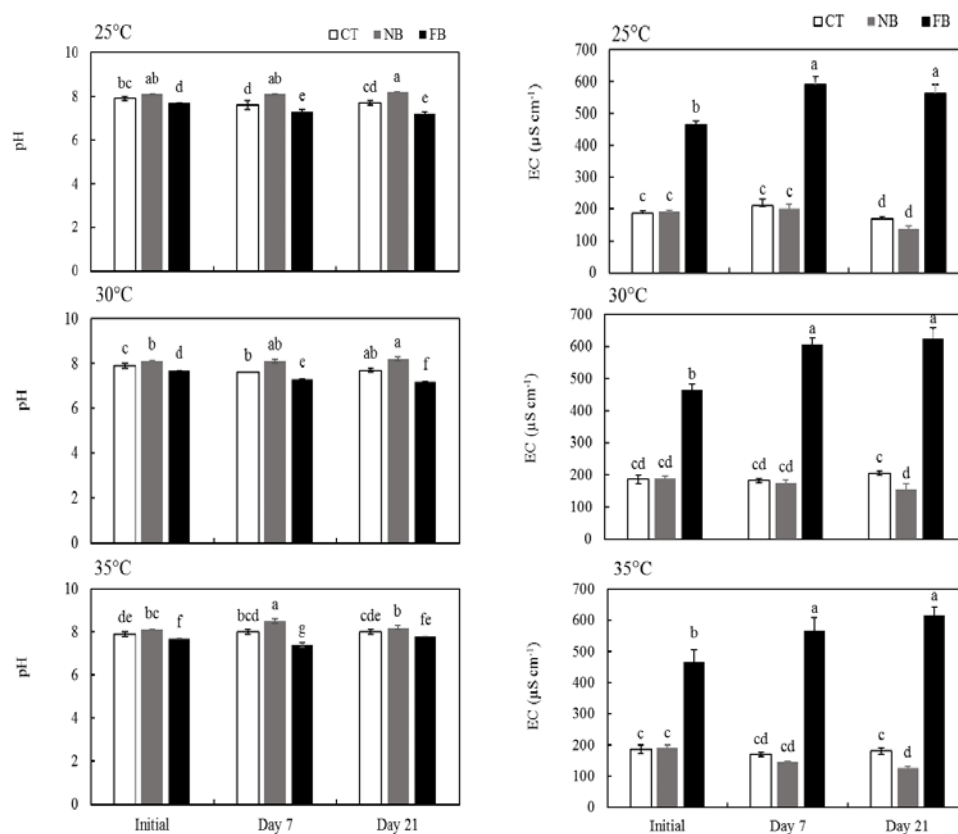


Figure 5.1. Soil pH (A) and EC (B) changes at different temperatures. Different letters (a, b, c, d, e and f) indicate significant differences on different days ($p < 0.05$). Bars indicate standard deviation ($n = 3$).

5.3.2. Changes in soil pH, EC, mineral N contents and DOC during incubation

The pH on day 21 was alkaline in all treatments and was higher in the NB treatment than in CT and FB treatments (Fig. 5.1a, $p < 0.05$). The functional biochar addition decreased soil pH by 0.2–1.1 units depending on temperature and increased soil EC (Fig. 5.1b, $p < 0.05$) while NB application had no effect on soil EC during the incubation period.

At the beginning of the experiment, 100 mg $\text{NO}_3^- \text{ kg}^{-1}$ dry soil was added to the soil samples. On day 7, NO_3^- contents in CT and FB treatments were not different from those prior to incubation while it was significantly decreased in the NB treatment. On day 21, the NO_3^- content continued to decrease to 42–59 mg kg^{-1} in the NB treatment (Table 5.3, $p < 0.05$) compared to the initial values. The NH_4^+ content in all treatments was reduced after 21 incubation days (Table 5.3, $p < 0.05$) compared to the initial.

In all treatments, DOC concentrations decreased and was the lowest in the FB treatment. Compared to the initial content, DTN decreased in the NB treatment, while it increased in the FB after 21 incubation days. No significant difference in DOC and DTN was observed at each temperature between prior to incubation and on day 21 (Table 5.3, $p < 0.05$).

5.3.3. N_2O and CO_2 emissions from different biochar materials

N_2O fluxes in all treatments were the highest on day 1 (Table 5.4), which is in agreement with the previous study (Harter *et al.*, 2014) and then decreased until the end of incubation (Fig. 5.2a, $p < 0.05$). The emissions of N_2O on day 1 were in the range of 2.1–7.0 mg N kg^{-1} from the NB, 3.6–10.2 mg N kg^{-1} from the FB and 4.4–28.6 mg N kg^{-1} from the CT (Table 5.4, $p < 0.05$). The emissions of N_2O on day 1 accounted for 35–49% of the total emission for 21 incubation days. Cumulative N_2O emissions from FB amended soil were higher than those from the NB regardless of temperature (Tables 5.4 & 5.5, $p < 0.05$). These outcomes were different from those predicted by the hypothesis because the low pH of FB as compared to NB could limit N_2 formation and therefore increased $\text{N}_2\text{O}/(\text{N}_2 + \text{N}_2\text{O})$ product ratios.

Table 5.3. Soil characteristics on day 21 of incubation at different temperatures.

		CT			NB			FB		
Prior to incubation	NO ₃ ⁻ (mg N kg ⁻¹)	104.7	±	2.2 ^a	109.4	±	3.3 ^a	109.0	±	6.1 ^a
	NH ₄ ⁺ (mg N kg ⁻¹)	3.0	±	0.1 ^a	2.3	±	0.2 ^b	2.4	±	0.3 ^b
	DOC (mg C kg ⁻¹)	69.7	±	3.7 ^a	70.4	±	5.9 ^a	53.7	±	7.5 ^b
	DTN (mg N kg ⁻¹)	103.8	±	3.5 ^a	108.4	±	2.0 ^a	38.8	±	3.1 ^b
25°C	NO ₃ ⁻ (mg N kg ⁻¹)	111.6	±	5.7 ^{aA}	59.3	±	2.1 ^{bA}	113.7	±	3.5 ^{aA}
	NH ₄ ⁺ (mg N kg ⁻¹)	0.9	±	0.1 ^{aA}	0.6	±	0.1 ^{bA}	0.5	±	0.1 ^{bA}
	DOC (mg C kg ⁻¹)	37.2	±	6.2 ^{aA}	48.9	±	6.2 ^{aA}	25.5	±	1.7 ^{bA}
	DTN (mg N kg ⁻¹)	114.9	±	4.4 ^{aA}	59.7	±	0.7 ^{bA}	53.9	±	4.2 ^{bA}
30°C	NO ₃ ⁻ (mg N kg ⁻¹)	109.2	±	2.0 ^{aA}	48.4	±	3.6 ^{bA}	107.7	±	7.3 ^{aA}
	NH ₄ ⁺ (mg N kg ⁻¹)	0.3	±	0.1 ^{bA}	0.2	±	0.1 ^{bA}	0.5	±	0.2 ^{aA}
	DOC (mg C kg ⁻¹)	31.0	±	3.1 ^{bA}	50.3	±	3.9 ^{aA}	29.0	±	2.1 ^{bA}
	DTN (mg N kg ⁻¹)	113.9	±	2.1 ^{aA}	43.9	±	1.6 ^{cA}	52.8	±	2.7 ^{bA}
35°C	NO ₃ ⁻ (mg N kg ⁻¹)	109.6	±	4.1 ^{aA}	42.3	±	0.8 ^{bA}	102.5	±	8.9 ^{aA}
	NH ₄ ⁺ (mg N kg ⁻¹)	0.5	±	0.1 ^{aA}	0.4	±	0.1 ^{abA}	0.3	±	0.1 ^{bA}
	DOC (mg C kg ⁻¹)	33.4	±	2.9 ^{aA}	40.6	±	7.6 ^{aA}	17.1	±	4.8 ^{bA}
	DTN (mg N kg ⁻¹)	115.8	±	4.0 ^{aA}	41.0	±	2.1 ^{cA}	51.0	±	1.1 ^{bA}

Data are means ± standard deviation. Different lowercase letters indicate difference among treatments. Different uppercase letters indicate difference among temperatures ($p < 0.05$, $n = 3$).

Table 5.4. N₂O and CO₂ fluxes on day 1 at three temperatures.

Temp.	N ₂ O (mg N kg ⁻¹ d ⁻¹)			CO ₂ (mg C kg ⁻¹ d ⁻¹)		
	CT	NB	FB	CT	NB	FB
25°C	6.5 ± 0.3 ^c	7.0 ± 0.3 ^c	10.2 ± 1.0 ^b	50.9 ± 1.4 ^{cd}	48.0 ± 3.9 ^{cd}	29.9 ± 11.9 ^d
30°C	4.4 ± 0.4 ^d	6.3 ± 0.1 ^c	6.9 ± 1.2 ^c	95.3 ± 3.7 ^b	63.6 ± 11.2 ^{bcd}	36.4 ± 9.5 ^d
35°C	28.6 ± 0.4 ^a	2.1 ± 0.2 ^e	3.6 ± 0.4 ^{de}	290.4 ± 23.5 ^a	76.6 ± 11.4 ^{bc}	60.3 ± 15.5 ^{cd}

Data are means ± standard deviation. Different letters indicate difference among treatments ($p < 0.05$, $n = 3$).

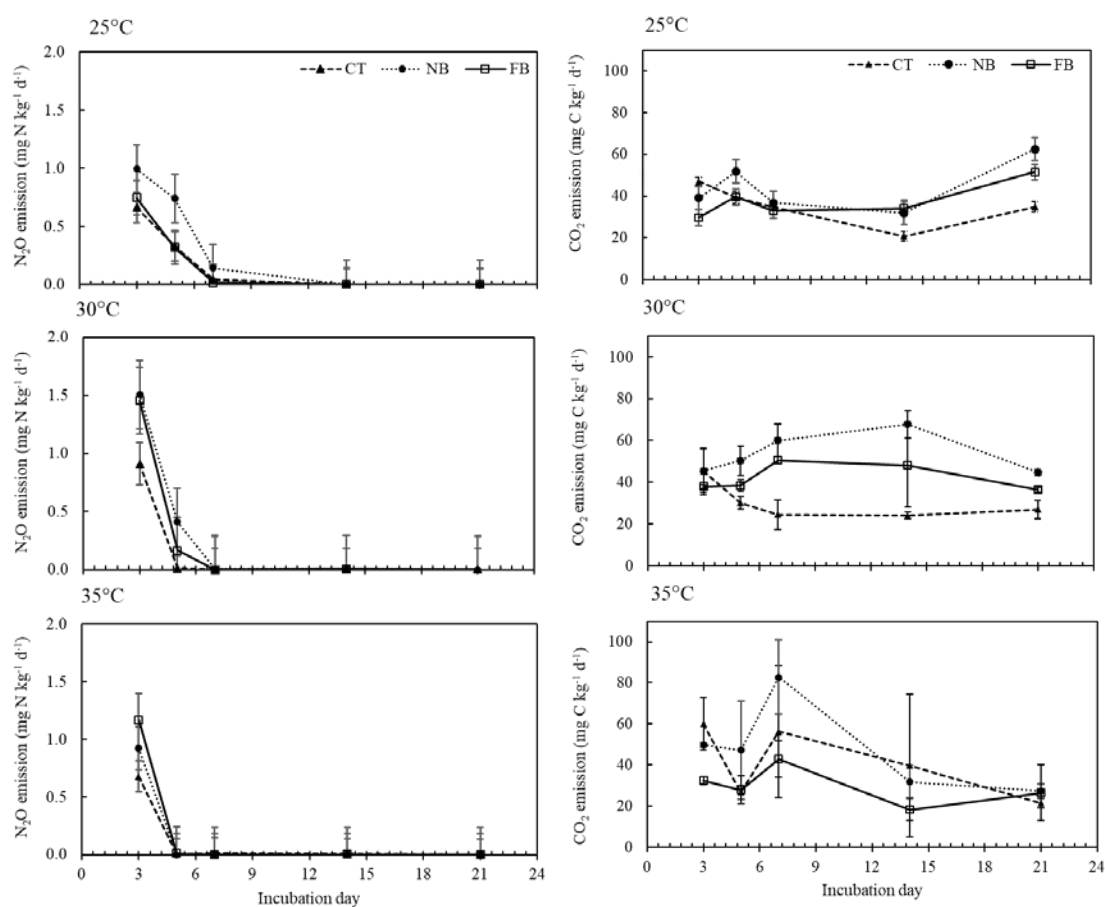


Figure 5.2. N₂O (a) and CO₂ (b) emissions in different treatments at each temperature from day 3 to day 21. Bars indicate standard deviation ($n = 3$).

Table 5.5. Cumulative N₂O and CO₂ emissions for 21 days of incubation.

Temp.	N ₂ O (mg N kg ⁻¹)			CO ₂ (mg C kg ⁻¹)		
	CT	NB	FB	CT	NB	FB
25°C	15.1 ± 1.0 ^c	18.1 ± 0.6 ^c	22.6 ± 1.8 ^b	696.8 ± 16.3 ^d	885.2 ± 134.4 ^{cd}	767.1 ± 12.9 ^{cd}
30°C	10.7 ± 0.7 ^d	16.4 ± 0.1 ^c	17.1 ± 2.5 ^c	713.2 ± 55.3 ^d	1219.5 ± 31.6 ^{ab}	916.6 ± 130.7 ^{bcd}
35°C	58.6 ± 0.7 ^a	6.1 ± 0.2 ^e	9.6 ± 0.6 ^d	1359.1 ± 240.9 ^a	1036.9 ± 88.9 ^{bc}	653.3 ± 86.2 ^d

Data are means ± standard deviation. Different letters indicate difference among treatments for each gas ($p < 0.05$, $n = 3$).

Fluxes of CO₂ were generally the largest on day 1 and continued until the end of the incubation.

CO₂ emissions from soil amended with FB and NB were respectively, 29.9–60.3 mg C kg⁻¹ and

48.0–76.6 mg C kg⁻¹ and from CT was 50.9–290.4 mg C kg⁻¹. In addition, FB had a neutral or negative effect on cumulative CO₂ emission as compared to NB after 21 incubation days (Table 5.5 and Fig. 5.2b, $p < 0.05$).

Different biochar materials had significant effects on both cumulative N₂O and CO₂ (Table 5.6, $p < 0.05$) but the N₂O and CO₂ responses to biochar materials were different. The FB had higher cumulative N₂O and lower cumulative CO₂ emissions than the NB.

5.3.4. High temperature effects on N₂O and CO₂ emissions

Amendment with biochar had negative or positive priming effects on cumulative N₂O and CO₂ fluxes as compared to the control, which was dependent on the temperature (Table 5.5, $p < 0.05$). At 25°C, N₂O fluxes were higher in the FB treatment than in NB and CT treatments. At 30°C, both biochar treatments had higher N₂O fluxes than the CT. At 35°C, biochar application reduced cumulative N₂O emission by 84% for FB and 90% for NB compared to the CT (Table 5.5, $p < 0.05$).

At 25°C, cumulative CO₂ emissions in all treatments were not significantly different but at 30°C, it was higher in the NB treatment than in the others. At 35°C, a significant reduction in cumulative CO₂ emissions was observed: 24% reduction for NB and 52% reduction for FB compared to the CT (Table 5.5, $p < 0.05$).

A two-way ANOVA showed that temperature had significant effects on both cumulative N₂O and CO₂ fluxes (Table 5.6, $p < 0.001$). When considering temperature effects on each amendment, there was a positive correlation between cumulative N₂O emission and temperature in the CT as it increased 10°C (Tables 5.5 & 5.6, $p < 0.05$), which is consistent with other studies (Cantarel *et al.*, 2011; Li *et al.*, 2016; Liang *et al.*, 2016; Zhang *et al.*, 2016a). On the contrary, a negative correlation between temperature and cumulative N₂O emission was observed in NB and FB treatments (Tables 5.5 & 5.6, $p < 0.05$). Cumulative CO₂ emission had a positive correlation as temperature of the CT increased 10°C, while there has no response of cumulative CO₂ emission to temperature change in NB and FB treatments (Tables 5.5 & 5.6, $p < 0.05$).

Table 5.6. Responses of N₂O and CO₂ emissions to temperature and biochar materials in a two-way ANOVA.

Factors	N ₂ O			CO ₂		
	<i>df</i>	<i>f</i>	<i>p</i>	<i>df</i>	<i>f</i>	<i>p</i>
Temperature (Temp)	2	166.8	< 0.0001	2	10.4	< 0.05
Biochar materials (Treatment)	2	391.2	< 0.0001	2	12.9	< 0.0001
Temp × Treatment	4	774.0	< 0.0001	4	17.3	< 0.0001

Table 5.7. *Q*₁₀ values of N₂O and CO₂ emissions at different temperatures.

N ₂ O	25–30°C	25–35°C
CT	0.50	3.89
NB	0.83	0.34
FB	0.58	0.42
CO ₂	25–30°C	25–35°C
CT	1.05	1.95
NB	1.90	1.17
FB	1.43	0.85

The *Q*₁₀ values of N₂O and CO₂ varied with the incubation temperature (Table 5.7). *Q*₁₀ values of CO₂ were higher than those of N₂O, except for the CT at 25–35°C. *Q*₁₀ values of N₂O and CO₂ of the biochar treatments at 25–30°C were higher than at 25–35°C. *Q*₁₀ values of CT at 25–35°C were the highest among the treatments.

5.4. Discussions

5.4.1. N₂O emissions from different biochar materials

Nitrous oxide emissions generally originate from denitrification or nitrification processes. The contribution of nitrification to N₂O emission under aerobic conditions is likely to be small (<0.2% for the majority of measurements) (Farquharson, 2016) and denitrification played a major role in N₂O production in the vegetable soil under the experimental conditions (Liu *et al.*, 2016). In addition, a low NH₄⁺ content compared to NO₃⁻ in all treatments indicated that N₂O emissions in this study were from denitrification. The main factors controlling denitrification and N₂O

production were pH and NO_3^- availability (Hermann Bothe *et al.*, 2006; Bremner and Shaw, 2009; Sun *et al.*, 2012). However, our results showed that only the NO_3^- content in the NB treatment was reduced with time (Table 5.3) and showed no change in the FB treatments (Table 5.3). N_2O emissions from NB and FB accounted for 6–17% and 9–21%, respectively, of the initial NO_3^- input, which was also highly correlated with increased soil pH after alkaline biochar application (NB) and NO_3^- reduction (Table 5.8, Fig. 5.1a), which are known to control denitrification in soil (Harter *et al.*, 2014).

High NO_3^- content in the FB-amended soil could be from nitrification via soil mineralization, converting NH_4^+ to NO_3^- (reduction of NH_4^+ content after 21 incubation days) and releasing N_2O and N_2 . The low pH of FB could limit the N_2 formation or may increase the N_2O product ratios (Cayuela *et al.*, 2013) ($\text{N}_2\text{O}/(\text{N}_2 + \text{N}_2\text{O})$), and explain the higher N_2O emissions from FB than from NB.

5.4.2. CO_2 emissions from different biochar materials

The lower CO_2 emissions in the FB treatment were probably due to the low DOC and TC contents in FB as compared to NB (Tables 5.1 & 5.3, $p < 0.05$). In addition, the effect of biochar addition on CO_2 emission was dependent on the characteristics of biochar, as observed by Spokas and Reicosky (2009). Different responses to soil CO_2 production from 16 different biochar materials (two biochars that suppressed CO_2 respiration, five chars that significantly stimulated CO_2 respiration compared to the soil controls and nine with no significant alteration) were shown although they were from the same feedstock and similar pyrolysis temperatures (Spokas and Reicosky, 2009). The role of biochar in CO_2 reduction was different depending on the temperature. At 25°C, there was no significant difference between the control and biochar-treated soils (Table 5.5, $p > 0.05$). At 30°C NB was a source of CO_2 emissions, and emitted CO_2 1.7 times greater than CT (Table 5.5, $p < 0.05$). However, at 35°C NB and FB were sinks of CO_2 , because they can absorb

CO₂ with reductions of 52% and 79%, respectively, compared with CT (Table 5.5, $p < 0.05$).

5.4.3. Effects of temperature on N₂O and CO₂ emissions

At 35°C, biochar addition reduced N₂O and CO₂ emissions while it had a neutral or positive effect at lower temperatures. The difference in temperature response between N₂O and CO₂ emissions may reflect the different rate of microbial processes mediating respiration and denitrification. In the laboratory, temperature is generally assumed to have an exponential relationship with the rate of soil N₂O emission below 30°C (Smith *et al.*, 1998). In addition, temperature effects may be complicated by accumulation of NO₂⁻ with the possibility of chemo-denitrification (Bailey, 1976), and repression of N₂ formation due to high NO₂⁻ or NO₃⁻ levels will reduce N₂O production. Keeney (1979) showed that the denitrification product (N₂O+N₂) at 25°C accounted for about 44% NO₃⁻ input, while at 40°C, nearly four times as much as gaseous N was evolved by 4 days at 25°C. Although, the N₂O/N₂ ratio has been shown to decrease with increasing temperature during denitrification (Keeney *et al.*, 1979), the higher N₂O emission in the soil without biochar amendment was not observed in the present study. In this study, we did not discover the mechanisms of increasing temperature effects on N₂O and CO₂ emissions with biochar amendment. Further study is required to identify simultaneous effects of biochar and temperature on N₂O and CO₂ emissions.

Once the temperature changed 10°C (25–35°C), N₂O emission from the CT was more sensitive to temperature change than from biochar-amended soil. However, little is known about the temperature sensitivity of N₂O emission from biochar-added soils. Most studies reported that N₂O emission is sensitive to temperature changes in different land use types and ecosystems. K.A. Smith *et al.*, (1998) indicated the order of N₂O emissions from different land use types and ecosystems: grazed grassland > grassland cut for conservation > potatoes > cereal crops and N₂O emissions from agroecosystems were generally higher than those from temperate natural ecosystems.

Table 5.8. Pearson's correlation for N₂O and CO₂ emissions and soil properties after 21 days of incubation in different treatments.

CT	N ₂ O	CO ₂	NO ₃ ⁻	NH ₄ ⁺	DOC	DTN	pH	EC
N ₂ O	-	0.930**	-0.117	0.323	-0.022	0.195	-0.080	-0.253
CO ₂	0.930**	-	-0.092	0.453	-0.184	0.351	0.051	-0.221
NO ₃ ⁻	-0.117	-0.092	-	0.240	0.119	0.827	-0.390	-0.163
NH ₄ ⁺	0.323	0.453	0.240	-	0.133	0.463	-0.538	-0.666
DOC	-0.022	-0.184	0.119	0.133	-	-0.007	-0.597	-0.360
DTN	0.195	0.351	0.827	0.463	-0.007	-	-0.203	-0.113
pH	-0.080	0.051	-0.390	-0.538	-0.597	-0.203	-	0.852
EC	-0.253	-0.221	-0.163	-0.666	-0.360	-0.113	0.852	-
NB	N ₂ O	CO ₂	NO ₃ ⁻	NH ₄ ⁺	DOC	DTN	pH	EC
N ₂ O	-	-0.063	0.811**	-0.358	0.624	0.703	0.571**	0.537
CO ₂	-0.063	-	-0.456	-0.470	0.159	-0.680	0.520	0.421
NO ₃ ⁻	0.811**	-0.456	-	0.028	0.400	0.910	0.081	0.152
NH ₄ ⁺	-0.358	-0.470	0.028	-	-0.400	0.190	-0.449	-0.580
DOC	0.624	0.159	0.400	-0.400	-	0.402	0.451	0.586
DTN	0.703	-0.680	0.910	0.190	0.402	-	-0.033	0.060
pH	0.571**	0.520	0.081	-0.449	0.451	-0.033	-	0.818
EC	0.537	0.421	0.152	-0.580	0.586	0.060	0.818	-
FB	N ₂ O	CO ₂	NO ₃ ⁻	NH ₄ ⁺	DOC	DTN	pH	EC
N ₂ O	-	0.316**	0.448	0.448	0.672	0.455	-0.838**	-0.418
CO ₂	0.316**	-	0.380	0.158	0.736	0.162	-0.744	0.112
NO ₃ ⁻	0.448	0.380	-	-0.322	0.459	0.075	-0.455	-0.696
NH ₄ ⁺	0.448	0.158	-0.322	-	0.306	-0.175	-0.413	0.169
DOC	0.672	0.736	0.459	0.306	-	0.182	-0.885	-0.107
DTN	0.455	0.162	0.075	-0.175	0.182	-	-0.401	-0.220
pH	-0.838**	-0.744	-0.455	-0.413	-0.885	-0.401	-	0.190
EC	-0.418	0.112	-0.696	0.169	-0.107	-0.220	0.190	-

**Significant at $p < 0.01$; *significant at $p < 0.05$, $n = 9$.

Their study also proved that the Q_{10} value for a sandy loam was 1.6 but ranged up to 12 for a clay loam soil at high water-filled pore space. The high Q_{10} values for N₂O emission related to the condition in which denitrification was likely to be the dominant mechanism (Smith *et al.*,

1998). The vegetable soil in this study was more sensitive to temperature change than the previous studies, whereas biochar treatments were less sensitive to temperature changes. The Q_{10} values of CO_2 at 5°C changes from 25°C to 30°C showed that CO_2 emissions from biochar-added soil were more sensitive to temperature change than the CT. However, when the temperature changes from 25°C to 35°C, CO_2 emission from the soil with biochar application is less sensitive to temperature change than the CT. The Q_{10} values in this study were lower than the previous study (Siriporn Wiriyatangsakul *et al.*, 2006).

5.5. Conclusions

The first objective was to determine the effects of different coffee waste biochar materials on the reduction of N_2O and CO_2 emissions and our results showed that application of normal biochar caused more CO_2 emission but less N_2O emission compared to functional biochar application. In N_2O and CO_2 response to temperature, amendment with two types of coffee waste biochar reduced N_2O and CO_2 emissions at 35°C compared to no biochar amendment. There was little difference between treatments with respect to N_2O and CO_2 emissions but the key factor responsible for this reduction at 35°C is unknown. It might be related to the soil-biochar mixture's immobilization of available soil N. Our findings showed that when increasing temperature, a positive correlation between temperature and N_2O and CO_2 emissions in the non-biochar amendment and a negative correlation between temperature and N_2O in the biochar treatments were observed. No correlation between CO_2 production and temperature was seen in the biochar treatments with increasing temperature.

This study did not examine the mechanism of biochar application effects on N_2O and CO_2 emissions when increasing temperature. Further studies need to be conducted to clarify the simultaneous mechanism of functional and normal biochar addition to soil on N_2O and CO_2 emissions at a specific temperature.

CHAPTER 6. GENERAL DISCUSSIONS AND CONCLUSIONS

6.1. Interactive effects of N input and temperature

Under aerobically incubation at 60% WHC, N₂O emissions from sandy loam soil were from autotrophic nitrification, nitrifier denitrification and/or coupled nitrification-denitrification. The contribution of the above-mentioned processes to the total N₂O emissions was known to be dependent on N input and temperature. Our results in the chapters 3 and 4 confirmed that the cumulative N₂O emissions from agricultural soil were significantly affected by N input sources, N application rates and temperature. In terms of temperature dependence, the cumulative N₂O emissions peaked at 25°C with different N input sources, (NH₄)₂SO₄ addition or different types of NH₄⁺-based compost (CC and SH). However, at higher temperatures, the lowest cumulative N₂O emissions were observed at 30°C with CC treatment, but at 35°C with SH treatment (chapter 4) and high NH₄⁺ (chapter 3). The lower soil pH in the SH treatments and high NH₄⁺ treatments could be the main reason for the same trend of temperature dependence of cumulative N₂O emissions from this sandy loam agricultural soil.

In addition, our findings reported that there has been an exponential relationship between the cumulative N₂O emissions from alkaline agricultural soil and increasing N application rates, from 30 to 120 mg NH₄-N kg⁻¹ as 1–4% CC treatment (chapter 4). With higher N content, the cumulative N₂O emissions did not increase exponentially with the increasing N application rates. The cumulative N₂O emissions peaked at 800 mg NH₄-N kg⁻¹ as (NH₄)₂SO₄ (chapter 3) and 2% SH (694 mg NH₄-N kg⁻¹) (chapter 4) and decreased to the lowest at 1200 mg NH₄-N kg⁻¹ as (NH₄)₂SO₄ (chapter 3) and 4% SH (1386 mg NH₄-N kg⁻¹) (chapter 4). It means that high NH₄-N application rates (> 2% SH or 800 mg NH₄-N kg⁻¹) would have limited N₂O emissions from this acidic agricultural soil. Parameters other than N availability would have reduced N₂O emissions. It is possible that N₂O was further reduced to N₂ or the interactive effects of higher temperature and the

highest N application rate would have enhanced the reaction from NO_2^- to NO_3^- , resulting in less NO_2^- and N_2O emissions (chapter 4).

The sandy loam soil in the experiments of chapters 3 and 4 was collected from the same field used for intensive vegetable cultivation in the Central Vietnam. However, characteristics of this soil were different depending on sampling time, especially, soil pH (alkaline, 7.8 in 2016 (chapter 4) and acidic, 6.3 in 2017 (chapter 3)) and higher soil NO_3^- in the 2017 than in the 2016. The different soil pH and soil NO_3^- would be the reasons for the different amount of NH_4^+ limiting N_2O emissions in the SH treatments and high $\text{NH}_4\text{-N}$ application rates.

6.2. Mitigation of N_2O and CO_2 emissions with biochar under temperature effects

Results obtained from Chapter 5 reported that increasing temperature from 25°C to 35°C, soil amendments with biochar decreased cumulative N_2O emissions. In addition, our findings showed that biochar addition could be an option for N_2O mitigation and a sink of CO_2 at the highest temperature, 35°C. However, at lower temperatures, amendment with biochar had neutral or positive effect on cumulative N_2O and CO_2 as compared to the control. It may be due to the different rate of microbial processes mediating respiration and denitrification, accumulation of NO_2^- and the soil-biochar mixture's mobilization of N availability.

Despite aerobically incubated at 60% WHC conditions, the predominant process occurred in the sandy loam soil added by biochar was denitrification, and nitrification and nitrification-derived N_2O was very small because of low NH_4^+ and high NO_3^- . It means that our findings were for the mitigation of N_2O and CO_2 emissions mostly from denitrification. It should be further studied to mitigate N_2O and CO_2 emissions from nitrification in the sandy loam agricultural soil.

6.3. Conclusions

Five-grams of air-dried sandy loam soil collected from a field used for intensive vegetable

cultivation during 2016–2017 were aerobically incubated for 28 days at 20°C, 25°C, 30°C and 35°C conditioned at 60% WHC with the addition of $(\text{NH}_4)_2\text{SO}_4$ or different types of NH_4^+ -based compost (CC and SH). Our study was designed to determine the effects of N input, N application rates and temperature on N_2O emissions from agricultural soil and their interactions. In addition, a mitigation study was carried out on this soil conditioned with $100 \text{ mg NO}_3^- \text{ kg}^{-1}$ at 60% WHC with the amendment of coffee waste biochar, normal biochar and high NO_3^- adsorption, functional biochar, to examine the mitigation of N_2O and CO_2 emissions at 5°C intervals between 25–35°C for 21 days.

Our findings showed that cumulative N_2O emissions from the sandy loam soil with NH_4^+ additions were from autotrophic nitrification, nitrifier denitrification and/or coupled nitrification-denitrification. The cumulative N_2O emissions from alkaline soil increased exponentially with the increasing N application rates, from 30 to $120 \text{ mg NH}_4^+ \text{ kg}^{-1}$. Higher NH_4^+ application rates limited cumulative N_2O emissions from both alkaline and acidic soil. The cumulative N_2O emissions peaked at $800 \text{ mg NH}_4^+ \text{ kg}^{-1}$ or 2% SH and then declined to $1200 \text{ mg N kg}^{-1}$ or 4% SH. In addition, N_2O emissions from alkaline agricultural soil were greater from compost treatments with less NH_4^+ and higher available carbon contents.

In terms of temperature dependence, the cumulative N_2O emissions from NH_4^+ -added soils for 28 days increased in the order of 35°C, 20°C, 30°C and 25°C. The cumulative N_2O emissions peaked at 25°C with different N input sources. Higher temperature decreased N_2O emissions from acidic soil with less NH_4^+ and higher available carbon. Especially, higher NH_4^+ application rates under higher temperature would have reduced N_2O emissions.

Biochar amendment had clear effects on mitigation of N_2O and CO_2 emissions from alkaline soil at the highest temperature. Normal biochar produced greater CO_2 and smaller N_2O emissions than the functional biochar did.

6.4. Implications for further study

Results obtained from a series of laboratory experiments provide a significant insight the effects of N input sources, N application rates and temperature on N₂O emissions from the sandy loam soil used for intensive vegetable cultivation. Our findings showed that cumulative N₂O emissions under aerobically incubation with high NH₄⁺ input were from autotrophic nitrification, nitrifier denitrification and/or coupled nitrification-denitrification. However, our designed experiments did not clarify the mechanisms related to the interactive effects of high NH₄⁺ and high temperature on N₂O production processes and their contributions to the total N₂O emissions. Microbial activities, nitrification-derived and denitrification-derived N₂O are a function of N input and temperature, and therefore should be further studied.

In addition, laboratory experiment related to biochar amendment was carried out under the conditions favorable for denitrification. Further studies should be designed for nitrification and nitrification-derived N₂O in the sandy loam soil.

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