



Communication

Aerated Irrigation and Pruning Residue Biochar on N₂O Emission, Yield and Ion Uptake of Komatsuna

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Abstract: After irrigation in intensive vegetable cultivation, the soil is filled with water leading to reduced oxygen content of the soil air which will affect vegetable growth and soil N₂O emission. In this study, the effect of aerated irrigation and residue biochar on soil N₂O emission, yield, and ion uptake of komatsuna grown in Andosol was explored. The experiment included four treatments; control (tap water irrigation), aerated water irrigation, pruning residue biochar with tap water irrigation, and a combination of aerated irrigation and biochar. The results showed that aerated irrigation had no effect on plant growth, but it also increased N₂O emission by 12.3% for several days after planting. Plant ion uptake was not affected by aerated irrigation. Biochar amendment increased shoot dry weight and significantly reduced soil N₂O emission by 27.9% compared with the control. Plant uptake of N and K also increased with biochar. This study showed that pruning residue biochar has the potential to mitigate N₂O emission while increasing vegetable growth and plant nutrient uptake. However, the study soil, Andosol, already has high soil porosity with low bulk density. Thus, further injection of air through irrigation showed no effect on plant growth but increased N₂O emission, hence soil aeration was not a limiting factor in Andosol.

Keywords: soil aeration; greenhouse gas emission; plant growth

1. Introduction

Nitrous oxide (N₂O) is a potent and long-lived atmospheric greenhouse gas and the single most important ozone-depleting compound emitted to the atmosphere [1]. Moreover, it has a global warming potential 296 times stronger than CO₂ [2]. Due to widespread use of synthetic nitrogen fertilizer, agricultural soil is the single largest source of global anthropogenic N₂O emission [3] and therefore, contributes significantly to increase N₂O concentrations in the atmosphere [4]. Hence, it is important to reduce soil N₂O emissions induced by N fertilizer to help mitigate anthropogenic contributions to global warming and ozone depletion.

Currently, research on N₂O emissions from agricultural soil are mostly focused on field crops and little has been reported from the soils of vegetable crops [5], especially in Asia. Intensive vegetable cultivation is likely to use a high N fertilizer with a high cropping index, which is considered to be an important source of N₂O [6]. Moreover, constant irrigation of vegetable crops influenced

organic matter decomposition, microbial biomass and activity, root biomass, soil aeration and nitrogen turnover, which in turn influence soil N₂O production [7,8]. Irrigation increases the soil water content surrounding the roots, reducing air-filled porosity. The root zone of the crop remains nearly saturated during and for some time after irrigation events, reducing availability and mobility of oxygen that remains trapped in soil pores, resulting in poor soil aeration in the crop root-zone during and following irrigation events [9,10]. To solve soil O₂ deficiency, aerated irrigation has been proposed as an effective tool to mitigate hypoxia in the crop root zone [11]. Aerating the soil through irrigation can affect crop growth and soil microbial activity [12,13]. Chen et al. [12] reported that aerating irrigation improved the growing environment in the root zone, promoting plant growth and thus increasing production and quality. The change of O₂ content in soil caused by aerating irrigation must inevitably affect the production and emissions of soil N₂O [5]. Due to its effect on O₂ diffusion, water filled pore space regulates the proportion of N₂O emission from nitrification and denitrification [14]. Machefert et al. [14] discussed that emission of NO should dominate in soils with good oxygen supply while N₂O may be the main product in moderately aerated soils. However, there is still limited information on the effect of aerated irrigation on soil N₂O emissions from vegetable soils.

Recently, biochar has received attention for possible use in agricultural soils. Conversion of crop residue into biochar and its application in soil as a soil amendment is a novel approach as a soil improver, as well as for carbon sequestration, improved soil fertility and crop production [15] and decreased greenhouse gas emission [16–19]. However, no difference or even an increase in soil N₂O emissions after biochar application was observed in other studies [20,21]. This was likely due to the variation in the physical and chemical characteristics of the different biochars, soil types and crop species [22,23]. In this study, biochar fabricated from pruning residues of orchards by the specific carbonization method (open burn kiln) was used to study its effect on vegetable growth and N₂O emission from volcanic ash soil.

Komatsuna (*Brassica rapa* var. *perviridis*) is a popular leafy vegetable grown in Japan. It requires the application of a high level of N fertilizers within a short growth cycle, contributing to N₂O emission to the atmosphere [24,25]. It is essential to investigate effective mitigation options of soil N₂O emission during intensive vegetable cropping while increasing vegetable yield under different management practices. In this study, a pot experiment was conducted to investigate the effects of soil aeration and/or biochar amendment on soil N₂O emission and yield of komatsuna. The objectives of this study were to (1) evaluate the effect of air nano-bubble water irrigation and biochar amendment on N₂O emissions during komatsuna growth and (2) to study their effects on soil properties, growth, and ion uptake of komatsuna. In this study, Andosol from volcanic ash, the most common arable soil in Japan, about 82% of vegetables are grown on Andosol in Ibaraki prefecture alone, was used.

2. Materials and Methods

A pot experiment was carried out at the Institute for Agro-Environmental Sciences, National Agriculture and Food Research Organization, Tsukuba, Ibaraki, Japan. The soil used in this study was collected in February 2018 from an upland field at the Horticultural Research Institute of the Agricultural Research Center in Ibaraki Prefecture, Ibaraki, Japan. The soil was classified as an Andosol, and soil properties are shown in Table 1. Biochar used in this study was produced from carbonization of pruning residues of the Japanese nashi pear tree under an open fire using an open burn kiln [18]. The biochar had a pH (H₂O) of 10.3, 5.7 g kg⁻¹ total N, 374 g kg⁻¹ total C, a surface area (Brunauer–Emmett–Teller Method) of 65.6 m² g⁻¹, 9.8% ash, 9.7% volatile matter, and 80.5% fixed C [18].

Table 1. Soil properties collected from an upland field at the Horticultural Research Institute, Agricultural Research Center, Ibaraki, Japan.

Parameter	Value
pH	5.3
EC (mS cm ⁻¹)	0.1
P ₂ O ₅ (mg 100 g ⁻¹)	4.1
K ₂ O (mg 100 g ⁻¹)	45.9
NO ₃ -N (mg 100 g ⁻¹)	2.4
NO ₂ -N (mg 100 g ⁻¹)	0.2
Total N (g kg ⁻¹)	3.7
Total C (g kg ⁻¹)	45
Bulk density (g cm ⁻³)	0.76
Coarse sand (%)	3.1
Fine sand (%)	23.6
Silt (%)	50.4
Clay (%)	22.9
Classification	Silk loam

Four treatments were assigned in a completely randomized design with three replications. The treatments were (1) control (no biochar amendment with tap water irrigation), (2) aerated water irrigation with nano-bubbles (AI), (3) pruning residue biochar with tap water irrigation (BC), and (4) a combination of AI and BC (AI + BC). The air nano-bubble water for aerated irrigation was generated using a commercially available nano-bubble generator that employs a two-phase flow swirling method (compact bubble generator manufactured by AQUAAIR Co., Ltd., Tokyo, Japan). Air was injected continuously for 30 min six times a day [26]. Fresh aerated water was immediately used for daily irrigation. The DO and pH value of air nano-bubble water were 8.04 mg L⁻¹ and 7.13 while those of tap water were 7.78 mg L⁻¹ and 7.32, respectively.

The biochar application rate was 60 g pot⁻¹ which was equivalent to 30 t ha⁻¹. For all treatments, N (urea), P (superphosphate), and K (muriate of potash) fertilizers were applied at the same rate of 120 kg ha⁻¹. All pots (1/5000 a Wenger pot, height 20 cm, and diameter 16 cm) were filled with about 3 kg of soil. All pots were placed on the ground under open-field conditions. To avoid precipitation, the pots were transferred to a greenhouse during rainfall events. Biochar and fertilizer were applied as a single dose and thoroughly mixed with top soil (0–15 cm soil layer) prior to seedling transplanting. Seeds of komatsuna were sown in plug trays in the greenhouse. Seedling emergence started four days after planting. One seedling of komatsuna (14-days old) was transplanted to each pot on 9 April 2018. The water content in the pots was maintained similarly for all treatments throughout the growing season. Irrigation was performed with the same amount of nano-bubble water or tap water three times per week to avoid soil surface drying. Komatsuna were harvested once they reached marketable size on 11 May 2018.

A closed chamber method was used to determine the N₂O flux from each pot. At the time of sampling, the pots were covered with a small chamber and gas samples were collected at 0, 10, and 20 min from the time the chambers were deployed. The chamber deployment duration was set according to Parkin and Venterea [27] and Rochette [28]. The sample gasses were then transferred to 15-mL vacuum glass vials with rubber stoppers. The collected air samples were analyzed for N₂O concentrations using a gas chromatograph (GC 2014, Shimadzu Corporation, Kyoto, Japan) equipped with an electron capture detector (ECD). The N₂O fluxes were calculated from the temporal increase of the gas concentration inside the chamber per unit time. Cumulative emissions of soil N₂O were calculated by multiplying the daily fluxes at each measurement for the time interval and then by summing up the obtained values.

Soil temperature at a depth of 10 cm was recorded at the time of air sampling. At harvest, shoot length and shoot weight were recorded. Top soil samples were collected after harvest of komatsuna for each pot to analyze soil pH, total N and C content. Total N and C contents were

analyzed by a NC analyzer ((Sumigraph NC-80; Sumika Chemical Analysis Service Co., Tokyo, Japan). The soil pH was determined in the supernatant suspension of 1:5 soil: water solution using a pH meter (FiveEasy, FE20, Mettler Toledo, Tokyo, Japan). N uptake (mg pot^{-1}) was calculated using the equation: $\text{N uptake (mg pot}^{-1}) = \text{dry matter weight (mg pot}^{-1}) \times \text{N content of shoot dry matter (\%)}$. Bulk density (0–5 cm soil depth) was determined by the core method using a sample ring of 5 cm^3 .

Soil porosity was calculated after analyzing soil samples using a Three Phase Meter, DIK 1120 (Daiki Rika Kogyo, Tokyo, Japan). For the quantification of ion content in the shoots, dried samples (0.2 g) were digested in concentrated nitric acid (HNO_3), and volumes made up to 10 mL with mili-Q water. Mineral element analysis of plant extracts was determined by atomic absorption spectrophotometer (Shimadzu AA-6300, Tokyo, Japan) and UV-Vis spectrophotometer (Shimadzu UV-1800, Tokyo, Japan).

The data collected were analyzed by analysis of variance (ANOVA) using CropStat 7.2 statistical software program (International Rice Research Institute, IRRI, Philippines). Treatment means were compared using the least significant difference (LSD) test at $P \leq 5\%$.

3. Results

3.1. Soil Properties and Soil Temperature Changes

Soil pH showed significant differences among the treatments after harvest of komatsuna (Table 2). Aerated irrigation and biochar, either alone or combined, showed significant increases in soil pH compared with control. However, the highest soil pH values were observed in treatments containing biochar. Soil pH increased by 0.22, 0.58, and 0.71 unit for AI, BC, and AI + BC treatments compared with control. There was no significant difference in soil total N content with aerated irrigation and/or biochar amendment (Table 2). The mean soil total N contents were 4.66, 4.82, 5.10, and 5.34 g kg^{-1} soil for control, AI, BC, and AI + BC, respectively. Soil total C content showed significant differences among the treatments (Table 2). Biochar with or without AI significantly increased total C by 72–79% compared with control soil. On the other hand, no difference in soil total C was observed between AI treatment and control soil. The soil C: N ratio was significantly affected by biochar amendment. High soil C: N ratios were observed in BC and AI + BC treatments compared with control. BC amendment decreased soil bulk density and increased soil porosity, because biochar itself had a much lower bulk density and higher porosity.

Table 2. Effect of aerated irrigation (AI) and/or biochar (BC) on properties of soil after harvest of Komatsuna. Values are mean \pm standard deviation ($n = 3$). Means followed by the same letters are not significantly different at the $P \leq 5\%$ level by the LSD test.

	pH (H_2O)	Total N (g kg^{-1} Soil)	Total C (g kg^{-1} Soil)	C: N Ratio	Bulk Density (g cm^{-3})	Soil Porosity (%)
Control	6.54 \pm 0.05 d	4.66 \pm 0.27 a	52.1 \pm 2.0 b	11.19 \pm 0.21 b	0.70 \pm 0.07	53.0 \pm 3.6
AI	6.76 \pm 0.07 c	4.82 \pm 0.16 a	53.5 \pm 0.4 b	11.12 \pm 0.29 b	-	-
BC	7.12 \pm 0.11 b	5.10 \pm 0.24 a	90.1 \pm 22.7 a	17.56 \pm 3.82 a	0.63 \pm 0.03	59.4 \pm 1.9
AI + BC	7.25 \pm 0.05 a	5.34 \pm 0.39 a	93.9 \pm 14.5 a	17.53 \pm 1.49 a	-	-
Analysis of variance P value						
Treatment	<0.01	>0.05	<0.05	<0.05	-	-

Soil temperature was low at the beginning of the growing period and subsequently increased (Figure 1). A large increase in soil temperature at nine days after transplanting was associated with warm air temperatures on that day. After that period, soil temperature fluctuated through 25 days and then declined until the end of the growing period.

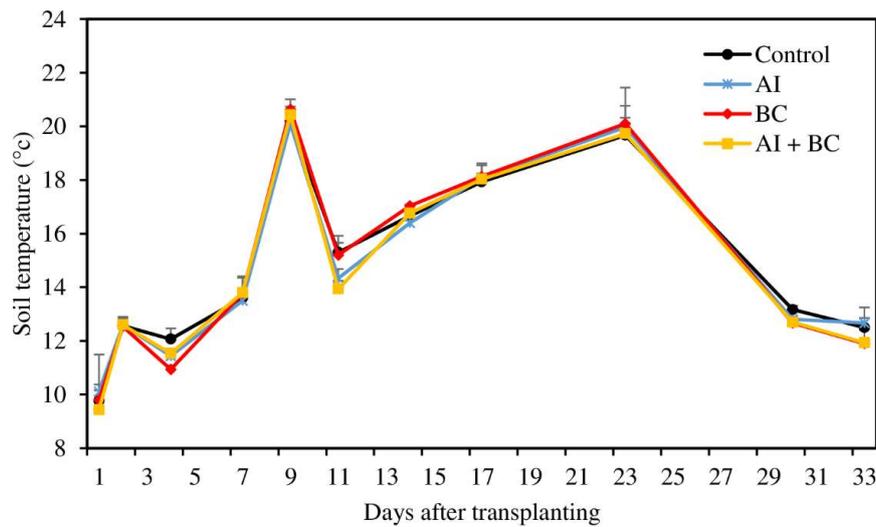


Figure 1. Variation in soil temperature during growing season of komatsuna. Error bars indicate standard deviation ($n = 3$). AI: aerated irrigation; BC: biochar.

3.2. N_2O Emissions

Soil N_2O emissions dramatically increased due to the application of N fertilizer, and the highest emissions occurred at nine days after transplanting of komatsuna (Figure 2). Then, a sharp decrease in soil N_2O emission was observed from 9 to 14 days, after which emissions remained steady with only small variation until the end of the growing period. Aerated irrigation showed the highest emission peak followed by the control and the lowest peak was observed in biochar treatment.

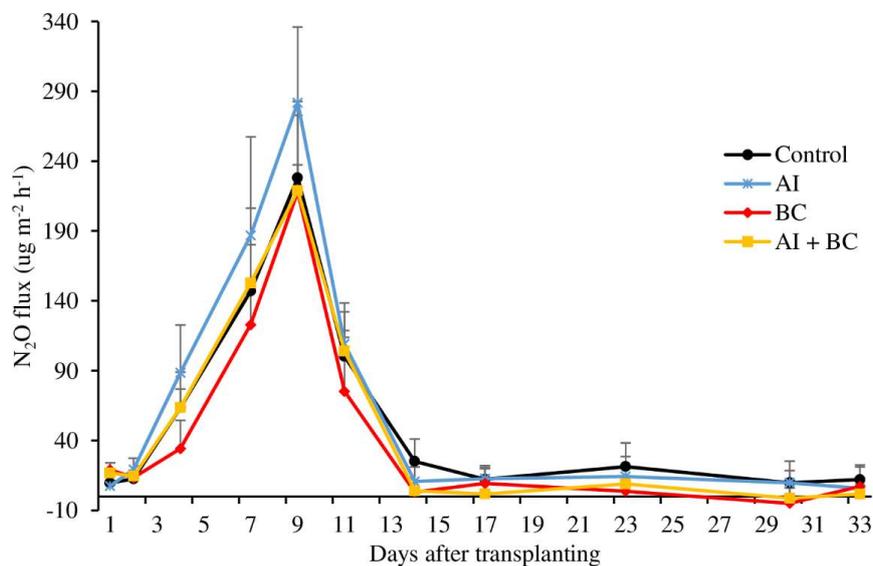


Figure 2. Soil N_2O emissions affected by aerated irrigation (AI) and/or biochar (BC) during the growing period of komatsuna. Error bars indicate standard deviation ($n = 3$).

A significant ($P < 0.05$) difference in cumulative N_2O emission was observed among the treatments (Figure 3). Cumulative N_2O emission for control, AI, BC, and AI + BC were 38.1 , 42.8 , 27.5 , and 30.7 mg m^{-2} , respectively. Biochar amendment was the only treatment that significantly affected N_2O emission, decreasing it by 27.9%.

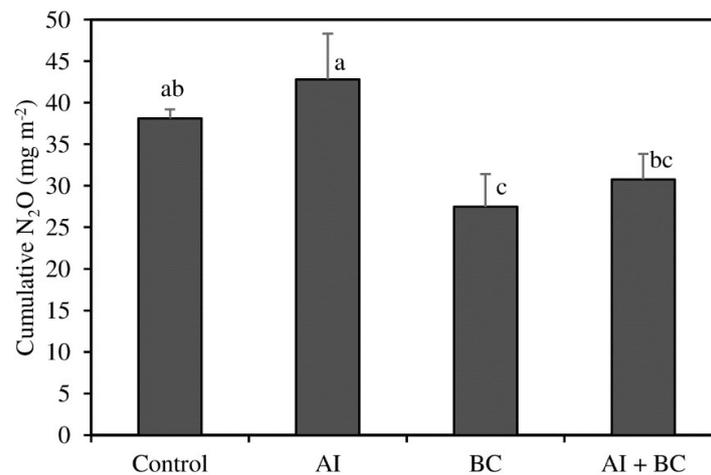


Figure 3. Cumulative N₂O emissions affected by aerated irrigation (AI) and/or biochar (BC). Error bars indicate standard deviation ($n = 3$). Means with the same letters are not significantly different at the $P \leq 5\%$ level by the LSD test.

3.3. Growth and Yield of Komatsuna

There were significant differences among the treatments in the shoot growth parameters of komatsuna at harvest (Table 3). AI treatment showed a lower shoot length and shoot fresh and dry weight compared with BC treatment, but neither differed from the control or AI + BC.

Table 3. Effect of aerated irrigation (AI) and/or biochar (BC) on shoot length, yield, and N content of komatsuna at harvest. Values are mean \pm standard deviation ($n = 3$). Means followed by the same letters are not significantly different at the $P \leq 5\%$ level by the LSD test.

	Shoot Length (cm)	Shoot Fresh wt. (g pot ⁻¹)	Shoot Dry wt. (g pot ⁻¹)
Control	23.6 \pm 2.2 ab	59.5 \pm 3.2 ab	7.20 \pm 0.08 b
AI	21.5 \pm 0.9 b	55.5 \pm 3.3 b	6.73 \pm 0.39 b
BC	25.3 \pm 1.2 a	65.6 \pm 0.8 a	7.80 \pm 0.08 a
AI + BC	24.5 \pm 1.9 ab	60.3 \pm 5.6 ab	7.54 \pm 0.36 ab
Analysis of variance P value			
Treatment	≤ 0.05	≤ 0.05	≤ 0.05

3.4. Ion Concentration and Uptake of Komatsuna

There were no significant treatment effects on shoot N, P, and Mg (Table 4). Shoot K and Ca contents were not affected by AI, but BC showed a higher shoot K content than control. Higher shoot Ca content was observed in the control compared with BC and AI + BC treatments.

Table 4. Effect of aerated irrigation (AI) and/or biochar (BC) on tissue ion concentration of komatsuna. Values are mean \pm standard deviation ($n = 3$). Means followed by the same letters are not significantly different at the $P \leq 5\%$ level by the LSD test.

	Concentration (mg g ⁻¹)				
	N	P	K	Mg	Ca
Control	16.72 \pm 2.7 a	0.64 \pm 0.06 a	13.9 \pm 2.7 b	2.16 \pm 0.05 a	20.9 \pm 0.2 a
AI	20.26 \pm 0.9 a	0.63 \pm 0.17 a	15.6 \pm 1.9 ab	2.16 \pm 0.21 a	21.0 \pm 3.1 a
BC	19.63 \pm 3.1 a	0.73 \pm 0.03 a	18.5 \pm 2.4 a	1.97 \pm 0.24 a	16.9 \pm 0.6 b
AI + BC	18.45 \pm 1.0 a	0.76 \pm 0.09 a	16.8 \pm 2.8 ab	2.04 \pm 0.21 a	15.7 \pm 1.2 b
Analysis of variance P value					
Treatment	> 0.05	> 0.05	≤ 0.05	> 0.05	≤ 0.05

N, P, K, and Mg uptake by komatsuna were not affected by AI treatment (Table 5). However, higher N and K, and lower Ca, uptake by komatsuna was observed for BC. A significantly lower Ca uptake was also observed for AI treatment compared with the control, and AI + BC Ca uptake was significantly lower than AI and BC alone.

Table 5. Effect of aerated irrigation (AI) and/or biochar (BC) on total mineral nutrient uptake of komatsuna. Values are mean \pm standard deviation ($n = 3$). Means followed by same letters are not significantly different at the $P \leq 5\%$ level by the LSD test.

	Uptake (mg pot^{-1})				
	N	P	K	Mg	Ca
Control	120.7 \pm 11.8 b	99.4 \pm 9.9 ab	100.5 \pm 20.9 b	15.6 \pm 0.7 a	150.5 \pm 3.0 a
AI	136.3 \pm 14.5 ab	89.9 \pm 18.0 b	104.2 \pm 11.6 ab	14.5 \pm 0.4 a	139.8 \pm 6.5 b
BC	152.9 \pm 12.9 a	121.9 \pm 6.1 ab	143.9 \pm 17.9 a	15.4 \pm 1.8 a	131.6 \pm 5.7 b
AI + BC	139.2 \pm 10.6 ab	122.8 \pm 10.1 a	127.4 \pm 26.9 ab	15.5 \pm 2.3 a	118.6 \pm 9.8 c
Analysis of variance P value					
Treatment	≤ 0.05	≤ 0.05	≤ 0.05	> 0.05	≤ 0.05

4. Discussion

4.1. Aerated Irrigation and Biochar on Yield and Ion Uptake of Komatsuna

The AI treatment had no effect on shoot fresh and dry weight of komatsuna compared with control (Table 3), though means were slightly lower than controls. Our results suggest that soil aeration was not a limiting factor for plant growth with Andosol which is known to exhibit high porosity and low bulk density (Table 2). Therefore, hypoxic stress (situations in which the oxygen concentration is a limiting factor) might not occur in this soil and further injection of air into the soil through irrigation showed no effect on plant growth. This result differed with the study of Ahmed et al. [29], who observed that air nano-bubbled into water not only had a negative effect on tomato growth also appeared to slow down the growth rates of the hypocotyl length compared to tap water. Li et al. [30] reported that, once aeration had eliminated hypoxic stress, more air injected resulted in a reduction in root to soil contact which had a negative effect on the rhizosphere, and decreased tomato fruit yields. In this study, we injected nanobubbles into the soil through irrigation which had long-term stability in water (e.g., up to 70 days according to Ushikubo et al. [31]). Due to high stability of nanobubbles and high aeration in AI-treatments, there might have been a reduction in root to soil contact according to Li et al. [30]. Du et al. [32] reported that high N application under aerated irrigation caused exorbitant plant N uptake which, combined with vigorous leaf transpiration, resulted in large water and nitrogen losses, thus reducing celery yield. In this study, only slightly higher N uptake was observed in the AI treatment. Biomass reduction might also be due to high nitrogen losses by increasing soil microbial abundance and enzyme activity under aerated irrigation [32].

Other studies have reported that oxygenation significantly improved crop yield compared with traditional irrigation [9,12]. Addition of hydrogen peroxide to irrigation water released oxygen to the root zone soil. It affected above ground biomass and increased in root mass, root length and soil respiration, and also enhanced water use efficiency for pod and lint yield as well as increased leaf photosynthetic rate [11].

In hydroponic culture, microbubbles have proven to facilitate the growth of lettuce [33] while nanobubbles were shown to enhance the growth of plants by improving the oxygen supply as nutrient elements [34]. Chen et al. [12] suggested that the benefits of oxygenation were notable not only for dicotyledonous cotton but also for monocotyledonous wheat and pineapple grown in red soil and black cracking clay soil. Carter et al. [35] and Czyn [36] reported that insufficient oxygenation of compacted soil may limit the development of root systems, plant growth and crop yield. Li et al. [30] observed that increasing aeration volume resulted in higher total fresh tomato fruit yield at 40 cm irrigation

line depth, but under the 15 cm line depth treatments yields first increased and then decreased with increasing aeration in silty clay loam soil. An increase in crop yield in their studies was due to a different soil aeration method and crops grown in clayey soil with high bulk density, while in this study, Andosol with low bulk density and high soil porosity was used (Table 2). Therefore, soil aeration was not a limiting factor in Andosol due to high soil porosity.

Increased agricultural productivity is a commonly reported benefit of biochar amendments to soils [37,38]. Our results showed that biochar amendment increased shoot dry weight compared with the control (Table 3). Biochar increased soil pH, total N, and soil porosity and decreased the bulk density of the soil (Table 2), which improved soil quality for plant growth. Zhang et al. [39] reported positive effects of lower bulk density due to BC amendment of soil which included nutrient cycling, retention of plant available water, reduced soil compaction, increased soil aeration, and increased crop yield.

Addition of biochar may have improved the availability of N and K due to its liming effect [40,41]. Many studies have reported that biochar improved soil physical and chemical properties and soil fertility via effects on the microbial community, thus increasing crop production [40,42,43]. However, Jeffery et al. [15] reported that high-nutrient biochar inputs stimulated yield substantially more than low-nutrient biochar in tropical soils, but arable soils in temperate regions are moderate in pH, higher in fertility, and generally receive higher fertilizer inputs, leaving little room for additional benefits from biochar. In this study, we observed higher shoot dry weight with a temperate soil and biochar amendment. However, the current result was only from the effect of fresh biochar and thus, it will be necessary to study the effect of biochar aging on vegetable growth and greenhouse gas emission during subsequent crop cycles in temperate soils.

N and K uptakes by komatsuna were increased by biochar amendment (Table 4). The increase in soil microbial activity under biochar amendment could also be one of the reasons for high nutrient uptake of komatsuna in biochar-treated soils. Van Zwieten et al. [44] and Chan et al. [45] also reported that high N uptake of vegetable crops grown in biochar-amended soils. Fresh biochar may contain significant amounts of soluble P and K [46], which contribute to the plant available pool upon incorporation in the soil, and thus increased plant uptake of K with BC amendment (Table 4). However, there was no effect on Mg uptake and lower Ca uptake was observed in biochar-treated soil. This result was in agreement with that of Albuquerque et al. [47], who also found decreased Ca after different biochar additions to a nutrient-poor, slightly acid soil. The increase in major nutrient uptake under biochar amendment indicated a potential effect of biochar for improving fertilizer use efficiency in vegetable soils.

Plant nutrient uptake in the aerated irrigations of AI and AI + BC treatments was not significantly different compared with control, except that Ca was reduced (Table 4). Morard et al. [48] stated that root oxygen deficiency caused a reduction in plant water and mineral uptake. However, in this study soil, due to high soil porosity with low bulk density, aerated irrigation to Andosol soil had no effect on macronutrient uptake compared with control.

4.2. Aerated Irrigation and Biochar on Soil N₂O Emissions

The results showed that application of nitrogen fertilizer caused an initial sharp increase in soil N₂O emissions. This result was in line with other studies [18,19,49]. The increase in N₂O emissions during the early growing period suggested that N fertilizer and decomposition of crop residue can provide a temporary abundance of C and N to microorganisms resulting in a rapid increase in N₂O emission [50]. The initial high N₂O flux during early growth especially the emission peak at 9 days may have partly been a result of the high soil temperature in that period (Figure 1).

In the present study, aerated irrigation had no overall effect on soil N₂O emission (Figure 3), but appeared to increase it at days 4-9 (Figure 2). Hou et al. [5] observed that the cumulative emission for N₂O increased by 10% in aerated irrigation compared with that of control. Hui et al. [51] also stated that there was no significant difference between aeration and non-aeration in soil N₂O emissions in the

spring-summer season, whereas aeration enhanced N_2O emissions significantly in the autumn-winter season. They discussed that the increased soil porosity and the reduced soil moisture due to aeration were bound to reduce soil N_2O emissions in the spring-summer season, while higher water-filled pore space under aerated irrigation during the autumn-winter period was beneficial to N_2O production. The soil oxygen content variation caused by aerated irrigation might affect the condition of nitrification and denitrification [14,52], which influences soil N_2O emissions [5,14]. Macheferet et al. [14] stated that, in soils with good oxygen supply, emission of NO should dominate while N_2O may be the main product in moderately aerated soils. Li et al. [53,54] found that aerated irrigation significantly increased soil catalase and urease activity and the activity was highest in the flowering period which favored high N_2O emissions from greenhouse tomatoes. Minamikawa et al. [26] working with potted rice observed that, although rice growth showed no difference between plants irrigated with nano-bubble water and control water, a significant reduction in cumulative CH_4 emission was observed in the nano-bubble water treatment. Increased in soil N_2O emissions from aerated irrigation treatment might be due to changes in soil microbial populations and activities under aerated conditions. Du et al. [32] showed that soil microbial abundance and enzyme activity were significantly higher in aerated irrigation compared with conventional irrigation. Improving soil aeration conditions can increase soil microbial abundance and enzyme activity [30], which in turn influences soil N_2O production. Due et al. [32] found that for the aerated irrigation treatment, soil nitrifiers and denitrifiers increased with increased N application and their abundances; a treatment with 250 kg N ha^{-1} were 2.9- and 1.7-fold greater than the nitrifier and denitrifier abundances in the CK treatment, respectively.

The high N_2O emission flux under AI treatment was observed during the early growing period (days 4-9) soon after basal application of fertilizer (Figure 2). This might be due to high availability of N fertilizer with increased microbial activity under aerated irrigation. Du et al. [32] reported that higher soil enzyme activity under aerated irrigation also favored urea hydrolysis and increased ammonium concentration in the soil, providing sufficient substrate for nitrification and thus a higher N loss would occur by means of denitrification. In this study, we used Andosol which was characterized with low bulk density and high soil porosity (Table 2). Further injection of air through aerated irrigation into the soil did not improve plant growth (Table 3) and nutrient uptake (Table 5), but increased soil N_2O production (Figure 2).

Although soil aeration increased N_2O emission, addition of pruning residue biochar significantly reduced N_2O emission by 27.9% compared with control (Figure 3). This result was in line with other studies [16-18,23]. Addition of biochar decreased soil bulk density and increased soil porosity (Table 2) compared with control, and therefore its addition might reduce soil denitrification. Downie et al. [55] reported that increased soil aeration upon biochar addition might also be responsible for reducing N_2O emissions. Rogovska et al. [56] also reported that biochar amendment decreased soil N_2O emissions by affecting soil physical properties, reducing soil compaction and bulk density. Another study also reported that an increase in soil porosity and aeration are major factors governing N_2O generation and diffusion [57]. However, biochar-related soil aeration (Table 2) might not be the sole reason for reducing N_2O emission in this study, because the same effect was not replicated by soil aeration through AI treatment. Therefore, there might also be other factors influencing reduction in N_2O emission by biochar amendment.

The reduction in N_2O emission by biochar amendment might also be due to an increase in soil pH (Table 2), since low pH prevents the assembly of functional N_2O reductase (N_2OR), the enzyme that reduces N_2O to N_2 during the process of denitrification [58,59]. Increased N_2OR activity due to higher pH by biochar might be one of the reasons for reduced N_2O emission from acid soils [19]. The underlying mechanism for a reduction in soil N_2O emission by biochar amendment was reported due to microbial reduction of N_2O to N_2 via *nosZ* gene-containing microorganisms [23].

Changes in soil C content and C: N ratio by biochar amendment (Table 2) might be an important parameter affecting soil N utilization and N_2O emission in this study. Geisseler et al. [60] and Guo et al. [61] found that the soil C: N ratio is a key parameter that determines pathways of soil N

utilization and thus impacts nitrification and denitrification. As the relative C content increases in soil, the N demand of microbes increases above the N availability and N becomes a limiting factor relative to C for nitrification or denitrification and, thus, N₂O emission becomes relatively low [62]. Therefore, a biochar-induced change in soil C and C:N ratio was probably the dominant factor for the reduction in soil N₂O emission (Table 2). Improved plant N uptake under biochar amendment might also be one of the reasons reducing soil N₂O emission (Table 4).

Although the combination of biochar and aerated irrigation (AI + BC) decreased N₂O emission by 19.5% compared with the control, the significant effect of biochar (27.9% reduction) was slightly diminished by adding AI. Biochar addition increased soil porosity and improved soil aeration (Table 2), but the further injection of air through aerated irrigation in the AI + BC treatment did not show a further reduction in N₂O; rather it partially reduced the significant effect of biochar. This might be due to the fact that aerated irrigation could potentially lead to N₂O production due to increased microbial population and activity, and provide a sufficient substrate for nitrification [32], thus N loss might have occurred by nitrification due to the biochar amendment in AI + BC treatment.

5. Conclusions

Our results indicated that aerated irrigation using nano-bubbles had a negative impact on komatsuna plant growth and soil N₂O emission from soil with low bulk density and high soil porosity. Therefore, it may be recommended that aerated irrigation should be practiced in soils with high clay content and high bulk density to mitigate N₂O emission while increasing vegetable crop yield. As expected, pruning residue biochar amendment significantly reduced soil N₂O emission. Changes in soil pH, soil C:N ratio, bulk density, and soil porosity under biochar amendment were recognized as the driving factor for N₂O emission change after application of pruning waste biochar. Moreover, biochar also enhanced vegetable growth and plant nutrient uptake of komatsuna. The results suggested that pruning residue biochar has the potential to mitigate N₂O emission while increasing vegetable growth and plant nutrient uptake from temperate soils. However, this study was conducted in a single growing season with limited replications, thus the findings cannot be extrapolated to broad conclusions. Further research is needed to investigate the effects of aerated irrigation using nano-bubbles and biochar on vegetable plant growth and N₂O emission from different soils.

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