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ORIGINAL PAPER

Nitrification, ammonia-oxidizing communities, and N_2O and CH_4 fluxes in an imperfectly drained agricultural field fertilized with coated urea with and without dicyandiamide

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Abstract Agricultural soil is a major source of nitrous oxide (N_2O), and the application of nitrogen and soil drainage are important factors affecting N_2O emissions. This study tested the use of polymer-coated urea (PCU) and polymer-coated urea with the nitrification inhibitor dicyandiamide (PCUD) as potential mitigation options for N_2O emissions in an imperfectly drained, upland converted paddy field. Fluxes of N_2O and methane (CH_4), ammonia oxidation potential, and ammonia-oxidizing bacteria (AOB) and ammonia-oxidizing archaea (AOA) abundances were monitored after the application of PCU, PCUD, and urea to upland soil. The results showed that urea application increased the ammonia oxidation potential and AOB and AOA abundances; however, the increase rate of AOB (4.6 times) was much greater than that of AOA (1.8 times). These results suggested that both AOB and AOA contributed to ammonia oxidation after fertilizer application, but the response of AOB was greater than AOA. Although PCU and PCUD had lower ammonia oxidation potential compared to urea treatment, they were not effective in reducing N_2O emissions. Large episodic N_2O emissions (up to 1.59 kg N ha^{-1} day^{-1}) were observed following heavy rainfall 2 months after basal fertilizer application. The episodic N_2O emissions accounted for 55–80 % of total N_2O emissions over the entire monitoring period. The episodic N_2O emissions following heavy rainfall would be a major source of N_2O in poorly drained agricultural fields. Cumulative CH_4 emissions ranged from −0.017 to −0.07 kg CH_4 ha^{-1}, and fertilizer and nitrification inhibitor application did not affect CH_4 oxidation.

Keywords Ammonia-oxidizing bacteria (AOB) · Ammonia-oxidizing archaea (AOA) · Nitrification · Polymer-coated fertilizer · Nitrification inhibitor · Nitrous oxide

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Introduction

Nitrous oxide (N_2O) is a greenhouse gas that also degrades the stratospheric ozone (Denman et al. 2007). The agricultural sector (soil and livestock) is a major source of N_2O and is estimated to emit 2.8 Tg N_2O–N per year, which accounts for 42 % of global anthropogenic N_2O emissions (Denman et al. 2007). Aerobic soil also acts as a sink for methane (CH_4), a greenhouse gas, through CH_4 oxidation by methanotrophs. Soil is estimated to oxidize 30 Tg CH_4 per year, which is about 5 % of the global CH_4 sink (Denman et al. 2007).

Nitrous oxide emissions are affected by many factors such as the amount and type of N fertilizer, temperature, soil texture, and soil pH (Bouwman et al. 2002; Baggs et al.

2010). Soil drainage is also an important factor affecting N_2O emissions (Bouwman et al. 2002; Skiba and Ball 2002). In Japan, poorly drained fields have traditionally been used as rice paddy fields. However, by 2008, about 30 % of these rice paddy fields had been converted to unflooded upland cropping fields (Ministry of Agriculture, Forestry and Fisheries 2009) because of the decline in rice consumption resulting from the westernization of diet. This land use change is expected to increase N_2O emissions from Japanese agricultural land because the mean fertilizer-induced N_2O emission factor (EF) from paddy fields is 0.31 % (Akiyama et al. 2005), substantially lower than the 0.64 % of mean EF for Japanese upland fields (Akiyama et al. 2005). In addition, the mean EF from poorly drained upland soils is 1.4 %, much higher than that from well-drained upland soils (0.32 %; Akiyama et al. 2006). In contrast, the conversion of rice paddy fields to unflooded upland cropping fields will greatly reduce CH_4 emissions because rice paddy fields are a source of CH_4, whereas aerobic soil is a sink for CH_4 (Nishimura et al. 2008).

The application of nitrogen (N) to soil in the form of chemical or organic fertilizers stimulates N_2O production, primarily via the microbial processes of nitrification and denitrification (Davidson 1991). It was believed that nitrification is performed by two groups of chemolithoautotrophic bacteria: the ammonia-oxidizing bacteria (AOB) and the nitrite-oxidizing bacteria (Hayatsu et al. 2008). However, recent studies showed that ammonia-oxidizing archaea (AOA) predominate among ammonia-oxidizing prokaryotes in soils (Leininger et al. 2006). Meanwhile, there is still a debate as to which microorganisms, AOB or AOA, are the main contributors to ammonia oxidation in soil (Di et al. 2010a, b). Also, the role of AOA in N_2O production in soil is unknown (Baggs and Philippot 2010; Di et al. 2010a, b), whereas N_2O production by marine AOA was recently reported (Santoro et al. 2011). Denitrification was traditionally believed to be processed by denitrifying bacteria; however, processes such as nitrifier denitrification, fungal denitrification, and co-denitrification were also recently found to be involved in the production of N_2O in soil (Baggs and Philippot 2010; Hayatsu et al. 2008). In addition, nitrate ammonification, methanotrophic nitrification, and the non-microbial process of chemodenitrification are also involved in the production of N_2O in soil, although the relative contributions of these processes are unclear (Baggs and Philippot 2010; Hayatsu et al. 2008).

Nitrification inhibitors delay the oxidation of ammonium in the soil (Weiske et al. 2001). Polymer-coated fertilizers release nutrients by diffusion through a semi-permeable polymer membrane, and the release rate can be controlled by varying the composition and thickness of the coating (thus also called slow-release or controlled-release fertilizer). According to the meta-analysis of field studies (Akiyama et al. 2010), nitrification inhibitors and polymer-coated fertilizers reduced N_2O emissions by an average of 38 and 35 %, respectively, compared with conventional fertilizers. In their analysis, however, the effects of polymer-coated fertilizers varied with soil and land use type, i.e., they were significantly effective in reducing emissions on imperfectly drained Gleysol grassland (77 %) but were not effective on well-drained Andosol upland fields.

Methane is oxidized by both methane monooxygenase (MMO) and ammonium monooxygenase (AMO) (Hanson and Hanson 1996). The close relationship between AMO and MMO activities implied that the addition of a nitrification inhibitor could reduce the activity of both enzymes (Bedard and Knowles 1989). Although Majumdar and Mitra (2004) found that dicyandiamide reduced CH_4 oxidation, several other studies have found that dicyandiamide does not affect CH_4 oxidation (Delgado and Mosier 1996; Jumadi et al. 2008; Weiske et al. 2001). Meanwhile, Aronson and Helliker (2010) analyzed published data and found that CH_4 uptake is inhibited by high rates (>100 kg N ha^{-1}) and stimulated by low rates of N application.

The aim of this study was to test PCU and PCUD as potential mitigation options for N_2O emissions after nitrogen fertilizer applications to an imperfectly drained, upland converted paddy field. The effects of PCU, PCUD, and urea on ammonia oxidation potential and AOB and AOA abundances were also investigated. Moreover, we investigated whether urea and nitrification inhibitor application affect CH_4 uptake by soil.

Materials and methods

Field management

The field study site was located at the National Institute for Agro-Environmental Sciences (NIAES), Tsukuba, Japan (36°01′ N, 140°07′ E). The field had been converted from a rice paddy to an upland field and used for the cultivation of upland crops (e.g., soybean) for 6 years; it was left fallow for a year before the experiment. The soil type of the experimental field was grey lowland soil (Fluvisols in FAO/UNESCO soil classification system). The top 5 cm of soil had the following properties: C, 1.70 %; N, 0.13 %; and pH (H_2O), 5.68. The particle size distribution was as follows: sand, 50.2 %; silt, 23.2 %; and clay, 26.6 % (sandy clay loam in the USDA classification system). Tile drains had been installed to a depth of approximately 0.5 m to improve drainage, but the field was still imperfectly drained.

The four treatments were defined by the fertilizers applied as follows:

1. Control—no N fertilizer.
2. Urea—Urea (non-coated) was used as conventional fertilizer treatment.

3. PCU—The urea used in this treatment was 70-day PCU (urea coated with polyolefin), which releases 80 % of its N within 70 days.
4. PCUD—The urea in this treatment was 70-day PCUD (urea with two layers of coatings: (1) the nitrification inhibitor dicyandiamide and (2) polyolefin). PCUD has recently become commercially available in Japan. The N content of dicyandiamide was 10 %.

The treatments were laid out in a randomized block design with three replicate plots of 18 m^2 (4×4.5 m).

Carrot (*Daucus carota* L.) was cultivated from June 16 to September 29, 2008. All plots received calcium superphosphate (150 kg ha^{-1} as P_2O_5) and potassium chloride (90 kg ha^{-1} as K_2O) as a basal application on June 16. An additional 80 kg ha^{-1} of potassium chloride as K_2O was applied to all plots on July 14 and September 2.

Except for the control plots, all plots received equal amounts of total N (250 kg ha^{-1}). To reflect the practices of local farmers, the amount and timing of urea applications followed the Ibaraki prefecture's fertilizer guidelines. According to those guidelines, 90 kg N ha^{-1} was applied as a basal fertilizer application on June 16 (BF) and an additional 80 kg N ha^{-1} was applied on July 14 (AF1) and September 2 (AF2), whereas all N was applied as a basal application on June 16 in the PCUD and PCU plots. Basal fertilizer was broadcast and then incorporated to a depth of approximately 10 cm, whereas the additional fertilizer applications were surface broadcast.

The soil volumetric water content was measured from 0- to 5-cm depth using EC-5 dielectric soil moisture sensors (Decagon Devices, Pullman, WA, USA). Water-filled pore space (WFPS) was calculated from the volumetric water content and soil bulk density (Carter and Ball 1993). The soil and air temperatures at a depth of 5 cm were monitored using ECT temperature sensors (Decagon Devices). Rainfall data were obtained from a weather station located within the NIAES.

Gas flux monitoring

Fluxes of N_2O and CH_4 from the soil surface were monitored from May 27, 2008 to March 6, 2009 in duplicate, i.e., two chambers for each treatment. The plots that received N were monitored using an automated gas sampling system (Akiyama et al. 2009), whereas the control plots were monitored manually. The system comprised six polycarbonate chambers connected to gas sampling units. Each chamber had a cross-sectional area of 8,100 cm^2 (90×90 cm) and a height of 45 cm. For flux measurement, the lid of each chamber was closed automatically for 30 min, during which time three headspace gas samples (at 0, 15, and 30 min) were collected and injected into evacuated glass vials by the automated sampling unit; the sampling resume was slightly modified from Akiyama et al. (2009). Samples were taken from 1600 to 1630 hours in order to obtain a daily average flux; this timing was adapted from a previous study of the diel fluctuation in N_2O flux from a nearby field (Akiyama and Tsuruta 2003). Measurements were taken on the N-treated plots every 3 days from May 27 to June 15, 2008; once a day from June 16 to November 5, 2008; and every 2 days from November 6 2008 to March 6, 2009. Gas flux from the control plots was manually sampled using cylindrical closed chambers (diameter, 25 cm; height, 10 cm) every 2 weeks. The fluxes were calculated from the changes of the gas concentrations during the sampling period according to Smith and Conen (2004).

The concentrations of N_2O and CH_4 were analyzed using a GC-2014 gas chromatograph (Shimadzu, Kyoto, Japan) with a HS-2B headspace autosampler (Shimadzu). Details of the combination of GC columns are presented in Sudo (2009). The headspace autosampler was modified for trace gas analysis by replacing the original syringe with a 2-mL gastight syringe (Pressure-Lok series A, VICI Precision Sampling, Baton Rouge, LA, USA) and the original heating unit was removed. Helium was used as the carrier gas. The N_2O concentration was determined with a CH_4- and N_2-doped ^{63}Ni electron capture detector at 340 °C. The CH_4 concentration was determined using a flame ionization detector. Standard gases (0.3, 0.5, 1, 2.5, and 5 μL L^{-1} N_2O and 2.01 μL L^{-1} CH_4) were analyzed before and after the analysis of samples every day. The coefficients of variation for repeated analyses of the standard gases (N_2O, 0.5 μL L^{-1}; CH_4, 2.01 μL L^{-1}) were 0.48 % for N_2O and 1.13 % for CH_4 (n=40 for each gas).

Soil sampling and mineral nitrogen measurement

Soil samples were taken periodically in triplicate. Surface soil (0–5 cm) was randomly collected from five points in each plot and mixed together in a plastic bag. Bulk soil samples were immediately transferred to the laboratory. Samples of fresh soil (10 g) were extracted with 100 mL KCl solution (100 g KCl per liter). The copper–cadmium reduction and diazotization method was used to analyze NO_3^- and the indophenol blue method used to analyze NH_4^+ using a TRRACS continuous flow analyzer (Bran+Luebbe, Norderstedt, Germany).

Ammonia oxidation potential

The ammonia oxidation potential of soils in the control and three fertilizer treatments was measured on five occasions: June 11, 2008 (5 days before BF application); June 19 and June 24, 2008 (3 and 8 days after BF); July 28, 2008 (14 days after AF1 to the urea treatment); and September 12, 2008 (10 days after AF2 to the urea treatment). Analysis

was performed using the shaken-slurry method (Belser and Mays 1980) within 24 h of soil sampling. From the 2-mm sieved bulk soil samples, 2.5 g of fresh soil was weighed into 50-mL plastic tubes treated with 10 mL of the reaction buffer consisting of 1 mM KH_2PO_4 (pH 7.2), 1 mM $(NH_4)_2SO_4$, and 10 mM $NaClO_3$. All tubes were shaken at 150 rpm on a shaker for 4 h at 25 °C. Aliquots of 1 mL were removed from each tube at 0, 2, and 4 h after the addition of the solution and centrifuged at 10,000×*g* and 4 °C for 10 min. The supernatant (0.1 mL) was added to a microplate and analyzed colorimetrically for NO_2^- (by the diazotization method) using a Viento multi-spectrophotometer (Dainippon Pharmaceutical Co., Ltd., Osaka, Japan). The NO_2^- pools increased linearly throughout the 4-h incubation period; therefore, the rate of nitrification in each soil sample was calculated by linear regression of the NO_2^- concentration against time. The ammonia oxidation rate in slurry represents a potential activity because we added $(NH_4)_2SO_4$ as the substrate of nitrification, and conditions in the field may not be as conducive to nitrification as a shaken (i.e., aerated) slurry incubated at 25 °C.

Quantification of *amoA* genes

Abundances of ammonia monooxygenase (*amoA*) genes of AOB and AOA were quantified on June 11, 2008 (5 days before BF application) and on September 12, 2008 (10 days after AF2 to the urea treatment) using a StepOnePlus Real-Time PCR system (Applied Biosystems, Foster City, CA, USA) with SYBR Premix Ex Taq polymerase (Takara Bio Inc., Shiga, Japan). DNA was extracted from 0.4 g of the soil sample (2-mm sieve) using a FastDNA SPIN Kit for soil (Qbiogene, Inc., Irvine, CA, USA) with a FastPrep Instrument (Qbiogene) in accordance with the manufacturer's instructions. Extracted DNA samples were further purified using a DNA Clean & Concentrator-25 kit (Zymo Research Corp., Orange, CA, USA), and then 80 μL of the purified soil DNA was obtained from each sample. All real-time PCR data were obtained from triplicate extractions of soil DNA with duplicate independent amplifications.

The primer pair amoA1F/amoA2R (Rotthauwe et al. 1997) was used to quantify AOB *amoA*. A 20-μL reaction mixture contained 10.0 μL of SYBR Premix Ex Taq (Takara Bio Inc.), 0.4 μmol of each of the two primers, 4 μg of bovine serum albumin (Takara Bio Inc.), 0.4 μL of ROX reference dye I (Takara Bio Inc.), and 1 μL of tenfold-diluted soil DNA. The thermal profile of the PCR was as follows: 2 min at 94 °C, 35 cycles of 30 s at 94 °C for denaturing, 30 s at 56 °C for annealing, and 30 s at 72 °C for extension.

To quantify AOA *amoA*, the primers amoA19IF (5′-ATGGTCTGGCTIAGACG-3′) and amoA643IR (5′-TCCCACTTIGACCAIGCGGCCATCCA-3′) were used. The PCR conditions were as described previously (Morimoto et al. 2011).

A standard curve for the quantification of AOB *amoA* was generated from tenfold dilutions (10^2–10^6 copies per microliter) of a pGEM-T Easy Vector System (Promega, Madison, WI, USA) containing the *amoA* fragment amplified from *Nitrosospira multiformis* ATCC25196 (accession no. U91603). Similarly, for the quantification of AOA *amoA*, we used clone S1001 (accession no. AB569307) containing an archaeal *amoA* fragment amplified from soil DNA as the standard. PCR efficiencies and coefficients of determination for the standard curves were respectively 91.0 % and r^2=0.998 for AOB *amoA* and 81.2 % and r^2=0.998 for AOA *amoA*.

Statistical analyses

The effects of different fertilizer treatments on N_2O and CH_4 emissions, ammonia oxidation potential, and AOB and AOA *amoA* gene copy numbers were evaluated using ANOVA followed by Tukey's multiple comparison test. The relationship between the percentage of WFPS and N_2O emissions was evaluated using regression analysis. All statistical analyses were performed using PASW Statistics, version 18.0 (SPSS Inc., New York, NY, USA).

Results

N_2O emissions and soil mineral nitrogen

In this study, fertilizer-induced N_2O emission factor (EF) ranged from 1.3 to 2.3 % (Table 1). In urea treatment, EF was close to the mean EF from poorly drained soils of Japanese agricultural fields (1.4 %, SD±0.95; Akiyama et al. 2006), whereas EFs of the PCU and PCUD treatments were much higher than this value.

The cumulative N_2O emissions over the entire measurement period in the PCU and PCUD treatments were not significantly different from that of the urea treatment (Table 1). Small N_2O peaks were observed following moderate rainfall (about 40 mm day^{-1}) after basal fertilizer application (PKs1 in Fig. 1). During this period, N_2O emissions from the four treatments were in decreasing order of urea > PCU > PCUD > control (Table 1); however, the difference was not significant. After the first additional fertilizer application to the urea plots, a small N_2O emission peak was observed after rainfall of 28 mm on July 18, but the peak value was smaller than that after basal fertilizer application (PK2 in Fig. 1).

Large episodic N_2O emissions were observed in the N-treated plots following heavy rainfall 2 months after basal fertilizer application (1 month after the first additional fertilizer application to urea plots; PKs3 in Fig. 1). The highest

Table 1 Cumulative N_2O and CH_4 emissions (means ± SD) from soil after the application of different N fertilizers in a poorly drained ex-paddy field used to cultivate carrots

	Total N application (kg N ha^{-1})	Total N_2O emissions (May 27, 2008–March 7, 2009; 285 days) (kg N ha^{-1})	N_2O emissions after basal fertilization (June 16–July 13, 2008; 28 days) (kg N ha^{-1})	Episodic period N_2O emissions (August 16–September 9, 2008; 25 days) (kg Nha^{-1})	Fertilizer-induced N_2O emission factor[a] (%)	Total CH_4 emissions (May 27, 2008–Mar 7, 2009; 285 days) (kg CH_4 ha^{-1})
Control	0	1.04±0.95[b] a	0.011±0.003 a	0.80±0.84 a	–	−0.0152±0.0169 a
Urea	250	4.30±2.93 a	0.462±0.446 a	2.34±2.31 a	1.3 a	−0.0428±0.0108 a
PCU	250	6.36±5.73 a	0.133±0.056 a	5.09±5.67 a	2.1 a	−0.0445±0.0133 a
PCUD	250	6.84±2.30 a	0.109±0.072 a	5.47±2.53 a	2.3 a	−0.0081±0.1973 a

Treatments were no-N control, urea, polymer-coated urea, and polymer-coated urea with dicyandiamide

PCU polymer-coated urea, *PCUD* polymer-coated urea with dicyandiamide

[b] Means in the same column followed by the same letter are not significantly different ($P \geq 0.05$) by Tukey's test

[a] Fertilizer-induced N_2O emission factor defined as the emission from fertilizer plots minus that of a zero-N control treatment, expressed as percent of N input

N_2O flux of 1.59 kg N ha^{-1} day^{-1} was observed in the PCUD treatment after 82.5 mm of rainfall on Aug 28 (Electronic supplementary material (ESM) Fig. 1). In all treatments, the NO_3^- content of the surface soil peaked before the episodic N_2O emissions and then decreased with rainfall and remained low during the episodic N_2O emissions (Fig. 2 and ESM Fig. 1). The cumulative N_2O emissions in the PCU and PCUD treatments during the episodic emissions were not significantly different from that of the urea treatment (Table 1). Emissions from the control plots were small (maximum, 0.17 kg N ha^{-1} day^{-1}) during the episodic N_2O emissions period, but accounted for 78 % of the total N_2O emitted (Table 1) from the control.

WFPS and N_2O emissions

During the episodic N_2O emissions, the N_2O flux increased exponentially with WFPS in all treatments (ESM Fig. 2). The coefficients of determination (r^2=0.517, 0.626, and 0.551 for PCU, PCUD, and the urea treatments, respectively) showed that the WFPS accounted for 52–63 % of the variance. The second additional fertilizer application in the urea plots on September 2, after the episodic N_2O emissions, led to only small N_2O emissions (PKs 4 in Fig. 1) when WFPS was approximately 75 %.

Ammonia oxidation potential and ammonia-oxidizing bacteria and archaea *amoA* abundances

Ammonia oxidation potential in the urea treatment was significantly higher than in the other treatments on June 24, July 28, and September 12 (P<0.05; Fig. 3). Ammonia oxidation potential in the urea treatment increased significantly (P<0.05) from a value of 5.89 nmol g^{-1} h^{-1} on June 11, measured prior to the basal fertilizer application, to 12.2 nmol g^{-1} h^{-1} on September 12 (Fig. 3).

AOA *amoA* gene copy numbers were greater than those of AOB (Fig. 4). On September 12, AOB *amoA* gene copy numbers in the urea treatments were significantly higher than those of the PUCD and control treatments (P<0.05). In contrast, AOA *amoA* gene copy numbers did not differ significantly between treatments before and after fertilizer application. In urea treatments, both AOB and AOA *amoA* gene copy numbers significantly increased from June 11 to September 12 (P<0.05), although the increase rate of AOB (4.6 times) was much greater than that of AOA (1.8 times). In the PCUD treatments, AOB *amoA* gene copy numbers significantly increased from June 11 to September 12; however, AOA *amoA* gene copy numbers were not significantly different (P<0.05). In PCU treatments, AOB and AOA *amoA* gene copy numbers were not significantly increased from June 11 to September 12 (P<0.05) owing to a large variation. In control treatments, AOB *amoA* gene copy numbers significantly decreased, whereas the change in AOA was not significant (P<0.05).

CH_4 flux

The patterns in CH_4 flux generally displayed a small range of variation, mostly representing small amounts of uptake but occasionally low levels of emissions (Fig. 5). Cumulative CH_4 emissions ranged from −0.07 to −0.01 kg CH_4 per hectare and did not differ significantly between treatments (Table 1).

Discussion

N_2O emissions and soil mineral nitrogen

The fact that the NO_3^- content of the surface soil (Fig. 2 and ESM Fig. 1) peaked before the episodic N_2O emissions (PKs3 in Fig. 1), and then decreased and remained low

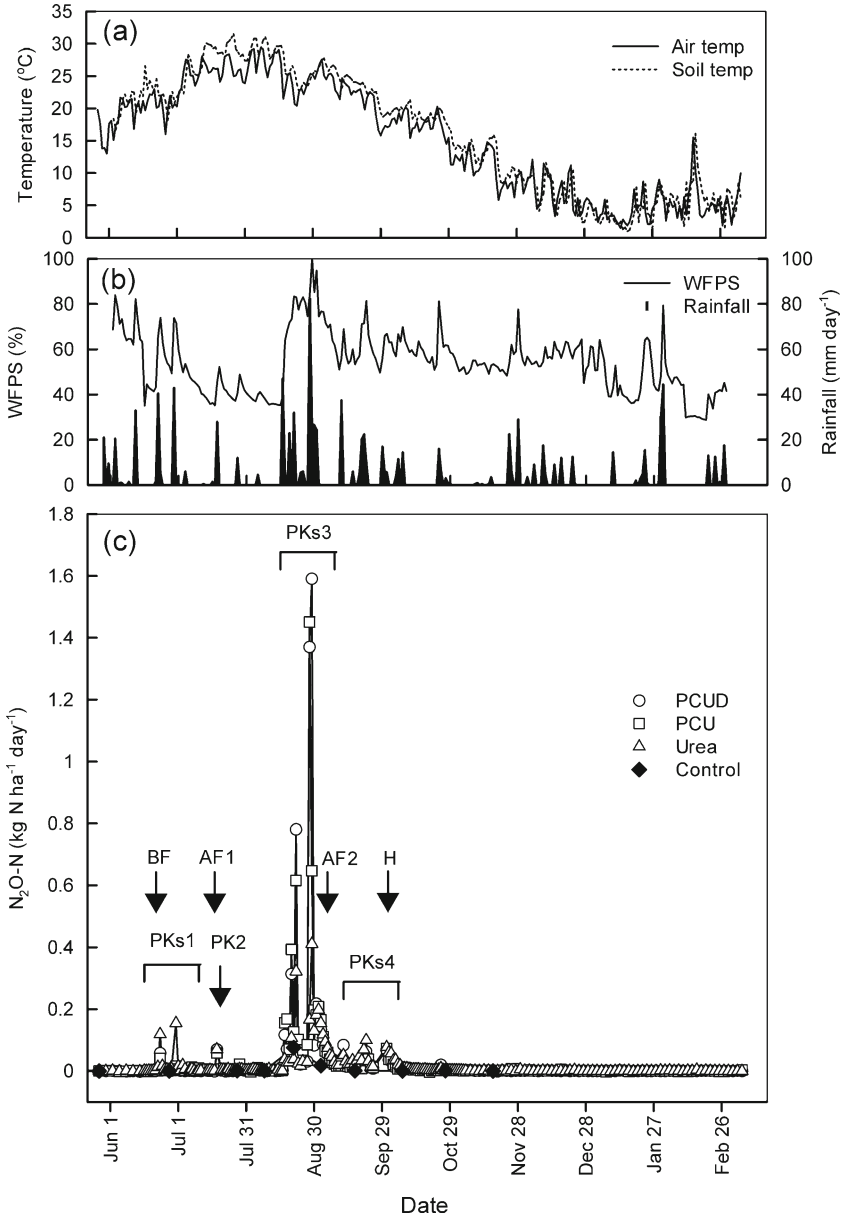

Fig. 1 Seasonal variations in soil and air temperatures (daily mean) (**a**); water-filled pore space (*WFPS*) at soil depths of 0–5 cm and rainfall (**b**); and N_2O flux (mean of duplicate determinations) after application of different N fertilizers in a poorly drained ex-paddy field used to cultivate carrots (**c**). The treatments were no-N control, urea, polymer-coated urea (*PCU*), and polymer-coated urea with dicyandiamide (*PCUD*). *BF* basal fertilizer application in the PCUD, PCU, and urea plots, *AF* additional fertilizer application in the urea plots, *H* harvest of carrots, *PK* N_2O peak. The measurement period was from May 27, 2008 to March 6, 2009

during the episodic N_2O emissions, indicated that NO_3^- was slowly accumulated by nitrification and was leached into the deeper soil layers (>5 cm) by the heavy rainfall. The episodic N_2O emissions were possibly produced by denitrification of the leached NO_3^- in the subsurface soil. High O_2 concentrations are known to suppress the activity and synthesis of the denitrification reductases, and the N_2O reductase is thought to be the most sensitive to O_2 (Otte et al. 1996). When aerobic soils become anaerobic, for example, following heavy rainfall, the NO_3^- and NO_2^- reductases are typically activated sooner than the N_2O reductase so that the denitrifier N_2O/N_2 ratio is higher for 1–2 days after rainfall (Knowles 1982; Otte et al. 1996). Morley et al. (2008) reported that all denitrification enzymes except the N_2O reductase remain active when re-exposed to O_2 after an anaerobic phase and suggested that short anoxic spells created by flooding and subsequent drainage will lead to large N_2O emissions.

In our study, the rate and timing of urea applications followed local guidelines. Consequently, the application method was different among treatments, i.e., urea was applied as split application, whereas PCU and PCUD were applied as basal applications. Basal fertilizer was applied by surface broadcasting and incorporation, whereas additional fertilizer was applied by surface broadcasting; therefore, the NH_4^+ and NO_3^- concentrations in the surface soil after the additional fertilizer applications were higher than those after basal fertilizer application (Fig. 2). This application method could also affect N_2O emissions.

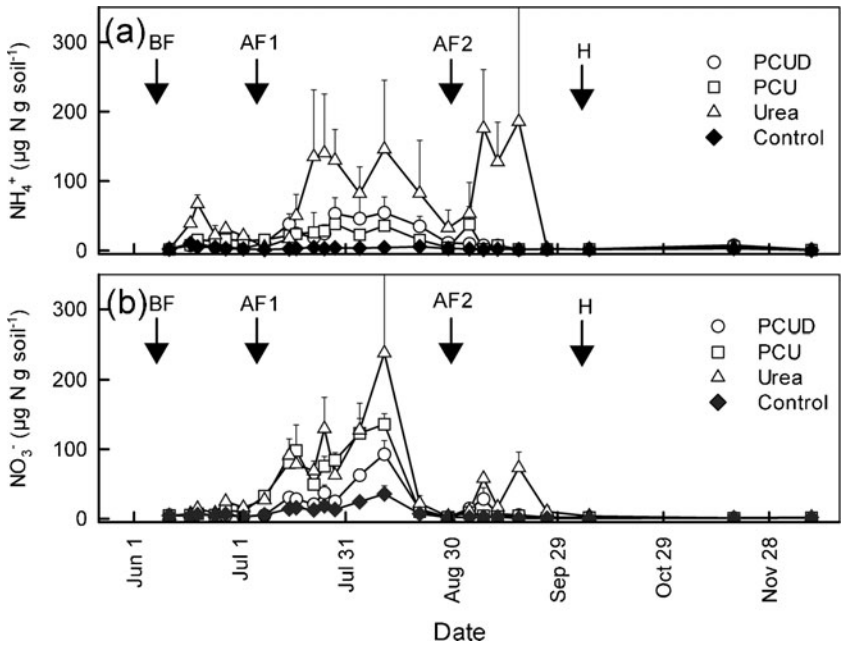

Fig. 2 Seasonal variations in NH_4^+ (**a**) and NO_3^- (**b**) in surface soil (0–5 cm, mean of triplicate determinations) after application of different N fertilizers in a poorly drained ex-paddy field used to cultivate carrots. The treatments were no-N control, urea, polymer-coated urea (*PCU*), and polymer-coated urea with dicyandiamide (*PCUD*). *BF* basal fertilizer application to PCUD, PCU, and urea plots, *AF* additional fertilizer application for urea plots, *H* harvest of carrots. The measurement period was from May 27, 2008 to March 6, 2009. Basal fertilizer was applied by surface broadcasting and incorporation, whereas additional fertilizer was applied by surface broadcasting (without incorporation)

WFPS and N_2O emissions

After basal fertilizer application, the value of N_2O emissions from the four treatments (PKs1 in Fig. 1) were in decreasing order of urea>PCU>PCUD>control (Table 1); however, the difference was not significant due to the large variation. Nitrous oxide is mainly produced by nitrification at lower WFPS (typically <70 %, depending on soil type), whereas denitrification becomes the main process at higher WFPS (Davidson 1991). WFPS during this period was relatively low, ranging from 35 to 74 %. At the same time, ammonia oxidation potential in the urea treatment became significantly higher than that of the other treatments on June 24 (8 days after BF application; Fig. 3). These results suggested that nitrification was an important pathway of N_2O emissions during this period, but denitrification may also have contributed to N_2O production just after the rainfall.

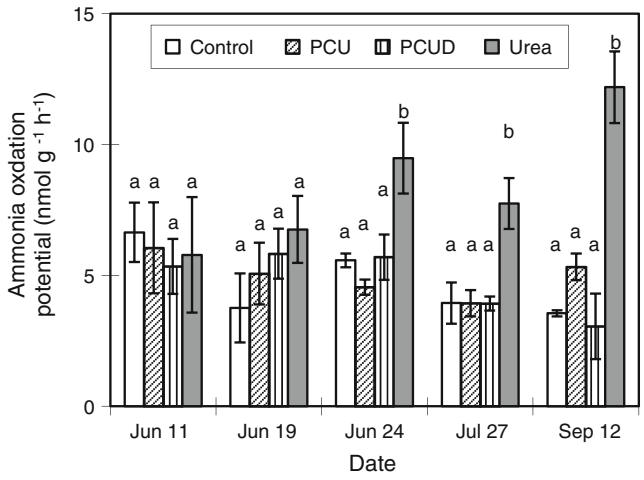

Fig. 3 Ammonia oxidation potential of soils. Sample was taken on five occasions: June 11, 2008 (5 days before BF application); June 19 and June 24, 2008 (3 and 8 days after BF); July 28, 2008 (14 days after AF1 to the urea treatment); and September 12, 2008 (10 days after AF2 to the urea treatment). The treatments were no-N control, urea, polymer-coated urea (*PCU*), and polymer-coated urea with dicyandiamide (*PCUD*). *Columns with the same letter* are not significantly different ($P \geq 0.05$) for the same sampling day by Tukey's test. *Error bars* indicate SD

Dobbie and Smith (2003) reported an exponential relationship between N_2O flux and WFPS in a grassland in the UK. We also found exponential relationships between N_2O flux and WFPS (ESM Fig. 2). Here, the WFPS increased to 100 % (Fig. 1a and ESM Fig. 1) following heavy rainfall on August 28, and the field was partly flooded from the evening of August 28 to the morning of August 29. Drainage of the surface-ponded water was slower in some plots (up to 20 h), but faster (<10 h) in other plots. This uneven drainage led to a large variation in WFPS and, thus, variation in the N_2O flux between plots. In this study, polymer-coated fertilizer with a nitrification inhibitor was tested as a mitigation option for N_2O emissions; therefore, we focused on investigating nitrification. However, our results showed that episodic N_2O emissions, of which denitrification is likely the main pathway, were much larger than N_2O emissions after

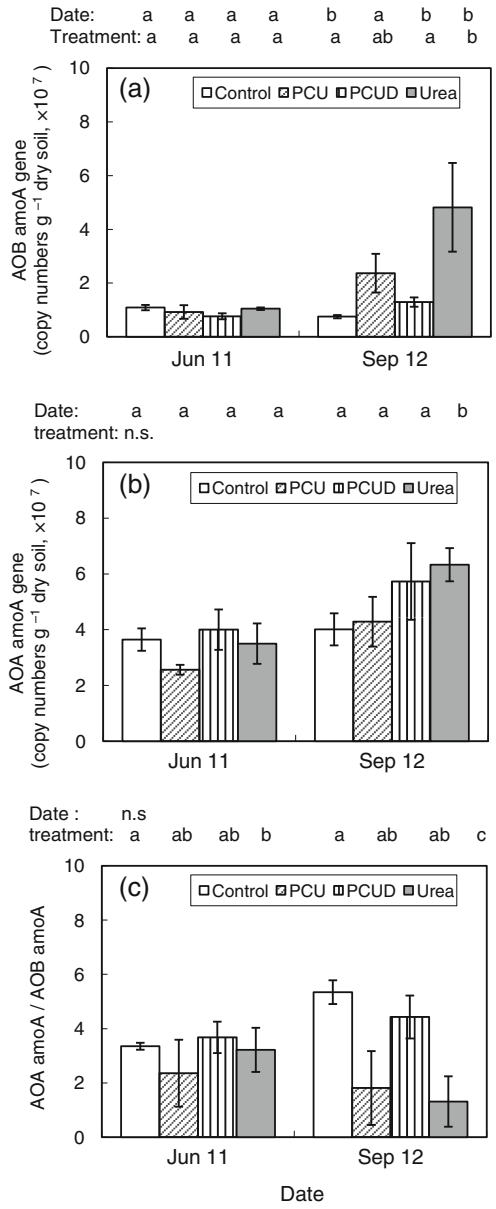

Fig. 4 Number of *amoA* gene copy numbers in soil among ammonia-oxidizing bacteria (*AOB*) (**a**) and ammonia-oxidizing archaea (*AOA*) (**b**) and the ratio of AOA *amoA* to AOB *amoA* before basal fertilizer application (June 11, 2008) and after basal fertilizer application (June 24, 2008) (**c**). Treatments were no-N control, polymer-coated urea (*PCU*), polymer-coated urea with dicyandiamide (*PCUD*), and urea. *Columns with the same letter* are not significantly different ($P<0.05$) between dates of same fertilizer treatments or between fertilizer treatments on the same sampling day by Tukey's test. *n.s.* no significant difference. *Error bars* indicate SD

fertilization. The main controlling factor of episodic N_2O emissions was WFPS rather than NO_3^- content; thus, polymer coating and nitrification inhibitor were not effective in reducing N_2O emissions during this period. Investigating denitrification in addition to nitrification is needed in future studies to link N_2O emissions and microbial pathways in situ.

Although many studies have reported increased N_2O emissions after rains, only a few have reported episodic emissions as high as those in our study. For example, using an automated flux monitoring system, Zheng et al. (2000) reported large N_2O emissions (about 10 mg N m^{-2} h^{-1}, 2.4 kg N ha^{-1} day^{-1}) at 99 % WFPS after heavy rainfall (82 mm day^{-1}) and also during the flooding of rice fields in a rice–wheat rotation cycle. Similarly, Ball et al. (2004), using an automated gas sampling system, reported epidemic N_2O emissions (up to 4.9 kg N ha^{-1} day^{-1}) from an imperfectly drained Gleysol grassland after heavy rain. These results indicate that this phenomenon is an important source of N_2O emissions from poorly drained agricultural fields, and because of its occurrence over short durations, it is possible that episodic N_2O emissions may have been missed in other studies. The identification of such episodic N_2O emissions requires daily monitoring. However, the typical measurement frequency used in common manual sampling methods is once or twice a week after fertilizer application, and even less frequently a month after fertilizer application, because it is generally considered that the bulk of the annual N_2O flux occurs during the first month (Dobbie and Smith 2003). In our study, however, the episodic N_2O emissions induced by heavy rainfall occurred 2 months after basal fertilizer application (1 month after the first additional fertilizer application to urea treatment), and the episodic N_2O emissions accounted for 55–80 % of total N_2O emitted over the entire monitoring period (Table 1). Therefore, missing the peak would have led to substantial underestimation of total N_2O emissions.

Low levels of N_2O emissions from the control plots during the episodic N_2O emissions period indicated that in addition to high WFPS, soil mineral N is required for high episodic N_2O emissions. Generally, WFPS, soil NO_3^- content, available C, and temperature are recognized to affect microbial denitrification (de Klein and Van Logtestijn 1996). In this study, available C and temperature were not changed between control and N fertilizer-applied plots; thus, these were not limiting factors during this period. Such high episodic N_2O emissions would occur only when none of the factors affecting microbial denitrification are limiting. Our results suggested that mitigating episodic N_2O emissions would greatly reduce annual N_2O emissions, and improving soil drainage, such as by the installation of effective tile drains, could be one option. de Klein and Ledgard (2005) estimated that optimizing drainage in poorly and imperfectly drained soils could reduce total direct and indirect N_2O emissions from New Zealand agriculture by 10 %.

Ammonia oxidation potential and abundances of ammonia-oxidizing bacteria and archaea

Our result that AOA was more abundant than AOB in the soil agreed with those of past studies (Di et al. 2010b; Chen

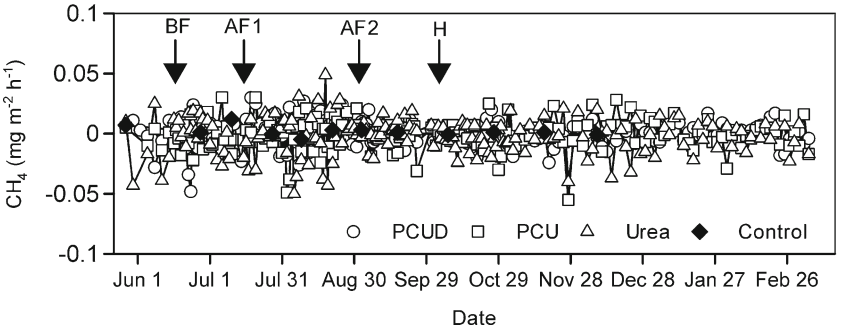

Fig. 5 Seasonal variations in CH_4 flux after application of different fertilizers. The treatments were no-N control, urea, polymer-coated urea (*PCU*), and polymer-coated urea with dicyandiamide (*PCUD*). *BF* basal fertilizer application to PCUD, PCU and urea plots, *AF* additional fertilizer application to urea plots, *H* harvest of carrots. Measurement period was from May 27, 2008 to March 6, 2009

et al. 2011; He et al. 2007; Leininger et al. 2006; Onodera et al. 2010; Shen et al. 2008). It has been suggested that AOB prefer high-NH_4^+ conditions, whereas AOA prefer low-NH_4^+ conditions (Erguder et al. 2009; Martens-Habbena et al. 2009; Valentine 2007). Di et al. (2009, 2010a) and Jia and Conrad (2009) reported that AOB play a more important role in nitrification in high-N agricultural soils than AOA. In our study, however, both AOB and AOA *amoA* gene copy numbers significantly increased from June 11 to September 12 ($P<0.05$) after urea application, although the increase rate of AOB (4.6 times) was much greater than that of AOA (1.8 times). These results suggested that, probably, both AOB and AOA are involved in ammonia oxidation after fertilizer application, but the response of AOB to fertilizer application was greater than AOA. Previous studies also reported that both AOB and AOA contributed to ammonia oxidation in agricultural soil (He et al. 2007; Morimoto et al. 2011; Schauss et al. 2009).

The significantly lower NH_4^+ and NO_3^- concentrations (Fig. 2) and ammonia oxidation potential (Fig. 4) after fertilizer application in the PCU and PCUD plots than in the urea plots ($P<0.05$) show that the polymer coating slowed the release of N, thus restraining the ammonia oxidation potential. In incubation and pot experiments on urine-treated soil, Di et al. (2009, 2010b) and O'Callaghan et al. (2010) reported that dicyandiamide significantly inhibited AOB population growth. In our study, however, the effect of dicyandiamide on additional reduction of the NO_3^- concentration and the AOB *amoA* gene copy numbers was not clear.

CH_4 fluxes

The CH_4 uptake in this field (−0.00081to −0.0045 kg CH_4 per hectare for 10 months; Table 1) was an order of magnitude less than that in a grey lowland soil in a nearby upland ex-paddy field (Nishimura et al. 2008). Dutaur and Verchot (2007) summarized global CH_4 uptake data and reported that the most important factor determining the CH_4 uptake rates is ecosystem type: uptake in agricultural soil is lower than in forest soil. The uptake rate in this study was in the lowest end of the range of reported CH_4 uptake rates for cultivated land (range, 0 to −4.23 kg CH_4 ha^{-1} $year^{-1}$; mean, −1.23 kg CH_4 ha^{-1} $year^{-1}$; Dutaur and Verchot 2007; note that the uptakes in our study are for 10 months). The low CH_4 uptake rate was probably due to the poor drainage of the field. In this study, neither the nitrification inhibitor dicyandiamide nor urea application affected CH_4 uptake to any measurable degree, probably because of the low range in CH_4 uptake rates.

The CH_4 uptake by soil generally decreases with increasing soil water content, with temperature generally having a secondary effect (Dalal et al. 2008). However, the variation in the rate of CH_4 uptake was too low to detect any relationships between it and WFPS and temperature.

Conclusion

The use of PCU and PCUD was tested as potential mitigation options for N_2O emissions in an imperfectly drained upland field. After basal fertilizer application in PCU, PCUD, and urea plots, small N_2O peaks were observed following moderate rainfall. Large episodic N_2O emissions associated with high WFPS caused by heavy rainfall indicated that denitrification was the main pathway for the episodic N_2O emissions and are a major source of N_2O in poorly drained agricultural fields. It is possible that N_2O emissions may have been underestimated in previous studies if the N_2O emission peak was missed due to inadequate sampling frequency. Mitigating these episodic N_2O emissions would significantly reduce annual N_2O emissions, and improving soil drainage, such as by the installation of effective tile drains, could be one option. Urea application significantly increased both AOB and AOA abundances, although the increase rate of AOB was much greater than that of AOA. Our results suggested that both AOB and AOA contributed to ammonia oxidation after fertilizer

application, but the response of AOB was greater than AOA. Although PCU and PCUD lowered ammonia oxidation potential compared to urea treatment, they were not effective in reducing cumulative N_2O emissions. Further research linking field-scale N_2O and CH_4 fluxes and microbial processes is needed to better quantify greenhouse gas fluxes from agricultural soils and to mitigate N_2O emissions.

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Conflict of interest The authors declare that they have no conflict of interest.

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