A review of denitrification on Australian dairy farms

Keith L. Weier & Peter R. Grace

Report prepared for Dairy Australia

2012

Disclaimer

Whilst all reasonable efforts have been taken to ensure the accuracy of the report entitled "A review of denitrification on Australian dairy farms", use of the information contained herein is at one's own risk. To the fullest extent permitted by Australian law, Dairy Australia disclaims all liability for any losses, costs, damages and the like sustained or incurred as a result of the use of or reliance upon the information contained herein, including, without limitation, liability stemming from reliance upon any part which may contain inadvertent errors, whether typographical or otherwise, or omissions of any kind.

Table of Contents

Aim

The review paper will attempt to source information on the following:-

(i) Collation and interpretation of denitrification data for dairy systems.
(ii) State of understanding of loss as N_2 and the ratio of loss as N_2O and N_2 and the potential to estimate N mass balance in dairy pastures.
(iii) Description of current measurement techniques N_2O and data quality for N_2O and N_2 in dairy systems.
(iv) Factors affecting the rate of denitrification transformations including fertiliser type, soil characteristics (e.g. soil moisture, organic C content and pools).
(v) Evaluation of the capacity to simulate the processes of N loss from denitrification using models specific to dairy systems.
(vi) Identification of practices associated with high or low denitrification loss, including use of inhibitors.
(vii) Recommendations for R & D needed to improve capacity to quantify and manage denitrification losses as a component of total dairy farm N balance, including using ^{15}N.

Executive Summary

The increasing use of nitrogen fertiliser by the Australian dairy industry has resulted in an increased N pool being available for loss from pastures by various pathways. Gaseous N loss via denitrification is one of these pathways, where microbial conversion of soil nitrate results in the emission of nitrous oxide (N_2O), a potent greenhouse gas, and dinitrogen (N_2). Although N_2O is lower in atmospheric concentration than CO_2, it has an estimated lifetime in the stratosphere greater than 100 years and a Global Warming Potential (GWP) 310 times higher than that of CO_2.

At present, 14.6% (79.5 Mt CO_2e) of Australia's greenhouse gas emissions are attributed to agriculture, of which 21.3% is N_2O. Nearly 73% of the national N_2O account comes from the agricultural sector.

The quantities of N_2O and N_2 emitted from pastures through biological denitrification are affected by the supply of carbon and nitrogen to the denitrifier population and the condition of the soil at that particular time. These include nitrate supply, a soluble carbon source, pH, soil temperature and water filled pore space.

This review was conducted to evaluate denitrification research within the dairy industry and to recommend procedures that could be used to mitigate emissions of N_2O and N_2 from dairy pastures.

Nitrous oxide emissions from N fertilised dairy pastures of southern Australian were estimated to range from 7-25 kg N ha^{-1} yr^{-1} whereas losses attributable to denitrification from ungrazed pastures were estimated to be as high as 20% of the N fertiliser applied. Studies in northern Victoria on flood-irrigated pastures found daily emissions of 4-170 g N_2O-N ha^{-1} day^{-1}, with the highest emission rates observed when N substrate, temperature and soil water filled pore space were at high levels.

Gaseous emissions from urine patches, containing an estimated 1000 kg N ha^{-1}, were found to range from 4.2-4.5 kg N_2O-N ha^{-1} over a measurement periods of 2 years. Further measurements from pastures, irrigated with dairy effluent, indicated that these losses could be higher than those found from pastures fertilised with inorganic N.

The presence of animals on pastures was found to have a deleterious effect on emissions, with denitrification losses of 52 g N_2O-N ha^{-1} recorded mainly due to a reduction in soil aeration. Gaseous N losses from a legume based pastures were 8 kg N ha^{-1} yr^{-1} from an estimated 108 kg N-fixed ha^{-1} yr^{-1}. However, there are few studies producing quantifiable results.

Whilst indirect losses of nitrate via leaching and run-off pathways are significant contributors to denitrification losses, there also exists the possibility of chemical denitrification of NH_4 to N_2 without proceeding through any biological process. Leaching losses, following urea application to pastures, were found to range from 13-31 kg N ha^{-1} yr^{-1}, with losses from legume pastures ranging from 15-35 kg NO_3^-N ha^{-1}. There was also a potential for leached N

to contaminate groundwater with average losses of 1-5 kg N ha^{-1} day^{-1} recorded from overlying pastures. Chemical denitrification was found to occur in swine lagoons under methanogenic conditions. There is also the possibility that this is occurring in effluent ponds on dairy farms.

The use of nitrification inhibitors to limit denitrification losses from urine patches has been extensively studied. Dicyandiamide (DCD), when added with urine to pastures, suppressed N_2O emissions by between 35% and 74%. Gaseous losses were found to be temperature dependent with larger losses recorded in spring. Under simulated conditions, DCD also reduced N_2O emissions from pastures which were subject to centre pivot irrigation.

Application of animal slurries and dairy effluent to pastures was found to supply between 50 and 240 kg N ha^{-1} yr^{-1} with the N concentration related to the quality of pasture grazed. Potential denitrification losses from this system have been estimated at 8.3% whilst lower values have been reported after the application of dairy shed effluent to pasture.

Measurement of denitrification losses in the field have been achieved through the use of various techniques. The fact that the main gaseous product from a denitrification event is di-nitrogen means that it is quickly diluted in the atmosphere, making exact quantification very difficult. Both static chambers and automatic have been successfully used to assess denitrification losses, but in the majority of cases only N_2O is quantifiable, unless acetylene inhibition is also used to block the conversion of N_2O to N_2.

Accurate quantification of denitrification using ^{15}N tracers still remains a challenge for researchers, in particular, direct field measurements. Quantification of both N_2O and N_2 using ^{15}N would allow for greater certainty to be applied to the emission factors for N_2O currently being used to estimate N_2O emissions from dairy farms, as well as in determining the environmental conditions which favour the production of unreactive N_2.

Mitigation of N_2O emissions from dairy pastures can be achieved through manipulation of current management practices. Applying N fertiliser at optimal rates, and integrating this rate with fixed and mineralised N will reduce N available for loss. Incorporation of N into the soil will further limit N loss processes. Where possible, application of nitrification inhibitors will regulate the N available for loss from urine patches and pastures. Applying dairy effluent to dry soil and avoiding grazing pastures when soils are wet are further possible avenues to achieve an N loss reduction.

Use of GPS guided centre pivot systems as a means of matching water use with soil type could produce a reduction in unwanted run-off. The use of grain or crops of low protein and high energy in a feeding program may result in a reduction in N content of animal waste excreted onto pasture.

In summary, future areas of research should include:

(i) Nitrogen supplied from mineralisation for each pasture soil needs to be established so that the nitrate present in the soil is known before either fertiliser or DCD application.

(ii) Increased emphasis on determining the textural, chemical and physical characteristics of the soils (and their variability) underlying dairy pastures as an aid in the determination of water filled pore space and mineralisable N. This information is critical in developing any predictive tools for reducing gaseous N losses.

(iii) The use of ^{15}N fertiliser in denitrification studies is required to accurately establish the proportion of gaseous emissions from dairy pastures entering the atmosphere as N_2O or N_2. Techniques are available to capture such data.

(iv) The use of ^{15}N in urine to assess the proportion of gases being emitted from urine patches as N_2O and N_2 through biological denitrification. Again, techniques are available for use in such a study.

(v) Soil N in the pasture from N_2 fixation needs to be measured either by ^{15}N dilution or using the acetylene reduction assay.

(vi) Accurate measurement of gaseous N losses from centre pivot irrigation of pasture using either water or dairy effluent. New methodologies may be required to acquire this information.

(vii) Measure gaseous emissions from ponds on dairy farms using techniques established in the United States, particularly targeting chemo-denitrification and anaerobic ammonium oxidation.

(viii) The increased use of simulation studies is critical to make full use of increasing amount of data which is available in N cycling and denitrification in Australia generally.

1 Introduction

The dairy industry is one of Australia's major rural industries. Based on a farmgate production of $3.9 billion in 2010/11, it ranks fourth behind the beef, wheat and horticultural industries. The industry continues to undergo significant change with a large proportion of levy investment (approximately 45%) going into research and development with the aim of improving farm profitability. A key part of this research is to improve the nutrient management that is occurring on all dairy farms (Dairy Australia 2010).

It has been demonstrated that nutrient use efficiency on most dairy farms is below 30% with surpluses of 250 kg of N being determined at the whole farm level (Gourley et al., 2011). This surplus is often caused by farms applying more fertiliser than is deemed necessary resulting in environmental, regulatory and efficiency implications.

Because of its high N content and relatively low cost, urea is the main N fertiliser broadcast onto dairy pastures. Consequently, large losses of N may occur through ammonia (NH_3) volatilisation. Conversion of the remaining ammonium (NH_4+) through nitrification results in the formation of NO_3^- which can then be lost either through leaching or denitrified to emit dinitrogen (N_2) and the greenhouse gas (GHG) nitrous oxide (N_2O).

Denitrification is a key process in the global nitrogen cycle as it removes reactive nitrogen by the production of N_2 thus closing the N cycle. Nitrous oxide is normally a precursor to the formation of N_2, with many environmental factors (e.g. soil pH, water, nitrogen and carbon availability) affecting the N_2O/N_2 production ratio. Acid soils emit more N_2O relative to N_2, with the opposite for soils with a pH > 6 (Rochester, 2003).

Nitrous oxide emissions account for 10% of global anthropogenic greenhouse gas (GHG) emissions on a CO_2 equivalent (CO_2e) basis, with the vast majority of them from agricultural practices (IPCC, 2007). In Australia, agriculture contributes 14.6% (79.5 Mt CO_2e) of total emissions reported in the National Greenhouse Gas Inventory and 72.6% of net N_2O emissions (Department of Climate Change and Energy Efficiency, 2012). With 21.3% (16.9 Mt CO_2e) of the agricultural emissions coming from N_2O, a large proportion is derived from animal excreta (30.8% or 5.2 Mt CO_2e) with synthetic fertilisers contributing a further 15.4% (2.6 Mt CO_2e).

On a typical dairy farm, approximately 61% of the GHG emissions arise from methane. Nitrous oxide accounts for a further 25% of emissions (11% from N fertiliser, dung and urine and 14% from indirect losses). Electricity production used on the farm contributes another 8% of emissions (Figure 1).

Since N_2O has an estimated lifetime in the stratosphere of >100 years, and a global warming potential (GWP) of 310, understanding the loss process and establishing mitigating procedures is essential in order to reduce the N_2O emissions from

agriculture. Management strategies to reduce N_2O should be equally applicable to reducing N_2 and increasing overall N use efficiency and overall profitability. To this end, the current state of knowledge of the denitrification process as it applies to Australian dairy farms will be reviewed.

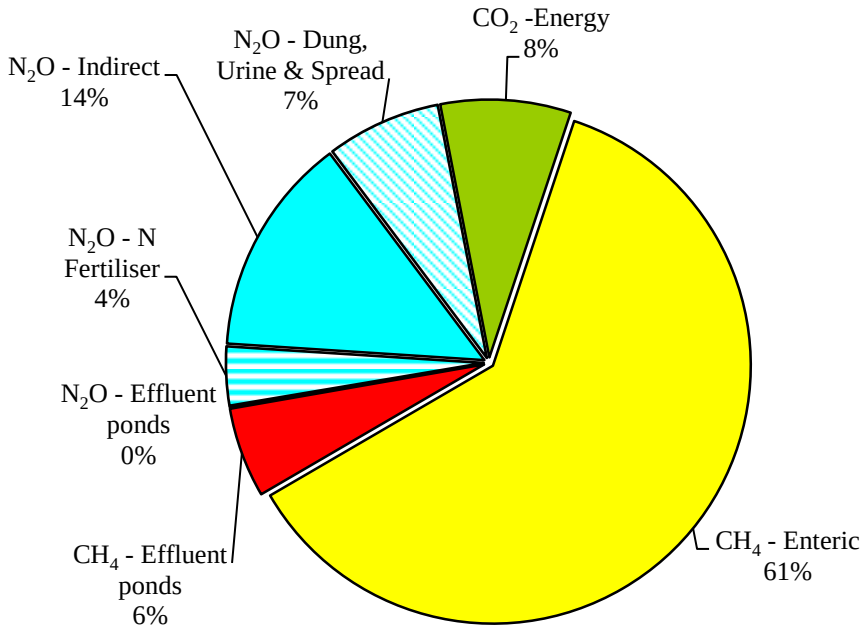

Figure 1. The output of methane and nitrous oxide emissions from a typical dairy farm in South Eastern Australia using DGAF (Eckard et al. 2011).

2 Information on current research into N losses on dairy farms

The current understanding of the denitrification process and its position in the field of N losses from dairy pastures needs to be known before proceeding with a program that will attempt to outline procedures which may mitigate this N loss.

Gaseous N emissions from dairy pastures have been studied under various conditions with results many and varied. Monaghan and Barraclough (1993) used ^{15}N to measure N_2O and N_2 emissions from undisturbed grassland soil treated with cow urine and held at specific water tensions in the laboratory. They found large and immediate emissions of N_2 following urine application to pasture. They attributed this to the solubilisation of soil organic matter by high pH and ammonia concentration.

Ledgard et al. (1996) studied inputs and N losses from dairy cow farmlets receiving 0, 225 and 360 kg N ha^{-1} during the first year of a grazing experiment. They found, denitrification losses were low (7-14 kg N ha^{-1} yr^{-1}), increasing with N application and occurring mainly in winter; volatile losses of NH_3 occurred directly after application of urea fertiliser; leaching loss was estimated to be 13, 18 and 31 kg N ha^{-1} yr^{-1}, respectively, in a year of low rainfall; N_2 fixation decreased with increasing application of N resulting in increased N losses; and 225 kg N ha^{-1} had little effect on N_2 fixation and on NO_3^- leaching.

A further study by Ledgard et al. (1999) found gaseous N losses by denitrification, from a fertilised white clover/ryegrass pasture, averaged from 5 to 25 kg N ha^{-1} yr^{-1} (Table 1).

Table 1. Nitrogen inputs/outputs (kg N ha^{-1} yr^{-1}) from a grazed pasture by N application (Ledgard et al. 1999) †

	0N	200N (L)	400N (L)	400N (H)
N Inputs:				
Fertilisers	0	215	413	411
N fixation	174	117	40	37
Purchased feed	3	4	3	69
Atmospheric deposition	2	2	3	2
Total Inputs	179	338	458	519
N losses/removals:				
Denitrification	5	15	25	24
Volatilisation	16	39	61	63
Leaching	40	79	150	133
Transfer to lanes/sheds	57	78	84	85
Milk/meat, surplus silage	81	110	126	126
Total Outputs	199	320	446	431
Nitrogen Balance	-20	18	12	81

†Mean annual N balances over 3 years for 3 farmlets receiving 0, 200, 400 and 400 kg N respectively, where L = 3.3 cows/ha and H = 4.4 cows/ha

Prasertak et al. (2001) found that 40% of the applied urea was lost from the soil-plant system in an ungrazed pasture in tropical Queensland with only 20% lost by volatilisation. As leaching or run-off losses were minimal, denitrification was assumed to account for the remaining 20%.

Gaseous N emissions from animal slurries or cattle urine have been studied over a number of years. Cattle grazing high-quality N pastures can return 25% and 55% of what they consume in dung and urine respectively (Fertilising Dairy Pastures Manual, 2005). In a further study, it was estimated that approx. 970 kg N ha^{-1} can be excreted in a urine patch as urea. Of this, only 33% was used for pasture growth while the

remainder was lost through leaching, denitrification and volatilisation. It has been estimated by Galbally et al. (2005) that between 4.2 and 4.5 kg N_2O-N ha^{-1} yr^{-1} could be lost via dentrification from a urine patch with a concentration of 1000 kg N ha^{-1}.

Ledgard et al. (1999) suggested adopting some intensive management practices such as feeding of grain or crops of low protein/high energy content to dairy herds (e.g. maize silage and cereal grains). This would lower the N content of dung and urine excreted onto the pasture and lower the quantity of N available for loss.

A study by Barton and Schipper (2001) found that soils irrigated with dairy farm effluent had a propensity to encourage greater emissions of N_2O than that emitted from an inorganic N source. They felt that the large available C source enhanced the denitrification process. This latter point was confirmed by Di et al. (2002) after applying ^{15}N labelled urine with dairy farm effluent to a grazed pasture. This study also found leaching from urine had a major overall effect on the quality of drainage water from dairying. Urine deposition on well aerated soils can lead to N_2O emissions via nitrification (Carter, 2007) but there was also the distinct possibility of large leaching losses of N.

Eckard et al. (2003) measured gaseous N losses from a grazed grass/clover pasture over 3 years, with and without 200 kg N ha^{-1}, applied as NH_4NO_3 and urea. It was found that NH_3 volatilisation was much greater from the urea treatment and denitrification losses slightly higher from the ammonium-nitrate treatment. This would not be unexpected. Although NH_4NO_3 applications would significantly reduce volatilisation, the economics of the proposal were not justified.

The effect of animal hooves on denitrification in a mixed ryegrass/clover pasture was studied by Menner et al. (2005). They found that animal treading caused a significant short-term increase in denitrification with a maximum loss of 52 g N_2O-N ha^{-1} day^{-1} after 8 days. After this period, denitrification rates decreased. They proposed that reduction in aeration and uptake of mineral N was responsible for the loss.

The above review of literature available on N losses occurring from dairy pastures seems to indicate that all forms of N loss pathways can operate in these fields. However, it would appear that studies specifically measuring the proportion of N gases being emitted as N_2O and N_2 cannot be identified. To this end, these pathways need to be quantified, the abiotic and biotic controlling factors identified, and procedures put in place to mitigate this major pathway of N loss.

3 The denitrification process

Denitrification, or dissimilatory nitrate reduction, is defined as the microbial reduction of NO_3 or NO_2 to gaseous nitrogen either as the oxide of nitrogen N_2O or molecular N_2. It is the principal pathway by which the greenhouse gas N_2O enters the atmosphere although, under certain conditions, N_2O can also be produced during

nitrification. Equation (1) outlines the sequential mineralisation, nitrification and denitrification of organic and inorganic nitrogen.

$$\begin{array}{ccccccccccc} & & & & & & N_2O & & & & \\ & & & & & & \uparrow & & & & \\ \text{Organic N} & \rightarrow & NH_4^+ & \rightarrow & NH_2OH & \rightarrow & NO_2^- & \rightarrow & NO_3^- & \rightarrow & NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2 \\ & & \uparrow & & & & & & & & \\ & & \text{Inorganic N} & & & & & & & & \end{array} \quad (1)$$

Mineralisation *Nitrification* *Denitrification*

Microbial denitrification is an alternative respiration process by bacteria in the soil that can use NO_3^- under oxygen (O_2) limited conditions. Most microbiologists identify denitrification as respiratory reduction coupled with electron transport phosphorylation using N oxides as electron acceptors. Although the complete denitrification path results in the reduction of NO_3^- to N_2, significant amounts of N_2O are produced as an obligate intermediate.

Denitrifying microorganisms are typical aerobic heterotrophic bacteria that have the capacity to reduce N oxides when O_2 is limited. The production of N_2O is controlled by the relative activity of reductase enzymes, irrespective of the microorganisms that occur. Such is the abundance of these organisms, that any restriction on denitrifying activity is moreso due to substrate limitation than the lack of the enzymes (Hutchinson, 1993).

4 Factors affecting biological denitrification

4.1 Nitrogen fertiliser

Urea is the principal form of nitrogen fertiliser applied to dairy farms. It has a nitrogen component of 46% and can be purchased at a low cost. However, broadcasting urea on to pastures with a high urease activity can lead to large initial losses of N through volatilisation (Harper et al., 1983). Transformation of the remaining urea is then likely to occur. Prasertsak et al. (2001) found that as much as 65% of the applied N was converted to NO_3^- eleven days after the application of urea to a dairy pasture in north Queensland. Provided all other controlling factors were present, denitrification can be expected to occur, with the concentration of NO_3^- in the soil having a major influence on the emission of both N_2O and N_2.

High NO_3^- concentrations generally inhibit the reduction of N_2O to N_2 by denitrifying microorganisms but this effect decreases as the concentration of nitrate decreases (Blackmer and Bremner, 1978; Bremner, 1997). This results in a greater production of N_2 as N_2O becomes the energy source for the denitrifiers in the absence of nitrate. However, this inhibitory effect increases with a decrease in soil pH.

A departure from the above has been suggested by Mosier et al. (1996). They suggested that soil management and cropping systems impact more on N_2O emissions than the mineral N source. Organic N sources such as animal manures and urine patches found in the dairy pasture system may induce larger emissions of N_2O than fertiliser N (Bouwman, 1990).

4.2 Carbon supply

The availability of organic matter as an energy source for the denitrifying bacteria is essential for the process of denitrification to proceed. However, the carbon source needs to be in a form that is readily available for use by the denitrifying bacteria. Thus, denitrification rates under anaerobic conditions correlate better with the soluble fraction of soil carbon than with the total soil carbon (Davidson et. al. 1987; Weier et al. 1998).

Organic soil amendments such as glucose, cellulose, succinate and methanol have also been found to stimulate denitrification although not all at the same rate (Burford and Bremner, 1975; Jacobsen and Alexander, 1980). More recent research seems to indicate a direct relationship between N_2O production and CO_2 emissions which means that a readily available energy source is present in the system for the denitrifier population (Weier et al., 1993a). However, Mathieu et al. (2006) found a positive correlation between N_2 and CO_2 which seems to confirm the importance of soluble C as the required energy source for the entire denitrifier population. For the intensive dairy pasture systems, available C sources are likely to be plentiful and not limit the production of N gases via denitrification.

4.3 Soil pH

Soil pH has a large influence on the proportions of N_2O and N_2 produced during the denitrification process. Focht and Verstraete (1977) suggested that the optimal rate for denitrification was between pH 7-8 while Koskinen and Keeney (1982) found N_2O to account for 83% of the gaseous N emitted at pH 4.6 and 5.4 when 90% of the NO_3^- was denitrified. As pH increased, the N_2O produced was readily reduced to N_2. Knowles (1981) suggested that the greater sensitivity of the nitrous oxide enzyme may be responsible for the decrease in N_2O emissions. This would agree with later findings that tend to suggest that, at low pH, the reduction of N_2O to N_2 is inhibited more than the reduction of NO_3^- to N_2O (Burford and Bremner, 1975).

Weier and Gilliam (1986) found a significant negative relationship between N_2O production and pH which showed that N_2O release had virtually ceased at pH 5.8. Di-nitrogen was thus the dominant gaseous product as pH increased above this value. Between pH 4.2 and 5.7, N_2O accounted for up to 92.5% of the NO_3^--N lost (Figure 2).

Further evidence of high pH affecting the products of N loss during denitrification was provided by Weier (pers. comm.) who found that 94% of N loss from a maize

field at pH 8.4 occurred as N_2. Rochester (2003) also found that, although the N_2O/N_2 ratio in alkaline soils was low, the N_2O produced still amounted to approx 2 kg N_2O-N ha^{-1}.

De Klein et al. (2001) suggested manipulating the end product of denitrification by enhancing the conversion of N_2O to N_2 as a means of reducing N_2O emissions. Van der Weerden et al. (1999) had earlier suggested that maintaining a soil pH at approx. 6.5 may help mitigate N_2O emissions by producing a low N_2O mole fraction.

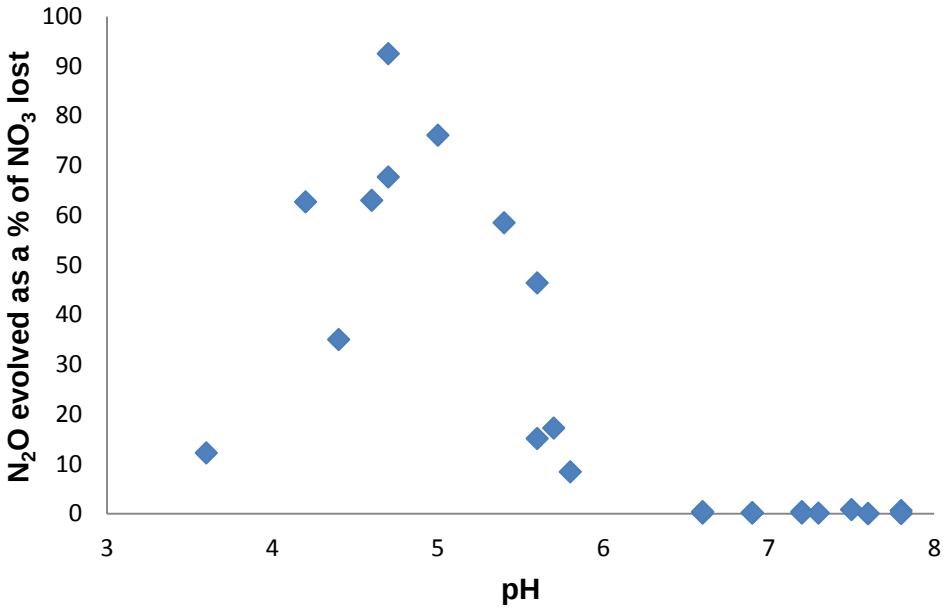

Figure 2. The relationship between N2O (as % of No3 lost) and pH after 21 days (date from Weier and Gilliam, 1986).

4.4 Water filled pore space

Soil moisture and aeration, here expressed as water filled pore space (WFPS) (2), has a significant effect on the N_2O emitted from both the nitrification and denitrification processes.

WFPS = [(gravimetric water content x soil bulk density/total soil porosity)] (2)

where soil porosity = [1 – soil bulk density/particle density]

and the particle density of soil = 2.65 Mg m^{-3}

The use of WFPS as a means for evaluating soil microbial activity with respect to soil moisture and aeration was first proposed by Linn and Doran (1984) when determining the contribution of nitrification and denitrification to gaseous N losses from soil. The

relative potential of WFPS as an indicator of aerobic and anaerobic activity in soil is illustrated in Figure 3.

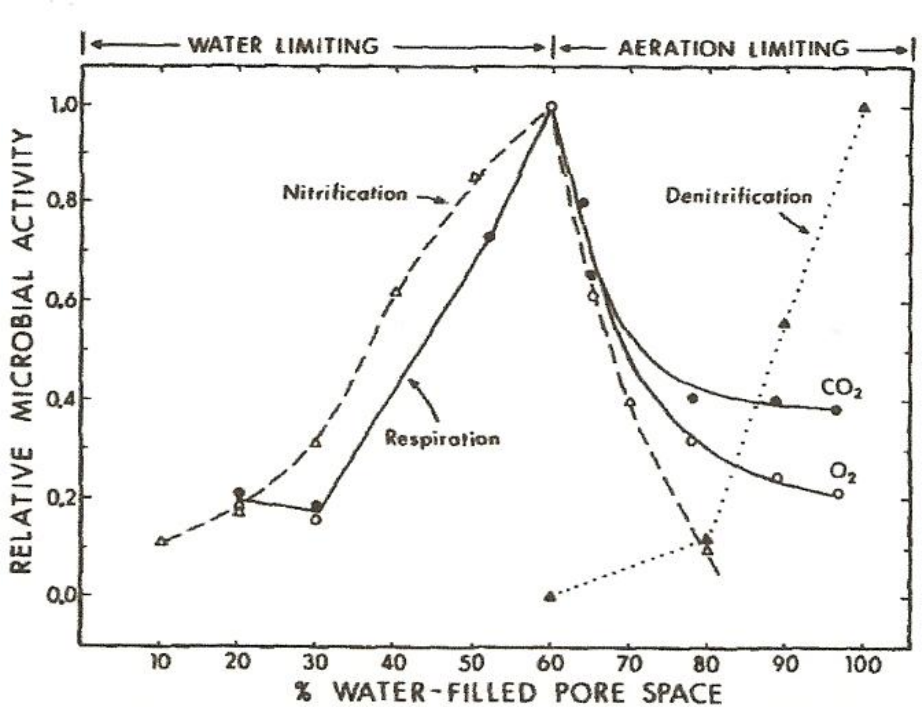

Figure 3. The relationship between water-filled pore space and relative amount of microbial nitrification (Greaves and Carter, 1920), denitrification (after Nommik,1956), and respiration [O_2 uptake (O-O) and CO_2 production (O-O) as determined by Linn and Doran, 1984].

Early studies by Bremner and Shaw (1958) found a definite relationship between soil water content and soil aeration, with denitrification ceasing below 60% water holding capacity and increasing rapidly above this value. As the water content of the soil increases, anoxic conditions increase, promoting the release of both N_2O and N_2 while also increasing the conversion rate of N_2O to N_2 (Weier et al., 1993b; Dalal et al., 2003).

Phillips et al. (2007) observed that N_2O emissions remained low immediately following irrigation when soils were saturated (WFPS) > 95%, but 2–3 days after irrigation, as soil moisture decreased below 95% WFPS, N_2O emissions increased rapidly and remained high for 1 to 2 days, followed by a gradual decrease to background levels at WFPS < 65%. The low N_2O emissions immediately following irrigation were most likely due to complete denitrification producing mainly N_2 emissions.

A decrease in the N_2O/N_2 ratio decreases can also occur in the presence of soluble C as it promotes the growth of soil microorganisms and increases the uptake of N_2O in soils (Blackmer and Bremner, 1976; Mathieu et al., 2006). Ruser et al. (2006) found N_2 to be the principal gaseous product at >75% WFPS when measuring the influence of compaction on denitrification.

The influence of soil texture and the associated WFPS on the occurrence of denitrification cannot be underestimated. Rowlings et al. (2012) found soil texture, water infiltration and drainage play a greater role in influencing fluxes of greenhouse gases in a rainforest ecosystem than either vegetation or seasonal change. Similarly, Eckard and Cullen (2011) found WFPS to be the principal factor controlling N_2O emissions from four dairy pasture sites in Victoria.

Soil types ranged from a freely-draining ferrosol (low WFPS) to an irrigated red chromosol dairy pasture (high WFPS). Anaerobic and aerobic microsites are also known to occur in coarse and fine textured soils in response to changing soil moisture conditions (Parkin, 1987; Weier et al., 1991). High rates of nitrification and denitrification are associated with these sites, in the latter case producing mean N_2O losses of 29 g N_2O-N ha^{-1}.

The underlying soil type on dairy farms needs to be assessed as different soil types will impact differently on a range of characteristics that affect the pathways of N loss from the farm. In order to minimise these losses, an understanding of these characteristics is essential if N use efficiency on dairy farms is to be increased.

4.5 Temperature

The activity and diversity of denitrifying microorganisms has been found to be related to their soil environment, with different populations active at different temperatures. Powlson et al. (1988) compared denitrification, at various temperatures, in an English temperate soil with that in an Australian sub-tropical soil. They found 92% of the NO_3^- had denitrified in the temperate soil after 7 days at 10^oC whilst 91% of the NO_3^- in the Australian soil still remained. Only at temperatures $>20^oC$ did all the remaining NO_3^- in the Australian soil denitrify.

Similarly, Malhi et al. (1990) found increased NO_3^- loss from Alberta soils as temperature rose from -4^0C to 40^0C with the greatest increase between 4 and 10^0C. However, neither of the above measured the gaseous loss occurring either as N_2 or N_2O. Broadbent and Clark (1965) suggested that the relative proportions of N_2O and N_2 in the gas produced during denitrification varied with temperature, with N_2O predominant at lower temperatures and N_2 at the higher temperatures.

However, Keeney et al. (1979) found N_2O emissions increase with increasing temperature, reaching a maximum at 37^oC. The latter was confirmed in a further study where they found 85% of the N evolved at 70^oC to be N_2O. Eckard et al. (2003) measured N_2O losses from N fertilised dairy pastures using the acetylene inhibition

method (Parkin et al., 1985). They found denitrification losses were larger in the winter period when soil temperatures were $<10^0$C. Higher soil moistures and lower oxygen availability may also have contributed to this loss.

However, field studies have also revealed that temperatures in tropical and subtropical areas have a limited effect on N losses via denitrification (Weier et al., 1991; 1998) with other factors such as available C and WFPS playing larger roles (Kiese and Butterbach-Bahl, 2002).

4.6 Effect of plant roots

Plant roots can have a significant effect on denitrification and the literature reports indicate their influence is quite complex. Smith and Tiedje (1979) found that the potential for denitrification in the rhizosphere was high when soil NO_3^- concentrations were high. At low NO_3^- concentrations, the planted soils denitrified at a lower rate than unplanted soils. Under controlled conditions, Brar (1972) found that living roots may aid denitrification by providing a readily available source of energy to the denitrifying bacteria.

Similarly, Bailey (1976) found that root exudates and sloughed off root cap cells increased microbial activity leading to increased NO_3^- reduction in the presence of the root. This disagrees with the later findings of Halder et al. (1986) who found no influence of root exudates on microbial denitrification in soils planted to maize and wheat. However, the quality and size of the plant root mass can influence the denitrification in soils by limiting the carbon available for the denitrifier population (Crush, 1998).

The cutting and removal of grass clippings results in an immediate increase in N_2O production from grassland. This is indicative of organic matter leaking out of roots once they are cut (Beck and Christensen, 1987). A further study by Klemedtsson et al. (1987), using barley plants, found that the plant increases the demand for oxygen in the soil and also provides an easily decomposable source of carbon, both of which impact on the denitrification rate.

Thus, in dairy pastures, where there is an extensive root system, a readily available source of carbon is present which, combined with factors such as N supply and soil texture, supply an environment where gaseous N losses could be quite large.

5 The N_2: N_2O ratio

To estimate the denitrification rate of soils both N_2O and N_2 must be known (Smith, 1990). By knowing the variation in the N_2/N_2O ratio, total denitrification (N_2+N_2O) can be estimated when only N_2O emissions are being measured.

Some of the factors known to affect the denitrification process were also found to influence the N_2/N_2O ratio. Temperature effects on the ratio have been reported by Keeney et al. (1979) and Avalakki et al. (1995). They found differing results, with the

former reporting an increase in the N_2O/N_2 ratio up to 37^oC and then decreasing while the latter reported an increase in the N_2O/N_2 with decreasing temperature.

High N_2O/N_2 ratios are commonly associated with soils of low pH (Rochester, 2003). Focht (1974) found that the decrease in the N_2O/N_2 ratio with increasing pH was attributed to a greater increase in the rate of N_2O reduction than N_2O production. Moraghan and Buresh (1977) reported that N_2O remained the dominant gaseous N product emitted at pH 6, but over 80% of the N_2O was reduced to N_2 at pH 8.

Firestone et al. (1980) found that soil acidity had little effect on the N_2/N_2O ratio in the absence of measurable quantities of NO_3^-. It was only in the presence of increasing concentrations of nitrate that increased N_2O production occurred at pH 4.9.

In a study on the interacting effects of C, NO_3^- and WFPS on biological denitrification, Weier et al. (1993b) found that the N_2/N_2O ratio was positively correlated with WFPS and available C and negatively correlated with soil NO_3^- level. They also concluded that an average N_2/N_2O ratio for estimation of denitrification from N_2O field measurements is not recommended because the ratio is influenced by the variation in environmental factors that affect gaseous N production. To overcome this problem, Mosier et al. (1996) suggested that a figure of 1.25(±1) % of applied inorganic N be used to calculate the N_2O lost from the fertiliser.

6 Indirect N loss processes

6.1 Chemo-dentrification

Volatilisation of ammonia (NH_3), after the application of urea fertiliser to crop or pasture soils, is the primary avenue of N loss for this fertiliser under alkaline conditions. However, under acid conditions, NH_4 and CO_2 are formed and nitrification can proceed since the oxidation of ammonium to nitrite by *Nitrosomonas* is an acid producing reaction (Chalk and Smith, 1983; Equation 3).

$$2NH_4^+ + 3O_2 \rightarrow 2NO_2^- + H_2O + 4H^+ \qquad (3)$$

Nitrite is somewhat reactive under acidic conditions, although nitrite decomposition will occur when the soil pH is alkaline (Smith and Chalk 1980). However, the accumulated nitrite may be unstable in the acidic portion of the soil environment, leading to gaseous loss of N (Hauck and Stephenson, 1965; van Cleemput, 1998). There exists the possibility of chemical conversion (chemo-denitrification) of NH_4 to N_2 without proceeding through any biological process. In this process, ammonium is converted to dinitrogen under anaerobic conditions using NO_2^- as the electron acceptor (Strous et al., 1997) and was seen as an avenue for removal of ammonium from waste waters (Equation 4).

$$NH_4^+ + NO_2^- \rightarrow N_2 + 2H_2O \quad (4)$$

Harper and Sharpe (1998) using micrometeorological methods, found that, when the largest amount of methanogenesis was occurring from swine lagoons, higher quantities of N_2 were evolved when compared to NH_3 and N_2O. Harper et al. (2000) suggested that the unaccounted for N lost from their swine lagoons may have been due to chemical denitrification, with NO_2^- again acting as the electron acceptor for reduction to N_2. Harper et al., (2012; in press) have also shown that at high rates of methanogenesis, a significant amount of NH_4^+ is converted to N_2.

However, as methanogenesis decreased, smaller emissions of N_2 occurred and higher rates of N_2O were produced. In a later studies, (Harper et al., 2010; Weaver and Harper, 2012) found that by removing organic matter from biofuel manure-processing lagoons, annual CH_4 emissions decreased by 47%. However, reduced methanogenesis resulted in a reduction in the chemical conversion of NH_4^+ to N_2 with the result that NH_3 emissions from biofuel farms increased by 46%.

Nitrogen estimated to be evolved as NH_3 was found to be converted to N_2, a factor that had not been previously considered in NH_3 estimates. Some estimates of N_2 emissions from the lagoons are given in Table 2.

Anaerobic ammonium oxidation (anammox) has also been found in a range of marine environments and may be responsible for up to 50% of the global removal of fixed nitrogen from the oceans (Dalsgaard et al., 2005). Beman et al, (2005) found microbial denitrification to be a powerful tool in decreasing N pollution in the Gulf of California from irrigation run-off.

There is a need to establish if anaerobic ammonium oxidation is occurring at the subsurface of animal slurries held in ponds on dairy farms. This may require the development of new techniques although it is possible that they could be available from researchers in the United States. This would then address an imbalance that may be occurring when evaluating inputs and outputs from a dairy farm.

Table 2. Monthly primary lagoon dinitrogen biological gas emissions. (reprinted from Weaver and Harper, 2012 ; in press, with permission)

	Biofuel		Control	
Month	Year 1[†]	Year 2[‡]	Year 1	Year 2
	kg N_2-N d^{-1} $lagoon^{-1}$ [§]			
April	27.3	22.3	25.9	37.1
May	24.4	36.5	24.2	51.3
June	16.3	45.8	14.2	56.1
July	19.0	47.2	18.0	60.1
August	36.7	45.5	31.2	48.1
September	47.4	37.9	49.9	33.2
October	44.6	36.1	40.5	32.5
November	37.7	21.2	43.9	22.2
December	19.6	15.9	36.6	22.7
January	17.7	12.5	31.5	21.5
February	19.1	11.4	36.2	22.8
March	20.0	12.5	40.5	22.7
Average	27.5	28.8	32.7	35.9
Standard Deviation[¶]	0.9	6.8	2.2	2.8

† Year 1: April 2004 to March 2005

‡ Year 2: April 2005 to March 2006

§ The lagoon size of each lagoon in the study is 1.69 ha. The emission of N_2 gas was calculated from the measured daily average of biogas collected from summa canisters and the daily percent composition of each gas measured from gas chromatography.

[¶] The standard deviation from the mean of the annual emissions of the three farms from each farm type.

6.2 Leaching

Leaching of NO_3^- is considered to be a major pathway for N loss from dairy pastures. Nitrogen added in the form of inorganic N, legume fixed N or N added from cow urine or cattle slurry are all potential point sources from which leaching may occur. Application of urea to established pastures usually undergoes a short period of large efflux especially during the warmer months (Harper et al., 1983; 1987). The remaining N can be subject to denitrification and leaching with N losses of 7-14kg N ha^{-1} yr^{-1} and 13-31 kg N ha^{-1} yr^{-1} respectively (Ledgard et al., 1996).

Nitrate loss from urine patches is also considered to be a major contributor to the total leaching losses observed from dairy pastures and thus has a large effect on drainage water quality (Ledgard et al., 1999; Di et al., 2002). Pakrou et al. (1997) even suggested that urine patches were the major point source of leached N in dairy pastures with values of 60 mg L^{-1} being obtained well into the grazing season.

Leaching of NO_3^- from legume based pastures has increased with the increase in fertiliser N application. N fixation decreases in the presence of large quantities of N resulting in increased nitrification with subsequent denitrification and leaching of NO_3^--N. Leaching losses of 15-35 kg NO_3^--N ha^{-1} have been obtained under grazed legume pastures under different climatic conditions (Fillery, 2001). Significant denitrification could also occur providing environmental conditions are favourable.

6.3 Groundwater

Surface based N fertiliser applications, mineralisation of soil organic matter and fixed N in pastures or beneath rainforest canopies, all provide opportunities for NO_3^- movement below the root zone and eventual contamination of the groundwater (Thorburn et al., 2003; Rowlings et al., 2012).

The effect of intensive dairying on the contamination of the underlying shallow groundwater was investigated by Stenger et al. (2008). Groundwater sampling at various depths had shown the presence of low NO_3^- concentrations. It was concluded that NO_3^- reduction through denitrification was occurring in this catchment in the vadose zone and/or in the shallow aquifer. Although no C source was identified, energy for the bacteria was supplied from leachate passing through the root zone. Pakrou et al. (1997) also found that leaching of NO_3^- from grazed pasture can be a significant source of pollution in the groundwaters of South Australia. They found average losses of between 1 and 5 kg N ha^{-1} day^{-1}.

Further evidence for denitrification occurring in groundwater can be found in studies by Exner (1994) and Weier et al. (1994). Exner used changes in the natural abundance ratio of the two stable isotopes of N, ^{14}N and ^{15}N, to identify the sources of NO_3^- contamination in the groundwater. She confirmed that denitrification was occurring in groundwater contaminated by human wastes and also in extremely shallow groundwater which was in contact with the lower soil horizons. Weier et al. (1994) found the addition of an energy source to NO_3^- contaminated groundwater can result in the remediation of the groundwater through biological denitrification.

Leaching of NO_3^- mineralised from litter deposited under rainforest can also result in contamination of the underlying groundwater. Soluble carbon leached from the soil following heavy rainfall can result in remediation of the groundwater through denitrification.

6.4 Nitrogen fixation

Pasture legumes have historically supplied much of the N needs of dairy pastures. Ledgard et al. (1999) estimated a perennial ryegrass/white clover pasture on a free draining soil could supply between 99 and 231 kg N ha^{-1} yr^{-1} through N_2 fixation but this declined to 15-44 kg N ha^{-1} when 400 kg N ha^{-1} yr^{-1} was applied. However, the 200 kg N ha^{-1} yr^{-1} treatment had little effect on N_2 fixation. Earlier research had indicated that N fertiliser was not required in clover based dairy pastures (Ellington,

1986) but this has since been negated by supplying N to these pastures when clover is not actively growing or fixing N (Eckard, 1998). As a consequence, N fertiliser use by dairy farmers has increased dramatically over the last 15 years (Eckard et al., 1997).

The quantity of N lost from legume-based pastures via biological denitrification is largely unknown (Fillery, 2001). The grazing animals can remove >60% of the N in the pastures, with the excreted N returned to the soil as urine and dung, the proportion of N being dependent on the quality of the pasture. Nitrogen inputs from intensively grazed legume based pastures in New Zealand suggest the contribution from N_2 fixation to be between 160-260 kg N ha^{-1} yr^{-1} with gaseous loss in the former case amounting to 20 kg N ha^{-1} yr^{-1} (Fillery, 2001).

However, the percentage lost due to denitrification was not calculated. Ledgard et al. (2009) found N_2O emissions to be greater from grass only pastures than from clover grass pastures after N fertiliser addition. They suggested that this occurrence may be due to additional specific N losses from the grass pasture that do not necessarily occur from the clover/grass pasture. In a UK study, white clover was found to provide the necessary N for pasture growth thus lowering the financial cost incurred through N fertiliser application (Andrews et al., 2007).

A study by Eckard et al. (2001) found N_2 fixation to contribute 108 kg N ha^{-1} yr^{-1} to a grazed dairy pasture with 8 kg N ha^{-1} yr^{-1} lost through denitrification. He also found that the N balance for this pasture suggested a likely depletion of N from the pasture. This suggests a need for the application of N fertiliser to the pasture at a rate to stimulate growth but not at a rate that would affect the N symbiosis or lead to increased losses of N via denitrification.

7 Laboratory measurement of denitrification

Denitrification rates can be measured using the acetylene (C_2H_2) inhibition technique (Duxbury and McConnaughey, 1986; Ryden et al., 1987). Intact soil cores are collected from the field and incubated in the laboratory (or in situ) using closed systems in which 10% of the air is replaced by C_2H_2. Acetylene inhibits the conversion of N_2O to N_2 with the total denitrification loss measured as N_2O (Balderston et al., 1976). Malone et al. (1998) compared the C_2H_2 inhibition technique with the ^{15}N technique and found that the flux measurements were not significantly different (3.9 v 4.3 nmol N g^{-1} hr^{-1}). Weier et al. (1993a) have successfully used the C_2H_2 inhibition technique to measure denitrification losses from Brigalow clay soils in central Queensland.

De Klein et al. (1994) and Watkins (2007) used the C_2H_2 inhibition technique to measure the effect of dicyandiamide (DCD) on denitrification rates from urine patches and from dairy farm soils. DCD was found to significantly lower N_2O emissions from urine patches while no significant decrease in denitrification rates was observed from the pasture soils. Luo et al. (1999; 2000) used the technique to measure denitrification losses from dairy pastures in New Zealand. They found that

denitrification losses induced by grazing were quite small while the annual loss from a legume-based dairy-farm pasture was 4.5 kg N ha^{-1}.

Studies in Canada (Paul and Zebarth (1997) used the technique to measure leaching and denitrification losses from the soil profile after application of dairy cattle manure. Researchers in Denmark (Maag and Vinther, 1996), Netherlands (van der Salm et al., 2007) and Japan (Deng et al., 2011) have used the C_2H_2 inhibition technique to measure denitrification losses from heavy clay soils and to develop a sustainable manure management system to reduce NO_3^- leaching and N_2O emissions from agricultural soils. This technique appears to be a research tool capable of returning quantifiable results on field denitrification rates when field conditions are such that field experimentation is not possible.

As an alternative to using C_2H_2 (a combustible gas), Butterbach-Bahl et al. (2002) developed a laboratory based N_2-free soil core incubation system, whereby a purge gas mixture of O_2 and He is used prior to direct measurements of both N_2 and N_2O. The N_2O/N_2 ratio from the laboratory incubations can be coupled with field observations of N_2O emissions from like soils to estimate N_2 production, thus providing an estimate of total denitrification (Scheer et al., 2009).

8 Field measurement of denitrification

8.1 Flow through chambers

Although open-flow and closed chambers were used as a method to measure N_2O emissions from the soil surface (Denmead, 1979; Denmead et al., 1979), they compete with static chambers as a measurement technique to monitor gaseous N emissions. For the closed system, air was drawn through a closed loop between the chamber air space and the gas analyser.

In the open system, outside air is drawn continuously through the chamber space and its N_2O enrichment or depletion measured. It was found that the closed system produced a low pressure within the chamber resulting in increased air being withdrawn from the soil. This led to incorrect estimates of the flux. This problem was overcome in the open system by adjusting the flow rate through the chamber. The open chamber was successfully used to measure N_2O exchange over 5 months in an unfertilised, mown, grass sward (Denmead et al., 1979). They found that exchange rates as small as 1 ng N m^{-2} sec^{-1} could be measured.

8.2 Static chambers

Initial in-field measurement of gaseous nitrogen emissions were made using static chambers either placed on the soil surface (Freney et. al., 1978) or pushed to a known depth into the soil (Weier et. al., 1996). These chambers were usually open-bottomed with gas samples drawn from the enclosed headspace over time. The concentrations of N_2O were calculated from a linear curve produced during the sampling. The main

disadvantage of static chambers is the inability to fully capture the highly episodic and diurnal nature of N emissions from denitrification. Chambers may be inaccessible and gas samples ideally need to be withdrawn from chambers every 6 or 8 hours over a 5 to 10 day period to cover any likely event of N loss (Weier, 1999). Both timing (during the day) and frequency of the gas sampling events have a major impact on the final estimate.

For example, Rowlings et al. (2010) reported that the error associated with the annual estimates of N_2O emissions from grazed sub-tropical pastures could be 10-16% if gas sampling and analysis was only performed every 3 days (compared to a daily sampling frequency). This error increased to 22-30% if sampling was only performed on a weekly basis.

Although the flow-through and static chamber methods were used over a number of years to measure gaseous N emissions quite successfully (Hutchinson and Mosier, 1981; Weier, 1999) and compared well with micrometeorological flux measurements (Laville et al., 1999; Smith et al., 1994), the high spatial and temporal variability associated with static chamber measurements, in particular, made extrapolation to the larger field scale difficult. This then led to a decrease in the use of both systems and a corresponding increase in the use of micrometeorological techniques for measuring gaseous N emissions.

8.3 Micrometeorological methods

Denmead (1983) states that micrometeorological techniques provide a method that does not disturb environmental or soil processes that influence gas exchange; allows continuous rapid measurement allowing for the inclusion of environmental effects; is suitable for measurement of a flux that is occurring over large areas; and minimises the sampling problem of point to point variation.

The major difficulty associated with this technique is that it requires a large experimental area and the very rapid and accurate determination of small gas concentrations. However, the move from static chamber measurements of N_2O flux to micrometeorology was a natural progression once the importance of quantifiable data for agro- ecosystems was established (Laville et al., 1999).

Denmead et al. (1998) described a mass balance technique for calculating gas production from a surface or volume in a small test plot. Gas concentrations were measured at four heights within the test plot. Gas production was calculated from the differences observed between fluxes integrated over downwind and upwind boundaries. They found the method suitable for flux measurement in situations where conventional micrometeorological methods cannot be used.

Denmead et al. (2000; 2010) again employed the mass balance approach to verify N_2O emissions from grazed pastures and sugarcane fields where values had been obtained from small enclosures and then extrapolated to field scale. They found that

the values obtained from the pasture using this technique produced even larger N_2O emissions than those previously reported from the same site. For the sugarcane site, N_2O emissions were about half those previously reported using short-term measurements.

Phillips et al. (2007) used a tuneable-diode laser (TDL) to measure N_2O concentrations in air sampled from flood-irrigated dairy pastures during a micrometeorological study in Victoria over 2 years. They found that daily emissions ranged from <4 to approximately 170 g N ha^{-1} day^{-1} with the highest emissions recorded when temperature was >15^o, N was present in the soil and WFPS ranged from 65-95%.

Variations on the above methods have also been used to monitor N_2O emissions. Smith et al. (1994) used different micrometeorological measurements (eddy correlation using TDLAS, flux gradients using FTIR, gas chromatography and TDLAS) to measure N_2O fluxes from an ungrazed field fertilised with 185 kg N ha^{-1}. All of the methods provided similar estimates of N_2O fluxes over the field, averaging 43-85 ng N_2O-N m^{-2} s^{-1}. Wagner-Riddle et al. (1996) have also used a TDL to measure N_2O emissions from a bare plot to evaluate the effect of management practices on the importance of the gas. They found peak N_2O emissions after irrigation and N fertiliser application at rates well above those for bare soil.

Although these methods are still the choice of many researchers, limitations on their use include a high deployment cost and technical difficulties associated with adverse weather conditions (Dalal et al., 2003). This has resulted in a move to fully automated field chambers where N_2O fluxes can be determined over larger areas, for greater time lengths and with gas sampling at regular time intervals. New fast response analyzers for N_2O measurement from Picarro which are suitable for micrometeorological studies will be compared to automated systems as part of an upcoming project in the Australia Department of Agriculture, Fisheries and Forestry (DAFF) Filling the Research Gap program.

8.4 Automatic chambers

Automatic chambers for continuous measurement of N_2O fluxes have been gradually adopted as a research tool for continuous monitoring of greenhouse gas emissions. Dobbie et al. (1999) measured N_2O fluxes from an intensively managed agricultural field using automatic chambers linked to a gas chromatograph fitted with an electron capture detector. Ambus and Robertson (1998) developed as system whereby CO_2 and N_2O fluxes were measured in air circulated between fully automated flow-through chambers and a photoacoustic infrared trace gas analyser (TGA).

More recently, Kelly et al. (2008) used a fourier transformed infrared (FTIR) spectrometer linked to automated chambers to measure N_2O emissions from surface applied urine with and without DCD addition. However, the development of a fully automated portable system for the determination of the greenhouse gases N_2O, CO_2

and CH_4 by Kiese and Butterbach-Bahl (2002) has provided researchers with a tool to continuously measure all three gases over time. The mobility of this system eliminates the need for expensive infrastructure and the ability to move between experimental sites (including on-farm) thus providing greater diversity in soil types, treatments and climates.

The system consists of a gas chromatograph equipped with a ^{63}Ni electron capture (ECD) and flame ionisation (FID) detectors for the analysis of N_2O and CH_4 respectively and a LICOR infrared gas analyser for the measurement of CO_2. The system has a computer-controlled gas sampling system all of which is housed in a water-proof trailer (Figure 4). Gaseous emissions are measured 8 times a day using 2500 cm^2 chambers. During sampling, the chambers are closed and opened for set times allowing for fluxes to be obtained at set intervals while minimizing any impact on the soil environment (Rowlings, 2010).

The system has been successfully used to measure N_2O and CO_2 emissions from soils in a tropical rainforest (Kiese and Butterbach, 2002) and monitor temporal variations in N_2O, CO_2 and CH_4 emissions in a subtropical rainforest (Rowlings et al., 2012) as well as irrigated cotton (Grace et al., 2010) and wheat (Scheer et al., 2012), pasture (Rowlings et al., 2010; Scheer at al., 2010; 2011) and tree crops (Rowlings et al., 2010).

These studies have consistently found that WFPS played a more important role in controlling greenhouse gas emissions than either vegetation or seasonal variability. These portable automated systems are now being used to assess greenhouse gas emissions in subtropical dairy systems near Gympie in Queensland, and temperate systems near Camden in New South Wales.

There is the potential for the inclusion of an in-line mass spectrometer within this system which will allow for the determination of N_2 loss via denitrification provided highly enriched ^{15}N fertiliser is used as the N fertiliser source. The easy deployment of the above system would suggest that the placement of such a collection device on dairy farm pastures could lead to quantifiable results being obtained for N_2O and N_2 emissions.

Figure 4. Portable automated greenhouse gas sampling unit as used at elected sites within the Nitrous Oxide Research Program in Australia.

9 Use of ^{15}N in field measurements

The stable isotope ^{15}N has been used extensively as a tracer for monitoring N movement and plant uptake of N from the soil. The natural abundance of ^{15} N is 0.366 atom % and must be enriched to as high as 99 atom % ^{15}N for use in agriculture and this adds a further cost to the procedure. Determination of the $^{14}N/^{15}N$ ratio using isotope ratio mass spectrometry (IRMS) provides measurement of the ^{15}N- labelled N_2 collected by gas sampling techniques. Output signals are generated proportional to the number of 3 types of molecules evolved: $^{28}N_2$, $^{29}N_2$ and $^{30}N_2$. If equilibrium exists between all 3 molecules, only the $^{28}N_2$ and $^{29}N_2$ signals need to be determined to calculate ^{15}N abundance.

An example of the latter can be found in a study by Vallis et al (1996). They used ^{15}N-labelled urea, applied as subsurface bands in sugarcane crops in southern Queensland and northern New South Wales, to measure N recovery by the crop. It was found that urea fertiliser supplied only 20-40% of the crop uptake in a given season. Total recovery by the crop in the soil-plant system averaged 56% at crop maturity.

Determination of gaseous products from denitrification using ^{15}N not only requires the measurement of the $^{14}N/^{15}N$ ratio but also the determination of the $^{28}N_2$, $^{29}N_2$ and the $^{30}N_2$ to calculate the ^{15}N abundance (Mosier and Schimel, 1993). This technique and

method of calculation has been used successfully in determining the proportion of gaseous N products emitted from denitrification studies in a sugarcane crop (Weier et al., 1998) and a maize crop Weier (pers. comm.). Both studies found the proportion of gases evolved differed markedly, with the majority of gas being emitted as N_2. The contributing factors to these results were high soil pH, an available C source, WFPS > 60% and the presence of soil NO_3^-.

Although there have been some studies using ^{15}N to follow N pathways in dairy pastures (Clough et al., 1998; 2006; Malone et. al., 1998; Munoz et. al., 2003) very few studies have been attempted using ^{15}N to follow and distinguish between gaseous N products from denitrification. Clough et al. (1998) found gaseous losses of N_2O to be <2% of N applied on application of ^{15}N-labelled urine over a 112 day measurement period, with the ratio of N_2/N_2O ranging from 6.2 to 33.2, indicating the majority of unrecovered N was due to N_2 loss.

Two options exist with respect to ^{15}N studies in dairy systems. First, the determination of total nitrogen ($N_2 + N_2O$) lost via denitrification (and assuming no volatile losses of NH_3) using a mass balance approach (i.e. denitrified N = N applied – N recovered in plant – N recovered in soil) can be undertaken quite inexpensively using static chambers and low enrichment ^{15}N fertilisers. This does require an accurate assessment of ^{15}N removed by the plant (throughout the season) and a deep soil profile analysis (0-100+ cm), including root material, at the end of the season. Animals need to be excluded from the ^{15}N application site and grazing simulated through artificial cuts taken when animals would normally be introduced during the season.

Second, both static and automatic chambers also provide the opportunity for direct gas measurement of $^{15}N_2O$ and $^{15}N_2$ if multiple gas samples are removed in the field during a gas sampling event and then analysed by IRMS in the laboratory. These tracer studies require gas sampling to be continued over a prolonged period (days to weeks) to ensure an accurate assessment of the N losses due to denitrification.

10 Use of nitrification inhibitors

The use of nitrification inhibitors to maintain the applied fertiliser source in the ammonium form for a longer period of time was first proposed by Goring in 1962. He found that nitrapyrin [2-chloro-6-(trichloromethyl)pyridine] delayed the conversion of NH_4^+ to NO_2^- but had little effect on the bacteria responsible for the conversion of NO_2^- to NO_3^-. When nitrapyrin was combined with urea and applied to soil, gaseous N loss and N leaching losses were reduced (Chancy and Kamprath, 1982).

The application of nitrapyrin with manure to field soils and laboratory incubated soils resulted in the effective inhibition of nitrification and a reduction in leaching (McCormick et al., 1983; Calderon et al., 2005). However, the effectiveness of nitrapyrin as a nitrification inhibitor has been brought into question (Hoeft, 1984; Weier and Gilliam, 1986). It was found that nitrapyrin was readily absorbed onto soil

colloids and soil organic matter thus decreasing its effectiveness. Therefore, any release of N_2O would be from denitrification as nitrapyrin does not affect the soil microorganisms responsible for denitrification (Henninger and Bollag, 1976).

The most potent inhibitor of nitrification is acetylene (Bremner and Blackmer, 1979; Walter et al., 1979) but, because of its gaseous nature, there was difficulty in maintaining the concentration of acetylene in the soil that was necessary to delay nitrification. The problem was somewhat overcome by the introduction of encapsulated calcium carbide (ECC) (Banerjee and Mosier, 1989; and Aulakh et al., 2001). They found reduced nitrification and increased recovery of N after the addition of ECC to N fertilised arable and flooded soils. The use of ECC in fertilised soils has been found to increase crop yields and improve the recovery of N.

There are other acetylene based nitrification inhibitors e.g. 2-ethynylpyridine and phenylacetylene that have proved effective in increasing recovery of N in laboratory and field studies (Freney et al., 1993) but neither have been tested in reducing N loss from urine and manure in dairy pastures. On that basis, neither could be considered for use in dairy pastures without further experimentation.

The nitrification inhibitor that has received most use in dairy pastures is dicyandiamide (DCD). De Klein and Logtestijn (1994) found that when DCD was added with urine to grassland soil, N_2O emissions were significantly reduced. Vallejo et al. (2001) also found DCD to reduce N_2O emissions but also found that water filled WFPS had a greater effect on denitrification.

Di and Cameron (2003) used DCD in a simulated grazed dairy pasture system under spray irrigation. They found that DCD applied immediately after urine application effectively reduced N_2O emissions. In a study in northern Victoria, Kelly et al. (2008) found DCD to reduce N_2O emissions from urine patches by 47% in spring and 27% in summer which suggests the inhibitor to be temperature dependent. In a later study, they found DCD inhibits N_2O emissions from urine patches by between 35% and 74% (Figures 5 & 6; Kelly, pers. comm.).

Further confirmation of the ability of DCD to control gaseous N emissions from urine patches has been supplied by Cookson and Cornforth (2002) and Di and Cameron (2008). They found NO_3^- concentrations in the soil to be reduced and N_2O emissions to be decreased from both nitrification and denitrification. More recently, 3,4-dimethylpyrazole phosphate (DMPP) was found to have the potential, in some soils, to inhibit nitrification by suppressing the ammonia oxidising bacteria eg *Nitrosomonas*, and therefore denitrification losses (Suter et al., 2010b). They had previously found that lower concentrations of DMPP were more effective than DCD at inhibiting N_2O emissions (Suter et al., 2010a).

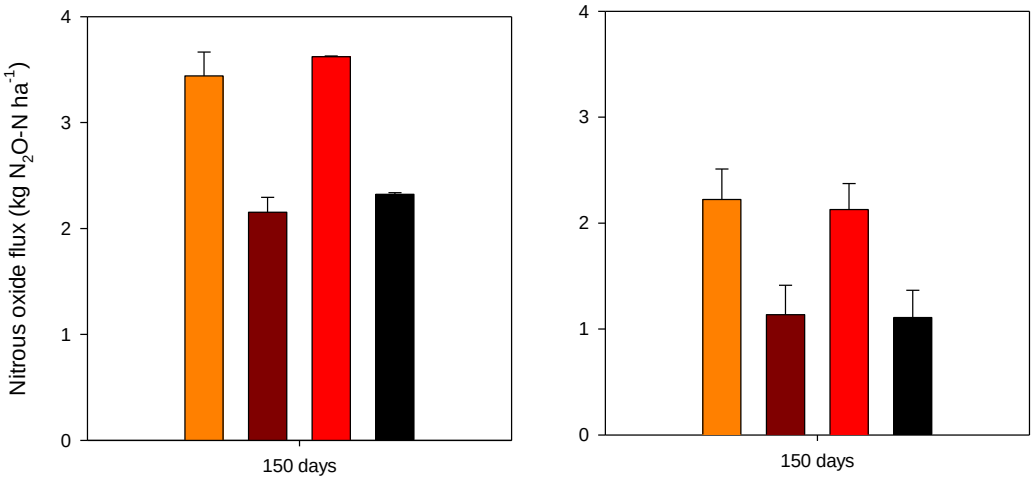

Figure 5. Nitrous oxide flux over 150 days *(Orange – urine applied day 29, brown – urine applied day 29 + DCD applied day 1, red – urine applied day 1, black – urine applied day 1 + DCD applied day 1).* [Application rates urine @ 1000 kg N/ha, DCD @ 10 kg a.i. / ha]. Time period of study, (a) 14 August 2009 – 15 April 2010, (b) 23 April 2010 – 22 September 2010.

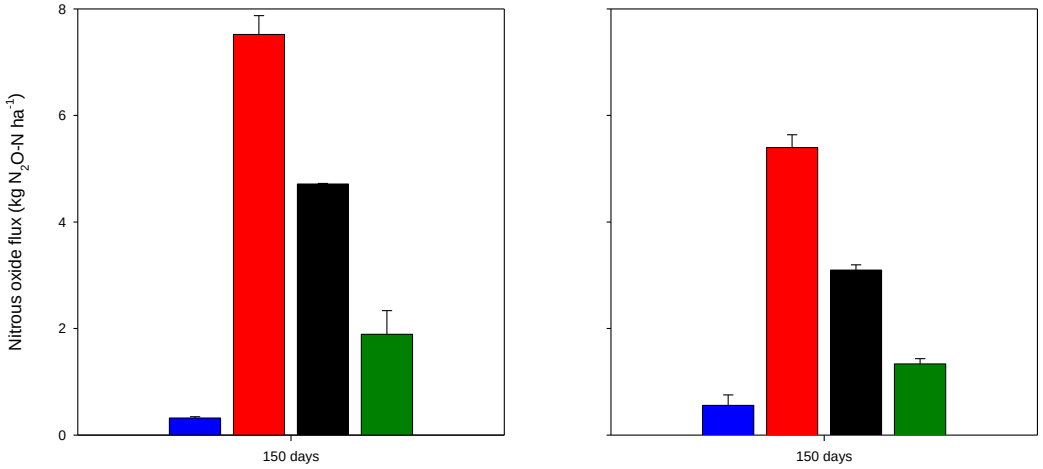

Figure 6. Nitrous oxide flux over 150 days *(Blue – nil applied, Red – urine applied 4 times to 25% of plot area, Black – urine applied 4 times to 25% of plot area + DCD applied day 1, Green – urine applied 4 times + DCD applied each urine application.* [Application rates urine @ 1000 kg N/ha, DCD @ 10 kg a.i. / ha]. Time period of study, (a) 23 September 2010 – 5 May 2011 – urine applied day 1, 29, 49, and 76, (b) 6 May 2011 – 12 October 2011 – urine applied day 1, 28, 112 and 133.

The effectiveness of the nitrification inhibitor DCD in reducing N_2O emissions from pasture and urine patches has been reasonably well established but its wider application may be hindered by its cost unless productivity benefits are clearly identified. The use of DCD with centre pivot irrigation of dairy effluent on pastures to reduce gaseous N emissions needs further research. Simulation studies may offer an avenue of research to evaluate this possible pathway for N loss.

11 Application of animal slurries and dairy effluent to pastures

The application of animal slurries and dairy farm effluent on pastures has been used as a method of recycling nutrients to the pastures. On an annual basis, irrigation of N from dairy farm ponds could supply between 50 and 240 kg N ha^{-1} yr^{-1} depending on the quality of pasture grazed (Whitehead, 1995). The use of centre pivots as a means of applying the effluent to pastures has resulted in technological advanced systems being developed. GPS is now attached to the pivot so that soils of different particle size will receive only the amount of water required for growth. Excess run-off is thus avoided and gaseous N losses reduced.

Although N losses via denitrification from animal excreta have not been extensively evaluated, Fillery (2001) suggests that denitrification losses from urine patches could be potentially as high as 25-30%. He also suggested that cattle defecating close to camping areas can cause large transfers of N within paddocks. He estimates that N transfer from pastures to laneways or to milking sheds can result in losses of approximately 55 kg N ha^{-1} yr^{-1}.

In an earlier study, Monaghan and Barraclough (1993) used a controlled ^{15}N study to estimate N losses from urine applications. They also found that denitrification losses could be as high as 65% and concluded that readily-oxidizable organic carbon may be the energy source for the denitrifying organisms. However, in well-aerated soils, nitrification was found to be the major contributor to N_2O loss from urine patches (Carter, 2007).

In an attempt to decrease N_2O emissions, de Klein and Logtestijn (1994) added dicyandiamide (DCD) with urine and found a significant decrease in N_2O losses. The use of cattle slurries as an organic N source for pastures has resulted in denitrification losses over a greater length of time when compared to mineral N applications (Ellis et al., 1998; Barton and Schipper, 2001). Stevens and Laughlin (2002) found the addition of cattle slurry with a NO_3^- fertiliser source stimulated gaseous N loss because of the creation of a readily decomposable source of C for the soil microorganisms. The maximum loss of $N_2O + N_2$ was 8.3%.

In contrast to this, Kester et al., (1997) found relatively low N_2O emissions when dairy shed effluent was applied to pasture. They surmised that the saturated water conditions (very high WFPS) reduced N_2O production by promoting the conversion of N_2O to N_2. Granli and Bockman (1994) and Dalal et al (2003) also found that the latter condition enhanced the completion of the denitrification sequence.

12 Modelling the denitrification process

With the high cost of field-based research, the loss processes in the N cycle, the high spatial and temporal variability of N losses, modelling is a logical and powerful tool for evaluating management interventions for increasing N use efficiency in dairy systems (Eckard et al., 2006).

Two categories of model complexity (or lack of) exist with respect to soil denitrification sub-models within crop/soil models. Mechanistic (or complex) approaches include explicit algorithms of microbial growth and/or gaseous diffusion. Examples of mechanistic models include DNDC (Li et al., 2000) and ECOSYS (Grant, 2001) and are characterised by a relatively large number of detailed parameters which can potentially be estimated in the laboratory under controlled conditions but are generally harder to determine in field situations. For example, in ECOSYS, microbial activity is based on six organic states within four organic matter-microbe complexes. Many of these models also utilise sub-daily time steps.

In contrast, empirical (or simplified) process models are normally easier to use and denitrification is determined by easily measurable parameters such as degree of saturation, soil temperature and nitrate content of the soil. Models in this category (which have been used in Australia) include DayCent (Del Grosso et al., 2000), DairyMod (Eckard et al., 2006; Johnson et al., 2008), APSIM (Keating et al., 2000) and DSSAT (Jones et al., 2003). Another model, WNMM (for Water and Nitrogen Management Model) (Li et al., 2008) may be considered a hybrid of these two approaches and has been successfully used to simulate N cycling and emissions from Victorian dairy pastures (Chen et al., 2010).

A trade-off therefore exists between the mechanistic models (with a potentially large error term due to the propagation of errors associated with a large number of parameters) and the more streamlined reductionist approach of empirical models which have less process based detail (and by circumstance less parameterisation) than the former.

For example, the gaseous N loss component of the DayCent model has been calibrated using a diverse set of soils in laboratory incubations with variable soil water contents to generate N loss equations with wide applicability. DayCent consistently predicts the seasonal pattern, or cumulative amount of N_2O emitted via both the nitrification and denitrification pathways (Del Grosso et al., 2008), but predictions on a daily basis are relatively poor.

This is not unexpected considering the relative simplicity of the plant growth and soil water and temperature algorithms in DayCent compared to some of the more popular crop production models (e.g. APSIM, DSSAT). However these latter aspects play major roles in the regulation of the key driving factors of denitrification i.e. water and N supply.

It is worth noting though that a new version of DayCent (Daily DayCent) with more detailed plant growth functions has successfully simulated N_2O emissions across a range of treatments in irrigated cropping systems of northern Australia (Scheer pers. comm.).

DayCent does have a rudimentary grazing function, but does not explicitly allow for the finer details of animal interaction, however, the underlying C/N cycling and gaseous N loss sub-models are transportable. Thorburn et al. (2010) translated DayCent algorithms into the modular APSIM framework and found it to accurately simulate N_2O emissions in high N input sugar cane systems of northern Australia.

Similar deficiencies in the accuracy of temporal simulations are evident in WNMM and the more process-orientated DNDC. Chen et al. (2010) report that WNMM was most reliable in estimating N_2O emissions from a urea and urine treated dairy pasture at a time scale of 35 days. Modified versions of the DNDC model for New Zealand (Saggar et al. 2007) and the United Kingdom (Wang et al., 2012) both report the relative success of the model in simulating seasonal emissions of N_2O, but an inability to estimate the magnitude of some high emissions events, or in fact missing high emissions entirely. This is not unique to DNDC, however, as all models (both mechanistic and empirical) in the complex area of denitrification must still be considered approximations only.

Whilst there still remains relatively large uncertainties in the development of the soil denitrification aspects of models generally, a major area of concern in grazing systems is how to handle the non-uniform movement of animals and their impact on soil structure and in particular the spatial distribution of N substrates (i.e. urine and manure) which contribute to gaseous N losses (Snow et al. 2009).

One model that currently allows for the explicit treatment of the impact of urine patches on whole system behaviour is DairyMod. The model's inorganic nitrogen dynamics include nitrification of ammonium, leaching and denitrification. The modelling approach utilises the single heterogeneous paddock function in EcoMod to capture the dynamics of urine patches on N transformation in the soil (Snow et al., 2009).

Paddocks are sub-divided into patches, corresponding to the proportion of the paddock that receives urine during grazing on a day. The location of the urine deposition can either follow a sequential rotation around all the patches or can be deposited randomly. The entire system dynamics are modelled explicitly for each patch. While mechanistically sound, this approach does present computational challenges in the large number of patches that must be simulated, but with current hardware this should not be considered an obstacle.

Models are only as good as the data that has been used in their development, and emission measurements of N_2O from grazing are extremely variable due to the uneven distribution of urine and manure (Wang et al., 2012). Increased availability of high

quality N_2O emission data as a direct consequence of the Australian government's Nitrous Oxide Research Program will provide the necessary high temporal resolution data for improving the current suite of simulation models which are potentially suitable for improving N use efficiency in Australia's dairy industry.

13 Mitigating N_2O emissions from dairy pastures

Increasing N fertiliser use by the dairy industry requires improved on-farm management practices whereby N use efficiency is increased and gaseous N emissions decreased. Appropriate management options to achieve the above have been proposed and include:-

(i) Application of fertiliser N at an annual rate of 200 kg N ha^{-1}, split into individual applications of 60 kg N ha^{-1}. Above this rate, environmental losses increase exponentially (Eckard, 1998). Measured rates should take into account the available mineral- N present in the soil.

(ii) Integration of the nitrogen fixed from clover based pastures with N fertiliser management (Eckard, 2001).

(iii) Applying fertiliser to match N use by plant demand, and switch from urea to diammonium phosphate (DAP) if the need arises (Peoples, 1995).

(iv) Applying other nutrients as required so that nutrient supply to pasture is balanced and N utilisation optimised (Dalal et al. 2003)

(v) Use of N fertilisers formulated with nitrification inhibitors (or alternatively as an inhibitor spray) to maintain the fertiliser in the ammonium form and reduce the nitrate available for gaseous loss. [Dicyandiamide (DCD) has been found to significantly reduce N_2O emissions from pastures and from urine patches (Di, 2008; Kelly et al., 2008)].

(vi) Avoiding surface application of all N fertilisers by incorporating N into the soil.

(vii) Avoiding grazing on wet soils. Soil compaction by grazing animals reduces the potential for drainage thus increasing the conditions which are conducive for N_2O loss.

(viii) Application of dairy effluent to dry soils to eliminate the possibility of denitrification occurring from saturated soils. If applying by centre pivot, the use of a GPS guided system would allow application rates to match soil types.

(ix) Adopting some intensive management practices by feeding grain or crops of low protein/high energy such as maize silage or cereal grains. This would reduce the N content of the dung and urine excreted onto the pasture (Ledgard et al. 1999).

(x) Introduce surface or subsurface drains on seasonally wet soils. Waterlogged soils will denitrify more than well drained soils, while improved drainage can reduce total denitrification losses (DeKlein and Eckard, 2008).

14 Recommendations for future research

The development of research programs aimed at producing management practices to reduce N_2 and N_2O emissions from dairy pastures must begin with a detailed study of the soil characteristics associated with the soil type underlying the dairy farm. Soil properties invariably control the factors responsible for N loss, with aerobic and anaerobic soil types influencing the occurrence of leaching, nitrification and denitrification respectively.

Having established the soil type underlying the dairy pasture, programs can be established to measure denitrification losses. Before this can occur, NO_3^- concentration in the soil as a result of nitrogen fixation and nitrogen mineralisation needs to be measured. This would allow for the calculation of the total concentration of N in the soil available for loss after N fertiliser application.

The N available for loss from urine patches also needs to be assessed so that a complete picture of N availability beneath the pasture is available when N fertiliser application rates are formulated.

Nitrogen fixation rates for clover based dairy pastures have not been measured with any degree of certainty. Techniques such as ^{15}N dilution and the acetylene reduction assay are available for producing results in this area but have received little attention. Eckard et al. (2001) suggests that about 7% from a fixed N total of 108 kg N ha^{-1} yr^{-1} may be lost via denitrification which is similar to a figure quoted by Fillery (2001) for a New Zealand study.

Some quantification of N_2 fixation values is needed so that a baseline figure can be used for quoting denitrification losses when balancing inputs and outputs from a dairy pasture.

Nitrogen mineralisation rates also need to be established for the pasture soils as a function of climate. If mineralisation has already produced high concentrations of NO_3^-, then the need for inhibition of N_2 and N_2O losses via denitrification becomes immaterial. Both laboratory and field methods are available for producing mineralisation rates, and should be used when considering available N concentration in the dairy pasture.

There is need to establish methodology for assessing N losses from dairy pastures when centre pivot irrigation is the principle method used for applying either water to N fertilised pasture or dairy effluent. Simulation of the above application method may offer a technique where quantifiable data could be obtained for use in calculations of N losses from the pasture.

Denitrification losses from dairy pastures have been measured using automatic chambers. However, losses have been based solely on N_2O emissions with no figures available for N_2 emissions. ^{15}N fertiliser has been used to calculate N_2 emissions from

sugar cane and maize fields (Weier et al., 1998) demonstrating the fact these techniques are available and potentially applied to measure the N_2 losses from dairy pastures. ^{15}N can also be applied with a urine solution to measure N_2 losses from this source.

If field rates involve the use of centre pivot irrigation, new systems may be required to allow for complete wetting of the soil surface in the chamber.

Gaseous efflux from effluent ponds on dairy ponds needs to be assessed. Research from the United States found that chemical denitrification was operating in swine lagoons with NH_4^+ converted to N_2 without any biological process. The N_2 emitted was related to the amount of methanogenesis occurring in the lagoon. Techniques for measuring this emission are available from researchers in the United States.

Summarising the above:-

(i) Nitrogen supplied from mineralisation for each pasture soil needs to be established so that the nitrate present in the soil is known before either fertiliser or DCD application.

(ii) Increased emphasis on determining the textural, chemical and physical characteristics of the soils (and their variability) underlying dairy pastures as an aid in the determination of water filled pore space and mineralisable N. This information is critical in developing any predictive tools for reducing gaseous N losses.

(iii) The use of ^{15}N fertiliser in denitrification studies is required to accurately establish the proportion of gaseous emissions from dairy pastures entering the atmosphere as N_2O or N_2. Techniques are available to capture such data.

(iv) The use of ^{15}N in urine to assess the proportion of gases being emitted from urine patches as N_2O and N_2 through biological denitrification. Again, techniques are available for use in such a study.

(v) Soil N in the pasture from N_2 fixation needs to be measured either by ^{15}N dilution or using the acetylene reduction assay.

(vi) Measure gaseous emissions from ponds on dairy farms using micrometeorological techniques established in the United States.

(vii) Accurate measurement of gaseous N losses from centre pivot irrigation of pasture using either water or dairy effluent. New methodologies may be required to acquire this information. These methods may also be useful for landscape assessment of emissions.

(viii) The increased use of simulation studies is critical to make full use of increasing amount of data which is available in N cycling and denitrification in Australia generally. A variety of models already exist in Australia which are either directly applicable to the dairy industry (DairyMod) or have the capability and flexibility (e.g. APSIM) to incorporate some of the more proven mathematical representations of the soil C and N cycles and N loss pathways which can be found in models like DayCent.

The Nitrous Oxide Research Program funded by DAFF and relevant Rural Development Corporations may be the appropriate place to establish programs to measure N_2 fixation and N mineralisation as well as programs to measure denitrification losses from dairy pastures using ^{15}N. An appropriate program also needs to be established to measure N losses from pastures under centre pivot irrigation.

15 Conclusions

We find, as a result of this review, there is significant research still required into denitrification losses from dairy pastures.

Increasing N fertiliser use has resulted in increased losses of N from dairy pastures and urine patches. Qualitative estimates of N_2O emissions ranged from 7-25 kg N ha^{-1} yr^{-1} for pastures and from 4.2 to 4.5 kg N_2O-N ha^{-1} for urine patches.

Nitrification inhibitors have been successful in reducing N_2O emissions from urine patches by as much as 74%.

Potential losses of N after dairy effluent or animal slurry may be as high as 8.3% although lower figures have also been reported. An emission factor of 1.25% has been proposed to calculate N_2O-N evolved per 100 kg fertiliser applied (Mosier et al., 1996). However, variations in this factor have been recorded, accentuating the need for quantitative figures to be available. Proven ^{15}N methods are available to produce such results and should be adapted by the industry research teams.

Most of the N_2O estimates have been made over a short duration which makes extrapolation of results to large areas less than ideal. The use of micrometeorological techniques and chamber methods offer the best solution to obtaining quantifiable data with the chamber method having the added benefit of using an in-line mass spectrometer for ^{15}N detection.

Indirect losses of N from dairy pastures contribute to the overall picture of N pathways operating on dairy farms and need to be assessed. Studies on urea volatilisation from pastures have resulted in the adaption of mitigation procedures to reduce this loss. Leaching losses still occur with losses ranging from 15-35 kg N ha^{-1} following fertiliser application to legume based pastures. The contamination of groundwater still persists when these situations exist. A clearer idea of the underlying soil texture may lead to better management of irrigation application.

Gaseous emissions from dairy ponds have not been measured. Techniques are available to measure these emissions, with data contributing to the N balance between N inputs and outputs.

Mitigation procedures have been proposed to limit N loss from dairy farm operations. These include reduction in N fertiliser application, making use of available N,

matching N supply with plant demand, avoiding surface application of N, avoiding grazing on wet soils and the use of nitrification inhibitors.

Changing feed supplements to those of grain or crops of low protein/high energy to reduce the N content of urine and dung excreted onto pasture would also aid in limiting N loss.

We suggest that future research on denitrification concentrate on obtaining quantitative estimates of N_2O and N_2 through the use of ^{15}N. Reduction in fertiliser N requirements through the prior knowledge of fixed and mineralised N is also needed as is the matching of pasture irrigation with soil type.

There has been extensive research on the use of nitrification inhibitors which needs to be promoted, with details of experimental procedures outlined for use by other researchers to enhance this promising area of emissions reduction.

A comprehensive research program linked to improved simulation modelling capability is needed to implement these recommendations to arrive at position where the pathways of N loss from dairy farms are quantified and N balances can be achieved with some certainty to ensure N use efficiency is optimised for maximum environmental and financial benefit.

Acknowledgements

We thank Dr Lowry Harper, Mr C Downie, Richard and Charles Bignell, Phil and Helen Beattie, Mr Kevin Kelly, Dr Sally Officer, Dr Helen Suter and Prof Deli Chen for taking time out of their busy schedules to discuss various aspects of the review with the authors.

References

Ambus P, Robertson GP (1998) Automated near-continuous measurement of carbon dioxide and nitrous oxide fluxes from soil. *Soil Science Society of America Journal* **62**, 394 - 400.

Andrews M, Scholefield D, Abberton M, McKenzie B, Hodge S, Raven J (2007) Use of white clover as an alternative to nitrogen fertiliser for dairy pastures in nitrate vulnerable zones in the UK: productivity, environmental impact and economic considerations. *Annals of Applied Biology* **151**, 11 - 23.

Aulakh MS, Kuldip-Singh, Doran J (2001) Effects of 4-amino1,2,4,-triazole, dicyandiamide and encapsulated calcium carbide on nitrification inhibition in a subtropical soil under upland and flooded conditions. *Biology and Fertility of Soils* **33**, 258 - 263.

Avalakki UK, Strong WM, Saffigna PG (1995) Measurements of gaseous emissions from denitrification of applied nitrogen-15. 2*. Effects of temperature and added straw. *Australian Journal of Soil Research* **33**, 89 - 99.

Bailey LD (1976) Effects of temperature and roots on denitrification in soil. *Canadian Journal of Soil Science* **56**, 79 - 87.

Balderston WL, Sherr B, Payne WJ (1976) Blockage by acetylene of nitrous oxide reduction in *Pseudomonas perfectomarinus. Applied and Environmental Microbiology* **31**, 504 - 508.

Banerjee NK, Mosier AR (1989) Coated calcium carbide as a nitrification inhibitor in upland and flooded soils. *Journal of Indian Society of Soil Science* **37**, 306 - 313.

Barton L, Schipper LA (2001) Regulation of nitrous oxide emissions from soils irrigated with dairy farm effluent. *Journal of Environmental Quality* **30**, 1881 - 1887.

Beck H, Christensen S (1987) The effect of grass maturing and root decay on N_2O production in soil. *Plant and Soil* **103**, 269 - 273.

Beman JM, Arrigo KR, Matson PA (2005) Agricultural runoff fuels large phytoplankton blooms in vulnerable areas of the ocean. *Nature* **434**, 211 -214.

Blackmer AM, Bremner JM (1976) Potential of soil as a sink for atmospheric nitrous oxide. G*eophysical Research Letters* **3**, 739 - 742.

Blackmer AM, Bremner JM (1978) Inhibitory effect of nitrate on the reduction of N_2O and N_2 by soil microorganisms. *Soil Biology and Biochemistry* **10**, 187 - 191.

Bouwman AF (1990) Exchange of greenhouse gasses between terrestrial ecosystems and the atmosphere. In 'Soils and the Greenhouse Effect' (Ed. Bouwman AF) pp. 61 - 127. John Wiley and Sons, New York.

Brar SS (1972) Influence of roots on denitrification. *Plant and Soil* **36**, 713 - 715.

Bremner JM, Shaw K (1958) Denitrification in soils: .II. Factors affecting denitrification. *Journal of Agriculture Science* **51**, 40 - 52.

Bremner JM, Blackmer AM (1979) Effects of acetylene and soil water content on emission of nitrous oxide from soils. *Nature* **280**, 380 - 381.

Bremner JM (1997) Sources of nitrous oxide in soils. *Nutrient Cycling in Agroecosystems* **46**, 7 - 16.

Broadbent FE, Clark FE (1965) Denitrification. In 'Soil Nitrogen' (Eds. Bartholomew WV, Clark FE) pp. 344 - 359. American Society of Agronomy, Madison, Wisconsin.

Burford JR, Bremner JM (1975) Relationships between the denitrification capacities of soils and total, water-soluble and readily decomposable soil organic matter. *Soil Biology and Biochemistry* **7**, 389 - 394.

Butterbach-Bahl K, Willibald G, Papen H (2002) Soil core method for direct simultaneous determination of N_2 and N_2O emissions from forest soils. *Plant and Soil* **240**, 105–116.

Calderon FJ, McCarty GW, Reeves JB (2005) Nitrapyrin delays denitrification on manured soils. *Soil Science* **170**, 350 - 359.

Carter, MS (2007) Contribution of nitrification and denitrification to N_2O emissions from urine patches. *Soil Biology and Biochemistry* **39**, 2091 - 2102.

Chalk PM, Smith CJ (1983) Chemodenitrification. In 'Gaseous Loss of Nitrogen from Plant-Soil Systems' (Ed's Freney JR, Simpson JR) pp. 65 - 89, Martinus Nijhoff/ Dr W. Junk Publishers, The Hague/ Boston/Lancaster.

Chancy HF, Kamprath EJ (1982) Effect of nitrapyrin on N response of corn on sandy soils. *Agronomy Journal* **74**, 565 - 569.

Chen D., Li Y, Kelly K, Eckard R (2010) Simulation of N_2O emissions from an irrigated dairy pasture treated with urea and urine in Southeastern Australia. *Agriculture, Ecosystems and Environment* **136**, 333 - 342.

Clough T, Kelliher FM, Wang YP, Sherlock RR (2006) Diffusion of ^{15}N-labelled N_2O into soil columns: a promising method to examine the fate of N_2O in subsoils. *Soil Biology and Biochemistry* **38** 1462-1468.

Clough T, Ledgard SF, Sprosen MS, Kear MJ (1998) Fate of ^{15}N labelled urine on four soil types. *Plant and Soil* **199**, 195-203.

Cookson WR, Cornforth IS (2002) Dicyandiamide slows nitrification in dairy cattle urine patches: Effects on soil solution composition, soil pH and pasture yield. *Soil Biology and Biochemistry* **34**, 1461 - 1465.

Crush JR (1998) Effect of different forage plants on denitrification potential of Horotiu soil. *New Zealand Journal of Agricultural Research* **41**, 421 - 426.

Dairy Australia Limited (2010) "Your Levy at Work Brochure".

Dalal RC, Wang W, Robertson GP, Parton WP (2003) Nitrous oxide emission from Australian agricultural lands and mitigation options: a review. *Australian Journal of Soil Research* **41**, 165 - 195.

Dalsgaard T, Thamdrup B, Canfield DE (2005) Anaerobic ammonium oxidation (anammox) in the marine environment. *Research in Microbiology* **156**, 457 - 464.

Davidson EA, Galloway LF, Strand MK (1987) Assessing available carbon: comparison of selected techniques across selected forest soils. *Communications of Soil Science and Plant Analysis* **18**, 45 - 64.

de Klein CAM, Eckard RJ (2008) Targeted Technologies for nitrous oxide abatement from Animal Agriculture. *Australian Journal of Experimental Agriculture* **48,** 14 - 20.

de Klein CAM, Sherlock RR, Cameron KC, van der Weerden TJ (2001) Nitrous oxide emissions from agricultural soils in New Zealand - a review of current knowledge and directions for future research. *Journal of the Royal Society of New Zealand* **31**, 543 - 574.

de Klein CAM, van Logtestijin RSP (1994) Denitrification and N_2O emission from urine-affected grassland soil. *Plant and Soil* **163**, 235 - 241.

Del Grosso SJ, Parton WJ, Mosier AR, Ojima DS, Kulmala AE, Phongpan S (2000) General model for N_2O and N_2 gas emissions from soils due to denitrification. *Global Biogeochemical Cycles* **14**, 1045 - 1060.

Del Grosso SJ, Halvorson, AD, Parton, WJ (2008) Testing DAYCENT model simulations of corn yields and nitrous oxide emissions in irrigated tillage systems in Colorado. *Journal of Environmental Quality* **37**, 1383-1389.

Deng M, Sonoko DK, Lee J, Hojito M, Yoh M (2011) Denitrification on Andosols in an intensive dairy farming region of central Japan. *Agriculture, Ecosystems & Environment* **144**, 330 - 337.

Denmead OT (1979) Chamber systems for measuring nitrous oxide emissions from soils in the field. *Soil Science Society of America Journal* **43**, 89 - 95.

Denmead OT, Freney JR, Simpson JR (1979) Studies of nitrous oxide emission from a grass sward. *Soil Science Society of America Journal* **43**, 726 - 728.

Denmead OT (1983) Meteorological methods for measuring gaseous losses of nitrogen in the field. In 'Gaseous Loss of Nitrogen from Plant-Soil Systems' (Ed's Freney JR, Simpson JR) Martinus Nijhoff/ Dr W. Junk, Publishers, Boston.

Denmead OT, Harper LA, Freney JR, Griffith DWT, Leuning R, Sharpe RR (1998) A mass balance method for non-intrusive measurements of surface-air trace gas exchange. *Atmospheric Environment* **32**, 3679 - 3688.

Denmead OT, Leuning R, Jamie I, Griffith DWT (2000) Nitrous oxide emissions from grazed pastures: measurement at different scales. *Chemosphere - Global Change Science* **2**, 301 - 312.

Denmead OT, Macdonald BCT, Bryant G, Naylor T, Wilson S, Griffith DWT, Wang WJ, Salter B, White I, Moody PW (2010) Emissions of methane and nitrous oxide from Australian sugarcane soils. *Agricultural and Forest Meteorology* **150**, 748 - 756.

Department of Climate Change and Energy Efficiency (2012) Australian National Greenhouse Gas Accounts. National Inventory Report 2010. Volume 1. The Australian Government Submission to the UN Framework Convention on Climate Change, April 2012. Department of Climate Change and Energy Efficiency, Canberra, ACT, Australia, 320pp.

Di HJ, Cameron KC, Silva RG, Russell JM, Barnett JW (2002) A lysimeter study of the fate of ^{15}N-labelled nitrogen in cow urine with or without farm dairy effluent in a grazed dairy pasture soil under flood irrigation. *New Zealand Journal of Agricultural Research* **45**, 235 - 244.

Di HJ, Cameron KC (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 HJ, Cameron KC (2008) Sources of nitrous oxide from N15-labelled animal urine and urea fertiliser with and without a nitrification inhibitor, dicyandiamide. *Australian Journal of Soil Research* **46**, 76 - 82.

Dobbie KE, McTaggart IP, Smith KA (1999) Nitrous oxide emissions from intensive agricultural systems: variations between crops and seasons, key driving variables, and mean emission factors. *Journal of Geophysical Research* **104**, 26891 - 26899.

Duxbury JM, McConnaughey PK (1986) Effect of fertilizer source on denitrification and nitrous oxide emissions in a maize field. *Soil Science Society of America Journal* **50**, 644 – 648.

Eckard RJ, Johnson I, Chapman DF (2006) Modelling nitrous oxide abatement strategies in intensive pasture systems. *International Congress Series* **1293**, 76-85.

Eckard RJ, McKenzie FR, Lane PR (1997) The 'Pendulum Paradigm' - trends in nitrogen fertiliser use on temperate grass/clover pastures. In 'Proceedings of the XVIII International Grasslands Congress, Canada, June 1997'. pp. 30.3 - 30.4. (Extension Service, Saskatchewan Agriculture and Food: Saskatoon, Saskatchewan).

Eckard RJ (1998) A critical review of research on the nitrogen nutrition of dairy pastures in Victoria. ILFR, The University of Melbourne and Agriculture Victoria, Ellinbank, NRE, pp. 1 - 31.

Eckard RJ, Chapman DF, White RE, Smith A, Chen D, Durling DA (2001) Nitrogen balances in high rainfall, temperate dairy pastures of south eastern Australia. In 'Proceedings of the XIX International Grasslands Congress, Sao Pedro, Brazil, February 2001'. pp. 169 - 170. (Brazilian Society of Animal Husbandry: Piracicaba, Brazil).

Eckard RJ, Chen D, White RE, Chapman DF (2003) Gaseous nitrogen loss from temperate perennial grass and clover dairy pastures in south-eastern Australia. *Australian Journal of Agriculture Research* **54**, 561 -570.

Eckard RJ, Cullen BR (2011) Impact of future climate scenarios on nitrous oxide emissions from pasture based dairy systems in south eastern Australia. *Animal Feed Science and Technology* **166-167**, 736 - 748.

Eckard R, Hegarty R, Thomas G (2011) Dairy Greenhouse Accounting Framework. Project No. UM10778.

Ellington A (1986) Nitrogen inputs and utilisation in leguminous pasture: a review of recent Australian literature. Department of Agriculture & Rural Affairs, Technical Report no. 128. AgDex 137/531. p. 19.

Ellis S, Yamulki S, Dixon E, Harrison R, Jarvis SC (1998) Denitrification and N_2O emissions from a UK pasture soil following the early spring application of cattle slurry and mineral fertiliser. *Plant and Soil* **202**, 15 - 25.

Exner ME (1994) Source identification of nitrate contamination in shallow groundwater in Nebraska, USA. In 'Water Down Under'. 21-25 November, Adelaide, Australia.

Fertilising Dairy Pastures Manual (2005) Nitrogenous Fertilisers, Section 12. Victorian Department of Primary Industries, Victoria State Government, Victoria.

Fillery IRP (2001) The fate of biologically fixed nitrogen in legume-based dryland farming systems: a review. *Australian Journal of Experimental Agriculture* **41** 361 - 381.

Firestone MK, Firestone RB, Tiedje JM (1980) Nitrous oxide from soil denitrification: factors controlling its biological production. *Science* **208**, 749 - 751.

Focht DD (1974) The effect of temperature, pH and aeration on the production of nitrous oxide and gaseous nitrogen - a zero-order kinetic model. *Soil Science* **118**, 173 - 179.

Focht DD, Verstraete W (1977) Biochemical ecology of nitrification and denitrification. In 'Advances in Microbial Ecology' (Ed. M. Alexander) pp. 135 - 214. (Plenum Press, New York).

Freney JR, Denmead OT, Simpson JR (1978) Soil as a source or sink for atmospheric nitrous oxide. *Nature* **273**, 530 - 532.

Freney JR, Chen DL, Mosier AR, Rochester IJ, Constable GA, Chalk PM (1993) Use of nitrification inhibitors to increase fertilizer nitrogen recovery and lint yield in cotton. *Fertiliser Research* **34**, 37 - 44.

Galbally I, Meyer M, Weeks I, Leuning R, Kelly K, Phillips F, Barker-Reid F, Gates W, Baigent R, Eckard R, Grace P (2005) A study of environmental and management drivers of non-CO_2 greenhouse gas emissions in Australian agro-ecosystems. *Environmental Sciences* 2, 133 - 142.

Goring CAI (1962) Control of nitrification by 2-chloro-6-(trichloromethyl) pyridine. *Soil Science* **93**, 211 - 218.

Gourley C, Gibson D, Smith A, Awty I and Dougherty W (2011) Accounting for Nutrients on Australian Dairy Farms. Final report.

Grant RF (2001) A Review of the Canadian Ecosystem Model ECOSYS. In (Eds. Shaffer, MJ, Ma L., Hansen S, pp 173-263, Lewis Publishers, Boca Raton.

Grace PR, Rowlings D, Rochester I (2010) Nitrous oxide emissions from irrigated cotton soils of northern Australia. In 'Proceedings 19th World Congress of Soil Science' (Eds. Gilkes RJ, Prakongkep N), IUSS, Brisbane.

Granli T, Bockman OC (1994) Nitrous oxide from agriculture. *Norwegian Journal of Agricultural Science* Supplement **12**, 1 - 128.

Halder K, Mosier A, Heinemeyer O (1986) The effect of growing plants on denitrification at high soil nitrate concentrations. *Soil Science Society of America Journal* **51**, 97 - 102.

Harper LA, Catchpoole VR, Davis R, Weier KL (1983) Ammonia volatilization, soil plant and microclimate effects on diurnal and seasonal fluctuations. *Agronomy Journal* **75**, 212 - 218.

Harper LA, De Visscher A, Weaver KH (2012) Anaerobic decomposition and conversion of animal waste nitrogen to nitrogen gas. *Journal of Environmental Quality* (In press).

Harper LA, Flesch TK, Weaver KH, Wilson JD (2010) The effect of biofuel production on swine farm methane and ammonia emissions. *Journal of Environmental Quality* **39**, 1984 - 1992.

Harper LA, Sharpe RR, Langdale GW, Giddens JE (1987) Nitrogen cycling in a wheat crop: Soil, plant, and aerial nitrogen transport. *Agronomy Journal* **79**, 965 - 973.

Harper LA, Sharpe RR (1995) Nitrogen dynamics in irrigated corn: soil-plant nitrogen and atmospheric ammonia support. *Agronomy Journal* **87**, 669 - 675.

Harper LA, Sharpe RR (1998) Ammonia emissions from swine waste lagoons in the Southeastern U.S. Coastal Plains. Final Grant Report to Division of Air Quality. North Carolina Department of Environment and Natural Resources, Raleigh, NC. USDA-ARS Agreement no. 58-6612-7M-022. North Carolina Dep. of Environment and Natural Resources, Raleigh, NC.

Harper LA, Sharpe RR, Parkin TB (2000) Gaseous nitrogen emissions from anaerobic swine lagoons: ammonia, nitrous oxide and dinitrogen gas. *Journal of Environmental Quality* **29**, 1356 - 1365.

Henniger NM, Bollag JM (1976) Effect of chemicals used as nitrification inhibitors on the denitrification process. *Canadian Journal of Microbiology* **22**, 668 - 672.

Hoeft RG (1984) Current status of nitrification inhibitor use in US agriculture. In 'Nitrogen in Crop Production' (Ed. Hauch RD) pp. 561 - 570, American Society of Agronomy, Madison, Wisconsin.

Hauck RD, Stephenson HF (1965) Nitrification of nitrogen fertilizers. Effect of nitrogen source, size and pH of the granule, and concentration. *Journal of Agriculture and Food Chemistry* **13**, 486 - 492.

Hutchinson GL, Davidson EA (1993) processes for production and consumption of gaseous nitrogen oxides in soil. In 'Agricultural ecosystem effects on trace gas and global climate change' (Ed's Peterson GAB, Luxmoore RJ) American Society of Agronomy, Madison.

Hutchinson GL, Mosier AR (1981) Improved soil cover method for field measurement of nitrous oxide fluxes. *Soil Science Society of America Journal* **45**, 311 - 316.

IPCC, 2007. Climate change 2007: the physical science basis. In 'Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change' (Ed's Solomon S, Gin D, Manning M, Chen Z, Marquis M, Averyt KB, Tignor M, Miller HL) p. 996, Cambridge University Press, Cambridge.

Jacobsen SN, Alexander M (1980) Nitrate loss from soil in relation to temperature, carbon source and denitrifier populations. *Soil Biology and Biochemistry* **12**, 501 – 505.

Johnson IR, Chapman DF, Snow VO, Eckard RJ, Parsons AJ, Lambert MG, Cullen BR (2008) DairyMod and EcoMod: biophysical pastoral simulation models for Australia and New Zealand. *Australian Journal of Experimental Agriculture* **48**, 621-631.

Jones JW, Hoogenboom G, Porter CH, Boote KJ, Batchelor WD, Hunt LA, Wilkens PW, Singh U, Gijsman AJ, Ritchie JT (2003). The DSSAT cropping system model. *European Journal of Agronomy*, **18**, 235 – 265.

Keating BA, Carberry PS, Hammer GL, Probert ME, Robertson MJ, Holzworth D, Huth NI, Hargreaves JNG, Meinke H, Hochman Z, McLean G, Verburg K, Snow V, Dimes JP, Silburn M, Wang E, Brown S, Bristow K, Asseng S, Chapman S, McCown RL, Freebairn DM, Smith CJ (2003) An overview of APSIM, a model designed for farming systems simulation, *European Journal of Agronomy* **18**, 267-288.

Kelly KB, Phillips FA, Baigent R (2008) Impact of dicyandiamide application on nitrous oxide emissions from urine patches in northern Victoria, Australia. *Australian Journal of Experimental Agriculture* **48**, 156 - 159.

Keeney DR, Fillery IR, Marx GP (1979) Effect of temperature on the gaseous nitrogen products of denitrification in a silt loam soil. *Soil Science Society of America Journal* **43**, 1124 - 1128.

Kester RA, Meijer ME, Libochant JA, de Boer W, Laanbroek HJ (1997) Contribution of nitrification and denitrification to the NO and N_2O emissions of an acid forest soil, a river sediment and a fertilised grassland soil. *Soil Biology and Biochemistry* **29**, 1655 - 1664.

Kiese R, Butterbach-Bahl K (2002) N_2O and CO_2 emissions from three different tropical forest sites in the wet tropics of Queensland, Australia. *Soil Biology and Biochemistry* **34**, 975 – 987

Klemdtsson L, Svensson BH, Rosswall T (1987) Dinitrogen and nitrous oxide produced by denitrification and nitrification in soil with and without barley plants. *Plant and Soil* **99**, 303 - 319.

Knowles R (1981) Denitrification. In 'Terrestrial Nitrogen Cycles' (Eds. Clark FE, Rosswall T) **33**, 315 - 329. (Ecological Bulletin, Stockholm).

Koskinen WC, Keeney DR (1982) Effect of pH on the rate of gaseous products of denitrification in a silt loam soil. *Soil Science Society of America Journal* **46**, 1165 - 1167.

Laville P, Jambert C, Cellier P, Delmas R (1999) Nitrous oxide fluxes from a fertilised maize crop using micrometeorological and chamber methods. *Agricultural and Forest Meteorology* **96**, 19 - 38.

Ledgard SF, Sprosen MS, Brier GJ, Nemaia EKK, Clark DA (1996) Nitrogen inputs and losses from New Zealand dairy farmlets, as affected by nitrogen fertilizer application: year one. *Plant and Soil* **18**, 65-69.

Ledgard, SF, Penno JW, Sprosen MS (1999) Nitrogen inputs and losses from clover/grass pastures grazed by dairy cows, as affected by nitrogen fertilizer application. *Journal of Agricultural Science* **132**, 215 – 225

Ledgard S, Schils R, Eriksen J, Luo J (2009) Environmental impacts of grazed clover/grass pastures. *Irish Journal of Agricultural and Food Research* **48,** 209 - 226.

Li C, Aber J, Stange F, Butterbach-Bahl K, Papen H (2000) A process-oriented model of N_2O and NO emissions from forest soils: 1. Model development. *Journal of Geophysical Research* **105**, 4369 - 4384

Li Y, Chen D, Barker-Reid F, Eckard RJ (2008). Simulation of N_2O emissions from rain-fed wheat and the impact of climate variation in southeastern Australia. *Plant and Soil* **309**, 239 - 251.

Linn DM, Doran J (1984) Effect of water-filled pore space on carbon dioxide and nitrous oxide production in tilled and non-tilled soils. *Soil Science Society of America Journal* **48**, 1267 - 1272.

Luo J, Tillman RW, Ball PR (1999) Grazing effects on denitrification in a soil under pasture during two contrasting seasons. *Soil Biology and Biochemistry* **31**, 903 - 912.

Luo J, Tillman RW, Ball PR (2000) Nitrogen loss through denitrification in a soil under pasture in New Zealand. *Soil Biology and Biochemistry* **32**, 497 - 509.

Maag M, Vinther FP (1996) Nitrous oxide emission by nitrification and denitrification in different soil types and at different soil moisture contents and temperatures. *Applied Soil Ecology* **4**, 5 - 14.

McCormick RA, Nelson DW, Sutton AL, Huber DM (1983) Effect of nitrapyrin in nitrogen transformations in soil treated with liquid swine manure. *Agronomy Journal* **75**, 947 - 950.

Malhi SS, McGill WB, Nyborg M (1990) Nitrate losses in soils: Effect of temperature, moisture and substrate concentration. *Soil Biology and Biochemistry* **22**, 733 -737.

Malone JP, Stevens RJ, Laughlin RJ (1998) Combining the 15N and acetylene inhibition techniques to examine the effect of acetylene on denitrification. *Soil Biology and Biochemistry* **30**, 31 - 37.

Mathieu G, Leveque J, Henault C, Milloux M-J, Bizouard F, Andreux F (2006) Emission and spatial variability of N_2O, N_2 and nitrous oxide mole fraction at the field scale, revealed with ^{15}N isotopic techniques. *Soil Biology and Biochemistry* **38**, 941 - 951.

Menner JC, Ledgard S, McLay C, Silvester W (2005) Animal treading stimulates denitrification in soil under pasture. *Soil Biology and Biochemistry* **37**, 1625 - 1629.

Monaghan RM, Barraclough D (1993) Nitrous oxide and dinitrogen emissions from urine-affected soil under controlled conditions. *Plant and Soil* **151**, 127- 138.

Moraghan JT, Buresh RJ (1977) Chemical reduction of nitrite and nitrous oxide. *Soil Science Society of America Journal* **41**, 47 - 50.

Mosier AR, Schimel DS (1993) Nitrification and Denitrification. In 'Nitrogen Isotope Techniques' (Ed's Knowles R, Blackburn TH) pp. 181 - 208, Academic Press Inc., San Diego.

Mosier AR, Duxbury JM, Freney JR, Heinemeyer O, Minami K (1996) Nitrous oxide emissions from agricultural fields: assessment, measurement and mitigation. *Plant and Soil* **181**, 95 - 108.

Munoz GR, Powell JM, Kelling KA (2003) Nitrogen budget and soil N dynamics after multiple applications of unlabelled or ^{15}Nitrogen-enriched dairy manure. *Soil Science Society of America Journal* **67**, 817 - 825.

Pakrou N, Dillon P, Stanger G (1997) Impact of pastoral land use on groundwater quality. Final Report LWRRDC Project No. CCW9, Water Industry Research Award. Centre for Groundwater Studies Report no. 64, South Australia.

Parkin TB, Sexstone AJ, Tiedje JM (1985) Comparison of field denitrification rates determined by acetylene-based soil core and nitrogen-15 methods. *Soil Science Society of America Journal* **49**, 94 - 99.

Parkin TB (1987) Soil microsites as a source of denitrification variability. *Soil Science Society of America Journal* **51**, 1194 - 1199.

Paul JW, Ziebarth BJ (1997) Denitrification and nitrate leaching during the fall and winter following dairy cattle slurry application. *Canadian Journal of Soil Science* **77**, 231 - 240.

Peoples MB, Mosier AR, Freney JR (1995) Minimizing gaseous loss of nitrogen. In 'Nitrogen Fertilizers in the Environment' (Ed. Bacon PE) pp. 565 - 602, Marcel Dekker Inc., New York.

Phillips FA, Leuning R, Baigenta R, Kelly KB, Denmead OT (2007) Nitrous oxide flux measurements from an intensively managed irrigated pasture using micrometeorological techniques. *Agricultural and Forest Meteorology* **143**, 92 - 105.

Powlson DS, Saffigna PG, Monique Kragt- Cottaar (1988) Denitrification at sub-optimal temperatures in soils from different climatic zones. *Soil Biology and Biochemistry* **20**, 719 - 723.

Prasertak P, Freney JR, Denmead OT, Saffigna PG, Prove BG (2001) Significance of gaseous nitrogen loss from a tropical dairy pasture fertilised with urea. *Australian Journal of Experimental Agriculture* **41**, 625 - 632.

Rochester IJ (2003) Estimating nitrous oxide emissions from flood-irrigated alkaline grey clays. *Australian Journal of Soil Research* **41**, 197 - 206.

Rowlings DW (2010) Influence of land use change on the biosphere-atmosphere-exchange of C and N trace gases in the humid, subtropical region of Queensland. PhD Thesis, Queensland University of Technology, Brisbane.

Rowlings DW, Grace PR, Kiese R, Weier, KL (2012) Environmental factors controlling temporal and spatial variability in the soil-atmosphere exchange of CO_2, CH_4 and N_2O from an Australian subtropical rainforest. *Global Change Biology* **18,** 726 - 738.

Rowlings D, Grace, PR, Kiese R, Scheer C (2010) Quantifying N_2O and CO_2 emissions from a subtropical pasture. In 'Proceedings 19th World Congress of Soil Science' (Eds. Gilkes RJ, Prakongkep N), IUSS, Brisbane.

Ruser R, Fiessa H, Russow R, Schmidt G, Buegger F, Munch JC (2006) Emission of N_2O, N_2 and CO_2 from soil fertilized with nitrate: effect of compaction, soil moisture and rewetting. *Soil Biology and Biochemistry* **38**, 263 - 274.

Ryden JC, Skinner JH, Nixon DJ (1987) Soil core incubation system for the field measurement of denitrification using acetylene-inhibition. *Soil Biology and Biochemistry* **19**, 753 - 757.

Saggar S, Giltrap DL, Li C, Tate K (2007) Modelling nitrous oxide emissions from grazed grasslands in New Zealand. *Agriculture, Ecosystems and Environment* **119**, 205 - 221.

Scheer C, Grace PR, Rowlings D (2012) Nitrous oxide emissions from irrigated wheat in Australia: Impact of irrigation management. *Plant and Soil* (published online).

Scheer C, Grace PR, Rowlings D, Kimber S, Van Zwieten L (2010) Greenhouse gas emissions from intensive pasture on ferrosol in Northern NSW, Australia: Impact of biochar amendment. In 'Proceedings 19th World Congress of Soil Science' (Eds. Gilkes RJ, Prakongkep N), IUSS, Brisbane.

Scheer C, Grace P, Rowlings D, Kimber S, Van Zwieten L (2011) Effect of biochar amendment on the soil-atmosphere exchange of greenhouse gases from an intensive subtropical pasture in northern New South Wales, Australia. *Plant and Soil* **345**, 47 - 58.

Scheer C, Wassmann R, Butterbach-Bahl K, Lamers J and Martius C (2009) The relationship between N_2O, NO, and N_2 fluxes from fertilized and irrigated dryland soils of the Aral Sea Basin, Uzbekistan. Plant and Soil **314**, 273 - 283.

Smith MS, Tiedje JM (1979) The effect of roots on soil denitrification. *Soil Science Society of America Journal* **43**, 951 - 955.

Smith CJ, Chalk PM (1980) Gaseous nitrogen evolution during nitrification of ammonia fertilizer and nitrate transformations in soils. *Soil Science Society of America Journal* **44**, 277 - 282.

Smith, KA (1990) Greenhouse gas fluxes between land surfaces and the atmosphere. *Progress in Physical Geography* **14**, 349 - 372.

Smith KA, Clayton H, Arah JRM, Christensen S, Ambus P, Fowler D, Hargreaves KJ, Skiba U, Harris GW, Wienhold FG, Klemedtsson L, Galle B (1994) Micrometeorological and chamber methods for measurement of nitrous oxide fluxes between soils and the atmosphere: overview and conclusions. *Journal of Geophysical Research* **99**, 16541 - 16548.

Snow VO, Johnson IR, Parsons AJ (2009) The single heterogeneous paddock approach to modelling the effects of urine patches on production and leaching in grazed pastures. *Crop and Pasture Science* **60**, 691-696.

Stenger R, Barkle G, Burgess C, Wall A, Clague, J (2008) Low nitrate contamination of shallow groundwater in spite of intensive dairying: the effect of reducing conditions in the vadose zone-aquifer continuum. *Journal of Hydrology (Wellington North*) **47**, 1 - 24.

Stevens RJ, Laughlin RJ (2002) Cattle slurry, applied before fertilizer nitrate, lowers nitrous oxide and dinitrogen emissions. *Soil Science Society of America Journal* **66**, 647 - 652.

Strous M, van Gerven E, Zheng P, Kuenen JG, Jetten MSM (1997) Ammonium removal from concentrated waste streams with the anaerobic ammonium oxidation (Anammox) process in different reactor configurations. *Water Research* **31**, 1955 - 1962.

Suter H, Chen D, Huilin L, Edis R, Walker C (2010a) Comparison of the ability of the nitrification inhibitors DCD and DMPP to reduce nitrification and N_2O emissions from nitrogen fertilisers. In 'Proceedings 19th World Congress of Soil Science' (Ed's Gilkes RJ, Prakongkep N) Brisbane, pp. 24 - 27.

Suter H, Chen D, Huilin L, Edis R, Walker C (2010b) Reducing N_2O emissions from nitrogen fertilisers with the nitrification inhibitor DMPP. In 'Proceedings 19th World Congress of Soil Science' (Ed's Gilkes RJ, Prakongkep N), Brisbane pp. 205 - 207.

Thorburn PJ, Biggs JS, Weier KL, Keating BA (2003) Nitrate in groundwaters of intensive agricultural areas in coastal Northeastern Australia. *Agriculture, Ecosystems & Environment* **94**, 49 - 58.

Thorburn PJ, Biggs JS, Collins K, Probert ME (2010) Using the APSIM model to estimate nitrous oxide emissions from diverse Australian sugarcane production system. *Agriculture, Ecosystems and Environment* **136**, 343-350.

Vallejo A, Diez JA, Lopez-Valdivia LM, Gasco A, Jimenez C (2001) Nitrous oxide emission and denitrification nitrogen losses from soils treated with isobutylenediurea and urea plus dicyandiamide. *Biology and Fertility of Soils* **34**, 248 - 257.

Vallis I, Catchpoole VR, Hughes RM, Myers RJK, Ridge DR, Weier KL (1996) Recovery in plants and soils of ^{15}N applied as subsurface bands of urea to sugarcane. *Australian Journal of Agricultural Research* **47**, 355 - 370.

van Cleemput O (1998) Subsoils: chemo-and biological denitrification, N_2O and N_2 emissions. *Nutrient Cycling in Agroecosystems* **52**, 187 - 194.

van der Salm C, Dolfing J, Heinen M, Velthof GL (2007) Estimation of nitrogen losses via denitrification from a heavy clay soil under grass. *Agriculture, Ecosystems & Environment* **119**, 311 - 319.

van der Weerden TJ, Sherlock RR, Williams PH, Cameron KC (1999) Nitrous oxide emissions and methane oxidation by soil following cultivation of two different leguminous pastures. *Biology and Fertility of Soils* **30**, 52 - 60.

Wagner-Riddle C, Thurtell GW, King KM, Kidd GE, Beauchamp EG (1996) Nitrous oxide fluxes and carbon dioxide fluxes from a bare soil using a micrometeorological approach. *Journal of Environmental Quality* **25**, 898 - 907.

Walter HM, Keeney DR, Fillery IRP (1979) Inhibition of nitrification by acetylene. *Soil Science Society of America Journal* **43**, 195 - 196.

Wang J, Cardenas LM, Misselbrook TH, Cuttle S, Thorman RE (2012) Modelling nitrous oxide emissions from grazed grassland systems. *Environmental Pollution* **162**, 223-233.

Watkins NL (2007) The ability of nitrification inhibitors to decrease denitrification rates in dairy farm soils. Thesis 'Master of Science in Earth and Ocean Sciences', The University of Waikato, New Zealand.

Weaver KH, Harper LA (2012) Effects on carbon and nitrogen emissions due to swine manure removal for biofuel production. *Journal of Environmental Quality* (In press).

Weier KL, Gilliam JW (1986) Effect of acidity on denitrification and nitrous oxide evolution from Atlantic coastal plain soils. *Soil Science Society of America Journal* **50,** 1202 - 1205.

Weier KL, MacRae IC, Myers RJK (1991) Seasonal variation in denitrification in a clay soil under a cultivated crop and a permanent pasture. *Soil Biology and Biochemistry* **23**, 629 - 635.

Weier KL, MacRae IC, Myers, RJK (1993a) Denitrification in a clay soil under pasture and annual crop: losses from ^{15}N-labelled nitrate in the subsoil in the field using C_2H_2 inhibition. *Soil Biology and Biochemistry* **25**, 999 - 1004.

Weier KL, Doran, JW, Power, JF, Walters, DT (1993b) Denitrification and the dinitrogen/nitrous oxide ratio as affected by soil water, available carbon and nitrate. *Soil Science Society of America Journal* **57**, 66 - 72.

Weier KL, Doran JW, Mosier AR, Power JF, Peterson TA (1994) Potential for bioremediation of high nitrate irrigation water via denitrification. *Journal of Environmental Quality* **23**, 105 - 110.

Weier KL, McEwan CW, Vallis I, Catchpoole VR, Myers RJK (1996) Potential for biological denitrification of nitrogen in sugarcane soils. *Australian Journal of Agricultural Research* **47**, 67 - 79.

Weier KL, Rolston DE, Thorburn PJ (1998) The potential for N losses via denitrification beneath a green cane trash blanket. In 'Proceedings of the Australian Society of Sugar Cane Technologists' **20**, 169 - 175.

Weier, KL (1999) N_2O and CH_4 emission and CH_4 consumption in a sugarcane soil after variation in nitrogen and water application. *Soil Biology and Biochemistry* **31**, 1931 - 1941.

Whitehead DC (1995) Grassland Nitrogen. CAB International, Wallingford, UK.