Jha N, Palmada, T, Berben P, Saggar S, Luo J, and McMillan A.M.S., 2016. Lime enhances denitrification rate and denitrifier gene abundance in pasture soils treated with urine and urine + DCD. In: *Integrated nutrient and water management for sustainable farming*. (Eds L.D. Currie and R.Singh). http://flrc.massey.ac.nz/publications.html. Occasional Report No. 29. Fertilizer and Lime Research Centre, Massey University, Palmerston North, New Zealand. 13 pages.

LIME ENHANCES DENITRIFICATION RATE AND DENITRIFIER GENE ABUNDANCE IN PASTURE SOILS TREATED WITH URINE AND URINE + DCD

Neha Jha[1], Thilak Palmada[1], Peter Berben[1], Surinder Saggar[1], Jiafa Luo[2] and Andrew M. S. McMillan[1]

[1] Ecosystems and Global Change, Landcare Research, Palmerston North 4442, New Zealand
[2]AgResearch, Hamilton, New Zealand

Abstract

Denitrification is one of the major soil processes that produce nitrous oxide (N_2O) in grazed pastures. Year-round grazing, animal excretion, heavy rainfall, and the use of nitrogen (N) fertilisers lead to high denitrification rates in grazed pastures. Complete denitrification produces harmless dinitrogen (N_2) as its end product, whereas N_2O (a potent greenhouse gas) is produced by incomplete denitrification. Soil pH is one of the key factors controlling denitrification end-products. Soil pH below 6.5 leads to higher N_2O emissions than N_2. Liming of soils to raise its pH and enhance denitrification activity has therefore been suggested to reduce N_2O to N_2. Application of the nitrification inhibitor dicyandiamide (DCD) to urine-affected soils offers another mitigation strategy to reduce N_2O production from soils.

We incubated two soils for 42 days at three liming levels amended with deionised water only, water + cattle urine (600 mg-N kg^{-1} soil), and water + cattle urine (600 mg-N kg^{-1} soil) + DCD (10 mg kg^{-1} soil) at near saturation soil water content, at 10°C and 15°C. We tested the influence of liming-induced pH increase on denitrification and denitrifier gene abundance in amended soils.

We observed higher denitrifier gene abundance in the lime-applied soils than in the soils where no lime was applied. We did not find any significant change in the denitrifier gene abundance with urine and urine + DCD amendments in two soils. The addition of urine along with water that created near-saturated conditions increased cumulative N_2O-emissions and denitrification in soils, and these increases were higher in the urine-amended limed soil than in the urine amended un-limed soils. DCD with urine reduced cumulative N_2O emission (62% for allophanic soil and 48% for fluvial soil at 15°C) and total denitrification (48% for allophanic soil and 40% for fluvial soil at 15°C) in urine-amended limed soil. Our results indicate liming could offer a mitigation option to increase denitrifier population in soils and reduce N_2O production from grazed pasture soils.

Introduction

Nitrification and denitrification are the chief sources of nitrous oxide (N_2O) emissions in grazed pastures. Denitrification acts as both a source and sink of N_2O. Complete denitrification produces harmless dinitrogen (N_2) as its end product, whereas N_2O is produced by incomplete denitrification as a potent greenhouse and ozone-depleting gas (Zumft, 1997). Various soil and environmental factors influence production of N_2O by nitrification and denitrification and further consumption of N_2O by denitrification, as reviewed by Saggar *et al.* (2013). Wet winter conditions, year round grazing, and deposition of animal excreta lead to high denitrification rates in New Zealand pastures (de Klein *et al.*, 2006; Luo *et al.*, 2000; Luo *et al.*, 2008). Soil pH controls the consumption of N_2O by affecting the activity of N_2O reductase, the enzyme responsible for the conversion of N_2O to N_2. Soil pH below 6.5 limits the activity of N_2O reductase and leads to more emission of N_2O than N_2. Soil pH has been found to be an important driver regulating denitrifier population in soils (Čuhel *et al.*, 2010; Enwall *et al.*, 2005). Thus alkaline pH of soil is favourable for reduction of N_2O to N_2.

Application of lime to soil is one of the effective ways to increase or maintain pH more than 6.5. Increased pH by liming has proved to increase numbers of denitrifying organisms (Nodar *et al.*, 1992), which may be helpful in reduction of N_2O. Enhanced pH with liming promotes production of N_2O and conversion of N_2O to N_2 and thus liming has been suggested as one of the possible mitigation options to reduce N_2O emissions (Clough *et al.*, 2003; 2004; Zaman *et al.*, 2007). Our understanding of the mechanism – how soil pH influences denitrifier gene abundance – is limited. It is also uncertain how changing soil pH impacts on the contribution of denitrifiers to net N_2O production, especially when there is urine deposition in grazed pastures.

Application of nitrification inhibitors, particularly dicyandiamide (DCD), to soil is another effective way of controlling N_2O emission from grazed pastures (Di & Cameron, 2006a, 2012). Nitrification inhibitors delay the conversion of ammonium (NH_4^+) to nitrite (NO_2^-) and then to nitrate (NO_3^-) often by weeks or months. Along with reducing nitrification by restricting NO_3^- availability, DCD could also be effective in indirectly controlling denitrification (Weiske *et al.*, 2001). Application of DCD to urine-affected soil has reportedly reduced N_2O emissions from urine patches by 60–85% (Di & Cameron, 2002, 2003, 2006b). Although there has been intensive study on the effect of DCD in successfully inhibiting nitrification, its additional effect on denitrification and the denitrifier population is not very well explored. Reports suggest the effects of DCD on denitrification are soil specific (Morales *et al.*, 2015a) and its effect on denitrifier (*nir*) gene abundance is selective (Di *et al.*, 2014; Wakelin *et al.*, 2013).

Due to the environmental conditions and management practices, grazed pasture soils in New Zealand exhibit high potential for N_2O emissions. Knowledge of the effect of pH in the presence of cattle urine and DCD on regulation of N_2O emission or conversion of N_2O to N_2 is an essential requirement for developing targeted strategies for lowering net emissions. Recent work in New Zealand soils indicated that N_2O consumption was modestly enhanced by liming and this effect was heavily modulated by temperature, urine addition, and soil type (McMillan *et al.*, 2016). Our objective here is to understand the influence of liming-induced pH changes on overall denitrification and denitrifier abundance in two contrasting dairy-pasture soils (allophanic and fluvial) under incubation conditions.

Materials and Methods

Collection of soil samples

We used an allophanic (Horotiu silt loam) and a fluvial (Manawatu fine sandy loam) soil for this study. Horotiu silt loam is classified as a Typic Orthic Allophanic soil, is well-drained and derived from largely volcanic alluvium, and has a high allophane content (Singleton, 1991). Manawatu fine sandy loam is classified as a Weathered Fluvial Recent soil in the New Zealand Soil Classification System (Hewitt, 1992). Collection of soil samples, liming application and their basic soil properties are described in McMillan *et al.* (2016). Liming rates were 0.0 (lime 0), 1.5 t ha^{-1} (lime 1) and 3.0 t ha^{-1} (lime 2) for the Manawatu soil and 0.0 (lime 0), 5.0 t ha^{-1} (lime 1) and 10.0 t ha^{-1} (lime 2) for the Horotiu soil.

Soil amendment and incubation

The two soils were treated with three levels of lime in triplicate and pre-incubated for 180 days. After pre-incubation and when the soil pH was stabilized, each soil and lime treatment were amended with only water, urine (600 mg N kg^{-1} soil) and urine (600 mg N kg^{-1} soil) + DCD (10 mg kg^{-1} soil) and incubated at near saturation at two temperatures, 10°C and 15°C for 42 days. The experimental conditions are described in Table 1

Table1. Description of Soil amendments and incubation conditions applied in the experiment.

Experimental Conditions		Soils					
		Allophanic			Fluvial		
Temperature	Amendments	Liming treatments			Liming treatments		
10°C	Control (only water, C)	Lime 0	Lime 1	Lime 2	Lime 0	Lime 1	Lime 2
	Urine (600 mg N kg^{-1} dry soil, U)	Lime 0	Lime 1	Lime 2	Lime 0	Lime 1	Lime 2
	Urine + DCD (10 mg DCD kg^{-1} dry soil, UI)	Lime 0	Lime 1	Lime 2	Lime 0	Lime 1	Lime 2
15°C	Control (only water, C)	Lime 0	Lime 1	Lime 2	Lime 0	Lime 1	Lime 2
	Urine (600 mg N kg^{-1} dry soil, U)	Lime 0	Lime 1	Lime 2	Lime 0	Lime 1	Lime 2
	Urine + DCD (10 mg DCD kg^{-1} dry soil, UI)	Lime 0	Lime 1	Lime 2	Lime 0	Lime 1	Lime 2

For gas sampling: Soil subsamples (50 g dry weight equivalent) were placed in plastic bottles (top radius of container r_1 = 2.3 cm, bottom radius of container r_2 = 2.9 cm, height of container = 7.5 cm, volume = 157.07 cm^3). Deionised water was added to increase soil water content (SWC) to near saturation.

For chemical and molecular analysis: Three field replicate soil samples (250 g each dry weight equivalent) for each treatment were taken in glass jars (volume = 500 ml). Deionised water was added to increase SWC to near saturation.

Soil Characteristics

The soils were analyzed for pH and mineral N content using standard laboratory protocols. Soil pH was measured in a 1:2.5 (w/w) soil to water mixture using a PHM 83 Autocal pH meter (Radiometer, Copenhagen, Denmark) after vigorous stirring and incubating the mixture overnight (Blakemore *et al.*, 1987).

DNA extraction from soils

DNA was extracted from 0.25 g of each replicate soil sample using the MoBio PowerSoil™ DNA Isolation Kit (MoBio, Solana Beach, CA, USA) following the manufacturer's instructions. DNA concentrations were determined and purity was confirmed by the ratio of

absorbance at 260 and 280 nm using a NanoDrop Spectrophotometer (Thermo Scientific, Wilmington, DE). DNA was stored at –20°C until analyzed.

Quantitative polymerase chain reaction (qPCR) of bacterial denitrifier genes

Quantification of bacterial *nir*S, *nir*K, and *nos*Z genes was accomplished using qPCR, as described previously(Deslippe *et al.*, 2014). The primers used were *nir*S Cd3aF, R3cd (Enwall *et al.*, 2010), *nir*K Copper 583F, 909R (Dandie *et al.*, 2011), and *nos*Z 2F, 2R (Henry *et al.*, 2006). Amplification efficiencies were within the expected range of values (E = 90–110%). The reactions were linear over seven orders of magnitude and sensitive down to 10^2 copies. Detailed reaction conditions are described in Morales *et al.* (2015b).

Nitrous oxide production

Nitrous oxide (N_2O) production was measured using the acetylene (C_2H_2) inhibition technique (Fedorova *et al.*, 1973). In brief, duplicate soil samples were incubated in 1-L glass jars, with gas tight lids fitted with a gas sampling port. One duplicate was treated with 10% purified C_2H_2 gas in the headspace. Gas samples were taken immediately, and again 24 hours later. At each sampling, the volume removed by sampling was replaced by the same volume of room air to maintain constant pressure in the jar headspace. Gas samples were collected on 1, 3, 7, 15, 30 and 42 days after application of amendments.

Gas samples were analyzed for N_2O in a Shimadzu GC 17A gas chromatograph (GC) (Shimadzu Corp., Japan) equipped with a back flush system. This GC had a sample loop, and a ^{63}Ni-electron capture detector (ECD) operating at column, injector and detector temperatures of 55, 75 and 330°C, respectively. Nitrogen (99.99 % purity) was used as the carrier gas and a makeup gas of 5% methane in argon for the ECD. Acetylene inhibits nitrification, but not denitrification, and so measures the N_2O released by denitrification (N_2O-A), while the N_2O released without C_2H_2 measures emissions from both nitrification and denitrification processes (N_2O-NA).

Cumulative N_2O measurements were calculated (for Day 0 to Day 42) using the trapezoidal rule for estimating area-under-the-curve (Purves, 1992).

The percentage reduction in N_2O produced with addition of DCD to urine was calculated using the following equation:

$$\text{Percent reduction in } N_2O = \left(\frac{Cum_U - Cum_{UI}}{Cum_U - Cum_{CT}}\right) \times 100$$

where, Cum_U, Cum_{UI} and Cum_{CT} correspond to the cumulative N_2O produced with and without C_2H_2 in the urine, urine + DCD and control treatments respectively.

Statistical Analysis

The effects of liming, urine ± DCD application, experimental conditions, and interactions on denitrification and denitrifier gene abundance were assessed using multiple-level analysis of variance (ANOVA) using a general linear model procedure in statistical software R (R Development Core Team, 2013). Tukey's Studentised Range Test at $\alpha = 0.05$ significance level was used *post hoc* to reveal significant differences among means.

Results and Discussion

Soil pH

Lime application to soils significantly increased their pH and even in the amended soils (water, urine and urine + DCD) the pH remained high in limed soil as compared to non-limed soil until the end of the incubation (42 days) (Table 2, Fig. 1).The pH increase in soils was in accordance with the lime application rate L0, L1, and L2. Lime ($CaCO_3$) in the presence of water neutralises H^+ ions which leads to a pH increase in soils (Page *et al.*, 2009).

The pH response was more consistent with the liming rate in the allophanic soil than in the fluvial soil at both incubation temperatures (Table 2) due to the high buffering capacity of the former (McMillan *et al.*, 2016). The pH of the incubated soils was higher in the fluvial soil than in the allophanic soil ($P < 0.05$) throughout the incubation. As compared with the control soils, addition of urine to soils resulted in significant ($P < 0.05$) pH increase; however in soils where DCD was added with urine, there was no significant effect on soil pH. Increase in pH with urine addition was associated with urea hydrolysis in soils. Urea hydrolysis in soils generally lasts for the first few days of urine application to soils (Zaman *et al.*, 2008). There was a sharp increase in pH with urine amendment, when urea hydrolysis was over, soil pH decreased and was constant throughout the incubation in both the soils (Fig. 1). Soil pH was significantly ($P < 0.05$) lower at 15°C than at 10°C.

Table 2. Mean changes in pH with liming and urine application to soils during the experiment ($n = 3$)

	Allophanic soil						Fluvial soil					
Lime treatment	Lime 0		Lime 1		Lime 2		Lime 0		Lime 1		Lime 2	
Pre-incubation												
Soil pH before lime application (–3 months)	5.75		5.75		5.75		5.10		5.10		5.10	
Soil pH at the start of experiment (day 0)	5.77		6.08		6.69		5.5		5.75		6.2	
Change in pH	0.02		0.33		0.94		0.4		0.65		1.1	
Incubation												
Temperature (°C)	10	15	10	15	10	15	10	15	10	15	10	15
pH in urine-only treatment on day 42	6.9	7.1	7.00	7.2	7.16	7.32	7.60	7.85	7.72	7.88	7.73	7.88
pH in urine + DCD treatment on day 42	7.00	7.00	7.00	7.3	7.2	7.4	7.63	7.88	7.72	7.87	7.76	7.88
pH change in urine only samples from day 0 to 42	1.13	1.33	0.92	1.12	0.47	0.63	2.1	2.35	1.97	2.13	1.53	1.68
pH change in urine + DCD samples from day 0 to 42	1.23	1.23	0.92	1.22	0.51	0.71	2.13	2.38	1.97	2.12	1.56	1.58

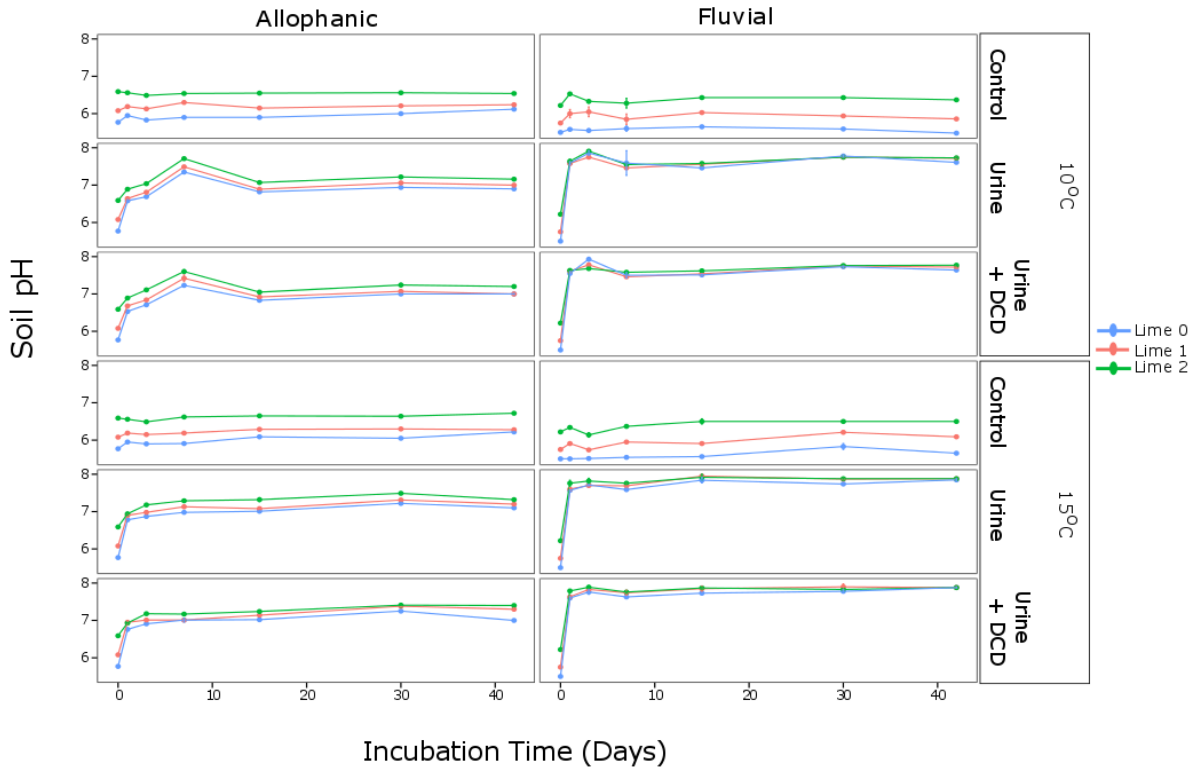

Figure 1. Soil pH (1:2.5 soil to water ratio) in allophanic (Horotiu silt loam) and fluvial (Manawatu sandy loam) soils for incubations at 10°C and 15°C. Data points are mean ± standard error of mean (n = 3). Lime 0 = no lime, Lime 1 = 1.5, and Lime 2 = 3.0 t ha^{-1} lime for fluvial soil; Lime 0 = no lime, Lime 1 = 5.0, and Lime 2 = 10.0 t ha^{-1} lime for allophanic soil.

Denitrifier gene abundance

Denitrifier gene abundance was higher in the allophanic soil than in the fluvial soil (P < 0.05) (Fig. 2). There was a significant (P < 0.05) effect of lime application on denitrifier gene abundance, with higher denitrifier gene copies in both the soils, especially in lime 2 treatments. We observed 48% increase in *nir* gene and 33% increase in *nos*Z gene abundance with liming, which is attributed to a favorable environment created by liming for denitrifiers and is also in accordance with earlier observations (Nodar *et al.*, 1992). Soil amendments with urine or urine + DCD significantly (P < 0.05) increased denitrifier gene abundances in the incubated soils. However, this increase was not significantly different among the two urine treatments (U and UI).

In the control samples (soils with no added urine) both *nir*S+K and *nos*Z gene abundances were significantly (P<0.05) correlated with their respective soil pH, r = 0.347 and 0.331 respectively for the two types of genes. Application of urine to soils increased their pH; denitrifier gene abundance remained constant with urine application and was also insensitive to the liming treatment. Previous studies have shown pH is a dominant factor influencing denitrifier gene abundance (Bárta *et al.*, 2010; Liu *et al.*, 2010).

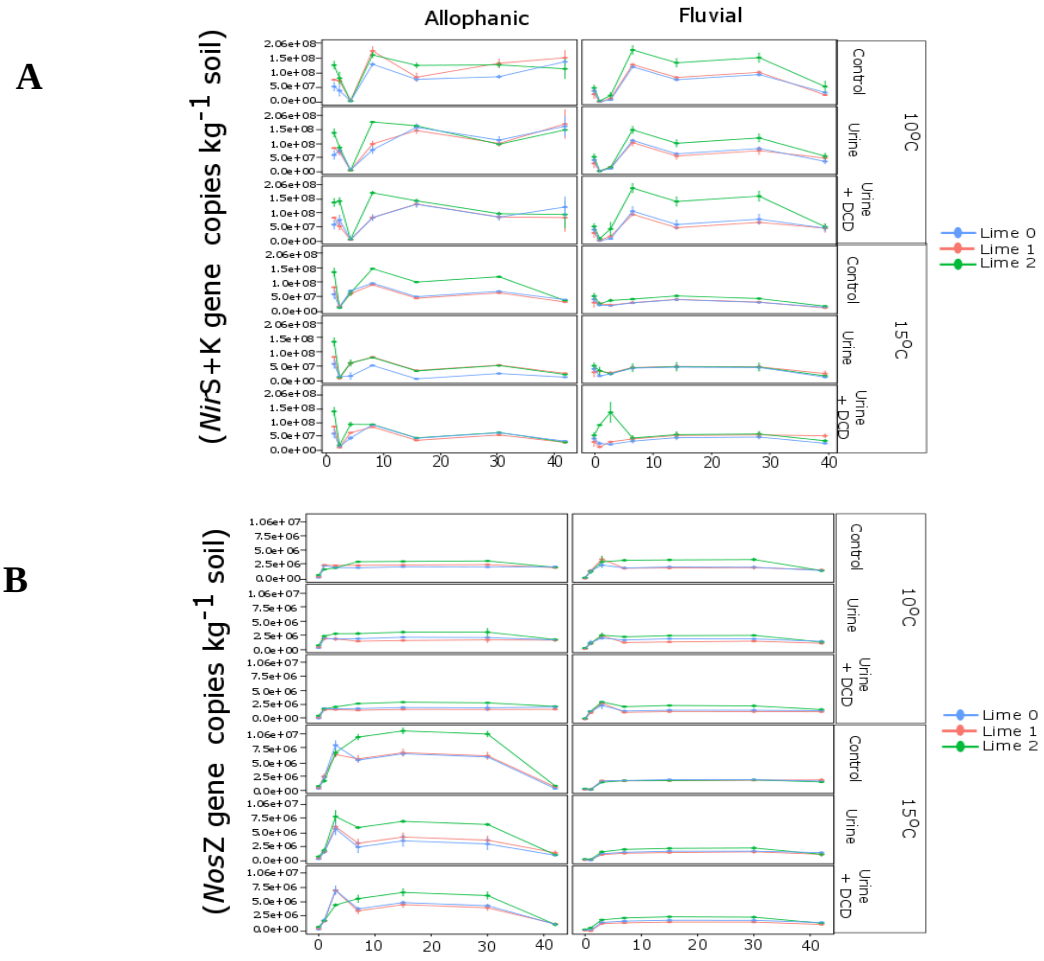

Figure 2. Denitrifier gene copies of (A) *nir*S+K gene and (B) *nos*Z gene in allophanic (Horotiu silt loam) and fluvial (Manawatu sandy loam) soils at 10°C and 15°C incubations Data points are mean ± standard error of mean (n = 3). Lime 0 = no lime, Lime 1 = 1.5, and Lime 2 = 3.0 t ha^{-1} lime for fluvial soil; Lime 0 = no lime, Lime 1 = 5.0, and Lime 2 = 10.0 t ha^{-1} lime for allophanic soil.

Cumulative N_2O and N_2O+N_2 (denitrification) emissions

Cumulative N_2O and N_2O+N_2 fluxes over 42 days incubation were significantly ($P < 0.05$) higher in the allophanic than in the fluvial soil (Fig. 3a). Allophanic soil with higher denitrifier gene abundance than the fluvial soil showed higher N_2O production and denitrification with liming than the fluvial soil.

Liming increased cumulative N_2O and N_2O+N_2 with the greatest cumulative N_2O+N_2 occurring in lime 2 soil followed by lime 1 and lime 0. Urine-amended limed soils produced significantly higher N_2O+N_2 than urine-amended un-limed soils. Liming caused greater cumulative N_2O emissions in the urine-amended soils. Increase in soil pH with liming and

further with amendment with cattle urine stimulated higher reductase enzyme activity (Šimek *et al.*, 2002) and thus higher denitrification in urine amended limed soils than the unamended or un-limed soils.

Urine application stimulated cumulative N_2O and N_2O+N_2 but addition of DCD to the urine-amended soil suppressed denitrification. Dicyandiamide suppressed cumulative N_2O by 62% for allophanic soil and 48% for fluvial soil at 15°C. There was 48% reduction in N_2O+N_2 through/when adding DCD with urine amendment in allophanic soil and 40% in urine-amended fluvial soil at 15°C (Table 2). Our results agree with the previous findings that reported higher N_2O production and denitrification in grazed pasture soils with urine application (Morales *et al.*, 2015a). Application of DCD with urine resulted in significantly lower N_2O emission and denitrification in both soils, which is in agreement with other studies (Di *et al.*, 2014; Morales *et al.*, 2015a). DCD inhibits nitrification and thus reduces the availability of NO_3-N content in soil, which might result in lower denitrification and N_2O emissions in DCD amended soils than in the non-DCD amendment. Indirect inhibition of denitrification with DCD was greater in the limed soils compared with un-limed soils.

The proportion of added urine-N denitrified was higher in the limed soils than the un-limed soils and in the urine-only treated soils than in the urine + DCD treated ones. This proportion was higher at 15°C than at 10°C. In the volcanic soil the effect of DCD in reducing denitrification was temperature dependent and lower at 10°C than at the 15°C. DCD was more effective in limed soils than in soils with no lime added (Table 3).

The ratio of $N_2O/(N_2O+N_2)$ was significantly ($P<0.05$) higher for the fluvial soil than for the allophanic soil, indicating higher denitrification of the available N in the alluvial soil than in the fluvial soil (Table 3). Liming of the soils and urine amendments also promoted more denitrification of N_2O than un-limed or un-amended soils. Soils with higher denitrifier gene (*nir*S+K and *nos*Z) abundance also produced greater amounts of N_2O through denitrification only than through nitrification + denitrification.

Table 3. Percent reduction in nitrous oxide emission with DCD addition to cattle urine without and with acetylene added. Data are mean (n = 3). DCD = dicyandiamide, Lime 0 = 0, Lime 1 = 1.5, and Lime 2 = 3.0 t ha^{-1} lime for fluvial soil; Lime 0 = 0, Lime 1 = 5.0, and Lime 2= 10.0 t ha^{-1} lime for the volcanic soil. Letter values indicate differences in mean using analysis of variance test for N_2O and N_2O+N_2 separately

	Nitrous oxide emission (N_2O)			Denitrification Rate (N_2O+N_2)		
Soil & incubation temperature	Lime 0	Lime 1	Lime 2	Lime 0	Lime 1	Lime 2
Allophanic 10°C	13.03^{e}	9.85^{e}	2.98^{f}	40.35^{BC}	42.10^{BC}	65.24^{A}
Allophanic 15°C	26.42^{d}	66.35^{a}	61.90^{a}	46.25^{B}	45.63^{B}	47.68^{B}
Fluvial 10°C	18.51^{d}	34.03^{c}	49.78^{bc}	25.17^{D}	39.05^{C}	33.43^{C}
Fluvial 15°C	44.71^{bc}	52.52^{b}	48.18^{bc}	41.43^{BC}	46.68^{B}	39.50^{C}

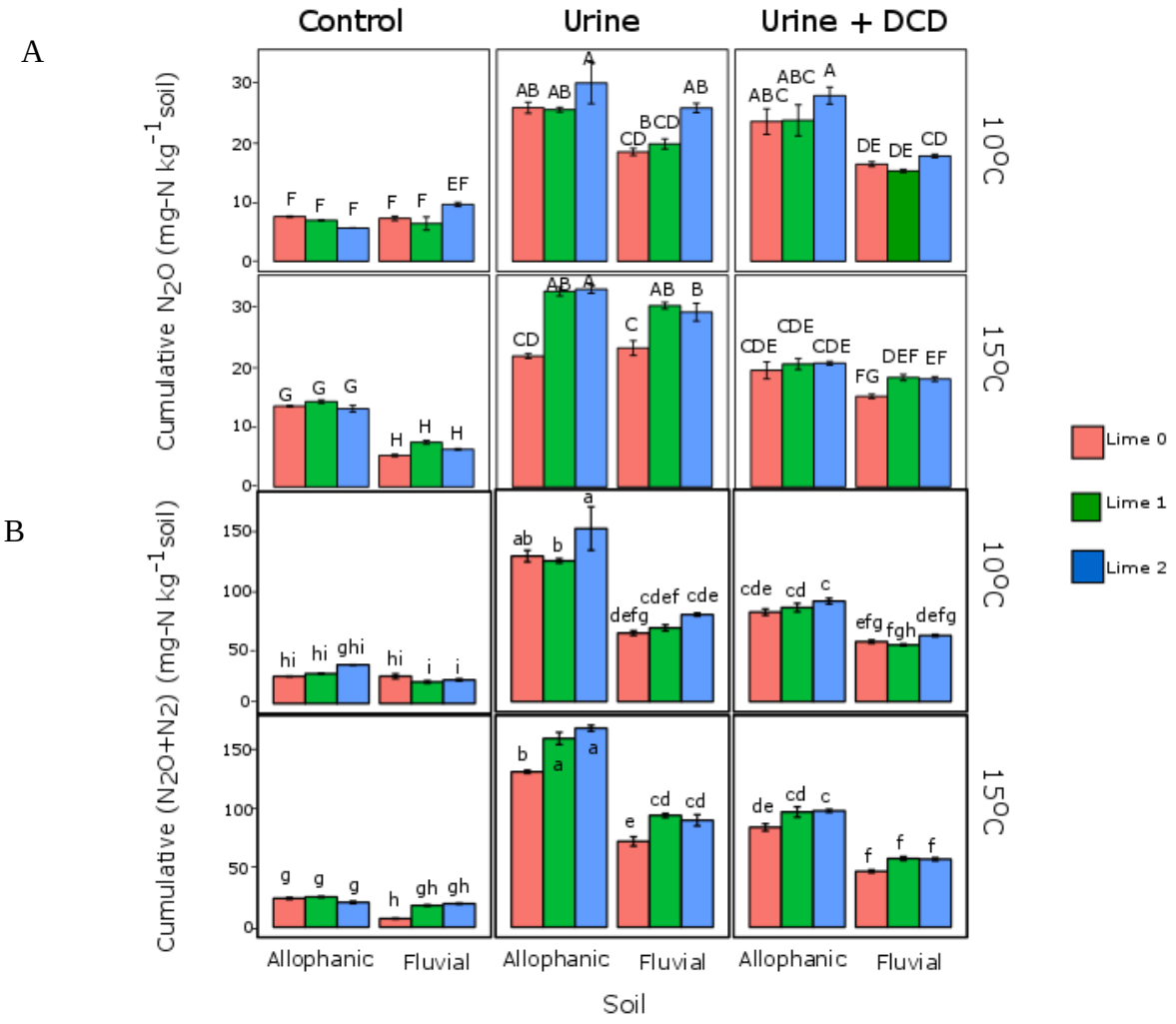

Figure 3. Cumulative nitrous oxide (N_2O) production (A) and denitrification (B), in allophanic (Horotiu silt loam) and fluvial (Manawatu sandy loam) soils incubated at 10°C and 15°C. Data points are mean ± standard error of mean (n = 3). Letter values indicate differences in mean using analysis of variance test. Lime 0 = no lime, Lime 1 = 1.5, and Lime 2 = 3.0 t ha^{-1} lime for fluvial soil; Lime 0 = no lime, Lime 1 = 5.0, and Lime 2 = 10.0 t ha^{-1} lime for allophanic soil.

Conclusions

Our study highlights the increase in denitrifier gene abundance and denitrification with lime application in two soils. Our study also confirms differences in denitrification rates in two contrasting soils, probably due to differences in their origin and the size of the denitrifier population. We noted denitrification as the main source of N_2O production when soils were amended with urine and urine + DCD. We also found that DCD was more pronounced in the reduction in N_2O production in the limed soils compared with the un-limed soils. Our results indicate that management practices such as liming on farm to increase the soil pH could offer a mitigation option to reduce N_2O production from grazed pasture soils, especially in soils with higher denitrifier populations.

Acknowledgement

We acknowledge the funding and support for this study by New Zealand Agricultural Greenhouse Gas Research Centre. We also acknowledge support from technical staff at AgResearch for collection of soil samples from Waikato, liming application and incubation for 3 months. We thank Dr. Kevin Tate for reviewing and Anne Austin for editing the manuscript.

References

Bárta, J., Melichová, T., Vaněk, D., Picek, T., Šantrůčková, H., 2010. Effect of pH and dissolved organic matter on the abundance of *nir*K and *nir*S denitrifiers in spruce forest soil. Biogeochemistry 101, 123-132.

Blakemore, L.C., Searle, P.L., Daly, B.K., 1987. Methods for chemical analysis of soils, NZ Soil Bureau. Department of Scientific and Industrial Research, Lower Hutt, New Zealand.

Clough, T.J., Sherlock, R.R., Kelliher, F.M., 2003. Can liming mitigate N_2O fluxes from a urine-amended soil? Soil Research 41, 439-457.

Clough, T.J., Kelliher, F.M., Sherlock, R.R., Ford, C.D., 2004. Lime and soil moisture effects on nitrous oxide emissions from a urine patch. Soil Science Society of America Journal 68, 1600-1609.

Čuhel, J., Šimek, M., Laughlin, R.J., Bru, D., Chèneby, D., Watson, C.J., Philippot, L., 2010. Insights into the effect of soil pH on N_2O and N_2 emissions and denitrifier community size and activity. Applied and Environmental Microbiology 76, 1870-1878.

Dandie, C.E., Wertz, S., Leclair, C.L., Goyer, C., Burton, D.L., Patten, C.L., Zebarth, B.J., Trevors, J.T., 2011. Abundance, diversity and functional gene expression of denitrifier communities in adjacent riparian and agricultural zones. FEMS Microbiology Ecology 77, 69-82.

de Klein, C.A.M., Smith, L.C., Monaghan, R.M., 2006. Restricted autumn grazing to reduce nitrous oxide emissions from dairy pastures in Southland, New Zealand. Agriculture, Ecosystems and Environment 112, 192-199.

Deslippe, J.R., Jamali, H., Jha, N., Saggar, S., 2014. Denitrifier community size, structure and activity along a gradient of pasture to riparian soils. Soil Biology and Biochemistry 71, 48-60.

Di, H.J., Cameron, K.C., 2002. The use of a nitrification inhibitor, dicyandiamide (DCD), to decrease nitrate leaching and nitrous oxide emissions in a simulated grazed and irrigated grassland. Soil Use and Management 18, 395-403.

Di, H.J., Cameron, K.C., 2003. Mitigation of nitrous oxide emissions in spray-irrigated grazed grassland by treating the soil with dicyandiamide, a nitrification inhibitor. Soil Use and Management 19, 284-290.

Di, H.J., Cameron, K.C., 2006a. The use of a nitrification inhibitor, dicyandiamide (DCD), to decrease nitrate leaching and nitrous oxide emissions in a simulated grazed and irrigated grassland. Soil Use and Management 18, 395-403.

Di, H.J., Cameron, K.C., 2006b. Nitrous oxide emissions from two dairy pasture soils as affected by different rates of a fine particle suspension nitrification inhibitor, dicyandiamide. Biology and Fertility of Soils 42, 472-480.

Di, H.J., Cameron, K.C., 2012. How does the application of different nitrification inhibitors affect nitrous oxide emissions and nitrate leaching from cow urine in grazed pastures? Soil Use and Management 28, 54-61.

Di, H.J., Cameron, K.C., Podolyan, A., Robinson, A., 2014. Effect of soil moisture status and a nitrification inhibitor, dicyandiamide, on ammonia oxidizer and denitrifier growth and nitrous oxide emissions in a grassland soil. Soil Biology and Biochemistry 73, 59-68.

Enwall, K., Philippot, L., Hallin, S., 2005. Activity and composition of the denitrifying bacterial community respond differently to long-term fertilization. Applied and Environmental Microbiology 71, 8335-8343.

Enwall, K., Throbäck, I.N., Stenberg, M., Söderström, M., Hallin, S., 2010. Soil resources influence spatial patterns of denitrifying communities at scales compatible with land management. Applied and Environmental Microbiology 76, 2243-2250.

Fedorova, R.I., Milekhina, E.I., Ilyukhina, N.I., 1973. On the possibilities of the 'gas exchange' method for the detection of extraterrestrial life: identification of nitrogen fixing microorganisms (Russian). Izevestiya Akademii Nauk SSSR - Seriya Biologicheskaya No. 6, 797-806.

Henry, S., Bru, D., Stres, B., Hallet, S., Philippot, L., 2006. Quantitative detection of the nosZ gene, encoding nitrous oxide reductase, and comparison of the abundances of 16S rRNA, *nar*G, *nir*K, and *nos*Z genes in soils. Applied and Environmental Microbiology 72, 5181-5189.

Hewitt, A., 1992. Soil Classification in New Zealand, DSIR Land Resource Scientific Report 19. Department of Scientific and Industrial Research.

Liu, B., Mørkved, P.T., Frostegård, A., Bakken, L.R., 2010. Denitrification gene pools, transcription and kinetics of NO, N_2O and N_2 production as affected by soil pH. FEMS Microbiology Ecology 72, 407-417.

Luo, J., Tillman, R.W., Ball, P.R., 2000. Nitrogen loss through denitrification in a soil under pasture in New Zealand. Soil Biology and Biochemistry 32, 497-509.

Luo, J., Ledgard, S.F., Lindsey, S.B., 2008. A test of a winter farm management option for mitigating nitrous oxide emissions from a dairy farm. Soil Use and Management 24, 121-130.

McMillan, A.M.S., Pal, P., Phillips, R.L., Palmada, T., Berben, P., Jha, N., Saggar, S., Luo, J., 2016. Can pH amendments in grazed pastures help reduce N_2O emissions from denitrification? – The effects of liming and urine addition on the completion of denitrification in fluvial and volcanic soils. Soil Biology and Biochemistry 93, 90-104.

Morales, S.E., Jha, N., Saggar, S., 2015a. Impact of urine and the application of the nitrification inhibitor DCD on microbial communities in dairy-grazed pasture soils. Soil Biology and Biochemistry 88, 344-353.

Morales, S.E., Jha, N., Saggar, S., 2015b. Biogeography and biophysicochemical traits link N_2O emissions, N_2O emission potential and microbial communities across New Zealand pasture soils. Soil Biology and Biochemistry 82, 87-98.

Nodar, R., Acea, M.J., Carballas, T., 1992. Microbiological response to $Ca(OH)_2$ treatments in a forest soil. FEMS Microbiology Letters 86, 213-219.

Page, K.L., Allen, D.E., Dalal, R.C., Slattery, W., 2009. Process and magnitude of CO_2, CH_4, and N_2O fluxes from liming of Australian acidic soils: a review. Australian Journal of Agricultural Research 47, 747-762.

Purves, E.D., 1992. Optimum numerical integration methods for estimation of area-under-the-curve (AUC) and area-under-the-movement-curve (AUMC). Journal of Pharmacokinetics and Biopharmaceutics 20, 211-226.

R Development Core Team, 2013. R: A Language and Environment for Statistical Computing, R Foundation for Statistical Computing, Vienna, Austria.

Saggar, S., Jha, N., Deslippe, J., Bolan, N.S., Luo, J., Giltrap, D.L., Kim, D.G., Zaman, M., Tillman, R.W., 2013. Denitrification and N_2O:N_2 production in temperate grasslands: Processes, measurements, modelling and mitigating negative impacts. Science of the Total Environment 465, 173-195.

Šimek, M., Jisova, L., Hopkins, D.W., 2002. What is the so-called optimum pH for denitrification in soil? Soil Biology and Biochemistry 34, 1227-1234.

Singleton, P.L., 1991. Soils of Ruakura- a window on the Waikato, DSIR Land Resources Scientific Report No.5, pp. 31-36.

Wakelin, S.A., Clough, T.J., Gerard, E.M., O'Callaghan, M., 2013. Impact of short-interval, repeat application of dicyandiamide on soil N transformation in urine patches. Agriculture, Ecosystems and Environment 167, 60-70.

Weiske, A., Benckiser, G., Herbert, T., Ottow, J., 2001. Influence of the nitrification inhibitor 3,4-dimethylpyrazole phosphate (DMPP) in comparison to dicyandiamide (DCD) on nitrous oxide emissions, carbon dioxide fluxes and methane oxidation during 3 years of repeated application in field experiments. Biology and Fertility of Soils 34, 109-117.

Zaman, M., Nguyen, M.L., Matheson, F., Blennerhassett, J.D., Quin, B.F., 2007. Can soil amendments (zeolite or lime) shift the balance between nitrous oxide and dinitrogen emissions from pasture and wetland soils receiving urine or urea-N? Australian Journal of Soil Research 45, 543-553.

Zaman, M., Nguyen, M.L., Blennerhassett, J.D., Quin, B.F., 2008. Reducing NH_3, N_2O and NO_3^--N losses from a pasture soil with urease or nitrification inhibitors and elemental S-amended nitrogenous fertilizers. Biology and Fertility of Soils 44, 693-705.

Zumft, W.G., 1997. Cell biology and molecular basis of denitrification? Microbiology and Molecular Biology Reviews 61, 533-616.