Nitrous oxide emissions from grazed grassland as affected by a nitrification inhibitor, dicyandiamide, and relationships with ammonia-oxidizing bacteria and archaea

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Abstract

Purpose Nitrous oxide (N_2O) is a potent greenhouse gas and, in grazed grassland systems where animals graze outdoor pastures, most of the N_2O is emitted from animal urine nitrogen (N) deposited during grazing. Recently, ammonia-oxidizing archaea (AOA) were found to be present in large numbers in soils as well in the ocean, suggesting a potentially important role for AOA, in addition to ammonia-oxidizing bacteria (AOB), in the nitrogen cycle. The relationship between N_2O emissions and AOB and AOA populations is unknown. The objective of this study was to determine the quantitative relationship between N_2O emissions and AOB and AOA populations in nitrogen-rich grassland soils.

Materials and methods We determined N_2O emissions and the abundance and activity of AOB and AOA in four different soils as affected by the applications of animal urine N and dicyandiamide (DCD) as a nitrification inhibitor. Relationships were then determined between N_2O emissions and the AOB and AOA populations.

Results and discussion Total N_2O emissions from the urine N applied at $1,000 \text{ kg N ha}^{-1}$ varied significantly in these soils, from 13.9 to $39.8 \text{ kg N}_2\text{O-N ha}^{-1}$. The DCD treatment reduced these N_2O emissions to between 2.8 and $15.3 \text{ kg N}_2\text{O-N ha}^{-1}$. The average emission factor of the urine N (EF_3) was 2.2% and this was decreased to 0.8%, representing a 64% reduction. Total N_2O emissions were significantly related to the *amoA* gene copy numbers of the AOB population and to the amount of nitrate-N in the soil, but not related to the abundance of AOA.

Conclusions These results demonstrate that the N_2O flux is mainly associated with the dynamics of the AOB population rather than the AOA in these nitrogen-rich grassland soils, and therefore, it is the AOB that should be the target of inhibition when mitigating N_2O emissions using nitrification inhibitors.

Keywords Ammonia-oxidizing archaea (AOA) • Ammonia-oxidizing bacteria (AOB) • Dicyandiamide • Grazed grassland • Greenhouse gas • Nitrification inhibitor • Nitrous oxide

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1 Introduction

Nitrification is a key biogeochemical process of the nitrogen (N) cycle which results in the oxidation of ammonia (NH_3) to nitrite (NO_2^-) and then to nitrate (NO_3^-). This process has major environmental and ecological consequences because it releases nitrous oxide (N_2O) which is a potent greenhouse gas and NO_3^- which can be leached to contaminate groundwater and surface waters. The long-term global warming potential of N_2O is 310 times that of carbon dioxide (CO_2) (IPCC 1995). N_2O is

released from both nitrification (Eq. 1) and denitrification (Eq. 2) in the soil:

Ammonia monooxygenase (AMO) is the key enzyme responsible for the first step of the nitrification process, the conversion of ammonia to hydroxylamine which is then converted to nitrite by hydroxylamine oxidoreductase. The AMO enzyme has three subunits which are encoded by the *amoA*, *amoB*, and *amoC* genes. Until recently, the first rate-limiting step of nitrification, the oxidation of NH_3 to hydroxylamine, is thought to be carried out mainly by autotrophic ammonia-oxidizing bacteria (AOB) of the β - and γ -subgroups of proteobacteria (Purkhold et al. 2000; Kowalchuk and Stephen 2001). Recently, however, the *amoA* gene has also been found in the domain Archaea, suggesting the possible presence of ammonia-oxidizing archaea (AOA; Venter et al. 2004; Könneke et al. 2005; Francis et al. 2005; Wuchter et al. 2006). Previously, archaea were thought to be extremophiles that lived in harsh environments, such as hot springs, but have recently been found in a broad range of habitats, such as soils and oceans. Archaea were found to be dominant among ammonia-oxidizing prokaryotes in a range of soils, suggesting that archaea may be the most abundant ammonia-oxidizing organisms in soil ecosystems on earth (Leininger et al. 2006; He et al. 2007; Prosser and Nicol 2008). However, the role of AOB and AOA in ammonia oxidation is not well understood and their relationships with N_2O emissions are unknown since they only indirectly affect the N_2O production processes.

In intensively grazed grassland, e.g., grazed dairy pastures, the largest N source for both NO_3^- leaching and N_2O emissions is the urine N excreted by the animal during grazing (Di and Cameron 2002a; Bolan et al. 2004; Di et al. 2007). Of the N ingested by the grazing animal, between 70% and 90% is excreted onto the pasture soil and about 80% is in the urine. The nitrogen loading rate under a single dairy cow urine patch can be as high as $1,000 \text{ kg N ha}^{-1}$ (Di and Cameron 2002a). About 25% of the grazed field may be covered by urine patches each year in a grazed dairy pasture, depending on the stocking rate and grazing period. Most of the N excreted in the urine is urea which, upon hydrolysis in the soil, is converted to NH_4^+ , thus available for nitrification. The nitrate ions produced through the nitrification process are subject to a number of reactions including plant uptake, leaching, and denitrification. Major efforts have been made to develop mitigation solutions for

N_2O emissions and NO_3^- leaching from urine patches in grazed grassland. One of the recent advances is the development of an agricultural spray containing a nitrification inhibitor to inhibit the conversion of NH_3 to NO_3^- (Di and Cameron 2002b). Nitrification inhibitors work by deactivating the AMO enzyme of soil ammonia-oxidizers responsible for the first step of the nitrification process, the conversion from ammonia to hydroxylamine (Amberger 1989). However, it remains unclear what role different ammonia-oxidizers (e.g., AOB and AOA) play in the production of N_2O in the high N loading urine patch soils and what relationships exist between the AOB and AOA abundance and N_2O emissions when nitrification inhibitors are applied. This knowledge is important for developing soil management technologies to mitigate N_2O emissions using nitrification inhibitors.

The objective of this research was to determine the quantitative relationship between N_2O emissions and AOB and AOA populations. In a series of field experiments, N_2O emissions were determined in a range of intensively grazed grassland soils sampled from across New Zealand as affected by applications of animal urine N as a nitrogen source and a nitrification inhibitor (dicyandiamide [DCD]). The abundance and activity of AOB and AOA populations in these soils were determined using quantitative polymerase chain reaction in a laboratory incubation study. The relationships between N_2O emissions and AOB and AOA populations were then determined. The hypothesis was that N_2O emissions as affected by the urine N and the DCD treatments would be related to AOB and AOA populations if they are functionally important for N_2O production in the soil. The AOB and AOA abundance has been reported previously (Di et al. 2009). This paper reports the N_2O emissions from the field studies as affected by the urine N and DCD treatments and relates them to AOB and AOA abundance as reported in Di et al. (2009).

2 Materials and methods

2.1 Soils and pasture

Four soils were used in this study; three were from different parts of New Zealand's South Island including Canterbury (CT), the West Coast (WC), and Southland (SL) and one from the Waikato (WK) in the northern part of New Zealand's North Island (Table 1). The four soils differ significantly in their properties. The CT Lismore soil (NZ Classification: Pallic Orthic Brown Soil, Hewitt 1998; USDA Soil Classification: Udic Haplustepl loamy skeletal, Soil Survey Staff 1998) is developed from gravely glacial outwash covered with varying depths of loess. It only has a shallow layer of fine soil at the surface, below which the

Table 1 Properties of soils used for the study

Location	Canterbury (CT) 43°45'54" S; 171°41'25" E	West Coast (WC) 42°42'12" S; 171°26'43" E	Southland (SL) 46°19'21" S; 168°16'02" E	Waikato (WK) 38°46'38" S; 175°18'26" E
Soil type	Lismore stony silt loam	Harihari recent silt loam	Mataura recent sandy loam	Horotiu silt loam
Particle size (%)				
Sand	10.5	4.2	69.1	20.4
Silt	66.0	93.6	29.0	73.8
Clay	23.5	2.2	1.9	5.8
pH (H ₂ O)	5.9	5.4	5.6	5.9
Organic C (g kg ⁻¹)	36.5	68.8	62.6	40.4
Total N (g kg ⁻¹)	3.5	5.6	5.7	3.5
Olsen P (mg kg ⁻¹)	53.0	46.5	21.1	16.9
CEC (cmol _c kg ⁻¹)	17.5	15.0	19.0	13.0
Exch. Ca ²⁺ (cmol _c kg ⁻¹)	10.3	5.0	6.7	7.1
Exch. Mg ²⁺ (cmol _c kg ⁻¹)	0.53	0.94	0.9	0.27
Exch. K ⁺ (cmol _c kg ⁻¹)	0.71	0.25	0.24	0.19
Base saturation (%)	66.0	40.4	42.5	60.6

gravel content increases significantly, thus making this soil very free-draining. The WC Harihari soil (Mottled Fluvial Recent; Aquents) is a silt loam soil with high silt content. The land at the site had been remodeled to create “humps” and “hollows” to increase drainage, which is typical of local practice. The lysimeters were collected at the “hump” or shoulder places. The SL Mataura soil is a recent sandy loam (Typic Fluvial Recent; Fluvents) developed in alluvium derived from schist and greywacke rock with high sand content. The WK Horotiu soil (Typic Orthic Allophanic Soil; Typic Udivitrand) is a free-draining soil developed from volcanic parent material. The pasture on all four soils was a mixture of perennial ryegrass (*Lolium perenne*) and white clover (*Trifolium repens*) used for dairy grazing.

Field experiments were conducted to determine N₂O emissions using large undisturbed soil monolith lysimeters (0.5 m diameter and 0.7 m deep) collected from each of the four sites. The collection of the lysimeters involved placing a metal cylinder casing on the soil surface, digging around the casing, and gradually pushing the casing down by small increments (Cameron et al. 1992). Once the casing had reached the desired depth, the soil monolith was cut at the base with a cutting plate, secured on the lysimeter casing and lifted out of the collection site. The gap between the soil core and the casing was sealed using petroleum jelly to stop edge-flow effects. The lysimeters from the three South Island sites were taken to and installed at a field lysimeter

facility at Lincoln University which is about 20 km south of Christchurch on the east coast of the South Island. The Horotiu soil lysimeters in the North Island were installed at a field lysimeter facility located on a research dairy farm just outside Hamilton in the Waikato region in the northern part of the North Island. The lysimeters were installed on either side of a trench with the soil surface of the lysimeters at the same level as the surrounding field. The space outside the lysimeter casings was backfilled with soil to the same level as the surface of the lysimeters and the surrounding field. The lysimeters were thus exposed to the same climatic conditions as the soil and pasture in the surrounding field.

2.2 Treatments on the lysimeters

For each soil type, there were two main treatments: urine and urine plus soil treatment with the nitrification inhibitor DCD; and each treatment had four replicates. Another lysimeter was used as a control for each of the three soils in the South Island to provide background N₂O emissions data. Previous studies have shown that background emissions from control soils were very low (Di et al. 2007). All the treatments, including the control, at the Waikato site in the North Island had four replicates. Fresh dairy cow urine was collected from lactating cows and analyzed for total N concentrations. The fresh cow urine with its N concentration standardized to 10.0 g L⁻¹ was applied to the

lysimeters at the equivalent rate of $1,000 \text{ kg N ha}^{-1}$ (19.6 g urine N per lysimeter, applied in 1.96 L of urine in a single application) in May to simulate a typical dairy cow urine patch (Di and Cameron 2002a; Haynes and Williams 1993). The DCD was dissolved in water and applied to the lysimeters at 10 kg ha^{-1} on the same day as the urine application in May. The DCD was applied again in July at the Waikato site and in August at the Lincoln University site. The two DCD applications, one in the autumn (May) and one in late winter/early spring (August), were designed to follow standard recommendations in New Zealand to reduce nitrate leaching during this high-drainage period from late autumn through winter to early spring. This is also the period when N_2O emissions are the highest due to wet soil conditions. Urea fertilizer was applied to all the lysimeters, including the controls, at the annual rate of 200 kg N ha^{-1} , split into eight applications at about monthly intervals starting from August (25 kg N ha^{-1} per application), following local farming practice. During the period of N_2O measurement (May–October), about 50 kg ha^{-1} of urea-N was applied because there was no fertilizer application during the winter months (May to July).

For the lysimeters from the three South Island sites installed at Lincoln University (CT, WC, and SL; see Table 1), two rainfall conditions were created to test the influence of rainfall inputs on N_2O emissions. This was done by applying simulated rainfall to supplement natural rainfall to create 1,100 and 2,200 mm of total rainfall per year. Simulated rainfall was applied using automated rainfall simulators. The irrigation water was deep groundwater (80 m depth) free from contamination (NO_3^- -N concentration $< 0.1 \text{ mg L}^{-1}$). The amount of rainfall applied varied in different seasons with a greater amount applied during the summer to meet the higher water demand by plants due to greater evapotranspiration. For the 1,100-mm condition, total rainfall during the winter was to reach the 75th percentile of rainfall records during this period, and summer rainfall was to equal the total water input of rainfall plus irrigation normally applied in the Canterbury region where this research was conducted. The amount of water applied for the 2,200-mm condition was simply to approximately double that of the 1,100-mm water input in the different seasons to create two significantly different rainfall treatments. These two rainfall treatments would cover the range of rainfall conditions in the main dairy farming regions of New Zealand.

At the Waikato site in the North Island, only one water input condition (the natural rainfall) was used due to resource constraints. Annual average rainfall at the site is about 1,200 mm per annum.

Pasture was harvested to typical grazing heights and removed before the application of the treatments and was cut at typical grazing intervals thereafter.

2.3 N_2O sampling and analysis

A standard closed chamber method similar to that described by Hutchinson and Mosier (1981) was used to determine N_2O emissions from the treated lysimeters. This is the same method that has been used to provide N_2O emissions data for the New Zealand national inventory. The gas chamber was constructed of a metal cylinder insulated with 2.5 cm thick polystyrene foam to avoid heating of the atmosphere in the chamber during sampling. During periods of N_2O measurement, the chamber was placed inside a small water trough which was mounted around the top of each lysimeter casing for gas sampling. At each sampling time, the chamber was placed on top of the lysimeters for a total of 40 min, and three samples (12 mL each), 20 min apart, were taken using a syringe through a rubber septum on top of the gas chamber. It has been shown that nitrous oxide fluxes may vary in response to temperature changes at different times of the day (Hatch et al. 2005). However, a local study has shown that the fluxes measured at midday represent a good daily average incorporating diurnal fluctuations (Barton et al. 2000; De Klein et al. 2003). Consequently, each sampling was carried out during the middle of the day between 1200 and 1400 hours when temperatures were very similar to daily means. Nitrous oxide was analyzed using gas chromatography (SRI 8610 gas chromatograph; SRI Instruments, California, USA) equipped with a ^{63}Ni electron capture detector. Sampling continued until N_2O levels reached the same levels as those in the controls.

2.4 Data analysis

Firstly, hourly N_2O fluxes were calculated using the slope of chamber headspace N_2O concentration change from the three samples collected on each sampling occasion (Hutchinson and Mosier 1981). Then daily emissions were calculated using those hourly fluxes, assuming that the fluxes measured represent the average hourly flux of that day. Cumulative emissions were then calculated by integrating the measured daily fluxes for the whole experimental measurement period. Analysis of variance for the N_2O emissions data from the urine and urine + DCD treatments was performed using GenStat (Version 11, Lawes Agricultural Trust) using a randomized block design.

2.5 AOB and AOA populations and nitrification rate

In order to explore the relationships between N_2O flux and AOB and AOA populations and nitrification rate in the soil, a companion laboratory incubation study was conducted to quantify the dynamics of AOB and AOA in the soil and nitrification rate. Details of the methods and results have been reported (Di et al. 2009) and only a brief summary is

provided here to aid in understanding the relationships between N_2O emissions and AOB and AOA. Soil samples (10 kg) were collected from 0 to 0.1 m depths at 10 random locations in a dairy farm from each region where the lysimeters were collected. The 10 samples were bulked into a single sample for each region and used for an incubation study. The soil samples were treated with urine N with and without DCD in the same rates as those in the lysimeter studies described above and were incubated under simulated autumn–winter–spring conditions (12°C) for 3 months at 80% soil moisture capacity. Subsamples were taken 1, 7, 21, 49, and 92 days after the application of the treatments and were analyzed for AOB and AOA populations using real-time polymerase chain reaction with primers targeting the

AOB and AOA *amoA* gene (Rotthauwe et al. 1997; Francis et al. 2005; Leininger et al. 2006). Subsamples of soil were taken at 1, 7, 14, 21, 28, 35, 49, 63, 77, and 92 days after the application of the treatments and were extracted with 2 M KCl to determine NO_3^- -N concentrations (Di et al. 2009).

3 Results

3.1 Temperature and rainfall conditions for N_2O measurements

At the Lincoln University lysimeter site, daily average air temperature varied from 0.8°C to 18.3°C during the period

Fig. 1 **a** Air temperature; **b** water inputs for the lower water regime; and **c** water inputs for the higher water regime for the South Island soils

of N_2O measurements (Fig. 1a). Although annual rainfall targets of 1,100 and 2,200 mm were set at the start of the project, the wetter than anticipated winter resulted in actual water input of 514 mm for the lower rainfall target for the 5-month N_2O measurement period and this was equivalent to about 1,250 mm per annum (thus slightly higher than the 1,100 mm target; see Fig. 1b). However, actual water input for the higher rainfall target condition was 615 mm for the N_2O measurement period and this was equivalent to about 1,550 mm per annum (thus lower than the 2,200 mm target for this period; see Fig. 1c). This was done to allow a higher amount of water applied during the summer period to meet the higher evapotranspiration losses in order to keep the annual total rainfall at 2,200 mm.

At the Waikato lysimeter site in the North Island, the daily average air temperature varied from a low of 3°C to a high of 16.3°C (Fig. 5a). The water input was 300 mm during the 82-day experimental period, equivalent to annual rainfall of about 1,300 mm (see Fig. 5b).

3.2 Daily N_2O emissions

At the Lincoln University lysimeter site in the South Island, peak N_2O -N emissions reached $1,234 \text{ g N}_2\text{O-N ha}^{-1} \text{ day}^{-1}$ for the urine treatment in the CT Lismore soil under the lower

rainfall condition (Fig. 2a). This was decreased to $623 \text{ g N}_2\text{O-N ha}^{-1} \text{ day}^{-1}$ with the application of DCD. For the Lismore soil under the higher rainfall condition, peak N_2O emissions reached $2,223 \text{ g N}_2\text{O-N ha}^{-1} \text{ day}^{-1}$ for the urine treatment (see Fig. 2b). This was decreased to $521 \text{ g N}_2\text{O-N ha}^{-1} \text{ day}^{-1}$ with the application of DCD. For the WC Harihari soil, the N_2O flux did not produce as distinctive a single N_2O peak as those found in the CT Lismore soil (Fig. 3a, b). However, the daily N_2O fluxes from the urine plus DCD treatment were lower than those of the urine treatment through most of the sampling period. For the SL Mataura soil, the peak N_2O flux reached $1,056 \text{ g N}_2\text{O-N ha}^{-1} \text{ day}^{-1}$ in the urine treatment for the lower rainfall condition (Fig. 4a), and this was decreased to $461 \text{ g N}_2\text{O-N ha}^{-1} \text{ day}^{-1}$ with the application of DCD. For the higher rainfall condition, the peak N_2O flux was $706 \text{ g N}_2\text{O-N ha}^{-1} \text{ day}^{-1}$ (see Fig. 4b) for the urine treatment, and this was decreased to $539 \text{ g N}_2\text{O-N ha}^{-1} \text{ day}^{-1}$ when DCD was applied.

For the Horotiu soil in the Waikato lysimeter site, peak N_2O flux reached $850 \text{ g N}_2\text{O-N ha}^{-1} \text{ day}^{-1}$ in the urine treatment (Fig. 5c). This was decreased to $162 \text{ g N}_2\text{O-N ha}^{-1} \text{ day}^{-1}$ when DCD was applied.

The N_2O fluxes in the controls were very low in all the four soils (see Figs. 2, 3, 4, and 5) which is consistent with findings in previous studies (Di et al. 2007).

Fig. 2 Daily N_2O emissions from the Canterbury Lismore stony silt loam soil under **a** lower rainfall and **b** higher rainfall conditions. Error bars indicate SEM

Fig. 3 Daily N_2O emissions from the West Coast Harihari recent silt loam under **a** lower rainfall and **b** higher rainfall conditions. Error bars indicate SEM

Fig. 4 Daily N_2O emissions from the Southland Mataka recent sandy loam soil under **a** lower rainfall and **b** higher rainfall conditions. Error bars indicate SEM

Fig. 5 **a** Daily average air temperature; **b** daily and cumulative water inputs; and **c** daily N_2O emissions from the Wai-kato Horotiu soil in the North Island. Error bars indicate SEM

3.3 Total N_2O emissions

Total N_2O emissions varied significantly between the different soils, with those in the urine treatment ranging from a low of $13.9 \text{ kg N}_2\text{O-N ha}^{-1}$ in the WC Harihari soil under the higher rainfall condition to a high of $39.8 \text{ kg N}_2\text{O-N ha}^{-1}$ in the CT Lismore soil under the higher rainfall condition (Table 2). The different water inputs did not result in significantly different total N_2O emissions ($P > 0.05$) due to relatively similar soil water conditions during the winter period (see Section 4). However, the DCD treatment did decrease the total N_2O emissions from all of the four soils. The emission factor ($\text{N}_2\text{O-N}$ emitted from the urine minus that from the control as a percentage of the urine N applied) from the urine (EF_3) varied from 1.4% to

3.0% (averaging 2.2%), and this was decreased to between 0.3% and 1.4% (averaging 0.8%) with the DCD treatment. Therefore, the DCD treatment resulted in a reduction of the average EF_3 by 64%. The percentage reduction of EF_3 by DCD under the higher rainfall condition was greater for two of the soils (CT and WC) and smaller for one soil (SL) compared to those under the lower rainfall condition.

3.4 Relationships between N_2O flux and AOB, AOA, and nitrification rate

The AOB and AOA abundance data reported in Di et al. (2009) were used to determine the relationships with N_2O emissions reported in this paper. The following significant exponential relationship was found between the total N_2O

Table 2 Total N₂O emissions and EF₃ as affected by soil, water input, and urine N and DCD treatments

Soil	Water inputs (mm/year)	Treatments	N ₂ O-N emissions (kg ha ⁻¹)	EF ₃ (%)	Percent reduction of EF ₃ by DCD
Canterbury	1,250	Control	1.07	— ^a	—
Canterbury	1,250	Urine	31.4 (2.6) ^b	3.0	—
Canterbury	1,250	Urine + DCD	15.3 (1.1)	1.4	53
West Coast	1,250	Control	1.04	—	—
West Coast	1,250	Urine	19.7 (3.0)	1.9	—
West Coast	1,250	Urine + DCD	8.8 (2.9)	0.8	58
Southland	1,250	Control	1.17	—	—
Southland	1,250	Urine	21.3 (8.9)	2.0	—
Southland	1,250	Urine + DCD	10.6 (1.6)	0.9	55
Canterbury	1,550	Control	1.26	—	—
Canterbury	1,550	Urine	39.8 (9.3)	3.9	—
Canterbury	1,550	Urine + DCD	11.9 (1.3)	1.1	72
West Coast	1,550	Control	0.38	—	—
West Coast	1,550	Urine	13.9 (3.0)	1.4	—
West Coast	1,550	Urine + DCD	4.9 (0.9)	0.4	71
Southland	1,550	Control	1.24	—	—
Southland	1,550	Urine	16.4 (2.1)	1.5	—
Southland	1,550	Urine + DCD	11.8 (0.7)	1.0	33
Waikato	1,300	Control	0.2 (0.09)	—	—
Waikato	1,300	Urine	16.6 (5.6)	1.6	—
Waikato	1,300	Urine + DCD	2.8 (0.4)	0.3	81
SED ^c for treatment			2.21		
Average EF ₃ from urine				2.2	
Average EF ₃ from urine + DCD				0.8	
Reduction of average EF ₃ by DCD (%)				64	

^a Not applicable^b Figures in the parentheses are standard errors of the mean (SEM). No SEM values in the controls for the Canterbury, Southland, and West Coast soils due to lack of replication^c Standard of the difference (degree of freedom=42)

emissions and the AOB populations after 49 days of incubation (when the AOB population peaked in most of the urine treatments; Di et al. 2009):

$$y = 23.4 - 24.3\text{EXP}(-0.031x) \quad (3)$$

$$R^2 = 0.47; P < 0.01$$

where y is the total N₂O emissions (in kilograms of N₂O-N per hectare) and x is the AOB *amoA* gene copy numbers (in million copies per gram of soil; Fig. 6a). However, no quantitative relationship was found between N₂O flux and AOA populations (see Fig. 6b). In addition, a significant relationship was found between total N₂O flux and NO₃⁻-N concentration in the soil after 49 days of incubation (see Fig. 6c):

$$y = 50.0 - 55.1\text{EXP}(-0.001x) \quad (4)$$

$$R^2 = 0.79; P < 0.01$$

where y is the total N₂O flux (in kilograms of N₂O-N per hectare) and x is the NO₃⁻-N concentration in the soil at

49 days of incubation (when AOB population peaked; Di et al. 2009).

4 Discussion

The finding of the *amoA* gene in archaea populations has raised the prospect of an important role for AOA in nitrification and thus the potential for N₂O production in different ecosystems (Könneke et al. 2005; Francis et al. 2005). Here, we show that N₂O emissions from the high-N loading urine patch soils in grazed grassland are mainly associated with the dynamics of the AOB populations rather than with that of the AOA despite the presence of AOA in these soils in large numbers. It has been suggested that AOA may be particularly adapted to unfavorable and low fertility environments (Mahmood and Prosser 2006; Valentine 2007; Schauss et al. 2009; Di et al. 2009). Therefore, in the soils that receive high rates of animal

Fig. 6 Relationships between the total N_2O flux and **a** soil AOB *amoA* gene copy numbers; **b** soil AOA *amoA* gene copy numbers; and **c** soil NO_3^- -N concentration after 49 days of incubation in the companion study

urine N in grazed grassland, while the AOB populations can adapt and flourish under high nutrient availability conditions, the growth and activity of the AOA populations are limited (Di et al. 2009). Therefore, AOA was only playing a limited role in N_2O emissions in the urine patch soils. However, further research is required to determine the role of AOA in N_2O production under a wide range of soil conditions.

The very good relationship ($R^2=0.79$; see Fig. 6c) between the N_2O flux and NO_3^- -N concentration in the soil suggests that NO_3^- , being both a product of the nitrification process and a substrate for the denitrification process, is an important indicator for N_2O emissions from urine patch soils in grazed grassland, accounting for about 79% of the variation of the N_2O emissions in these soils. The amount of variation of N_2O emissions accounted for by AOB abundance is lower ($R^2=0.47$). This is not surprising considering that N_2O is produced by both nitrification and denitrification (Eqs. 1 and 2, respectively) in the soil and is

affected by a number of factors. In addition, the AOB abundance was measured under laboratory controlled conditions, whereas the N_2O emissions were measured under field conditions. The soil samples used for the incubation study were also collected separately at different times from the lysimeters.

This study also shows that the DCD nitrification inhibitor technology was highly effective in reducing N_2O emissions from all four soils under different environmental conditions. The average reduction of 64% of the average urine N EF_3 was similar to that (70%) reported previously (Di et al. 2007). The effectiveness of DCD was not significantly affected by the amount of rainfall applied. The annual rainfall conditions created for this study covered those of most of the dairy farming regions of New Zealand which are rain fed or irrigated. This study and those reported by Di et al. (2007) clearly show that DCD is highly effective in reducing N_2O emissions in six different soils from five key dairy regions across New Zealand.

The reduction of the EF_3 in the SL Mataura soil under the higher rainfall condition was much smaller than in the other soils and compared with previous studies (Di et al. 2007). It is not clear what was causing this smaller reduction because DCD was shown to be effective in inhibiting the nitrification process and the AOB populations in this soil (Di et al. 2009). Similarly, it is not clear why daily N_2O flux was higher in the lower rainfall than in the higher rainfall treatments in this soil (see Fig. 4).

It has been shown previously that water is an important factor for N_2O emissions from agricultural soils as denitrification, which produces N_2O , is favored under wet (and anaerobic) soil conditions (e.g., Machefert et al. 2002; Baggs et al. 2003; Dobbie and Smith 2003). However, it is probably not surprising that the amount of water input did not have a significant impact on the amount of N_2O emitted in the present study because the experiments were conducted during the wet autumn–winter–spring period when the soil water content was close to field capacity for all the soils. Therefore, even the lower rainfall condition did not create a dry enough soil condition to provide a spectrum of soil moisture conditions to give significant influence of soil water-filled pore space on N_2O emissions.

The average EF_3 for the urine N of 2.2% without the use of DCD derived from this study was higher than the New Zealand country-specific value of 1%. Our previous study showed that the EF_3 from the urine N varied from 0.1% to 2.0% in different soils, averaging 0.9% (Di et al. 2007). The higher EF_3 value in the present study was because of the wet soil conditions under which these studies were conducted which favored higher N_2O emissions. This value, therefore, does not represent the average EF_3 as it does not include those measured during the drier seasons of the year. However, it is particularly encouraging to see the effective performance of DCD in reducing N_2O emissions under the relatively high rainfall conditions because the wet autumn–winter–early spring season is when N_2O emissions are the highest due to the wet conditions.

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