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ORIGINAL ARTICLE

Effects of charcoal addition on N_2O emissions from soil resulting from rewetting air-dried soil in short-term laboratory experiments

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Abstract

Laboratory experiments were conducted to examine the effect of charcoal addition on N_2O emissions resulting from rewetting of air-dried soil. Rewetting the soil at 73% and 83% of the water-filled pore space (WFPS) caused a N_2O emission peak 6 h after the rewetting, and the cumulative N_2O emissions throughout the 120-h incubation period were 11 ± 1 and 13 ± 1 mg N m^{-2}, respectively. However, rewetting at 64% WFPS did not cause detectable N_2O emissions (-0.016 ± 0.082 mg N m^{-2}), suggesting a severe sensitivity to soil moisture. When the soils were rewetted at 73% and 78% WFPS, the addition of charcoal to soil at 10 wt% supressed the N_2O emissions by 89% . In contrast, the addition of the ash from the charcoal did not suppress the N_2O emissions from soil rewetted at 73% WFPS. The addition of charcoal also significantly stimulated the N_2O emissions from soil rewetted at 83% WFPS compared with the soil without charcoal addition ($P < 0.01$). Moreover, the addition of KCl and K_2SO_4 did not show a clear difference in the N_2O emission pattern, although Cl^- and SO_4^{2-}, which were the major anions in the charcoal, had different effects on N_2O-reducing activity. These results indicate that the suppression of N_2O emissions by the addition of charcoal may not result in stimulation of the N_2O-reducing activity in the soil because of changes in soil chemical properties.

Key words: denitrification, K fertilization, liming, N_2O-reducing activity, rewetting effect.

INTRODUCTION

N_2O is an important greenhouse gas produced in soil (Bouwman 1990). It has a destructive potential in the stratospheric ozone layer (Crutzen 1981). Nitrification and denitrification are the most important biological processes in the production of N_2O in soil. Denitrification is identically the sole process associated with N_2O reduction (Zumft 1997).

In a previous study, we examined the effects of soil amendments (liming material, inorganic salts and charcoal) on the N_2O-reducing activity of denitrifying communities (Yanai *et al.*). We found that charcoal and its ash, which had a high content of alkali and inorganic salts (Nerome *et al.* 2005), seemed to promote the growth activity and N_2O-reducing activity of denitrifying communities and that liming and anions affected these activities more than cations. These results suggest that N_2O emissions from soil can be affected by certain soil amendments because of the modifying activity of N_2O reduction, assuming that N_2O emissions from soil through denitrification depend on the balance of the N_2O-producing and N_2O-reducing activity of denitrifying communities (Cavigelli and Robertson 2001). In fact, Inubushi *et al.* (1999) and Azam and Müller (2003) observed stimulation of N_2O emissions from soil by the addition of NaCl in a laboratory incubation study, and this result can be explained by the suppression of N_2O-reducing activity by Cl^- or Na^+ (Yanai *et al.*). In contrast, the effects of liming on N_2O emissions from soil are inconsistent in field studies (Borken and Brumme 1997; Butterbach-Bahl *et al.* 1997; Klemedtsson *et al.* 1997; Mosier *et al.* 1998; Tokuda and Hayatsu 2004; Wang *et al.* 1997) and in laboratory incubation studies (Borken *et al.* 2000; Clough *et al.* 2003; Clough *et al.* 2004; Khalil *et al.* 2003).

Pulses of N_2O emission have been observed in field studies following irrigation and precipitation events

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(e.g. Kusa *et al.* 2006; Ruser *et al.* 2001) and have been reproduced in laboratory incubation experiments as a rewetting of dry soil (Rudaz *et al.* 1991). Rudaz *et al.* (1991) and Ruser *et al.* (2006) investigated the contribution of nitrification and denitrification to the production of N_2O emitted after rewetting using the C_2H_2 addition method and ^{15}N tracing technique, respectively, and concluded that the N_2O was mainly produced through denitrification. In the present study, to examine the relationship between enhancing the N_2O-reducing activity of denitrifying communities and N_2O emissions from soil, we examined the effects of charcoal and anion species on N_2O emissions caused by the rewetting of air-dried soil in the laboratory.

MATERIALS AND METHODS

Soil samples and charcoal

The soil samples examined were the same as those used in our previous study (Yanai *et al.*). Soil sampling was conducted at the Field Museum Tsukui, the Field Science Center of Tokyo University of Agriculture and Technology, Tokyo, Japan. Soil samples were collected from a grassland field in which one side was planted with *Sorghum bicolor* (L.) Moench and the other with *Sorghum sudanense* (Piper) on April 2004 and March 2005, respectively. The soil is classified as Typic Hapludand, and the soil texture is loam to clay loam at a depth of 0–40 cm, with a granular structure (Kurokawa, pers. comm.). After collection, moist soil samples were passed through a 2-mm mesh sieve, and part of the sample was then air-dried. Selected physico-chemical properties of the soil samples are listed in Table 1. Soil pH (H_2O) value was determined in a 1:2.5 air-dried soil (weight) to deionized water (volume) ratio. Total carbon and nitrogen contents of the soils were determined using the dry combustion method using a CN CORDER MT-700 (Yanaco, Kyoto, Japan). Water soluble organic C and NO_3^--N contents were determined using a TOC meter (TOC-V_{CSH}, SHIMADZU Co. Ltd., Kyoto, Japan) and an ion chromatograph (LC-20AT, SHIMADZU Co. Ltd., Kyoto, Japan), respectively, in 1:10 extracts (air-dried soil to deionized water w/v) at 240 rpm for 30 min. The population density of denitrifiers in the air-dried soil samples was determined using the most probable number method in five replicates of 10-fold serial dilution (Tiedje 1994). Maximum water-holding capacity (MWHC) was determined using the Hilgard method. Particle density was determined using the pycnometer method (Blake and Hartge 1986). Water-filled pore space (WFPS) was calculated as follows:

$$\text{WFPS} = (\text{Gravimetric water content}/\rho H_2O) \cdot (\text{Bulk density}/\text{Porosity})$$

where Porosity = 1 – (Bulk density/Particle density). In the present study, we set the density of water (ρH_2O) at 1 g cm^{-3}.

The charcoal, which was made from municipal biowaste, was provided by JFE Holdings. The physico-chemical properties of the charcoal are listed in Yanai *et al.* and its potential usefulness for cultivation was demonstrated by Nerome *et al.* (2005). Some selected physico-chemical properties of the charcoal and its ash, which was obtained by heating at 700°C for 4 h (as the test of weight loss-on-ignition; LOI), are listed in Table 2. Charcoal and ash pH (H_2O) values were determined in a 1:5 air-dried material to deionized water ratio (w/v). The MWHC and particle density were determined as described above. Anion

Table 1 Selected physico-chemical properties of the air-dried soils examined in this study (oven-dry basis)

Abbreviation of soil name	Date of sampling	pH (H_2O)	Total C (mg C g^{-1})	Total N (mg N g^{-1})	C/N ratio	WSOC	NO_3^--N	Denitrifiers (log MPN g^{-1})	Water content	MWHC	Bulk density	Specific gravity	Porosity (cm^3 cm^{-3})
						(µg g^{-1})			(g H_2O g^{-1})		(g cm^{-3})		
TG2004	Apr. 2004	6.0	69.6	5.62	12.4	155	45.8	6.5	0.15	1.21	0.58	2.05	0.72
TG2005	Mar. 2005	5.4	70.3	5.45	12.9	74	6.4	6.2	0.13	1.11	0.59	2.03	0.71

MPN, most probable number; MWHC, maximum water-holding capacity; WSOC, water soluble organic carbon.

Table 2 Selected physico-chemical properties of charcoal and its ash examined in this study

	pH (H_2O)[†]	LOI (%)	Water content (g H_2O g^{-1})	MWHC (g H_2O g^{-1})	Bulk density (g cm^{-3})	Particle density (g cm^{-3})	Anion content (µmol g^{-1})[‡]		
							Cl^-	NO_3^-	SO_4^{2-}
Charcoal	9.3	38	0.14	1.38	0.50	1.64	510	0	9
Ash	11.6	–	0.03	–	0.46	–	1240	2	80

[†] 1:5 ratio. [‡] Air-dried material basis in Yanai *et al.* LOI, weight loss-on-ignition; MWHC, maximum water-holding capacity; –, not determined.

contents (Cl^-, NO_3^- and SO_4^{2-}) in these materials were determined using an ion chromatograph (LC-20AT, SHIMADZU Co. Ltd. Kyoto, Japan) in 1:20 extracts (air-dried material to deionized water w/v) at 240 rpm for 30 min.

Measurement of N_2O emissions from soil after rewetting

To simulate the thin surface layer in arable fields, where the soil could be subjected to air-drying following continuous clear weather, 30 g of sieved air-dried soil was placed in a Petri dish (1.3 cm height and 8.5 cm diameter) without compaction. As a result, the thickness of the soil was approximately 0.8 cm. To simulate the condition of the soil during or immediately after precipitation, distilled water was added into the soil samples in the Petri dishes to more than 70% of their MWHC (equivalent to 64% of the water-filled pore space [WFPS]). Immediately after rewetting the soil sample, N_2O emissions were periodically measured using the closed-chamber method (Hutchinson and Mosier 1981). A clear glass bell-jar (14 cm width, 26 cm height, 2.32 L) was used as a gas-tight chamber to monitor the concentration change in the headspace gas. The inlet and outlet of the bell-jar were sealed with a rubber stopper and a rubber septum, respectively, and the bottom part of the bell-jar was tightly attached with a ground glass-plate using a high vacuum-sealing compound (HIVAC-G, Shin-Etsu Chemical Co. Ltd., Tokyo, Japan). A pressure-controlling bent (Hutchinson and Mosier 1981) was installed at the rubber stopper in the inlet and gas samples were collected through the rubber septum installed at the outlet. After placing the chamber onto the soil sample, headspace gas was withdrawn five times at 2 or 8 min intervals, depending on the rate of concentration change. N_2O concentration in the collected gas sample was analyzed using a gas chromatograph (GC-14A, SHIMADZU, Kyoto, Japan) equipped with an electron capture detector and a stainless steel column packed with Porapak-Q (80/100 mesh, 3 mm diameter, 2 m length). The column and detector temperatures were kept at 90°C and 330°C, respectively. Argon containing 5% CH_4 was used as a carrier gas at a flow rate of 23 mL min^{-1}. The N_2O emission rate was calculated using the linear regression method (Hutchinson and Mosier 1981). After measurement of the N_2O emission rate, the chamber was removed and the soil sample was left at room temperature (approximately 20–28°C) without a lid on the Petri dish. The water content was maintained during the incubation period by adding distilled water. N_2O emissions were monitored until the first peak of N_2O emissions disappeared.

As a preliminary experiment, we examined the effect of moisture content after rewetting on the N_2O emissions from soil (TG2005). Distilled water was added into the soil samples at 17.0, 19.9 and 23.1 mL to adjust the rewetted condition to 70, 80 and 91% MWHC, equivalent to 64, 73 and 83% WFPS, respectively. This experiment was conducted in triplicate.

Effect of soil amendments on N_2O emissions from soil resulting from rewetting

Based on the result of the preliminary experiment (Fig. 1), the moisture content after rewetting was adjusted to more than 73% WFPS in this study. First, to simulate precipitation in grassland amended with charcoal in the surface layer, 2 mm-sieved charcoal was mixed with soil (TG2004) in three of six Petri dishes at 10 wt% (equivalent to 13 vol%) before rewetting, and the N_2O emissions were compared with the remaining three Petri dishes as the non-added control. The soil samples were added with distilled water to moisten the soil of the non-added control at 78% WFPS. Second, to test whether the effect of the charcoal addition on the N_2O emission results from the stimulation of N_2O-reducing activity by pH increase, the charcoal or its ash was mixed with soil (TG2005) in three of nine Petri dishes before rewetting, and three Petri dishes as the non-added control. The amounts of added charcoal and its ash were determined in order to set soil pH (H_2O) at 6.0, and the rate of addition was 8.2 and 1.6 wt%, equivalent to 9.7 and 2.0 vol%, respectively. The soil samples were rewetted by adding distilled water, which was necessary to

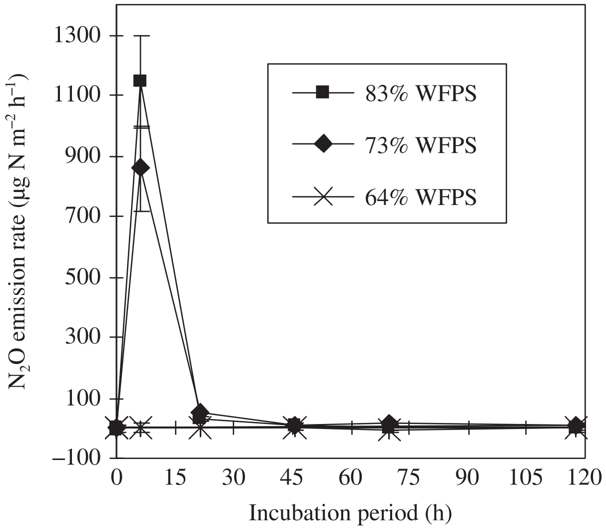

Figure 1 Effect of rewetting on N_2O emissions from soil (TG2005). An air-dried soil sample was rewetted using distilled water at 64 (×), 73 (◆) and 83% (■) of the water-filled pore space (WFPS) and incubated at room temperature. The values shown are the mean ± standard deviation of three replicates. The cumulative N_2O emissions during the 120-h incubation period at a rewetting level of 64, 73 and 83% WFPS were -0.016 ± 0.082, 11 ± 1 and 13 ± 1 mg N m^{-2} (equivalent to -0.003 ± 0.03, 2.3 ± 0.3 and 2.8 ± 0.4 µg N g^{-1}soil), respectively.

moisten the soil of the non-added control at 73% WFPS. Third, to estimate the interaction between the rate of charcoal addition and the moisture content after rewetting, N_2O emissions were compared with three levels of charcoal additions (0, 2 and 8.2 wt%, equivalent to 0, 2.4 and 9.7 vol%, respectively) in triplicate. Distilled water was added to the soil samples to moisten the soil of the non-added control (0% charcoal) at 83% WFPS. Finally, as Cl^- and SO_4^{2-} were not only the major anion species of the charcoal (Table 2), but also were applied into arable fields through fertilization, we examined the effect of anion species of K solution on N_2O emissions after rewetting. Of the nine Petri dishes containing the soil samples (TG2005), distilled water, 10 mmol L^{-1} KCl and 5 mmol L^{-1} K_2SO_4 solution were each added to three dishes to adjust to 73% WFPS of the soil, and the N_2O emissions were compared. The concentration of K solution was decided based on the concentration of K in a commercial liquid fertilizer (Otsuka Chemical Co. Ltd., Osaka, Japan), and the estimated load of Cl^- and SO_4^{2-} added with the charcoal, ash and K solution is listed in Table 3.

Calculation of the cumulative N_2O emission and statistical analysis

The cumulative N_2O emissions were estimated using the linear trapezoidal method, and the value was expressed as an arithmetic mean and standard deviation (SD). The level of significance of the treatments was examined using an unpaired *t*-test for TG2004 and by ANOVA followed by Tukey's multiple comparison tests for TG2005 ($P < 0.05$). If one of the mean values of the triplicates appeared to lose normality (mean – 2SD < 0), the original data were log-transformed before comparison (Bland and Peacock 2002).

RESULTS

N_2O emissions from soil after rewetting

N_2O emissions were not detected after rewetting at 64% WFPS, but were detected after rewetting at 73% and 83% WFPS, and the cumulative N_2O emissions throughout the 120-h incubation period at room temperature were –0.016 ± 0.082, 11 ± 1 and 13 ± 1 mg N_2O-N m^{-2} (–0.003 ± 0.03, 2.3 ± 0.3 and 2.8 ± 0.4 μg N_2O-N g^{-1}soil), respectively (Fig. 1). Rewetting over 73% WFPS triggered N_2O emissions, but there were no significant differences in the cumulative N_2O emissions between soils rewetted at 73% and 83% WFPS ($P = 0.180$).

Effects of charcoal addition on N_2O emissions after rewetting at 73% WFPS for TG2004

The highest N_2O emission rate was observed 30 h after rewetting, and the values were 2620 ± 460 and 383 ± 74 μg N m^{-2} h^{-1} in the treatments without and with charcoal addition, respectively (Fig. 2). The addition of

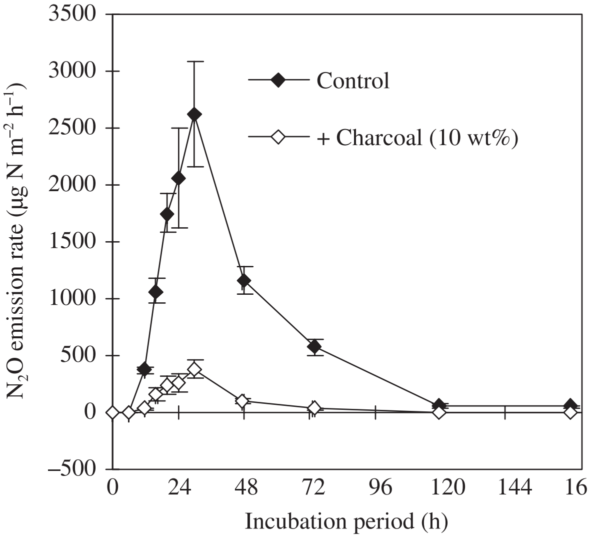

Figure 2 Effect of charcoal addition on N_2O emissions from soil (TG2004) rewetted at 78% of the water-filled pore space of the soil. The values shown are the mean ± standard deviation of three replicates. The cumulative N_2O emissions during the 168-h incubation period for the non-added control and the 10 wt% charcoal addition were 105 ± 14 and 11.1 ± 2.4 mg N m^{-2} (equivalent to 19.9 ± 2.7 and 2.1 ± 0.5 μg N g^{-1} soil), respectively.

Table 3 Estimation of Cl^- and SO_4^{2-} load onto soil (TG2005) by the addition of charcoal, ash and K solution

Application rate or concentration		Added into soil (μmol g^{-1} soil)		Concentration in soil solution (mmol L^{-1}) at			
				73% WFPS		83% WFPS	
		Cl^-	SO_4^{2-}	Cl^-	SO_4^{2-}	Cl^-	SO_4^{2-}
Charcoal	2 wt%	10	0.2	NA	NA	10	0.2
Charcoal	8.2 wt%	42	0.7	46	0.8	40	0.7
Ash	1.6 wt%	20	1.3	23	1.5	NA	NA
KCl	10 mmol L^{-1}	7.5	0	8.5	0	NA	NA
K_2SO_4	5 mmol L^{-1}	0	3.7	0	4.3	NA	NA

NA, not applicable with respect to the objectives of this study; WFPS, water-filled pore space.

charcoal decreased the N_2O emission peak by 85% of that of the control without charcoal. The cumulative N_2O emissions were 105 ± 14 and 11.1 ± 2.4 mg N m^{-2} (19.9 ± 2.7 and 2.1 ± 0.5 μg N g^{-1} soil) in the treatments without and with charcoal addition, respectively. The charcoal addition significantly decreased N_2O emissions by 89% of the control value without charcoal ($P < 0.01$).

Effects of liming (pH 6.0) with charcoal and its ash on N_2O emissions after rewetting at 73% WFPS for TG2005

The highest N_2O emission rate was observed at 12 h after rewetting in the non-added control and the ash-added soil (Fig. 3), but N_2O emissions were kept at a low level in the charcoal-added soil throughout the observation period (72 h). The cumulative N_2O emissions throughout the 72-h incubation period in the non-added control, ash-added and charcoal-added soils were 4.1 ± 1.9, 4.3 ± 1.2 and 0.8 ± 0.7 mg N m^{-2} (0.9 ± 0.4, 1.0 ± 0.3 and 0.2 ± 0.2 μg N g^{-1} soil), respectively. Charcoal addition decreased N_2O emissions by 80% of the value of the non-added control ($P < 0.05$), whereas ash addition did not.

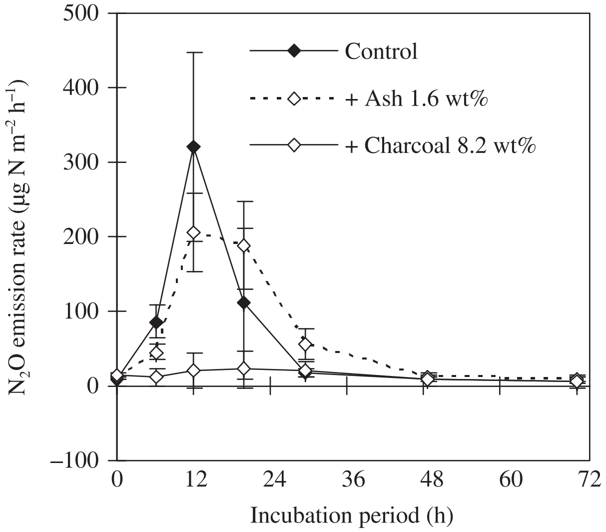

Figure 3 Effect of liming by using charcoal and its ash on N_2O emissions from soil (TG2005) rewetted at 73% of its water-filled pore space. The values shown are the mean ± standard deviation of three replicates. The cumulative N_2O emissions during the 72-h incubation period for the non-added control, ash-amended soil and charcoal-amended soil were 4.1 ± 1.9, 4.3 ± 1.2 and 0.8 ± 0.7 mg N m^{-2} (equivalent to 0.9 ± 0.4, 1.0 ± 0.3 and 0.2 ± 0.2 μg N g^{-1}soil), respectively.

Effects of charcoal addition on N_2O emissions after rewetting at 83% WFPS for TG2005

The N_2O emission rate at 6 h after rewetting was lower in the 2 and 8.2 wt% charcoal added-soils than in the non-added control, while the N_2O emission rate more than 12 h after the rewetting was higher in the 2 and 8.2 wt% charcoal added-soil than in the non-added control (Fig. 4). The cumulative N_2O emissions throughout the 72-h incubation period in the non-added control, and in the 2 and 8.2 wt% charcoal-added soil were 6.8 ± 0.9, 10.0 ± 0.8 and 10.3 ± 0.6 mg N m^{-2} (1.5 ± 0.2, 2.2 ± 0.2 and 2.4 ± 0.1 μg N g^{-1}soil), respectively. The addition of charcoal at 2 and 8.2 wt% significantly increased N_2O emissions by 47% and 51% of the values of the non-added control, respectively ($P < 0.01$).

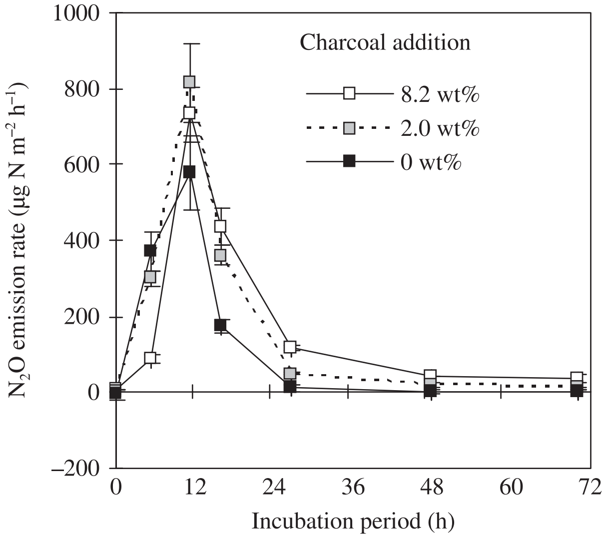

Figure 4 Effect of charcoal addition on N_2O emissions from soil (TG2005) rewetted at 83% of its water-filled pore space. The values shown are the mean ± standard deviation of three replicates. The cumulative N_2O emissions during the 72-h incubation period for the non-added control and the 2 and 8.2 wt% charcoal additions were 6.8 ± 0.9, 10.0 ± 0.8 and 10.3 ± 0.6 mg N m^{-2} (equivalent to 1.5 ± 0.2, 2.2 ± 0.2 and 2.4 ± 0.1 μg N g^{-1}soil), respectively.

Effects of KCl and K_2SO_4 on N_2O emissions after rewetting at 73% WFPS for TG2005

The highest N_2O emission rate was observed 12 h after rewetting (Fig. 5). The mean N_2O emission rate was higher in 10 mmol L^{-1} KCl than in 5 mmol L^{-1} K_2SO_4 and the non-added control, but considerable variability was observed in the KCl-added soil. The cumulative N_2O emissions throughout the 72 h incubation in the non-added control, 10 mmol L^{-1} KCl and 5 mmol L^{-1} K_2SO_4 were 2.9 ± 0.6, 5.3 ± 6.1 and 4.4 ± 1.5 mg N m^{-2} (0.6 ± 0.1, 1.2 ± 1.3, 1.0 ± 0.3 μg N g^{-1} soil) with CV values of 21, 115 and 34% (17, 108 and 30%), respectively. There were no significant differences between the control and the 10 mmol L^{-1} KCl ($P = 0.9986$) or 5 mmol L^{-1} K_2SO_4 additions ($P = 0.8559$), or between the 10 mmol L^{-1} KCl and 5 mmol L^{-1} K_2SO_4 additions ($P = 0.8794$).

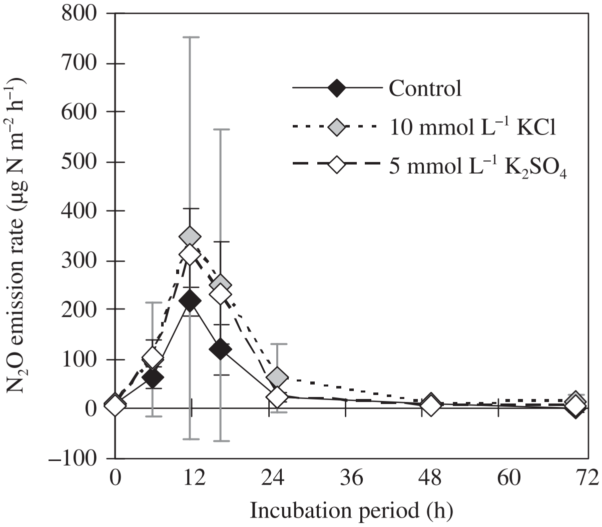

Figure 5 Effect of Cl$^-$ and SO$_4^{2-}$ of K salts on N$_2$O emissions from soil (TG2005) rewetted at 73% of its water-filled pore space. An air-dried soil sample was rewetted using distilled water (control) or a K solution and incubated at room temperature. The values shown are the mean ± standard deviation of three replicates. The cumulative N$_2$O emissions during the 72-h incubation period for the non-added control, the 10 mmol L^{-1} KCl-added soil and the 5 mmol L^{-1} K$_2$SO$_4$ added soil were 2.9 ± 0.6, 5.3 ± 6.1 and 4.4 ± 1.5 mg N m^{-2} (equivalent to 0.6 ± 0.1, 1.2 ± 1.3 and 1.0 ± 0.3 μg N g^{-1} soil), respectively.

DISCUSSION

The present study demonstrated that rewetting of air-dried soil at 73% WFPS caused significant N$_2$O emissions (Fig. 1), and the N$_2$O emissions were suppressed by the addition of charcoal (Figs 2,3). This suppression of the N$_2$O emissions was first considered to be a liming effect because charcoal has alkali (Table 2) and it had the potential to increase the N$_2$O-reducing activity of denitrifying communities (Cavigelli and Robertson 2000), which might cause a decrease in N$_2$O emissions (Cavigelli and Robertson 2001). Therefore, liming resulting from the ash was expected to have a similar potential for promoting N$_2$O-reducing activity to the charcoal itself. We checked the soil pH (1:2.5 ratio) after the observation of N$_2$O emission from soils to which charcoal and its ash had been added and there were no significant differences between these amendments (5.7 ± 0.03 and 5.6 ± 0.01, respectively, $P = 0.08$), but these treatments were significantly different from the non-added control (4.9 ± 0.2, $P < 0.01$, $n = 3$). However, the addition of ash did not suppress N$_2$O emissions (Fig. 4). Moreover, the suppressive effects of charcoal addition on N$_2$O emissions were not observed when the soils were rewetted at 83% WFPS (Fig. 4). These results indicate that soil pH amendments, which are intended to stimulate the N$_2$O-reducing activity, may not explain the suppression of the N$_2$O emissions from soil rewetted at 73% WFPS (Figs 2,3). In addition, irrespective of the inhibitory effects of Cl$^-$ and the stimulatory effects of SO$_4^{2-}$ on N$_2$O-reducing activity of denitrifying communities (Yanai *et al.*), there were no clear differences in the N$_2$O emissions when KCl and K$_2$SO$_4$ were added to the soils (Fig. 5). This finding could result from the use of concentrations (5 and 10 mmol L^{-1}) that were too low to affect the denitrifying communities (Table 3) because the effects of Cl$^-$ and were detected at more than 40 mmol L^{-1} in the liquid medium in our previous study (Yanai *et al.*). Nevertheless, these results suggested that amelioration of the chemical properties of soil in order to stimulate the N$_2$O-reducing activity may not be related to the suppression of the N$_2$O emissions from soil rewetted at 73% WFPS (Figs 2,3).

Increases in N$_2$O emission rates with increasing soil water contents have been reported from laboratory and field studies and have been attributed to increasing denitrifying activity induced by decreased O$_2$ diffusion into the soil (Ruser *et al.* 2006 and references therein). In the present study, we observed a similar trend, namely, that N$_2$O emissions increased with increases in the water content of soil by rewetting at 73% and 83% WFPS, whereas significant N$_2$O emissions were not detected by rewetting at 64% WFPS (Fig. 1). This result suggests that a decrease in the moisture conditions from 83% to 73% WFPS did not affect the denitrifying communities, while a decrease from 73% to 64% WFPS may result in a significant decrease in the anoxic microsites, which results in the suppression of denitrification. Thus, undetectable N$_2$O emissions from soil rewetted at 64% WFPS may not be the result of complete denitrification, including N$_2$O reduction to N$_2$, but, rather, to insufficient development of anoxic microsites in the soil to trigger denitrification. Possibly, this was caused by the soil sample TG2005, which had less denitrification activity because of a lower population density of denitrifiers, soil pH, and the amount of substrate compared with the soil sample TG2004 (Table 1). In addition, the decay of the N$_2$O emission rate in the later incubation period after rewetting may be the completion of N$_2$O production (stepwise reductions of NO$_3^-$, NO$_2^-$, and NO) rather than the kinetic equilibration of N$_2$O production and reduction followed by N$_2$ production. Ruser *et al.* (2006) observed few N$_2$ emissions after rewetting, indicating a low or undetectable contribution of N$_2$O-reducing activity in the later incubation period after rewetting. Therefore, the N$_2$O-reducing activity of denitrifying communities may not significantly affect N$_2$O emissions after rewetting of air-dried soil, suggesting that the suppressive effect of the charcoal addition on N$_2$O emissions (Figs 2,3) might result from inhibition of N$_2$O-producing activity of denitrifying communities.

Although there was no direct evidence to show a linkage between the addition of charcoal and the suppression of N_2O emissions from soil (Figs 2,3), the added charcoal itself probably absorbed water and improved the aeration of the soil, leading to a suppression of N_2O production (stepwise reduction of NO_3^-, NO_2^- and NO) similar to the soil rewetted at 64% WFPS (Fig. 1). In fact, the charcoal examined was made up of porous particles, whereas the ash was nearly pulverized. Such differences in the size and structure possibly affect the water absorption capacity of these materials, and may consequently cause differences in the soil aeration, the denitrification process, and N_2O emissions from soil, although the charcoal addition did not significantly affect the MWHC or the particle density (data not shown). Hence, the significant increases in N_2O emissions by the addition of charcoal to soil rewetted at 83% WFPS (Fig. 4) can be interpreted as an interaction between the insignificant improvement of the aeration of the soil and the stimulation of the N_2O-producing activity resulting from neutralization (e.g. Cavigelli and Robertson 2000).

Charcoal was examined in this study because of its potential use for soil amendments in temperate regions (Nerome *et al.* 2005) and in the tropics (Glaser *et al.* 2002; Yamato *et al.* 2006). Although any extrapolation of the findings from this short-term laboratory study to a long-term field scale should be conducted with caution, field applications of charcoal possibly suppress N_2O emissions from arable soil, depending on the moisture or aeration conditions of the soil. In contrast, our understanding of the process of suppressing N_2O emissions from soil by charcoal is still preliminary. Therefore, further studies are necessary to understand both the mechanisms and possible side-effects of charcoal addition to soil on the suppression of N_2O emissions from soil, such as the activity of NO_3^- assimilation, NO_2^- accumulation in soil or NO_x emissions from soil.

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REFERENCES

Azam F, Müller C 2003: Effect of sodium chloride on denitrification in glucose amended soil treated with ammonium and nitrate nitrogen. *J. Plant Nutr. Soil Sci.*, **166**, 594–600.

Blake GR, Hartge KH 1986: Particle density. *In* Physical and Mineralogical Methods. Ed. A Klute, pp. 377–382, Soil Science Society of America, Madison.

Bland M, Peacock J 2002: Normal distribution. *In* Statistical Questions in Evidence-based Medicine. Ed. K Adachi, pp. 79–85, Shinoharashinsha Press, Tokyo (in Japanese).

Borken W, Brumme R 1997: Liming practice in temperate forest ecosystems and the effects on CO_2, N_2O and CH_4 fluxes. *Soil Use Manage.*, **13**, 251–257.

Borken W, Grundel S, Beese F 2000: Potential contribution of *Lumbricus terrestris* L. to carbon dioxide, methane and nitrous oxide fluxes from a forest soil. *Biol. Fertil. Soils*, **32**, 142–148.

Bouwman AF 1990: Exchange of greenhouse gases between terrestrial ecosystems and the atmosphere. *In* Soils and the Greenhouse Effect. Ed. AF Bouwman, pp. 61–127. John Wiley, New York.

Butterbach-Bahl K, Gasche R, Breuer L, Papen H 1997: Fluxes of NO and N_2O from temperate forest soils: Impact of forest type, N deposition and of liming on the NO and N_2O emissions. *Nutr. Cycl. Agroecosys.*, **48**, 79–90.

Cavigelli MA, Robertson GP 2000: The functional significance of denitrifier community composition in a terrestrial ecosystem. *Ecology*, **81**, 1402–1414.

Cavigelli MA, Robertson GP 2001: Role of denitrifier diversity in rates of nitrous oxide consumption in a terrestrial ecosystem. *Soil Biol. Biochem.*, **33**, 297–310.

Clough TJ, Kelliher FM, Sherlock RR, Ford CD 2004: Lime and soil moisture effects on nitrous oxide emissions from a urine patch. *Soil Sci. Soc. Am. J.*, **68**, 1600–1609.

Clough TJ, Sherlock RR, Kelliher FM 2003: Can liming mitigate N_2O fluxes from a urine-amended soil? *Aust. J. Soil Res.*, **41**, 439–457.

Crutzen PJ 1981: Atmospheric chemical processes of the oxides of nitrogen, including nitrous oxide. *In* Denitrification, Nitrification and Atmospheric Nitrous Oxide. Ed. CC Delwiche, pp. 17–44. John Wiley, New York.

Glaser B, Lehmann J, Zech W 2002: Ameliorating physical and chemical properties of highly weathered soils in the tropics with charcoal – A review. *Biol. Fertil. Soils*, **35**, 219–230.

Hutchinson GL, Mosier AR 1981: Improved soil cover method for field measurement of nitrous oxide fluxes. *Soil Sci. Soc. Am. J.*, **45**, 311–316.

Inubushi K, Barahona MA, Yamakawa K 1999: Effects of salts and moisture content on nitrous oxide emission and nitrogen dynamics in Yellow soil and Andosol in model experiments. *Biol. Fertil. Soils*, **29**, 401–407.

Khalil MI, Van Cleemput O, Rosenani AB, Fauziah CI, Shamshuddin J 2003: Nitrous oxide formation potential of various humid tropic soils of Malaysia: A laboratory study. *Nutr. Cycl. Agroecosys.*, **66**, 13–21.

Klemedtsson L, Klemedtsson AK, Moldan F, Weslien P 1997: Nitrous oxide emission from Swedish forest soils in relation to liming and simulated increased N-deposition. *Biol. Fertil. Soils*, **25**, 290–295.

Kusa K, Hu R, Sawamoto T, Hatano R 2006: Three years of nitrous oxide and nitric oxide emissions from silandic andosols cultivated with maize in Hokkaido, Japan. *Soil Sci. Plant Nutr.*, **52**, 103–113.

Mosier AR, Delgado JA, Keller M 1998: Methane and nitrous oxide fluxes in an acid Oxisol in western Puerto Rico: Effects of tillage, liming and fertilization. *Soil Biol. Biochem.*, **30**, 2087–2098.

Nerome M, Toyota K, Islam T-MD *et al.* 2005: Suppression of bacterial wilt of tomato by incorporation of municipal biowaste charcoal into soil. *Soil Microorganisms*, **59**, 9–14 (in Japanese with English summary).

Rudaz AO, Davidson EA, Firestone MK 1991: Sources of nitrous oxide production following wetting of dry soil. *FEMS Microbiol. Ecol.*, **85**, 117–124.

Ruser R, Flessa H, Russow R, Schmidt G, Buegger F, Munch JC 2006: Emission of N_2O, N_2 and CO_2 from soil fertilized with nitrate: Effect of compaction, soil moisture and rewetting. *Soil Biol. Biochem.*, **38**, 263–274.

Ruser R, Flessa H, Schilling R, Beese F, Munch JC 2001: Effect of crop type-specific soil management and N fertilization on N_2O emissions from a fine-loamy soil. *Nutr. Cycl. Agroecosys.*, **59**, 177–191.

Tiedje JM 1994: Denitrifiers. *In* Microbiological and Biochemical Properties. Eds RD Weaver, JS Angle and PS Bottomley, pp. 245–267, Soil Science Society of America, Madison.

Tokuda S, Hayatsu M 2004: Nitrous oxide flux from a tea field amended with a large amount of nitrogen fertilizer and soil environmental factors controlling the flux. *Soil Sci. Plant Nutr.*, **50**, 365–374.

Wang YP, Meyer CP, Galbally IE, Smith CJ 1997: Comparisons of field measurements of carbon dioxide and nitrous oxide fluxes with model simulations for a legume pasture in southeast Australia. *J. Geophys. Res.*, **102**, 28 013–28 024.

Yamato M, Okimori Y, Wibowo IF, Anshori S, Ogawa M 2006: Effects of the application of charred bark of *Acacia mangium* on the yield of maize, cowpea and peanut, and soil chemical properties in South Sumatra, Indonesia. *Soil Sci. Plant Nutr.*, **52**, 489–495.

Yanai Y, Hatano R, Okazaki M, Toyota K. Chemical factors affecting the N_2O-reducing activity of denitrifying communities – Analysis of the C_2H_2 inhibition-based N_2O production curve of soil.

Zumft WG 1997: Cell biology and molecular basis of denitrification. *Microbiol. Mol. Biol. Rev.*, **61**, 533–616.