

Nitrate leaching and nitrous oxide emissions related to bacteria and not to archaea in nitrogen rich grassland soils

H.J. Di^{A,F}, K.C. Cameron^A, J.P. Shen^B, J.Z. He^B, C.S. Winefield^C, M. O'Callaghan^D and S. Bowatte^E

^ACentre for Soil and Environmental Quality, PO Box 84, Lincoln University, Lincoln 7647, Christchurch, New Zealand.

^BResearch Centre for Eco-Environmental Sciences, Chinese Academy of Sciences, 18 Shuangqing Road, Beijing 100085, China.

^CFaculty of Agriculture and Life Sciences, PO Box 84, Lincoln University, Lincoln 7647, Christchurch, New Zealand.

^DAgResearch Ltd, Lincoln Research Centre, Private Bag 4749, Christchurch, New Zealand.

^EAgResearch Ltd, Grasslands Research Centre, Tennent Drive, Private Bag 11008, Palmerston North 4442, New Zealand.

^FCorresponding author, Email Hong.Di@lincoln.ac.nz

Abstract

The oxidation of ammonia (NH₃) to nitrate (NO₃⁻) is a key process in the global nitrogen (N) cycle which has major ecological and environmental implications both in influencing nitrous oxide (N₂O) emissions and NO₃⁻ leaching. We investigated the relationships between nitrification, NO₃⁻ leaching and N₂O emissions with ammonia oxidizing bacteria (AOB) and archaea (AOA) in nitrogen rich grassland soils. Both AOA and AOB were detected in large numbers in these grassland soils. The AOB abundance grew by 3.2 to 10.4 fold and activity increased by 177 fold in response to the addition of a urine-N substrate, and the AOB growth was significantly inhibited by a nitrification inhibitor, dicyandiamide (DCD). However, neither the AOA abundance, nor activity, increased with the application of an ammonia substrate. DCD decreased NO₃⁻ leaching by 59% and decreased N₂O emissions by 64% from animal urine-N patches. Significant quantitative relationships were found between the AOB abundance and the nitrification rate, NO₃⁻-N leaching losses, and N₂O emissions, whereas no such relationships were found with AOA. These findings suggest that nitrification and thus NO₃⁻ leaching and N₂O emissions are driven by bacteria rather than archaea in these nitrogen rich grassland soils.

Key Words

Ammonia oxidizing bacteria, ammonia oxidizing archaea, nitrification, nitrification inhibitor, nitrate leaching, nitrous oxide emissions, grazed grassland soil.

Introduction

Nitrification is a key biogeochemical process of the nitrogen (N) cycle which results in the oxidation of ammonia (NH₃) to nitrite (NO₂⁻) and then to nitrate (NO₃⁻). This process has major environmental and ecological consequences, because it releases nitrous oxide (N₂O) which is a potent greenhouse gas and NO₃⁻ which can be leached to contaminate groundwater and surface waters. The long-term global warming potential of N₂O is c. 310 times that of carbon dioxide (CO₂). Nitrate can contribute to surface water eutrophication and is deemed harmful to human health if present at high concentrations in the drinking water. Until recently, the oxidation of NH₃ to NO₂⁻, was thought to be carried out mainly by autotrophic ammonia-oxidizing bacteria (AOB). Recently, however, the ammonia monooxygenase (AMO) genes have also been found in the domain Archaea, suggesting a potentially important role for ammonia-oxidizing archaea (AOA) (e.g. Leininger *et al.* 2006; He *et al.* 2007; Prosser and Nicol 2008). However, the role of AOB and AOA in ammonia oxidization is not well understood and the relationships between the populations of AOB and AOA and nitrification rate, N₂O emissions and NO₃⁻ leaching are unknown.

In intensively grazed grassland, e.g. grazed dairy pastures, the largest N source for both NO₃⁻ leaching and N₂O emissions is the urine-N excreted by the animal during grazing (Di and Cameron 2002a). The nitrogen loading rate under a dairy cow urine patch can be as high as 1000 kg N/ha (Di and Cameron 2002a). Most of the N excreted in the urine is urea which, upon hydrolysis in the soil, is converted to NH₄⁺, and is thus available for nitrification. One of the recent advances in the mitigation of NO₃⁻ leaching and N₂O emissions from grazed pastures is the development of an agricultural spray containing a nitrification inhibitor (NI) to inhibit the conversion of NH₃ to NO₃⁻ (Di and Cameron 2002b). However, it remains unclear what role different ammonia-oxidizers (e.g. AOB and AOA) play in nitrification in the soil and what group of ammonia oxidizers are in fact inhibited by the NIs. The objectives of this research were to: (i) study AOB and AOA populations in a range of intensively grazed grassland soils across New Zealand; (ii) determine their relationships with nitrification rate, N₂O emissions and NO₃⁻ leaching losses; and (iii) determine the effect of dicyandiamide (DCD) nitrification inhibitor on these populations and processes.

Materials and methods

Determination of AOB and AOA populations

Soil samples (0-0.1 m depth) were taken from six different sites across New Zealand: Northland (NL), Waikato (WK), and Rotorua Lakes (RL) in the North Island, and Canterbury (CT), West Coast (WC) and Southland (SL) in the South Island and were used in an incubation study under controlled temperature (12°C) and moisture (80% soil water holding capacity) (Details of the soil classifications and properties are found in Di *et al.* 2009a; 2009b; 2009c). The experiment consisted of six soils and each soil had the following treatments: A: Control; B: 50 kg urea-N + 1000 kg urine-N per ha; C: 50 kg urea-N + 1000 kg urine-N + 10 kg DCD per ha. Each treatment was replicated four times. Treatment B was designed to simulate a situation under a dairy cow urine patch in a grazed pasture where the N input from the urine was equivalent to 1000 kg N/ha, and 50 kg of fertilizer N was also applied to the grazed field, including urine patch areas. DCD (dicyandiamide) is a nitrification inhibitor used here to determine its effect on AOB and AOA populations and on nitrification rate. Soil samples were taken at different intervals after the application of the treatments for the determination of the *amoA* gene copy numbers of the AOB and AOA populations. Sub-samples were also extracted with 2 M KCl to determine NO₃⁻ concentrations and thus give a measure of nitrification rate.

Determination of nitrate leaching and nitrous oxide emissions

Large undisturbed soil monolith lysimeters (0.5 m diameter and 0.7 m deep) were collected and used to determine NO₃⁻ leaching and N₂O emissions (Di *et al.* 2009b; 2009c). The lysimeters were 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 lysimeters were exposed to the same climatic conditions as the soil and pasture in the surrounding field. Two rainfall conditions (1260 mm and 2145 mm p.a.) were created using a rainfall simulation system in order to test the influence of rainfall inputs on NO₃⁻ leaching and N₂O emissions. Pasture was harvested at typical grazing heights and removed before the application of the treatments and was subsequently cut at typical grazing intervals thereafter. Leachates from the lysimeters were collected as required and analysed for NO₃⁻, nitrite (NO₂⁻), and NH₄⁺ concentrations. A standard closed chamber method was used to determine N₂O emissions from the treated lysimeters (Di *et al.* 2009c).

Results

Abundance of AOA and AOB and nitrification rate

Both AOA and AOB *amoA* genes were detected in large numbers but they varied widely in the six different soils (Di *et al.* 2009a). Phylogenetic analysis showed that all the AOB clones recovered were closely related to the *Nitrosospira* species and no clones were closely aligned with the *Nitrosomonas* species. A majority of the AOA clones identified were closely aligned with the soil clade. Interestingly, a number of the clones were affiliated with cluster Marine, suggesting similarity with AOA of marine origin.

Whilst the AOB populations in the Controls (no nitrogen substrate or NI) of the six soils remained relatively stable over the three month incubation period, they increased by 3.2, 5.7, 9.4, 10.4, 4.7 and 5.4 fold following the application of the urine-N substrate in the NL, WK, RL, CT, WC and SL soils, respectively (Figure 1a). When the nitrification inhibitor, DCD, was applied, the AOB population growth was significantly inhibited (Figure 1a). In contrast, the AOA population abundance remained largely unchanged in all the soils irrespective of the urine-N and inhibitor treatments (Figure 1b). Therefore, in all the soils the AOA populations did not grow with the supply of the large dose of urine-N substrate.

Following the application of the urine-N to the WK soil, the AOB RNA copy numbers increased by more than 177 times, from 1.1x10² in the Control soil to 2.4x10⁴ in the urine-N treated soil (Di *et al.* 2009a). When DCD was applied, the AOB activity was significantly inhibited, with the RNA copy numbers remaining the same as that in the Control. In contrast, the AOA activity did not change with the urine-N application, with the RNA copy numbers remaining the same in the Control, Urine and Urine + DCD treatments. In the Urine treatment, the AOA RNA copy number was only 2% that of the AOB.

In all six soils, the addition of the urine-N substrate significantly increased the nitrification rate, as indicated by the rising NO₃⁻-concentrations, but the nitrification rates were reduced by the NI treatments (Figure 1c).

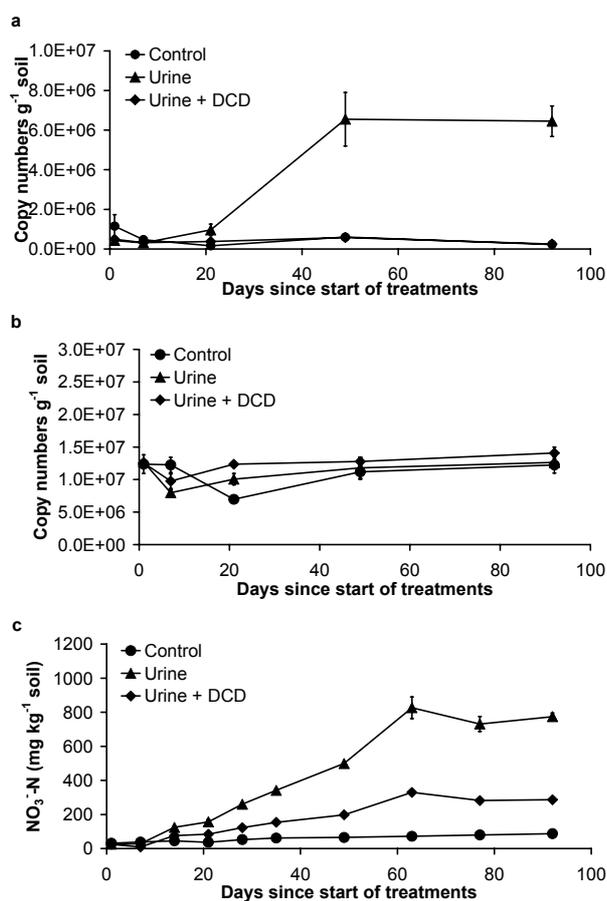


Figure 1. (a): AOB population abundance; (b): AOA population abundance; and (c): Nitrate-N concentration in the soil, in the Waikato Horotiu soil. Similar trends were found in the other soils.

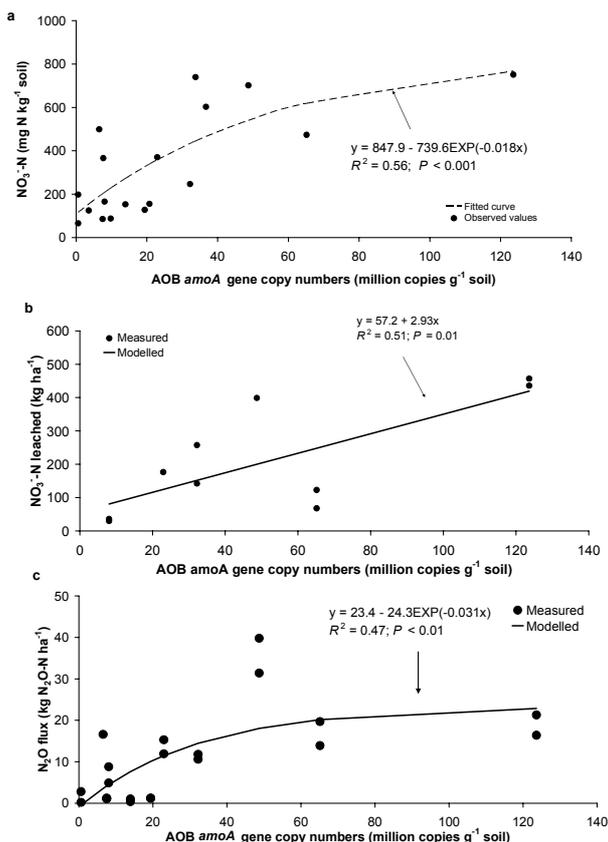


Figure 2. Relationships between AOB abundance and (a) Nitrification rate; (b) Nitrate-N leaching losses; and (c) Nitrous oxide emissions.

Nitrate leaching and nitrous oxide emissions

Under the 1260 mm rainfall treatment, total NO_3^- -N leaching losses in the Urine treatment ranged from a low of 122.9 kg NO_3^- -N/ha in the WC Harihari soil to a high of 435.8 kg NO_3^- -N/ha in the SL Mataura soil. These were significantly decreased to between 35.8 and 176.5 kg NO_3^- -N/ha when DCD was applied ($P < 0.05$). The application of DCD therefore reduced the NO_3^- leaching losses by between 56 to 71%. Under the 2145 mm rainfall condition, the total NO_3^- -N leaching losses in the Urine treatment varied from 67.7 kg NO_3^- -N/ha in the WC Harihari soil to 457.0 kg NO_3^- -N/ha in the SL Mataura soil. These losses were decreased to 29.7 and 257.4 kg NO_3^- -N/ha with the application of DCD. The application of DCD therefore reduced these NO_3^- leaching losses by between 44 to 56%. The difference in the amount of NO_3^- -N leached between the two rainfall conditions was not statistically significant ($P > 0.05$). The average reduction in NO_3^- -N leaching loss by the DCD treatment under both rainfall conditions was 59%.

Total N_2O emissions varied significantly between the different soils, with those in the urine treatment ranging from a low of 13.9 kg N_2O -N/ha in the WC Harihari soil under the higher rainfall condition to a high of 39.8 kg N_2O -N/ha in the CT Lismore soil under the higher rainfall condition. The different water inputs did not result in significantly different total N_2O emissions ($P > 0.05$). However the DCD treatment decreased the total N_2O emissions from all of the four soils. The emission factor 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%.

Relationships between ammonia oxidizers and nitrogen losses

Regression analysis showed a significant exponential relationship between the NO_3^- -N concentration and the abundance of the AOB population (Di *et al.* 2009a) (Figure 2a). Therefore, as the AOB population abundance increased the nitrification rate increased exponentially. However, no quantitative relationship was found between the NO_3^- -N concentration and the abundance of AOA populations. A significant relationship

was found between total NO_3^- -N leaching loss from both rainfall treatments of the soils and the AOB *amoA* gene copy numbers (Di *et al.* 2009b) (Figure 2b), but no relationship was found with the AOA populations. A significant exponential relationship was also found between the total N_2O emissions and the AOB populations after 49 days of incubation (when the AOB population peaked in most of the urine treatments) (Di *et al.* 2009c) (Figure 2c) but no quantitative relationship was found with AOA populations.

Conclusions

AOB and AOA populations were present in large numbers in the New Zealand grassland soils. However, in the nitrogen rich urine patch soils, only the AOB population grew and activity increased when supplied with the high dose of ammonia substrate in the urine. The AOB population and activity were also significantly inhibited by the nitrification inhibitor DCD. The AOA population abundance and activity did not grow when supplied with the high dose of ammonia substrate (urine). The nitrification inhibitor DCD significantly decreased nitrate leaching by an average of 59% under two contrasting rainfall conditions (1260 mm and 2145 mm p.a.), and significantly decreased nitrous oxide emissions by an average of 64%. Significant quantitative relationships were found between the nitrification rate, nitrate leaching losses and nitrous oxide emissions with AOB abundance, but not with the AOA abundance. These results suggest that nitrification, and thus nitrate leaching and nitrous oxide emissions, are driven by AOB rather than AOA in these nitrogen rich grassland soils with animal urine-N inputs.

Acknowledgments

We thank the New Zealand Foundation for Research, Science and Technology (FRST), Ravensdown Fertiliser Co-operative Ltd, Ministry of Agriculture and Forestry (MAF), and the Pastoral21 Consortium for funding, Emily Gerard and Shona Brock of AgResearch and Jie Lei, Steve Moore, Carole Barlow, Trevor Hendry, Neil Smith, Nigel Beale, and Roger Atkinson of Lincoln University, for technical support.

References

- Di HJ, Cameron KC (2002a) Nitrate leaching in temperate agroecosystems: sources, factors and mitigating strategies. *Nutrient Cycling in Agroecosystems* **64**, 237-256.
- Di HJ, Cameron KC (2002b) The use of a nitrification inhibitor, dicyandiamide (DCD), to reduce nitrate leaching from cow urine patches in a grazed dairy pasture under irrigation. *Soil Use and Management* **18**, 395-403.
- Di HJ, Cameron KC, Shen JP, Winefield CS, O'Callaghan M, Bowatte S., He J.Z. (2009a) Nitrification driven by bacteria and not archaea in nitrogen rich grassland soils. *Nature Geoscience*. **2**, 621-624.
- Di HJ, Cameron KC, Shen JP, He JZ, Winefield CS (2009b) Nitrate leaching from grazed grassland as affected by a nitrification inhibitor, dicyandiamide, and relationships with ammonia oxidizing bacteria and archaea. *Soil Use and Management* **25**, 454-461.
- Di HJ, Cameron KC, Sherlock RR, Shen JP, He JZ, Winefield CS (2009c) Nitrous oxide emissions related to bacteria and not to archaea in nitrogen rich grassland soils. *Journal of Soils and Sediments* DOI 10.1007/s11368-009-0174-x.
- He JZ, Shen JP, Zhang LM, Zhu, YG, Zheng YM, Xu MG, Di HJ (2007) Quantitative analyses of the abundance and composition of ammonia-oxidizing bacteria and ammonia-oxidizing archaea of a Chinese upland red soil under long-term fertilization practices. *Environmental Microbiology* **9**, 2364-2374.
- Leininger S, Urich T, Schloter M, Schwark L, Qi J, Nicol GW, Prosser JI, Schuster SC, Schleper C (2006) Archaea predominate among ammonia-oxidizing prokaryotes in soils. *Nature* **442**, 806-809.
- Prosser JI, Nicol GW (2008) Relative contributions of archaea and bacteria to aerobic ammonia oxidation in the environment. *Environmental Microbiology* **10**, 2931-2941.