ORIGINAL ARTICLE

Effects of charcoal addition on N\textsubscript{2}O 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\textsubscript{2}O 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\textsubscript{2}O emission peak 6 h after the rewetting, and the cumulative N\textsubscript{2}O emissions throughout the 120-h incubation period were 11±1 and 13±1 mg N m\textsuperscript{−2}, respectively. However, rewetting at 64\% WFPS did not cause detectable N\textsubscript{2}O emissions (−0.016±0.082 mg N m\textsuperscript{−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 N\textsubscript{2}O emissions by 89\%. In contrast, the addition of the ash from the charcoal did not suppress the N\textsubscript{2}O emissions from soil rewetted at 73\% WFPS. The addition of charcoal also significantly stimulated the N\textsubscript{2}O emissions from soil rewetted at 83\% WFPS compared with the soil without charcoal addition (P<0.01). Moreover, the addition of KCl and K\textsubscript{2}SO\textsubscript{4} did not show a clear difference in the N\textsubscript{2}O emission pattern, although Cl\textsuperscript{−} and SO\textsubscript{4}\textsuperscript{2−}, which were the major anions in the charcoal, had different effects on N\textsubscript{2}O-reducing activity. These results indicate that the suppression of N\textsubscript{2}O emissions by the addition of charcoal may not result in stimulation of the N\textsubscript{2}O-reducing activity in the soil because of changes in soil chemical properties.

Key words: denitrification, K fertilization, liming, N\textsubscript{2}O-reducing activity, rewetting effect.

INTRODUCTION

N\textsubscript{2}O 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\textsubscript{2}O in soil. Denitrification is identically the sole process associated with N\textsubscript{2}O reduction (Zumft 1997).

In a previous study, we examined the effects of soil amendments (liming material, inorganic salts and charcoal) on the N\textsubscript{2}O-reducing activity of denitrifying communities (Yanai \textit{et al.}). We found that charcoal and its ash, which had a high content of alkali and inorganic salts (Nerome \textit{et al.} 2005), seemed to promote the growth activity and N\textsubscript{2}O-reducing activity of denitrifying communities and that liming and anions affected these activities more than cations. These results suggest that N\textsubscript{2}O emissions from soil can be affected by certain soil amendments because of the modifying activity of N\textsubscript{2}O reduction, assuming that N\textsubscript{2}O emissions from soil through denitrification depend on the balance of the N\textsubscript{2}O-producing and N\textsubscript{2}O-reducing activity of denitrifying communities (Cavigelli and Robertson 2001). In fact, Inubushi \textit{et al.} (1999) and Azam and Müller (2003) observed stimulation of N\textsubscript{2}O emissions from soil by the addition of NaCl in a laboratory incubation study, and this result can be explained by the suppression of N\textsubscript{2}O-reducing activity by Cl\textsuperscript{−} or Na\textsuperscript{+} (Yanai \textit{et al.}). In contrast, the effects of liming on N\textsubscript{2}O emissions from soil are inconsistent in field studies (Borken and Brunme 1997; Butterbach-Bahl \textit{et al.} 1997; Klemedtsson \textit{et al.} 1997; Mosier \textit{et al.} 1998; Tokuda and Hayatsu 2004; Wang \textit{et al.} 1997) and in laboratory incubation studies (Borken \textit{et al.} 2000; Clough \textit{et al.} 2003; Clough \textit{et al.} 2004; Khalil \textit{et al.} 2003).

Pulses of N\textsubscript{2}O emission have been observed in field studies following irrigation and precipitation events...
(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$_2$O emitted after rewetting using the C$_2$H$_2$ addition method and $^{15}$N tracing technique, respectively, and concluded that the N$_2$O was mainly produced through denitrification. In the present study, to examine the relationship between enhancing the N$_2$O-reducing activity of denitrifying communities and N$_2$O emissions from soil, we examined the effects of charcoal and anion species on N$_2$O 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 samples examined were the same as those used in our previous study (Yanai et al., 2001) and have been reproduced in laboratory incubation experiments as a rewetting of dry soil (Rudaz et al., 1991). Soil samples and charcoal were collected from a grassland 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 ($\text{H}_2\text{O}$) 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-VCSH, 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 Hartz 1986). Water-filled pore space (WFPS) was calculated as follows:

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

where Porosity = 1 – (Bulk density/Particle density). In the present study, we set the density of water (pH$_2$O$_2$) 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 ($\text{H}_2\text{O}$) 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)

<table>
<thead>
<tr>
<th>Abbreviation of soil name</th>
<th>Date of sampling</th>
<th>pH ($\text{H}_2\text{O}$)</th>
<th>Total C (mg C g$^{-1}$)</th>
<th>Total N (mg N g$^{-1}$)</th>
<th>C/N ratio</th>
<th>WSOC (µg g$^{-1}$)</th>
<th>NO$_3$-N (µg g$^{-1}$)</th>
<th>Denitrifiers (log MPN g$^{-1}$)</th>
<th>Water content MWHC (g H$_2$O g$^{-1}$)</th>
<th>Bulk density (g cm$^{-3}$)</th>
<th>Specific gravity</th>
<th>Porosity</th>
</tr>
</thead>
<tbody>
<tr>
<td>TG2004</td>
<td>Apr. 2004</td>
<td>6.0</td>
<td>69.6</td>
<td>5.62</td>
<td>12.4</td>
<td>155</td>
<td>45.8</td>
<td>6.5</td>
<td>0.15</td>
<td>1.21</td>
<td>0.58</td>
<td>2.05</td>
</tr>
<tr>
<td>TG2005</td>
<td>Mar. 2005</td>
<td>5.4</td>
<td>70.3</td>
<td>5.45</td>
<td>12.9</td>
<td>74</td>
<td>6.4</td>
<td>6.2</td>
<td>0.15</td>
<td>1.11</td>
<td>0.59</td>
<td>2.03</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th></th>
<th>pH ($\text{H}_2\text{O}$)$^1$</th>
<th>LOI (%)</th>
<th>Water content ($\text{g H}_2\text{O g}^{-1}$)</th>
<th>MWHC ($\text{g H}_2\text{O g}^{-1}$)</th>
<th>Bulk density (g cm$^{-3}$)</th>
<th>Particle density (g cm$^{-3}$)</th>
<th>Anion content ($\mu$mol g$^{-1}$)$^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Charcoal</td>
<td>9.3</td>
<td>38</td>
<td>0.14</td>
<td>1.38</td>
<td>0.50</td>
<td>1.64</td>
<td>510</td>
</tr>
<tr>
<td>Ash</td>
<td>11.6</td>
<td>–</td>
<td>0.03</td>
<td>–</td>
<td>0.46</td>
<td>–</td>
<td>1240</td>
</tr>
</tbody>
</table>

Cl$^-$ | NO$_3$ | SO$_4$$^2$ |
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>9</td>
<td>–</td>
</tr>
</tbody>
</table>

$^1$1:5 ratio. $^2$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\(_2\)O 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\(_2\)O 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\(_2\)O 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\(_2\)O emission rate was calculated using the linear regression method (Hutchinson and Mosier 1981). After measurement of the N\(_2\)O 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\(_2\)O emissions were monitored until the first peak of N\(_2\)O emissions disappeared.

As a preliminary experiment, we examined the effect of moisture content after rewetting on the N\(_2\)O 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\(_2\)O 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\(_2\)O 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\(_2\)O emission results from the stimulation of N\(_2\)O-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\(_2\)O) 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\(_2\)O 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\(_2\)O 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.](image-url)
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

Table 3 Estimation of Cl⁻ and SO₄²⁻ load onto soil (TG2005) by the addition of charcoal, ash and K solution

<table>
<thead>
<tr>
<th>Application rate or concentration</th>
<th>Added into soil (µmol g⁻¹ soil)</th>
<th>Concentration in soil solution (mmol L⁻¹) at 73% WFPS</th>
<th>Concentration in soil solution (mmol L⁻¹) at 83% WFPS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cl⁻</td>
<td>SO₄²⁻</td>
<td>Cl⁻</td>
</tr>
<tr>
<td>Charcoal 2 wt%</td>
<td>10</td>
<td>0.2</td>
<td>NA</td>
</tr>
<tr>
<td>Charcoal 8.2 wt%</td>
<td>42</td>
<td>0.7</td>
<td>46</td>
</tr>
<tr>
<td>Ash 1.6 wt%</td>
<td>20</td>
<td>1.3</td>
<td>23</td>
</tr>
<tr>
<td>KCl 10 mmol L⁻¹</td>
<td>7.5</td>
<td>0</td>
<td>8.5</td>
</tr>
<tr>
<td>K₂SO₄ 5 mmol L⁻¹</td>
<td>0.4</td>
<td>3.7</td>
<td>0</td>
</tr>
</tbody>
</table>

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 $105 \pm 14$ and $11.1 \pm 2.4 \text{ mg N m}^{-2}$ ($19.9 \pm 2.7$ and $2.1 \pm 0.5 \text{ g N g}^{-1} \text{ soil}$) 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 $4.1 \pm 1.9$, $4.3 \pm 1.2$ and $0.8 \pm 0.7 \text{ mg N m}^{-2}$ ($0.9 \pm 0.4$, $1.0 \pm 0.3$ and $0.2 \pm 0.2 \text{ g N g}^{-1} \text{ soil}$), 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.

**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 $6.8 \pm 0.9$, $10.0 \pm 0.8$ and $10.3 \pm 0.6 \text{ mg N m}^{-2}$ (equivalent to $1.5 \pm 0.2$, $2.2 \pm 0.2$ and $2.4 \pm 0.1 \text{ g N g}^{-1} \text{ soil}$), 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 ($P < 0.01$).

**Effects of KCl and K$_2$SO$_4$ 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$_2$SO$_4$ 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$_2$SO$_4$ were $2.9 \pm 0.6$, $5.3 \pm 6.1$ and $4.4 \pm 1.5 \text{ mg N m}^{-2}$ ($0.6 \pm 0.1$, $1.2 \pm 1.3$, $1.0 \pm 0.3 \text{ g N g}^{-1} \text{ soil}$) 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$_2$SO$_4$ additions ($P = 0.8559$), or between the 10 mmol L$^{-1}$ KCl and 5 mmol L$^{-1}$ K$_2$SO$_4$ additions ($P = 0.8794$).
the addition of ash did not suppress N_2O emissions (Fig. 4). These treatments were significantly different from the 5.7 and 5.6 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_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 equilibra-
Although there was no direct evidence to show a linkage between the addition of charcoal and the suppression of N$_2$O 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$_2$O 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$_2$O 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$_2$O emissions by the addition of charcoal to soil rewetted at 83% WFPS (Fig. 4) can be interpreted as an interaction between the significant improvement of the aeration of the soil and the stimulation of the N$_2$O-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$_2$O emissions from arable soil, depending on the moisture or aeration conditions of the soil. In contrast, our understanding of the process of suppressing N$_2$O 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$_2$O 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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