NITROUS OXIDE AND CARBON DIOXIDE EMISSIONS FROM AGRICULTURAL SOIL AMENDED WITH DIFFERENT TYPES OF BIOCHAR AT THREE TEMPERATURES

Ngoc Tuong Van HOANG¹ and Morihiro MAEDA²*

¹Graduate School of Environmental and Life Science, Okayama University (3-1-1 Tsushima-Naka, Kita-Ku, Okayama 700-8530, Japan) E-mail: tuongvan1508@gmail.com
²Professor, Graduate School of Environmental and Life Science, Okayama University (3-1-1 Tsushima-Naka, Kita-Ku, Okayama 700-8530, Japan) *Corresponding author E-mail: mun@cc.okayama-u.ac.jp

A laboratory experimental study was conducted to investigate the effects of different coffee waste biochar materials at three temperatures on nitrous oxide (N₂O) and carbon dioxide (CO₂) emissions from agricultural soil in Central Vietnam. Soil amended with 2% normal biochar, 2% functional biochar (with a high NO₃-N adsorption capacity of 20 g N kg⁻¹) or no addition was adjusted at 60% water holding capacity, conditioned with 100 mg N-NO₃ kg⁻¹ dried-soil and aerobically incubated at 25°C, 30°C or 35°C for 21 days. N₂O and CO₂ emissions were measured on days 1, 3, 5, 7, 14 and 21. Results showed that the addition of normal biochar caused more CO₂ emission but less N₂O emission compared to the functional biochar application. At 25°C, biochar amendment had a neutral or positive effect on N₂O and CO₂ emissions. At 35°C, biochar amendment reduced N₂O and CO₂ emissions compared to the control. However, there was little difference in N₂O and CO₂ emissions among biochar treatments. In addition, with increasing temperature, a positive correlation between temperature and N₂O and CO₂ emissions with the non-biochar amendment and a negative correlation between temperature and N₂O with the biochar treatments were observed. This is because temperature probably affects the rate of microbial processes mediating respiration and denitrification and the soil-biochar mixture’s mobilization of N availability.

Key Words: carbon dioxide, functional biochar, nitrous oxide, normal biochar, temperature.

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 years¹¹. Agriculture is a source of N₂O, CO₂ and CH₄, and accounted for 10–12% CO₂-equivalent to total global greenhouse gas (GHG) emissions¹⁵. Intensive vegetable cropping systems often receive excess nitrogen (N) application and result in greater N₂O emissions and water quality deterioration¹⁰⁻¹².

Biochar is considered a useful soil amendment to improve soil properties, increase carbon sequestration and reduce GHG emissions from soil⁹. Amendment of soil with biochar reduced N₂O emissions by 54%⁷ and 76%⁸. However, there have been studies reporting no difference or even an increase in N₂O emissions after biochar application⁸⁹. 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 change⁹.

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 application¹³ and high temperature¹⁴. 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\(^\text{15}\). Soil respiration is also stimulated by a temperature increase. Panosso et al. (2011) showed that CO\(_2\) 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 CO\(_2\)\(^\text{14,16}\) and N\(_2\)O\(^\text{14}\) have not been well documented. Little is known about high temperature effects on N\(_2\)O and CO\(_2\) 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 NO\(_3\)-N, probably due to excess use of N fertilizer. Normal biochar and a biochar with high NO\(_3\)-N adsorption capacity, a functional biochar produced from coffee waste, were used as soil amendments to evaluate the ability to mitigate N\(_2\)O emissions from the Vietnamese agricultural soil. The functional biochar was reported to adsorb about 20 g N-NO\(_3\) kg\(^{-1}\)\(^\text{17}\) and we hypothesized that functional biochar produces less N\(_2\)O 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 N\(_2\)O and CO\(_2\) emissions and 2) investigate the response of N\(_2\)O and CO\(_2\) emissions from agricultural soil to different temperatures.

2. MATERIALS AND METHODS

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\(^{-1}\); NO\(_3\)-N, 41 mg N kg\(^{-1}\); NH\(_4\)-N, 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 cmolc kg\(^{-1}\).

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\(^\text{7}\). The functional biochar was reported to have a NO\(_3\) adsorption capacity of 20 g kg\(^{-1}\)\(^\text{17}\). The mean particle diameter and specific surface area of the functional biochar were 938 μm and 94.7 m\(^2\) g\(^{-1}\) on a dry-matter basis, respectively. The normal biochar was produced by burning the coffee grounds at 110°C and carbonizing at 600°C for 1 h\(^\text{17}\). Characteristics of the raw and biochar materials are presented in Table 1.

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\(^\text{18}\). After adding biochar, soil microcosms were adjusted to 60% water holding capacity (WHC) and conditioned with 100 mg N-NO\(_3\) kg\(^{-1}\) dry soil (adding 0.44 mL of KNO\(_3\) 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.

| Materials        | pH   | EC (μS cm\(^{-1}\)) | CEC (cmolc 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).
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 N\textsubscript{2}O and CO\textsubscript{2} 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\textsuperscript{-1} soil h\textsuperscript{-1}) were calculated by using the following equation:

\[ V_i = \rho \times C \times (V_i + V_i \times \alpha) \times 273/(W \times 273 + T)/t \]

where \( V_i \) is N\textsubscript{2}O or CO\textsubscript{2} emission (mg kg\textsuperscript{-1} h\textsuperscript{-1}), \( t \) is closing time before collecting gas, \( \rho \) is the density of N\textsubscript{2}O-N (1.25 kg m\textsuperscript{-3}) or CO\textsubscript{2}-C (0.5357 kg m\textsuperscript{-3}) at 25°C, \( C \) is the N\textsubscript{2}O or CO\textsubscript{2} concentration (ppm), \( V_i \) (m\textsuperscript{3}) is the head volume, \( V_L \) (m\textsuperscript{3}) is the volume of the liquid phase, \( \alpha \) 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 (NO\textsubscript{3}-N, NH\textsubscript{4}-N) on days 0, 7 and 21 of incubation.

The temperature sensitivity coefficient, \( Q_{10} \), was calculated using this equation:\n
\[ Q_{10} = (R_0/R_1)^{(10(T_2/T_1))} \]

where \( T_2 \) and \( T_1 \) are the incubation temperatures (°C); \( R_0 \) and \( R_1 \) are the emission rates of \( T_1 \) and \( T_2 \), respectively.

### 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 plus, 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.

### Statistical analysis

Analysis of variance (ANOVA) with Tukey’s HSD posthoc test was used to examine the significant differences (\( p < 0.05 \)) in N\textsubscript{2}O and CO\textsubscript{2} emissions, pH, EC, NO\textsubscript{3}-N, NH\textsubscript{4}-N, 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\textsubscript{2}O and CO\textsubscript{2} emissions. Pearson’s correlation was performed for cumulative N\textsubscript{2}O as the main factor with dependences of CO\textsubscript{2} emissions, pH, EC, NO\textsubscript{3}-N, NH\textsubscript{4}-N, DTN and DOC among treatments after 21 incubation days. All statistical calculations were performed using SPSS software (SPSS 16.0).

### 3. RESULTS

#### 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 2, \( p < 0.05 \)) compared to the control. Initial NO\textsubscript{3}-N and NH\textsubscript{4}-N were not significantly different in the FB while they were lower in the NB than the CT (Table 2, \( p < 0.05 \)).

\begin{table}[h]
\centering
\begin{tabular}{|l|c|c|c|}
\hline
Treatments & pH & EC & NO\textsubscript{3}-N & NH\textsubscript{4}-N \\
\hline
Soil (CT) & 7.8 ± 0.1 & 116 ± 4 & 12.0 ± 0.4 & 2.6 ± 0.2 \\
Soil mixed with 2% normal biochar (NB) & 7.9 ± 0 & 131 ± 3 & 10.8 ± 0.4 & 2.4 ± 0.1 \\
Soil mixed with 2% functional biochar (FB) & 7.5 ± 0 & 504 ± 18 & 11.4 ± 0.3 & 2.5 ± 0 \\
\hline
\end{tabular}
\caption{Properties of initial soil amended with/without biochar}
\end{table}
### Table 3

Soil characteristics on day 21 of incubation at different temperatures

|          | CT        | NB        | FB        |
|----------|-----------|-----------|-----------|
| **Prior to incubation** |           |           |           |
| NO3-N (mg N kg⁻¹) | 104.7 ± 2.2a | 109.4 ± 3.3a | 109.0 ± 6.1a |
| NH4-N (mg N kg⁻¹) | 3.0 ± 0.1a  | 2.3 ± 0.2b  | 2.4 ± 0.3b  |
| DOC (mg C kg⁻¹)  | 69.7 ± 3.7a | 70.4 ± 5.9a | 53.7 ± 7.5b |
| DTN (mg N kg⁻¹)  | 103.8 ± 3.5a | 108.4 ± 2.0a | 38.8 ± 3.1b |
| **25°C** |           |           |           |
| NO3-N (mg N kg⁻¹) | 111.6 ± 5.7aA | 59.3 ± 2.1bA | 113.7 ± 3.5aA |
| NH4-N (mg N kg⁻¹) | 0.9 ± 0.1aA | 0.6 ± 0.1bA | 0.5 ± 0.1bA |
| DOC (mg C kg⁻¹)  | 37.2 ± 6.2aA | 48.9 ± 6.2aA | 25.5 ± 1.7bA |
| DTN (mg N kg⁻¹)  | 114.9 ± 4.4aA | 59.7 ± 0.7bA | 53.9 ± 4.2bA |
| **30°C** |           |           |           |
| NO3-N (mg N kg⁻¹) | 109.2 ± 2.0aA | 48.4 ± 3.6bA | 107.7 ± 7.3aA |
| NH4-N (mg N kg⁻¹) | 0.3 ± 0.1bA | 0.2 ± 0.1bA | 0.5 ± 0.2aA |
| DOC (mg C kg⁻¹)  | 31.0 ± 3.1bA | 50.3 ± 3.9aA | 29.0 ± 2.1bA |
| DTN (mg N kg⁻¹)  | 113.9 ± 2.1aA | 43.9 ± 1.6bA | 52.8 ± 2.7bA |
| **35°C** |           |           |           |
| NO3-N (mg N kg⁻¹) | 109.6 ± 4.1aA | 42.3 ± 0.8bA | 102.5 ± 8.9aA |
| NH4-N (mg N kg⁻¹) | 0.5 ± 0.1aA | 0.4 ± 0.1abA | 0.3 ± 0.1bA |
| DOC (mg C kg⁻¹)  | 33.4 ± 2.9aA | 40.6 ± 7.6aA | 17.1 ± 4.8bA |
| DTN (mg N kg⁻¹)  | 115.8 ± 4.0aA | 41.0 ± 2.1bA | 51.0 ± 1.1bA |

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

### Fig. 1

Soil pH (A) and EC (B) changes at different temperatures (25°C, 30°C and 35°C)

Different letters (a, b, c, d, e and f) indicate significant differences on different days (p < 0.05). Bars indicate standard deviation (n = 3).
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. 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. 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 NO$_3$-N kg$^{-1}$ dry soil was added to the soil samples. On day 7, NO$_3$-N 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$_3$-N content continued to decrease to 42–59 mg kg$^{-1}$ in the NB treatment (Table 3, *p* < 0.05) compared to the initial values. The NH$_4$-N content in all treatments was reduced after 21 incubation days (Table 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 3, *p* < 0.05).

| Temperature | 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$^c$                   | 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).

Fig. 2 N$_2$O (a) and CO$_2$ (b) emissions in different treatments at each temperature from day 3 to day 21. Bars indicate standard deviation (*n* = 3).
NITROUS OXIDE AND CARBON DIOXIDE EMISSIONS FROM AGRICULTURAL SOIL AMENDED WITH DIFFERENT TYPES OF BIOCHAR AT THREE TEMPERATURES

Table 5 Cumulative N$_2$O and CO$_2$ 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$^d$ | 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$^{bd}$ |
| 35°C  | 58.6 ± 0.7$^a$ | 6.1 ± 0.2$^e$ | 9.6 ± 0.6$^d$ | 1359.1 ± 240.9$^b$ | 1036.9 ± 88.9$^{be}$ | 653.3 ± 86.2$^d$ |

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

N$_2$O and CO$_2$ emissions from different biochar materials

N$_2$O fluxes in all treatments were the highest on day 1 (Table 4), which is in agreement with the previous study$^{19}$ and then decreased until the end of incubation (Fig. 2a, $p < 0.05$). The emissions of N$_2$O 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 4, $p < 0.05$). The emissions of N$_2$O on day 1 accounted for 35–49% of the total emission for 21 incubation days. Cumulative N$_2$O emissions from FB amended soil were higher than those from the NB regardless of temperature (Tables 4 & 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 N$_2$O/(N$_2$ + N$_2$O) product ratios.

Fluxes of CO$_2$ were generally the largest on day 1 and continued until the end of the incubation. CO$_2$ 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 CO$_2$ emission as compared to NB after 21 incubation days (Table 5 and Fig. 2b, $p < 0.05$).

Different biochar materials had significant effects on both cumulative N$_2$O and CO$_2$ (Table 6, $p < 0.05$) but the N$_2$O and CO$_2$ responses to biochar materials were different. The FB had higher cumulative N$_2$O and lower cumulative CO$_2$ emissions than the NB.

High temperature effects on N$_2$O and CO$_2$ emissions

Amendment with biochar had negative or positive priming effects on cumulative N$_2$O and CO$_2$ fluxes as compared to the control, which was dependent on the temperature (Table 5, $p < 0.05$). At 25°C, N$_2$O fluxes were higher in the FB treatment than in NB and CT treatments. At 30°C, both biochar treatments had higher N$_2$O fluxes than the CT. At 35°C, biochar application reduced cumulative N$_2$O emission by 84% for FB and 90% for NB compared to the CT (Table 5, $p < 0.05$).

At 25°C, cumulative CO$_2$ 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 CO$_2$ emissions was observed: 24% reduction for NB and 52% reduction for FB compared to the CT (Table 5, $p < 0.05$).

A two-way ANOVA showed that temperature had significant effects on both cumulative N$_2$O and CO$_2$ fluxes (Table 6, $p < 0.001$). When considering temperature effects on each amendment, there was a positive correlation between cumulative N$_2$O emission and temperature in the CT as it increased 10°C (Tables 5 & 6, $p < 0.05$), which is consistent with other studies$^{20,21,22}$. On the contrary, a negative correlation between temperature and cumulative N$_2$O emission was observed in NB and FB treatments (Tables 5 & 6, $p < 0.05$). Cumulative CO$_2$ emission had a positive correlation as temperature of the CT increased 10°C, while there has no response of cumulative CO$_2$ emission to temperature change in NB and FB treatments (Tables 5 & 6, $p < 0.05$).

The $Q_{10}$ values of N$_2$O and CO$_2$ varied with the incubation temperature (Table 7). $Q_{10}$ values of CO$_2$ were higher than those of N$_2$O, except for the CT at 25–35°C. $Q_{10}$ values of N$_2$O and CO$_2$ 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.
4. DISCUSSIONS

**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) and denitrification played a major role in N₂O production in the vegetable soil under the experimental conditions. In addition, a low NH₄-N content compared to NO₃-N in all treatments indicated that N₂O emissions in this study were from denitrification. The main factors controlling denitrification and N₂O production were pH and NO₃-N availability. However, our results showed that only the NO₃-N content in the NB treatment was reduced with time (Table 3) and showed no change in the FB treatments (Table 3). N₂O emissions from NB and FB accounted for 6–17% and 9–21%, respectively, of the initial NO₃-N input, which was also highly correlated with increased soil pH after alkaline biochar application (NB) and NO₃-N reduction (Table 8, Fig. 1a), which are known to control denitrification in soil (Naik et al., 2013). High NO₃-N content in the FB-amended soil could be from nitrification via soil mineralization, converting NH₄-N to NO₃-N (reduction of NH₄-N content after 21 incubation days) and releasing N₂O and N₂. The low pH of FB could limit the N₂ formation or may increase the N₂O product ratios (N₂O/(N₂ + N₂O)), and explain the higher N₂O emission from FB than from NB.

**CO₂ emissions from different biochar materials**

The lower CO₂ emissions in the FB treatment were probably due to the low DOC and TC contents in FB as compared to NB (Tables 1 & 3, p < 0.05). In addition, the effect of biochar addition on CO₂ emission was dependent on the characteristics of biochar, as observed by Spokas and Reicosky (2009). Different responses to soil CO₂ production from 16 different biochar materials (two biochars that suppressed CO₂ respiration, five chars that

| Factors                        | N₂O      | CO₂       |
|--------------------------------|----------|-----------|
|                               | df | f    | p     | df | f    | p     |
| 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 |

**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 (Brown et al., 2008). In addition, temperature effects may be complicated by accumulation of NO₂ with the possibility of chemo-denitrification (Spokas and Rsonsky, 2009), and repression of N₂ formation due to high NO₂ or NO₃ levels will reduce N₂O production.

**Table 6** Responses of N₂O and CO₂ to temperature and biochar materials in a two-way ANOVA

**Table 7** $Q_{10}$ Values of N₂O and CO₂ emissions for 21 incubation days at different temperatures

|        | N₂O     | CO₂     |
|--------|---------|---------|
|        | 25–30°C | 25–35°C |
| CT     | 0.50    | 3.89    |
| NB     | 0.83    | 0.34    |
| FB     | 0.58    | 0.42    |
|        | 25–30°C | 25–35°C |
| CT     | 1.05    | 1.95    |
| NB     | 1.90    | 1.17    |
| FB     | 1.43    | 0.85    |
Keeney (1979) showed that the denitrification product (N₂O+N₂) at 25°C accounted for about 44% NO₃-N 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, 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. Their study also proved that the Q₁₀ 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 Q₁₀ values for N₂O emission related to the condition in which denitrification was likely to be the dominant mechanism. 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 Q₁₀ values of CO₂ at 5°C changes from 25°C to 30°C showed that CO₂ emissions

**Significant at p < 0.01; *significant at p < 0.05, n = 9.
from biochar-added soil were more sensitive to temperature change than the CT. However, when the temperature changes from 25°C to 35°C, CO₂ 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 [1].

5. CONCLUSIONS

The first objective was to determine the effects of different coffee waste biochar materials on the reduction of \(\text{N}_2\text{O}\) and \(\text{CO}_2\) emissions and our results showed that application of normal biochar caused more \(\text{CO}_2\) emission but less \(\text{N}_2\text{O}\) emission compared to functional biochar application. In \(\text{N}_2\text{O}\) and \(\text{CO}_2\) response to temperature, amendment with two types of coffee waste biochar reduced \(\text{N}_2\text{O}\) and \(\text{CO}_2\) emissions at 35°C compared to no biochar amendment. There was little difference between treatments with respect to \(\text{N}_2\text{O}\) and \(\text{CO}_2\) 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 \(\text{N}_2\text{O}\) and \(\text{CO}_2\) emissions in the non-biochar amendment and a negative correlation between temperature and \(\text{N}_2\text{O}\) in the biochar treatments were observed. No correlation between \(\text{CO}_2\) production and temperature was seen in the biochar treatments with increasing temperature.

This study did not examine the mechanism of biochar application effects on \(\text{N}_2\text{O}\) and \(\text{CO}_2\) emissions when increasing temperature. Further studies need to be conducted to clarify the simultaneous mechanism of functional and normal biochar addition to soil on \(\text{N}_2\text{O}\) and \(\text{CO}_2\) emissions at a specific temperature.

ACKNOWLEDGEMENTS

We would like to thank Satoshi Hayashi and Riei Yokoyama at Nissoku Corporation, Okayama, Japan for providing coffee waste biochar. We also thank Takuya Fujimura for his assistance in soil analysis.

REFERENCES

1) Metz B., Davidson O. R., Bosch P. R., Dave R. & Meyer L. A.: Climate Change 2007: Synthesis Report. Contribution of working groups I, II and III to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. 2007.
2) Smith, P., Metz B. & Davidson O. R.: Contribution of Working Group III to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. In: Climate Change Mitigation (eds B. Metz, O.R. Davidson, P.R. Bosch, R. Dave & L.A. Meyer). Cambridge University Press, pp. 498-540, 2007.
3) Khai N. M., Ha P. Q. & Öborn I.: Nutrient flows in small-scale peri-urban vegetable farming systems in Southeast Asia - A case study in Hanoi. Agriculture, Ecosystems & Environment, Vol. 122, pp. 192-202, 2007.
4) Wang H. J., Huang B., Shi X. Z. et al.: Major nutrient balances in small-scale vegetable farming systems in peri-urban areas in China. Nutrient Cycling in Agroecosystems, Vol. 81, pp. 203-218, 2008.
5) Zhang M., Chen Z. Z., Li Q. L., Fan C. H. & Xiong Z. Q.: Quantitative relationship between nitrous oxide emissions and nitrogen application rate for a typical intensive vegetable cropping system in Southeastern China. Clean - Soil, Air; Water, Vol. 44 (12), pp. 1725-1732, 2016.
6) Xie T., Sadasivam B. Y., Reddy K. R., Wang C. & Spokas K.: Review of the effects of biochar amendment on soil properties and carbon sequestration. Journal of Hazardous, Toxic, and Radioactive Waste, Vol. 20, pp. 1-14, 2016.
7) Cayuela M. L., van Zwieten L., Singh B. P., Jeffery S., Roig A. & Sánchez-Monedero M. A.: Biochar's role in mitigating soil nitrous oxide emissions: A review and meta-analysis. Agriculture, Ecosystems & Environment, Vol. 191, pp. 5-16, 2014.
8) Sánchez-García M., Roig A., Sánchez-Monedero M. A. & Cayuela M. L.: Biochar increases soil \(\text{N}_2\text{O}\) emissions produced by nitrification-mediated pathways. Frontiers in Environmental Science, Vol. 2, pp. 1-10, 2014.
9) Spokas K. A. & Reicosky D. C.: Impacts of sixteen different biochars on soil greenhouse gas production. Annals of Environmental Science, Vol. 3, pp. 179-193, 2009.
10) Yoo G. & Kang H.: Effects of biochar addition on greenhouse gas emissions and microbial responses in a short-term laboratory experiment. Journal of Environmental Quality, Vol. 41, pp. 1193-1202, 2012.
11) Zhang, H., Voroney R. P. & Price G. W.: Effects of biochar amendments on soil microbial biomass and activity. Journal of Environmental Quality, Vol. 43, pp. 2104-2114, 2014.
12) Li B., Bi Z. & Xiong Z.: Dynamic responses of nitrous oxide emission and nitrogen use efficiency to nitrogen and biochar amendment in an intensified vegetable field in southeastern China. GCB Bioenergy, Vol. 9, pp. 400-413, 2016.
13) García-Marco S., Ravella S. R., Chadwick D., Vallejo A., Gregory A. S. & Cárdenas L. M.: Ranking factors affecting emissions of GHG from incubated agricultural soils. European Journal of Soil Science, Vol. 65, pp. 573-583, 2014.
14) Liang L. L., Grantz D. A. & Jenerette G. D.: Multivariate regulation of soil \(\text{CO}_2\) and \(\text{N}_2\text{O}\) pulse emissions from agricultural soils. Global Change Biology, Vol. 22, pp. 1286-1298, 2016.
15) Benoit M., Garnier J. & Billen G.: Temperature dependence of nitrous oxide production of a luvisolic soil in batch experiments. Process Biochemistry, Vol. 50, pp. 79-85, 2015.
16) Signor D., Cerri C. E. P. & Conant R.: N₂O emissions due to nitrogen fertilizer applications in two regions of sugarcane cultivation in Brazil. Environmental Research Letters, Vol. 8, pp. 1-9, 2013.

17) Yokoyama J. T, Hayashi S., Nakanishi M. & J. T.: Nitrate nitrogen adsorption of the functional charcoal prepared from vegetable waste. Proceedings of International Symposium on EcoTopia Science 2007, pp. 161-165, 2007.

18) Shackley S., Sohi S., Ibarrola R. et al.: Biochar, tool for climate change mitigation and soil management. Geoenvironmenting responses to climate change: selected entries from the Encyclopedia of Sustainability Science and Technology, pp. 73-140, Springer, New York, 2013.

19) Harter J., Krause H.-M., Schuettler S. et al.: Linking N₂O emissions from biochar-amended soil to the structure and function of the N-cycling microbial community. International Society for Microbial Ecology, Vol. 8, pp. 660-674, 2014.

20) Cantarel A. A. M., Bloor J. M. G., Deltroyn N. & Soussana J. F.: Effects of climate change drivers on nitrous oxide fluxes in an upland temperate grassland. Ecosystems, Vol. 14, pp. 223-233, 2011.

21) Zhang J., Peng C., Zhu Q. et al.: Temperature sensitivity of soil carbon dioxide and nitrous oxide emissions in mountain forest and meadow ecosystems in China. Journal of Atmospheric Environment, Vol. 142, pp. 340-350, 2016.

22) Farquharson R.: Nitrification rates and associated nitrous oxide emissions from agricultural soils - a synopsis. Soil Research, Vol. 54, pp. 469-480, 2016.

23) Liu R., Hu H., Suter H. et al.: Nitrification is a primary driver of nitrous oxide production in laboratory microcosms from different land-use soils. Frontiers in Microbiology, Vol. 7, pp. 1-10, 2016.

24) Bremner J. M. & Shaw K.: Denitrification in soil. II. Factors affecting denitrification. Journal of Agricultural Science, Vol. 51, pp. 40-52, 2009.

25) Hermann Bothe, Stuart Ferguson & William E. N.: Biology of the Nitrogen Cycle: COST edition, Elsevier, The Netherlands, 2006.

26) Sun P., Zhuge Y., Zhang J. & Cai Z.: Soil pH was the main controlling factor of the denitrification rates and N₂/N₂O emission ratios in forest and grassland soils along the Northeast China Transect (NECT). Soil Science and Plant Nutrition, Vol. 58, pp. 517-525, 2012.

27) Cayuela M. L., Sánchez-Monedero M. A., Roig A., Hanley K., Enders A. & Lehmann J.: Biochar and denitrification in soils: when, how much and why does biochar reduce N₂O emissions? Scientific Reports, Vol. 3, pp. 1-7, 2013.

28) Smith K. A., Thomson P. E., Clayton H., McTaggart I. P. & Conen F.: Effects of temperature, water content and nitrogen fertilisation on emissions of nitrous oxide by soils. Atmospheric Environment, Vol. 32, pp. 3301-3309, 1998.

29) Bailey L. D.: Effects of temperature and root on denitrification in a soil. Canadian Journal of Soil Science, Vol. 56, pp. 79-87, 1976.

30) Keeney D. R., Fillery J. R. & Marx G. P.: Effect of temperature on the gaseous nitrogen products of denitrification in a silt loam soil. Soil Science Society of America Journal, Vol. 43, pp. 1124-1128, 1979.

31) Siriporn Wiriyatangsakul, Amnat Chidthaisong, Sudarat Tripetchkul & Limtong P.: Effects of moisture and temperature on respiration in tropical forest and agricultural soils. Kasetsart J. (Natural Science), Vol. 40, pp. 395-409, 2006.

(Received: April 20, 2017; Accepted: November 29, 2017)