THE INFLUENCE OF SOIL PARAMETERS AND DENITRIFIERS ON N_2O EMISSIONS IN NEW ZEALAND DAIRY-GRAZED PASTURE SOILS

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ABSTRACT

Denitrification is a primary source of nitrous oxide (N_2O) production found globally in temperate grasslands and in New Zealand pasture soils. The various reductase enzymes coded by specific denitrifier genes, and influenced by soil and environmental factors, regulate the N_2O production and reduction during the denitrification process. An understanding of the soil and environmental factors that have the potential to enhance the activity of denitrifiers reducing N_2O to dinitrogen (N_2) contributes to the development of novel and effective N_2O mitigation technologies.

This thesis attempts to address a critical gap in our understanding of the role of bacterial denitrifier communities and their abundance in denitrification under various field-moist and incubation conditions in New Zealand dairy-grazed pasture soils. This thesis consists of a literature review (Chapter 2) that describes the biochemical and molecular aspect of denitrification, and identifies key factors that affect the process and the denitrifier community structure and its abundance. It addresses the advantages and limitations of available techniques to measure denitrification, the denitrifier community structure and abundance, and also any available mitigation options to reduce denitrification losses. The review concludes by identifying gaps in our knowledge of the denitrifier communities, and their abundance and activities in New Zealand soils.

The acetylene (C_2H_2) inhibition technique (AIT) was standardised (Chapter 3) for the measurements of denitrification enzyme activity (DEA) and denitrification rate (DR) used during the research. Similarly, terminal restriction fragment length polymorphism (T-RFLP) and quantitative polymerase chain reaction (qPCR) were standardised to assess denitrifier community structure (numbers of *nir*S+*nir*K and *nos*Z gene T-RFs) and abundance (*nir*S+*nir*K and *nos*Z gene copy numbers) in soils.

Soil samples were obtained from 10 sites with known management histories, representing dairy farms across the North and South Islands of New Zealand, to determine the soil factors contributing to spatial variability in DEA, DR and denitrifier community structure and abundance within and across dairy-grazed pastures (Chapter 4). Despite the spatial variability at each site, the DEA results show large differences in potential denitrification in these soils. The outcome of this study painted an overall picture of the distribution of denitrification enzyme

activities in the various New Zealand dairy-grazed pasture soils and the biochemical characteristics and presence of denitrifiers related to denitrification in contrasting soils.

The bacterial denitrifier genes richness correlated significantly ($P < 0.05$) to Olsen P, microbial biomass C (MBC), and NH_4^+-N contents of soil, whereas these gene abundances correlated to MBC and NO_3^--N contents in soils, thus confirming inherent soil characteristics that influence denitrifier populations in soils. *Nir*S and *nir*K gene copy numbers correlated positively with N_2O emissions (from nitrification and denitrification). There was no clear relationship between *nos*Z gene copy numbers and denitrification rate in the field-moist soils, which could be due to less anaerobic condition for denitrifiers to carry denitrification.

To determine the effect of increasing soil water content (SWC) on changes in denitrification, 5 soils contrasting in DEA were incubated at field capacity (FC) and saturation SWC (Chapter 5). The measured DRs were higher in soils incubated at saturation, ranging from 21.5 to 73.9 μg N_2O-N kg^{-1}soil hr^{-1}, than in soils incubated at FC, in which DR varied from 0.8 to 50.4 μg N_2O-N kg^{-1}soil hr^{-1}. Although the direction of change in denitrification with the increase in SWC was similar among the soils, the magnitude of increase was variable among the five soil types. This variability was mainly driven by their inherent biochemical (NO_3^--N, TC, TN, MBC, Olsen P, DEA,) and molecular characteristics (denitrifier richness and denitrifier abundance).

A subsequent incubation experiment was conducted to investigate the effect of water only, cattle urine, and cattle urine with added nitrification inhibitor dicyandiamide (DCD) on denitrification and numbers of denitrifier gene T-RFs and their copy numbers in three pasture soils with contrasting DEA. The results of this incubation are described in Chapters 6 and 7. The results described in Chapter 6 explore the effect of saturation (only water addition) on DRs, denitrifier gene richness, and denitrifier gene copy numbers in three soils. At saturation soil water content in the incubated soils, the increases or decreases in DRs with incubation time were variable in three soils and depended on their TC, TN, Olsen P, MBC, and denitrifier gene richness. It is inferred from the results that, with increasing SWC, a denitrifier community of a constant size was maintained in the incubated soils.

The results in Chapter 7 describe the effect of urine with and without DCD on denitrification, denitrifier richness, and denitrifier gene abundance. There were changes in soil pH, NO_3^--N, NH_4^+-N, soluble C, and microbial biomass C in soils with urine application. The

numbers of denitrifier gene T-RFs and copy numbers either remained unchanged or were lower under urine + DCD treatments than urine only and related to N_2O productions (from both nitrification and denitrification) during the incubation.

There was an overall increase in DR with application of urine and urine + DCD to soils. Comparatively higher N_2O-NA (N_2O productions from non- acetylene jars) were observed in urine-only treatments than in urine + DCD. The cumulative N_2O-NA with the addition of urine and urine + DCD were variable among the three soils. During 4 weeks incubation the N_2O-NA ranged from 20.9 to 26.7 mg N_2O-N kg^{-1} soil in urine treatments and from 19.6 to 21.7 mg N_2O-N kg^{-1} soil in urine + DCD treatments in three soils. The proportion of total N denitrified during the entire incubation ranged from 6.3 to 22.4 % in urine treatments and 3.6 to 5.6 % in urine + DCD treatments in three soils.

Denitrifier gene richness and gene copy numbers during incubation helped identify overall changes in the denitrifier community with the application of treatments and link these changes to N_2O-NA across various soils. Overall, in the urine-applied samples the N_2O-NA (positively) was significantly correlated with their DEA, MBC, Olsen P, and numbers of denitrifier gene richness and denitrifier gene copy numbers.

The information obtained in this research helped enhance the understanding of the variability in denitrification and the denitrifier community in the New Zealand dairy pasture soils. The molecular measurements of the soils helped identify differences in N_2O productions in soils at similar incubation conditions. Soils with low denitrifier richness and, more importantly, lower abundance of complete denitrifiers had limited capacity to denitrify available inorganic N. Measurements of DEA, denitrifier richness, and denitrifier abundance in soils, along with their N_2O productions (with and without acetylene), indicated the inherent potential of soils to carry denitrification. Under urine application, soil with the most abundant denitrifier population and the highest DEA responded to added treatment quickly and produced more N_2O-NA and N_2O-A than the other two soils did. On the other hand, soils with lower DEA and less abundant denitrifier community showed lower N_2O production (with and without acetylene) even under anaerobic condition than did other soils.

DEDICATION

This work is dedicated to my parents, my husband, and my parents-in-law

for their consistent encouragement and support

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PUBLICATIONS AND PRESENTATIONS

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6. Morales SE, **Jha N**, Saggar S **(2015)** Impact of urine and DCD application on microbial communities in dairy-grazed pasture soils. Soil Biology and Biochemistry 88, 343-353.

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15. Morales S.E, **Jha N**, Saggar S **(2014)** (Abstract and poster) Impact of urine and DCD application on microbial communities in dairy-grazed pasture soils.15th International Symposium on Microbial Ecology, 24-29 August, Seoul. South-Korea.

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LIST OF ABBREVIATIONS

AN	Avogadro's Number
ANOVA	Analysis of variance
AOA	Ammonia oxidising archaea
AOB	Ammonia oxidising bacteria
bp	Base pairs
BSA	Bovine serum albumin
C	Carbon
Cd	Cadmium
C_2H_2	Acetylene
cnorB/cnorBp	Cytochrome nitric oxide reductase enzyme
CO_2	Carbon-dioxide
Cp	Crossing Point
CT	Control
Cu	Copper
DCD	Dicyandiamide
DEA	Denitrification enzyme activity
DNA	Deoxyribonucleic acid
DNRA	Direct nitrate reduction to ammonia
dNTPs	Deoxinucleotide triphosphates
DR	Denitrification rate
ds	double stranded
ECD	Electron capture detector
EDTA	Ethylenediaminetetraacetic acid
Eq	Equation
F	Forward
FAM	6 carboxyfluorescein
Fe	Iron
GC	Gas Chromatograph
GHG	Greenhouse gas
H^+	Hydrogen ions
H'	Shannon Diversity Index
HR	Horotiu silt loam
hrs	hours
HSD	Honestly significant difference
IGEPAL	octylphenoxypolyethoxyethanol
IPCC	Intergovernmental panel for climate change
J	Pielou's Coefficient of Evenness
K_2SO_4	Potassium Sulphate
KCl	Potassium chloride
LM	Listmore stony silt loam
MF	Mayfield silt loam
Mf.E	Ministry of Environment
$MgCl_2$	Magnesium chloride

Mn	Manganese
Mo	Molybdenum
MPN	Most Probable Number
mRNA	Messenger ribonucleic acid
MS	Microsoft
MW	Manawatu fine sandy loam
MWEI	Manawatu fine sandy loam (Effluent irrigated)
N	Nitrogen
N_2	Dinitrogen
N_2O	Nitrous oxide
N_2OR	Nitrous oxide reductase
NAR	Nitrate reductase
NEB	New England Biolabs
NH_3	Ammonia
NH_4^+	Ammonium
NIR	Nitrite reductase
Nir	Nitrite reductase gene
*Nir*K	Copper containing nitrite reductase gene
*Nir*S	Cytochrome-cd1 nitrite reductase gene
NO	Nitric oxide
NO_2^-	Nitrite
NO_3^-	Nitrate
NOR	Nitric oxide reductase
*Nos*Z	Nitrous oxide reductase gene
NZ	New Zealand
O_2	Oxygen
OH	Otorohanga silt loam
OH^-	Hydroxyl ion
OTUs	Operational taxonomic units
P	Phosphorus
PCA	Principal components analysis
PCR	Polymerase chain reaction
PL	Paparua silt loam (Lincoln)
PS	Paparua silt loam (Springston)
Q_{10}	Temperature coefficient
qPCR	Quantitative polymerase chain reaction
R	Reverse
RNA	Ribonucleic acid
s	second
S	Sulphur
SWC	Soil water content
TAE	Tris-ascetic acid EDTA
Taq	*Thermus aquaticus*
TeK	Te Kowhai silt loam
TET	Tetrachlorofluorescein
TM	Tokomaru silt loam

T-RFLP	Terminal restriction fragment length polymorphism
T-RFs	Terminal restriction fragments
Tris-HCl	2-amino-2-(hydroxymethyl)-1,3-propandiol Hydrochloric acid
U	Cattle urine only
UI	Cattle urine +DCD
UV	Ultra violet