

ORIGINAL ARTICLE

Effects of charcoal addition on N₂O emissions from soil resulting from rewetting air-dried soil in short-term laboratory experimentsYosuke YANAI¹, Koki TOYOTA² and Masanori OKAZAKI²¹Graduate School of Bio-Applications and Systems Engineering and ²Institute of Symbiotic Science and Technology, Tokyo University of Agriculture and Technology, Tokyo 184-8588, Japan**Abstract**

Laboratory experiments were conducted to examine the effect of charcoal addition on N₂O emissions resulting from rewetting of air-dried soil. Rewetting the soil at 73% and 83% of the water-filled pore space (WFPS) caused a N₂O emission peak 6 h after the rewetting, and the cumulative N₂O 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 N₂O 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% suppressed the N₂O emissions by 89%. In contrast, the addition of the ash from the charcoal did not suppress the N₂O emissions from soil rewetted at 73% WFPS. The addition of charcoal also significantly stimulated the N₂O emissions from soil rewetted at 83% WFPS compared with the soil without charcoal addition ($P < 0.01$). Moreover, the addition of KCl and K₂SO₄ did not show a clear difference in the N₂O emission pattern, although Cl[−] and SO₄^{2−}, which were the major anions in the charcoal, had different effects on N₂O-reducing activity. These results indicate that the suppression of N₂O emissions by the addition of charcoal may not result in stimulation of the N₂O-reducing activity in the soil because of changes in soil chemical properties.

Key words: denitrification, K fertilization, liming, N₂O-reducing activity, rewetting effect.

INTRODUCTION

N₂O 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 N₂O in soil. Denitrification is identically the sole process associated with N₂O reduction (Zumft 1997).

In a previous study, we examined the effects of soil amendments (liming material, inorganic salts and charcoal) on the N₂O-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 N₂O-reducing activity of denitrifying

communities and that liming and anions affected these activities more than cations. These results suggest that N₂O emissions from soil can be affected by certain soil amendments because of the modifying activity of N₂O reduction, assuming that N₂O emissions from soil through denitrification depend on the balance of the N₂O-producing and N₂O-reducing activity of denitrifying communities (Cavigelli and Robertson 2001). In fact, Inubushi *et al.* (1999) and Azam and Müller (2003) observed stimulation of N₂O emissions from soil by the addition of NaCl in a laboratory incubation study, and this result can be explained by the suppression of N₂O-reducing activity by Cl[−] or Na⁺ (Yanai *et al.*). In contrast, the effects of liming on N₂O emissions from soil are inconsistent in field studies (Borken and Brumme 1997; Butterbach-Bahl *et al.* 1997; Klemmedtsson *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 N₂O 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 N₂O emitted after rewetting using the C₂H₂ addition method and ¹⁵N tracing technique, respectively, and concluded that the N₂O was mainly produced through denitrification. In the present study, to examine the relationship between enhancing the N₂O-reducing activity of denitrifying communities and N₂O emissions from soil, we examined the effects of charcoal and anion species on N₂O 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 (H₂O) 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 NO₃⁻-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:

$$\text{WFPS} = (\text{Gravimetric water content}/\rho_{\text{H}_2\text{O}}) \cdot (\text{Bulk density}/\text{Porosity})$$

where Porosity = 1 – (Bulk density/Particle density). In the present study, we set the density of water ($\rho_{\text{H}_2\text{O}}$) 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 (H₂O) 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 ₂ O)	Total C (mg C g ⁻¹)	Total N (mg N g ⁻¹)	C/N ratio	WSOC (µg g ⁻¹)	NO ₃ ⁻ -N (log MPN g ⁻¹)	Denitrifiers (log MPN g ⁻¹)	Water content		Bulk density (g cm ⁻³)	Specific gravity	Porosity (cm ³ cm ⁻³)
									(g H ₂ O g ⁻¹)	MWHC			
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 ₂ O) [†]	LOI (%)	Water content (g H ₂ O g ⁻¹)	MWHC (g H ₂ O 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⁻, NO₃⁻ and SO₄²⁻) 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 N₂O 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, N₂O 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. N₂O 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% CH₄ was used as a carrier gas at a flow rate of 23 mL min⁻¹. The N₂O emission rate was calculated using the linear regression method (Hutchinson and Mosier 1981). After measurement of the N₂O 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. N₂O emissions were monitored until the first peak of N₂O emissions disappeared.

As a preliminary experiment, we examined the effect of moisture content after rewetting on the N₂O 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 N₂O 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 N₂O 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 N₂O emission results from the stimulation of N₂O-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 (H₂O) 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

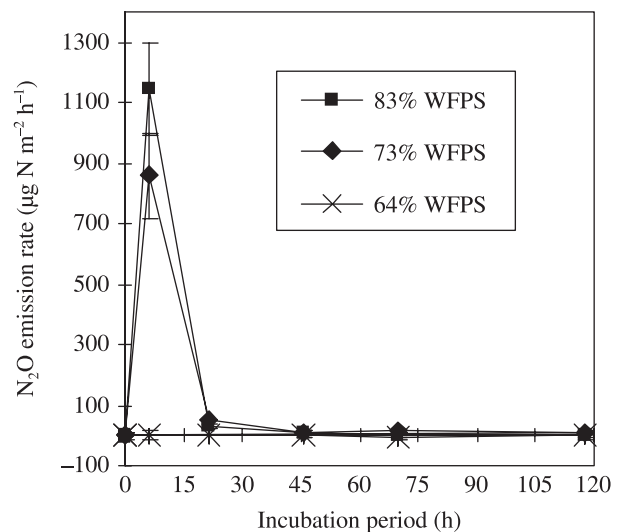


Figure 1 Effect of rewetting on N₂O emissions from soil (TG2005). An air-dried soil sample was rewetted using distilled water at 64 (×), 73 (◆) and 83% (■) of the water-filled pore space (WFPS) and incubated at room temperature. The values shown are the mean ± standard deviation of three replicates. The cumulative N₂O 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, N₂O 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 SO₄²⁻ 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 N₂O 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 K₂SO₄ solution were each added to three dishes to adjust to 73% WFPS of the soil, and the N₂O 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 SO₄²⁻ added with the charcoal, ash and K solution is listed in Table 3.

Calculation of the cumulative N₂O emission and statistical analysis

The cumulative N₂O 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

N₂O emissions from soil after rewetting

N₂O emissions were not detected after rewetting at 64% WFPS, but were detected after rewetting at 73% and

83% WFPS, and the cumulative N₂O emissions throughout the 120-h incubation period at room temperature were -0.016 ± 0.082 , 11 ± 1 and 13 ± 1 mg N₂O-N m⁻² (-0.003 ± 0.03 , 2.3 ± 0.3 and 2.8 ± 0.4 µg N₂O-N g⁻¹ soil), respectively (Fig. 1). Rewetting over 73% WFPS triggered N₂O emissions, but there were no significant differences in the cumulative N₂O emissions between soils rewetted at 73% and 83% WFPS ($P = 0.180$).

Effects of charcoal addition on N₂O emissions after rewetting at 73% WFPS for TG2004

The highest N₂O 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

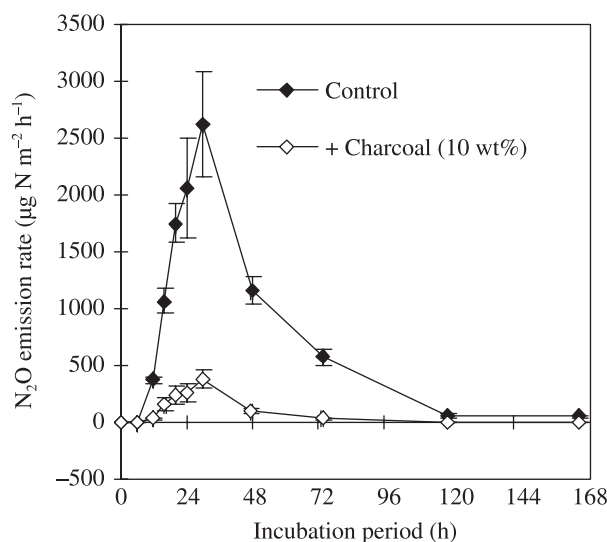


Figure 2 Effect of charcoal addition on N₂O emissions from soil (TG2004) rewetted at 78% of the water-filled pore space of the soil. The values shown are the mean \pm standard deviation of three replicates. The cumulative N₂O emissions during the 168-h incubation period for the non-added control and 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 SO₄²⁻ 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⁻		
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 ₂ SO 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 N₂O emission peak by 85% of that of the control without charcoal. The cumulative N₂O emissions were 105 ± 14 and 11.1 ± 2.4 mg N m⁻² (19.9 ± 2.7 and 2.1 ± 0.5 $\mu\text{g N g}^{-1}$ soil) in the treatments without and with charcoal addition, respectively. The charcoal addition significantly decreased N₂O emissions by 89% of the control value without charcoal ($P < 0.01$).

Effects of liming (pH 6.0) with charcoal and its ash on N₂O emissions after rewetting at 73% WFPS for TG2005

The highest N₂O emission rate was observed at 12 h after rewetting in the non-added control and the ash-added soil (Fig. 3), but N₂O emissions were kept at a low level in the charcoal-added soil throughout the observation period (72 h). The cumulative N₂O 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 $\mu\text{g N g}^{-1}$ soil), respectively. Charcoal addition decreased N₂O emissions by 80% of the value of the non-added control ($P < 0.05$), whereas ash addition did not.

Effects of charcoal addition on N₂O emissions after rewetting at 83% WFPS for TG2005

The N₂O emission rate at 6 h after rewetting was lower in the 2 and 8.2 wt% charcoal added-soils than in the

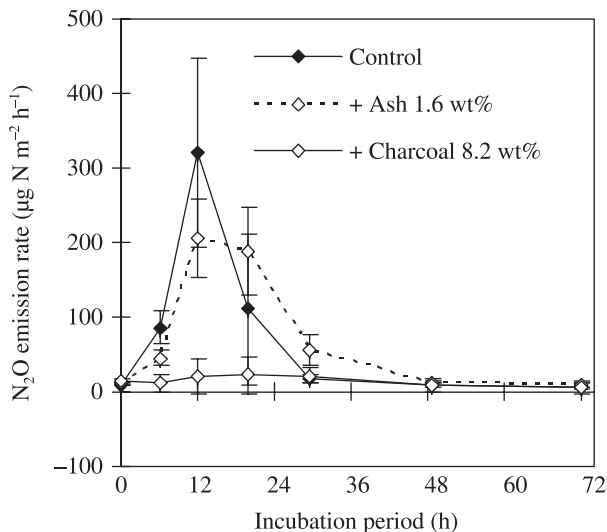


Figure 3 Effect of liming by using charcoal and its ash on N₂O emissions from soil (TG2005) rewetted at 73% of its water-filled pore space. The values shown are the mean \pm standard deviation of three replicates. The cumulative N₂O emissions during the 72-h incubation period for the non-added control, 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 $\mu\text{g N g}^{-1}$ soil), respectively.

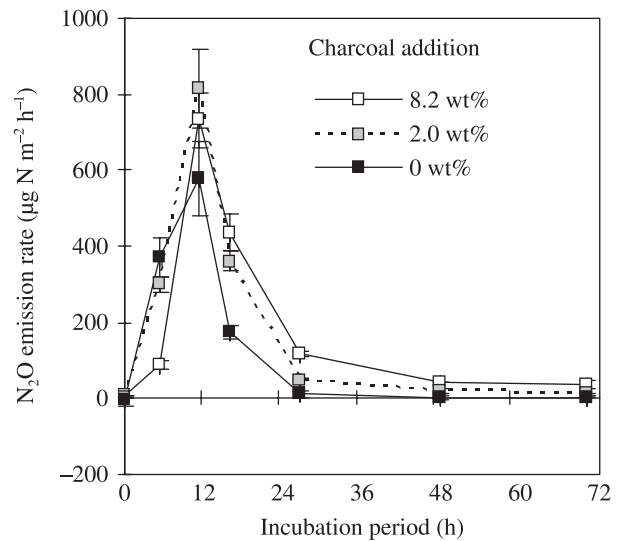


Figure 4 Effect of charcoal addition on N₂O emissions from soil (TG2005) rewetted at 83% of its water-filled pore space. The values shown are the mean \pm standard deviation of three replicates. The cumulative N₂O emissions during the 72-h incubation period for the non-added control and the 2 and 8.2 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 $\mu\text{g N g}^{-1}$ soil), respectively.

non-added control, while the N₂O 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 N₂O 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 $\mu\text{g N g}^{-1}$ soil), respectively. The addition of charcoal at 2 and 8.2 wt% significantly increased N₂O emissions by 47% and 51% of the values of the non-added control, respectively ($P < 0.01$).

Effects of KCl and K₂SO₄ on N₂O emissions after rewetting at 73% WFPS for TG2005

The highest N₂O emission rate was observed 12 h after rewetting (Fig. 5). The mean N₂O emission rate was higher in 10 mmol L⁻¹ KCl than in 5 mmol L⁻¹ K₂SO₄ and the non-added control, but considerable variability was observed in the KCl-added soil. The cumulative N₂O emissions throughout the 72 h incubation in the non-added control, 10 mmol L⁻¹ KCl and 5 mmol L⁻¹ K₂SO₄ 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 $\mu\text{g N g}^{-1}$ 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 K₂SO₄ additions ($P = 0.8559$), or between the 10 mmol L⁻¹ KCl and 5 mmol L⁻¹ K₂SO₄ additions ($P = 0.8794$).

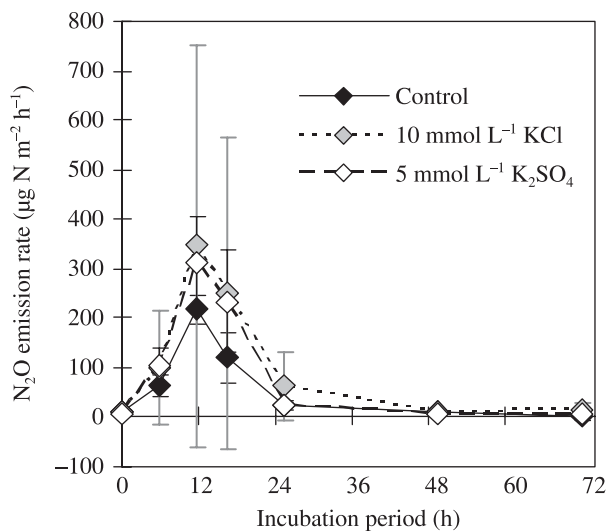


Figure 5 Effect of Cl^- and SO_4^{2-} of K salts on N_2O emissions from soil (TG2005) rewetted at 73% of its water-filled pore space. An air-dried soil sample was rewetted using distilled water (control) or a K solution and incubated at room temperature. The values shown are the mean \pm standard deviation of three replicates. The cumulative N_2O 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_2SO_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 $\mu\text{g N g}^{-1}$ soil), respectively.

DISCUSSION

The present study demonstrated that rewetting of air-dried soil at 73% WFPS caused significant N_2O emissions (Fig. 1), and the N_2O emissions were suppressed by the addition of charcoal (Figs 2,3). This suppression of the N_2O emissions was first considered to be a liming effect because charcoal has alkali (Table 2) and it had the potential to increase the N_2O -reducing activity of denitrifying communities (Cavigelli and Robertson 2000), which might cause a decrease in N_2O emissions (Cavigelli and Robertson 2001). Therefore, liming resulting from the ash was expected to have a similar potential for promoting N_2O -reducing activity to the charcoal itself. We checked the soil pH (1:2.5 ratio) after the observation of N_2O 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_2O emissions (Fig. 4). Moreover, the suppressive effects of charcoal addition on N_2O 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_2O -reducing activity, may not explain the suppression of the N_2O 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_2O -reducing activity of denitrifying communities (Yanai *et al.*), there were no clear differences in the N_2O emissions when KCl and K_2SO_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 SO_4^{2-} 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_2O -reducing activity may not be related to the suppression of the N_2O emissions from soil rewetted at 73% WFPS (Figs 2,3).

Increases in N_2O 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_2O emissions increased with increases in the water content of soil by rewetting at 73% and 83% WFPS, whereas significant N_2O 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_2O emissions from soil rewetted at 64% WFPS may not be the result of complete denitrification, including N_2O 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_2O emission rate in the later incubation period after rewetting may be the completion of N_2O production (stepwise reductions of NO_3^- , NO_2^- , and NO) rather than the kinetic equilibration of N_2O 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_2O -reducing activity in the later incubation period after rewetting. Therefore, the N_2O -reducing activity of denitrifying communities may not significantly affect N_2O emissions after rewetting of air-dried soil, suggesting that the suppressive effect of the charcoal addition on N_2O emissions (Figs 2,3) might result from inhibition of N_2O -producing activity of denitrifying communities.

Although there was no direct evidence to show a linkage between the addition of charcoal and the suppression of N₂O 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 N₂O production (stepwise reduction of NO₃⁻, NO₂⁻ 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 N₂O 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 N₂O 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 N₂O-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 N₂O emissions from arable soil, depending on the moisture or aeration conditions of the soil. In contrast, our understanding of the process of suppressing N₂O 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 N₂O emissions from soil, such as the activity of NO₃⁻ assimilation, NO₂⁻ accumulation in soil or NO_x emissions from soil.

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