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Returning Tea Pruning Residue and Its Biochar Had a Contrasting Effect on Soil N₂O and CO₂ Emissions from Tea Plantation Soil

Aung Zaw Oo ^{1,*} , Shigeto Sudo ^{1,*}, Khin Thuzar Win ², Akira Shibata ³, Tomohito Sano ⁴  and Yuhei Hirono ⁴

¹ Institute for Agro-Environmental Science (NIAES), National Agriculture and Food Research Organization (NARO), 3-1-3 Kannondai, Tsukuba, Ibaraki 305-8604, Japan

² Central Regional Agricultural Research Center, National Agriculture and Food Research Organization (NARO), 2-1-18 Kannondai, Tsukuba 305-8666, Japan; khinthuzarwin@gmail.com

³ Ritsumeikan University OIC Research Organization, 2-150 Iwakura-cho, Ibaraki, Osaka 567-8570, Japan; akira118@aria.ocn.ne.jp

⁴ Institute of Fruit Tree and Tea Science, National Agriculture and Food Research Organization (NARO), 2769 Kanaya-Shishidoi, Shimada, Shizuoka 428-8501, Japan; sanotomo@affrc.go.jp (T.S.); hirono@affrc.go.jp (Y.H.)

* Correspondence: aungzawoo@gmail.com or aungzawoo@affrc.go.jp (A.Z.O.); ssudo@affrc.go.jp (S.S.)

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Abstract: A laboratory incubation experiment is conducted for 90 days under controlled conditions where either pruning residue or its biochar is applied to determine which application generates the lowest amount of greenhouse gas from tea plantation soil. To study the effect of incorporation depth on soil N₂O and CO₂ emissions, experiment 1 is performed with three treatments: (1) control; (2) tea pruning residue; and (3) residue biochar mixed with soil from two different depths (0–5 cm and 0–10 cm layers). In experiment 2, only the 0–10 cm soil layer is used to study the effect of surface application of tea pruning residue or its biochar on soil N₂O and CO₂ emissions compared with the control. The results show that biochar significantly increases soil pH, total C and C/N ratio in both experiments. The addition of pruning residue significantly increases soil total C content, cumulative N₂O and CO₂ emissions after 90 days of incubation. Converting pruning residue to biochar and its application significantly decreases cumulative N₂O emission by 17.7% and 74.2% from the 0–5 cm and 0–10 cm soil layers, respectively, compared to their respective controls. However, biochar addition increases soil CO₂ emissions for both the soil layers in experiment 1. Surface application of biochar to soil significantly reduces both N₂O and CO₂ emissions compared to residue treatment and the control in experiment 2. Our results suggest that converting pruning residue to biochar and its addition to soil has the potential to mitigate soil N₂O emissions from tea plantation.

Keywords: residue management; greenhouse gas; mitigation; tea plantation

1. Introduction

Nitrous oxide (N₂O) is a potent greenhouse gas (GHG) and the single most important ozone depleting compound currently emitted to the atmosphere [1]. Atmospheric N₂O concentrations have increased by 19% since pre-industrial times, with an average increase of 0.77 ppbv yr⁻¹ for the period 2000–2009 [2]. Agricultural soil is the single largest source of global anthropogenic N₂O emissions, accounting for approximately 59% of anthropogenic emissions [3]. Agriculture is a sector with considerable mitigation potential [4], which could change the position of agriculture from the large emitter to a much smaller emitter or even a net sink with the greatest mitigation contribution originating from soil C sequestration [5]. N₂O emissions from agriculture can be tackled by improving

N fertilizer and manure management technology, practicing cover crops, reduced till or no till, and crop residue and biochar amendment.

Crop residues are a major component of biomass production in agriculture. Globally, 3.8 billion tonnes of crop residues are produced annually from cereal, sugar, legumes, tuber and oil crops [6]. As a common soil management practice, returning residues to soil can improve soil physical, chemical and biological properties [7], but also influence GHG emissions from soil to the atmosphere. Soil incorporation of crop residues results in the emissions of GHG mainly CO₂ and N₂O from soil [8].

N₂O emission factors for crop residues varied largely from 0.62% to 2.8%, indicating that there is a huge uncertainty of soil N₂O emissions following crop residue incorporation [9]. Studies have reported that returning crop residues may increase N₂O emissions [10,11]. However, other studies observed the opposite results [12,13]. The effect of crop residues incorporation on soil GHG fluxes is not clear and depends on the C/N ratio of the crop residues [10–14]. Although, returning crop residues by incorporation or surface residues retention on cropping soils offers the potential of soil C sequestration, it can be offset if crop residues amendment increases the emissions of GHG from soils.

In recent years, biochar production from crop residues and its application to soil has been proposed as novel approach to sequester atmospheric CO₂ in terrestrial ecosystems and reduce GHG emissions from soil [15]. Although there is a negative impact of biochar production emission including the fuel required to pick up the pruning residues and the reapplication of biochar, production emission can be compensated by C sequestration. The application of biochar to soils has been regarded as a promising new approach for GHG mitigation because biochar is highly resistant to degradation in the soil environment [16]. Studies have reported that biochar amendment affects C and N turnover by influencing microbial community structure and biomass [17], and hence alters CO₂ and N₂O emissions from soil [18]. Biochar addition has been reported to have positive, negative, or negligible effects on soil N₂O emissions [19–24]. This apparent inconsistency might be due to differences in biochar types and properties of the soils used. Consequently, biochar addition can also markedly affect soil CO₂ emission [16]. To date, no consensus exists on net impact of biochar amendment on N₂O and CO₂ emissions in agricultural soils.

Tea (*Camellia sinensis*), a leaf-harvested crop, is cultivated widely in Japan. Tea plants are pruned in October and the pruning residues are left between the rows and later incorporated into the soil by tillage together with applied N fertilizer. In some cases, surface application of N fertilizer is done without mixing with the soil. Many studies reported that N₂O emission rates in tea fields were much higher than those in other upland and paddy fields [15,25,26]. Akiyama et al. [27] reported that the mean fertilizer-induced emission factor of N₂O in tea fields was much higher as compared to other upland and paddy fields. Returning tea residue to soil after pruning might contribute to high N₂O emissions from tea plantation soil. Therefore, to understand the potential effect of returning tea pruning residue on soil GHG emissions, laboratory experiment was conducted to evaluate: (1) the effect of tea pruning residue or its biochar incorporation depth on soil N₂O and CO₂ emissions from two soil layers; and (2) the effect of surface application of pruning residue or its biochar on soil GHG emissions from tea plantation soil. We hypothesized that: (1) returning residue to soil may influence soil N₂O and CO₂ emissions; and (2) an alternative way of making biochar from pruning residue and its addition may be a potential way to reduce GHG emissions from tea plantation soil.

2. Materials and Methods

2.1. Soil and Biochar

The soil used in this study was collected from a tea plantation at the Institute of Fruit Tree and Tea Science, National Agriculture and Food Research Organization (NARO), Shizuoka, Japan (34°48' N, 138°08' E). The soil type is classified as red-yellow soil. Soil samples were collected at a depth of 0–5 and 0–10 cm from the multiple points of a selected field. The soil was thoroughly mixed, air-dried and sieved at 2 mm to obtain a composite sample for the incubation study. The soil is composed of

19% sand, 33% silt, and 48% clay and other soil physiochemical properties are shown in Table 1. In tea plantation, the width between rows was 0.3 m, and the width of the canopy of tea plants was 1.5 m. The N fertilizer application rate in tea plantation was 510 kg N ha⁻¹ yr⁻¹ with four split applications. Ammonium sulfate fertilizer was used as N source. The fertilizer was applied in the form of bands with widths of 0.3 m between the rows, which is the conventional practice in tea cultivation. Therefore, the side dressing rate of 63 kg N ha⁻¹ before soil sampling was equivalent to the application rate of 378 kg N ha⁻¹. Since soil samples were collected 33 days after side dressing of N fertilizer with the rate of 63 kg N ha⁻¹, no further addition of N fertilizer was done in this incubation study.

Table 1. Properties of soil and biochar.

Materials	Total C (g kg ⁻¹)	Total N (g kg ⁻¹)	C/N Ratio	NH ₄ ⁺ (mg kg ⁻¹)	NO ₃ ⁻ (mg kg ⁻¹)	pH (H ₂ O)	EC (μS cm ⁻¹)	Sand (%)	Silt (%)	Clay (%)
Soil	57.7	6.5	8.9	259.1	107.5	3.08	748	19	33	48
	Total C (g kg ⁻¹)	Total N (g kg ⁻¹)	C/N Ratio	pH (H ₂ O)	Surface area (BET) (m ² g ⁻¹)		EC (mS cm ⁻¹)			
Biochar	482.4	19.2	25.2	10.2	2.7		7.3			

Biochar was produced from pruning residue of tea plants. In tea plantation, autumn skiffing was done in October and pruning residues were left on between the rows. Pruning residue of tea plants was about 4 t ha⁻¹ in dry weight. Biochar was produced from carbonization of tea pruning residues under open fire using open burn kiln [24]. Pyrolysis temperature was approximately 500–600 °C and biochar yield was about 30% on a dry weight basis with this method. Biochar was air dried and ground to pass a 2-mm sieve. Properties of the biochar are shown in Table 1.

2.2. Experimental Design and Incubation Study

Two laboratory incubation experiments were conducted separately to evaluate the effect of pruning residue or its biochar on soil N₂O and CO₂ emissions from different soil mixing layers (Experiment 1) and to determine the effect of surface application of residue or its biochar (Experiment 2) on GHG emissions from tea plantation soil (Table 2). The soils used in both experiments were from the same location in the same field.

Table 2. Summary of two incubation experiments. (GHG: greenhouse gases; WFPS: water filled pore space).

Items	Experiment 1			Experiment 2		
Objective	To study the effect of incorporation depth on soil GHG emissions			To study the effect of surface application on soil GHG emissions		
Soil layer used	Two different depths: 0–5 cm and 0–10 cm soil layers			Only 0–10 cm soil layer		
Treatments	(1) Control	(2) Pruning residue	(3) Residue biochar	(1) Control	(2) Pruning residue	(3) Residue biochar
Incubation condition	80% WFPS and 25 °C			80% WFPS and 25 °C		

Experiment 1: Tea plantation soil with a depth of 0–5 and 0–10 cm was used. For each treatment, 75 g dry soil was added to a polypropylene jar (750 mL). The soil moisture was adjusted to 80% water filled pore space (WFPS) by carefully spraying deionized water on to the soil. Then, the soils were pre-incubated at 25 °C and 80% WFPS for 7 days in an incubator in the dark to revive soil microbial activity. The treatments were: (1) control; (2) pruning residue 4 g kg⁻¹ soil; and (3) residue biochar 4%. The residue application rate was based on the amount of crop residue after tea pruning. Tea pruning residue contained total N 29.8 g kg⁻¹ and total C 463.6 g kg⁻¹ with C/N ratio of 15.6. Under actual field conditions, application was done between the rows and complete mixing of soil and residue or biochar was not possible. Therefore, residue and biochar were lightly mixed with different soil depths of 0–5 cm and 0–10 cm soil layers in this study. Separate sets of treatments were prepared to analyze for soil pH.

Experiment 2: Tea plantation soil with a depth of 0–10 cm was used to investigate the effect of surface application of pruning residue or its biochar on N₂O and CO₂ emissions. A total of 75 g of air-dried soil was added to a polypropylene jar (750 mL) and pre-incubated in the dark at 25 °C and 80% WFPS. After pre-incubation, pruning residue 4 g kg⁻¹ soil or biochar 4% was spread on the soil surface uniformly.

Both experiments were laid out in a completely randomized design with three replications. No additional N fertilizer was applied in both experiments since soil used in this study was collected 33 days after N fertilizer application in the field. The jars were incubated aerobically for 90 days at a constant temperature of 25 °C in an incubator. To prevent moisture loss, aluminum sheets were placed over the top of each jar, and pinholes were pierced to allow gas exchange. The moisture content of the soil was maintained at 80% WFPS throughout the experiment by weighing the jars twice a week and adding deionized water if needed.

2.3. Gas Sampling and Analysis

The air samples were collected on 0, 1, 2, 3, 4, 7, 11, 14, 18, 25, 28, 32, 36, 39, 43, 47, 50, 57, 63, 68, 75, 83 and 90 days of incubation. Before sampling, the jars were thoroughly flushed with ambient air and left opened for approximately 30 min to equilibrate with the atmosphere [23]. The jars were then sealed for 30 min using lids that had a rubber septum for gas sampling. Gas samples were drawn from the incubation jar using 50-mL syringe and then transferred to 15 mL vacuum glass vials with butylene rubber stoppers. The concentration of N₂O and CO₂ were analyzed using an automated analysis system for three gases of CO₂, CH₄, and N₂O. This system consists of two gas chromatographs (GC 2014, Shimadzu Corporation, Kyoto, Japan), of which one has both a thermal conductivity detector (TCD) and a flame ionization detector (FID), and the other has an electron capture detector (ECD). This system can analyze 80 samples consecutively with a modified automated headspace sampler (HSS-2B, Shimadzu, Kyoto, Japan) [28]. The difference in gas concentrations between the atmosphere and samples was used to determine the total emissions. The cumulative gas emission from each jar was calculated by integrating emissions over the 90 days of incubation.

2.4. Soil Analysis

At the end of incubation, soil total N and total C contents were analyzed by using a NC analyzer (Sumigraph NC-80; Sumika Chemical Analysis Service Co., Tokyo, Japan). Soil pH was measured in the supernatant suspension of 1:5 soil: H₂O solution using a pH meter (FiveEasy, FE20, Mettler Toledo, Tokyo, Japan).

2.5. Statistical Analysis

The effect of pruning residue and its biochar applications on soil properties and cumulative gas emissions were tested by the analysis of variance (ANOVA) using CropStat 7.2 statistical software program. The treatment mean comparisons at 5% level of probability by Tukey's HSD test and principle component analysis were done using XLSTAT Version 2016 (Addinsoft Company, New York, NY, USA).

3. Results

3.1. N₂O Emissions

The initial soil N₂O emission before treatment application ranged from 0.7 to 2.2 µg N kg⁻¹ h⁻¹ in both experiments (Figure 1). In all the treatments, high N₂O emission fluxes were observed on Day 1 of incubation and then decreased immediately upon continued incubation in both experiments. Pruning residue amendment showed the highest emission peak followed by the control and the lowest peak was observed in biochar amendment.

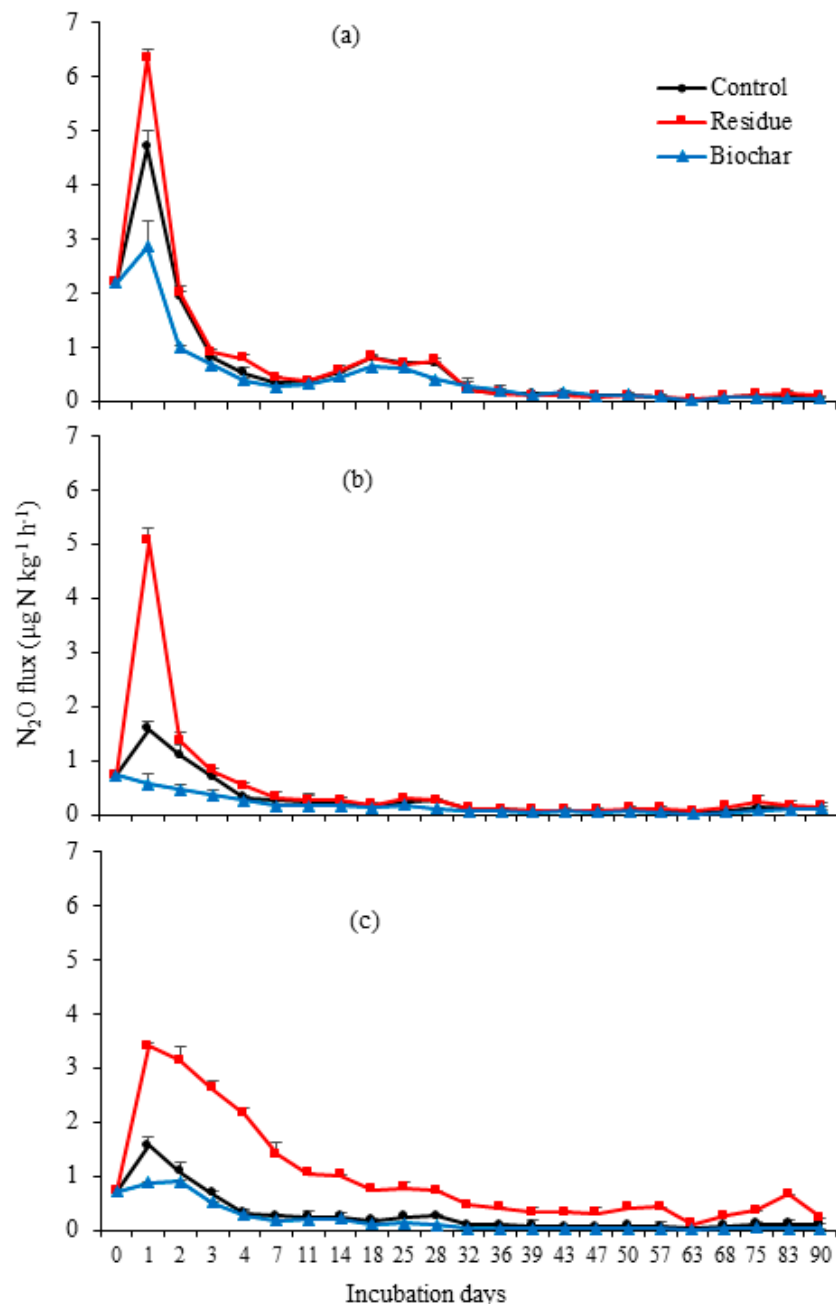


Figure 1. Soil N₂O emissions during 90 days incubation period: (a) 0–5 cm soil layer; (b) 0–10 cm soil layer; and (c) surface application. Error bars indicate standard deviation ($n = 3$).

After 90 days of incubation, soil N₂O emission from different soil layers were significantly ($p < 0.01$) affected by pruning residue amendment in experiment 1 (Figure 2). The mean cumulative soil N₂O emissions for residue and control treatments were 1.49 and 1.27 mg N kg⁻¹ soil for the 0–5 cm soil layer, and 1.06 and 0.61 mg N kg⁻¹ soil for the 0–10 cm soil layer, respectively. Cumulative N₂O emission from residue amendment significantly ($p < 0.01$) increased by 18% and 74% for the 0–5 cm and 0–10 cm soil layer, respectively, compared to their respective controls.

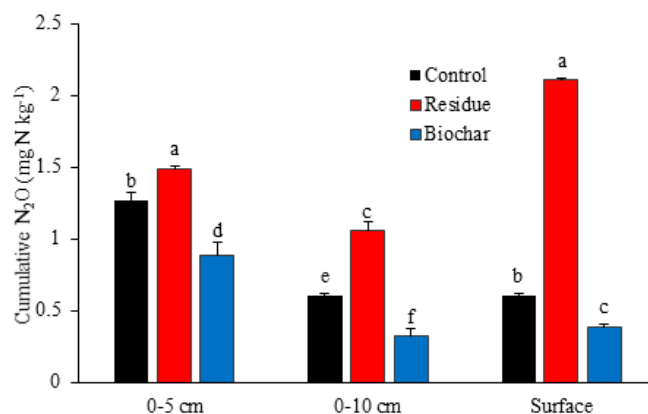


Figure 2. Cumulative N₂O emissions after 90 days of incubation. Error bars indicate standard deviation ($n = 3$). The same letter above or within bars for the 0–5 and 0–10 cm soil layers, and above the bars for surface application indicates that bar mean values are not significantly different at the 5% level by Tukey’s HSD test.

However, the addition of residue biochar significantly ($p < 0.01$) decreased N₂O emission from both the soil layers compared with residue and control treatments in experiment 1 (Figure 2). The mean cumulative soil N₂O emission for biochar treatment was 0.89 mg N kg⁻¹ soil for the 0–5 cm layer and 0.33 mg N kg⁻¹ soil for the 0–10 cm soil layer. The rate of emission from biochar amendment significantly ($p < 0.01$) declined by 17.7% for the 0–5 cm soil layer and 74.2% for the 0–10 cm soil layer compared to their respective controls. When comparing soil N₂O emissions from two different soil layers, top soil layer (0–5 cm) under control or amended with treatments resulted high cumulative emissions compared to those of the 0–10 cm soil layer.

Surface application of pruning residue and its biochar showed an opposite effect on cumulative N₂O emission after 90 days of incubation (Figure 2). The mean cumulative soil N₂O emissions for the control, residue, and biochar were 0.61, 2.11, and 0.39 mg N kg⁻¹ soil, respectively. Surface application of pruning residue significantly ($p < 0.05$) increased N₂O emission by 247% compared to the control. Biochar from pruning residue and its surface application significantly ($p < 0.05$) declined soil N₂O emission by 35.8% compared to the control. When comparing the methods of treatment application to the same soil layer (0–10 cm layer), surface application of residue showed relatively high soil cumulative N₂O emission compared to incorporation of treatment. However, surface application of biochar reduced soil N₂O emission compared to biochar incorporation.

3.2. CO₂ Emissions

The initial soil CO₂ emission before treatment application ranged from 4.3 to 5.1 mg C kg⁻¹ h⁻¹ in both experiments (Figure 3). In all treatments, the highest CO₂ fluxes occurred within the first seven days of incubation and then there were gradual decline in CO₂ fluxes over time in both experiments. Among the treatments, the highest emission peak was observed in residue treatment followed by the biochar and the lowest emission peak was observed in the control. After 90 days of incubation, soil CO₂ emissions from different soil layers were significantly ($p < 0.01$) affected by residue amendment in experiment 1 (Figure 4). The mean cumulative CO₂ emissions for residue and control treatments at the end of the incubation period were 13,947 and 10,931 mg C kg⁻¹ soil for the 0–5 cm soil layer, and 10,363 and 6001 mg N kg⁻¹ soil for the 0–10 cm soil layer, respectively. Cumulative CO₂ emissions increased by 28% and 72% for the 0–5 cm and 0–10 cm soil layer, respectively, compared to their respective controls. Biochar amendment also showed significant ($p < 0.01$) increase in CO₂ emissions from both the soil layers in experiment 1 (Figure 4). The mean cumulative CO₂ emission from biochar were 11,710 mg C kg⁻¹ soil for the 0–5 soil layer and 7136 mg C kg⁻¹ soil for the 0–10 cm soil layer. The cumulative CO₂ emissions from biochar amendment increased by 7% and 19% for the 0–5 cm

and 0–10 cm soil layer, respectively, compared to their respective controls. When comparing soil CO₂ emissions from two different soil layers, top soil layer (0–5 cm) under the control or amended with treatments resulted high cumulative emission compared to those of the 0–10 cm soil layer.

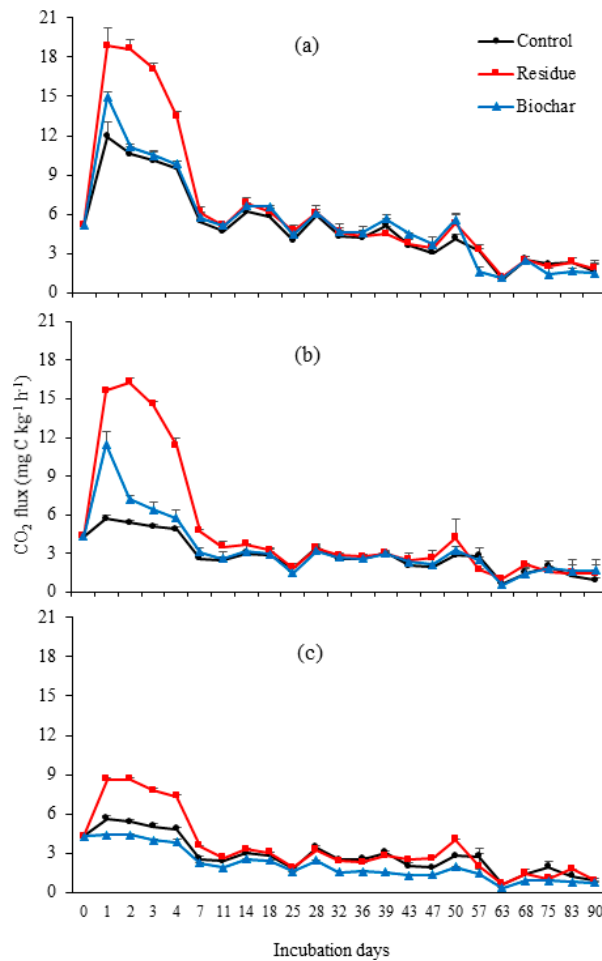


Figure 3. Soil CO₂ emissions during 90 days incubation period: (a) 0–5 cm soil layer; (b) 0–10 cm soil layer; and (c) surface application. Error bars indicate standard deviation (*n* = 3).

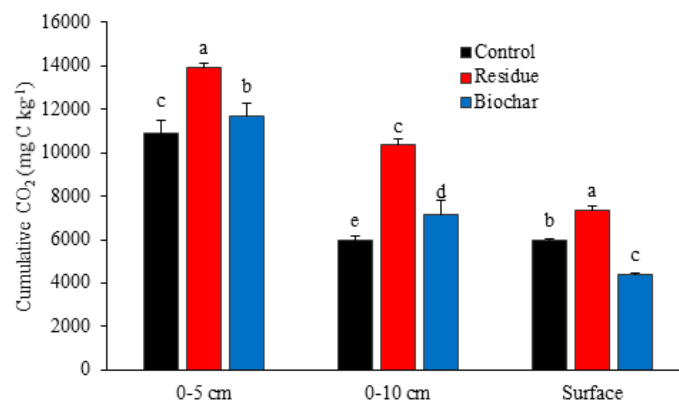


Figure 4. Cumulative CO₂ emissions after 90 days of incubation. Error bars indicate stand deviation (*n* = 3). The same letter above or within bars for the 0–5 and 0–10 cm soil layers, and above the bars for surface application indicates that bar mean values are not significantly different at the 5% level by Tukey’s HSD test.

After 90 days of incubation, soil CO₂ emission was significantly ($p < 0.05$) affected by surface application of pruning residue and its biochar (Figure 4). The mean cumulative soil CO₂ emissions for the control, residue, and biochar were 6001, 7361 and 4399 mg C kg⁻¹ soil, respectively. Cumulative CO₂ emission from surface application of pruning residue significantly ($p < 0.05$) increased by 23% compared to the control. Conversely, surface application of biochar significantly ($p < 0.05$) declined soil CO₂ emission by 60% compared to the control. When comparing the methods of treatment application to the same soil layer (0–10 cm layer), surface application of either residue or biochar showed low soil cumulative CO₂ emissions compared to incorporation of treatment.

3.3. Soil Properties

The initial soil pH before treatment application was 4.5 for the 0–5 cm soil layer and 3.4 for the 0–10 cm soil layer (Figure 5). Increase in soil pH was observed on Day 4 of incubation in all treatments for both soil layers and then no further significant changes in soil pH occurred but with some fluctuation until the end of the incubation period. Biochar amendment showed the highest soil pH throughout the incubation period for both the soil layers. After 90 days of incubation, soil pH for the control, pruning residue, and biochar were 4.56, 4.60, and 4.73 for the 0–5 cm soil layer and 3.55, 3.58, and 4.03 for the 0–10 cm soil layer, respectively. Soil pH increased by 0.17 and 0.48 units for the 0–5 cm and 0–10 cm soil layer, respectively, following biochar amendment. Soil pH increased by pruning residue was relatively small at the end of incubation for both the soil layers.

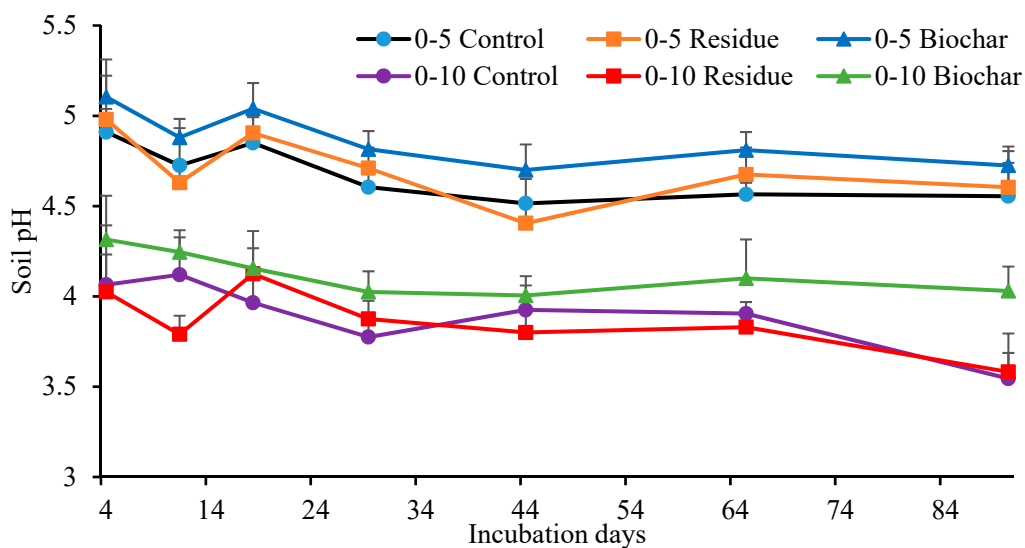


Figure 5. Changes in soil pH during 90 days incubation period. Error bar indicates standard deviation ($n = 3$). 0–5, the 0–5 cm soil layer; 0–10, the 0–10 cm soil layer.

After 90 days of incubation, there was no significant ($p > 0.05$) difference in soil total N content by either residue or biochar application for both the soil layers in experiment 1 (Figure 6a). The mean soil total N contents for the control, residue, and biochar were 26.1, 25.5, and 24.9 g kg⁻¹ soil for the 0–5 cm soil layer, and 15.6, 15.7, and 17.4 g kg⁻¹ soil for the 0–10 cm soil layer, respectively. Soil total N content was also not affected ($p > 0.05$) by surface application of pruning residue and its biochar in experiment 2 (Figure 6a). The relative high soil N content was observed in residue amendment (17.9 g kg⁻¹ soil) followed by biochar (16.8 g kg⁻¹ soil) and the lowest was observed in the control (15.5 g kg⁻¹ soil).

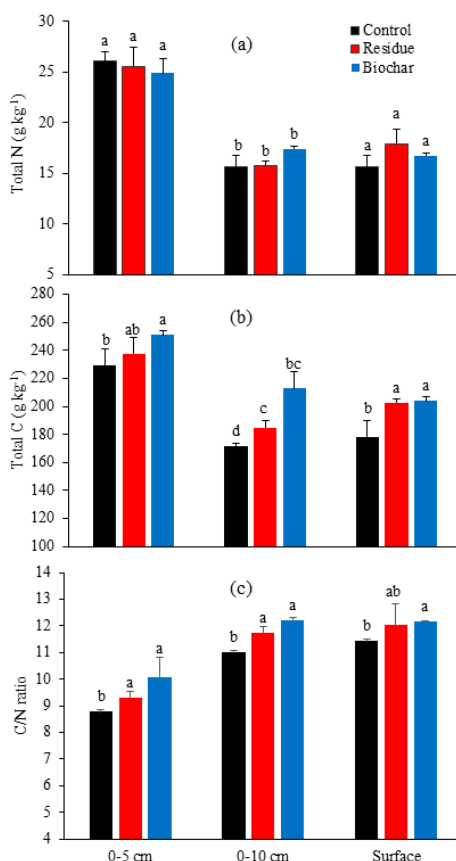


Figure 6. (a) Soil total N; (b) total C; and (c) C/N ratio after 90 days of incubation. Error bars indicate standard deviation ($n = 3$). The same letter above or within bars for the 0–5 and 0–10 cm soil layers, and above the bars for surface application indicates that bar mean values are not significantly different at the 5% level by Tukey's HSD test.

After 90 days of incubation, soil total C content was significantly ($p < 0.05$) affected by residue amendment in experiment 1 (Figure 6b). The mean soil total C contents from residue and control at the end of the incubation period were 237.7 and 229.2 g kg⁻¹ soil for the 0–5 cm soil layer, and 184.7 and 171.6 g kg⁻¹ soil for the 0–10 cm soil layer, respectively. Residue amendment increased soil total C content by 4% and 8% for the 0–5 cm and 0–10 cm soil layer, respectively, compared to their respective controls. Biochar amendment significantly ($p < 0.01$) increased soil total C content after 90 days of incubation in experiment 1 (Figure 6b). The mean soil total C contents for biochar and control were 250.9 and 229.2 g kg⁻¹ soil for the 0–5 cm soil layer, and 212.7 and 171.6 g kg⁻¹ soil for the 0–10 cm soil layer, respectively. Biochar amendment increased soil total C content by 9.5% and 23.9% for the 0–5 cm and 0–10 cm soil layer, respectively, compared to their respective controls. Soil total C content was also significantly ($p < 0.05$) affected by surface application of pruning residue and its biochar after 90 days of incubation in experiment 2 (Figure 6b). The mean soil total C contents for the control, residue, and biochar were 178.3, 202.7, and 203.7 g kg⁻¹ soil, respectively. Surface application of residue and its biochar increased soil total C content by 13.7% and 14.3%, respectively, compared to the control.

After 90 days of incubation, soil C/N ratio was significantly ($p < 0.05$) affected by residue and its biochar amendment in experiment 1 (Figure 6c). Residue amendment increased soil C/N ratio by 6.9% and 7.3% for the 0–5 cm and 0–10 cm soil layer, respectively. Biochar also increased soil C/N ratio by 16.1% and 11.9% for the 0–5 cm and 0–10 cm soil layer, respectively, compared to the control. In experiment 2, surface application of residue and its biochar significantly ($p < 0.05$) increased soil C/N ratio by 5.2% and 7.0%, respectively, compared to the control.

3.4. Correlation of Cumulative Gas Emissions and Soil Properties

A principal component analysis (PCA) was performed for both experiments to explore the soil properties that related with GHG emissions (Figure 7). A vector represented each variable, and the length of each vector indicated the strength of its contribution. The relative importance of each variable can be estimated from the perpendicular projection of each sample to its respective vector. The two main axes (F1 and F2) indicated the total variance of the data explained in the PCA. The first principal component explained about 51.9%, 63.6% and 57.1% of the observed variations, while the second component accounted for 24.5%, 26.5% and 32.9% for the 0–5 cm soil layer (Figure 7a), 0–10 cm soil layer (Figure 7b), and surface application (Figure 7c), respectively. In experiment 1, soil N_2O emission was inversely related with soil pH and C/N ratio for the 0–5 cm soil layer and with soil pH, total N and C/N ratio for the 0–10 cm soil layer (Figure 7a,b). In experiment 2, soil N_2O emission was also inversely related with soil pH and C/N ratio (Figure 7c).

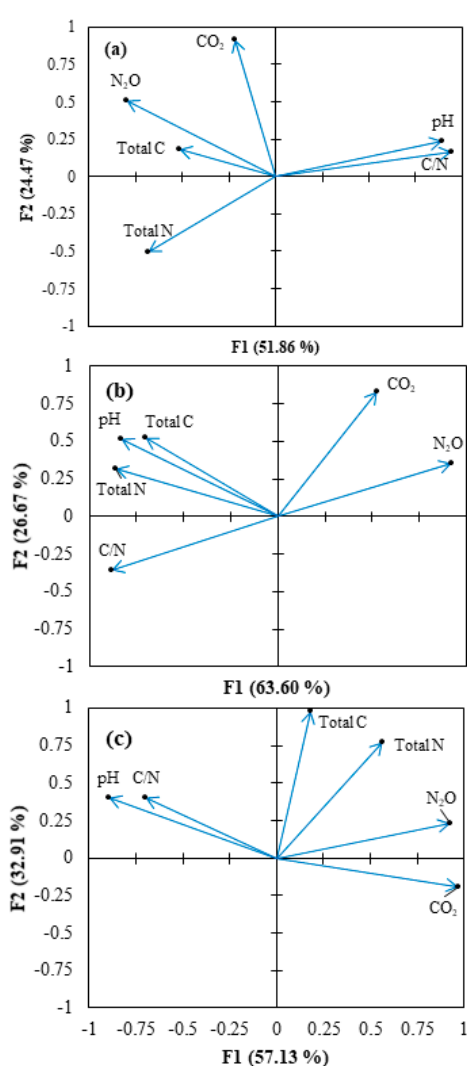


Figure 7. Correlation triplot based on a principle component analysis (PCA) using cumulative emissions and soil properties; a PC2 versus PC1 loadings for the included variables: (a) 0–5 cm soil layer; (b) 0–10 cm soil layer; and (c) surface application. Arrows indicate vectors for both gas emissions and soil properties, with longer arrows indicating higher influence for those parameters. The direction of an arrow indicates the steepest increase in the variable and the length indicates the strength relative to the other variables.

4. Discussion

4.1. N₂O Emissions

Returning crop residue is a common practice in agricultural system that consequently influences soil N₂O emissions [29]. In this study, cumulative N₂O emissions were higher in tea pruning residue amended soil compared to the control for both the soil layers in experiment 1 (Figure 2). This result was in accordance with previous studies [10,11,30,31]. N₂O emissions from agricultural soils in Europe were 12 times higher in residue amended soil due to increase denitrification stimulated by the added substrate and the creation of anaerobic micro sites by increased soil respiration [32]. A meta-analysis conducted by Chen et al. [9] also reported that crop residue amendment generally enhanced soil N₂O emissions compared with unamended controls. However, other studies observed the opposite effect on soil N₂O emissions after residue amendment [12,13,33]. According to the results of Wu et al. [34], the residue C/N ratio is a good predictor of soil N₂O emissions. Chen et al. [9] reported that residue amendment generated significantly positive effects on soil N₂O emissions when C/N ratios of crop residues were <45, slightly positive effects for C/N ratios of 45–100, and slightly negative effects for C/N ratios >100. Wu et al. [34] observed that rapeseed cake with low C/N ratio showed high N₂O emissions while wheat straw with high C/N ratio showed lower cumulative N₂O emissions from red soil. Muhammad et al. [35] discussed that incorporation of plant residues enhanced N₂O emissions and this enhancement was quantitatively dependent on C/N ratio of the residues, lower C/N ratio of the residues inducing higher concentration of dissolved organic carbon and larger amount of N₂O emission. In this study, high cumulative N₂O emissions from pruning residue amended soil might be due to its relatively low C/N ratio (15.6). When the C/N ratio of incorporated crop residue is small, the availability of N being greater, first for nitrification and then for denitrification [36]. Incorporation of pruning residue might provide a source of readily available C and N in the soil, and subsequently influences the N₂O emissions from amended soil. Chen et al. [9] also reported that residue with relative low C/N ratio can provide sufficient N which further stimulate nitrification and/or denitrification and thereby enhancing soil N₂O emission compared to unamended soils.

After 90 days of incubation, surface application of pruning residue also increased N₂O emission by 247% compared to the control in experiment 2 (Figures 1c and 2). Similar results were observed when residues were mixed with different layers of soils in experiment 1 (Figures 1a,b and 2) which might be because of its relative low C/N ratio. Baggs et al. [10] reported that, when straw with a small C/N ratio is present on the soil surface, N immobilization probably will not occur, more N will be available for nitrification and denitrification processes and higher N₂O emissions may occur. The presence of straw with high C/N ratio on the soil surface may increase N immobilization and thus decrease the denitrification reactions and soil N₂O emissions [10]. Signor and Cerri [37] discussed that the maintenance of residue on the soil surface affects the N mobilization and immobilization and, consequently, the N availability in the soil, as well as nitrification and denitrification processes. In this study, surface application of pruning residue might reduce soil evaporation and affect soil N cycling by favoring more denitrification for N₂O production. Therefore, returning tea pruning residue by either incorporation or surface application to soil favored high cumulative N₂O emissions from tea plantation soil.

Due to increase in soil N₂O emissions from returning pruning residue, conversion of residue to biochar and its application might be one of the alternative ways to reduce N₂O emission from tea plantation soil. However, biochar addition has been reported to have positive, negative, or negligible effects on soil N₂O emissions [19–24]. This apparent inconsistency might be due to differences in biochar types and properties of the soils used. The results of this study showed that residue biochar addition to different soil layers significantly decreased cumulative N₂O emissions from tea plantation soil compared to the control and residue treatment in experiment 1 (Figures 1a,b and 2). The reduction in N₂O emission by biochar amendment might be due to increase in soil pH, as found in this study (Figure 5). This result was also supported by correlation analysis that N₂O emission was

inversely related with soil pH in this experiment (Figure 7a,b). Tokuda and Hayatsu [38] reported a negative exponential relationship between soil pH and N₂O emission potential in tea plantation soil. The N₂O emissions can be decreased compared to the control due to an increase in soil pH by biochar amendment [20,39]. The likely reason is that low pH prevents the assembly of functional N₂O reductase (N₂OR), the enzyme that reduces N₂O to N₂ in denitrification [40]. Increased N₂OR activity due to pH rise by biochar amendment might be one of the reasons for reducing N₂O emission from tea plantation soil. Due to the retention of water in their fine pores and its capacity to create local alkaline conditions, biochar particles might also act as hotspots for complete denitrification [22,41]. It is thus likely that, in these microsites, the intermediary product N₂O might be completely reduced to N₂.

Since biochar can significantly change the soil C availability and the C/N ratio [41], changes in soil C/N ratio may be one of the important parameters affecting soil N utilization and N₂O emission. Previous studies discussed that the soil C/N ratio is a key parameter that determines pathways of soil N utilization and thus impacts nitrification and denitrification [42,43]. The reduction in N₂O emission by biochar amendment might be because of an increase in soil C/N ratio as found in this study (Figure 6c). This result was also supported by correlation analysis that N₂O emission was inversely related with soil C/N ratio in this experiment (Figure 7). Ernfors et al. [44] and Oo et al. [24] also reported that soil C/N ratio was negatively correlated with N₂O emission, although no significant correlation was observed by Feng and Zhu [45]. Previous studies indicated that as the relative C content increases in soil, a higher proportion of ammonium is immobilized (or assimilated) by microbes instead of being nitrified (or mineralized), leading to a decrease in soil inorganic N and suppression of N₂O emission [42]. The N demand of microbes increases above N availability when the soil C/N ratio increases and N becomes the limiting factor relative to C for nitrification or denitrification and thus N₂O emission becomes relatively low [45].

After 90 days of incubation, surface application of residue biochar significantly reduced soil cumulative N₂O emission by 35.8% compared to the control in experiment 2 (Figure 2). When pruning residue was returned to the soil as surface application, soil N₂O was relatively higher compared to the control and biochar amendment which was due to its low C/N ratio. Our previous study also showed that surface application of high rate (B10%) of pruning waste biochar to Japanese pear orchard soil significantly reduced N₂O emission [39]. Decreased in N₂O emission under surface application of biochar might be because of its high C/N ratio compared to C/N ratio of pruning residue. Baggs et al. [10] discussed that C/N ratio of the residue present on the soil surface is a key factor affecting soil N₂O emissions. The results of this short-term study suggest that converting pruning residue to biochar and its application either incorporation or surface, has the potential to mitigate soil N₂O emissions from tea plantation soil.

4.2. CO₂ Emissions

A significantly higher cumulative CO₂ emission was observed from pruning residue amended soil compared to the control for both the soil layers in experiment 1 (Figure 4). Increase in CO₂ emission was also observed from the surface application of pruning residue compared to the control in experiment 2 (Figure 4). Other studies also reported high CO₂ emission upon returning crop residues [8,32,35]. Possible reason for high emission might be that residue provided more easily degradable and potentially more soluble C for microbial activity. The increased CO₂ emissions in the crop residue treatments confirmed that the added C substrate is immediately broken down by soil microbes after incorporation [32,35]. Hadas et al. [46] reported that the C mineralization of residues in the early stage of decomposition is influenced by the amount of soluble C present in the added plant materials.

After 90 days of incubation, soil cumulative CO₂ emission was significantly higher upon biochar amendment compared to the control for both the soil layers in experiment 1 (Figure 4). The results of meta-analysis conducted by He et al. [47] also showed that biochar application significantly increased soil CO₂ fluxes by 22.14%. They discussed that the stimulation of soil CO₂ fluxes might be associated

with the higher soil organic C status and the more active soil microbial activities since biochar application enhanced soil organic C and soil microbial biomass C [48]. The increase in CO₂ production upon biochar amendment was most likely due to mineralization of labile C fractions added with the biochar [49,50]. Chia et al. [51] discussed that biochar increased soil surface area due to pore structures which promotes microbial activity and increases CO₂ emission.

Although high emission was observed over the control, soil cumulative CO₂ emission was significantly lower upon biochar amendment compared to the residue treatment (Figure 4). Hernandez-Soriano et al. [52] also observed that biochar amendment significantly reduced soil CO₂ emissions compared to the application of raw materials. Application of biochar can reduce bioavailability of soluble organic substrate by organic matter sorption to biochar and physical protection which slows down mineralization and decomposition of soil organic matter and thus reduces soil CO₂ emission [53]. Lehmann et al. [54] reported that a change in microbial abundance and community structure due to biochar presence may affect not only biochar mineralization itself but also mineralization of existing soil carbon.

After 90 days of incubation, surface application of residue biochar significantly reduced soil cumulative CO₂ emission compared to the control in experiment 2 (Figure 4). The reduction in CO₂ emission upon biochar application on the soil surface has also been reported by Oo et al. [39]. When pruning residue was returned to the soil as surface application, soil CO₂ was relatively higher compared to the control and biochar treatment. Hernandez-Soriano et al. [52] demonstrated that soil amended with biochar maintains C mineralization rates comparable to non-amended soil while significantly reducing CO₂ emissions compared to the application of raw materials. Under surface application of biochar in this study, labile C added with the biochar might not directly available for mineralization and therefore reducing soil CO₂ emission. Another possible reason of decrease in CO₂ emission from biochar treatment might be due to its adsorption capacity for CO₂ [55]. Although surface application of biochar is not a common agricultural practice in tea plantation, this theoretical study aimed at focusing on mechanisms and the effect of biochar application on the soil surface on soil GHG emissions. Our results suggested that surface application of residue biochar has the potential to reduce CO₂ emission from tea plantation soil.

5. Conclusions

Returning pruning residue by either incorporation or surface application significantly increased CO₂ and N₂O emissions from tea plantation soil. Conversion of tea pruning residue to biochar and its incorporation to soil significantly reduced N₂O emission from tea plantation soil. However, residue biochar amendment to soil still increased soil CO₂ emission. Surface application of residue biochar significantly reduced both soil N₂O and CO₂ emissions. The results show that conversion of pruning residue to biochar and its application has the potential to mitigate N₂O emission compared to raw residue amendment. Instead of returning tea pruning residue to soil, use of its biochar as surface application is recommended to mitigate both N₂O and CO₂ emissions from tea plantation soil. However, this study only investigated a short time frame and further research is needed to investigate the effect of residue biochar returned to tea plantation soil on N₂O and CO₂ emissions under field conditions.

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