

# Effect of biogas digested slurry based-biochar and digested liquid on N<sub>2</sub>O, CO<sub>2</sub> flux and crop yield for three continuous cropping cycles of komatsuna (*Brassica rapa* var. *perviridis*)

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**Abstract** Biogas production generates digested slurry, as a byproduct, which can be used as fertilizer after its conversion into digested liquid and biochar. A microcosm-based study was conducted to evaluate the effects of chemical fertilizer (CF), digested liquid (DL) and varying concentrations of biogas digested slurry based-biochar along with DL on N<sub>2</sub>O flux, CO<sub>2</sub> flux, soil chemical properties and crop yield for three continuous cropping cycles of komatsuna (*Brassica rapa* var. *perviridis*) from April to July 2013. Analyses revealed that DL-treated soils released almost equal cumulative amounts of N<sub>2</sub>O and CO<sub>2</sub> as soils treated with CF. The soil mineral-N contents were also similar for the DL- and CF-treated soils while DL application increased the soluble organic carbon (SOC) content of the soil compared to CF treatment. The application of slurry-based biochar increased N<sub>2</sub>O and CO<sub>2</sub> flux, which, in turn, appeared to depend upon biochar concentration. The application of biochar probably increased the nitrification rate as biochar-treated soils had higher values of NO<sub>3</sub><sup>-</sup>-N and lower values of NH<sub>4</sub><sup>+</sup>-N compared to soils not treated with biochar at most of the observations. The SOC content was also the highest in biochar-treated soils. The overall crop yield for three cropping cycles was the highest in DL and biochar at low application rate (BL), and it was lower in CF, biochar at medium (BM) and high (BH) application

rate. This study indicates that the application of DL could be an effective strategy to minimize the use of CF, without affecting N<sub>2</sub>O flux, CO<sub>2</sub> flux, soil mineral N, and increasing crop productivity. The effects of slurry-based biochar on greenhouse gases flux and crop yield depends on the application rate of biochar.

**Keywords** Agriculture · Biochar · CO<sub>2</sub> flux · Crop yield · Digested liquid · N<sub>2</sub>O flux

## Introduction

The increase in energy demand has driven the need to consider biofuels as an alternative to fossil fuels (Singla et al. 2012). Great attention has been paid to anaerobic digestion of animal wastes and plant residues, because it produces renewable energy in an environmental friendly manner in many countries, including Japan, India, China and Europe (Albuquerque et al. 2011). The increase in biofuel production has simultaneously increased the formation of byproducts (Alotaibi and Schoenau 2013) such as the digested slurry that remains after biogas production. This slurry can be used as a soil amendment after its conversion into digested liquid (DL) (Singla et al. 2013; Singla and Inubushi 2014b) and biochar (Singla and Inubushi 2013, 2014a). The use of these as soil amendments might reduce the use of chemical fertilizers (Singla et al. 2013) that would also solve the problem of disposal of digested slurry.

Apart from water vapours and methane, carbon dioxide (CO<sub>2</sub>) and nitrous oxide (N<sub>2</sub>O) are two important greenhouse gases (GHG) (IPCC 2013). The concentrations of CO<sub>2</sub> and N<sub>2</sub>O in the atmosphere have increased from pre-industrial levels of 280 ppm and 270 ppb to about 391 ppm and 324 ppb, respectively, in 2011 (IPCC 2013). Although the atmospheric concentration of N<sub>2</sub>O is much lower than that of CO<sub>2</sub>, it has an almost 298 times higher global warming potential (IPCC 2013). Agricultural soil is a major source of

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$\text{N}_2\text{O}$  (Ebid et al. 2008).  $\text{N}_2\text{O}$  is produced in the soil mainly by two contrasting microbial processes: (a) nitrification of ammonium-N ( $\text{NH}_4^+\text{-N}$ ) to nitrate-N ( $\text{NO}_3^-\text{-N}$ ) and (b) denitrification of  $\text{NO}_3^-\text{-N}$  to  $\text{N}_2\text{O}$  and ultimately to  $\text{N}_2$ . Nitrification is more active under aerobic soil conditions while denitrification is dominant under anaerobic conditions (Inubushi et al. 1996). There are reports of increase in  $\text{N}_2\text{O}$  emission just after the application of N fertilizers because the nitrification process is active after  $\text{NH}_4^+\text{-N}$  application to agricultural soils (Amkha et al. 2009; Singla et al. 2013). Usually, most plants cannot use the whole amount of the applied N fertilizer, and the residual N can be leached into groundwater as nitrate ( $\text{NO}_3^-$ ), evolved as  $\text{N}_2\text{O}$  or volatilized as ammonia ( $\text{NH}_3$ ) (Amkha et al. 2009). Komatsuna is one of the popular leafy vegetables grown in Japan, and it requires the application of large amounts of N fertilizers within a short period, contributing towards  $\text{N}_2\text{O}$  emission to the atmosphere (Ebid et al. 2008; Amkha et al. 2009; Singla et al. 2013).

Biochar, which is the product of the thermal degradation of organic materials in the absence of air (pyrolysis), is used as a soil amendment (Lehmann et al. 2011). Many studies have reported the beneficial effects of biochar in improving soil quality and crop productivity (Jones et al. 2012; Kammann et al. 2012; Wang et al. 2012; Saarnio et al. 2013; Singla and Inubushi 2013, 2014a). Its effect is more significant for soils with low fertility and/or with low pH value, because of their lower nutrient retention capacity (Lehmann 2007). The observed effects on soil fertility are mainly due to a pH increase in acid soils or improved nutrient retention through cation adsorption (Lehmann et al. 2011). Asai et al. (2009) and Wang et al. (2012) reported a decreased crop yield by the application of biochar without N-fertilizer compared to biochar with N-fertilizer treatment, thus indicating that the addition of an N-source with biochar seems to be essential for the crop improvement. There has also been increasing attention to the possibility of mitigating climate change by diverting C into the agricultural soils through the application of biochar (Lehmann et al. 2011).

Still, there is contradiction over the effect of biochar on GHG fluxes. Some of the studies suggest that the application of biochar can decrease  $\text{N}_2\text{O}$  emissions (Singh et al. 2010; Bruun et al. 2011; Kammann et al. 2012; Wang et al. 2012) and increase  $\text{CO}_2$  emission (Bruun et al. 2011, 2012; Jones et al. 2011, 2012; Wang et al. 2012). However, some of the published reports also state that the application of biochar may increase  $\text{N}_2\text{O}$  emission (Yanai et al. 2007; Singh et al. 2010; Bruun et al. 2011; Kammann et al. 2012; Saarnio et al. 2013; Xie et al. 2013) or may not cause any significant change in  $\text{N}_2\text{O}$  emission (Kammann et al. 2012; Xie et al. 2013). It appears that the amount of  $\text{N}_2\text{O}$  and  $\text{CO}_2$  emissions will depend on the physical and chemical properties of the biochar, the raw material used for biochar preparation, the type of the soil, microbiological activity and composition, and the water

and fertilizer management (Yanai et al. 2007; Kammann et al. 2012; Saarnio et al. 2013). Moreover, studies on the effect of biogas digested slurry-based biochar on  $\text{N}_2\text{O}$  emissions are almost not available in the published literature. However, our previous reports (Singla and Inubushi 2013, 2014a) showed the influence of biogas digested slurry-based biochar on  $\text{N}_2\text{O}$  emissions under waterlogged conditions, which may not be strongly conclusive for  $\text{N}_2\text{O}$  emissions under different conditions. In paddy fields, the soil is water-saturated and remains under anaerobic conditions for a long time, and N dynamics are presumed to be distinctly different from grasslands or other arable land which are under aerobic conditions most of the time (Win et al. 2009). In another study with komatsuna (Singla et al. 2013), DL as N-fertilizer increased crop yield without increasing cumulative  $\text{N}_2\text{O}$  emissions and without changing soil mineral-N contents compared to chemical fertilizer.

On the basis of available reports, we aimed at the replacement of mineral fertilizer, reduction of  $\text{N}_2\text{O}$  emissions and increase of crop yields with the application of DL and biochar. It was hypothesized that the addition of biochar to the komatsuna-cultivated soils should affect  $\text{N}_2\text{O}$  and  $\text{CO}_2$  emissions, and it should depend on the amount of biochar added. A microcosm experiment with three consecutive komatsuna crops was conducted to evaluate the effects of DL or chemical fertilizer (CF) as N fertilizer, and the varying concentrations of biochar as C source along with DL on (a)  $\text{N}_2\text{O}$  and  $\text{CO}_2$  flux, (b) soil chemical properties, (c) crop yield.

## Materials and methods

### Experimental design

The experiment was conducted at the soil science experimental field, Graduate School of Horticulture, Chiba University, Matsudo, Japan from April to July 2013. Komatsuna was cropped in three consecutive cropping cycles (30 days each) in plastic containers (42 cm length  $\times$  32 cm width  $\times$  30 cm depth) filled with almost 32 kg of soil to create a soil depth of 20 cm. The soil (sand-dune Regosol) was collected from Kujukuri, Chiba, Japan and had the following physico-chemical properties: sand 97.3 %; silt 2.7 %; and clay <0.01 % (Singla and Inubushi 2013); pH ( $\text{H}_2\text{O}$ ) 6.6; electrical conductivity (EC) ( $\text{H}_2\text{O}$ ) 21.4  $\text{mS m}^{-1}$ ; total C (TC) 1.19 %; total N (TN) 0.11 %; ammonium-N ( $\text{NH}_4^+\text{-N}$ ) 6.5  $\mu\text{g g}^{-1}$  dry soil (ds); and nitrate-N ( $\text{NO}_3^-\text{-N}$ ) 5.4  $\mu\text{g g}^{-1}$  ds (Singla et al. 2013). Biochar and DL were obtained from Yamada Biomass Plant, Chiba, Japan. The production procedure and pyrolysis temperature for biochar production from the solid portion of digested slurry were those described by Singla and Inubushi (2013, 2014a). Yamada Biomass Plant uses 4 t  $\text{day}^{-1}$  of cattle wastes mainly cow manure and 0.6 ton  $\text{day}^{-1}$  of food

processing residues to produce 0.1 t day<sup>-1</sup> of biogas. This process also generates 3.8 t day<sup>-1</sup> of digested slurry. Biochar is prepared from half-dried solid portion of slurry which usually has water content of 45 %. Overheated steam is provided for carbonization of this solid portion. Carbonization and pyrolysis is done at 330 °C which is followed by an output process temperature at 370 °C. The distillation of liquid portion is done to get DL. Usually, 250 L of dehydrated filtrate liquid is filled into distillation machine. The distillation process is stopped after getting out of 228 L distilled water. So, 22 L of DL is formed from 250 L of dehydrated filtrate liquid. The resulting DL is stored at 4 °C until the application. The chemical properties of the DL are as follows: pH (H<sub>2</sub>O) 6.35; EC (H<sub>2</sub>O) 12,500 mS m<sup>-1</sup>; TC 0.83 %; TN 1.45 %; NH<sub>4</sub><sup>+</sup>-N 14,400 µg ml<sup>-1</sup>; NO<sub>3</sub><sup>-</sup>-N 2.30 µg ml<sup>-1</sup>; P<sub>2</sub>O<sub>5</sub> 15.40 µg ml<sup>-1</sup>; and K<sub>2</sub>O 33.80 mg ml<sup>-1</sup>. The chemical properties of biochar are mentioned in Table 1.

Six treatments in triplicate were as follows: (1) control with no applied N; (2) chemical fertilizer (CF) (8:8:8 N/P/K, mainly consisting of (NH<sub>4</sub>)<sub>2</sub>HPO<sub>4</sub> and KCl; Daihosuka Co. Ltd., Tokyo, Japan) at 120 kg NPK ha<sup>-1</sup>; (3) digested liquid (DL) as N source at 120 kg N ha<sup>-1</sup>; (4) biochar low (BL): DL same as no. 3 + biochar (7.72 g container<sup>-1</sup>) at 180 kg C ha<sup>-1</sup> (C content of biochar: N content of DL, C/N ratio 1.5); (5) biochar medium (BM): DL same as no. 3 + biochar (15.44 g container<sup>-1</sup>) at 360 kg C ha<sup>-1</sup> (C/N ratio 3.0); (6) biochar high (BH): DL same as no. 3 + biochar (30.88 g container<sup>-1</sup>) at 720 kg C ha<sup>-1</sup> (C/N ratio 6.0). The detailed description of each treatment is mentioned in Table 2. Calcium superphosphate was used as P source to reach a total concentration of P at 120 kg P ha<sup>-1</sup> in each treated soil except for no external application of P in treatment no. 2 and 6. Potassium chloride was used as K source to reach a total concentration of K at 120 kg K ha<sup>-1</sup> in the control soil. The whole quantity of biochar was incorporated into the soil as basal dose 2 days prior to seedling transplantation of 1st cropping; whereas P and K in the control soil, CF in treatment no. 2 and DL in

treatment nos. 3–6 were applied in two split doses 2 days prior to seedling transplantation of first two croppings: 50 % before 1st cropping and 50 % before 2nd cropping. The 3rd cropping in each container was carried without any fertilizer amendment to see the residual effects of each amendment. Six seedlings (14 days old) for each cropping cycle were transplanted to the containers. Each treated container was kept inside the glass house without air temperature regulator.

To monitor the soil moisture in each container, the water-mark soil moisture sensor (model no. 6450 WD, Spectrum Technologies, Inc., Osaka, Japan) was placed vertically at 0–8 cm soil depth at the day of the first and the second inorganic and organic fertilizer application and kept in place until the end of the experiment. Soil moisture (0–8 cm soil depth) and soil temperature (0–5 cm soil depth) were recorded by a digital thermo recorder (model no. TR-71Ui, T and D Corporation, Nagano, Japan). The water content in each container was maintained at 60 % of the water holding capacity (WHC) of soil throughout the entire experiment (Singla et al. 2013).

#### Gas sampling and analysis

The gas samples from each container were collected at 2 to 7-day intervals by the closed chamber (height 20 cm; and diameter 17.4 cm) method (Singla et al. 2013). The base of the chamber was settled in the center of each container on the day of fertilizer application and kept in place until the end of the experiment. At each measurement, three samples were taken from the sampling chamber into disposable syringes at 0, 10, and 20 min after putting the top on the chamber base; then, samples were immediately transferred into pre-evacuated vials (Singla et al. 2013). Collected air samples were analysed for N<sub>2</sub>O and CO<sub>2</sub> concentration by gas chromatographs (GC-14B; Shimadzu, Kyoto, Japan) equipped with ECD and TCD, respectively (Singla and Inubushi 2013). The N<sub>2</sub>O flux (µg m<sup>-2</sup> h<sup>-1</sup>) and CO<sub>2</sub> flux (mg m<sup>-2</sup> h<sup>-1</sup>) were calculated from the temporal increase in gas concentration inside the chamber per unit time. Cumulative N<sub>2</sub>O and CO<sub>2</sub> evolutions were calculated by multiplying the daily fluxes at each measurement with the time interval and summing up the obtained values (Singla et al. 2013).

#### Soil sampling, chemical analyses, and plant biomass analysis

Soil samples (in triplicate) were collected from the 0–20 cm layer at day 5, 30, 37, 62, and 92 of the experiment and analysed for NH<sub>4</sub><sup>+</sup>-N and NO<sub>3</sub><sup>-</sup>-N contents after extraction with 1 M KCl solution (1:5; soil:solution) by using the nitroprusside (Anderson and Ingram 1989) and hydrazine-reduction methods (Carole and Scarigelli 1971), respectively. The SOC content of soil was determined using a TOC analyzer (TOC-5000, Shimadzu, Kyoto, Japan) after soil

**Table 1** Chemical properties of biogas digested slurry based biochar

Parameters	Value	Parameters	Value
pH (H <sub>2</sub> O)	8.81	Cd (mg kg <sup>-1</sup> )	0.4
EC (H <sub>2</sub> O) (mS m <sup>-1</sup> )	386	Cr (mg kg <sup>-1</sup> )	3.7
Total C (%)	31.72	Cu (mg kg <sup>-1</sup> )	76
Total N (%)	3.4	Pb (mg kg <sup>-1</sup> )	2.5
NH <sub>4</sub> <sup>+</sup> -N (µg g <sup>-1</sup> )	19.90	Hg (mg kg <sup>-1</sup> )	0.1
NO <sub>3</sub> <sup>-</sup> -N (µg g <sup>-1</sup> )	2.20	Mo (mg kg <sup>-1</sup> )	0.02
P <sub>2</sub> O <sub>5</sub> (%)	6.4	Ni (mg kg <sup>-1</sup> )	2.1
K <sub>2</sub> O (%)	2.2	Zn (mg kg <sup>-1</sup> )	317
H (%)	2.5	B (mg kg <sup>-1</sup> )	5
Total mg (%)	2.2	Cl (mg kg <sup>-1</sup> )	10
As (mg kg <sup>-1</sup> )	0.5	Na (mg kg <sup>-1</sup> )	25

**Table 2** Total application rate ( $\text{g m}^{-2}$ ) and sources of N, P, K and C in each treatment

Treatments	Total N ( $\text{NH}_4^+\text{-N}$ ) application ( $\text{g m}^{-2}$ ); source	Total P ( $\text{P}_2\text{O}_5$ ) application ( $\text{g m}^{-2}$ ); source	Total K ( $\text{K}_2\text{O}$ ) application ( $\text{g m}^{-2}$ ); source	Total C application ( $\text{g m}^{-2}$ ); source
Control	— <sup>a</sup>	12; calcium superphosphate	12; potassium chloride	— <sup>a</sup>
CF	12; mineral fertilizer	12; mineral fertilizer	12; mineral fertilizer	— <sup>a</sup>
DL	12; DL	12; calcium superphosphate	28; DL	— <sup>b</sup>
BL	12; DL	12; biochar + calcium superphosphate	28; DL	18; biochar
BM	12; DL	12; biochar + calcium superphosphate	28; DL	36; biochar
BH	12; DL	12; biochar	28; DL	72; biochar

<sup>a</sup>No external addition

<sup>b</sup>The small fraction of C of the DL was not taken into consideration as DL was applied in DL, BL, BM and BH treatments

CF chemical fertilizer, DL digested liquid, BL biochar at low concentration, BM biochar at medium concentration, BH biochar at high concentration

extraction with 0.5 M  $\text{K}_2\text{SO}_4$  (1:5; soil:solution) (Vance et al. 1987). The dry weight of the harvested shoots was measured after drying the plant material at 80 °C for 7 days in an air-oven. Yield ( $\text{g m}^{-2}$ ) was calculated by summing up the dry weight of three crops.

### Statistical analyses

Significance of treatments was tested by one-way analysis of variance (ANOVA), and Tukey's HSD test (at  $P < 0.05$ ) was applied for the differences in mean values. All the statistical analyses were completed using SPSS Statistics 20 (IBM, New York, USA).

## Results

### $\text{N}_2\text{O}$ and $\text{CO}_2$ flux

The highest  $\text{N}_2\text{O}$  fluxes occurred during the initial phase after fertilizer amendments (Fig. 1a) also affected by the soil temperature (Fig. 1c). Biochar-treated soil emitted the highest amount of  $\text{N}_2\text{O}$ , and it significantly increased with the increase in biochar concentration (Table 3). The DL- and CF-treated soils showed a very similar emission pattern and an equal cumulative  $\text{N}_2\text{O}$  evolution (Table 3). During the 2nd cropping cycle, all amended soils behaved in the same way regarding magnitude and occurrence of  $\text{N}_2\text{O}$  emissions, whereas no longer peak was observed during the 3rd cropping cycle in either soil.

In contrast to the  $\text{N}_2\text{O}$  flux, the  $\text{CO}_2$  flux in each treatment was to a higher extent influenced by the soil temperature, irrespective of the timing of fertilizer application (Fig. 1b, c). Soil temperature was highest during the 3rd cropping cycle, and the highest  $\text{CO}_2$  fluxes could accordingly be observed during that period. Similar to the  $\text{N}_2\text{O}$  emissions, DL- and CF-treated soils showed a similar emission pattern during each

cropping cycle. Also, their cumulative  $\text{CO}_2$  evolution during 92 days of the experiment was equal and significantly higher ( $P < 0.05$ ) than in the control soil (Fig. 1b). The seasonal and cumulative  $\text{CO}_2$  evolution was highest ( $P < 0.05$ ) in biochar-treated soils (Fig. 1b), and it increased with the increase in the concentration applied (Table 3).

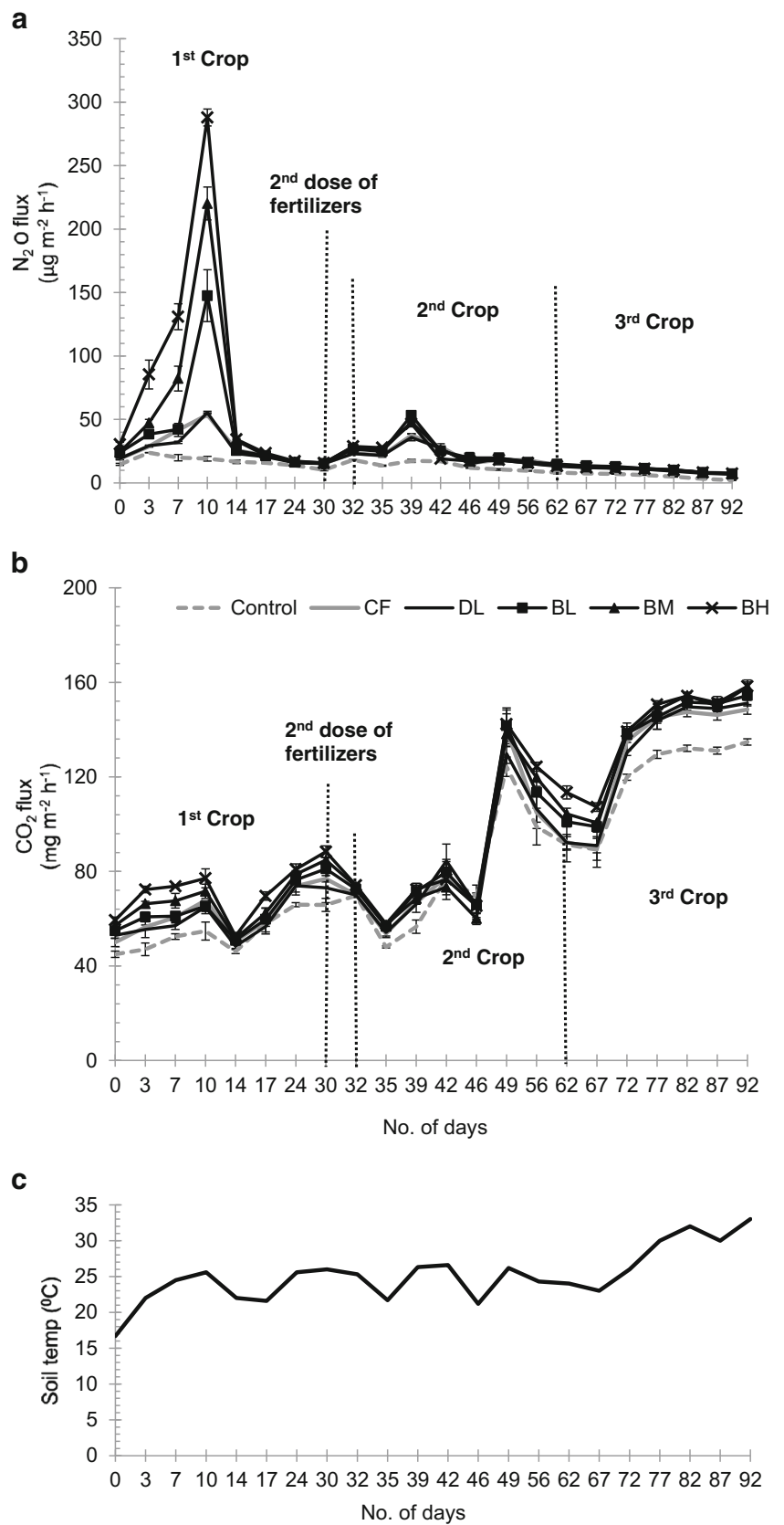
### Soil mineral-N and SOC dynamics

The  $\text{NH}_4^+\text{-N}$  content of biochar-treated soils was lower than in CF- and DL-treated soils at day 5, 37, and 62; while no significant difference was observed between the amended soils at day 30 and 92 (Fig. 2a). The  $\text{NO}_3^-\text{-N}$  content of biochar-treated soils was higher than in CF- and DL-treated soils at day 5, 30, 37 and 62; while no significant difference was observed between the amended soils at the last sampling day (Fig. 2b). The  $\text{NO}_3^-\text{-N}$  content was always higher than  $\text{NH}_4^+\text{-N}$  content regardless of the treatments (Fig. 2a, b). The SOC content of each treated soil was highest at the beginning of the experiment and decreased with time. The SOC content of biochar-treated soils was significantly higher ( $P < 0.05$ ) than in all other treatments at each measurement, and it increased with the increase in biochar concentration except for BM and BH at the last sampling day (Fig. 2c). The SOC content of the DL-treated soil was significantly higher than in CF-treated soil, except at day 30.

### Plant biomass and crop yield

Each N treatment provided higher plant biomass and crop yield than the control containers (Table 3). The application of biochar had negative impacts on plant biomass and crop yield, depending on the application rate. The BL- and DL-treated containers had the significantly highest plant biomass in the 2nd cropping cycle and also the highest ( $P < 0.05$ ) overall crop yield compared to the control, CF, BM and BH. The BM-treated containers had lower plant biomass and crop yield compared to DL and BL treatments; while BM and BH

**Fig. 1** Changes in N<sub>2</sub>O (mean±SD, n=3) (a), CO<sub>2</sub> (mean±SD, n=3) (b) and soil temperature (c) across the different treatments in komatsuna vegetated soils. Bars represent SD



**Table 3** Cumulative N<sub>2</sub>O and CO<sub>2</sub> evolution and plant yield due to application of the chemical fertilizer, digested liquid and varying concentrations of biochar

Treatments	Cumulative N <sub>2</sub> O evolution (mg m <sup>-2</sup> )	Cumulative CO <sub>2</sub> evolution (g m <sup>-2</sup> )	Dry weight (g plant <sup>-1</sup> )			Yield <sup>a</sup> (g m <sup>-2</sup> )
			1st crop	2nd crop	3rd crop	
Control	25.1±0.3 e	186.8±2.3 e	3.13±0.17 c	4.07±0.14 d	2.63±0.08 b	366±05 d
CF	42.9±0.8 d	204.3±2.1 d	5.29±0.13 b	6.37±0.14 bc	3.35±0.07 a	559±08 c
DL	41.1±0.1 d	203.1±1.2 d	6.04±0.44 a	7.71±0.88 a	3.39±0.27 a	638±54 ab
BL	52.3±1.8 c	212.0±1.2 c	6.28±0.32 a	7.89±0.44 a	3.76±0.04 a	667±29 a
BM	63.6±1.4 b	215.5±0.7 b	5.99±0.24 ab	6.11±0.20 c	3.63±0.23 a	585±15 c
BH	77.9±2.1 a	223.5±1.5 a	6.05±0.20 a	6.49±0.22 b	3.79±0.40 a	608±23 bc

CF chemical fertilizer, DL digested liquid, BL biochar at low concentration, BM biochar at medium concentration, BH biochar at high concentration

<sup>a</sup>Yield was calculated by summing up the dry weight of three crops

Different letters in each column denote significant differences ( $p < 0.05$ ) among the treatments according to a Tukey's HSD test. Mean value±standard deviation ( $n=3$ )

had equal plant biomass compared to CF treatment. The plant biomass in each container was the highest in the 2nd cropping cycle, and it was the lowest in the 3rd cropping cycle.

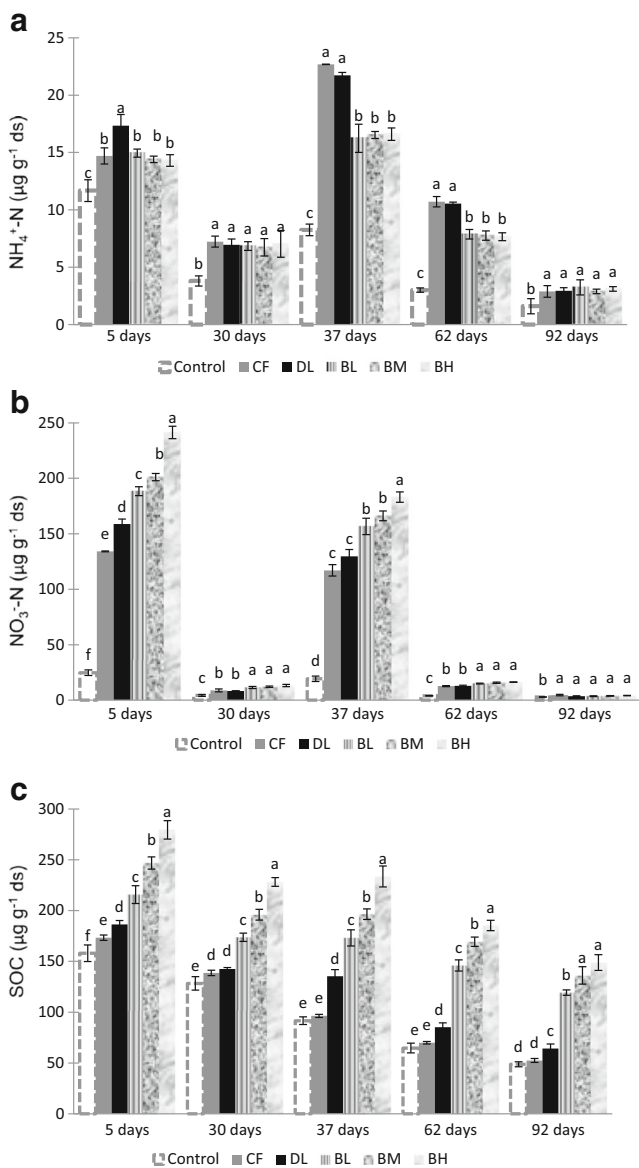
## Discussion

### N<sub>2</sub>O flux, CO<sub>2</sub> flux, soil mineral-N and SOC dynamics

N<sub>2</sub>O is produced in soil mainly by the microbial processes of nitrification and denitrification under the control of many factors, including mineral N and available C contents, O<sub>2</sub> partial pressure, soil moisture, pH and temperature, etc. (Inubushi et al. 1996; Hayakawa et al. 2009). Nitrification is the main process for N<sub>2</sub>O production at 60–80 % WHC, while denitrification is the major contributor at 90–100 % WHC (Inubushi et al. 1996). Nitrification is presumed to be the major contributor for the higher N<sub>2</sub>O evolution in the initial phase after the N application in each treatment (Fig. 1a) since the WHC of the soil was set to 60 % throughout the entire experiment. The increase in NO<sub>3</sub><sup>-</sup>-N contents (Fig. 2a, b) of soils also indicate that nitrification mainly occurred in the initial stages and could have contributed to the higher N<sub>2</sub>O evolution in the initial than later stages. Organic materials, such as sewage sludge compost, animal manure, crop residues, municipal solid wastes and compost can enhance emission of N<sub>2</sub>O to the atmosphere compared to inorganic or mineral fertilizers (Hayakawa et al. 2009; Alotaibi and Schoenau 2013). The increase in NO<sub>3</sub><sup>-</sup>-N and decrease in NH<sub>4</sub><sup>+</sup>-N contents of biochar-treated soils compared to biochar non-treated soils (Fig. 2a, b) indicated that nitrification rate was increased under biochar application which also increased N<sub>2</sub>O evolution with respect to biochar non-treated soils (Fig. 1a). In a recent microcosms study, it was observed that nitrification rate was increased by biochar application especially during the initial

phase of the experiment because of increase in the abundance of nitrifiers (Song et al. 2014). Other reports have also observed the increase in N<sub>2</sub>O flux after applying biochar (Yanai et al. 2007; Bruun et al. 2011; Saarnio et al. 2013).

The decrease in NH<sub>4</sub><sup>+</sup>-N and NO<sub>3</sub><sup>-</sup>-N content of soils at 30, 62, and 92 days indicates that probably denitrification also occurred along with nitrification. Another reason for decrease in mineral-N content of soils could also be due to plant uptake. Muller et al. (2004) reported that NO<sub>3</sub><sup>-</sup>-N reduction through denitrification is the predominant mechanism responsible for N<sub>2</sub>O production even under aerobic soil conditions. Thus, despite favourable conditions for nitrification, temporary anaerobic microsites may have been present causing denitrification in the later stages (Cannavo et al. 2004). Nitrification may be stimulated around the soil-pellet interface, where conditions are aerobic, and denitrification may be stimulated within the pellets, where anaerobic conditions prevail. It usually happens because of presence of moisture which convert microaerophilic zone into anaerobic zone (Cabrera et al. 1994). Organic fertilizers supply not only N but also organic C, which can enhance denitrification activity in soil by stimulating heterotrophic microbial activity (Velthof et al. 2002). This mechanism generally involves the growth of microbes that are adapted to respond quickly to newly available C sources, remineralizing soil nutrients and co-metabolizing more refractory organic matter in soil (Kuzayakov 2010). However, C/N ratio in the DL was low (0.57) in the present study, and similar to other studies (Alotaibi and Schoenau 2013; Singla et al. 2013), addition of material with a low C/N ratio will not be enough to affect N mineralization and increase N<sub>2</sub>O and CO<sub>2</sub> emissions compared to inorganic fertilizers (Fig. 1a, b). Biochar has been reported to produce positive and negative impacts on N<sub>2</sub>O emission (Yanai et al. 2007; Bruun et al. 2011; Jones et al. 2012; Kammann et al. 2012; Saarnio et al. 2013). Changes in the mineralization rate of organic matter in soil, and the decomposition of biochar itself may also affect N cycling in soil. Bruun et al. (2012)



**Fig. 2** Changes in exchangeable  $\text{NH}_4^+\text{-N}$  (mean  $\pm$  SD,  $n=3$ ) (Fig. 2a),  $\text{NO}_3^-\text{-N}$  (mean  $\pm$  SD,  $n=3$ ) (Fig. 2b) and SOC (mean  $\pm$  SD,  $n=3$ ) (Fig. 2c) contents of soils across the different treatments. Bars represent SD

found that biochar from fast pyrolysis led to net immobilization of mineral N whereas biochar from slow pyrolysis (like in our study) led to net N mineralization. These could be the possible reasons for significantly increasing  $\text{N}_2\text{O}$  evolution with the increase in biochar concentration (Fig. 1a; Table 3). It is well known that multiple factors contribute to GHG emissions in biochar-treated soils. Besides the physical and chemical properties, GHG emissions largely depend on the production procedure of the biochar, the type of the biomass used for pyrolysis, chemical properties of biochar and soil properties (Lehmann 2007). The raw material used for biochar preparation in the study of Bruun et al. (2012) was wheat straw which reduced  $\text{N}_2\text{O}$  emission under fast pyrolysis. The raw material of biochar preparation for increasing  $\text{N}_2\text{O}$  emission was

manure (Singh et al. 2010) and corn in the study of Xie et al. (2013). The observations of Singh et al. (2010) could be more closely related with our study due to closeness in the material used for biochar preparation. So, the raw material and slow pyrolysis conditions for biochar preparation increased gases production in the present study (Fig. 1a, b).

The important reason for increasing  $\text{CO}_2$  production by biochar application is assumed to be the increase in the soil respiration rate which has been attributed to microbial decomposition of labile components of biochar or potentially to an abiotic release of C (Zimmerman et al. 2011; Jones et al. 2012). Many studies have shown an increase in SOC contents after biochar application (Kolb et al. 2009; Prabha et al. 2013; Xie et al. 2013). Some researchers have suggested that biochar additions to soil have the potential to induce a positive priming effect with an increase in the decomposition of resident soil organic matter (SOM). In a 10-year buried bag incubation, Wardle et al. (2008) found little observable biochar degradation over the incubation period, but found that biochar appeared to stimulate the decomposition of the soil humus. The effect of biochar on the mineralization of SOM depends on the production temperature: biochar produced at low temperatures (250–400 °C) stimulate C mineralization whereas biochar produced at high temperatures (525–650 °C) suppress C mineralization in the soil (Saarnio et al. 2013). It has been observed that biochar produced at high temperatures had a more pronounced degree of aromaticity and a lower carboxyl group content which results in greater chemical recalcitrance and stability compared to biochar produced at low temperatures (Bruun et al. 2008). Biochar produced at low temperatures contain incomplete pyrolyzed carbohydrates (Bruun et al. 2011). Our study fit in this conclusion as our biochar was produced at rather low temperature (330–370 °C) and could be stimulating C mineralization, thus enhancing  $\text{CO}_2$  evolution (Fig. 1 b; Table 3). Bruun et al. (2011) found that the addition of N-rich biogas slurry did not increase  $\text{CO}_2$  production compared to unamended soils, which was increased by increasing biochar concentration as shown in our study. Decomposition of incomplete pyrolyzed carbohydrates and C availability resulting from biochar addition increased  $\text{CO}_2$  production (Fig. 1b).

#### Plant biomass and crop yield

Biochar can increase (Kammann et al. 2012; Rajkovich et al. 2012; Saarnio et al. 2013; Singla and Inubushi 2014a) or decrease (Zwieten et al. 2010; Rajkovich et al. 2012; Xie et al. 2013) crop yield depending on the raw material and pyrolysis conditions used for its preparation, soil type, crop type and many other environmental factors (Kolb et al. 2009; Bruun et al. 2012). The inputs of organic C can stimulate soil microbial activity, and thus nutrient recycling (Antil et al. 2009). Additionally, the activities of certain enzymes e.g., alkaline

phosphatase, aminopeptidase, *N*-acetylglucosaminidase and urease have been reported to increase with organic matter additions to soil (Goyal et al. 1999; Bailey et al. 2010). The same was found for DL treatment to soil (Singla et al. 2013; Singla and Inubushi 2014b) and BL treatment in the present study. Both treatments increased crop yield (Table 3) compared to CF treatment (Fig. 2c).

Some researchers have observed that biochar application to soil increased crop yield and decreased N<sub>2</sub>O evolution (Kammann et al. 2012; Saarnio et al. 2013). Interestingly, it was also observed that if inputs of biochar increased N<sub>2</sub>O evolution, then it did not increase the yield (Xie et al. 2013). It was found true in our study for BM and BH treatment. Both treatments produced lesser yield compared to DL and BL treatment; but both were not significantly different from CF treatment (Table 3). Rajkovich et al. (2012) reported a decrease in yield from higher application of biochar prepared from dairy manure, paper sludge and food waste, while Zwieten et al. (2010) reported varying results with paper mill waste based-biochar depending on the crop type. The higher N<sub>2</sub>O-N losses through nitrification/denitrification (Xie et al. 2013) could be one of the probable reasons for not increasing the yield with higher concentrations of biochar (Fig. 1a). Adsorption of mineral-N to biochar surface could be another mechanism responsible for the decrease of plant yield with high concentrations of biochar (Steiner et al. 2008), since it may reduce N uptake by plants (Rajkovich et al. 2012; Xie et al. 2013). It is also known that N is the most important nutrient limiting plant growth in farming systems (Ebid et al. 2008; Amkha et al. 2009; Singla et al. 2013). This also explains the lowest crop yield in the control (Table 3). Another possible reason for affecting the plant growth by higher application of biochar could be the presence of Na in the biochar (Table 1). The reduction of plant growth by Na is due to the increase in osmotic potential with reduction in water uptake by plant (Rajkovich et al. 2012).

## Conclusion

The effects of digested liquid on N<sub>2</sub>O and CO<sub>2</sub> evolution and on the soil mineral-N content were almost equal to that of the chemical fertilizer. However, the digested liquid-treated soils showed higher soluble organic C content and overall yield than the chemical fertilizer treated soils. This indicates that komatsuna cropping under fertilization with the digested liquid can be a possible solution for fertilizer management even if a field experiment is needed to confirm these results. The effects of biochar were variable depending on its application rate. Each application rate of biochar increased soluble organic C content of soil, N<sub>2</sub>O and CO<sub>2</sub> evolution. Biochar at low

application rate did not affect the crop yield, which was decreased at medium and high application rates.

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