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Technical Report

Validating the cost/benefits of improved fertiliser practices and
quantifying nutrient loads and pathways from irrigated dairy
pastures in the Wet Tropics and the Burnett-Mary regions
RRRD055



David Rowlings, Jack Koci, Paul N Nelson and Rick Kowitz

Validating the cost/benefits of improved fertiliser practices and quantifying nutrient loads and pathways from irrigated dairy pastures in the Wet Tropics and the Burnett-Mary regions (Project RRRD055)

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Acronyms

DoE	Department of the Environment
GBR	Great Barrier Reef
GBRMPA	Great Barrier Reef Marine Park Authority
NRM	Natural Resource Management
QDAFF	Queensland Department of Agriculture, Fisheries and Forestry
RRRC	Reef and Rainforest Research Centre Limited
RRRD	Reef Rescue Research & Development

Abbreviations

DMPP	3, 4-dimethylpyrazole phosphate
ANOVA	Analysis of Variance
BOM	Bureau of Meteorology
CEC	Cation exchange capacity
ET_c	Crop evapotranspiration
DM	Dry matter
ET	Evapotranspiration
NI	Nitrification inhibitor
DON	Disolved organic Nitrogen
N	Nitrogen
N₂O	Nitrous oxide
N₂	Dinitrogen
NO₃⁻	Nitrate
NH₄⁺	Ammonium
C	Carbon
P	Phosphorus
PO₄	Phosphate
CP	Crude protein
ET₀	Reference evapotranspiration
K_s	Water stress coefficient

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Executive Summary

Background

In developing this project, the dairy industry identified a number of key priorities where further research and development would assist the industry to improve water quality outcomes for the Great Barrier Reef Lagoon. These included the following:

- Improved quantification of nutrient inputs and outputs at a whole-of-farm and paddock scale to improve nutrient use efficiency and reduce risks of nutrient loss from dairy farms.
- Regional evaluation of nitrogen stabilisation, modified fertiliser products, and delivery systems (e.g. slow release fertilisers, nitrogen (N) stabilisation and liquid nitrogen).
- Quantifying the costs and benefits of specific practices in relation to water quality outcomes and improved nutrient use efficiency.
- Development of a practical tool to assist on-farm soil nutrient management decisions, and to identify nutrient balancing issues and opportunities for improving nutrient use efficiency and identifying potential risks.
- Determining the level to which Farm Management System (FMS) practices would deliver on NRM Water Quality targets.

Through a strategic partnership between the Australian Government's Caring for Our Country Reef Rescue program, Queensland Dairyfarmers' Organisation, Queensland University of Technology, James Cook University, and Incitec Pivot Fertilisers, it was possible to combine a number of these research priorities into one project.

The aim of these investigations was to determine the cost/benefit of using urea treated with a nitrification inhibitor compared to using standard urea in terms of reduced nitrogen losses through leaching and runoff, and the potential production efficiency gains. The contribution of Incitec Pivot Fertilisers made it possible to value-add to the water quality research with an investigation of gaseous losses of nitrogen, therefore completing the nitrogen loss pathways from dairy pastures.

Until now there has been little quantification of N loss from fertiliser application on dairy farms in tropical and sub-tropical Queensland, and the management practices that might reduce these losses. Such research is vital, given the proximity of farming systems to sensitive environmental areas, such as the Great Barrier Reef World Heritage Area, and the increasing economic constraints on dairy production in the tropics.

This research was conducted on two commercial irrigated dairy farms, situated at Gympie and Ravenshoe. Management practices (e.g. grazing, fertiliser application and irrigation) were performed as per normal farming operations and are common to many dairy production systems in the Great Barrier Reef catchments.

Key findings

This study investigated the effect of fertiliser application rate and a nitrification inhibitor (DMPP) on N losses and pasture growth, on two dairy farms within the Australian tropics. The experiments were conducted on commercially operating dairy farms near Ravenshoe in

North Queensland, and Gympie in southern Queensland, where all management practices (e.g. grazing, fertiliser application and irrigation), were performed as per normal practice. While undertaking such a field trial presented several challenges, the results show promising avenues for improving N use efficiency and decreasing losses of N to the environment.

While it was not possible to accurately allocate losses to particular mechanisms, N losses at the Ravenshoe Red Ferrosol appeared to be mainly as a result of deep drainage, whereas runoff and denitrification dominated losses in the heavier textured Gympie soil. At the Gympie site, the annual nitrogen use budget was dominated by the unusual climatic conditions rather than any potential management decisions. Optimal pasture growth was limited in what is traditionally the prime growing season by the extended and unseasonal dry period from August to mid-January, which accentuated losses from the extreme rain event at the end of January and skewed nutrient losses substantially towards runoff. In such extreme climatic events there is little that good management can achieve that will alleviate such losses. However improved nitrogen use efficiency (NUE) can still be achieved during the drier, cooler fertilisation period by the closer matching of N fertiliser application to plant N demand.

A clear response to fertiliser rate, and the effectiveness of the DMPP inhibitor were demonstrated during the fertilisation period across a range of parameters (yield, N removed, NUE), though N₂O losses were unaffected at Ravenshoe. Pasture yield was almost as high in the low N + DMPP treatment as it was in the high N treatment. NUE and the effect of DMPP was substantially improved at the lower rate of N application suggesting that N was not the limiting factor for production, and indeed soil water was in deficit for long periods of the experiment at both sites. As such reducing N application during periods of high water deficit is an effective strategy for improving NUE.

The results indicate that fertiliser N inputs can be reduced from the standard commercial rates with no economic or yield penalty when urea is used in conjunction with DMPP. Halving the fertiliser rate when an inhibitor was used, reduced the amount of N potentially lost to the environment by more than half. DMPP did not significantly reduce N₂O emissions at either site, though a trend towards lower emissions was observed at Gympie. In the low N treatments most of the N was utilised by the pasture. In the high N rate treatments, however, there were large losses of N that were unaccounted for possibly through denitrification to N₂.

The following key recommendations can be drawn from this research:

1. The increase in pasture crude protein (CP) content associated with DMPP application suggests that farmers can apply urea at lower rates to achieve up to 19% CP. In this way, less N is applied to maintain the same level of production by dairy cows. The cost of DMPP is approximately 20% more than conventional Urea so this additional cost needs to be accounted.
2. Management of N fertiliser application rates according to soil moisture deficits, irrigation availability and predicted precipitation could substantially reduce N losses. This allows for a closer matching of plant N demand to fertiliser supply. In periods of adequate to excess soil moisture, the use of the DMPP inhibitor increases NUE efficiency.

3. Before the observed improvements in agronomic NUE lead to higher overall farm NUE, cows grazing pasture with higher CP may require rumen fermentable carbohydrate (RFC) supplements. Feeding RFC supplements, in combination with increased stocking rates, provides an opportunity for harnessing agronomic gains in NUE and increasing milk production.
4. The higher pasture N concentration can be balanced by supplementing cow feed with fCHO in combination with an increased stocking rate. This approach to harnessing improvements in agronomic NUE leaves the rate of N application unchanged but increases farm production.

Benefits and application of the work

At both sites there was a significant increase in biomass with DMPP applied at half the industry standard rate compared to urea at the same rate. At Gympie the yield was 20% higher than in the urea only treatment and similar to that observed from the higher N rate treatments. At Ravenshoe the biomass from the lower rate when combined with DMPP was greater than the higher N rate treatments.

These findings will be of most interest for farmers looking to maximize their productivity at the lowest possible cost. The use of lower rates of urea nitrogen fertiliser should also reduce the potential risk of losses via other pathways, including leaching and runoff.

Dairy farmers and service providers are already benefiting from the research through the extension of these findings through field days, fact sheets and dairy industry publications. Industry recommended practices and priorities for future investment will be reviewed when the research results are conclusive.

Future directions

This research has contributed to our understanding of nutrient pathways in irrigated tropical and subtropical dairy pastures, and will help to quantify the amounts of nitrogen and phosphorus fertiliser lost through the various pathways. The research has also measured the value of the DMPP inhibitor in reducing losses. However, it is recognised that these findings are highly dependent on seasonal conditions. Differences in some results for the two sites will provide additional insights into the climate, soil and management factors affecting N loss from dairy systems and in developing recommendations for good practice to improve NUE in tropical and subtropical dairy farms.

Now that the methodology is well established, there would be considerable value to the dairy industry in continuing this research over a longer period of time to understand the impacts of seasonal variability on these investigations, and to measure the effect of using additional improved practices and technology to further reduce losses and improve fertiliser efficiency.

There is an additional need to examine animal management practices that allow farmers to harness improvements in agronomic NUE derived from optimised fertiliser management. Factors such as pasture utilisation rates and ruminant N nutrition also need to be considered in a holistic approach to dairy N management.

Introduction

Dairy production is one of rural Australia's major industries, with annual farmgate production of \$3.9 billion. Dairy also represents one of the most intensive and nitrogen (N) loaded production systems in the high rainfall regions of tropical and subtropical Queensland. Application rates of up to 400 kg N ha⁻¹ year⁻¹ ensure year round production is maintained. Farm level surpluses of 250 kg of N per year have been documented. High fertiliser costs and variable commodity prices have increased economic constraints on production; increasing the need for improved N use efficiency (NUE). This project investigated the fate of applied N fertiliser to irrigated dairy farms, with emphasis on improving NUE to reduce losses to environmental areas and maintain farm profitability.

The balance between N inputs to the soil-plant-animal system and outputs in produce and N losses is a major factor determining the productivity of grazed pastures (Scholefield and Oenema, 1997; Pakrou and Dillon, 2000). In many grazed pasture systems, there often exists an imbalance between N inputs and outputs. The increasing use of N fertilisers, in excess of the pasture requirement, is a major factor responsible for this imbalance. Within Australia, improved pastures are the third largest consumer of N fertiliser (7%) after cereal (66%) and sugarcane crops (9%; Chudleigh and Simpson, 2001). The NUE of Australian pasture-based dairy farms ranges between 14-50% with a median of 25% (Gourley *et al.*, 2012), the latter marginally lower than those of 33% and 30% recorded for cereal production (Raun and Johnson, 1999) and sugarcane (Basanta *et al.*, 2003; Prasertsak *et al.*, 2002) respectively. Therefore the dairy industry represents a major opportunity for reducing environmental N losses, including run-off and leaching losses to sensitive water catchments and coastal lagoons adjacent to the Great Barrier Reef World Heritage Area (Brodie *et al.*, 2008; Thorburn *et al.*, 2011). Given the low N-use efficiency of the dairy industry, such reductions will also enhance the economics of production.

Loss of N originating from fertiliser applied to agricultural land is a globally significant social, environmental, ecological and economic issue (Di and Cameron 2002a; Eickhout *et al.* 2006; Mosier and Kroeze 2000; Spiertz 2009). Groundwater contamination, arising from leaching of NO₃⁻ past the root zone, poses a serious health threat to populations reliant on groundwater as a drinking source (Singh and Sekhon, 1979; Thorburn *et al.*, 2003; Rivett *et al.*, 2008). NO₃⁻ carried by overland runoff (Ng Kee Kwong *et al.*, 2002; Udawatta *et al.*, 2006) and groundwater flow (Wriedt *et al.*, 2007) can detrimentally affect aquatic ecosystems through a decline in water quality and eutrophication (Hessen *et al.*, 1997; Howarth, 2008). Gaseous losses of N as NH₃ and N₂O contribute to acid rain, ozone depletion and global warming (Bolan *et al.*, 2004; Robertson and Vitousek, 2009). From an economic perspective, the loss of N adds a substantial financial cost to primary producers (Oenema and Pietrzak, 2002; Chen *et al.*, 2008; Arriaga *et al.*, 2009; Cichota and Snow, 2012).

In general, the focus of management practices to improve NUE is to increase the pasture's ability to compete with N loss processes. One relatively simple management practice is to match the rate and timing of fertiliser application to pasture requirement. It is common practice however, for farmers to apply N fertiliser in excess of crop requirements, to ensure N requirements are being met (Sommer *et al.*, 2004; Weier and Grace, 2012). Further, it is a common assumption that the more N fertiliser applied, the greater the pasture production,

which will lead, for example in dairy systems, to higher milk production, and inevitably a greater profit (Staines *et al.*, 2011). However there are numerous soil, plant and animal biophysical constraints to this simplistic approach which are explained in detail in the appendixes. Rate response trials specific to soil type and environmental conditions provide the only means of determining optimal fertiliser application rates.

Another management practice which is gaining increasing recognition as an effective way of improving fertiliser NUE, is the use of enhanced efficiency fertilisers. These can take the form of a physical barrier such as a slow release granule coating or more targeted chemicals that affect key enzymes in N loss mechanisms. A relatively new enhanced efficiency fertiliser on the commercial market is nitrification inhibitor 3, 4-dimethylpyrazole phosphate (DMPP, commercial name ENTEC®). DMPP is a chemical compound that slows down the oxidation of ammonium (NH_4^+) to NO_2^- in the soil by inhibiting the activities of *Nitrosomas* bacteria (Prasad and Power, 1995; Zerulla *et al.*, 2001; Chien *et al.*, 2009). Early studies into the use of DMPP across a range of agricultural systems are promising and the major advantage DMPP offers over other commercially available products is that only low concentrations of the active compound are required to inhibit nitrification (Zerulla *et al.*, 2001). DMPP has been shown to retain N in the root zone for a longer period, providing more time for plant uptake, and reducing N loss (Moir *et al.*, 2007; O'Connor *et al.*, 2012; Subbarao *et al.*, 2012). The effectiveness and duration of this action however depends on climatic conditions, site characteristics and the agricultural crop (Zerulla *et al.*, 2001; Barth *et al.*, 2008; Chen *et al.*, 2010).

The key objective of the project was to quantify N and P losses from conventionally fertilised tropical dairy pastures and to investigate the potential of mitigating these losses. Two mitigation strategies were examined, the reduced N application rates and the use of the nitrification inhibitor DMPP.

Rationale

The rationale behind this study is based on the following premises:

- While NO_3^- leaching and N_2O emission following fertiliser application to grazed pastures have been extensively studied in temperate regions, relatively few studies have been conducted in tropical or sub-tropical environments.
- While the use of DMPP has been extensively studied in temperate regions, relatively little research has been conducted in the tropical or sub-tropical environments.
- While dairy pastures are a relatively minor land use in the Wet Tropics and Gympie region, the amounts of N fertiliser used are large, so reduction in losses could have an effect disproportionate to the size of the industry.
- The proximity of farming systems in north-east Queensland to sensitive environmental areas, such as the Great Barrier Reef World Heritage Area, and the poor quality of some surface and groundwater in the region, necessitates research into farming practices to reduce N losses in water.
- High fertiliser costs and low milk prices have increased economic constraints on dairy production in the tropics, increasing the need for improved N fertiliser use efficiency (Grimshaw, 1995).

Project Objectives

The objectives of the project were to:

1. Determine if nitrogen leaching losses from dairy pastures can be reduced if conventional urea fertiliser applications are combined with a nitrification inhibitor.
2. Determine if run-off losses of sediment, nitrogen (N) and phosphorus (P) can be reduced if conventional urea fertiliser applications are combined with a nitrification inhibitor.
3. Assess the cost/benefits of using urea treated with a nitrification inhibitor compared to standard urea in terms of both water quality benefits and farm productivity.
4. Extend learnings from the on-farm research to the wider industry through field days and publications.
5. Review/modify the industry current recommended practices as a result of the findings, and to determine priorities for investment.

In summary, the key goal of the research was to quantify N and P losses from a conventionally fertilised tropical and subtropical dairy pastures and to investigate the potential for mitigating these losses. The research focussed on two mitigation strategies – reduced nitrogen application rates using conventional urea fertiliser and the use of the urea coated with the nitrification inhibitor, DMPP.

Methods

Study sites

The trials for this project were set up on two contrasting soil types: (1) a deep Red Ferrosol at Ravenshoe on the Atherton Tablelands, Far North Queensland; and (2) a Red Dermosol at Gympie in south-east Queensland. Treatments were coordinated over the two sites with experimental design and measurement protocols modified to fit with dairy farm management practices and climate characteristic of each site. The consistent focus across the two sites was testing the impact of rate of fertiliser application and the impact of a nitrification inhibitor on N losses. Treatments at both sites therefore included two levels of urea fertiliser – a high rate consistent with industry standard; and a low rate equivalent to half industry standard – and applications with and without the nitrification inhibitor, DMPP. Measurements included rainfall and temperature, run-off, leaching, soil moisture and pasture yields. Differences in the experiments reflected farm management practices, measurement equipment and seasonal variations and while these made interpretation of results somewhat more complex, consistent trends were seen. Further, understanding reasons for any variations in results provided additional insights into the variability in response and the level of confidence in extrapolating results from the trials more broadly to tropical and sub-tropical dairy systems.

For clarity, the experimental design and results are presented separately for the Ravenshoe site and the Gympie site, with interpretation of the findings and implications for improved management of N fertiliser on northern Australian dairy farms integrated in the discussion and conclusion sections.

Ravenshoe site, far north Queensland

Location, climate and soils

The study site was located on the RASCHODA dairy farm at Ravenshoe State School (17°36'35"S, 145°29'27"E) on the edge of the township of Ravenshoe. Ravenshoe is situated on the southern Atherton Tablelands of Far North Queensland, at an elevation of 912 m above sea level. This area falls within the Wet Tropics bioregion. The dairy industry on the Tablelands occurs south of Kairi, mainly within the Malanda, Milla Milla and Ravenshoe districts and includes approximately 60 farms (Ruth Chalk, pers. comm.).

Ravenshoe has a subtropical climate, with hot, humid summers and mild, relatively dry winters. Rainfall is distinctly seasonal, with more than 70% falling in the summer months of December to March. The winter months, May through October, are considerably drier, receiving less than a quarter of total annual rainfall. Mean annual rainfall at Ravenshoe is 1228 mm (Table 1). Long term temperature records for Ravenshoe are not available, however a 24-year (1973-1997) record for nearby Koombooloomba Dam (26 km southwest of Ravenshoe, elevation 760 m) show mean daily maximum and minimum temperatures of 27.9°C to 19.1°C respectively in the warmest month of January, and 19.5 °C to 11.5 °C respectively in the coldest month of July (Figure 1). Light frosts can be expected 3-4 times per year, with more severe frosts occurring on average once every three years (Heiner and Grundy, 1994).

Table 1: Annual and monthly rainfall statistics (mm) for Ravenshoe (1928-2011). Source: BOM, 2012.

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Annual
Mean	223.9	284.3	240.8	90.3	56.6	38.1	25.2	21.8	19.3	28.0	75.7	124.0	1228.0
Minimum	17.0	16.0	14.0	1.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	10.1	58.1
Maximum	1290.0	948.0	816.7	332.0	174.6	129.5	83.9	103.1	145.0	150.0	250.4	429.8	4853.0

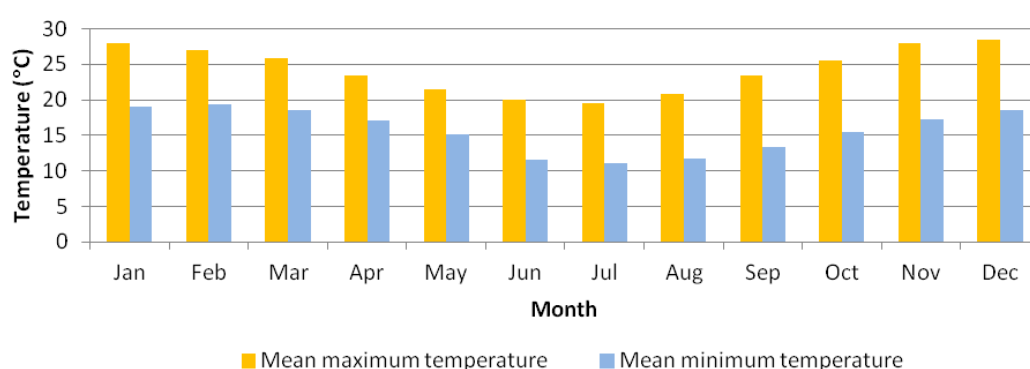


Figure 1: Mean daily minimum and maximum temperatures for Koombooloomba Dam (1973 – 1997).

The geology of the area is predominantly comprised of the Atherton Basalts, part of the Atherton Basalt Province (Best, 1962). It is dominated by sub aerial basaltic lava flows, of Pliocene-Quaternary age. A combination of varying climate, different geologies and large scale *volcanic* activity have shaped the geomorphology of the Tablelands (Malcom *et al.*,

1999). The landforms around Ravenshoe would generally be classified as low and high hills with slopes ranging from undulating to steep (Heiner and Grundy, 1994). Climate, particularly rainfall and temperature, have exerted a major influence on the soil development processes in the area (Heiner and Grundy, 1994).

The soil at the study site is classified as Pin Gin series (Malcom *et al.*, 1999). The Pin Gin series is a Red Ferrosol, formed through the extensive weathering of older basalt flows. Soil profiles are generally very deep and well drained, consisting of pedal dark or red clay A horizons, over pedal red clay B2 horizon. The soil reaction trend is acid (Malcom *et al.*, 1999).

The study site drains into North Cedar Creek, which flows into the Millstream, a major tributary of the Herbert River. The Herbert River flows for over 340 km in a generally south-easterly direction before entering the Coral Sea, and the Great Barrier Reef Marine Park, near Lucinda. North Cedar Creek provides the main source of irrigation water for the farm.

River flow is suggested to be derived mostly from groundwater, with large amounts of surface runoff generated only during extreme events (Cook *et al.*, 2001). The aquifers underlying the study site could be described as generally unconfined, layered systems, containing several basalt flows separated by palaeo-weathering surfaces and minor alluvial gravels of palaeo-drainage channels (Locsey, 2004). They are characterised by low total dissolved solids, relatively high recharge rates, high horizontal flow rates and short water residences times (Cook *et al.*, 2001). Cook *et al.* (2001) estimated the age of the groundwater's to range from 5 to 30 years, based on CFC-11 (chlorofluorocarbon) dating methods.

Experimental design

The study was designed as a 2x2 factorial experiment (i.e. 4 treatments) with blocks. One factor was urea (46% N, 0% P, 0% K) application rate, with two levels; a high rate which is the industry standard of 'one bag of urea per acre', equivalent to 124 kg urea ha⁻¹ application⁻¹ or 57 kg N ha⁻¹ application⁻¹, and a low rate which is half the industry standard (62 kg urea ha⁻¹ application⁻¹ or 28 kg N ha⁻¹ application⁻¹). Urea is applied during the dry season, following each grazing event. There are typically around 8 applications per year, resulting in an annual fertiliser application rate of 500 kg N ha⁻¹ at the industry standard rate. The other factor was the addition of the nitrification inhibitor, DMPP, with the treatment levels being with or without. DMPP was supplied by Incitec Pivot (marketed as Urea with ENTEC®), and came pre-formulated on urea granules at a rate of 4.86 g DMPP per kg of urea. Each treatment had four replicate plots, giving 16 plots in total (Figure 2). The design was partially randomised within blocks but was not completely randomised, to facilitate spreading of fertiliser using commercial equipment.

The whole trial was set up in two locations; the 'Tropical Trial' was located in paddocks dominated by tropical grass species including setaria (*Setaria sphacelata*) and kikuyu (*Pennisetum clandestinum*) and the 'Ryegrass Trial' was in paddocks having a mixture of tropical species and ryegrass (*Lolium spp.*) (Figure 3). These two trials were identical in design except that the Tropical Trial took up 4 paddocks whereas the Ryegrass Trial took up two paddocks.



Figure 2: Experimental layout of the Tropical Trial (upper) and Ryegrass Trial (lower). Note that symbols are not to scale. Numbers along the left side show distance (m) of fertiliser runs from fence line.

The Tropical Trial was set up in early February 2012. This involved the installation of runoff plots, a weather station, soil water content and temperature probes and suction lysimeters. Baseline soil and pasture sampling were also undertaken and a ¹⁵N urea labelling experiment was started. Data from the Tropical Trial was used in the calculation of the water balance model and estimation of NO₃⁻ leaching over the entire trial period, and to also investigate the treatment effect on pasture productivity and gaseous emissions of N during the summer growing season.

The 'Ryegrass Trial' was set up in late May 2012 in paddocks into which ryegrass had been sown ('green struck') in late April 2012. Data from the Ryegrass Trial was used to investigate the treatment effect on pasture productivity and gaseous emissions of N during the winter growing season.



Figure 3: Location of the Tropical Trial and Ryegrass Trial on the RASCHODA farm.

The study was designed to follow normal farm management practices in all aspects except for the experimental treatments. Paddocks were grazed for 24 hours, approximately every 21 days, by 47 head of cattle, comprising Holstein and Jersey cows. Fertiliser (according to treatments described above) was applied immediately following grazing. Paddocks were then irrigated with approximately 25 mm of water, via a travelling irrigator. This amount was determined to minimise deep drainage, based on earlier soil water content monitoring carried out by an irrigation consultant. A single application of Impact 67S, (N 13%, P 11%, K 15%, S 5%) was applied to all paddocks at a rate of 25 kg ha⁻¹ in late March.

Fertiliser was spread using the farm's normal system; a Rondini SPT500 fertiliser spreader (Daken, Acacia Ridge, Queensland), behind a small tractor travelling at approximately 7 km hour⁻¹ (Figure 4). The tractor run spacing and rate settings traditionally used on the farm, were utilised in this study until a fertiliser spreader calibration was undertaken (16th August 2012). The spreader was calibrated by placing collection tubs side by side across the width of a plot, and weighing the collected fertiliser. The calibration revealed a highly uneven lateral distribution, accentuated in the middle of the plots, where pasture plots and gas chambers were located, due to overlapping applications (Figure 5). To try and achieve a more uniform distribution, the spacing of the runs were adjusted (Figure 6).

The first and last fertiliser treatment applications were on 15/5/2012 and 15/1/2013 (total of 12 applications) in the Tropical Trial and 27/6/2012 and 27/9/2012 (total of 5 applications) in the Ryegrass trial (Table 2).

Table 2. Summary timetable for experimental treatments at the Ravenshoe site.

	Month (2012)												Month (2013)				
	J	F	M	A	M	J	J	A	S	O	N	D	J	F	M	A	M
Weather, soil water																	
Tropical fertiliser applic.					1	1	2	1	2	1	1	2	1				
Tropical pasture growth			1		2	1						1	2	1	2	1	
Tropical ¹⁵ N trial																	
Tropical soil																	
Ryegrass fertiliser applic.						1	1	1	2								
Ryegrass pasture growth					1	1	1	2	1	2							
Ryegrass ¹⁵ N trial																	
Ryegrass soil																	
Gas emission																	



Figure 4: Fertiliser spreader and tractor

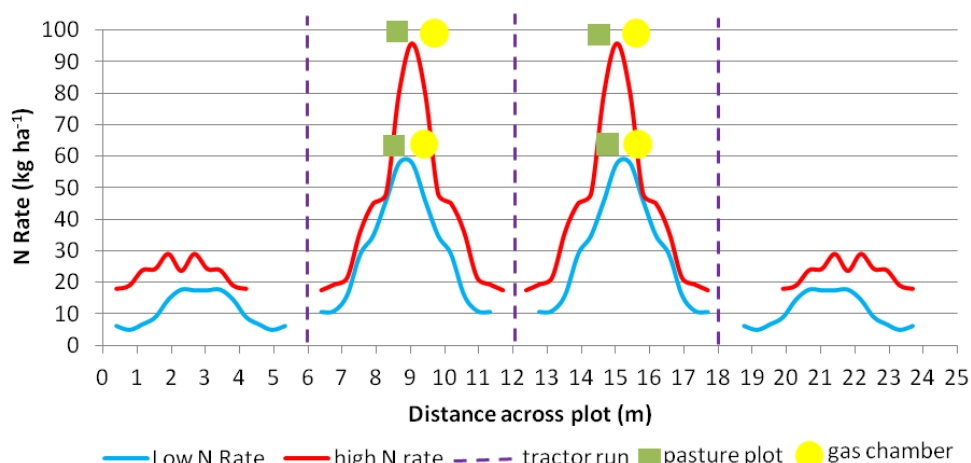


Figure 5: Initial lateral distribution of fertiliser application rate in Ryegrass Trial. Note, the same tractor run spacings were used in the Tropical Trial.

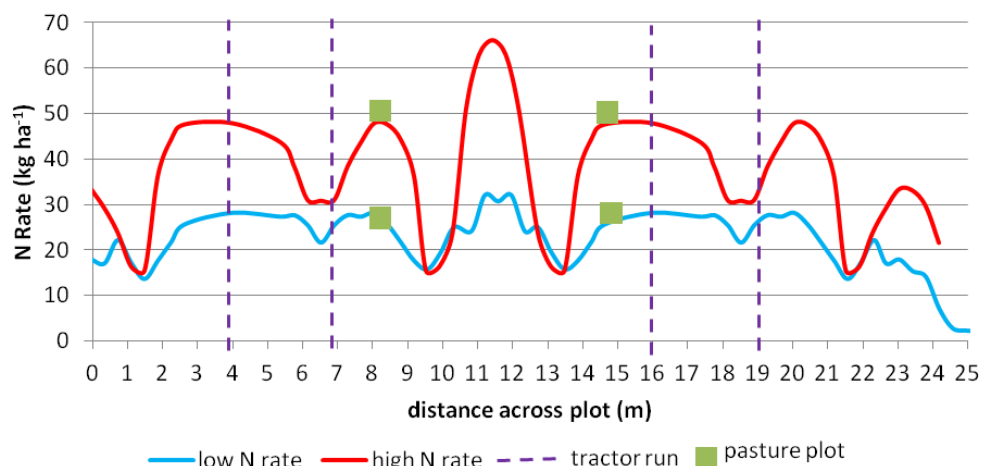


Figure 6: Adjusted lateral distribution of fertiliser application rate.

Weather data

A WeatherMaster 2000 weather station (Envirodata, Warwick, Queensland) provided wind speed, wind direction, air temperature, relative humidity, evaporation, solar radiation and rainfall + irrigation data at daily, hourly, fifteen minute and one minute intervals (Figure 7). A Texas Electronics TR-525M Rain Gauge Tipping Bucket Rainfall Sensor (Texas Electronics, Dallas, Texas), fitted with a HOBO UA-003-64 Pendant Logger (Onset, Computer Corporation,

Pocasset, Massachusetts), provided a backup measure of rainfall + irrigation. Campbell CS616-L water content reflectometers and Campbell 107-L temperature probes provided soil water content and soil temperature down a vertical profile at 10-minute intervals, with the data recorded by Campbell CR1000 datalogger (Campbell Scientific, Logan, Utah). The soil water content reflectometers and temperature probes were located at 0.05, 0.15, 0.35 and 0.7 m depth. An access tube for a Sentec Diviner capacitance sensor (Sentek Pty Ltd, Stepney, South Australia) was located in each trial plot of the Tropical Trial, providing an intermittent (approximately monthly) measure of soil water content within each trial plot.

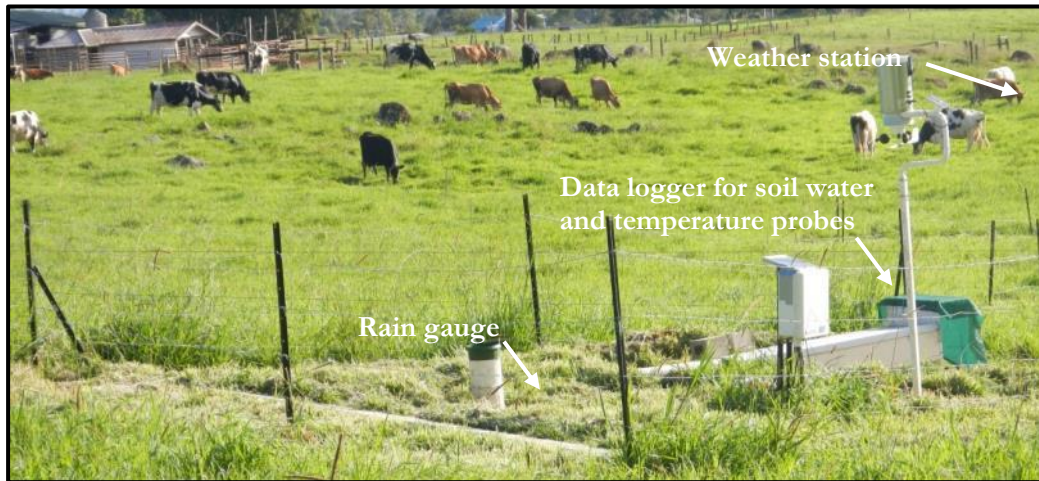


Figure 7: Weather station, soil water and temperature probe and rain gauge.

Water measurements

An annual water balance summary was calculated using the HowLeaky water balance model (McClymont D.J. *et al.*, 2011). HowLeaky is a decision support software used to describe the impacts of alternative land-uses on water quality and water balance (evaporation, transpiration, runoff and deep drainage). Input parameters included climate, irrigation, soil, and vegetation data. A full description of the input parameters used in the model is included in the Appendix.

Deep drainage was also estimated for the whole monitoring period, and for individual leachate sampling events, using Equation 4. For leachate sampling events 1 and 2, the water balance was calculated for the period from when soil water content started to rise to when it returned to the original level. For leachate sampling event 5, the water balance was calculated for each day a sample was retrieved.

$$DD = R + I - ET - RO + \Delta S \quad (1)$$

Where;

DD = deep drainage

R = measured rainfall

I = measured irrigation

ET = estimated evapotranspiration

RO = measured runoff

ΔS = measured change in storage (calculated as the change in soil water content, expressed as a depth of water and summed over 0.7 m, between the first day and the last day of the water balance calculation).

Daily evapotranspiration (ET) at the study site was estimated using meteorological data from the weather station, soil water content data, and pasture growth data. Daily reference ET (ET_0) was calculated by the weather station using the Penman-Monteith equation (Allen *et al.*, 1998) and the radiation, air temperature, humidity and wind speed collected. ET_0 is measure of the evaporative demand of the atmosphere from a reference surface (Allen *et al.*, 1998). The reference surface closely resembles an extensive surface of green grass of uniform height, actively growing, completely shading the ground and with adequate water. To calculate potential crop ET (ET_c), under standard conditions (crops grown in large fields under excellent agronomic and soil water conditions), ET_0 is multiplied by a crop coefficient (K_c). In this study, ET_c was assumed to equal ET_0 , as the crop was a fertilised and irrigated pasture of fairly uniform height, completely shading the ground. ET was calculated by multiplying ET_c by a crop water stress coefficient (K_s).

K_s was calculated using the relationship between pasture growth and soil water content measured in the Ryegrass trial. The maximum value of K_s was assumed to be 0.9 during the sampling period when growth was fastest (19/06/12 – 16/07/12). During that period, the mean soil water content at 0.15 m depth was $0.44 \text{ m}^3 \text{ m}^{-3}$. It was assumed that K_s declined in proportion to pasture growth rate, and this decline was related to soil water content at 0.15 m. Pasture growth rates for three sampling periods (between 17/07/2012 - 20/09/2012), were calculated as a proportion of the maximum growth rate. This proportion was used to derive K_s values for each sampling period. Pasture growth rate (and therefore K_s) was assumed to be zero when soil water content at 0.15 m was $0.2 \text{ m}^3 \text{ m}^{-3}$ (permanent wilting point, Correa *et al.*, 2006). The relationship between K_s and average soil water content during the corresponding sampling period was adequately described using a linear regression ($r^2 = 0.94$), (Figure 8). The resulting equation was used to calculate K_s from daily soil water content data. At water contents $> 0.48 \text{ m}^3 \text{ m}^{-3}$, K_s was set to 1. Daily ET was then estimated, by multiplying daily ET_0 by daily K_s . The calculation of K_s was based on the assumption that plant transpiration decreases as soil water content decreases (Sinclair and Ludlow, 1986; Allen *et al.*, 1998). Also, the period of maximum pasture growth was assumed to be indicative of high evapotranspiration rates (Hillel, 1997).

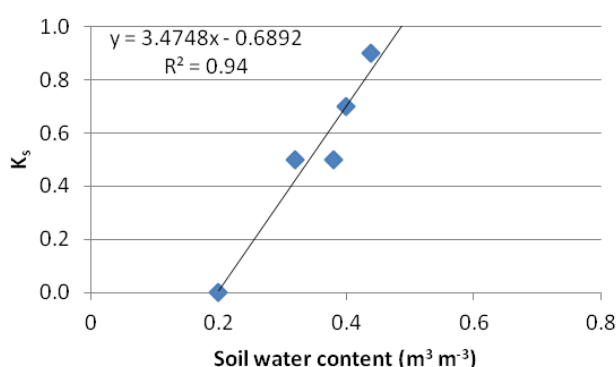


Figure 8: Relationship between water stress coefficient (K_s) and soil water content.

Leaching

To measure the concentration of N and P in water draining below the root zone, suction lysimeters were constructed. The construction of the lysimeters was similar to that described by Bajracharya & Homagain (2006). The lysimeters consisted of a hi-flow (size 4, 28% water absorption) ceramic tensiometer tip (Cooinda Ceramics, Bayswater, Victoria)

securely attached to the base of a 1.5 m length of 40 mm diameter electrical conduit and sealed with araldite. All lysimeters were tested for air-tightness of seal, vacuum integrity and water suction capacity by drawing a partial vacuum in a bucket of water.

One suction lysimeter was installed in each plot in the Tropical Trial. A 50-mm diameter push tube corer was used to make a hole to 1.1 m depth. To reduce possible smearing resulting from the drilling process, the surface of the hole was 'roughed up' using a wire brush. A thick slurry using local topsoil and water filled the hole to halfway. The lysimeter was pushed through the slurry, so that the centre of the tensiometer tip sat at 1 m depth. To avoid preferential flow down the drilled hole, bentonite was packed around the conduit, 0.3 m below the surface.

Lysimeters were sampled by applying a partial vacuum and extracting the soil solution (Figure 9). A rubber stopper, connected to a hand-held vacuum pump via a 0.5-m length of rubber tubing was securely fitted to the conduit. A partial vacuum of 70-80 kPa was drawn and left for approximately 24 hours. After 24 hours, a 2-m length of 2-mm irrigation pipe was fed to the bottom of the tensiometer tip. One end of the irrigation pipe was fitted into a tightly sealed conical flask. The conical flask had an outlet to which a 0.3-m length of rubber tubing connected a hand held vacuum pump. To extract the solution, a partial vacuum was again drawn until all of the water inside the tensiometer tip had been removed.

The suction lysimeters were sampled a total eleven times (Table 3). The samples were kept frozen until analysis. The 2012 leachate samples were sent to TropWater Analytical Services (Townsville, Queensland). The samples were analysed for total N, $\text{NH}_4^+\text{-N}$, $\text{NO}_2^-\text{-N}$ and $\text{NO}_3^-\text{-N}$ using OI Analytical Flow IV Segmented Flow Analysers, according to the methodology described in American Public Health Association (APHA, 2005). The 2013 leachate samples were sent to the Department of Science, Information Technology, Innovation and the Arts Water Chemistry Centre (DSITIA, Brisbane, Queensland). These samples were analysed for $\text{NH}_4^+\text{-N}$, $\text{NO}_x\text{-N}$ and $\text{PO}_4^{3-}\text{-P}$ using dissolved segmented flow analysis, total N (Kjeldahl), total P (Kjeldahl), electrical conductivity and pH.

During the largest leaching event, LS5, samples were collected on 5 days. On 4 of those days (23-26/1/2013) samples were collected from at least 3 plots of all four treatments, allowing statistical analysis of the results. Solute concentrations for each plot on each day of that 4-day period were multiplied by the drainage volume for that day and the resulting amounts were added together for each plot, giving an amount of solute leached during the course of the event for each plot. The calculation assumed that drainage volume was the same for all plots. On 23/1/2013 two samples were taken from most plots; one in the morning and one in the afternoon. For the purpose of calculating drainage fluxes, the mean of those two concentrations was used. For pH and EC, values for each day were averaged for each plot.

Table 3: Leachate sampling events

Date	Suction App.	Sample event	Leachate retrieved
17/04/2012		LS1	Yes
23/05/2012		LS2	Yes
5/11/2012		LS3	No
2/01/2013		LS4	No
21/01/2013		LS5	Yes
22/01/2013		LS5	Yes
23/01/2013		LS5	Yes
24/01/2013		LS5	Yes
25/01/2013		LS5	Yes
26/01/2013		LS5	Yes
21/02/2013		LS6	Yes

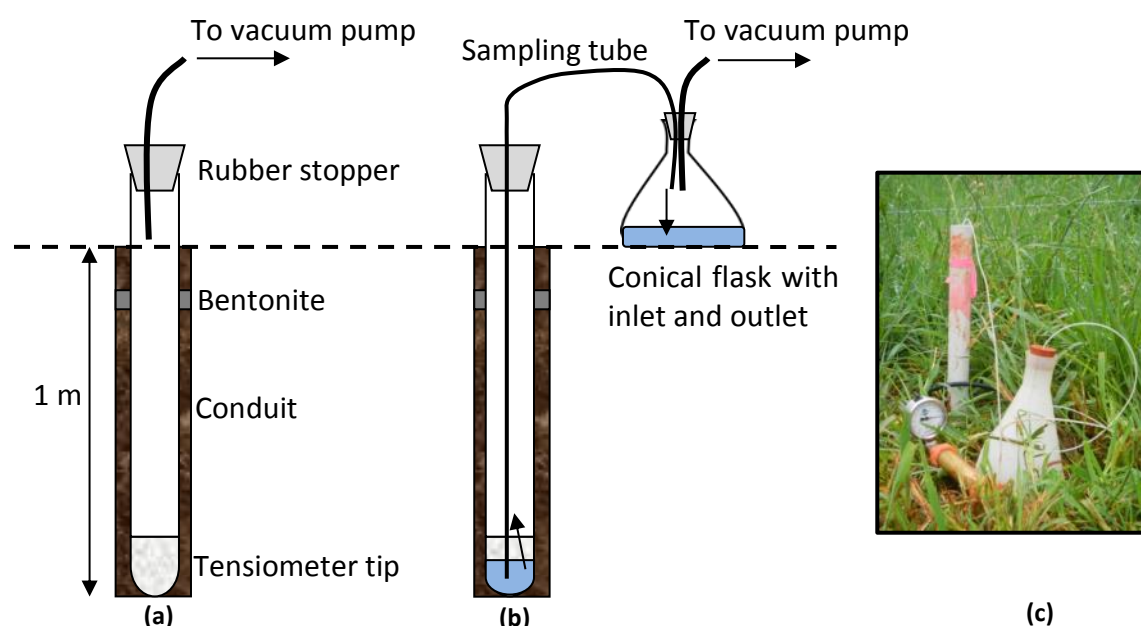


Figure 9: (a) Installation of lysimeter into the soil (b and c) Extracting leachate sample. Adapted from Bajracharya & Homagain (2006). Note, diagram is not to scale.

Runoff

Runoff plots were installed in the Tropical Trial in order to measure surface runoff and the volume and N and P loads therein. Runoff data was also used in the water balance model. The equipment used to measure runoff was designed by Cyril Ciesiolka (Consultant for Ninderry Associates, Toowoomba, Queensland).

A Nikon DTM-352 total station (Nikon Surveying Instrument, Westminster, Colorado) was first used to undertake a 20 m x 10 m gridded survey of the Tropical Trial. The survey data was imported into ArcGIS version 9.2, and interpolated to produce a topographic map using simple spline methods (Figure 10). The topographic map was used to identify the approximate location of areas suitable for four runoff plots, where slope across the plots was equal to approximately 9%. A Dumpy Level and tape measure were used to precisely locate the position of the runoff plots in the field.

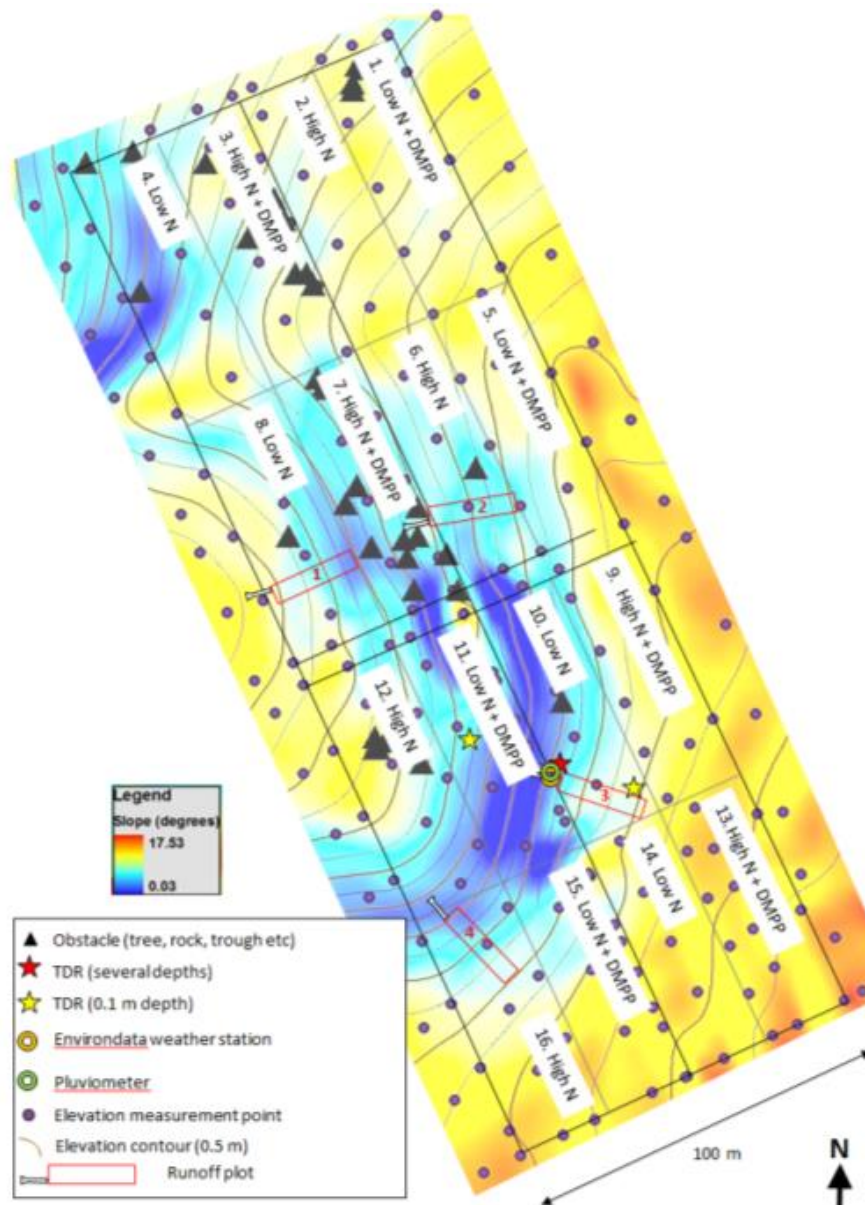


Figure 10: Location of runoff plots in relation to slope (Tropical Trial).

In two replicate plots of two treatments (high N rate and low N rate), one runoff plot ($22.13 \text{ m} \times 4.52 \text{ m} = 100 \text{ m}^2$) was installed, running up/down the slope (Figure 11). Runoff plots 1, 2, 3 and 4 were located in main plots 8, 6, 10 and 16, respectively. The standardised size of the runoff plots allowed for the direct application of the Universal Soil Loss Equation (USLE) (USLE, Wischmeier and Smith, 1960) for calculating sediment loads. Surface runoff from outside the plot was excluded via rubber bunding material (200 mm deep, 20 mm thick) protruding approximately 100 mm out of the ground. Runoff from within the plot area was collected via troughing flush with the soil surface. The collected water was funnelled through PVC pipe to a manifold box which emptied into a tipping bucket. An automatic logger, connected to the tipping bucket via a reed switch, recorded the timing and number of tips. This data was transferred to a computer using a Hobo U-DTW-1 Waterproof Shuttle and analysed using Hoboware 3.3.0 software. A digital counter, also connected to the tipping bucket via a reed switch provided a backup means of recording the number of tips. Every second tip, water was collected in a 20 L plastic jerry can.



Figure 11: Installation of runoff plots.

Runoff water samples were collected once, following a significant rainfall event in January 2013. During the rainfall event, water was sampled from the jerry can and immediately frozen. Samples were sent to DSITIA and analysed for $\text{NH}_4^+\text{-N}$, $\text{NO}_x\text{-N}$ and $\text{PO}_4^{3-}\text{-P}$ using dissolved segmented flow analysis, total N (Kjeldahl), total P (Kjeldahl), electrical conductivity and pH.

Soil sampling and analysis

Soil was sampled using a Christie Hydraulic Soil Corer (Christie Engineering, Horsley Park, New South Wales) using a tube with 37-mm diameter cutting edge (Figure 12). In the Tropical Trial, soil was sampled in February 2012 (prior to fertiliser application), November 2012 (after 9 fertiliser applications) and in April 2013 (after 12 fertiliser applications, at the end of the summer growing season). In the Ryegrass Trial, soil was sampled in September 2012 (after 3 fertiliser applications) and in November 2012 (after 8 fertiliser applications, at the end of the winter growing season).

In each trial plot, soil was sampled at 0.0-0.1, 0.1-0.25, 0.25-0.4, 0.4-0.7, 0.7-1.0, 1.0-1.3 m depth. The first time soil was sampled in the Tropical and Ryegrass Trials, a single core was taken from each trial plot. After air drying and grinding to pass a 2-mm sieve, two 50 g subsamples from two trial plots, with the same treatment, at the same depth, were combined for analysis. On all other soil sampling occasions, two cores from within the same trial plot, at the same depth, were combined on site. After air drying and grinding to pass a 2 mm sieve, 50 g subsamples from each individual trial plot were submitted for analysis. 4 separate cores were taken in the Tropical Trial for bulk density calculation.

On three occasions (9/2/2012, 8/11/2012 and 20/4/2013), soil samples were taken from the Tropical Trial, as specified above, and analysed for bulk density by oven-drying (105°C for 24 hours) and weighing.

$$\text{Bulk Density (g cm}^{-3}\text{)} = \frac{\text{Dry soil weight (g)}}{\text{Soil volume (cm}^3\text{)}}$$

Soil chemical analyses were carried out by Nutrient Advantage Laboratories (Melbourne, Victoria). Samples were analysed for $\text{NH}_4^+\text{-N}$ (2M KCl), $\text{NO}_3^-\text{-N}$ (2M KCl), chloride (water extraction), pH (1:5 water), pH (1:5 soil: 0.01M CaCl_2), and electrical conductivity (1:5 soil: water). Samples from the 0.0-0.1 m depth were also analysed for phosphorus (Colwell, 0.5M NaHCO_3 , pH 8.5). Colour was determined using the Munsell Soil Colour Charts (2009). Field texture was determined using the methodology described by McDonald and Isbell (2009).

Soil samples were also analysed for total C and N contents and natural abundance of ^{15}N and ^{13}C expressed $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$. Small subsamples (1.5 g) were oven dried at 60°C for 24 hours, before being finely milled in a bench-top ring mill using an agate head. The agate head was acid washed between samples with clean sand and ~ 3 ml of 1M hydrochloric acid. The head was then rinsed once with hot water, once with deionised water, dried and wiped out with methanol. The fine milled aliquots were then dried a second time, at 60°C for 48 hours just prior to analysis. At the Cairns Analytical Unit of James Cook University (Cairns, Queensland), soil samples collected in February and September of 2012 were analysed by elemental analyser/continuous-flow isotope ratio mass spectrometry (EA/CF-IRMS) using an ECS 4010 CHNSO elemental analyser (Costech Analytical Technologies Inc., Valencia, California) fitted with a Zero Blank autosampler (Costech Analytical Technologies Inc.) coupled via a ConFloIV interface (Thermo Fisher Scientific, Waltham, Massachusetts) to a ThermoFisher Scientific DeltaVPLUS isotope ratio mass spectrometer. $\delta^{15}\text{N}$ results were reported as per mil (‰) deviations from the $\delta^{15}\text{N}$ (air) reference standard. $\delta^{13}\text{C}$ results were reported as per mil (‰) deviations from the $\delta^{13}\text{C}$ VPDB reference standard. The precision (standard deviation) on internal standards of $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ was 0.2‰ and 0.1‰ respectively. Soil samples collected in November 2012 and April 2013 were analysed for total C and N content at the University of New South Wales XRF Laboratory (Kensington, New South Wales), using a LECO TruSpec Analyser: CN Module.



Figure 12: Soil sampling using a push-tube soil rig.

Gaseous N losses

Emissions of N_2O , CO_2 , NH_3 and CH_4 from the soil surface were measured using the closed chamber technique (Hutchinson and Mosier, 1981; Menéndez *et al.*, 2006; see Figure 13). Only N_2O and CO_2 data is presented. Cylindrical PVC chambers, with a height of 125 mm and radius of 75 mm, were inserted 50 mm into the soil. A well-sealed lid, with a capillary vent tube to allow ambient pressure fluctuation, was placed over the chamber. In a closed loop system, air from the headspace of the chamber was pumped through a 2-m length of Teflon tubing to a photoacoustic multi-gas analyser (INNOVA model 1412, LumaSense Technologies, Ballerup, Denmark). The INNOVA was factory calibrated and set to compensate for the cross-interference between measured gases and water vapour. The

instrument sampled continuously, with a 5-second sample integration time. The increase in concentration was generally linear over the period 1-15 minutes, so linear regression of concentration against time, along with chamber volume, was used to calculate the flux. The gas flux was converted to an amount emitted per hectare per day, and cumulative gas emissions were calculated. Three separate gas emission experiments were undertaken.

Experiment 1 was conducted in the Ryegrass trial in July/August 2012. In this experiment, two chambers were installed in each plot, one day prior to tractor fertiliser application. Immediately following installation, emissions from each chamber were measured. The following day, fertiliser was spread via a tractor and immediately irrigated with 25 mm of water. Emissions were measured daily over the following 7 days, and again 17 days after fertiliser application. To calculate emissions between days 7 and 17, a linear interpolation was applied. Results suggested that uneven distribution of fertiliser in these large trial plots may have influenced gas emissions, so a smaller experiment (Experiment 2) was conducted, in which application rates were strictly controlled.

Experiment 2 was conducted in the Ryegrass Trial in September 2012. In this experiment, known amounts of fertiliser were applied precisely by hand to the chamber and surrounding area (radius of 0.1 m). Only the high rate $\pm$ DMPP treatments were investigated, with 4 chambers installed in each treatment, spaced 3 m apart. Emissions were measured one hour prior to fertiliser application, then again at 1, 4, 21, 24 and 27 hours respectively. The hand-spread experiment was undertaken over 24 hours, as the previous trial showed that most of the emission occurred during that period. Results from Experiment 2 suggested large diurnal variability in emissions, so a third experiment (Experiment 3) was conducted to better quantify this variability. Experiment 3 also investigated the treatment effect on emissions two months after fertiliser application had ceased.

Experiment 3 was conducted in the Tropical Trial in March 2013. In this experiment, only N_2O and CO_2 emissions were measured and a second order polynomial regression was fitted to the data. Two chambers were installed in each plot, one day prior to emission measurement. An additional 'temporal' chamber was installed in a high N – DMPP treatment (plot 6) and a low N + DMPP treatment (plot 11). Emissions were measured over two days, with replicates 1 and 2 measured on the first day and replicates 3 and 4 measured on the second day. The timing of measurement for each treatment was reversed between days to account for diurnal variability in emissions throughout the day. Additionally, emissions from the 'temporal' chamber in plot 6 and 11 were the first, middle and last to be measured on each day.

Soil water content and temperature at a depth of 0 – 0.12 m were measured at the same time as gas emission measurements, using Campbell Scientific Hydrosense II (Campbell Scientific, Logan, Utah). For Experiment 3, a soil sample (0-0.1 m depth) was taken from inside each chamber after the emission measurements were completed and analysed for pH, EC, extractable NO_3^- , extractable NH_4^+ and total C and N.



Figure 13: Measurement of gaseous emissions using the INNOVA gas analyser (in vehicle) and closed chamber.

Pasture biomass and N uptake

Pasture samples were taken from the Ryegrass Trial during the winter growing season (May 2012 - October 2012), and from the Tropical Trial during the summer growing season (May-June 2012 and December 2012 – April 2013). In both trials, $8 \times 0.5 \text{ m}^2$ ($1 \text{ m} \times 0.5 \text{ m}$) quadrats spaced 9 – 18 m apart, along two transects, were located in each trial plot. Immediately prior to grazing, the pasture was clipped to height of 50 mm, using a motorised hedge trimmer (Figure 14). The clipped pasture was immediately dried for 24 hours at 70°C , before being weighed. Pasture dry matter (DM) yield was then calculated. A 500-g combined subsample from all of the pasture plots within each trial plot was ground to $< 2 \text{ mm}$. A small subsample (1.5 g) was analysed for N and C content and the natural abundance of ^{15}N and ^{13}C , expressed as $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$, using the same methodology described for soil C and N analysis. The effect of the treatments on DM yield and total N, for each cut, and also cumulatively, was tested using two-way ANOVA.

A rising plate meter was used as a quick, non-destructive method of estimating pasture biomass, at many more points than the quadrat cut method. Prior to clipping the pasture plots, pasture height was estimated using a rising plate meter (Figure 15) (Earle and McGowan, 1979). A two-way ANOVA was used to test the effect of the treatments on pasture height for each sampling event (see section 2.12). The relationship between pasture height and pasture yield was also determined. Additionally, to provide greater spatial coverage of pasture height, a height reading was taken every meter within each trial plot, along the same transects as pasture production plots.



Figure 14: Pasture sampling.

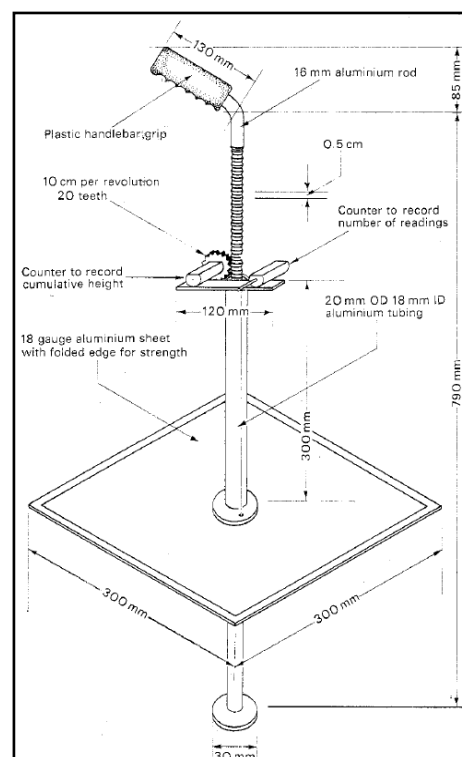


Figure 15: The Ellinbank Pasture Meter.
Source: Earle and McGowan (1979).

N balance measurements and calculations

A partial N balance was constructed for each treatment for one year, using the Ryegrass Trial values for May to October and the Tropical Trial values for November to April. The time course of excess N in the field, available for loss to the atmosphere or hydrosphere, was calculated by adding the N added in fertiliser and subtracting the N taken off in harvested pasture during each month. The calculation is an underestimate of excess N as it does not account for N recycled back into the fields as manure and urine. However, it was used as a means of comparing the treatments. An estimate of the annual N balance was made using the same data for fertiliser and pasture uptake, assuming that: 1/3 of the N taken up by pasture was returned to the field (as manure and urine); the loss of N in deep drainage during LS2 and LS5 was representative (in terms of amount of N per unit of water drained) of the loss in deep drainage throughout the year; runoff measured in the plots with no DMPP added was representative of that in the plots with DMPP added; soil storage did not change; and emission of N_2O averaged $20 \text{ g N ha day}^{-1}$.

To provide a better understanding of the fate of fertiliser N, a one-off application of ^{15}N -enriched urea was made to microplots, and pasture and soil were analysed for ^{15}N over the following three months. The ^{15}N -enriched urea (20 atom % ^{15}N) was applied to one unconfined circular microplot, 1 m^2 in area, located within each of the main trial plots in both the Tropical Trial and the Ryegrass Trial (Hauck et al. 1994). The ^{15}N -enriched urea was applied in solution using a hand-operated sprayer, at the same rate as the remainder of the trial plot (ie. 6.18 or $12.36 \text{ g urea m}^{-2}$, $\pm$ DMPP). A buffer of normal urea, extending 0.5 m away from the edge of the microplot was applied at the same time. The applications were made on 3/5/2012 in the Tropical Trial and 11/8/2012 in the Ryegrass Trial. Prior to

application, the grass was clipped to a height of 5 cm. Immediately following urea application, 15 mm of irrigation water was applied. Pasture was sampled from these plots at the same time as the other pasture cuts (a total of 4 times). Three months after application of the ^{15}N -enriched urea (10/8/2012 in Tropical Trial and 7/11/2012 in Ryegrass Trial), the soil was sampled using a hydraulically driven soil corer with 37-mm diameter cutting tip. In the Tropical Trial, soil samples were taken at the depths of 0.00-0.02, 0.10-0.12, 0.25-0.27, 0.40-0.42, 0.70-0.72 and 1.00-1.02 m and soil from the intervening increments was re-inserted into the hole. In the Ryegrass trial, soil samples were taken at 0-0.1, 0.1-0.25, 0.25-0.4, 0.4-0.7, 0.7-1.0 and 1.0-1.3 m depth. In the Ryegrass Trial it was difficult to separate pasture and soil near the soil surface, so separate samples were taken in this zone. In each microplot, one quadrat 10 cm x 20 cm, encompassing pasture 0-5 cm above the soil surface, and roots and soil 0-2 cm below the soil surface was excavated. The samples were oven dried for 48 hours at 70 °C, then separated into pasture (including roots retained on a 2-mm sieve), and soil (including roots that passed through the sieve). Soil and pasture samples were analysed for ^{15}N content at the Queensland University of Technology, with every 12th sample analysed in duplicate. The recovery of the labeled N in the pasture and soil was determined using a two-component mixing model, which simplifies to the equation

$$N_l = N_s \cdot \frac{R_s - R_u}{R_l - R_u} \quad \text{Equation 1}$$

where N_l is the amount of N that came from the labeled pool, N_s is the total amount of N in the sample, R_s is the ratio of ^{15}N to total N in the sample (expressed as atm %), R_u is the ratio of ^{15}N to total N in the unlabelled pool (expressed as atm %) and R_l is the ratio of ^{15}N to total N in the labeled pool (expressed as atm %). Equation 1 is commonly called the Ndff equation (Barracough 1995). The ratio of ^{15}N to total N in the unlabelled pool (R_u) was taken to be that of the soil, analysed outside of the labeled microplots. That natural abundance had been reported using $\delta^{15}\text{N}$ notation, which was converted to atm % by

$$R_u = \frac{100 \cdot AR(D_u/1000+1)}{1+AR(D_u/1000+1)} \quad \text{Equation 2}$$

where AR is the absolute ratio (mole fraction) of ^{15}N in air, which is 0.0036764 and D_u is the $\delta^{15}\text{N}$ of the unlabelled pool. For the plant samples, the unlabelled pool was assumed to be the original soil N at 0-0.1 m depth. For the soil samples, the unlabelled pool was assumed to be the original soil N at the sampled depth. Of the 32 results (16 plots in two trials), 5 had total recoveries in pasture plus soil greater than 100%. Losses in these plots were assumed to be zero.

Statistical methods

The field trials were designed in a way that all results could be analysed as a two-way ANOVA. The two-way ANOVA is a parametric statistical test which allows the simultaneous analysis of the effect of two factors (or independent variables), on population means (Zar, 1999). It is particularly useful, given that both the 'main effects' of each factor, and the 'interaction effects' of the two factors on the dependent variable, are simultaneously tested. In this study, a fixed-effects model, or model 1 ANOVA was designed. The model is said to have fixed effects, because the levels of each factor have been specifically chosen (Zar, 1999). Application rate had two levels (high and low), and DMPP had two levels (with or without). Three main hypotheses were tested: (1) the main effect of fertiliser rate, (2) the

main effect of DMPP addition, and (3) the interaction effect of fertiliser rate and DMPP addition.

Data from each sampling event were entered into SPSS version 21 (IBM Corp, Armonk, New York). The data was first checked for normality and the presence of outliers using the 'Explore' command. This generated descriptive statistics (including mean, standard deviation, minimum and maximum values, skewness, kurtosis), and the Shapiro-Wilk test of normality. Histograms, boxplots, and normal Q-Q plots were also generated to visualise the data distribution. If data was found to have a moderate positive skewness and/or outliers (as assessed by inspection of boxplots for values greater than 1.5 box-lengths from the edge of the box), a square root transformation was applied. After transformation, the data was again checked for normality and outliers.

The General Linear Model – 'Univariate' command was used to conduct the two-way ANOVA. This generated descriptive statistics (including the mean, standard deviation and number of observations), Levene's Test of Equality of Error Variances ($\alpha = 0.05$), and profile plots. Of most interest was the 'Tests of Between-Subject Effects', which included significance values ($\alpha = 0.05$), for each of the 'main effect' factors and the 'interaction effect' term.

Gympie site, south-east Queensland

Location, climate and soils

The study site is located on a privately owned dairy farm 10 km due east of Gympie, 180 km north of Brisbane (Latitude: 26.19° S; Longitude: 152.74). The farm runs between 200-240 milking cows under management typical of northern dairy production systems. Management consists of a pasture based rotationally grazed production system divided into an annual winter/spring and a summer/autumn management cycle. Around the beginning of May, the existing summer dominated pastures are heavily grazed, slashed and mulched with a flail mulcher to reduce biomass load and vigour of the summer pasture. Annual ryegrass (*Lolium multiflorum*) seed and 100 kg of DAP (18% N) is then surface spread across the pasture up to a week later before a final pass with the flail mulcher.

The annual ryegrass is typically grazed and fertilised with urea on a 3 week cycle until October, and irrigation applied as required. Fertiliser rates vary between individual farms but are in line with the traditional bag-to-the-acre regime of 40-45 kg N ha⁻¹ per application. With increasing temperatures towards the end of spring the ryegrass naturally finishes and the summer kikuyu (*Pennisetum clandestinum*) and star grass (*Cynodon dactylon*) dominate. No fertiliser is applied during the summer dominated growing season. The paddocks of approximately 1 ha are grazed with the entire milking herd for the 6-8 hours between milkings.

The climate at the site is humid subtropical, with the nearby town of Gympie having a mean annual precipitation of 1133 mm (1870-2013) (Bureau of Meteorology, 2009). Rainfall is summer dominated, with over 55% of the rainfall falling between December and March (Figure 16). Rainfall variation is extremely large with the in-situ weather recording a total of just 330 mm of rain in the 6.5 months between 1 June 2012 and 23 January 2013, compared

to 900 mm from the 23 January to 28 February 2013. Mean temperatures range from a minimum of 19°C to a maximum of 31°C in the summer and from 7°C to 22°C in the winter. Frosts are common in June and July but can also occur infrequently in May, August and September.

The soil at the site is a clay loam texture increasing to clay below 250 mm and classified as a Red Dermosol (Isbell, 2002). An initial soil survey conducted prior to the experiment detected no significant moisture, soil carbon or nitrogen gradients across the site. The surface soil (0-100 mm) has a pH of 6.0 and a total organic carbon content of 4.7%. Initial soil analysis revealed live roots to at least 1400 mm.

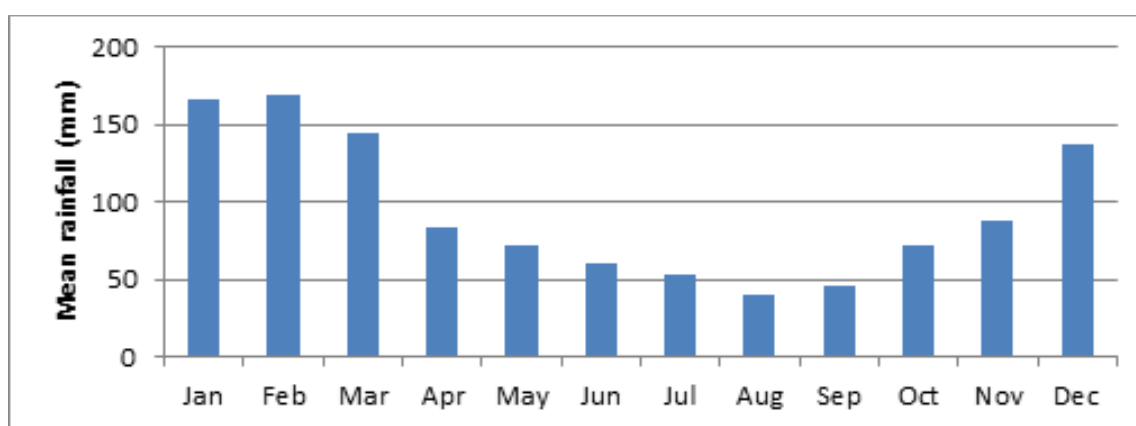


Figure 16: Mean (1870-2012) monthly rainfall for Gympie, Queensland.

Experimental design

The experiment was established in late November 2011 and ran until late April 2013, collating 18 months of N use data. The experiment was divided into two periods; 3 months of solely summer pasture management between February 2011 and April 2012, and 12 months of an N rate and inhibitor response between April 2012 and April 2013. The summer pasture experimental period commenced after an initial run-in period to test the methodology. No fertiliser was applied to the summer pasture. The N rate and inhibitor trial commenced with the establishment of the annual ryegrass in April 2013 and treatments were monitored for 12 months through the winter/spring ryegrass season and the subsequent summer pasture management period.

An 80 m x 65 m area of uniform slope, pasture composition and management history was selected for the location of the experimental plots (Figure 17). The experimental design consisted of 16 randomised, 30 m long by 6 m plots running in 2 rows parallel to the slope. A 5 m buffer zone and a 600 mm deep drainage trench were installed between the rows to avoid run-on from the above plots while a 2 m buffer zone was allowed between adjacent plots. Two tipping bucket runoff plots were established in the design.

The last fertiliser application of the summer pasture experiment occurred on the 29th November 2011 at the rate of 35 kg N ha⁻¹. The fertiliser N rate and inhibitor response experiment consisted of 3 replicates of the following 4 treatments, with an additional 0N plot included as a runoff plot:

- 0 kg N ha
- 23 kg N ha (lower farmer practice) - **UREA-LOW**

- | | |
|--------------------------------------|--------------------|
| - 23 kg N ha + DMPP inhibitor | - DMPP-LOW |
| - 45 kg N ha (upper farmer practice) | - UREA-HIGH |
| - 45 kg N ha + DMPP inhibitor | - DMPP-HIGH |

The decision on the fertiliser rates to use was guided by the local “farmer practice” rate and the experience of local agronomists from Queensland DAFF of 2 kg N day⁻¹ for high treatment and 1 kg N day⁻¹ for low. A decision was made to work at the lower end of the range to maximize the likelihood of a response to the inhibitor.

No additional N fertiliser or irrigation was added to the summer pasture, which was grazed 11 times between early December 2011 and April 2012. The annual ryegrass was oversown directly into the summer pasture as is the common practice in the region. Ryegrass seed was mixed with 100 kg DAP (18% N) and surface broadcast over the plots on the 26/04/2012 (no N was added to the zero N treatments). The summer kikuyu and star grass dominated pasture was intensively grazed overnight with 240 head of cattle and hard mulched the following day, leaving a residue height of less than 2 cm. Plots were then watered overnight with 20 mm of irrigation.

Plots were grazed a total of 14 times between the 26 June 2012 and 9th April 2013 at approximately 3-week intervals. Plots were fertilised the day following grazing and received 7 applications of N at the treatment rates between the 9 June 2012 and the 17 October 2012. To ensure the even distribution of fertiliser, the 30 m x 6 m plots were evenly subdivided into 20, 3 m x 2 m subplots. Pre-weighed bags of fertiliser were then hand spread evenly over each subplot to the desired rate. Plots were subsequently irrigated no later than 3 days after fertilisation.

Weather data

Two tipping bucket pluviometers were installed at the site (HOBO RG3, Onset Computer Corporation, Bourne, MA and Rain Collector II, Davis Instruments, Hayward, CA, USA) and logged at 5 minute intervals. These were supplemented by a 300 mm diameter meteorological manually measured rainfall collector. Site temperature was measured at 30 minute intervals using 3 temperature loggers (Hobo Pendant temperature/ light data logger, Onset Computer Corporation, Bourne, MA, USA) located in the shade, 1 m off the ground.

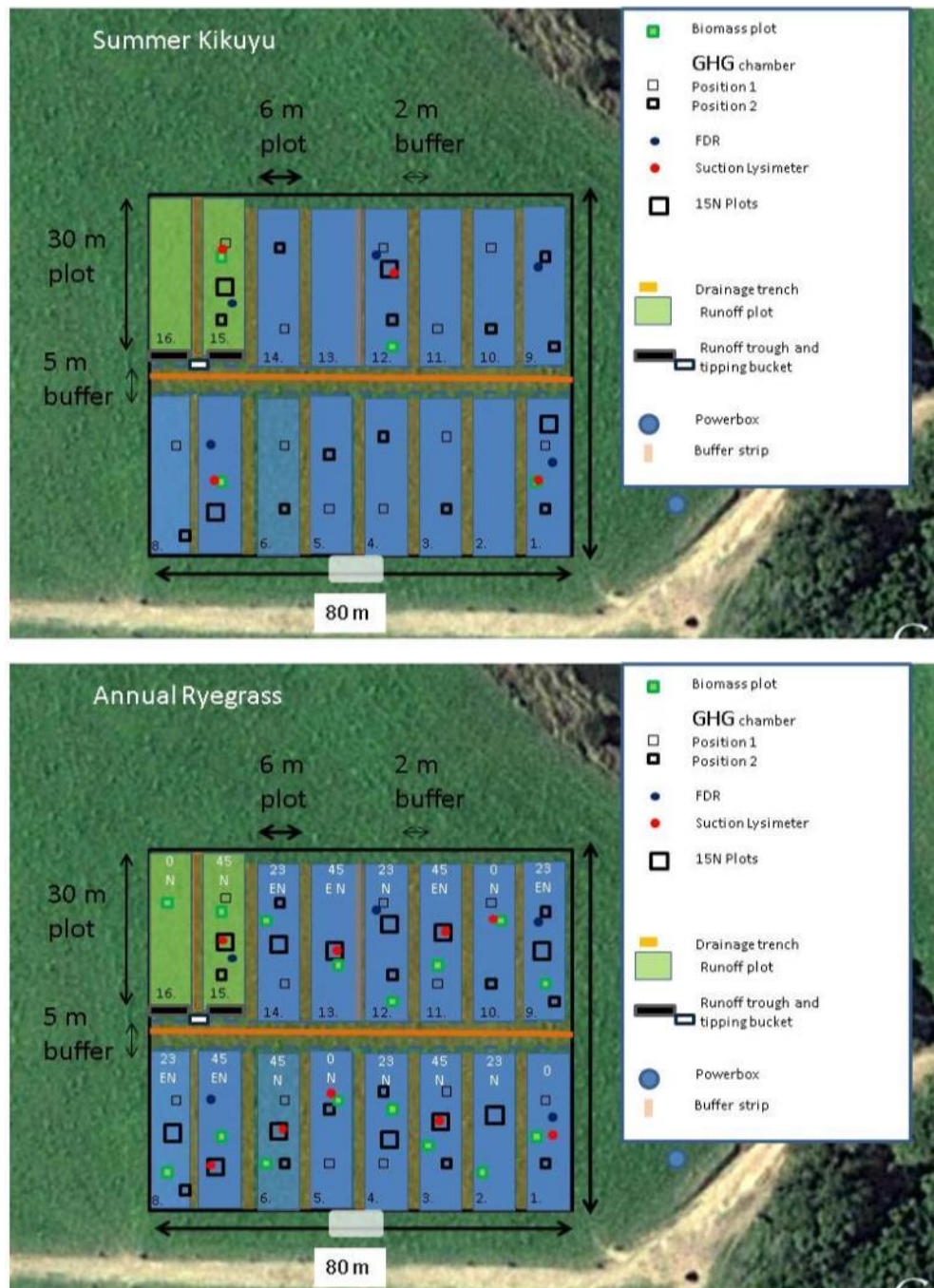


Figure 17: Plot layout design for the summer pasture and N rate and inhibitor experimental periods on a sub-tropical dairy farm near Gympie, Queensland.

Soil sampling and analysis

Soil samples were collected between each grazing event (2-3 week intervals) and bulked from 3 random locations per plot. Deep soil cores were collected at the beginning and end of each management season and sampled at 100, 200, 300, 500, 700, 1000 and 1400 mm depth. Subsamples of each sample were dried at 105 °C for determination of gravimetric soil moisture content, and the remained further subsampled and dried at 60 °C and 40 °C. The 60 °C treated samples were passed through a 2 mm sieve, ground to 2 µm and analysed by dry combustion (CNS-2000, LECO Corporation, St Joseph, MI, USA) for total C and N content. The 40 °C subsample was sent to Nutrient Advantage Laboratory Services, Werribee, Victoria and analysed for NO_3^- and NH_4^+ (2M KCl extract) and Cowell P.

Soil water content was logged continuously in one replicate plot per treatment at 30 minute intervals using frequency domain reflectometry (FDR) probes (Triscan Solo, Sentek, Stepney, South Australia), with sensors installed at 100, 200, 400, 700, 1000, 1300 and 1600 mm soil depth. Sensors were calibrated at each depth for both gravimetric soil water content and nitrate. Additional 30 minute soil moisture readings from an alternate plot per treatment (0-100 mm depth) were collected using time domain reflectometry (TDR) probes. Soil temperature was measured from a PT100 probe inserted at 100 mm soil depth and logged at 5 minute intervals.

Water measurements – Leaching

Nitrogen transport down the soil profile was estimated using suction-cup lysimeters (Sentek solutesamples, Stepney, SA). Two lysimeters were installed in near each of the 4 replicate ¹⁵N subplots in the summer pasture experiment at 40 cm and 60 cm depth. This was repeated in the N rate and inhibitor trial with lysimeters installed in each of the 0N and 45N treatment replicate plots. Leachate was collected after significant rainfall events and analysed for total nitrate and dissolved organic carbon.

Water measurements – Run-off

Surface runoff volume and rates, together with nutrient and sediment loads were investigated from two tipping bucket run-off collectors during the fertiliser rate and inhibitor trial. One plot was left unfertilised as a control and the other received the UREA-HIGH treatment. In June 2012, 150 mm wide edging was buried 100 mm deep around each plot leaving a 13 m X 32 m long catchment area (416 m²).

The collectors consisted of a 200 mm deep, 12 m long trough buried perpendicular to the slope flush with the soil surface. The troughs drained through two, 100 mm diameter PVC pipes into a 1.5 m long by 1 m wide by 200 mm high manifold fitted with a slotted 50 mm high overflow to allow even distribution of water into the 10 L PVC tipping bucket. The tipping bucket was connected to a reed-switch and logged via a data logger (Hobo Pendant logger, Onset Computer Corporation, Bourne, MA), and a manual counter.

At the outlet of the PVC tubes, a 200 x 300 mm glass container was placed and the sample hose from a 24-sample autosampler (ISCO 3700, Teledyne Isco, Inc, Lincoln, NE, USA) secured. The autosampler was connected to the tipping buckets reed switch and programmed to take samples at between 5 and 20 tip intervals depending on the potential size of events. Once activated, the sampler collected a 700 mL into a polypropylene bottle. Samples were collected the next day when possible. An aliquot was transferred to a 120 mL HDPE bottle and acidified to pH < 2 with 1.25 mL/L concentrated H₂SO₄. A second aliquot was transferred into an unacidified 120 mL HDPE bottle, and both samples frozen. Total oxidised N (NO_x-N) was determined by analysing the unacidified sample for NO₂⁻ and NO₃⁻ by ion chromatography using a Dionex RF2100 ion chromatograph (Thermo Fisher Scientific, Australia). The limit of reporting (LOR) was 0.369 mg NO₂⁻/L with a detection limit of 0.111 mg NO₂⁻/L and 0.466 mg NO₃⁻/L with a method detection limit of 0.14 mg NO₃⁻/L, respectively. However, it is assumed that NO₃⁻ is the dominant form of NO_x in the samples. Total Kjeldahl Nitrogen (TKN) was determined in the preserved water sample using U.S. EPA method 351.2 Revision 2, and a copper (II) catalyst (O'Dell, 1993). This method converts all organic N to ammonia, and also includes free ammonia. The concentration of TKN in the

resulting solution was then measured colorimetrically. Quantification was based on standard curves, which were calibrated in a range of 0.1–4.0 mg N/L, with a detection limit of 0.035 mg N/L. The total N concentration was then calculated as the product of NO_x + TKN. Ammonium N (NH₄-N) was determined colorimetrically in the same aliquot, using U.S. EPA method 350.1 Revision 2 (O'Dell, 1993c), with a standard calibration range of 0.02–2.0 mg N/L and a detection limit of 0.007 mg N/L. A 0.45 µm mixed cellulose filter was used on all samples prior to analysis to prevent instrument interference with particulates. Samples exceeding the standard calibration range were diluted with the relevant matrix. Analytical techniques and equipment have not been changed in the course of the study, and quality control standards and repeat analysis of samples in the analytical process were used to determine reproducibility of measurements of all determinants. All colorimetry analysis was performed on a discrete analyser (AQ2+, SEAL Analytical, USA). Sediment load was determined by filtering samples through pre-weighed [DW3] 45 micron filter paper, oven drying at 60 °C and weighing.

Gaseous N losses

Automated chambers were deployed across the plot in 2 campaigns coinciding with the summer pasture and N rate and inhibitor trials. Chambers were installed in early 2012 and measured from February to April in the summer pasture trial. Chambers were removed immediately prior to each grazing and the animals were allowed to access the bases. Chambers were re-installed in the ON, UREA-LOW, DMPP-LOW and UREA-HIGH plots on 26 April 2012 with complimentary funding from Dairy Australia and the Department of Agriculture, fisheries and forestry (DAFF) *Filling the Research Gap*. The automated chambers monitored emissions from three replicate plots for 12 months until April 2013. Chambers were swapped between the two bases at 3-week intervals or following grazing. A 75 cm² wire cage was installed around each base to avoid livestock impacts over the experiment. The automated system consists of pneumatically operated static chambers, linked to an automated sampling system, an in situ gas chromatograph and an infrared gas analyser. The clear acrylic glass chambers covered a surface area of 0.25 m² (500 mm x 500 mm), have a height of 150 mm, a volume of 37.5 dm³ and are secured to stainless steel bases inserted permanently into the soil to a depth of 100 mm. A tipping bucket rain gauge (Davis Instruments Corp. CA, USA) connected to the system allows for automated opening of the lids during rainfall events.

Nitrous oxide and CH₄ concentrations are determined using a gas chromatograph (SRI GC8610, Torrance, CA, USA) equipped with ⁶³N Electron Capture Detector (ECD) for N₂O and a Flame Ionisation Detector (FID) for CH₄. Carbon dioxide is measured continuously with a non-dispersive infrared CO₂ analyser (LI-820; LI-COR, Lincoln Nebraska, USA). To minimise interference from moisture vapour and CO₂ on N₂O measurement, a precolumn filled with sodium hydroxide coated silica was installed ahead of the analytical column and changed regularly.

A full measurement cycle for flux determination commenced with lid closure and finished when the lids opened 60 minutes later. During this time each chamber is sequentially sampled for three minutes followed by a known calibration standard (0.5 ppm N₂O, 3.7 ppm CH₄, 800 ppm CO₂, Air Liquide Australia). Samples pass through the 3 ml sample loop of two separate (ECD, FID) 8-port valves before injection into the respective carrier streams. The LI-

820 is connected to one valves waste vent and logged at 1 Hz. This process was repeated at 15 minute intervals, sampling each chamber four times over the closure period. The lids remained open for a further 120 minutes before the commencement of the next cycle, allowing 8 flux measurements for each chamber to be obtained per day.

Pasture biomass and N uptake

Biomass cuts were taken from 1 m² subplots from which cattle were excluded via a wire mesh exclusion cage. One subplot per replicate treatment was installed in the N rate and inhibitor trial. For comparison, two additional, randomly selected 0.25 m² areas in each plot were also harvested. Pasture was cut using electric hand shears to a uniform height of 40 mm. Samples were then bulked, oven dried at 60 °C and analysed for dry weight and total N and C using dry combustion (CNS-2000, LECO Corporation, St Joseph, MI, USA). Additional biomass was collected from each 1m² ¹⁵N plot prior to each grazing.

N balance – ¹⁵N recovery

At the establishment of the N rate and inhibitor trial, 1m² subplots were installed in each treatment replicate (with the exception of the 0 N replicates). The subplots were bounded by 150 mm deep PVC edging to limit lateral N movement in the surface soil. A solution of 10% atom enrichment ¹⁵N labeled urea was dissolved in 1L of deionised water and applied to the plots at the designated rates for each treatment. The ¹⁵N applications coincided with the plot fertiliser application, each plot receiving 7 applications between 9 June 2012 and 17 October. For the inhibitor treatments, a 5% DMPP solution was first added to the urea before being dissolved in water. Plots were caged off to remove livestock for the entire experiment. Irrigation was applied immediately after application to avoid volatilisation losses.

Pasture biomass cuts were collected prior to each grazing interval until 9 April 2013. Pasture cutting occurred 12 times over the year in conjunction with grazing, though poor pasture growth prevent sampling on the 3/12/2012. Samples were dried at 60 °C, weighed and ground to 2 µm and analysed using an isotope ratio mass spectrometer (20-22 IRMS, Sercon Ltd, Crewe, UK) for % recovery at QUT's Central Analytical Research Facility.

On 9 April 2013, a 55 cm x 55 cm section in the center of each 1 m² plot was harvested to ground level and the litter (0-2 cm) layer carefully removed. The soil was then excavated into a plastic tub to 100 mm and 200 mm intervals. The major roots in the top 100 mm were then removed, the soil thoroughly mixed and subsampled. Three, 50 mm diameter deep soil cores were then collected across each section and bulked at 300, 500, 700, 1000 and 1400 mm depth intervals. Pasture, litter, roots and soil were then taken back to QUT laboratory, a soil subsample dried at 105 C for water content and the remained dried at 60 °C. Roots were carefully washed to remove excess soil before drying. To avoid cross contamination, samples were ground in order of increasing enrichment and the grinding apparatus carefully cleaned with ethanol and DI water between samples from different treatments. Samples were then analysed on the IRMS for % ¹⁵N recovery and δ¹⁵N using equations 1 and 2.

Results

Ravenshoe site, far north Queensland

Site weather data

Over the 14-month study period, climate at the study site was similar to long-term averages. Monthly rainfall was below average in 8 months and above average in 6 months (Figure 18). Maximum monthly rainfall occurred in January 2013 (390 mm), and minimum rainfall occurred in August 2012 (2 mm). Daily air temperature at the study site averaged 20.4°C, with a maximum of 36.7°C occurring in late-January 2013 and a minimum of -1.8°C occurring in mid-August 2012 (Figure 19). The minimum air temperature resulted in a heavy frost across most of the farm.

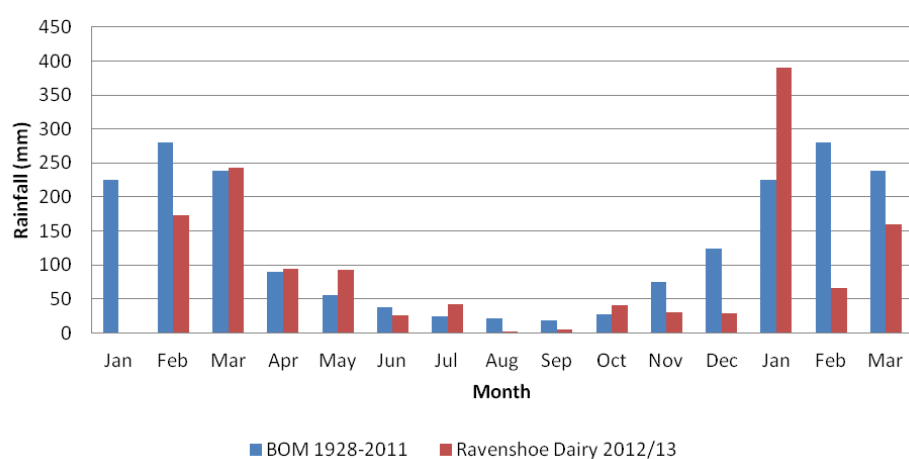


Figure 18: Ravenshoe Dairy monthly rainfall (February 2012-March 2013) and mean monthly rainfall at Ravenshoe (1928-2012, Kuradilla St. source: BOM, 2012).

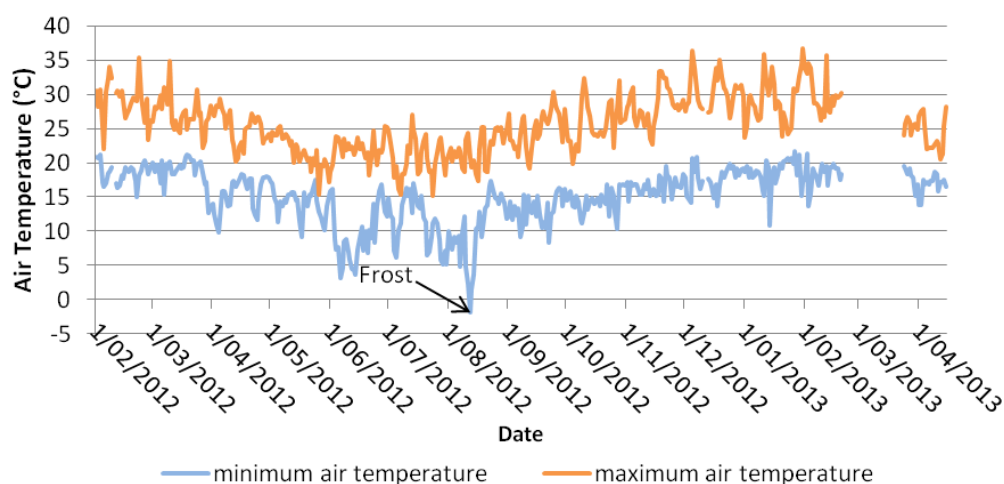


Figure 19: Daily minimum and maximum air temperature at the study site.

N losses – run-off and leaching

The water balance was dominated by rainfall and transpiration, with little runoff (Table 4). Annual rainfall was 1310 mm, with an additional 264 mm of irrigation water applied between May and December. Evapotranspiration was the dominant water output. Of that,

HowLeaky allocated 889 mm (56.5% of annual rainfall + irrigation) to transpiration and the remainder to soil evaporation. Estimates of ET and deep drainage differed significantly between the 'measured' and 'modelled' values, but the modeled runoff corresponded closely to the measured runoff. Runoff was 6 mm in both cases.

Soil water content responded quicker to rainfall and irrigation events at the surface than at depth (Figure 20). The change in soil water content was most pronounced at the surface and became more subtle with depth. Throughout most of the irrigation season (May-September), soil water content at the bottom of the root zone (0.7 m) varied little ($0.35 - 0.42 \text{ m}^3 \text{ m}^{-3}$). With warming temperatures through September, soil water content at 0.7 m fell to $0.28 \text{ m}^3 \text{ m}^{-3}$.

Leachate samples were collected during four deep drainage events, which ranged in size from 36 to 217 mm (Table 5 and Table 6). Deep drainage during the largest event, LS5, followed a similar pattern to rainfall. Drainage was low at the start of the event (8.8 mm rainfall, 6.4 mm drainage per day), peaked in the middle of the event (144.4 mm rainfall, 71.8 mm drainage per day), and gradually declined as rainfall declined.

The concentration of solutes in leachate was generally greater than that of rainfall, and varied through the year (Table 7). Nitrate concentration was particularly variable, increasing by two orders of magnitude between the first and last events. NH_4^+ -N concentrations were similar between events, being similar to NO_3^- -N concentrations in the earlier, smaller events. In the earlier events, total N was analysed in addition to mineral N forms. If the difference between total N and mineral N is taken as organic N, then organic N was a significant component of leachate in those events, making up 66-80% of the total. In those early events NO_2^- and NO_3^- were also analysed separately. The concentration of NO_2^- -N was negligible compared to NO_3^- -N, so NO_x -N can be considered equivalent to NO_3^- -N for all samples. Concentrations were very variable between plots during LS5, with standard deviations similar in magnitude to the means. Total solute concentration (EC) tended to decline during the event.

Estimated N leaching was low for leachate sampling events 1 and 2, but substantial during sampling event 5, due to the higher concentrations and volumes involved. The mean amount of total N leached below the root zone prior to fertiliser application (LS1) was $0.570 \text{ kg N ha}^{-1}$, increasing to $1.909 \text{ kg N ha}^{-1}$ after one fertiliser application. The mean amount of NO_3^- -N leached below the root zone prior to fertiliser application was $0.113 \text{ kg NO}_3^- \text{ N ha}^{-1}$, increasing to $0.230 \text{ kg NO}_3^- \text{ N ha}^{-1}$ after one fertiliser application. Mean NO_3^- -N therefore accounted for <15% of the total N leached below the root zone, after one fertiliser application. 21.5% of the total N leached was in mineral N form.

In LS5, lean losses in deep drainage were lowered by reducing N rate and by application of DMPP, but variation between plots was large (Table 8), so the effects of the treatments were not significant. There was however a significant positive effect of DMPP on leachate pH.

An estimate of N loss in deep drainage, averaged across treatments, was made by assuming that the weighted mean N concentration in the 4 sampled events was representative of deep drainage throughout the year. The total estimate deep drainage N loss calculated in this way was 77, 50, 99 and 61 kg N ha^{-1} for the Low N, Low N + DMPP, High N and High N +

DMPP treatments, respectively, which represented 30, 20, 19 and 12 % of the fertiliser inputs.

Runoff was sufficient to collect samples only during the large rainfall event in January 2013. Runoff samples were collected from all four plots on 23 and 24/1/2013. The samples from were combined for the two days. Concentrations differed substantially between plots with the same treatment (Table 10), but estimated losses of N and P per hectare were low (Table 11), irrespective of treatment.

Table 4: Annual water balance summary. For the 'Measured' values, drainage was calculated by difference. For the 'Modelled' values, ET, runoff and drainage were calculated using HowLeaky.

Source	Rainfall	Irrigation	ET	Runoff	Δ Storage	Drainage
	mm					
Measured (1/2/12-15/4/13)	1476	355	972	6	4	857
Measured (1/5/12-30/4/13)	964	355	679	4	-16	620
Modelled (1/1/12-31/12/12)	1310	264	1225	6	0	356

Table 5: Total water balance for LS1, LS2 and LS6. Note % of rainfall + irrigation does not add up to 100% due to Δ Storage.

Sampling Event and Date	Rainfall mm	Irrigation mm	ET mm	Runoff mm	Δ Storage mm	Drainage mm
LS1 10/04/12 - 28/04/12	93.0	0.0	39.8 (42.8)	0.4 (0.4)	-8.7 (9.4)	44.2 (47.5)
LS2 14/05/12 - 12/06/12	105.0	61.6	64.1 (38.4)	0.6 (0.3)	-29.4 (17.6)	72.6 (43.6)
LS6 27/01/13 - 21/02/13	51.4	0.0	55.2	0.1	40.6	36.7

Table 6: Daily water balance for LS5. Value in brackets indicate % of rainfall + irrigation inputs. Note % of rainfall + irrigation does not add up to 100% due to Δ Storage.

Date	Rainfall mm	Irrigation mm	ET mm	Runoff mm	Δ Storage mm	Drainage mm
20/01/13	8.8	0.0	2.2 (25.1)	0.0 (0.0)	-0.2 (2.7)	6.4 (72.2)
21/01/13	39.6	0.0	2.2 (5.6)	0.2 (0.4)	-28.4 (71.8)	8.8 (22.2)
22/01/13	32.7	0.0	2.2 (6.8)	0.1 (0.3)	-17.1 (52.3)	13.3 (40.6)
23/01/13	144.4	0.0	2.2 (1.5)	0.6 (0.4)	-70 (48.4)	71.6 (49.6)
24/01/13	82.7	0.0	2.2 (2.7)	0.4 (0.5)	-30.0 (36.3)	50.1 (60.6)
25/01/13	13.9	0.0	2.2 (15.9)	0.2 (1.1)	19.4 (139.3)	30.9 (222.3)
26/01/13	15.5	0.0	2.2 (14.3)	0.1 (0.3)	22.6 (146.)	35.9 (231.5)
Total	337.6	0.0	15.5 (4.6)	1.4 (0.4)	-103.8 (30.7)	216.9 (64.3)

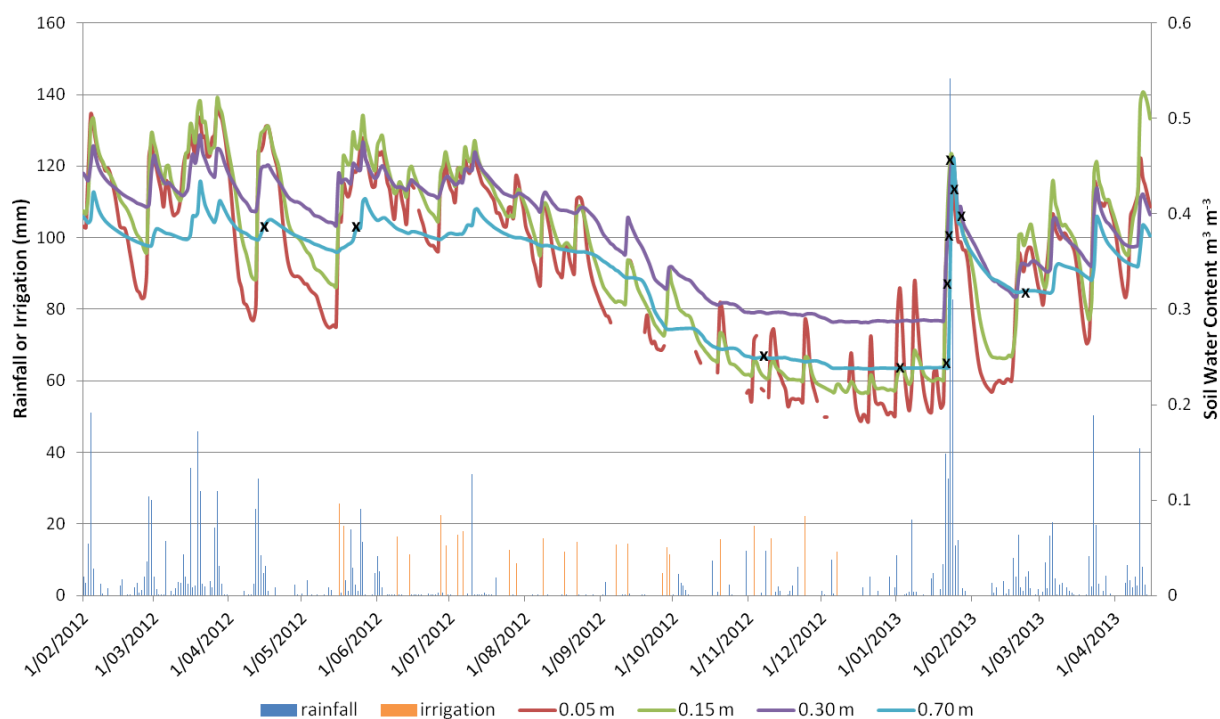


Figure 20: Daily rainfall, irrigation and soil water content at several depths. 'x' (on the 0.7 m soil water content line) shows the time at which leachate samples were taken.

Table 7: Mean concentration of solutes in rainfall and leachate during each event sampled, for each treatment.

Sampling date	Event no.	Treatment	n	pH	EC $\mu\text{S cm}^{-1}$	NH_4^+	NO_2^-	NO_3^-	NO_x	Total N	Phosphate
						mg N L^{-1}					mg P L^{-1}
27/03/2012	Rain		1	6.36	5	0.106	0.002	0.095	0.097	0.315	-
23/01/2013	Rain		1	5.60	<5	0.008	-	-	0.002	-	0.038
17/04/2012	LS1	Low N - DMPP	1	-	-	0.068	0.000	0.074	0.074	0.661	-
17/04/2012	LS1	Low N + DMPP	0	-	-	-	-	-	-	-	-
17/04/2012	LS1	High N - DMPP	1	7.56	174	0.454	0.001	0.237	0.238	2.031	-
17/04/2012	LS1	High N + DMPP	2	-	-	0.107	0.002	0.558	0.560	2.243	-
23/05/2012	LS2	Low N - DMPP	1	-	-	0.362	0.037	0.372	0.409	2.377	-
23/05/2012	LS2	Low N + DMPP	1	-	-	0.296	0.000	0.173	0.173	3.091	-
23/05/2012	LS2	High N - DMPP	1	-	-	0.206	0.006	0.240	0.246	2.313	-
23/05/2012	LS2	High N + DMPP	1	-	-	0.140	0.001	0.554	0.555	3.314	-
22-26/01/2013	LS5	Low N - DMPP	21	7.2	325	0.083	-	-	15.838	-	0.021
22-26/01/2013	LS5	Low N + DMPP	16	7.6	195	0.150	-	-	9.625	-	0.019
22-26/01/2013	LS5	High N - DMPP	16	7.2	283	0.497	-	-	20.650	-	0.005
22-26/01/2013	LS5	High N + DMPP	15	7.6	199	0.802	-	-	11.359	-	0.008
21/02/2013	LS6	Low N - DMPP	0	-	-	-	-	-	-	-	-
21/02/2013	LS6	Low N + DMPP	0	-	-	-	-	-	-	-	-
21/02/2013	LS6	High N - DMPP	3	7.2	293	0.242	-	-	29.500	-	0.038
21/02/2013	LS6	High N + DMPP	0	-	-	-	-	-	-	-	-

Table 8. Estimated amount of N and P lost in deep drainage during the leaching events sampled.

Sample Event	Drainage $\text{m}^3 \text{ha}^{-1}$	Treatment	n	NH_4^+	NO_x	Total N	Phosphate
				kg N ha^{-1}			g P ha^{-1}
LS1	443	Low N - DMPP	1	0.03	0.03	0.29	-
		Low N + DMPP	0	-	-	-	-
		High N - DMPP	1	0.20	0.11	0.90	-
		High N + DMPP	2	0.05	0.25	0.99	-
LS2	728	Low N - DMPP	1	0.26	0.30	1.73	-
		Low N + DMPP	1	0.22	0.13	2.25	-
		High N - DMPP	1	0.15	0.18	1.68	-
		High N + DMPP	1	0.10	0.40	2.41	-
LS5	1885	Low N - DMPP	21	0.14	29.85	-	39.5
		Low N + DMPP	16	0.32	18.14	-	35.6
		High N - DMPP	16	0.69	38.93	-	9.0
		High N + DMPP	15	1.44	21.41	-	15.8
LS6	367	Low N - DMPP	0	-	-	-	-
		Low N + DMPP	0	-	-	-	-
		High N - DMPP	3	0.18	10.83	-	13.9
		High N + DMPP	0	-	-	-	-

Table 9. Quantities of N and P leached, and mean pH and EC, during leaching event LS5 (on 23-26/1/2013).

Treatment	Plot	pH	EC $\mu\text{S cm}^{-1}$	NH_4^+	NO_x	Min. N	Phosphate
				kg N ha^{-1}			g P ha^{-1}
Low N - DMPP	4	7.58	133	0.08	5.24	5.32	25.0
	8	7.03	98	0.16	0.62	0.78	25.3
	10	7.01	521	0.24	63.40	63.64	106.6
	14	7.39	504	0.09	45.18	45.3	5.1
	<i>mean</i>	7.25	314	0.14	28.61	28.75	40.5
	<i>sd</i>	0.24	199	0.06	26.53	26.56	39.1
Low N + DMPP	1	7.90	224	0.98	18.52	19.50	110.0
	5	7.43	346	0.09	56.41	56.49	2.6
	11	7.83	100	0.15	3.13	3.27	6.0
	15	7.40	110	0.05	0.89	0.94	39.2
	<i>mean</i>	7.64	195	0.32	19.74	20.05	39.5
	<i>sd</i>	0.23	100	0.39	22.23	22.22	43.2
High N - DMPP	2	7.50	119	0.06	12.81	12.87	20.2
	6	7.28	217	0.06	39.18	39.24	7.0
	12	7.40	521	2.40	73.68	76.08	4.7
	16	6.80	196	0.25	25.00	25.26	3.7
	<i>mean</i>	7.24	263	0.69	37.67	38.36	8.9
	<i>sd</i>	0.27	153	0.99	22.79	23.69	6.6
High N + DMPP	3	7.90	159	0.44	18.06	18.50	4.2
	7	7.33	80	0.07	0.45	0.52	13.6
	9	7.43	138	0.06	2.35	2.40	23.3
	13	7.83	405	5.20	62.27	67.48	17.3
	<i>mean</i>	7.62	195	1.44	20.78	22.23	14.6
	<i>sd</i>	0.25	124	2.18	24.91	27.05	6.9

Table 10. Concentrations of nutrients in the runoff sampled during the largest runoff event, in January 2013.

Date	Treatment	plot	pH	EC	NH ₄ ⁺	NO _x	Total Kjeldahl N	Phosphate P	Total Kjeldahl P
				µS cm ⁻¹	mg N L ⁻¹	mg N L ⁻¹	mg N L ⁻¹	mg P L ⁻¹	mg P L ⁻¹
23-24/01/2013	Low N - DMPP	8	7.2	56	0.82	1.33	2.49	0.03	0.08
23-24/01/2013	Low N - DMPP	10	7.7	136	6.27	0.33	15.70	0.52	0.90
23-24/01/2013	High N - DMPP	6	6.8	78	0.25	3.07	11.80	0.41	1.75
23-24/01/2013	High N - DMPP	4	6.9	56	1.35	0.22	8.16	0.25	0.50

Table 11. Estimated amount of N and P in runoff during the monitoring period, assuming 5.35 mm of runoff.

Sample Event	Treatment	NH ₄ ⁺	NO _x	Total Kjeldahl N	Phosphate P	Total Kjeldahl P
		g N ha ⁻¹	g N ha ⁻¹	g N ha ⁻¹	g P ha ⁻¹	g P ha ⁻¹
LS5	Low N - DMPP	190	44	487	15	26
LS5	High N - DMPP	43	88	534	17	60

N losses – gaseous

In Experiment 1, N₂O and CO₂ emissions were significantly affected by the treatments. Mean N₂O emission was greatest the day after fertiliser application (78.5 g N ha⁻¹ day⁻¹) and steadily declined back to baseline levels (1.9 g N ha⁻¹ day⁻¹) by day 17 (Figure 21). Total N₂O emitted after fertiliser application was greatest for the high N + DMPP treatment (407.6 g N ha⁻¹ or 0.58 % of N applied) and lowest for the low N - DMPP treatment (142.3 g N ha⁻¹ or 0.27 % of N applied) (Table 12). Cumulative N₂O emission was significantly higher for treatments with DMPP than treatments without ($p < 0.001$). N rate and the interaction effect were not found to significantly effect N₂O emissions. Total CO₂ emitted after fertiliser application was greatest for the high N - DMPP treatment (940.2 kg C ha⁻¹) and lowest for the low N – DMPP treatment (729.9 kg C ha⁻¹). The main effects of N rate and DMPP and their interaction were significant ($p < 0.05$).

During Experiment 1, soil temperature averaged 17.1°C with a minimum and maximum of 15.0°C and 20.1°C respectively. Due to equipment malfunction, soil water content could not be measured. Data from the Tropical Trial suggested soil water content at 0.15 m over the measurement period would be in the range of 0.3-0.6 m³ m⁻³.

In the more carefully controlled Experiment 2, in which fertiliser was spread by hand, there were no significant effects of treatments on N₂O and CO₂ emissions. One to 4 hours after fertiliser application, N₂O emissions were greater for the + DMPP treatment than for the – DMPP treatment, whereas 21 – 27 hours after fertiliser application, the effects of the treatments reversed (Figure 22). On both days of measurement, N₂O emissions appeared to increase throughout the day. Cumulatively, there was no statistically significant difference in N₂O emission between the treatments ($p = 0.994$). On both days of measurement, CO₂ emissions were greater for the + DMPP treatment than for the – DMPP treatment, although cumulatively, the difference was not significant ($p = 0.915$). Similar to N₂O, CO₂ emissions appeared to increase throughout the day.

During Experiment 2, soil temperature averaged 19.7°C, with a minimum and maximum of 17.8°C and 22°C respectively. After irrigation, soil water content averaged 0.40 m³ m⁻³, with a minimum of 0.33 m³ m⁻³ and a maximum of 0.47 m³ m⁻³.

In Experiment 3, where fertiliser had not been applied for over two months, there were no significant effect of treatments on N_2O emissions ($p>0.05$), however there was an interaction effect between N rate and DMPP ($p<0.05$). Mean N_2O emission was greatest for the high N + DMPP treatment (10 g N ha^{-1}) and lowest for the low N – DMPP treatment (0.0 g N ha^{-1}) (Table 12). Mean CO_2 emission was greatest for the low N – DMPP treatment ($106.0 \text{ kg C ha}^{-1} \text{ day}^{-1}$) and lowest for the high N – DMPP treatment ($77.1 \text{ kg C ha}^{-1} \text{ day}^{-1}$).

During Experiment 3, soil temperature averaged 23.6°C , with a minimum and maximum of 21.5°C and 32.7°C respectively. Soil water content averaged $0.38 \text{ m}^3 \text{ m}^{-3}$, with a minimum and maximum of $0.27 \text{ m}^3 \text{ m}^{-3}$ and $0.44 \text{ m}^3 \text{ m}^{-3}$, respectively. There were no significant correlations between N_2O or CO_2 emission and the measured soil variables, except for a positive correlation ($r=0.64$) between N_2O emission and soil chloride content.

Emissions of N_2O and CO_2 varied diurnally. In the high N – DMPP treatment, on both days of measurement, N_2O emissions were relatively high in the morning ($27.3 - 67.0 \text{ g N ha}^{-1} \text{ day}^{-1}$), decreased in the middle of the day ($7.3 - 8.0 \text{ g N ha}^{-1} \text{ day}^{-1}$) and increased in the afternoon ($64.2 - 67.0 \text{ g N ha}^{-1} \text{ day}^{-1}$) (Figure 22). In the low N + DMPP treatment, on both days of measurement, emissions were much lower and varied less ($-4.8 - 10.1 \text{ g N ha}^{-1} \text{ day}^{-1}$). CO_2 emissions in the both the high N – DMPP treatment and low N + DMPP treatment followed very similar diurnal patterns. On the first day of measurement, CO_2 emissions were lowest in the morning ($100.5 - 108.9 \text{ kg C ha}^{-1} \text{ day}^{-1}$) and afternoon ($102.0 - 107.6 \text{ kg C ha}^{-1} \text{ day}^{-1}$) and greatest in the middle of the day ($117.0 - 117.9 \text{ kg C ha}^{-1} \text{ day}^{-1}$). On the second day of measurement, CO_2 emissions increased from $55.9 - 63.4 \text{ kg C ha}^{-1} \text{ day}^{-1}$ in the morning, to $93.4 - 107.3 \text{ kg C ha}^{-1} \text{ day}^{-1}$ in the middle of the day to $121.7 - 124.9 \text{ kg C ha}^{-1} \text{ day}^{-1}$ in the afternoon.

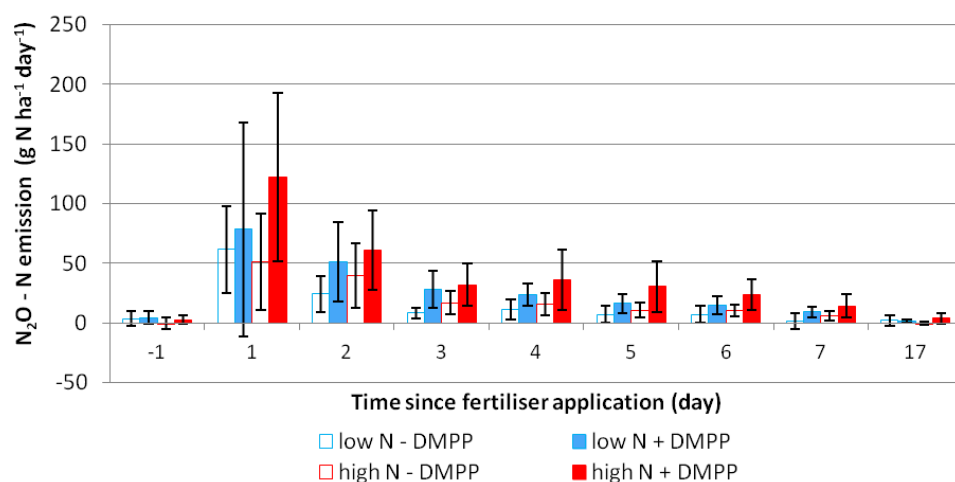
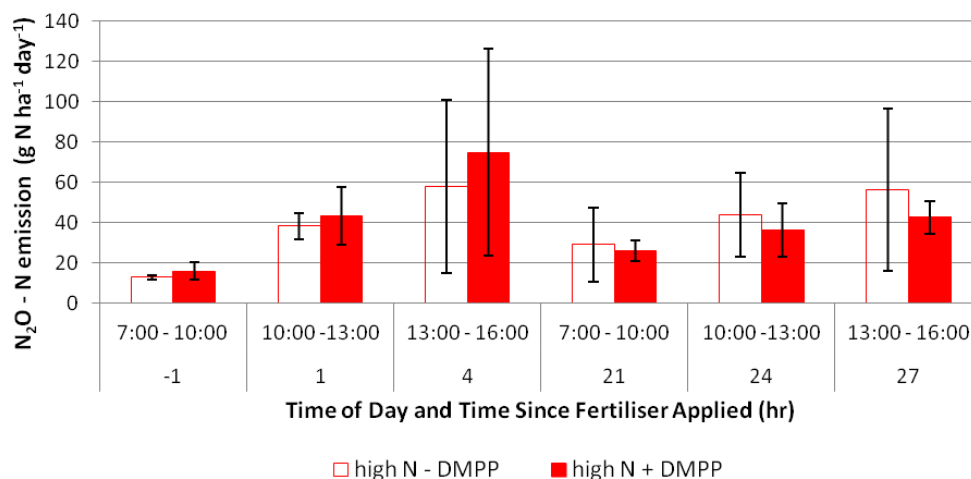


Figure 21: Mean N_2O emission in Ryegrass Trial (Experiment 1). Error bars represent standard deviation from mean.

Table 12: Cumulative N₂O and CO₂ emission in Ryegrass Trial over 17 days (Experiment 1).

Treatment	N ₂ O-N (g N ha ⁻¹)					CO ₂ -C (kg C ha ⁻¹)			
	Mean	% of N App.	Std. Dev.	Min.	Max.	Mean	Std. Dev.	Min.	Max.
low N - DMPP	142.3	0.27	92.8	-45.0	279.9	729.9	106.5	586.6	898.0
low N + DMPP	273.2	0.52	160.5	111.9	604.4	754.3	90.8	624.1	906.5
high N - DMPP	179.5	0.25	103.0	77.2	323.6	940.2	135.6	805.1	1124.5
high N + DMPP	407.6	0.58	190.9	73.9	651.2	732.0	137.4	595.1	1009.5

Figure 22: Mean N₂O emission in Ryegrass Trial (Experiment 2). Error bars represent standard deviation from mean.Table 13: Cumulative N₂O and CO₂ emission in Ryegrass Trial over 27 hours (Experiment 2).

Treatment	N ₂ O-N (g N ha ⁻¹)					CO ₂ -C (kg C ha ⁻¹)			
	Mean	% of N App.	Std. Dev.	Min.	Max.	Mean	Std. Dev.	Min.	Max.
high N - DMPP	39.8	0.06	37.3	8.6	92.7	56.4	44.0	-1.8	97.3
high N + DMPP	40.0	0.06	32.3	12.9	85.7	60.9	67.1	-20.0	144.1

Table 14: Mean and Standard Deviation of N₂O and CO₂ Emission (Experiment 3).

Treatment	N ₂ O-N (g N ha ⁻¹ day ⁻¹)				CO ₂ -C (kg C ha ⁻¹ day ⁻¹)			
	Mean	Std. Dev.	Min.	Max.	Mean	Std. Dev.	Min.	Max.
low N - DMPP	0.0	12.9	-20.0	20.0	106.0	22.9	75.1	146.6
low N + DMPP	5.0	9.3	-10.0	20.0	84.5	26.3	45.5	119.5
high N - DMPP	7.5	8.9	-10.0	20.0	77.1	21.7	53.0	117.2
high N + DMPP	10.0	33.7	-30.0	70.0	102.5	32.3	73.4	161.9

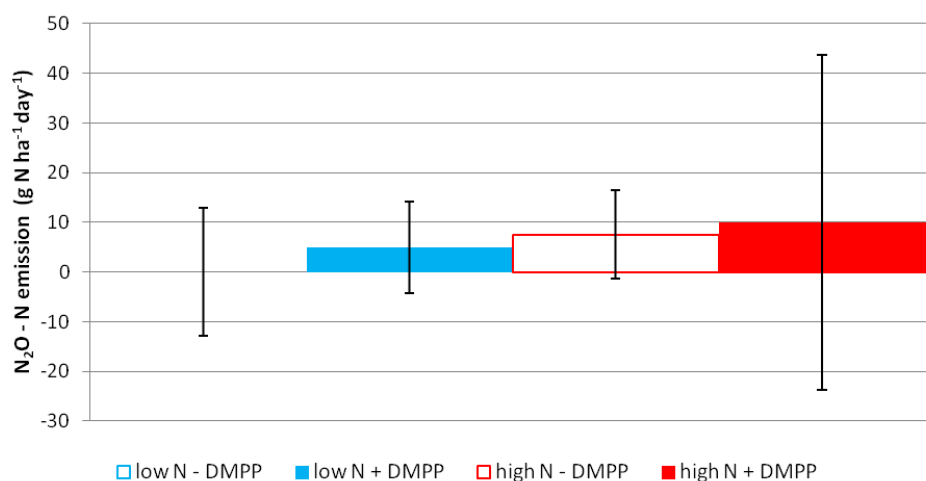


Figure 23: Mean N_2O emission in Tropical Trial (Experiment 3). Error bars represent standard deviation from mean.

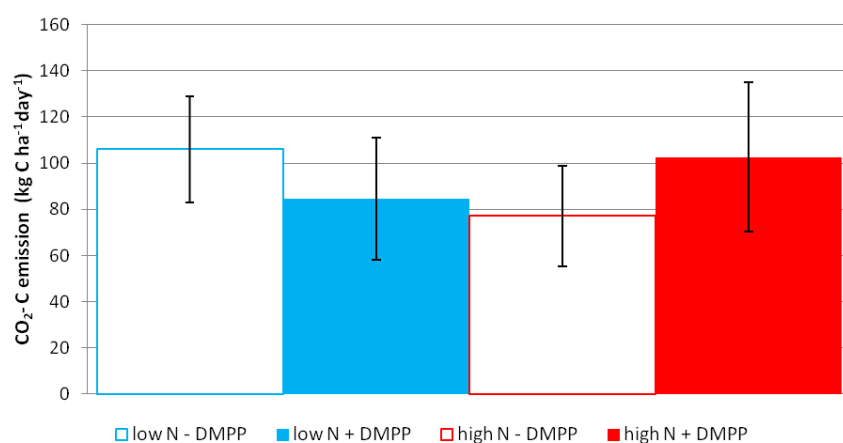


Figure 24: Mean CO_2 emission in Tropical Trial (Experiment 3). Error bars represent standard deviation from mean.

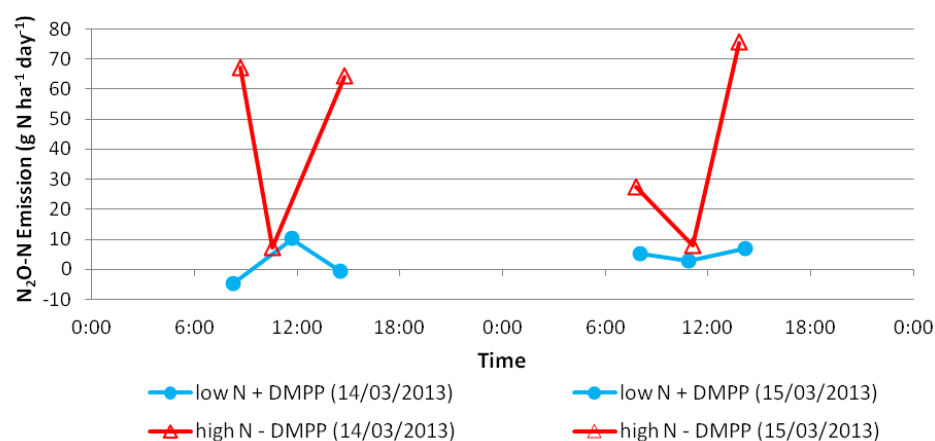


Figure 25: Diurnal variation in N_2O emission in Tropical Trial (Experiment 3).

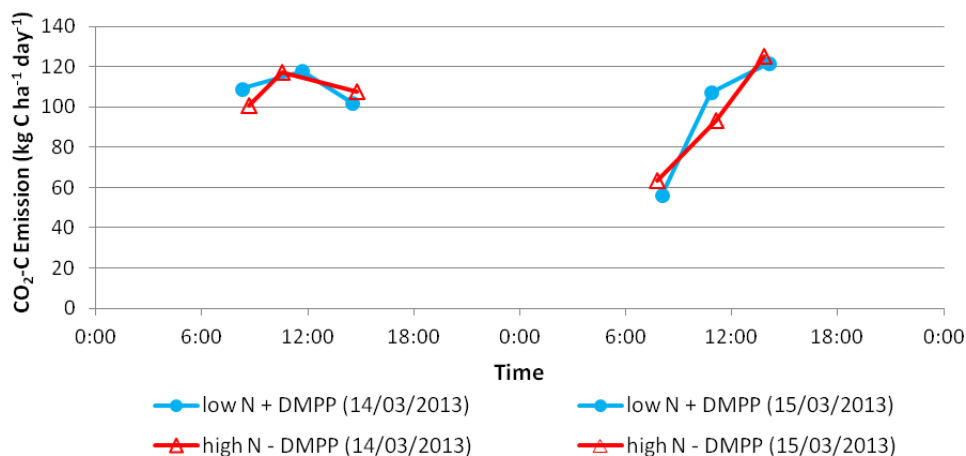


Figure 26: Diurnal variation in CO₂ emission in Tropical Trial (Experiment 3).

Soil Nitrogen

The soil was dark reddish brown clay to clay loam, with a slightly acidic-neutral pH, low EC and quite high N and P contents at the surface (Table 15). Soil pH (1:5 water) was slightly acidic at the surface (6.2 – 5.8), increasing to a maximum of 7.1 – 6.8 at 0.7 – 1.7 m depth. Soil pH (1:5 CaCl₂) was slightly more acidic (6.6 – 5.2). Electrical conductivity (EC) was low (mean 0.05 dS/m) and generally decreased with depth. NH₄⁺-N content was greatest in the top 0.1 m (28.0 – 19.5 mg kg⁻¹) and decreased with depth (0.8 – 1.2 mg kg⁻¹ at 1.7 m). NO₃⁻-N content was also greatest in the top 0.1 m (30.0 – 15.8 mg kg⁻¹) and generally decreased with depth (< 1 mg kg⁻¹ at 1.7 m). However in two of the profiles, NO₃⁻-N concentration increased below 1 m (18.0 – 7.2 mg kg⁻¹ at 1.7 m). Cl⁻ content was more variable down the profile, with the concentration ranging from 50.5 – < 10 mg kg⁻¹. Colwell P in the top 0.1 m ranged from 86.5 – 31.5 mg kg⁻¹.

After three fertiliser applications in the Ryegrass Trial, there were significant treatment effects on soil mineral N contents. Mean NH₄⁺-N concentration in the top 0.1 m was highest for the high N + DMPP treatment, followed by the low N + DMPP treatment, high N – DMPP treatment and the low N – DMPP treatment (20.0, 18.0, 17.5 and 14.0 mg kg⁻¹ respectively) (Figure 27). There were statistically significant ($p < 0.05$) effects on mean NH₄⁺-N concentration of N rate and DMPP, but not their interaction. Mean NH₄⁺-N concentration decreased with depth to <3.0 mg kg⁻¹ at 1.3 m. In the top 0.1 m, mean NO₃⁻-N concentrations appeared to be higher for low N + DMPP and high N + DMPP treatments (114.5 and 94.0 mg kg⁻¹ respectively) than the high N – DMPP and low N – DMPP treatments (29.5 and 20.0 mg kg⁻¹ respectively) (Figure 28). However, this apparent effect of DMPP was not significant ($p=0.101$). Neither were there significant effects of the N rate ($p=0.891$) or the N rate x DMPP interaction ($p= 0.710$). Mean NO₃⁻-N concentration decreased with depth to < 3.0 mg kg⁻¹ at 1.3 m.

Table 15: Baseline soil analysis (Tropical Trial).

Treatment	Depth (m)	pH (1:5 Water)	pH (1:5 CaCl ₂)	EC (dS m ⁻¹)	Cl ⁻ (mg kg ⁻¹)	NO ₃ ⁻ - N (mg kg ⁻¹)	NH ₄ ⁺ - N (mg kg ⁻¹)	P - Col (mg kg ⁻¹)
low N - DMPP	0.0 - 0.1	5.8	5.2	0.11	18.5	22.5	28.0	48.0
	0.1 - 0.25	5.9	5.3	0.04	11.0 ^x	7.5	9.7	
	0.25 - 0.4	6.5	6.0	0.03	21.0 ^x	1.7	3.2	
	0.4 - 0.7	6.5	6.2	0.03	21.0 ^x	1.7 ^y	1.4	
	0.7 - 1.0	6.9	6.4	0.02	15.0 ^x	1.0 ^{yy}	1.1	
	1.0 - 1.3	7.0	6.5	0.02	18.0 ^x	1.0 ^{yy}	1.0	
	1.3 - 1.7	6.9	6.5	0.03	13.0 ^x	1.0 ^{yy}	1.2	
low N + DMPP	0.0 - 0.1	6.2	5.6	0.17	29.0	30.0	26.0	86.5
	0.1 - 0.25	6.1	5.4	0.11	31.5	17.6	10.2	
	0.25 - 0.4	6.3	5.7	0.09	38.0	13.6	4.4	
	0.4 - 0.7	6.6	6.3	0.05	33.0	15.0 ^y	1.5	
	0.7 - 1.0	6.7	6.5	0.04	20.5	13.0 ^y	1.2	
	1.0 - 1.3	6.8	6.5	0.05	50.5	17.0 ^y	0.8	
	1.3 - 1.7	6.8	6.5	0.06	41.5	18.0 ^y	0.8	
high N - DMPP	0.0 - 0.1	6.2	5.5	0.09	12.0 ^x	15.8	19.5	31.5
	0.1 - 0.25	6.2	5.4	0.05	15.0 ^x	3.1	3.9	
	0.25 - 0.4	6.6	6.0	0.03	12.0 ^x	2.4	2.7	
	0.4 - 0.7	6.9	6.3	0.03	13.0 ^x	1.4 ^y	1.5	
	0.7 - 1.0	7.1	6.6	0.03	11.0 ^x	1.0 ^{yy}	0.9	
	1.0 - 1.3	7.0	6.6	0.03	13.0 ^x	1.0 ^{yy}	0.7	
	1.3 - 1.7	7.0	6.5	0.03	15.0	1.0 ^{yy}	0.9	
high N + DMPP	0.0 - 0.1	6.2	5.6	0.12	15.0	22.5	26.5	76.0
	0.1 - 0.25	6.3	5.7	0.06	17.0	9.1	6.5	
	0.25 - 0.4	6.4	5.9	0.04	11.0 ^x	2.9	2.9	
	0.4 - 0.7	6.8	6.4	0.04	16.0 ^x	1.2	1.6	
	0.7 - 1.0	7.0	6.6	0.03	11.0 ^x	1.0 ^{yy}	1.0	
	1.0 - 1.3	6.9	6.4	0.04	11.0 ^x	1.1 ^y	0.7	
	1.3 - 1.7	6.8	6.4	0.05	12.5 ^x	7.2 ^y	1.4	
Average		6.6	6.1	0.05	19.5	8.3	5.7	60.5
Minimum		5.8	5.2	0.02	<10.0	<1.0	0.7	31.5
Maximum		7.1	6.6	0.17	50.5	30.0	28.0	86.5
Std. Deviation		0.4	0.5	0.04	10.5	8.6	8.5	25.3

Electrical conductivity (EC), chloride (Cl⁻), Colwell phosphorus (P-Col)

Values reported are the average of two combined sub-samples from the same treatment;

^x indicates one of the two values averaged were reported as being less than the detection limit for Cl⁻: 10.0 mg kg⁻¹^y indicates one of the two values averaged were reported as being less than the detection limit for NO₃⁻: 1.0 mg kg⁻¹^{yy} indicates both of the values averaged were reported as being less than the detection limit NO₃⁻

If either of the values were below the detection limit, the detection limit value was used in the average

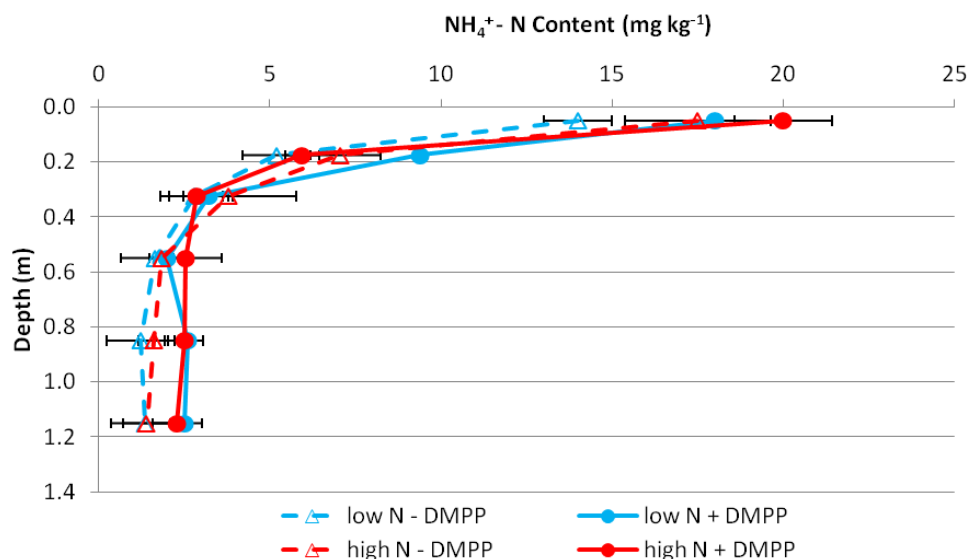


Figure 27: Mean NH_4^+ -N concentration in soil profile after 3 fertiliser applications (Ryegrass Trial). Error bars represent standard deviation from the mean.

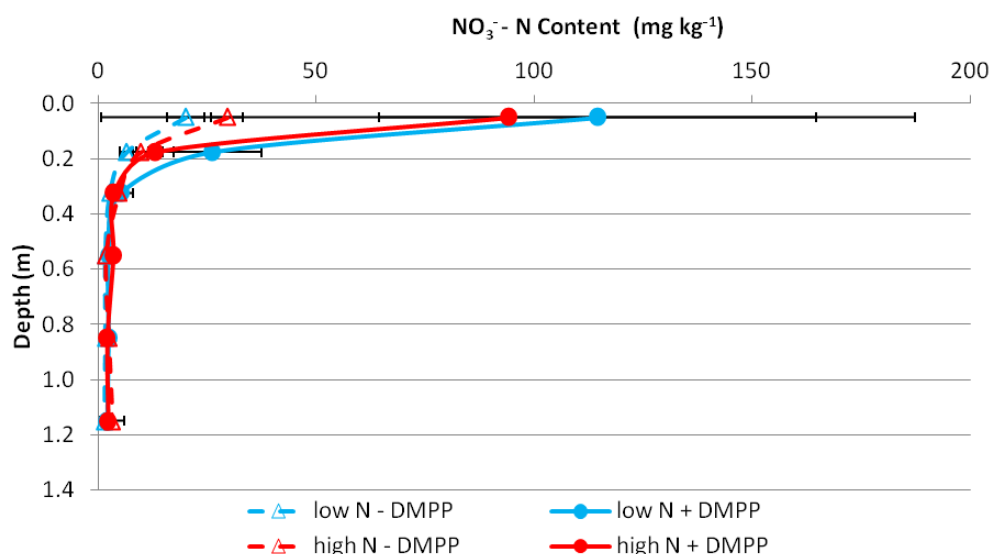


Figure 28: Mean NO_3^- -N concentration in soil profile after 3 fertiliser applications (Ryegrass Trial). Error bars represent standard deviation from the mean.

Pasture Response

Pasture yield and N content

Cumulative pasture yield in the Ryegrass Trial at the end of the 6 sampling events, was greatest for the high N + DMPP treatment, followed by the low N + DMPP, high N - DMPP, and low N - DMPP treatments (Table 14). Cumulatively, the main effects of N rate and DMPP were both significant ($p < 0.01$), whereas their interaction was not ($p = 0.195$) (Table 16). However, the effects of the treatments differed between sampling dates (Figure 29). There was no significant difference in yield between treatments ($p < 0.05$) in the first sampling event. After the second sampling event, the treatments with DMPP had a significantly higher yield than treatments without DMPP ($p < 0.01$). N rate also had a significant ($p < 0.05$) positive effect on yield. Over the next 3 sampling events, as temperature and pasture production fell, the difference in yield between treatments generally narrowed, and by the 5th and 6th

sampling event, there was no statistically significant difference ($p < 0.05$) between treatments.

Cumulative pasture yield in the Tropical Trial at the end of 6 sampling events, was greatest for the low N + DMPP treatment, followed by the high N – DMPP, high N + DMPP and low N – DMPP treatments (Table 16). Cumulatively, there was no significant difference in pasture yield between the low and high N rate ($p = 0.74$) (Table 17). The treatments with DMPP however, had a significantly higher yield than treatments without DMPP ($p < 0.01$). The interaction between N rate and DMPP was found to significantly effect cumulative pasture yield ($p < 0.01$). However, the effects of treatments differed between sampling dates (Figure 30). For the first 3 sampling events, where pasture growth was low, the main effects were not significant ($p > 0.05$), however the interaction effect was ($p < 0.01$). In the fourth sampling event, following the first significant summer rain, both the main effects and the interaction effect were significant ($p < 0.01$). In the fifth sampling event, where pasture growth was greatest, the effect of DMPP, and the interaction effect were significant ($p < 0.05$), however the effect of N rate was not ($p = 0.108$). Data from the sixth sampling event was omitted from analysis due to unreliable data. By the seventh sampling event, where pasture growth had slowed, the effect of DMPP was significant ($p < 0.01$), however the effect of N rate, and the interaction effect was not.

Pasture N content in the Ryegrass Trial was influenced by N rate and DMPP. After the first sampling event, N content was greatest for the low N + DMPP treatment (3.06%) and lowest for the low N – DMPP treatment (2.57%) (Table 18). After the third sampling event, mean pasture N content was greatest for the high N + DMPP treatment (3.56%) and lowest for the low N – DMPP treatment (2.88%). After the fifth sampling event, mean pasture N content was greatest for the high N – DMPP treatment (3.60%) and lowest for the low N – DMPP treatment (2.92%). Mean N content increased from the first to the fifth sampling event in all treatments. The effect of N rate was not significant for the first sampling event ($p = 0.438$), but was significant for the third and fifth ($p < 0.05$) (Table 19). The effect of DMPP was significant for the first and third sampling event ($p < 0.05$), but not the fifth ($p = 0.150$). The interaction effect was not significant for any sampling event.

Pasture N content in the Tropical Trial was significantly influenced by N rate in both cuts and not significantly influenced by DMPP in either cut (Table 18). As for the Ryegrass Trial, N content increased with time.

Pasture height

The trend in mean pasture height in the Ryegrass Trial with each sampling event was similar to mean pasture yield, with the treatments with DMPP exhibiting a greater mean height than treatments without DMPP (Figure 31). This difference was statistically significant ($p < 0.001$) for sampling events 2, 3 and 6. The difference between N rates was statistically significant ($p < 0.05$) only for sampling event 2. A linear regression of pasture height and pasture DM yield produced an r^2 of 0.57 (Figure 32).

Table 16: Cumulative mean pasture yield (kg DM ha⁻¹).

	Ryegrass Trial		Tropical Trial	
	Low N	High N	Low N	High N
DMPP -	3112	3690	3453	5101
DMPP +	3756	3978	5320	4356

Table 17: Significance (p-value) of main and interaction effects for cumulative pasture weight (DM).

Effect	Ryegrass Trial	Tropical Trial
N Rate	0.007	0.074
DMPP	0.004	0.006
N Rate x DMPP	0.195	0.000

Table 18: Mean pasture N content (%).

	Ryegrass Trial						Tropical Trial			
	Sampling Event						Sampling Event			
	1		3		5		4		7	
	Low N	High N	Low N	High N	Low N	High N	Low N	High N	Low N	High N
DMPP -	2.57	2.82	2.88	3.34	2.92	3.60	2.75	3.29	3.05	3.61
DMPP +	3.06	2.99	3.31	3.56	3.41	3.53	2.99	3.47	3.33	3.56

Table 19: Significance (p-value) of main and interaction effects for pasture N content.

	Ryegrass Trial			Tropical Trial	
	1	3	5	4	7
N Rate	0.438	0.030	0.013	0.000	0.042
DMPP	0.012	0.044	0.150	0.093	0.508
N Rate x DMPP	0.159	0.481	0.064	0.785	0.364

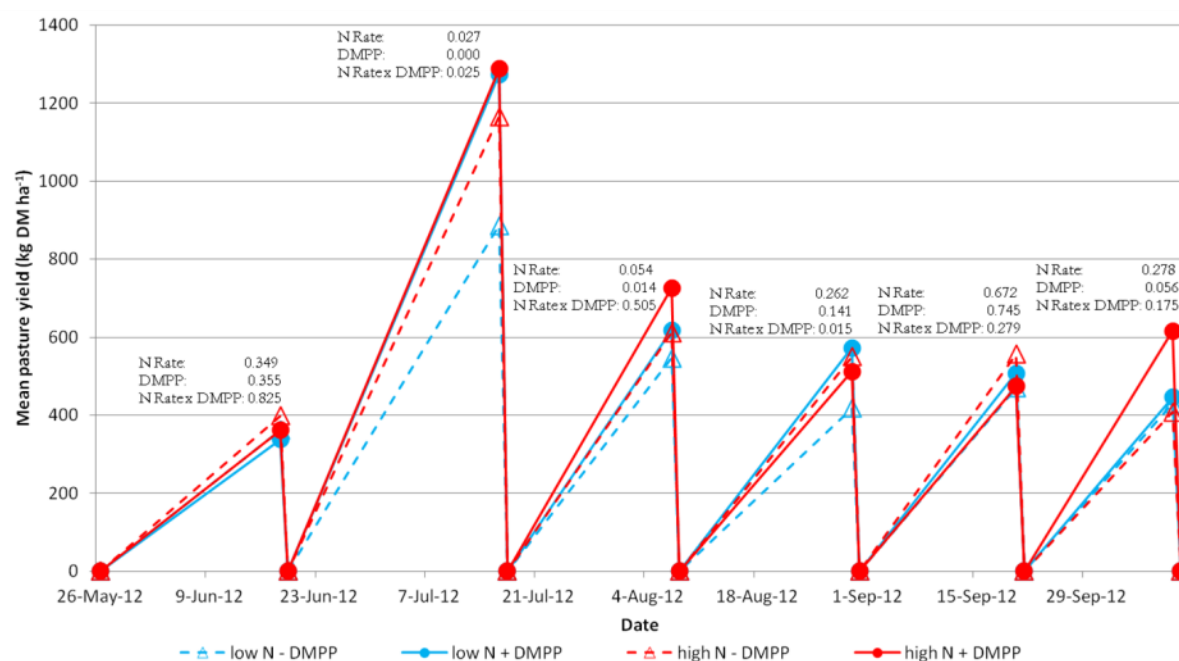


Figure 29: Mean pasture yield (DM) for each sampling event with significance (p-value) for main and interaction effect at each sampling event given (Ryegrass Trial).

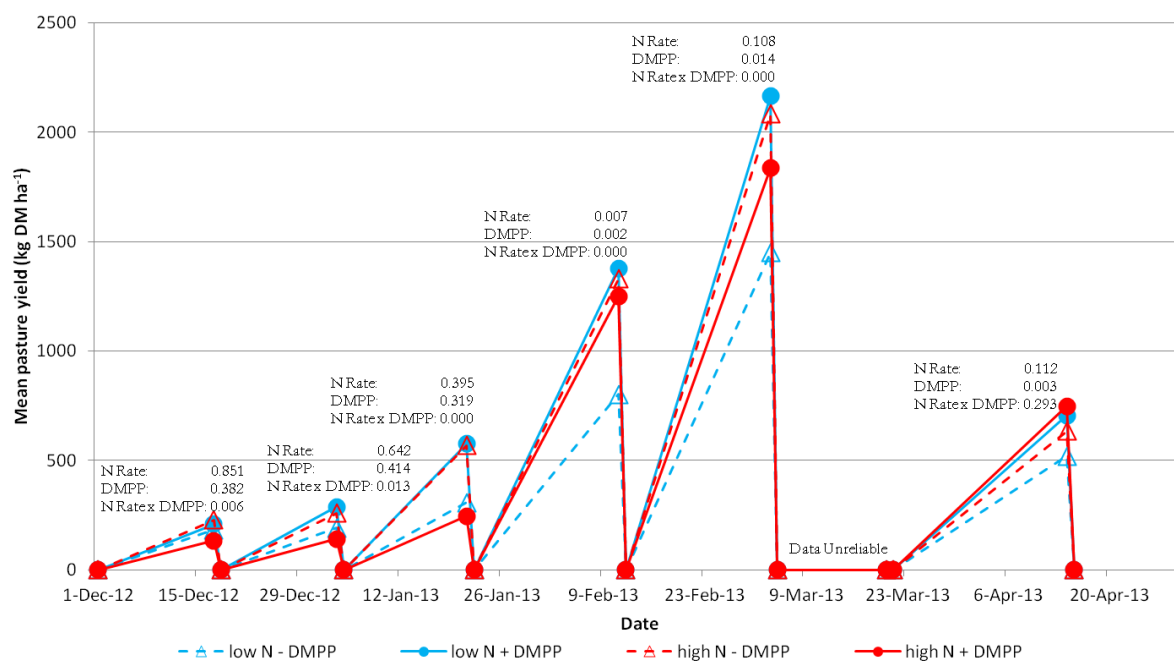


Figure 30: Mean pasture yield (DM) for each sampling event with significance (p-value) for main and interaction effect at each sampling event given (Tropical Trial).

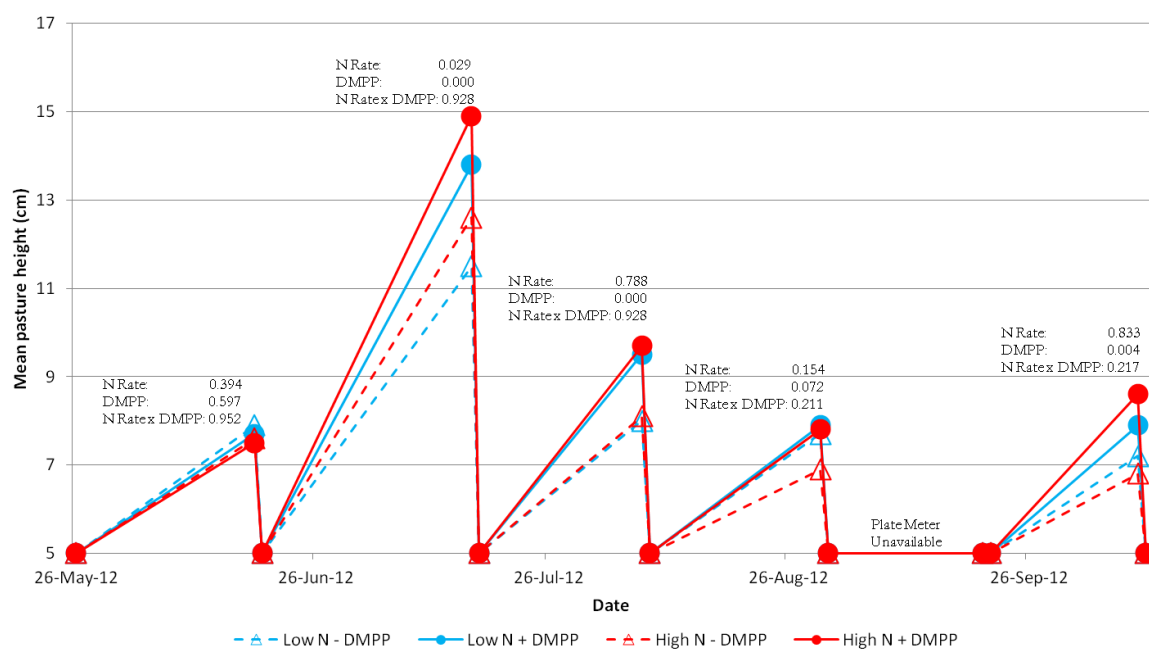


Figure 31: Mean pasture height for each sampling event with significance (p-value) for main effects and interaction effect at each sampling event given (Ryegrass Trial).

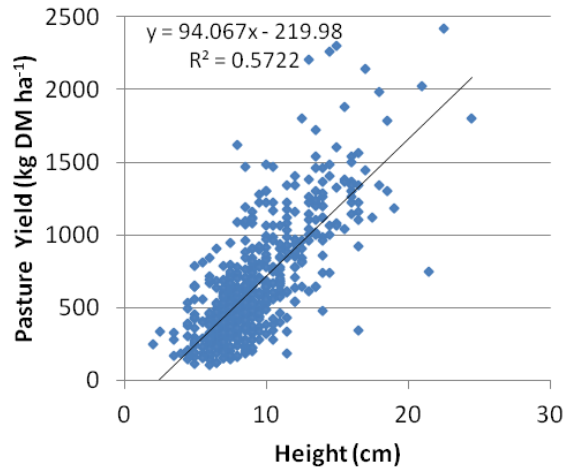


Figure 32: Relationship between pasture height and pasture yield (DM)

N balance

The partial N balance calculation showed that halving the fertiliser rate more than halved the amount of N available to be lost to the environment, especially when DMPP was also applied (Figure 33). DMPP decreased excess N at the low N rate but not at the high rate. The cumulative excess increased with time until the wet season, when fertiliser application was stopped. The annual balance estimate suggested large unaccounted-for losses in the High N treatments (Table 20). Possible explanations are gaseous losses of N that were not measured (eg. N₂, NO) or underestimates of the other losses.

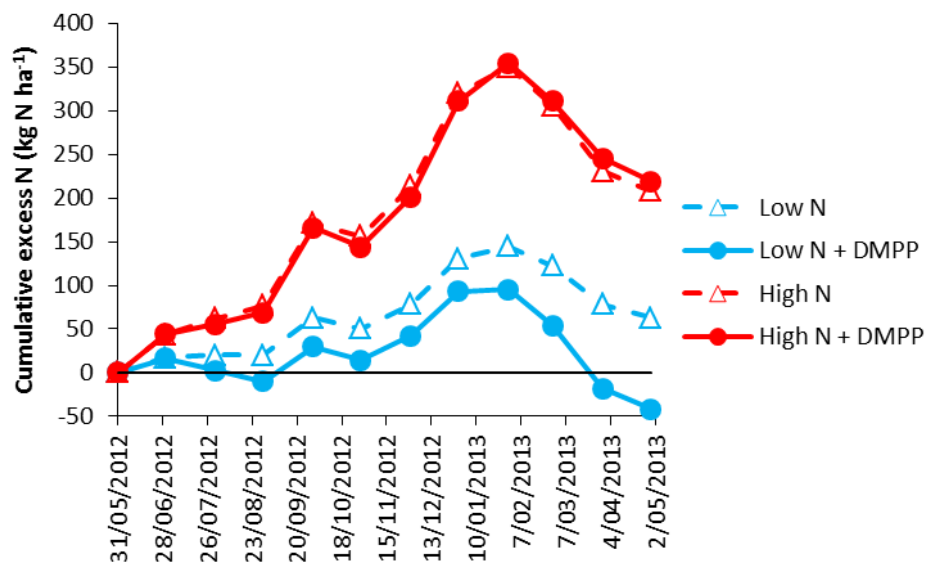


Figure 33. Cumulative excess N (fertiliser N added – pasture N harvested) over the course of a year.

Table 20. Estimate of inputs (fertiliser) and outputs of N over the course of a year.

	Fertiliser	Pasture offtake¹	Deep drainage²	Runoff	N₂O emission	Unaccounted loss
	kg N/ha					
Low N	254	128	77	0.5	7.3	42
Low N + DMPP	254	197	50	0.5	7.3	-1
High N	513	203	99	0.5	7.3	203
High N + DMPP	513	196	61	0.5	7.3	248

In the ¹⁵N labeling trials, total recovery of the N added in the labeled fertiliser, and hence the loss of added N (calculated by difference), was similar in the two trials. However, the proportion recovered in pasture versus soil was quite different (Table 21). Uptake by pasture was much higher in the Ryegrass Trial (27-40%) than in the Tropical Trial (6-12%), reflecting the difference in pasture growth between the two trials during the period of the labeling experiment. In both trials, less than 10% of the N in the pasture (collected over 4 harvests) was derived from the labeled fertiliser application.

The treatments did not have a significant effect on recovery of labeled fertiliser in pasture, nor on the amount to N lost (by difference) (Table 22). However, in both trials the proportion of fertiliser N recovered in the soil was significantly lower in the high N treatments than the low N treatments. That can be seen by the concentration of fertiliser-derived N in the soil, which was similar for the two N rates (Figure 34). N contents were below the detection limit below 0.7 m depth.

Table 21: Fate of fertiliser three months after application (¹⁵N labelling experiment).

	Tropical Trial			Ryegrass Trial		
	Pasture offtake	Soil	Lost	Pasture offtake	Soil	Lost
	% of that added			% of that added		
Low N	9.1	72.9	19.6	39.4	37.7	23.3
Low N + DMPP	6.2	66.6	27.2	27.2	45.0	17.8
High N	12.0	36.1	52.0	39.4	31.7	21.5
High N + DMPP	8.3	50.5	41.5	30.3	22.5	39.9

Table 22. Significance (p values) of treatment effects on recovery of applied fertiliser (¹⁵N labelling experiment).

	Tropical Trial			Ryegrass Trial		
	Pasture	Soil	Lost	Pasture	Soil	Lost
N rate	0.378	0.039	0.097	0.855	0.012	0.341
DMPP	0.238	0.673	0.869	0.295	0.853	0.602
N rate x DMPP	0.883	0.375	0.489	0.872	0.115	0.342

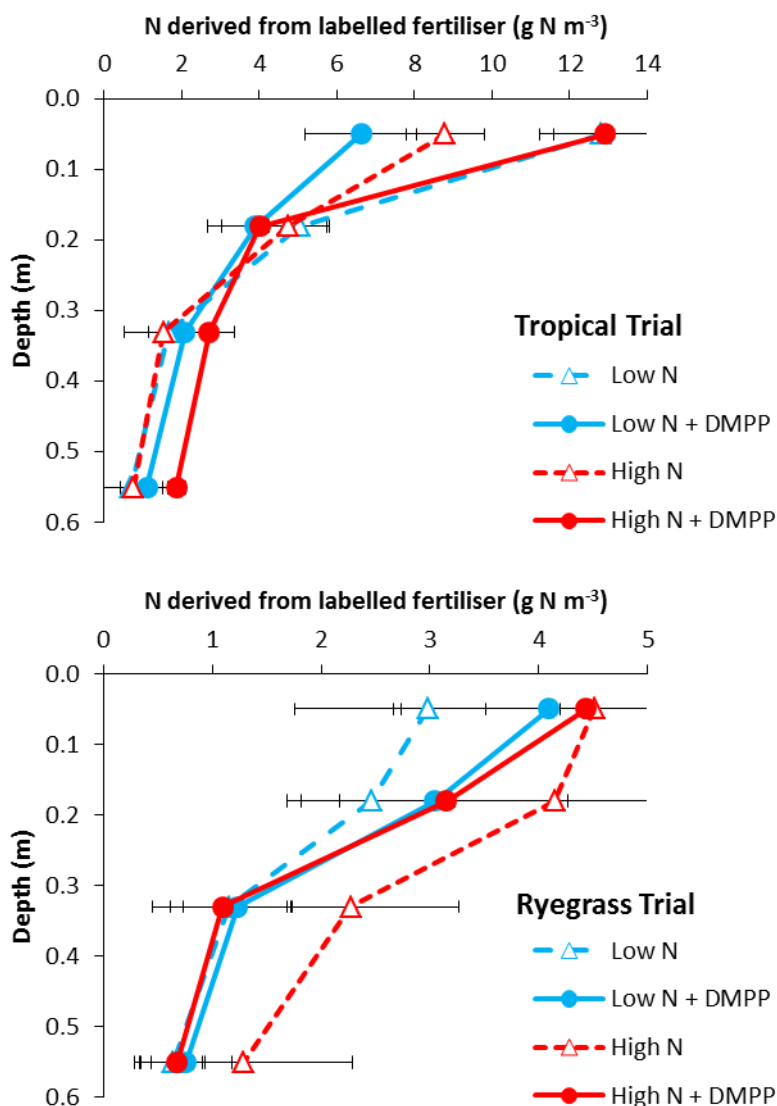


Figure 34: Distribution of fertiliser-derived N down the profile 3 months after application, in the Tropical Trial (upper) and Ryegrass Trial (lower). Error bars show standard deviation.

Gympie site, South-east Queensland

Site weather data

The 2012-13 season was characterised by extreme climatic conditions. A total of 1375 mm of rain was recorded during the measurement year, 18% higher than the 1133 mm mean annual precipitation. Despite a wet start, rainfall during and immediately following the fertilisation period (June to October) was unusually low. Below average rainfall was recorded from all months between August until late January, and below the 5th percentile for August and December (Figure 35). Rainfall was supplemented with 200 mm of irrigation during fertilisation but ceased in late November due to water shortages. This resulted in poor summer pasture growth with widespread pasture death and a subsequent lengthening of the typical grazing interval between November and January (Figure 36).

In comparison, in the 6 weeks from late January to early March over 1000 mm of rain had fallen at the site, 80% of the annual precipitation. This has resulted in widespread flooding

and difficulties in accessing the site (Figure 35, Figure 36), although no equipment was damaged during this extreme weather event.

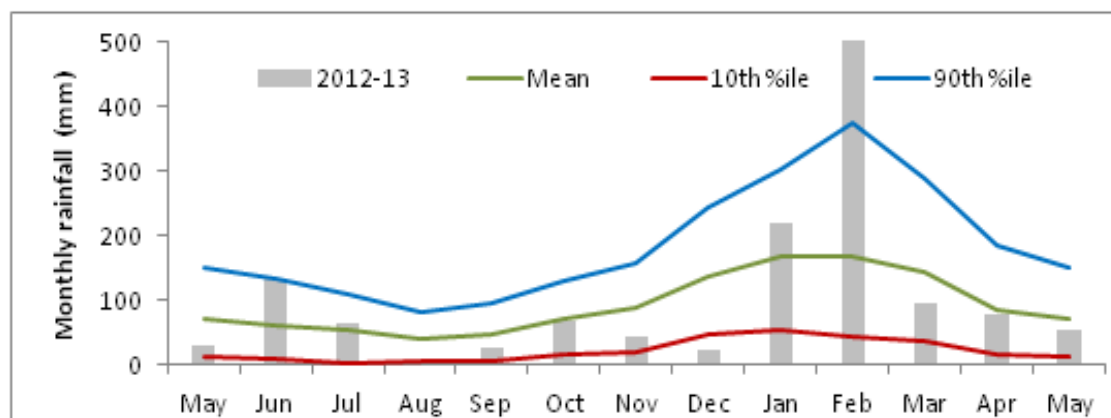


Figure 35: Monthly rainfall recorded at the Gympie site for the 2012-2013 measurement year as well as the mean, 10th percentile and 90th percentile (1870-2013) taken from the nearby BOM climate station.



Figure 36: Pasture death due to unseasonably low rainfall, December 2012. Sole bridge to access site following floods in February 2013.

Soil Mineral Nitrogen

Despite the addition of up to 333 kg N ha^{-1} over the course of the experiment no clear influence of fertilisation on the mineral N pool was evident. No significant difference ($P > 0.05$) was evident between the DMPP and urea treatments for either NO_3^- or NH_4^+ . Variability between and within treatments was lowest during the spring ryegrass period and increased greatly over the summer. Nitrate dominated the mineral fraction of the soil N pool during the wetter periods after sowing and late summer while NH_4^+ was dominant in the spring. Concentrations of NO_3^- in both high N treatments increased substantially during the early January to over 50 kg N ha^{-1} and by late January 2013 all treatments ranged between $30\text{--}60 \text{ kg NO}_3^- \text{ N ha}^{-1}$.

Profile mineral N concentrations were collected at each plot immediately prior to ryegrass seeding and are averaged in Figure 38 along with the profiles from each treatment at the end of the experiment in April 2013. Mineral N at depth from pre-fertilisation displayed a

similar concentration profile to the post experiment 0N treatment, with surface NO_3^- below 20 kg N ha^{-1} dropping to below 5 at depth. This pattern was similar across the remainder of the treatments, with NO_3^- concentrations between 35 and 55 kg N ha^{-1} recorded in the top 10 cm dropping rapidly to below 10 kg N ha^{-1} at depth. Ammonium N by comparison increased with depth across all treatments (including the zero N), with up to 73 kg N ha^{-1} measured between 50 and 100 cm soil depth.

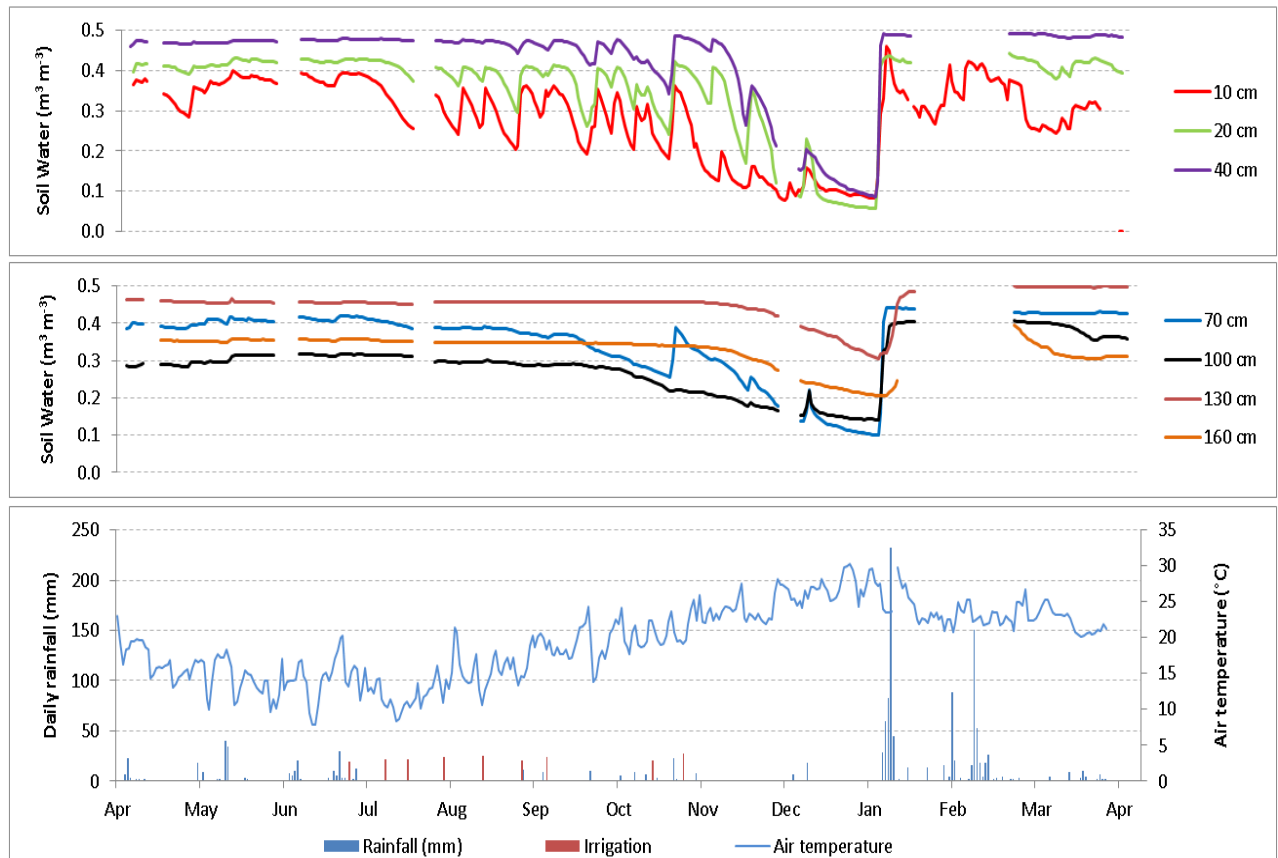


Figure 37: Daily rainfall, irrigation, air temperature and soil water content to 160 cm. “x” represents timing of leachate sample collection at 40 cm and 70 cm depths.

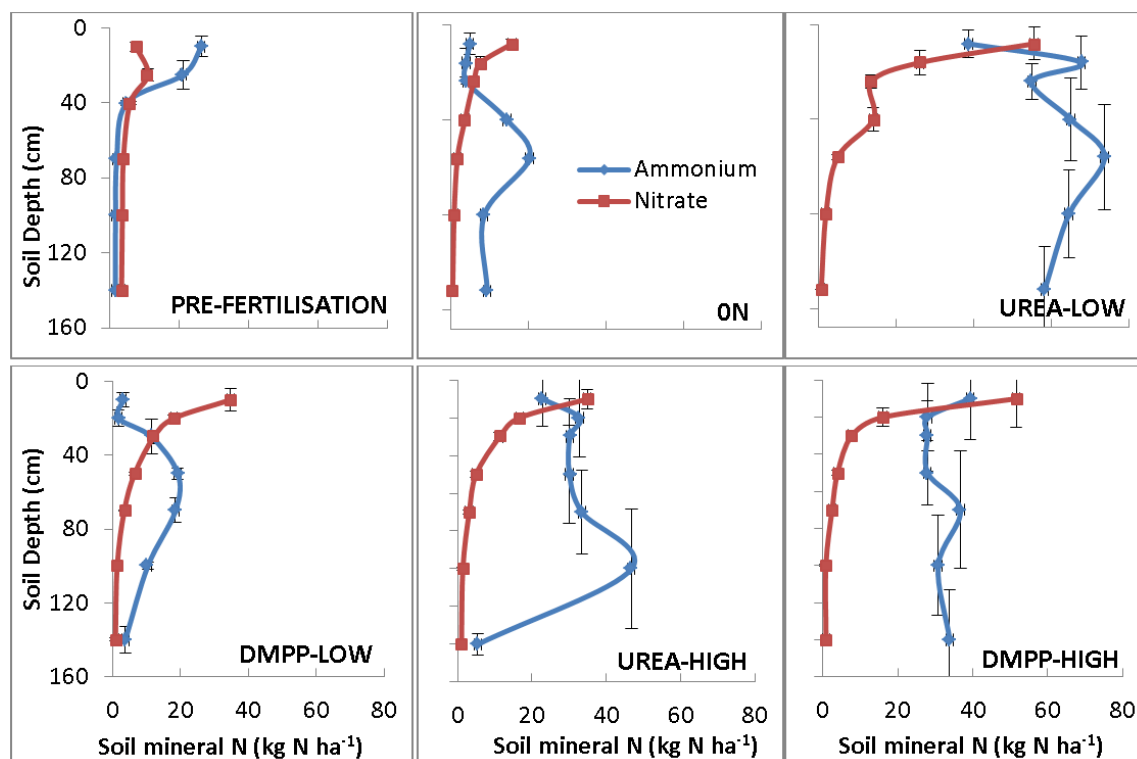


Figure 38: Soil profile mineral nitrogen (Nitrate and ammonium) from the commencement (pre-fertilisation, 26/04/2012) and at the cessation (9/04/2013) of the experiment for each treatment from a dairy pasture at Gympie, Queensland.

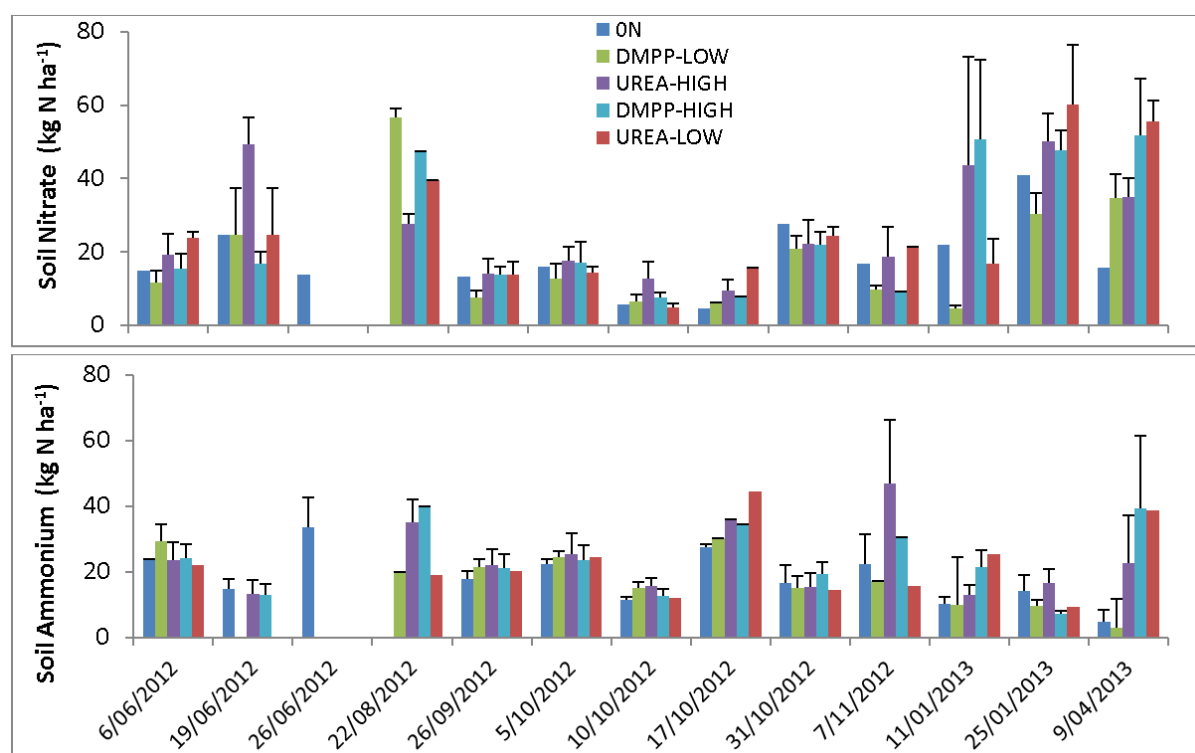


Figure 39: Soil nitrate and ammonium N (0-10 cm) from each treatment over the 26th April 2012 - 9th April 2013 measurement period from the dairy farm at Gympie.

N Losses – run-off and leaching

Runoff

Surface runoff events measured by the tipping bucket plots were recorded 5 times over 26 April 2012 to 9 April 2013 measurement period. All events occurred well outside the fertilisation period and no significant difference was observed between the two plots in either runoff volume or nutrient loads. Average values from the two plots were therefore used in any calculations.

A total of 598 mm of rainfall was lost as surface runoff, 44% of the total (Table 23). Highest losses occurred in late January and February when almost 1000 mm fell in a six-week period. The largest individual rain events of 360 mm resulted in runoff losses of 328 mm, 91% of the rain that fell.

Two large runoff events were recorded in late February and early March (Figure 40). Following the large rain event in late January, soil water in the upper soil layers was rapidly depleted below 27% VWC despite a further 16 mm from consistent light rain. An additional 62 mm of rain fell on the 18/02/2013 before runoff occurred. A total of 30 mm of runoff was recorded during this event, 22% of the 140 mm that fell over the 2 days. In comparison the large, 240 mm rain event 6 days later initiated runoff after only 30 mm. Over 95% of the rain that fell ran off. A maximum runoff rate of 25 mm per hour was recorded during this event.

Nutrient concentrations were collected for 3 of the 5 events. Highest NO_3^- and PO_4 concentrations occurred at the start of the large 27 January 2013 event. The autosampler during this period was set to collect one subsample for approximately every 1 mm m^2 of runoff. Twenty-four samples were collected by the autosampler during the initial runoff period. Concentrations varied widely but generally increased from 5 to 39 mg L^{-1} of NO_3^- -N over the first 14 mm of runoff before decreasing to 25 mg L^{-1} after 24 mm. Losses of total and dissolved (PO_4) P averaged 1.6 mg PO_4 -P L^{-1} over the same period. Due to the extremely large rain event this only represented 7% of total runoff and equipment failure prevented a bulk sample from being collected. The total N loss from other events was calculated by multiplying the average nutrient load per L by the total volume recorded by the tipping bucket runoff plots. To generate a total loss from the January event a polynomial curve was fitted to the limited data which estimated N contents dropped to near zero after 37 mm of runoff whereas P losses were assumed constant. This resulted in an estimated 26 kg N and 10 kg P ha^{-1} . However it must be noted the considerable error associated with this estimate.

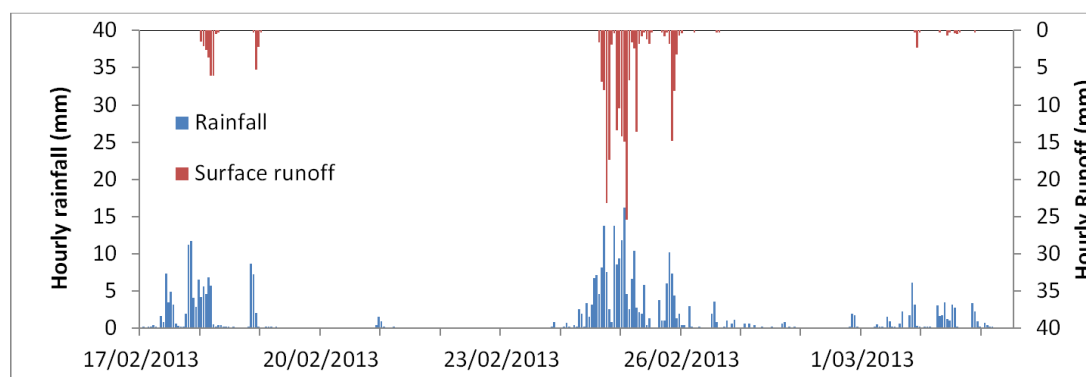


Figure 40: Hourly rainfall (mm) and surface runoff for 2 large runoff events in February 2013 from the dairy pasture at Gympie, Queensland.

Table 23: Total rainfall, surface runoff and nutrient concentrations and load measured from the tipping bucket runoff plots at Gympie (n=2).

Event date	Rain (mm)	Runoff event length (hours)	Mean runoff intensity (mm hr ⁻¹)	Max runoff intensity (mm hr ⁻¹)	Total runoff (mm m ²)	Rain lost as runoff (%)	NO ₃ ⁻ (mg N L ⁻¹)	NH ₄ ⁺ (mg N L ⁻¹)	Total N lost (kg N ha ⁻¹)	Total P lost (kg P ha ⁻¹)
3/06/2012	75	3.5	2.1	3.3	7.4	10	2.6	0.5	0.23	0.33
27/01/2013	360	48*	6.8	-	328.1	91	5.6-39.3	0.0	26^^	9.8^^
18/02/2013	140	24	1.2	6.1	30.2	22	0.5 ⁺	0.0 ⁺	0.23	0.69
25/02/2013	240	47	4.8	25.4	228	95	0.5	0.2	1.28	5.22
2/03/2013	53	18	0.2	2.3	4.4	8	0.5 ⁺	0.3 ⁺	0.03	0.10
Annual	1375 [#]				598	44			27.7	16.2

* estimated event length based on rainfall data, ⁺ estimated nutrient concentrations based on similar events, [#] annual rainfall for the Gympie site. ^^ estimated from NO₃⁻ concentrations generated during the first 7% of the event only.

Leaching

Leachate was collected twice before the commencement of the experiment in March 2012 to establish background soil nitrate values and 5 times over the 12 month measurement period. Sampling followed significant rain events or irrigation, though adequate soil water was only able to be collected from a limited number of lysimeters 4 out of the 5 times. No samples were collected from mid-September to late January due to the large soil water deficient at depth (Figure 37).

Concentrations of NO₃⁻ were consistently below the detection limit prior to fertilisation and in the 0N plots (Table 24). Concentrations increased to above 2.9 mg N L⁻¹ at 40 cm following commencement of fertilisation and were consistently lower at 60 cm. No clear treatment effect was observed. Highest NO₃⁻ concentrations were recorded following the large January rainfall event when greater than 10 mg N L⁻¹ was recorded from several lysimeters though results varied widely (0.6 – 20.9 mg N L⁻¹) at this time.

Table 24: NO₃⁻ concentrations in the soil water at 40 cm and 60 cm depth collected using suction cup lysimeters.

Sampling date	Treatment	n	NO ₃ ⁻ (mg N L ⁻¹)	
			40 cm	60 cm
9/03/2012	ON	6	0.01	0.01
20/03/2012	ON	6	0.01	0.01
14/07/2012	ON	2	-	0.02
	DMPP-LOW	2	-	1.64
	UREA-HIGH	4	2.23	1.63
	DMPP-HIGH	2	-	2.10
1/08/2012	ON	1	-	0.02
	DMPP-LOW	1	-	1.64
	UREA-HIGH	3	2.93	1.63
	DMPP-HIGH	1	-	2.10
27/08/2012	ON	2	0.05	0.01
	DMPP-LOW	2	-	0.01
	UREA-HIGH	4	2.33	2.20
	DMPP-HIGH	4	0.10	0.04
12/09/2012	-	-	-	-
25/01/2013	DMPP-LOW	-	-	-
	UREA-HIGH	2	4.78	11.08
	DMPP-HIGH	4	5.53	8.06
29/01/2013	DMPP-LOW	4	3.80	2.31
	UREA-HIGH	4	10.74	1.82
	DMPP-HIGH	4	2.89	1.79

N losses – gaseous

Annual losses of N₂O-N for 26 April 2012 to 9 April 2013 measurement period totalled between 1350 and 2190 g ha⁻¹ (Figure 41). All treatments emitted substantial N₂O-N prior to the first treatment fertiliser application on 9 June, accounting for around 20% of the annual losses (Table 25). Post-fertilisation (9 November 2012 to 9 April 2013) emissions accounted for the largest proportion N₂O losses, up to 58% of the annual total. The emission pulse observed in late January following 400 mm of rainfall, 12 weeks after the last fertilisation, dwarfed early pulses. Little treatment effect was observed during this large N₂O emission pulse.

A significant difference ($P > 0.05$) between the different rates on N₂O losses was observed only during the fertilisation period with fertilisation increasing N₂O emissions from the ON by 62% and 257% for the UREA-LOW and UREA-HIGH rates, respectively. Emissions from the DMPP-LOW treatment appeared 7% lower than UREA-LOW but the difference was not significant ($P < 0.05$).

Nitrous oxide emissions over the spatial summer study varied widely between individual chambers with average chamber emissions ranging from below 50 to almost 200 µg m² hr⁻¹ (Figure 43). The highest flux of over 900 µg m² hr⁻¹ (equivalent to 225 g N₂O-N ha day⁻¹) was recorded from one chamber in late March following a 205 mm rain event.

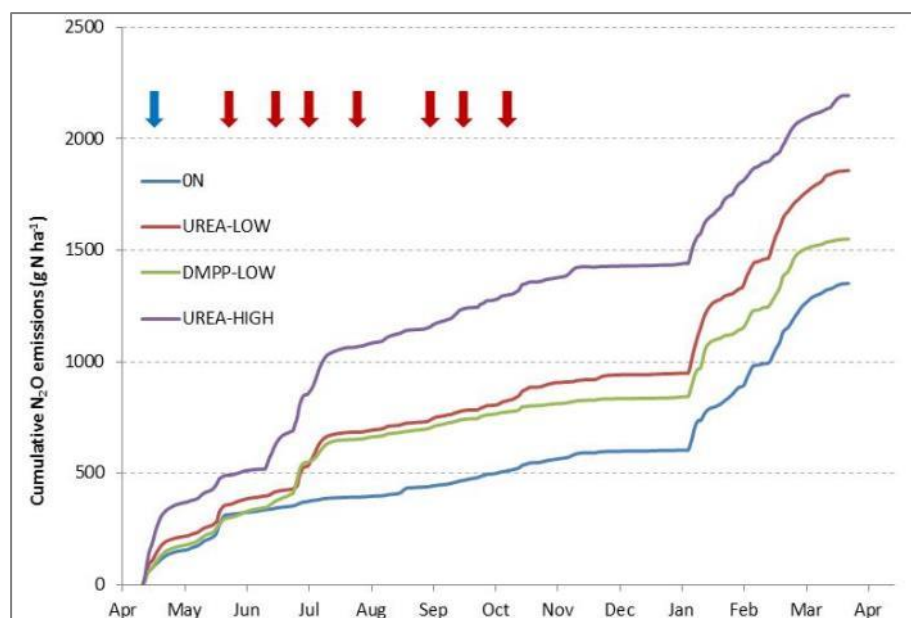


Figure 41: Cumulative emissions of N₂O (g N ha⁻¹) from the 26th April 2012 - 9th April 2013 measurement year. The blue arrow indicates ryegrass seeding and the addition of 18 kg N ha⁻¹ starter fertiliser; red arrows fertiliser applied at the relevant treatment rates.

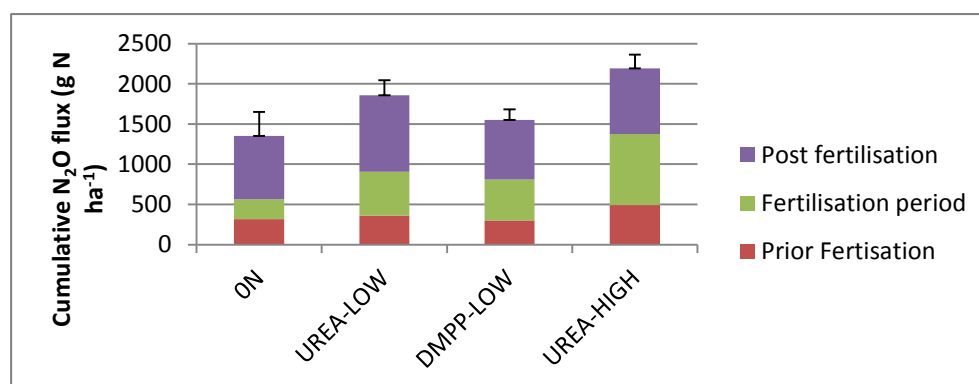


Figure 42: Cumulative totals of N₂O emissions (g N ha⁻¹) for total annual (26th April 2012 - 9th April 2013), prior to fertilisation (26th April 2012 - 9th June 2012), fertilisation (9th June - 11th November) and post fertilisation (11th November - 9th April) measurement periods.

Table 25: Cumulative N₂O emissions and significance ($P > 0.05$) for the fertiliser rate and inhibitor trial at Gympie, Queensland. Emissions are divided into total annual (26th April 2012 - 9th April 2013), prior to fertilisation (26th April 2012 - 9th June 2012), fertilisation (9th June - 11th November) and post fertilisation (11th November - 9th April) measurement periods. Like letters indicate results are not significantly different.

Measurement period flux [g N-N ₂ O ha ⁻¹ season ⁻¹]	Treatment			
	ON	UREA-LOW	DMPP-LOW	UREA-HIGH
Cumulative N ₂ O Flux	1351 ± 301 ^A	1857 ± 186 ^{AB}	1550 ± 131 ^{AB}	2193 ± 168 ^B
Pre-fertilisation N ₂ O Flux	316 ± 45 ^A	360 ± 54 ^{AB}	300 ± 22 ^A	492 ± 75 ^B
Fertilisation period N ₂ O Flux	247 ± 270 ^A	545 ± 134 ^A	511 ± 112 ^A	883 ± 132 ^B
Post fertilisation N ₂ O Flux	789 ± 184 ^A	952 ± 163 ^A	739 ± 60 ^A	819 ± 56 ^A

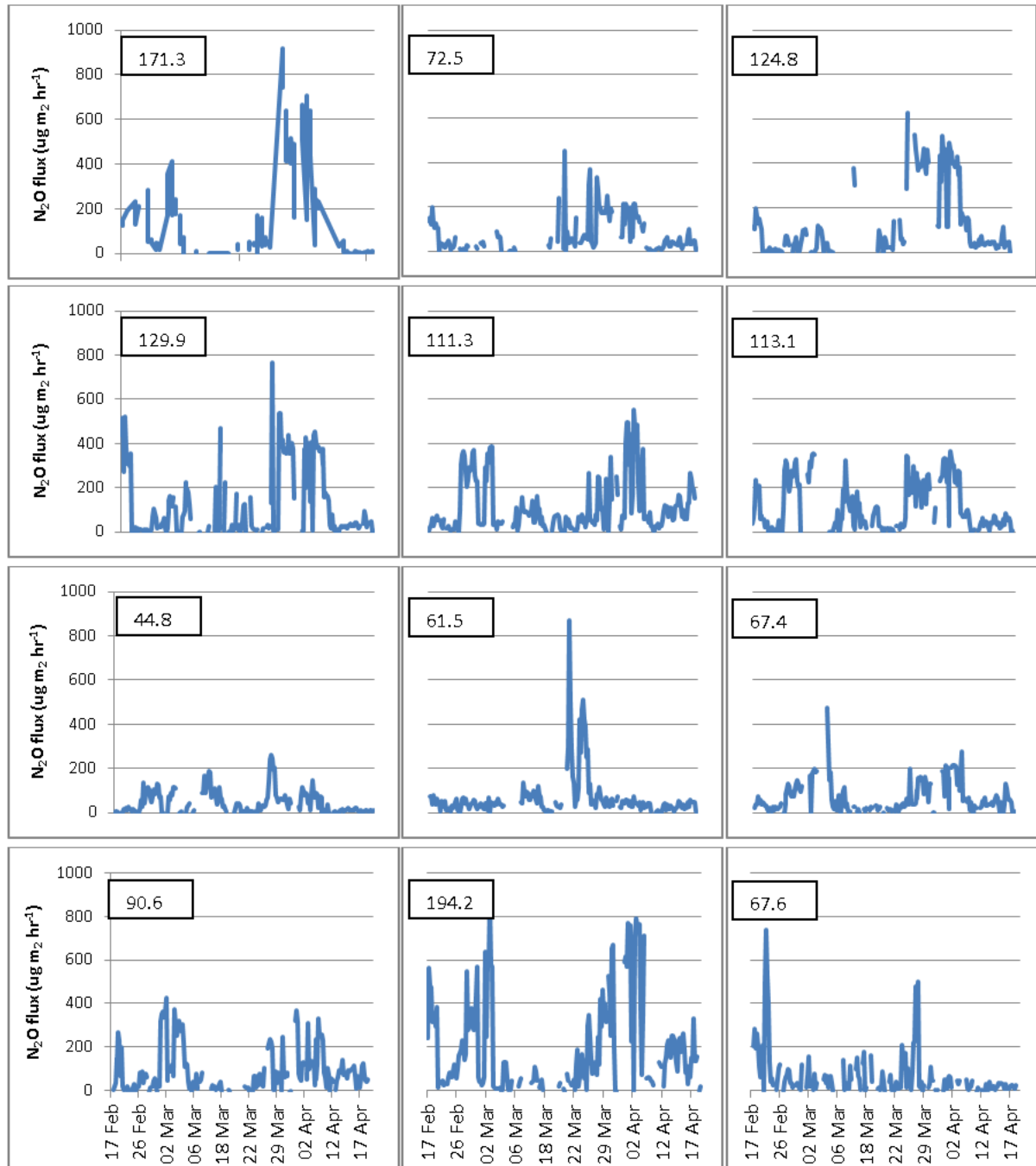


Figure 43: Spatial variability of N₂O emissions from the dairy summer pasture between February and April 2012 from Gympie, Queensland. Each graph represents hourly N₂O flux (µg N₂O-N m² hr⁻¹). Boxed text equals the mean flux from each chamber over the measurement period.

Pasture Biomass

For ease of analysis and interpretation the measurement period was divided into two distinct periods: the fertilisation periods between planting in April 2012 and mid-November and post fertilisation period between November 2012 and April 2013. These periods correlated with ryegrass being the dominant pasture species when fertiliser was applied and kikuyu and *Cynodon spp.* (African Stargrass) species dominating over the summer.

The annual ryegrass pasture was slow to establish following surface seeding on 26 April. Unseasonable warm and wet climatic conditions prior to planting resulted in higher than usual growth of the summer perennials. This limited the effectiveness of the growth restricting (heavy grazing followed by mulching) measures utilised on the perennial pasture to allow for the ryegrass establishment. As such, competition with the still active perennial pasture restricted growth of the newly planted ryegrass. Optimal ryegrass growth didn't occur until mid-June following the first harvest when cold weather ceased the growth of the summer perennials. By mid-October the summer perennials dominated the pasture composition as increasing temperatures and decreasing available moisture restricted ryegrass growth.

A linear response of pasture DM yield to increasing urea fertiliser application was observed during the fertilization period. The high rate of N produced a 75% increase in biomass production compared to the ON treatment (Table 26). Growth rates peaked in early September before leveling off as soil moisture became limiting (Figure 44). Very little impact on yield was observed between the UREA-HIGH (45 kg N ha⁻¹) fertiliser treatment and the DMPP-HIGH inhibitor treatment, though a significant ($P>0.05$) increase was observed in the reduced rate. Biomass in the DMPP-LOW (23 kg N ha⁻¹) treatment was over 18% higher than in the urea only treatment and was similar to that observed from the highest N rate treatments.

Pasture growth was highest between 6 and 26 September with a maximum daily growth of 60 kg DM ha⁻¹ recorded from both high N treatments. Little rainfall was recorded during this period though the application of almost 50 mm of irrigation and adequate subsoil moisture maintained optimum conditions. Towards the end of October growth rates declined sharply as the summer pastures began to dominate in conjunction with an increase in the soil moisture deficient. The further reduction in rainfall and lack of available irrigation supply restricted pasture growth in all treatments to almost zero from late October to mid-January. No DM cuts were made during this period. Pasture growth responded rapidly following the late January rain event, though no treatment effect was evident during this time.

The cumulative nitrogen harvested in the pasture DM followed a similar response to pasture growth (Figure 45). Nitrogen DM removal at the end of the fertilisation period was highest in UREA-high treatment, followed by the DMPP-high and DMPP-low. Although not significantly different ($P>0.05$), substantially more (>42%) N was removed from the DMPP-low treatment compared to UREA-LOW. As with the DM response, no clear treatment effect was observed during the summer period, with similar N removal from the DMPP-LOW, DMPP-HIGH and UREA-HIGH on an annual basis.

The proportion of pasture N uptake from the applied fertiliser pool (Figure 46) was determined using the ^{15}N isotope tracer. With the exception of the UREA-LOW treatment, the proportion of N derived from the fertiliser pool varied greatly over the fertilisation period. Excluding the first DM cut on the 26 June, the lowest fertiliser utilization from the DMPP treatments occurred between the July and August cuts. The highest proportion was taken up by the UREA-HIGH treatment in late September when up to 60% of the plant N was fertiliser derived. This also corresponded with the highest growth rates and high crude protein (CP) concentration (Figure 47). Pasture fertiliser uptake declined rapidly following the cessation of fertilisation, though was still contributing between 6 and 13% of the removed N over 200 days later.

The optimal dietary N requirement for dairy pastures when pasture CP is not considered limiting was determined by Tamminga *et al.* 1992 and Van Vuuren *et al.* 1993 to be between 15 and 19% CP. Crude protein (%N x 6.25) during the fertilisation period was either at the upper end or above this range. The exception to this was in late November when DM production had virtually ceased due to low soil water and an unexplained dip early September. Protein levels throughout the summer were at the lower end of the recommended range and dropped sharply following the large rain events in February, before rising again in March and April. No significant difference ($P>0.05$) was determined between treatments though CP content in the UREA-HIGH was consistently larger.

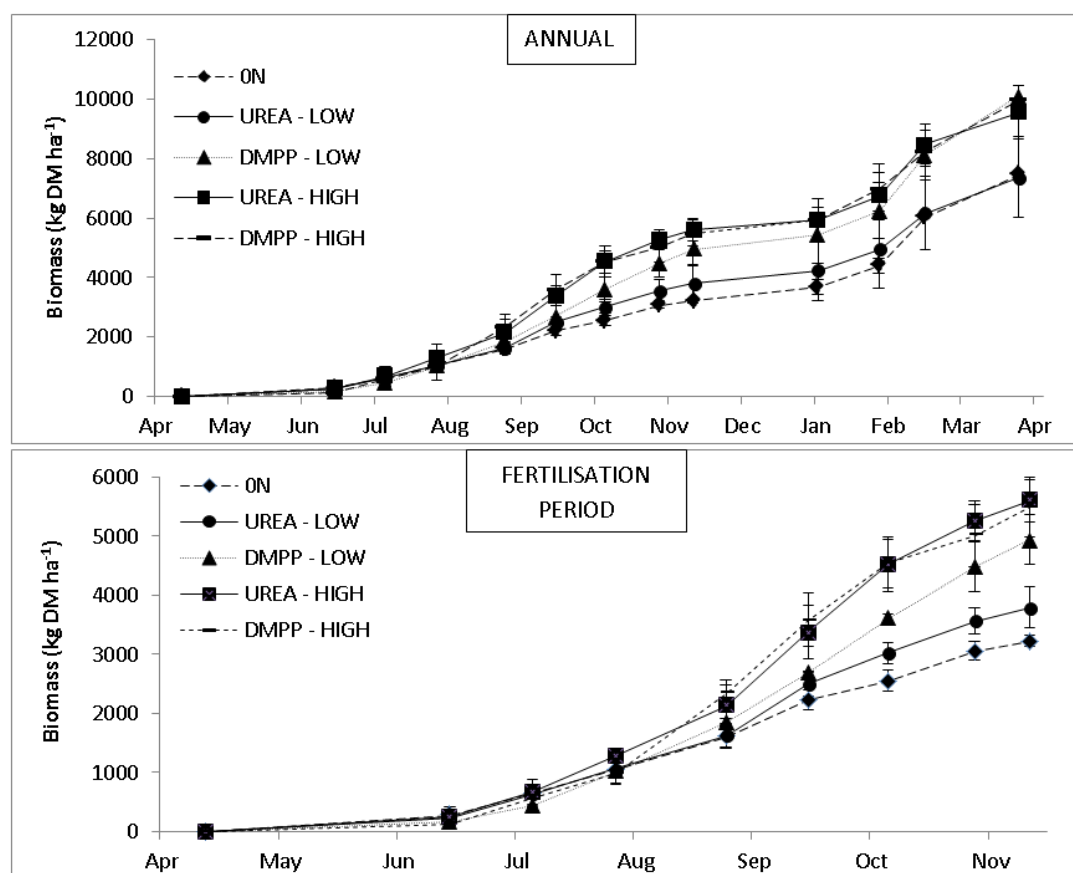


Figure 44: Cumulative pasture biomass production from the 26th April 2012 - 9th April 2013 measurement year and the ryegrass fertilisation period (June-October).

Table 26: Cumulative dry matter (DM) yield and significance ($P > 0.05$) for the fertiliser rate and inhibitor trial at Gympie, Queensland. DM yields are divided into total annual (26th April – 9th April 2013) fertilization (24th May – 11th November) and post-fertilisation (11th November – 9th April) measurement periods. Like letters indicate results are not significantly different.

Measurements	Treatment				
	ON	UREA-LOW	DMPP-LOW	UREA-HIGH	DMPP-HIGH
Fertiliser added [kg N ha ⁻¹]	0	179	179	333	333
DM - Fertilisation [Mg ha ⁻¹]	3.2 ± 0.2 ^A	3.8 ± 0.2 ^A	4.9 ± 0.4 ^B	5.6 ± 0.4 ^B	5.5 ± 0.5 ^B
DM - Post Fertilisation [Mg ha ⁻¹]	4.2 ± 0.3 ^A	4.8 ± 0.1 ^A	7.5 ± 0.1 ^B	6.4 ± 0.7 ^{AB}	6.0 ± 1.0 ^{AB}
DM – annual [Mg ha ⁻¹]	7.5 ± 0.3 ^A	8.6 ± 0.1 ^A	12.5 ± 0.3 ^B	12.0 ± 0.9 ^B	11.4 ± 0.5 ^B

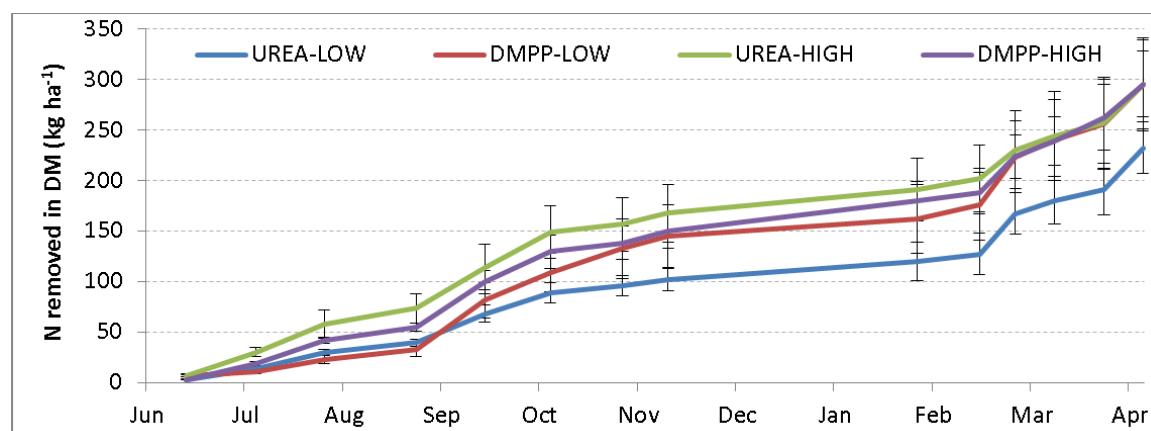


Figure 45: Total N removed in the pasture dry matter (DM) over the 26th April 2012 - 9th April 2013 measurement period from the dairy farm at Gympie.

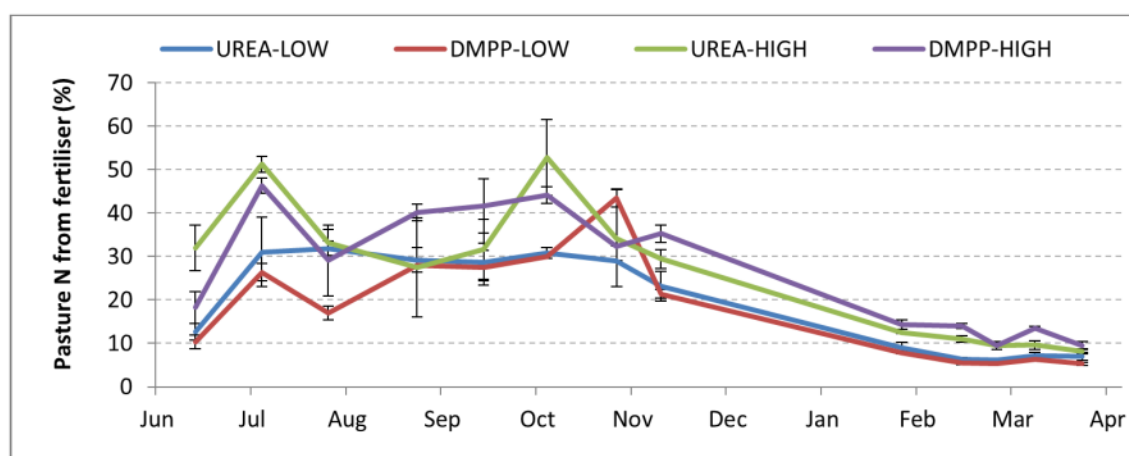


Figure 46: Total N removed in the pasture dry matter (DM) over the 26th April 2012 - 9th April 2013 measurement period from the dairy farm at Gympie.

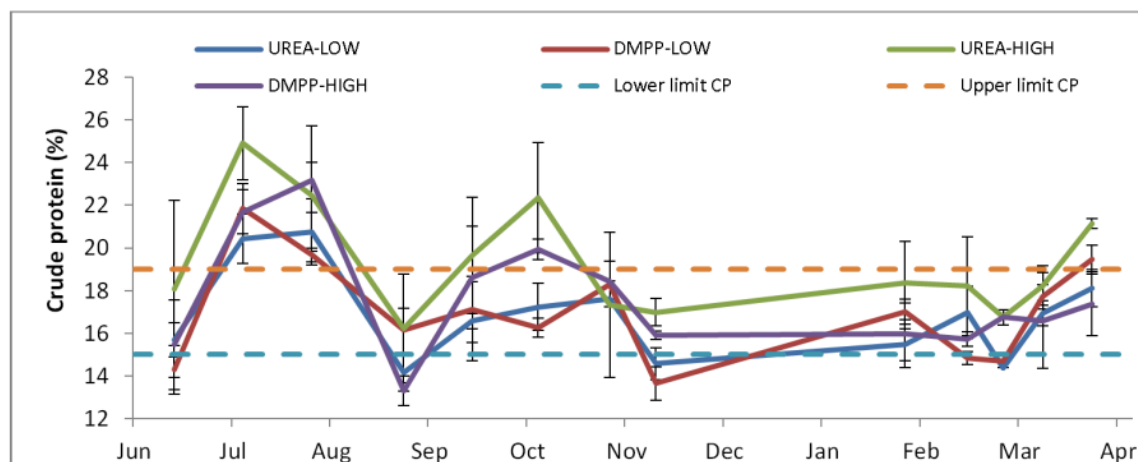


Figure 47: Estimated pasture crude protein (CP%) for the Gympie dairy site from the 26th April 2012 - 9th April 2013 measurement year. Upper and lower CP limits are based on recommended dietary N requirements for dairy cows (Tamminga *et al.* 1992, Van Vuuren *et al.* 1993).

Fertiliser N recovery

The summary of the ^{15}N fertiliser recovery is given in

Table 27. The nitrogen use efficiency (NUE) of the DMPP inhibitor was greater at both rates, though was only statistically higher ($P > 0.05$) than urea at the lower DMPP rate. The DMPP-LOW rate had the highest NUE of 30.6%, followed by the UREA-LOW (25%), DMPP-HIGH (23%) and UREA-HIGH (20%) respectively. A substantial proportion of the applied fertiliser remained in the soil pool in all treatments.

The highest soil recovery was from the UREA-LOW treatment where up to 37% of the applied fertiliser N was retained, though variability was high. Over 85 kg N ha⁻¹ of the applied fertiliser was measured in the soil pool from the DMPP-HIGH treatment. The vast majority of this was associated with the top 10 cm of soil, which contributed over 75% of the soil fertiliser N pool in all treatments (Figure 48). None of the applied ^{15}N fertiliser was recovered below 70 cm and the highest recovery at 50 cm was 2.2 kg N from the UREA-HIGH treatment. Surface litter and large roots also contributed to the fate of the applied fertiliser N, accounting for up to 10% and 4.5 % respectively.

Total N recovery in the pasture DM, litter, roots and soil was highest in the UREA-LOW treatment, closely followed by the DMPP-LOW treatment. This represented 73% and 68% of the applied N respectively and was substantially higher than the both high fertiliser treatments where recoveries were around 50%. As such, highest losses came from these treatments, with 154 kg ha⁻¹ of N unaccounted for in the UREA-HIGH treatment (49% of total), 131 kg N ha⁻¹ in the DMPP-HIGH (42%), and 51 and 43 kg N ha⁻¹ in the UREA-LOW and DMPP-LOW treatments.

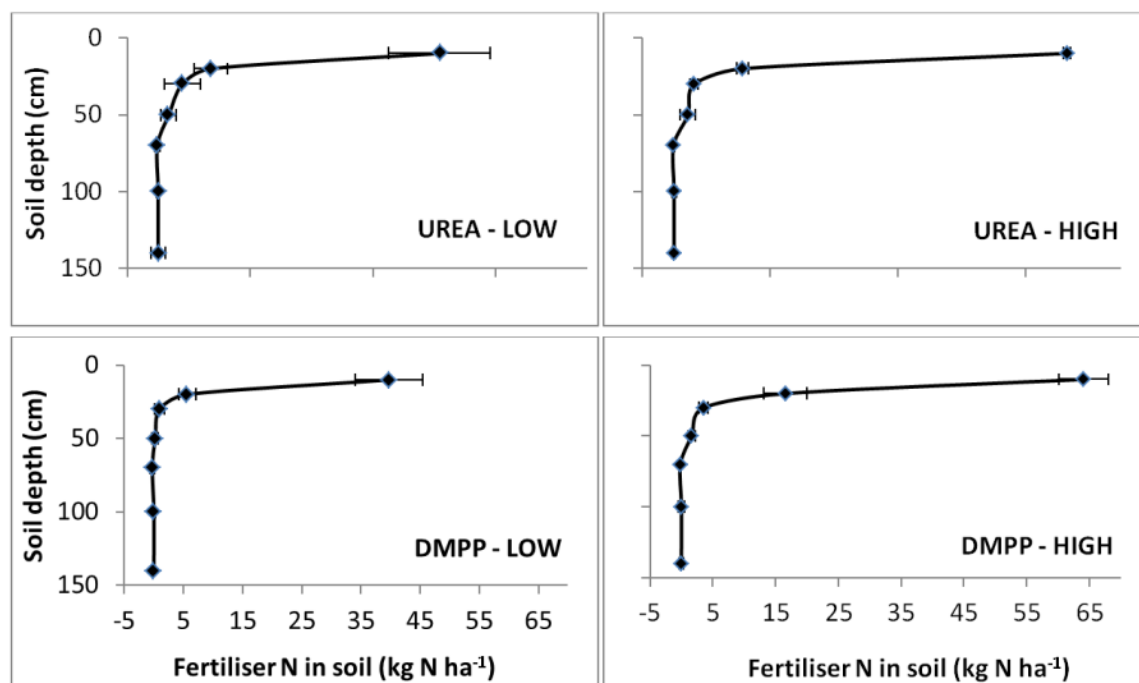


Figure 48: Soil recovery of fertiliser applied N from the UREA-LOW, DMPP- LOW, UREA-HIGH and DMPP-HIGH treatments using ^{15}N abundance at the end of the 12 month study on April 9th 2013.

Table 27: Fate of applied fertiliser as determined by ^{15}N isotope recovery over the 26th April 2012 - 9th April 2013 measurement period from the dairy farm at Gympie. N losses were determined by subtracting the sum of recovered N in DM, litter, roots and soil from the total N added.

	UREA-LOW		DMPP-LOW		UREA-HIGH		DMPP-HIGH	
	kg N ha^{-1}	%	kg N ha^{-1}	%	kg N ha^{-1}	%	kg N ha^{-1}	%
<i>Fertiliser N added</i>	161		161		315		315	
Pasture DM	39.6 ± 4.1	25.0	49.3 ± 5.5	30.6	61.76 ± 7.2	19.6	73.2 ± 4.4	23.2
Surface litter (0-2cm)	17.1 ± 8.7	10.6	12.6 ± 3.0	7.8	19.2 ± 4.6	6.1	19.8 ± 7.4	6.3
Large roots (0-20 cm)	1.6 ± 0.1	1.0	1.9 ± 0.1	1.2	2.3 ± 0.5	0.7	4.5 ± 0.8	1.4
Soil (0-160 cm)	60.0 ± 17.0	37.3	46.3 ± 9.0	28.0	77.5 ± 4.0	24.1	85.7	26.6
Total Recovery	118.3 ± 30.0	73 ± 19	110.1 ± 17.6	68 ± 11	160.8 ± 18.1	51 ± 6	183.2 ± 12.6	58 ± 4
Losses	42.7 ± 30.0	27 ± 19	50.9 ± 17.6	32 ± 11	154.2 ± 18.1	49 ± 6	131.8 ± 12.6	42 ± 4

Table 28: Summary of N losses (kg N ha⁻¹) from a fertilised dairy pasture near Gympie, Queensland.

	ON	UREA-LOW	DMPP-LOW	UREA-HIGH	DMPP-HIGH
N losses*	-	43	51	154	132
Runoff	28	28	28	28	28
Leaching	minimal	minimal	minimal	minimal	minimal
N ₂ O	1.4	1.9	1.6	2.2	-
Total loss	29.4	29.9	29.6	30.2	30.2
Unknown	-	13	21	124	102

* as determined by the ¹⁵N isotopic tracer study.

Discussion

Water N losses

Ravenshoe

At the Ravenshoe site, ET and deep drainage were the most significant outputs of the soil water balance, while runoff was insignificant. ET was the dominant process, accounting 70.5% of rainfall+irrigation at the Ravenshoe site. This indicates that the fairly even distribution of rainfall+irrigation over time maintained sufficient water in the root zone for plants to efficiently transpire. Deep drainage was estimated to account for 37.2% of rainfall+irrigation. While this was high compared to runoff (< 1% of rainfall+irrigation), other studies in the Wet Tropics have found deep drainage to account for a far greater proportion of the water balance. For example, Prove et al. (1997) found drainage below 0.6 m to account for up to 78% of the total water balance under pasture in the Johnstone River Catchment. It must be noted, rainfall (mean of 2717 mm) was significantly higher in Prove et al. (1997), compared to rainfall+irrigation in this study (949.8 mm), and included the wet season months of December-April. Under these conditions, deep drainage is expected to be higher. In the Wet Tropics, deep Ferrosol profiles (1 – >10 m) (Cotching, 1995), which have generally high infiltration rates and hydraulic conductivities (Bonell and Gilmour, 1978; Bonell *et al.*, 1983), together with high rainfall intensities, provide favourable conditions for deep drainage (Rasiah and Armour, 2001). Runoff on the other hand is minimised, as water is able to rapidly infiltrate the soil. Only during extreme rainfall events, such as those associated with cyclones, will the infiltration capacity of Ferrosol soil be exceeded, and runoff produced (Bonell and Gilmour, 1978; Bonell *et al.*, 1981). In the Wet Tropics, favourable deep drainage conditions in Ferrosol soils increase the susceptibility of N to leaching loss.

Only in one leaching event was sufficient sample collected for the effects of the treatments to be analysed statistically. Although leaching loss appeared to be correlated with N rate, and decreased by addition of DMPP, the effects were not significant due to high inter-plot variability. Such variability is often a problem when using suction cups to collect samples. Interestingly, the results from the early leaching events indicated that <15% of the total N leached after one fertiliser application was in the NO_3^- form, suggesting most of the N leached was organic. This was surprising, given that leaching loss of N from agricultural lands is generally associated with NO_3^- (van Kessel *et al.*, 2009). However, there is an increasing recognition that the leaching of dissolved organic nitrogen (DON) can be an important N loss pathway from agricultural systems (Murphy *et al.*, 2000; van Kessel *et al.*, 2009). In light of these findings, future leachate sampling and analysis should include more detailed investigations of the DON component of leached N, as this could potentially be a significant N loss pathway under pasture in the Wet Tropics. Nevertheless, the results suggest negligible NO_3^- leaching occurred over the dry season (maximum of 0.2 kg N ha⁻¹). These results are in line with those of Prove et al. (1997) who found mineral nitrogen measured in drainage water under pasture to indicate little N moved below 0.6 m. Further evidence for negligible NO_3^- leaching in present study was that after three fertiliser applications, the soil NO_3^- -N concentration below the root zone was low (<3 mg kg⁻¹). These results are in line with those of Rasiah and Armour (2001), who found soil NO_3^- -N concentration below the root zone of fertilised pasture near Malanda, to be below the detection limit (<0.1 mg kg⁻¹).

While the loss of fertiliser NO_3^- appeared to be low, it must be kept in mind that rainfall was substantially below long-term averages for most of the wet season, except for January due to the one large event that occurred. Therefore, to quantify the effects of the treatments on N leaching, the experiment should be carried out over a longer period.

Gympie

In contrast to the Ravenshoe site the major water loss mechanism at the Gympie site was runoff. This was dominated by two extremely large runoff events in late January and February, which together accounted for over 40% of the annual water budget (excluding irrigation). This is at the upper range of the 15-59% of annual rainfall lost as runoff described by Ciesiolka et al. (1995) at a nearby pineapple farm but much higher than the 0.4 – 11.5% lost from dairy pastures in southern Australia (Fleming and Cox, 2001).

High NO_3^- and total organic N concentrations were measured at the beginning of the January runoff event but were close to zero in the runoff event of February event one month later. This can partially be attributed to the high soil nitrate level measured and the poor condition of the pasture immediately prior to the January event. In comparison, by late February the rapidly growing pasture would have taken up any excess nitrate limiting potential losses. Due to the difficulties in accessing the site to retrieve samples during these extreme rainfall events (up to 5 days), there is a large potential for errors in sample analysis. In the case of NO_3^- however, this typically leads to a decrease in N concentration of the sample so any errors typically underestimate losses.

The two major runoff events occurred over 100 days after the last application of the fertilisation treatments. It is therefore not unexpected that there was no difference evident in nutrient load between the HIGH-UREA and ON treatment. Whatever fertiliser N remained in the soil at the time of these events was insignificant compared to that released by mineralisation from the organic matter during the preceding dry period. Indeed no treatment difference was detected in the mineral N contents at by this time.

Leaching on the other hand was considered negligible at the Gympie site. Although the experiment commenced with a full soil moisture profile, only the late January and February rain events caused soil water movement below 1 m in the medium clay subsoil. While some movement of nitrate to 60 cm depth was evident in the soil from the suction lysimeters, the deep soil sampling at the commencement and end of the fertiliser rate experiment indicated only insignificant concentrations of nitrate below 1 m. This is supported by the lack of any of the ^{15}N isotope tracer found at depth. The decline in soil moisture values during the dry periods suggests the pasture is access water and nutrients from at least 160 cm soil depth. Maximum rooting depths of kikuyu have been reported to exceed 2 m (Nie *et al.*, 2008) so NO_3^- below 60 cm is still within the active root zone.

Gaseous N losses

Ravenshoe

At the Ravenshoe site, the total amount of N_2O -N emitted during the 'tractor-spread' experiment was quite low (0.27-0.58% of applied N). Interestingly, despite being low, N_2O – N emissions were consistently higher for treatments with DMPP, at both the high and low N

rate, and cumulatively, the difference was statistically significant ($p < 0.001$). This was surprising, given the extensive literature showing that DMPP reduces emissions of N_2O (e.g. Linzmeier *et al.*, 2001; Weiske *et al.*, 2001; Suter *et al.*, 2010). Several possible explanations for the anomalous result are discussed below.

Firstly, it is important to consider soil water content and temperature at the time of measurement, as these are the main soil properties influencing DMPP's ability to reduce N_2O emission (Chen *et al.*, 2010; Menéndez *et al.*, 2012). The literature generally suggests the ability of DMPP to reduce N_2O emissions is lower at high soil water contents and temperatures. For example, in an incubation experiment on a clay loam soil from Victoria, Chen *et al.* (2010) found DMPP to slow nitrification appreciably at 15°C when the soil was at 40% and 60% water filled pore space (WFPS), but was less effective at 25°C and 60% WFPS. For the duration of the tractor-spread experiment, soil water content ranged from 0.2-0.6 $m^3 m^{-3}$, while soil temperature averaged 15 - 20°C. Within this range, DMPP is expected to be effective in reducing N_2O emissions. For example, in an incubation experiment on Pin Gin soil, Suter *et al.* (2010) found DMPP to reduce N_2O emission by 93% at a temperature of 25°C, and a soil water content of 60% WFPS. It is important to point out, higher reductions in the N_2O emissions are often observed in laboratory incubation experiments compared to field studies, due to the more even distribution of the inhibitor within a smaller soil volume (Chen *et al.*, 2010). Nevertheless, it remains unlikely soil moisture or temperature is responsible for the observed result.

In addition to soil water content and temperature, the adsorption of DMPP to inorganic soil constituents is important in influencing the efficiency of DMPP. In short term incubation experiments, Barth *et al.* (2001b) found nitrification to be less inhibited in soils with higher clay contents, in which DMPP may be adsorbed to inorganic soil constituents. Given the high clay content of the soil at the study site, it is possible DMPP was so strongly adsorbed to clay particles that it was unable to effectively inhibit nitrification. Although plausible, this would still not explain why N_2O emissions were higher in DMPP treatments. It must be noted, because of its soil-adsorption behaviour, DMPP is rarely subject to translocation in the soil profile, and that separation of the active substance from the applied NH_4^+ is unlikely (Barth *et al.*, 2001a).

Although not stated in the literature, it is possible that under certain environmental conditions, the inhibitory effect of DMPP on the organisms and enzymes involved in nitrification may be delayed until later in the nitrification process. For example, if the inhibitor came into effect during the oxidation of NO_2^- to NO_3^- , then there would be ample opportunity for N_2O loss during the earlier stages of nitrification. While research suggests DMPP inhibits only the first stage of nitrification, the oxidation of NH_3 (Weiske *et al.*, 2001; Li *et al.*, 2008; Kleineidam *et al.*, 2011), there remains much uncertainty about the specific mode of action the inhibitor has on the organisms and enzymes involved (McCarty, 1999). No attempt has been made in this study to quantify this suggestion, and it is obviously an avenue for further research.

The most plausible explanation for the anomalous result was that an uneven number of urea granules were applied to each chamber during tractor fertiliser application. As shown in the fertiliser calibration, there was an inherent degree of variation in the rate of fertiliser

application when spread via a tractor. For example surface topography, tractor travelling speed, wind speed and its direction can all influence fertiliser application rate.

To investigate this possibility further, a second shorter gas experiment was undertaken, at an increased temporal resolution and with strictly controlled fertiliser application rate. In this experiment, no statistically significant difference in cumulative N_2O -N emission between the treatments was observed ($p=0.795$). Interestingly, the second gas experiment still did not show a reduction in N_2O -N emission as a result of applying DMPP. Another interesting finding from the hand-spread experiment was an apparent diurnal pattern in N_2O emission that was not shown by the tractor-spread experiment. N_2O emissions were found to be lowest in the morning and increase throughout the day. Variations in soil temperature and water content throughout the day are suggested to account for this temporal variability (Blackmer *et al.*, 1982; Dalal *et al.*, 2003).

To fully understand the effects of DMPP and fertiliser application rate on N_2O emissions in this system, there is clearly a need for further research. Of particular importance will be to replicate the gas emission experiments, with timing of emission measurements taking into account the peak of emissions during the first day and the apparent diurnal fluctuations. Furthermore, application rate in the vicinity of the chambers should be strictly controlled and sufficient chambers should be used to account for spatial variability (Dalal *et al.*, 2003; Saggar *et al.*, 2004; Turner *et al.*, 2008).

Gympie

Nitrous oxide emissions over the measurement period were at the lower range of reported losses for temperate dairy systems (Veldkamp *et al.*, 1998; Eckard *et al.*, 2003). Considering the amount of nitrogen applied to the pasture emissions were lower than expected for tropical pastures. For example, Rowlings *et al.*, (2010) measured up to $2.1 \text{ kg N}_2\text{O-N ha}^{-1} \text{ year}^{-1}$ from a nearby unfertilized subtropical *Setaria spaciolata* and *Trifolium repens* pasture. This is only marginally lower than the $2.2 \text{ kg N ha}^{-1} \text{ year}^{-1}$ measured from the highest N rate, which received 333 kg N. This directly reflects the low soil moisture conditions experienced over the fertilisation period. Although emissions responded to fertilisation events in June and July when soil moisture was high, subsequent events from August to October resulted in virtually no response. The small (20-25 mm) irrigation applications immediately following fertiliser applications had no influence on emissions. The emission pulse observed in late January following 400 mm of rainfall, 12 weeks after the last fertilisation, dwarfed early pulses. Little treatment effect was observed during this large N_2O emission pulse.

Despite the unseasonably low soil water contents, soil NO_3^- concentrations increased by an average of 20 and 40 kg N ha^{-1} respectively for the UREA-HIGH and the DMPP-HIGH treatments between early November 2012 and early January 2013. This increased further following 95 mm of steady rain over 2 days prior to the large January 2013 flood event. The high soil moisture and temperature conditions promoted the rapid nitrification of any accumulated NH_4^+ over the preceding extended dry period to produce a flush of highly mobile NO_3^- in the surface soil. Together with the reduced capacity of NO_3^- uptake by the inactive pasture, this resulted in ideal conditions for large N losses following the additional 314 mm of rain. As such an estimated 28 kg N was lost over the following week in the surface runoff and an additional 190 to 310 g N- N_2O , up to 70% of total annual losses.

The high N₂O fluxes observed in the spatial study also demonstrate the large potential for losses over the summer periods. As with the large losses over the same period in 2012-2013, rapid mineralisation and subsequent nitrification produced large quantities of NO₃⁻ that is rapidly converted to N₂O during large rain events. While high spatial variability is inherent in all N₂O studies (Turner *et al.*, 2008), variability of losses from the spatial study was driven in part by animal behaviour. This study allowed the cows to graze the chamber bases (which were fenced off in the fertiliser rate trial), resulting not only in increased N inputs from manure and urine (up to 650 kg ha⁻¹, de Klein *et al.* 2003), and in localised compaction (Livesley *et al.*, 2008) but also in preferential grazing. Due to its higher nutritional value (CP's >20%, 11-16% for kikuyu and *Cynodon* respectively, Bogdan 1977, Fulkerson et al, 1998), the more N vigorous kikuyu was preferentially grazed in favour of the less vigorous star grass. This potentially increased N₂O losses from the *Cynodon* patches due to a combination of both higher soil water and nitrate contents. This is also evident from the large variability in the in-plot sampled mineral N that was observed across all treatments during the summer of the fertiliser rate trial.

A substantial proportion of the annual N₂O losses occurred immediately following ryegrass seeding and prior to the treatment applications. To ensure ryegrass establishment, the existing perennial pastures are heavily mulched to reduce competition. This large input of highly mineralisable organic matter into the soil released a flush of mineral nitrogen well in excess of the 18 kg N ha⁻¹ added as a starter fertiliser. The low water use and soil N uptake ability by the newly established ryegrass pasture resulted in high potential losses during this period. Both the rate of N uptake and plant water use rapidly increased once the ryegrass was fully established and emissions of N₂O subsequently dropped.

In contrast to the Ravenshoe site the DMPP created a 20% reduction in N₂O emissions compared to the urea at the low application rate. However due to large inter-treatment variability in the urea treatment this reduction was not significantly different. As the fertiliser was applied directly to the chambers from pre-weighed satchels this variability most likely resulted from the uneven establishment of the ryegrass into the mulched summer perennials, and the inherent variability in soil process described above. Moreover, the unusually dry conditions over the majority of the fertilisation period would limit the effectiveness of the DMPP inhibitor, which primarily reduces emissions from denitrification (Chen et al. 2010; Menéndez et al. 2012). Under wetter seasonal conditions, DMPP inhibitors may prove to be an effective N₂O mitigation strategy.

Pasture growth and uptake of N

Ravenshoe

At the Ravenshoe site, cumulative pasture yield was significantly higher ($p < 0.01$) for treatments with DMPP, than treatments with standard urea applications. Soil analysis showed that the DMPP successfully delayed the bacterial oxidation of NH₄⁺ to NO₃⁻; there was a significantly higher ($p < 0.05$) mean NH₄⁺-N concentration in the top 0.1 m after 3 fertiliser applications for treatments with DMPP than for those without. The mean pasture N content after 3 fertiliser applications was also significantly higher ($p < 0.05$) for treatments with DMPP. The results of this study are in line with several others that have observed

increased productivity in a variety of agricultural and horticultural crops as a result of DMPP (Zerulla *et al.*, 2001; Pasda *et al.*, 2002; Roco and Blu, 2006). The slowed production of NO_3^- might be expected to reduce N losses and thereby increase plant uptake. However, significant losses during the study period were not measured, therefore the effect on pasture growth was not due to reduced losses, but rather to something else.

The effect of DMPP on pasture growth appeared to be due to enhanced uptake of N due to greater availability of NH_4^+ in the root zone, although the influence of N and P present in the DMPP product is unclear. The results suggest the pasture is growing better by taking up N in the NH_4^+ form rather than the NO_3^- form. In plants, NH_4^+ is known to be more efficiently metabolized than NO_3^- because it does not need to be reduced when incorporated into amino-acids or other organic compounds (Haynes and Goh, 1978; Díez-López and Hernaiz, 2008). The N and P present in the DMPP may also have influenced pasture yield. At the high application rate, an additional 1 kg N ha^{-1} , and 1.2 kg P ha^{-1} was applied during each application. While no studies have investigated the influence of N and P derived from the DMPP compound on pasture yield, it may be significant. Further pasture nutrient analysis and studies of DMPP decomposition would help to elucidate possible effects of DMPP on nutrient supply.

Cumulative pasture yield was significantly higher ($p < 0.05$) for treatments fertilised with the high N rate than the low N rate. This suggests that halving the industry standard fertiliser application rate may supply less N than required for optimum plant growth. Interestingly however, the results show cumulative pasture yield to be higher for the low rate + DMPP treatment ($3752 \text{ kg DM ha}^{-1}$), than high rate - DMPP treatment ($3732 \text{ kg DM ha}^{-1}$). These results suggest that by increasing the amount of NH_4^+ available, DMPP can more than compensate for the lower rate of N applied. From an economic perspective, this is an important finding. The low rate + DMPP produced a high yield at the lowest cost ($\$30 \text{ t yield}^{-1}$). N losses would also presumably be lower with a lower rate of applied N.

It is interesting to note that the effects of the treatments differed between sampling dates. For example, while the main effects of N rate and DMPP on pasture yield were both significant ($p < 0.05$ and 0.001 respectively) after the second sampling, the significance generally narrowed with each subsequent sampling. This suggests the main effects had a greater influence on pasture yield when growth was greater, earlier in the season (perhaps in response to higher temperatures and higher soil water contents). The interaction between N rate and DMPP did not have a significant effect on pasture N content for either sampling event; the effect of DMPP on pasture N content did not depend on N rate and vice versa.

Gympie

Dry matter yields at the Gympie site were over 30% higher across all treatments compared to Ravenshoe despite the lower fertiliser application rates. Both sites however followed similar yield responses to the different treatments, with significantly higher yields at the lower DMPP rates (during the ryegrass fertilisation). Dry matter yield followed a linear response to urea fertiliser application rate from the Gympie site (Figure 49). This reflected results reported from Lowe *et al.* (2005) who measured DM yields from 7 months of annual ryegrass ranging from 3.9 t DM ha^{-1} without N fertiliser to $21.1 \text{ t DM ha}^{-1}$ with the application of $100 \text{ kg N ha}^{-1} \text{ month}^{-1}$. These same authors reported that 90% of the maximum

DM yield for annual ryegrass in the subtropics of Australia is achieved by applications of between 50–85 kg N ha⁻¹ month⁻¹. Moreover, N yield rose linearly as the rate of N application was increased from 0 to 150 kg N ha⁻¹ month. Interestingly no difference was observed between the urea and DMPP at the higher rate indicating soil water, rather than nitrogen, was the limiting factor to production.

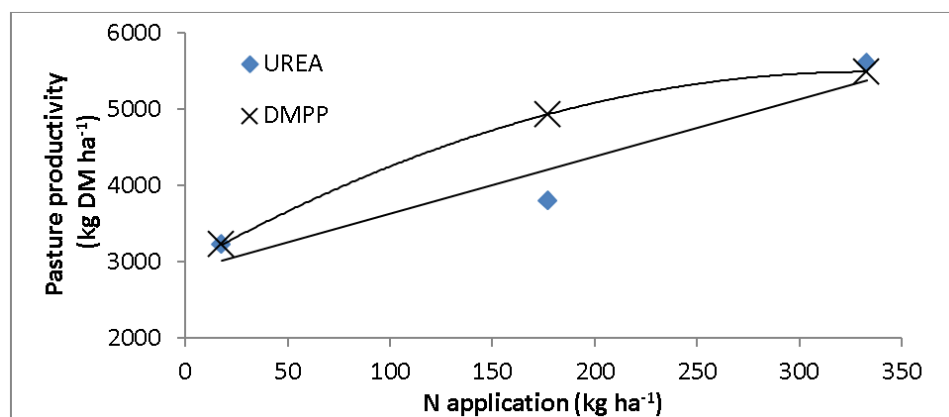


Figure 49. Response of DM yield to increased fertiliser application rate and DMPP inhibitors on a dairy pasture at Gympie, Queensland.

The highest growth rates at the Gympie site (between late August and October) corresponded to the lowest soil nitrate values as any nitrified N was rapidly taken up by the ryegrass. Ammonium N was the dominant form of N during this period suggesting ryegrass is more selective than the tropical perennial species in the form of N it takes up. Lowest NO₃⁻ concentrations were recorded over the 3 weeks following the late September fertilisation. This matched some of the highest crude protein levels and fertiliser recoveries observed throughout the measurement year. During this period, a combination of adequate soil moisture content, favourable temperatures and the well-established ryegrass plants allowed for maximum NUE.

Nitrogen Use Efficiency

The nitrogen cycle from the dairy pasture at Gympie was dominated by fertiliser application during the ryegrass season and by microbial mineralisation of the organic matter through the summer months. Up to 10 tonnes of total N (organic and inorganic) was measured in the top 30 cm of soil and an additional 90 kg in the surface litter. While some of this is tied up in the stable organic carbon matrixes in the soil, a substantial proportion would be available for microbial mineralisation. Nitrogen use efficiency is often tied to the size of the N cycle (Robertson and Groffman, 2007). Larger N cycles generally have higher potentials of N loss as the “leakiness” of the system increases. Historic fertiliser recommendations of up to 500 kg N year are common in subtropical dairy systems (Eckard *et al.* 1989, Lowe *et al.* 2005). Additional N inputs from supplementary feed excreted as manure and urine can also add significantly to the N budget.

The amount of N removed in the DM yield was substantially higher than fertiliser N inputs from both low N treatments (160 and 130% for DMPP and Urea respectively) and around 90% for the high N treatments. This is a much higher recovery than the 30–60% reported from previous work in subtropical annual ryegrass (Lowe *et al.* 2005) and the 40–50% from

topical grasses (Henzell, 1963). The use of the ^{15}N isotopic tracer however allowed for the fate of the applied fertiliser N to be examined in detail. The proportion N removed in the DM that was sourced from the added fertiliser was typically only between 30 and 40%. This suggests the remaining 60-70% was sourced from the large mineralisable N pool. Moreover, only 20-30% of the added fertiliser N was removed in the biomass over the entire measurement year, with a further 7-11% accounted for in the coarse roots and litter. As this didn't accumulate in the mineral fraction of the soil, the vast majority of the remaining applied fertiliser N ended up in the organic soil pool.

Total annual losses of the ^{15}N isotopic tracer were almost 50% for the high N treatments and up to 30% for the low N. Although the DMPP significantly increased ($P>0.05$) N recovery from the DM at the low N rate, no difference between treatments was evident for total losses. Between 40 and 150 kg of N was unaccounted. The majority of the measured N losses occurred outside the fertilisation period. The largest single loss was associated with the late January rain event as both runoff and N_2O . An estimated 29 kg N and 10 kg P ha^{-1} was lost in this single runoff event, the majority of it as dissolved NO_3^- and PO_4 . By contrast N_2O losses were small (<2 kg). During large rain events gaseous N losses typically occur from microbial denitrification, which concurrently produces both N_2O and N_2 . Di-nitrogen is notoriously difficult to measure directly in the field, though laboratory studies suggest the ratio of N_2 to N_2O during denitrification can be as exceed 200:1 if available carbon and NO_3^- are not limiting (Weier *et al.*, 1993b; Dannenmann *et al.*, 2008). This suggests that denitrification was a major N loss pathway from the dairy pasture when NO_3^- concentrations are high.

Research implications for Dairy farmers

At the Gympie site, the application of the low (23 kg N ha^{-1} respectively) urea plus DMMP treatment increased ($P>0.05$) pasture DM yields by 30% above that of the pasture fertilised with the same rates of N as urea respectively. When the significantly ($P > 0.05$) similar pasture crude protein contents of the urea and urea plus DMMP treatments are taken into account, the total yield of N ha^{-1} increased by over 42%. Moreover, the ^{15}N study component of the present trial demonstrated that a higher ($P>0.05$) proportion of plant N originated from urea N applied with DMMP. Thus, from a purely agronomic perspective, *urea plus DMMP improved N-use efficiency (NUE)*: the addition of DMMP was associated with a higher pasture N concentration and yield. *Nevertheless, these agronomic improvements in NUE become less certain when considered in terms of ruminant production.*

NUE by ruminants is determined by the availability of rumen fermentable carbohydrates (RFC) relative to rumen fermentable N (RFN). When adequate RFC is available, RFN is converted to microbial protein-N before its subsequent absorption in the small intestine. By contrast, if RFN is too high relative to RFC the former accumulates as ammonia-N in the rumen before its absorption into the blood and subsequent conversion to urea-N by the liver. This urea leaves the animal in milk and urine. An inefficient conversion of RFN to microbial protein-N in the rumen potentially loses any gains made in NUE at the agronomic level.

The NRC (1989) recommendations for the N requirements dairy cows range from 14.5% to 15.8% CP for a 600kg Friesian cow producing 23 L to 30 L milk/day respectively. In studies that measure the NUE of dairy cows grazing ryegrass pasture of different N concentrations, N becomes excessive for cows consuming 17 kg DM/day when it exceeds between 15 (Van Vuuren, 1993) to 19% CP (Tamminga, 1992). Insufficient RFC relative to RFN above these concentrations resulted in a linear decrease in NUE by these cows. Since, in the present study, increases in CP content resulted in concentrations above 19% one cannot assume that the application of DMPP improved NUE from a ruminant production perspective. Indeed, in an experiment at Mutdapilly, in South East Queensland, Moss et al. (1992) measured no response in milk production when protein rich supplements were fed to cows grazing fertilised annual ryegrass.

The above considerations caution against assuming that higher N yield, pasture N content or N recoveries equate to improved farm NUE. It seems as though several animal management practices require modification if the additional N captured in plant DM is to contribute to improvements in overall NUE. In this regard, farmers may harness the agronomic improvements in NUE associated with DMPP application by using one of three management strategies:

1. The increases in pasture CP content associated with DMPP application in both the northern and southern sites of this study suggest that farmers can apply urea at lower rates to achieve the recommended N requirements for dairy cows producing up to 27 L/cow per day. In this way, less N is applied to maintain the same level of production by dairy cows.
2. The higher pasture N concentration can be balanced by supplementing cow feed with RFC in combination with an increased stocking rate. This approach to harnessing improvements in agronomic NUE leaves the rate of N application unchanged but increases farm production.
3. The adequate levels of pasture N for cow requirements in the present study may caution farmers against feeding expensive protein supplements to dairy cows. Not only would this reduce unnecessary feed costs, but it would reduce the quantity of urinary N deposited on pasture.

Economic analysis

Economic analysis demonstrated the high urea treatment to be marginally more cost effective than both the DMPP high and DMPP low treatments at the applied rates (Table 29). The low DMPP treatment yielded a profit margin 5.5 times higher than the straight urea. At the higher rate DMPP incurred no additional yield benefit and as such was more expensive than straight urea. However, using the DM response to fertiliser rate curves developed in Figure 49, to achieve 90% of “optimal” ($45 \text{ kg Urea-N ha}^{-1}$) an additional 31 kg N ha^{-1} is required (equivalent to 25 kg N ha^{-1} per application). This is equivalent to a 66% increase in profit over urea application at the same rate. The use of the nitrification inhibitor DMPP is therefore a viable option for reducing N losses and increasing profitability when combined with optimised N application rates.

Table 29. Economic analysis of the costs/benefits of the different fertiliser applications. * DM yield increase above 0N. *Based on a conversion of 1.2L/milk per kg DM (Fulkerson 2008). #Using a milk price of 50c L⁻¹. ** Fertiliser cost delivered to farm gate in southern Queensland.

Treatment	DM yield increase* (kg ha ⁻¹ yr ⁻¹)	Additional milk production ⁺ (L milk)	Additional income [#] (ha)	Fertiliser cost ^{**} (t)	Fertiliser cost (ha ⁻¹ yr ⁻¹)	Gross profit (ha ⁻¹ yr ⁻¹)
UREA-LOW	600	720	\$360	\$536	\$202	\$132
DMPP-LOW	1700	2040	\$1020	\$694	\$261	\$758
UREA-HIGH	2400	2882	\$1440	\$536	\$388	\$1032
DMPP-HIGH	2300	2760	\$1380	\$694	\$502	\$856

Benefits and application

The use of the nitrification inhibitor DMPP has been shown to be an effective strategy for reducing N losses while maintaining or enhancing pasture yields and profitability. By closely matching plant N demand with fertiliser supply significant reductions in fertiliser application are possible, reducing N losses into the environment and increasing farm profitability.

Conclusions

Pasture yield at both measurement sites was almost as high in the low N + DMPP treatment as it was in the high N treatment. This result indicates that fertiliser N inputs can be halved from the commercial rate with no yield penalty, if DMPP is applied. DMPP significantly increased pasture yield and N content at the low N rate.

At the Gympie site, NUE dynamics of the fertilised pasture could be divided into two distinct phases, the winter ryegrass fertilisation and the summer post fertilisation period. The annual NUE budget was dominated by the unusual climatic conditions rather than any potential management decisions. Optimal pasture growth was limited in what is traditionally the prime growing season by the extended and unseasonal dry period from August to mid-January, which accentuated losses from the extreme rain event at the end of January and skewed nutrient losses substantially towards runoff. In such extreme climatic events there is little good management can achieve that will alleviate such losses. However improved NUE efficiency can still be achieved during the fertilisation period by the closer matching of N fertiliser application to plant N demand.

A clear response to fertiliser rate and the effectiveness of the DMPP inhibitor were demonstrated during the fertilisation period across a range of parameters (yield, N removed, NUE, N₂O emissions). NUE and the effect of DMPP was substantially improved at the lower rate of N application suggesting that N was not the limiting factor for production, and indeed soil water was in deficient for long periods of the experiment. As such reducing N application during periods of high water deficient may be an effective strategy for improving NUE.

Halving the fertiliser rate more than halved the amount of N available to be lost to the environment, especially when DMPP was also applied. While it was not possible to accurately allocate losses to particular mechanisms, at the Ravenshoe Red Ferrosol most appeared to be lost in deep drainage, whereas runoff and denitrification dominated losses in the heavier textured Gympie soil. DMPP did not significantly reduce N₂O emissions at either site, though a reduction was observed at Gympie. In the low N treatments most of the N was utilised by the pasture. However, in the high N rate treatments there were large losses of N that were not accounted for.

Future Directions

Further research is required to validate the preliminary pasture growth results presented in this study. For dairy producers, measurements of the pasture yield response to inhibitors are required for multiple years to account for climatic variation. Before recommendations are made to farmers, a more detailed cost-benefit analysis, which incorporates other measures of pasture quality, is required. Research should focus on not only maximising yields and reducing N losses directly from pastures, but examine the system holistically. This is of particular importance if improved N management only increases the crude protein in pastures to above the dietary intake requirements of dairy cows. In this scenario, the potential exists to simply move from N losses directly associated with fertiliser application to indirect losses from increased levels of cow excreted N.

Future research could also include the undertaking of similar trials in other production systems that drain into the Great Barrier Reef (e.g. sugarcane, bananas). This will help to improve our understanding of N loss under fertilised crops in the Wet Tropics, and the effectiveness of management practices seeking to reduce those losses. Further, given that DMPP is not yet commercially available in north Queensland, significant further research, in a variety of agricultural settings, is required to evaluate its potential.

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Appendix 1: Summary of literature

Introduction

The objective of this introductory chapter is to place this project within a broader research context, by discussing the nitrogen (N) cycle in grazed pastures, the loss of N from fertiliser application, and the management practices seeking to reduce those losses. Finally, the aims of the project are given.

Grazed pastures and the N cycle

In grazed pasture, N cycles through the atmosphere, soil, plants and grazing animals, undergoing transformations between organic, mineral and gaseous forms (Figure 1). The major N inputs to the soil-plant-animal system, include the fixation of atmospheric dinitrogen (N_2) by leguminous plant species, the mineralisation of soil organic matter, plant residues and grazing animal excreta (dung and urine), the addition of feed supplements and increasingly N fertiliser application (Ledgard *et al.*, 1999; Bolan *et al.*, 2004; Gourley *et al.*, 2012). The major N outputs from grazed pastures include plant uptake, live weight gain and milk production (Ledgard *et al.*, 1997; Gourley *et al.*, 2007; Weier and Grace, 2012). Unfortunately, there often exists an imbalance between N inputs and outputs, resulting in a large N pool that can be lost through various transformation pathways. These pathways include nitrification, denitrification and ammonia (NH_3) volatilisation (Gourley *et al.*, 2007; Weier and Grace, 2012). The following section provides an overview of the processes involved in N cycling in grazed pastures.

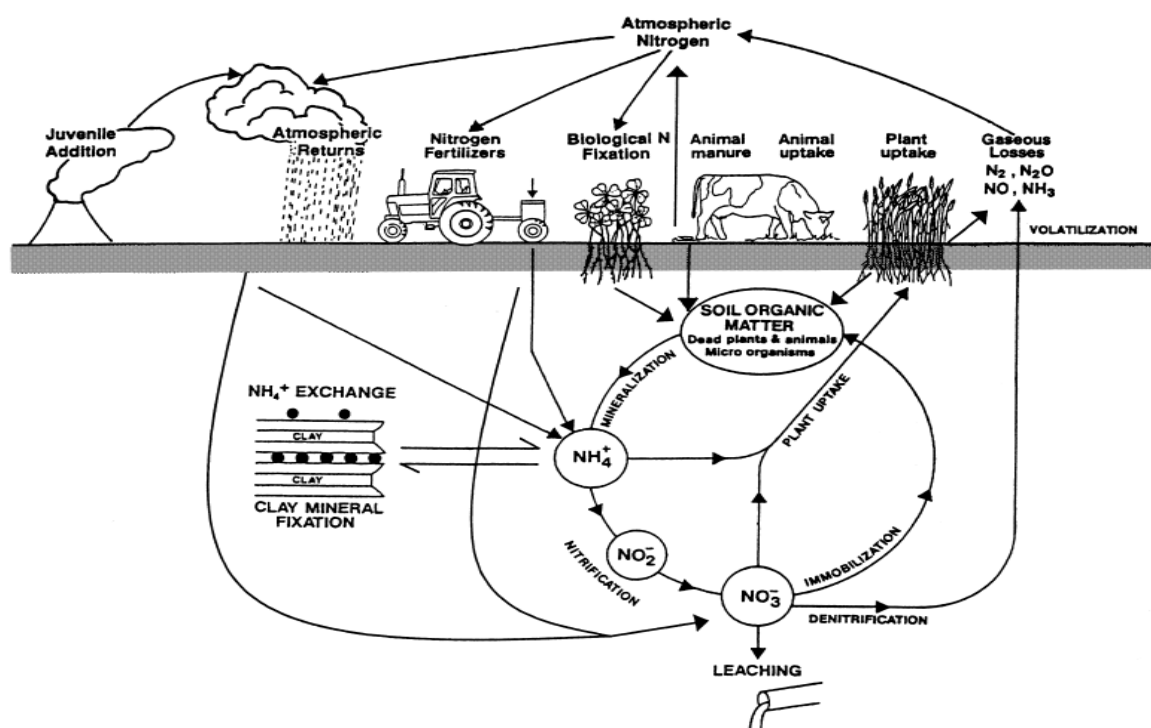


Figure 1: The N cycle in managed pastures. Source: Di and Cameron (2002a).

N fixation

N fixation is the process by which the triple bond of atmospheric N₂ gas is reductively broken to generate ammonium (NH₄⁺) (Cabello *et al.*, 2009). The symbiotic relationship between leguminous plant species such as white clover (*Trifolium repens*) and a group of bacteria known as rhizobia, provides the major source of fixed N (Ledgard, 2001). Factors influencing N fixation include the species and abundance of legume, soil and climatic conditions, nutrient supply and grazing management (Menneer *et al.*, 2004; Unkovich, 2012). In Australian dairy pastures, annual N fixation rates are generally low (< 50 kg ha⁻¹), largely due to the low legume pasture content (< 30% of total pasture biomass production) (Unkovich, 2012).

Mineralisation and immobilisation

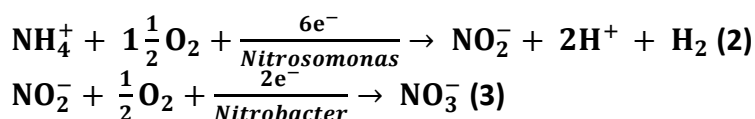
Mineralisation (or ammonification) is an enzymatic microbial process by which organic N, (derived from soil organic matter, plant residues and animal excreta), is converted into inorganic or mineral forms such as NH₄⁺ or NH₃ (Jarvis *et al.*, 1996; Schimel and Bennett, 2004). The converse process is the immobilization of inorganic N into organic forms, as microorganisms incorporate N into their cellular components (Chalk *et al.*, 1990; Bruun *et al.*, 2006). The balance of mineralisation vs. immobilisation depends largely on the carbon (C) to N ratio of the organic residues, the availability of mineral N and the degree of microbial activity (Bengtsson *et al.*, 2003).

Fertiliser application

To ensure high productivity is maintained, N fertilisers are increasingly applied to grazed pastures (Prasertsak *et al.*, 2001a; Dalal *et al.*, 2003; Eckard *et al.*, 2003). For example, more than 60% of dairy farms in south-east Australia apply up to 200 kg N ha⁻¹ year⁻¹ (Eckard, 1998), while in the north Queensland dairy industry, application rates of up to 500 kg N ha⁻¹ year⁻¹ ensure year-round production is maintained (Teitzel *et al.*, 1991; Prasertsak *et al.*, 2001a). Unfortunately, the efficiency of the applied fertiliser is often low, and plant uptake can be < 50% of the N applied (Baligar and Bennett, 1986; Prasertsak *et al.*, 2001b; Chen *et al.*, 2008; Chien *et al.*, 2009). The production of nitrate (NO₃⁻) during nitrification, N₂O during both nitrification and denitrification and gaseous NH₃ during NH₃ volatilisation, are key processes responsible for low fertiliser use efficiency in grazed pastures.

Nitrification

Nitrification is the aerobic oxidation of NH₄⁺ to NO₃⁻ (Equation 1 and 2) (Jarvis *et al.*, 1996). The process is classically carried out by two different groups of chemolithoautotrophs, known as ammonium-oxidising bacteria, and nitrite-oxidising bacteria (Cabello *et al.*, 2009). The *Nitrosomonas* species represent the ammonium-oxidising bacteria, and are responsible for the conversion of NH₄⁺ to nitrite (NO₂⁻), while the *Nitrobacter* species represent the nitrite-oxidising bacteria, and are responsible for the conversion of NO₂⁻ to NO₃⁻ (Sahrawat, 2008). These autotrophic bacteria use the oxidation of NH₄⁺ or NO₂⁻ as a sole energy source, and carbon dioxide (CO₂) as a main source of C (Kowalchuk and Stephen, 2001; Cabello *et al.*, 2009).



Nitrification is a complex process requiring several enzymes (Figure 2), and is discussed in detail by Cabello et al. (2009). Briefly, NH_4^+ is firstly oxidised to hydroxylamine through a reaction involving the ammonia monooxygenase enzyme. Hydroxylamine is then converted into NO_2^- by the hydroxylamine oxidoreductase enzyme. Finally, NO_2^- is oxidised to NO_3^- by nitrite oxidoreductase. It is important to note that N_2O , which is commonly associated with denitrification, is often a by-product of nitrification. Here, NO_2^- , and possibly hydroxylamine, are utilised as alternative electron acceptors, thereby being reduced to N_2O (Dalal *et al.*, 2003). N_2O production by nitrification is now considered to be equally as important as denitrification (Mosier *et al.*, 1996).

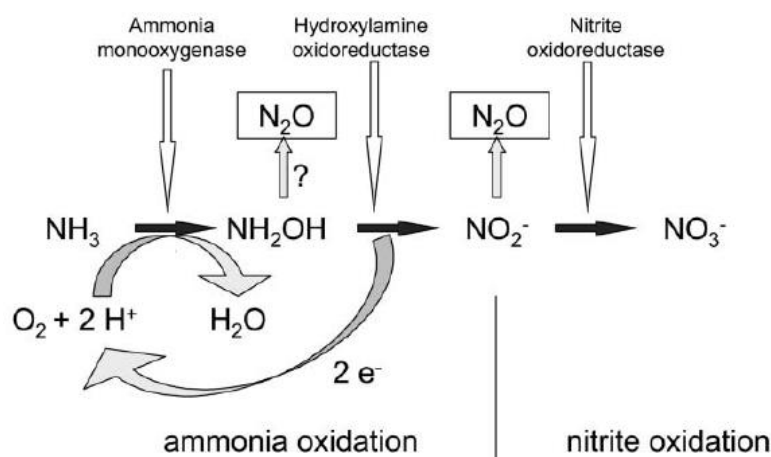


Figure 2: Pathways and enzymes involved in nitrification. Source: Wrage et al. (2001).

The rate of nitrification depends primarily upon NH_4^+ availability, soil aerobicity (and thus soil texture, structure and water content), temperature, pH and the populations of nitrifying bacteria. The availability of NH_4^+ to the nitrifying bacteria is the most important physical factors influencing nitrification (Davidson and Hackler, 1994). The rate of nitrification generally increases with increasing NH_4^+ concentration (Malhi and McGill, 1982; Davidson and Hackler, 1994). Processes which may limit the availability of NH_4^+ , include the adsorption of organic N and NH_4^+ by clay minerals within the soil matrix (Baldock and Skjemstad, 2000; Wang *et al.*, 2003), and also the biological immobilization of NH_4^+ (Wheatley *et al.*, 2001; Bengtsson *et al.*, 2003).

Nitrification proceeds in well aerated soils, with the maximum rate achieved when air-filled porosity is around 60% (Brady and Weil, 2008). Soil aerobicity is related to soil texture, structure and water content (Jarvis *et al.*, 1996). Soil texture and structure modify the environmental conditions (such as pore size distribution, gas exchange, mobility of cells and diffusion of substrates) under which nitrification take place (Thomsen *et al.*, 2003). Soil water content affects the diffusion of atmospheric air into the soil (Sahrawat, 2008), and also microbial activity (Stark and Firestone, 1995). Nitrification is therefore generally more rapid in coarse than fine textured soils (Strong *et al.*, 1999), when water content is at or near field capacity (Malhi and McGill, 1982; Zaman *et al.*, 1999; Sahrawat, 2008).

In response to temperature, the rate of nitrification generally follows a bell-shaped curve, with maximum rates occurring at 30 - 35°C (Sahrawat, 2008). The relationship between temperature and nitrification rate has however been found to vary between climates (Mahendrappa *et al.*, 1966; Malhi and McGill, 1982), with several studies (e.g. Sabey *et al.*,

1954; Frederick, 1955; Justice and Smith, 1961; Myers, 1975) suggesting that nitrifying bacteria in tropical climates require and tolerate higher temperatures than nitrifying bacteria in more temperate climates.

Soil pH affects not only the chemical form, concentration and availability of N compounds, but also influences bacterial cell growth, activity and diversity (Nicol *et al.*, 2008). Nitrification generally occurs within a soil pH range of 5 to 10, with the optimum pH being approximately 8 (Sahrawat, 1982; Gilmour, 1984; Goodroad and Keeney, 1984; Bramley and White, 1989; Kyveryga *et al.*, 2004; Sierra, 2006).

The populations of nitrifying bacteria are responsible for the rate-limiting step of nitrification in most ecosystems (Kowalchuk and Stephen, 2001; Chu *et al.*, 2008). Much research has gone into understanding the population size and nitrification potential of these organisms (e.g. Belser, 1979; Darrah *et al.*, 1985; Martikainen, 1985; Chu *et al.*, 2008) and their molecular microbial ecology (e.g. Kowalchuk and Stephen, 2001). NH_4^+ availability, soil aerobicity, temperature and pH are among the most important factors which regulate the population size, growth, activity and diversity of nitrifying bacteria and hence the rate of nitrification in soil (Belser, 1979).

Denitrification

Denitrification is the stepwise reduction of NO_3^- or NO_2^- , to N gases that include nitric oxide (NO), N_2O and N_2 , by denitrifying microbes (Knowles, 1982; Del Grosso *et al.*, 2000). The microbes gain energy by coupling N oxide reduction with the oxidation of organic matter through phosphorylation (Barton *et al.*, 1999; Bolan *et al.*, 2004). Most denitrifying bacteria are chemoheterotrophs which can use NO_3^- as their primary electron acceptor in the absence of oxygen (Knowles, 1982). The enzymes that catalyse reduction reactions include NO_3^- reductase, NO_2^- reductase, NO reductase and N_2O reductase (Figure 3) (Wrage *et al.*, 2001). The processes involved in denitrification are reviewed comprehensively by Knowles (1982). Weier and Grace (2012) provide a recent review of denitrification on Australian dairy farms.

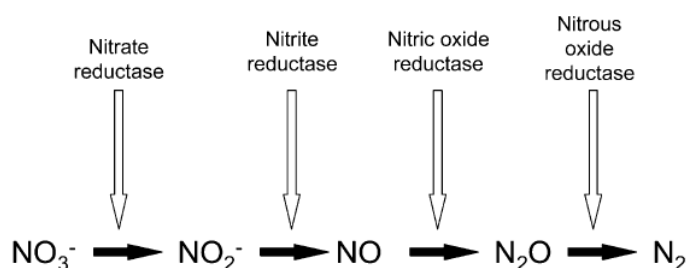


Figure 3: Pathways and enzymes involved in denitrification. Source: Wrage *et al.* (2001).

Coupled nitrification-denitrification and nitrifier denitrification are two additional terms worth mentioning. Coupled nitrification-denitrification is a term used to stress that NO_2^- or NO_3^- produced during nitrification can be utilised by denitrifiers (Figure 4) (Wrage *et al.*, 2001). This occurs in soils where conditions are favourable for both nitrification and denitrification (e.g. Khdyer and Cho, 1983). Nitrifier denitrification, often confused with coupled nitrification-denitrification, is a pathway of nitrification, in which the oxidation of NH_4^+ to NO_3^- is followed by the reduction of NO_2^- to N_2O and N_2 (Wrage *et al.*, 2001). Little is known about the nitrifier-denitrification pathway. Wrage *et al.* (2001) provides a detailed review. The remainder of this review will focus simply on denitrification.

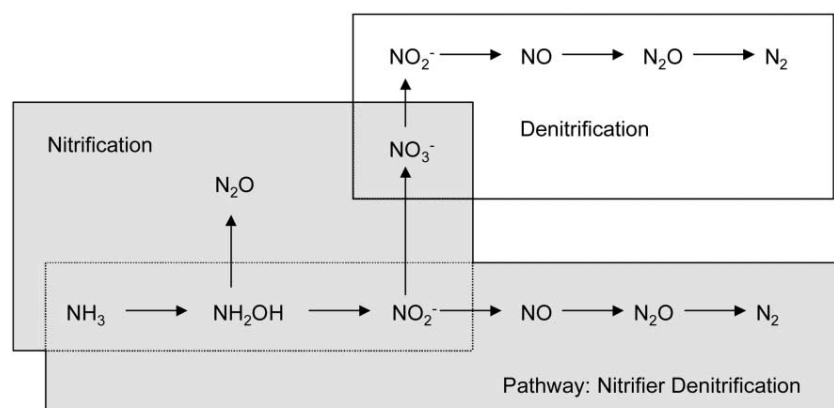


Figure 4: Relationships between nitrification and denitrification pathways. Source: Wraage et al. (2001).

The main factors affecting denitrification include the availability of oxygen, NO_3^- and organic C, pH, soil temperature and water content. The availability of oxygen affects both the growth and activities of denitrifying organisms (Bonin *et al.*, 1989; Thomas *et al.*, 1994). NO_3^- or oxides of N are required as terminal electron acceptors, while organic carbon is required as an electron donor (Knowles, 1982). pH affects the rate of denitrification, with optimum denitrification taking place at a pH of 7-8 (Thomas *et al.*, 1994). Soil temperature influences the degree of microbial activity (Bolan *et al.*, 2004). Finally, soil water content affects both soil aeration and substrate movement and hence the availability of oxygen (De Klein and Van Logtestijn, 1996; Luo *et al.*, 1999). Many studies have found a positive relationship between denitrification rate and; NO_3^- concentration (e.g. Colbourn, 1992; Luo *et al.*, 1999), carbon availability, pH (Weier *et al.*, 1993a; Thomas *et al.*, 1994), temperature (e.g. Stanford *et al.*, 1975; De Klein and Van Logtestijn, 1996; Luo *et al.*, 1999) and water content (e.g. Ruz-Jerez *et al.*, 1994; De Klein and Van Logtestijn, 1996; Luo *et al.*, 1999), and a negative relationship between the rate of denitrification and oxygen concentration (e.g. Parkin and Tiedje, 1984; Thomas *et al.*, 1994).

NH₃ volatilisation

NH_3 volatilisation is defined as the exchange of NH_3 gas from the soil to the atmosphere following hydrolysis of urea (Bremner and Douglas, 1971; Gioacchini *et al.*, 2002; Cichota and Snow, 2012). Urea is the most commonly applied N fertiliser, largely due to its high N content and relatively low cost (Weier and Grace, 2012). Upon surface application (either through deposition of excreta or fertiliser), urea is hydrolysed by the urease enzyme to ammonium carbonate $[(\text{NH}_4)_2\text{CO}_3]$, which, due to its instability, quickly dissociates into NH_4^+ and carbonate (CO_3^{2-}) (Bolan *et al.*, 2004). The CO_3^{2-} ions react with water to release hydroxyl ions, increasing the pH around the site of hydrolysis, causing the NH_4^+ dissociate to NH_3 (Equation 3) (Bolan *et al.*, 2004; Sommer *et al.*, 2004). Given the appropriate environmental conditions, urea hydrolysis is generally quite rapid, taking place within 3-4 hours of application (Whitehead and Raistrick, 1993). The processes involved in NH_3 volatilisation are reviewed comprehensively by Sommer, Schjoerring & Denmead (2004) and Bolan *et al.* (2004). Cichota and Snow (2012) provide a recent review of NH_3 volatilisation on Australian dairy farms.



The major factors affecting the extent and rate of NH_3 volatilisation include soil properties

such as pH, cation exchange capacity (CEC) and soil moisture, and climatic factors such as temperature and wind speed. pH affects the equilibrium between NH_4^+ and NH_3 (Sommer *et al.*, 2004); an increase in pH will cause more NH_4^+ to dissociate to NH_3 , and hence increase the amount of NH_3 volatilisation (Bolan *et al.*, 2004). Several studies have demonstrated the positive relationship between pH and NH_3 volatilisation (e.g. Ernst and Massey, 1960; O'Toole *et al.*, 1985; Zhenghu and Honglang, 2000), with the potential for significant NH_3 volatilisation at pH values > 7 (Sommer *et al.*, 2004). CEC affects the availability of NH_3 for volatilisation; the higher the CEC, the greater the amount of NH_4^+ ions adsorbed to the negatively charged cation exchange sites (Bolan *et al.*, 2004). Several studies have demonstrated a negative relationship between CEC and NH_3 emission (O'Toole *et al.*, 1985; Whitehead and Raistrick, 1990; Zhenghu and Honglang, 2000). Soil water content affects the rate at which urea hydrolysis takes place; hydrolysis increases with increasing water content (Black *et al.*, 1987; Al-Kanani *et al.*, 1991).

A positive relationship between NH_3 volatilisation and temperature and wind speed has been demonstrated in several studies (e.g. Sommer *et al.*, 1991). Increasing temperature increases the production of NH_3 present (increased microbial activity) and decreases the solubility of NH_3 in water (Sommer *et al.*, 2004). Increasing wind speed increases the diffusion of NH_3 away from the site of volatilisation, lowering the concentration of NH_3 at the soil-air interface which stimulates further volatilisation (Huijsmans *et al.*, 2003).

N balance

The balance between N inputs to the soil-plant-animal system and outputs in produce and N losses is a major factor determining the productivity of grazed pastures (Scholefield and Oenema, 1997; Pakrou and Dillon, 2000). In many grazed pasture systems, there often exists an imbalance between N inputs and outputs. Ledgard *et al.* (1999) undertook a comprehensive three-year N balance study on clover/grass pastures grazed by dairy cows in New Zealand. In farmlets fertilised with $400 \text{ kg N ha}^{-1} \text{ year}^{-1}$, the output of N (in milk, meat and feed) relative to the total N input averaged 26%, and this was comparable to intensively managed dairy farms in England and the Netherlands (14-20%). Several N balance studies on Australian dairy farms (e.g. Eckard *et al.*, 2001; Gourley *et al.*, 2012) gave results similar to those reported by Ledgard *et al.* (1999), generally showing greater inputs than outputs. The increasing use of N fertilisers, in excess of the pasture requirement, is a major factor responsible for this imbalance. It is also why N losses from the grazed pasture system are becoming an increasingly important issue (Gourley *et al.*, 2012).

N loss from fertiliser application to grazed pastures

The major N loss processes in grazed pasture include NO_3^- leaching, N_2O emission and NH_3 volatilisation. Runoff and erosion also contribute to N loss, however they are not considered major pathways in grazed pasture (Weier, 1994). Most of the research on N loss from fertiliser application to grazed pastures has been conducted in temperate area, and very few studies have been undertaken in the Wet Tropics of north-east Queensland.

N loss through NO_3^- leaching

The leaching loss of N from agricultural lands is generally associated with NO_3^- , because it is the dominant form of N and has a high solubility (van Kessel *et al.*, 2009). NO_3^- leaching can be described as the movement of NO_3^- below the crop root zone (Di and Cameron, 2002a).

The conversion of NH_4^+ to NO_3^- during nitrification significantly increases the susceptibility of N to leaching loss (Wu *et al.*, 2007). While in the NH_4^+ form, N is relatively immobile, with the NH_4^+ ion strongly adsorbed to the cation exchange sites (Sahrawat, 1982; Di and Cameron, 2002a; Yu *et al.*, 2007). The negatively charged NO_3^- ions are repelled rather than adsorbed by the negatively charged colloids dominating most soils (Brady and Weil, 2008), making the N very mobile (Wu *et al.*, 2007). In fertilised pastures, total annual NO_3^- leaching rates range from 0 to > 200 kg N ha⁻¹ (Table 1). N losses through of NO_3^- -N leaching range from 0 - > 20% of the applied N, with the amount leached generally increasing with increasing fertiliser application rate.

Recent research on the fate of applied fertiliser N to croplands on the Ferrosols of far north Queensland has shown that the NO_3^- leaching below the crop root-zone is a major N loss pathway (Rasiah and Armour, 2001). However, Ferrosol soils generally have a high anion exchange capacity at depth, which allows for the adsorption of the NO_3^- ion to clay minerals (Rasiah and Armour, 2001; Rasiah *et al.*, 2003a; Donn and Menzies, 2005). NO_3^- leached below the root zone may therefore accumulate at depth (Rasiah and Armour, 2001; Rasiah *et al.*, 2003a). It may also enter deep groundwater (Thorburn *et al.*, 2003), enter lateral flow that discharges into creeks and streams and/or denitrify within the profile (Rasiah *et al.*, 2003b; Rasiah *et al.*, 2005).

Table 1: Selected studies on leaching losses of N from fertiliser applied to pasture.

Location	Soil type	Fertiliser application		NO ₃ ⁻ leaching			Reference
		Type	Rate (kg N ha ⁻¹)	kg N ha ⁻¹ year ⁻¹	% of N Applied	Conc. (mg N L ⁻¹)	
New Zealand	Stony free draining	Urea	0	4.8	-	-	Di & Cameron (2002a)
New Zealand	Silt loam	Urea	200	17.1	6*	-	Ledgard et al. (1996)
			0	13	-	6.4	
			225	18	2*	10.1	
New Zealand	-	Urea	360	31	5*	30	Ledgard, Penno & Sprosen
			0	20-74	-	-	
			200	78-101	29-14*	-	
New Zealand	Sandy soil	Urea	400	100-204	20-33*	-	Silva et al. (1999)
			0	3.2	-	1	
			200	8	28*	2.6	
North Queensland	Krasnozem	Urea	400	17.4	4*	5.3	Prasertsak et al. (2001)
			115	0	-	-	
			0	2.2	-	-	
South-east Australia	-	Urea	200	3.5	0.7*	-	Eckard et al. (2001)
			0	2.2	-	-	
New Zealand	Sandy loam	Ammonium chloride	400	28 - 48	8.7 - 9.8	8.3 - 10	Di et al. (1998)
United Kingdom	Clay loam	Ammonium	200	38.5	19	-	Scholefield et al. (1993)
		Nitrate	400	133.8	33	-	

* leaching losses above those in the control (0 kg N ha⁻¹ year⁻¹) as a percentage of total N applied

N loss through N₂O emission

As discussed above, N₂O emission can be a by-product of both nitrification and denitrification processes. Gaseous emission of NO and N₂ are also produced during denitrification; however are extremely difficult to measure and are rarely reported, and so will not be discussed in this study. Several studies have investigated N₂O emission from fertilised pasture systems under variety of conditions (Table 2). Total annual N₂O emissions range from 0 to > 30 kg N ha⁻¹. N losses through N₂O emission range from 0 - > 20% of the applied N, with the amount of N₂O emitted generally increasing with increasing fertiliser application rate.

In the Wet Tropics, very few studies have measured N₂O emissions from fertiliser application to grazed pastures, although several studies have indirectly estimated it. For example, in their study of the fate of urea applied to dairy pasture near Milla Milla, Prasertsak et al. (2001a) concluded that 20% of the applied N was lost by denitrification, as the remaining 80% was accounted for in plant uptake, soil N and NH₃ volatilisation, and there was no evidence for leaching or runoff.

Table 2: Selected studies on N₂O emission from fertiliser applied to pasture.

Location	Soil type	Fertiliser application		N ₂ O emission		Reference
		Type	Rate (kg N ha ⁻¹)	kg N ha ⁻¹ year ⁻¹	% of N Applied	
New Zealand	Silt loam	Urea	0	7	-	Ledgard et al. (1996)
			225	11	1*	
			360	14	2*	
New Zealand	-	Urea	0	3 - 7	-	Ledgard, Penno & Sprosen (1999)
			200	11 - 25	4 - 9*	
			400	15 - 30	3 - 6*	
New Zealand	Alluvial	Urea	400	3 - 19	1 - 5	Ruz-Jerez, White & Ball (1994)
North Queensland	Krasnozern	Urea	115	-	20	Prasertsak et al. (2001)
South-east Australia	-	Urea	0	8	-	Eckard et al. (2001)
			200	14	3*	
South-east Australia	Hydrosol	Urea	200	13	7	Eckard et al. (2003)
Spain	Clayey loam	Calcium	145	25	17	Estavillo et al. (1994)
		Ammonium				
United Kingdom	Loam	Nitrate	290	37	13	Ryden (1983)
		Ammonium	0	2	-	
		nitrate				
			250	11	4*	
			500	29	5*	

* N₂O emissions above those in the control (0 kg N ha⁻¹ year⁻¹) as a percentage of total N applied

N loss through NH₃ volatilisation

N loss through NH₃ volatilisation is generally associated with the deposition of dung and urine (Vallis *et al.*, 1982; Bolan *et al.*, 2004). However, in situations where urea is not incorporated into the soil soon after application, NH₃ volatilisation can be a significant N loss pathway. For example, in a micrometeorological study on a pasture soil from Milla Milla, Prasertsak *et al.* (2001a) found 20% of the applied N was lost through NH₃ volatilisation. Total annual NH₃ volatilisation rates from fertiliser application to grazed pastures ranges from 0 to > 50 kg N ha⁻¹ (Table 3). Those losses correspond to 0 to > 50% of the applied N, with the amount of NH₃ volatilisation generally increasing with increasing fertiliser application rate. In this study, NH₃ emissions are expected to be low, given that the urea is irrigated in with 20 – 25 mm of water, immediately following application. Incorporation of the urea into the soil (~50 mm) soon after application is known to drastically reduce NH₃ volatilisation (Fenn and Miyamoto, 1981; Black *et al.*, 1987; Holcomb *et al.*, 2011).

Table 3: Selected studies on NH₃ volatilisation from fertiliser applied to pasture.

Location	Soil type	Fertiliser Application		NH ₃ volatilisation		Reference
		Type	Rate (kg N ha ⁻¹)	kg N ha ⁻¹ year ⁻¹	% of N Applied	
Canada	Clay loam/fine sandy loam	Urea	100	-	20 - 50	Rawluk, Grant & Racz (2001)
Netherlands	Calcareous clay	Calcium	550	42	8	Bussink (1992)
		Ammonium	550	39	7	
		Nitrate	250	8	3	
New Zealand	Silt loam	Urea	30	-	12	Black et al. (1985)
			60	-	20	
			100	-	25	
			200	-	35	
New Zealand	Silt loam	Urea	0	15	-	Ledgard et al. (1996)
			225	45	13*	
			360	63	13*	
New Zealand	-	Urea	0	15 - 17	-	Ledgard, Penno & Sprosen (1999)
			200	30 - 45	8 - 14*	
			400	63 - 74	12-14*	
North Queensland	Krasnozem	Urea	115	-	20	Prasertsak et al. (2001)
South-east Australia	Hydrosol	Urea	200	57	29	Eckard et al. (2003)
South-east Australia	-	Urea	0	10	-	Eckard et al. (2001)
			200	24	7*	
Switzerland	Mollic Gleysol	Ammonium	20	1	6	Herrmann et al. (2001)
		Nitrate	40	2	4	
United Kingdom	-	Urea	70	4 - 14.4	6 - 21	Ryden et al. (1987)
			100	20 - 36	20 - 36	

* NH₃ volatilisation above those in the control (0 kg N ha⁻¹ year⁻¹) as a percentage of total N applied

Implication of N loss and effects of management practices

Loss of N originating from fertiliser applied to agricultural land is a globally significant social, environmental, ecological and economic issue (Di and Cameron 2002a; Eickhout et al. 2006; Mosier and Kroeze 2000; Spiertz 2009). Groundwater contamination, arising from leaching of NO₃⁻ past the root zone, poses a serious health threat to populations reliant on groundwater as a drinking source (Singh and Sekhon, 1979; Thorburn *et al.*, 2003; Rivett *et al.*, 2008). NO₃⁻ carried by overland runoff (Ng Kee Kwong *et al.*, 2002; Udawatta *et al.*, 2006) and groundwater flow (Wriedt *et al.*, 2007) can detrimentally affect aquatic ecosystems through a decline in water quality and eutrophication (Hessen *et al.*, 1997; Howarth, 2008). This is particularly relevant in the catchments adjacent to the Great Barrier Reef World Heritage Area (Brodie *et al.*, 2008; Thorburn *et al.*, 2011). Gaseous losses of N as NH₃ and N₂O contribute to acid rain, ozone depletion and global warming (Bolan *et al.*, 2004; Robertson and Vitousek, 2009). From an economic perspective, the loss of N adds a substantial financial cost to primary producers (Oenema and Pietrzak, 2002; Chen *et al.*, 2008; Arriaga *et al.*, 2009; Cichota and Snow, 2012).

A number of management practices aimed at reducing N loss and improving N fertiliser use efficiency exist (see for e.g. reviews by Cuttle and Scholefield, 1995; Monaghan *et al.*, 2007; Chen *et al.*, 2008; Chien *et al.*, 2009). In general, the focus of management practices is to increase the pasture's ability to compete with N loss processes. One relatively simple management practice is to match the rate and timing of fertiliser application to pasture requirement. Another management practice, which is gaining increasing recognition as an effective way of improving N fertiliser use efficiency, is the addition of nitrification inhibitors (NI) to the fertiliser.

Matching fertiliser application to pasture demand

Matching fertiliser application to pasture demand is critically important for maintaining high productivity in managed pastures, while minimizing production costs and the potential loss of N from the soil-pasture system. It is common practice however, for farmers to apply N fertiliser in excess of crop requirements, to ensure N requirements are being met (Sommer *et al.*, 2004; Weier and Grace, 2012). Further, it is a common assumption that the more N fertiliser applied, the greater the pasture production, which will lead for example, to higher milk production, and inevitably a greater profit (Staines *et al.*, 2011). While this may be economically rational from a farmers point of view, there are a number of reasons why applying more fertiliser than required, does not necessarily translate into increased productivity. Among other reasons, such as timing and frequency of fertiliser application, matching application rate to pasture demand is critical for minimizing potential N loss and production costs. Rate response trials specific to soil type and environmental conditions provide the only means of determining optimal fertiliser application rates.

Rate response trials on the Atherton Tablelands were mostly carried out in the 1970's and 80's (e.g. Teitzel, 1979; Standley *et al.*, 1981; Davison *et al.*, 1985a; Davison *et al.*, 1985b; Cowan *et al.*, 1987; Davison *et al.*, 1987). The results of these trials generally suggested peak pasture production is achieved with around 300 kg N ha⁻¹ year⁻¹, with the greatest response to the first 200 kg N ha⁻¹ (Teitzel *et al.*, 1991; Teitzel, 1992). There have been no recent rate response trials undertaken on the Atherton Tablelands; although some efficient north Queensland dairy farmers have found that halving the industry standard application rate (500 kg N ha⁻¹ year⁻¹) does not reduce productivity (Russell Fry, pers. comm.). Clearly there is a need for more up-to-date research, so farmers can be sure the management practices they implement will achieve maximum pasture production at lowest cost.

Nitrification inhibitors

NI are chemical compounds that slow down the oxidation of NH₄⁺ to NO₂⁻ in the soil by inhibiting the activities of *Nitrosomas* bacteria (Prasad and Power, 1995; Zerulla *et al.*, 2001; Chien *et al.*, 2009). More specifically, these compounds inhibit the activity of monooxygenase enzyme (Figure 5) (McCarty, 1999). In recent years, substantial progress has been made toward understanding the specific mode of action of different NI, although there remains much uncertainty (McCarty, 1999). Regardless of the specific mode of action, NI can potentially result in N being retained in the root zone for a longer period, providing more time for plant uptake, and reducing N loss (Moir *et al.*, 2007; O'Connor *et al.*, 2012; Subbarao *et al.*, 2012).

Nitrification

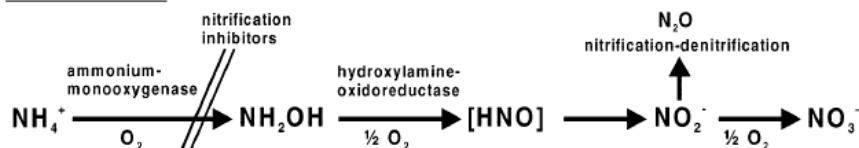


Figure 5: NI blocking NH_3 oxidation. Source: Weiske et al. (2001).

An extensive range of NI are now commercially available (Prasad and Power, 1995). Dicyandiamide (DCD) and nitrapyrin (N-Serve) are two of the more commonly used compounds. In managed pastures, these compounds have been found to reduce N_2O losses by up to 80% (McTaggart *et al.*, 1997; Williamson and Jarvis, 1997; Bolan *et al.*, 2004; Zaman *et al.*, 2009; Luo *et al.*, 2010), decrease NO_3^- leaching losses by up to 80% (Di and Cameron, 2004) and increase pasture dry matter yield by up to 35% (Williamson *et al.*, 1998; Moir *et al.*, 2007). It is important to note that most of the research in managed pasture has focused on the effect of the NI on reducing N losses from the deposition animal excreta (mostly urine), as this is where most N is lost. See for example the work of Di and Cameron (2002b; Di and Cameron, 2003; Di and Cameron, 2004; Di and Cameron, 2005; 2007) and Moir, Cameron and Di (2007). There has been relatively little research on the effectiveness of NI for reducing N loss directly from the fertiliser applied to pasture.

A relatively new NI on the commercial market is 3, 4-dimethylpyrazole phosphate (DMPP, commercial name ENTEC®). DMPP is a heterocyclic N compound containing two adjacent ring N atoms (Figure 6) (Weiske *et al.*, 2001; Zerulla *et al.*, 2001). Similar to other classes of NI, heterocyclic N compounds slow down the nitrification process by inhibiting the activity of monooxygenase enzyme during the NH_3 oxidation (Weiske *et al.*, 2001). Little is known about the specific mode of action that heterocyclic N compounds have on NH_3 oxidation, although evidence suggest the inhibitory influence may be related to the presence of ring N (McCarty and Bremner, 1989; McCarty, 1999).

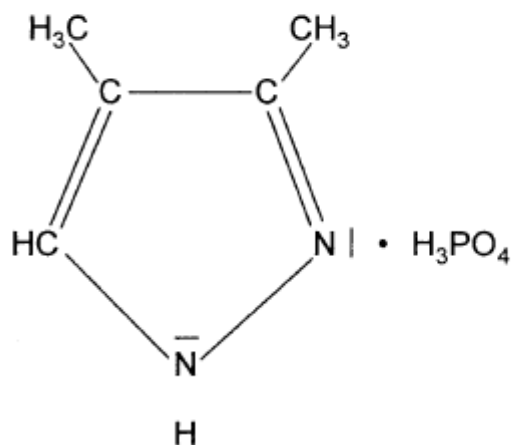


Figure6: Chemical structure of DMPP. Source: Zerulla et al. (2001).

Research suggests DMPP inhibits only the first stage of nitrification, NH_3 oxidation, while the next step, NO_2^- oxidation, is unaffected (Li *et al.*, 2008; Kleineidam *et al.*, 2011; Yang *et al.*, 2012). For example, in a study on the effect of DMPP on populations of nitrifying organism and enzyme activities in rice-oilseed rape cropping systems, Li *et al.* (2008) found the population of NH_3 oxidising bacteria decreased by 24.5 – 30.9% in treatments treated with

DMPP, while the nitrite oxidising bacteria and the hydroxylamine reductase remained almost unaffected.

The major advantage DMPP offers over other commercially available products is that only low concentrations of the active compound are required to inhibit nitrification (Zerulla *et al.*, 2001). For example, an application of 0.5–1.5 kg DMPP ha⁻¹ (depending on the amount of applied N) is sufficient under field conditions to reliably inhibit nitrification over a period of 4–10 weeks (Zerulla *et al.*, 2001). The duration of action depends on climatic conditions, site characteristics and the cultivated crop (Zerulla *et al.*, 2001; Barth *et al.*, 2008; Chen *et al.*, 2010). DMPP has been submitted to extensive standard toxicology and ecotoxicology tests, which have revealed no side-effects (Zerulla *et al.*, 2001).

HowLeaky Parameters

Climate parameter

A climate parameter file, spanning the 1-01-2012 to the 30-04-13, was created utilising data (maximum and minimum temperature and radiation) from the weather station located on site. Where data from the weather station was missing or unavailable, patched point data from SILO (Jeffrey et al., 2001) was used. Soil evaporation data was derived entirely from SILO.

Irrigation parameter

An irrigation parameter file was created utilising data from the weather station.

Soil parameter

The soil parameter file was downloaded directly from the HowLeaky field studies database (McClymont et al., 2013). This data was derived from Cogle et al (2011) study of runoff, soil loss, and nutrient transport from cropping systems on red Ferrosols in tropical northern Australia.

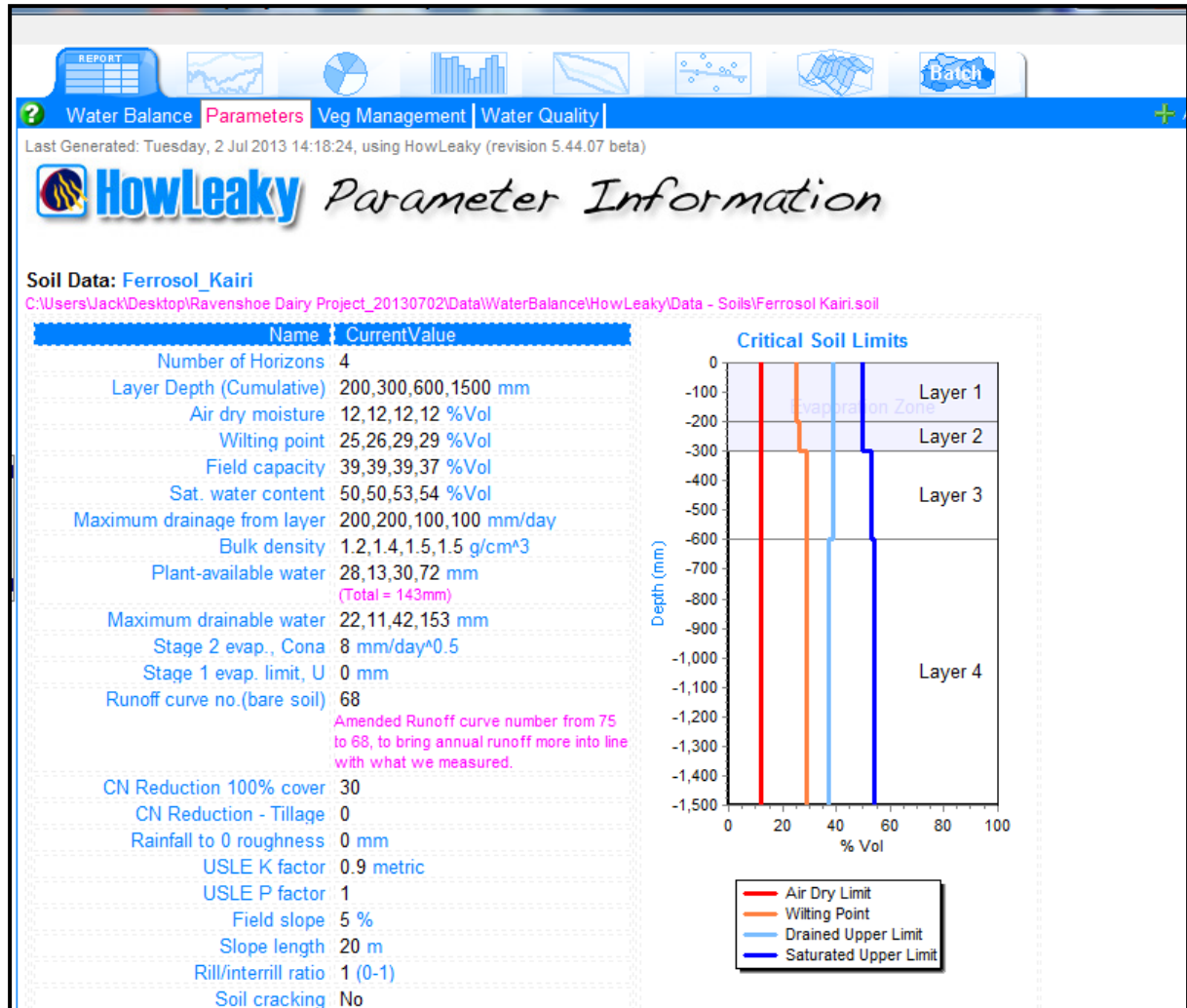


Figure 7: Screenshot of soil parameter information

Vegetation

The vegetation parameter file was downloaded directly from the HowLeaky field studies database (McClymont et al., 2013). This data was derived from Cogle et al (2011) study of runoff, soil loss, and nutrient transport from cropping systems on red Ferrosols in tropical northern Australia.

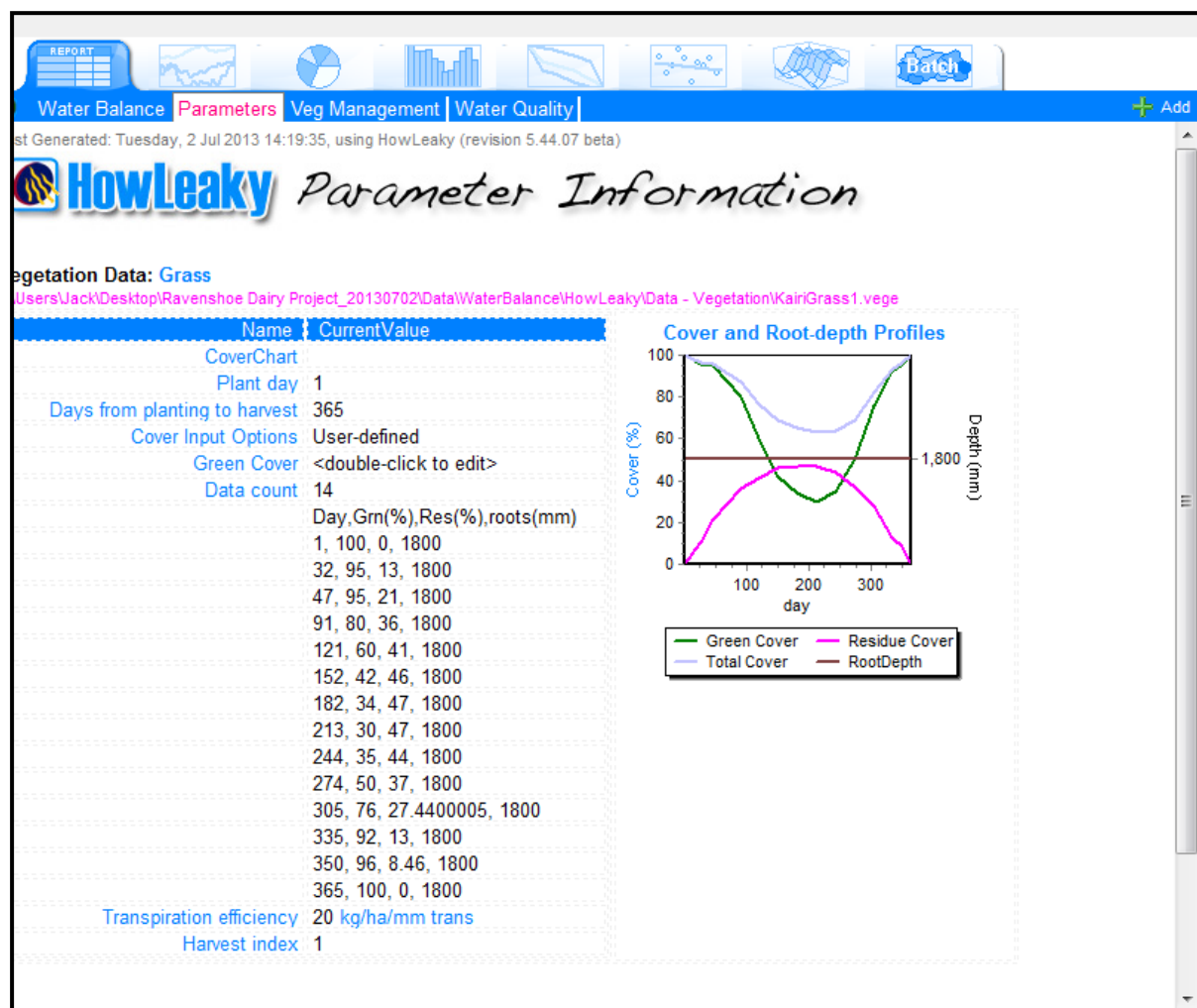


Figure 8: Screenshot of vegetation parameter information

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