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**CRDC**

**Rural R&D for Profit Program**

# **More Profit from Nitrogen**

RRDP1715 (July 2016 – May 2020)

**Improving dairy farm nitrogen efficiency using  
advanced technologies**

**Final Report**

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**The University of Melbourne**



THE UNIVERSITY OF  
**MELBOURNE**



**Dairy  
Australia**

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Provide details of all personnel involved in the project.

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# Plain English Executive Summary

## Program objectives and methods

The objectives of the research conducted were to understand the contributions of soil mineral N to pasture production with a view to be able to better manage this N and improve the efficiency of N fertiliser utilisation in HRZ dairy pastures of southern Australia. To achieve this objective, studies of seasonal pasture responses to N at 5 rates of urea-N (0, 20, 40, 60 and 80 kg N/ha) were conducted under dryland and irrigated conditions with a longer-term irrigation trial (2.5 years) and shorter-term dryland trial (1 year) at Allansford, and a second short-term dryland trial conducted at a satellite site (Cooriemungle, autumn 2019). All trials were replicated small plot trials with regular fertiliser application following each pasture harvest (at 3-leaf stage) to mimic commercial grazing rotations. Pasture biomass, herbage N content, soil moisture, temperature and mineral N, and climate data were collected regularly over the course of each trial. The impact of urease and nitrification inhibitors on N dynamics and biomass production were also assessed in the field at three N rates (10, 20 and 40 kg N/ha). <sup>15</sup>N microplot studies were used to determine the fate of applied fertiliser N, and the source of N taken up by the pasture using soil from the trial sites and from key dairy soils of the region.

Advanced technologies were investigated as tools to assist making N fertiliser decisions. Two drone-based campaigns were held (Dec 2017 and Feb 2019), where hyperspectral imagery was used to determine differences in pasture biomass and N content. In addition, two campaigns were run using hand-held sensors (a Soil Plant Analysis Development (SPAD) meter and VNIR spectrometer). The hand-held unit was used to determine changes in spectra from pasture over two growth cycles (winter: 17<sup>th</sup> and 25<sup>th</sup> May, 4<sup>th</sup> and 18<sup>th</sup> June 2018, and summer: 3<sup>rd</sup> and 14<sup>th</sup> Jan, 7<sup>th</sup>, 15<sup>th</sup> and 19<sup>th</sup> Feb 2019).

The field trials were supported by laboratory incubation experiments on mineralisation rates from pasture soils collected in the region, under different climatic conditions. A mineralisation calculator was developed based on a biophysical model (WNMM), to provide another tool to assist farmers with decision making. This calculator was refined based on discussions with advisor groups.

Losses of N as N<sub>2</sub>O and N<sub>2</sub> were measured and predicted based upon laboratory measurement of the N<sub>2</sub>:N<sub>2</sub>O emission ratio from soils, combined with field-based spot measurements of N<sub>2</sub>O emissions.

## Key Findings and implications

The key findings of the project, and the implications for industry are;

- Seasonal responses to N were clear, with generally little response in autumn due to limited water availability under both dryland and irrigated systems. Where irrigation management led to greater autumn soil profile water, a good response to N was seen. Improved irrigation management at the edges of the dryland growing season (early irrigation startup in spring when soils are drying, and longer irrigation in autumn) could improve pasture productivity and NUE.
- Mineralisation contributed substantial amounts of plant available N in the soil, particularly under dryland conditions and following the summer where pasture uptake of N was limited.
- The recovery of fertiliser N in the plant was low (19-30%) following each fertilisation event, and the majority of the N taken up by the plant came from the soil (>70%). We assume that the N from applied fertiliser not recovered in the plant was immobilised and then released from the soil organic matter pool over time for plant uptake. This leads to good recovery of N unless there are major losses as ammonia, from denitrification, or via leaching of nitrate, which we predict were minimal at our site. After 8-12 months, around 33-49 % of the applied fertiliser had been utilised by the pasture 26-78% was recovered in the soil and roots and up to 41% was unaccounted for.

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- Variations in N use efficiency (NUE) occurred between seasons, and indicate that the fertilisation rates should be variable to match the seasonal N response. NUE was higher with lower N inputs, but the reduced pasture productivity at these lower N rates, and the impact on farm feed requirements needs to be considered.
- A modelling approach is a viable tool for predicting mineralisation and the seasonal pattern in mineralisation rates under dryland and irrigated conditions was identified. The key drivers of mineralisation were identified as future temperature and N rate. The mineralisation calculator was viewed as a useful tool to educate advisors on the drivers of mineralisation, however its usefulness will depend on its ability to cope with the high level of climate variability.
- Using remote sensing approaches to predict pasture production and N content are valid. Complications in pasture systems exist due to the variety of species and leaf architecture. Measurements prior to 3-leaf stage are considered to be most useful. Climate conditions experienced in southwest Victoria made use of the remote sensing approach challenging as cloud cover varied dramatically over the course of a day.
- Overall the use of currently available enhanced efficiency fertiliser had limited impact on pasture dry matter production and nutrient cycling which is partly due to climate and water management, although productivity benefits were seen during one winter period where reduced inputs were possible with use of DMPP coated urea, compared to granular urea. Limited ammonia loss is expected to occur under well managed irrigated pastures, and the dryland site was limited to one year of data, plus N fertilizer was only applied to the dryland site when there was good active pasture growth and the growth responses were likely to be reliable.

Reducing N inputs during seasons where soil stored N levels are high, such as at the autumn break on dryland pastures, will benefit the industry by reducing the input cost of fertiliser, and the potential off-site environmental impacts, supporting the commitments of the Australian dairy industry to improve the efficiency of N use for pasture production.

#### **Future work**

The project identified the contributions of mineral N to pasture nutrition, the impacts of growing season on pasture productivity and N uptake, and the variation in NUE that occurs over the year. The season 'shoulders' (early autumn, late spring) have greatest uncertainty for dairy farmers, particularly under dryland conditions. The good N response under irrigation seen in autumn 2019 coincided with higher soil moisture deep in the profile (> 20 cm depth).

Further research is warranted in the following areas;

- Understanding the implications of maintaining deep soil moisture at the 'shoulders' of the growing season, to determine the benefits and risks associated with extended irrigation in these HRZ regions, particularly as climate variability increases.
- Gaining a greater understanding of the fate of N fertilizer applied under marginal growing conditions (start and finish of the dryland growing season) when pasture growth responses are likely to be low and uneconomic would further identify how NUE can be improved.
- Identification of the key loss pathways associated with the unaccounted for N
- Determining the contribution of fertiliser N to future pasture growth (extent and longevity) under a continuous fertiliser application strategy
- Development of remote sensing capabilities for areas with challenging climates.

The project acknowledges the financial support from the Australian Government Department of Agriculture as part of its Rural R&D for Profit program, Dairy Australia, and The University of Melbourne.

# Abbreviations and glossary

DM: Dry matter

DMPP: Dimethylpyrazole phosphate, a nitrification inhibitor.

EEF: Enhanced efficiency fertiliser, includes urease inhibitor and nitrification inhibitor treatments

HRZ: High rainfall zone

N: Nitrogen

N<sub>2</sub>: Dinitrogen gas

N<sub>2</sub>O: Nitrous oxide

NBPT: N-(n-butyl) thiophosphorictriamide, a urease inhibitor

NUE: Nitrogen Use Efficiency

NU<sub>A</sub>E: Agronomic N utilisation efficiency of fertiliser calculated as harvested product per unit applied N, where  $NU_{A}E = (\text{grass yield at } N_x - \text{grass yield at } N_0) / \text{kg of N applied at } N_x$

θ<sub>v</sub>: Volumetric water content (m<sup>3</sup> m<sup>-3</sup>)

WFPS: Water filled pore space

# 1 Project rationale

## Identified industry need

The project was undertaken to gain a better understanding of nitrogen (N) cycling and utilisation in rainfed and dryland dairy pasture production systems in the high rainfall zone (HRZ) of southern Australia, and to develop a tool that farmers could use to make fertiliser decisions with confidence. Under irrigated production systems, high rates of N (e.g. 480 kg N/ha annually) are applied to maximise pasture growth. Under dryland systems, less N is applied (e.g. 200 kg N/ha/year) due to a shorter growing season. Both irrigated and dryland pastures are considered to have high inputs of N compared to other grazing systems (e.g. cattle, sheep). In addition to high inputs, the estimated recovery of the applied N fertiliser is considered to be low (~ 40%), indicating that there are currently some major inefficiencies in the system.

Under both irrigated and dryland production systems, seasonal variations in pasture responses, as dry matter (DM) production per unit of N applied, are seen, but the industry continues to apply N largely at a standard rate after each grazing (e.g. 40 kg N/ha). This response variation means that growers can be wasting money in some seasons (e.g. autumn) where there is little response to fertiliser inputs, or not making the most from growing conditions (e.g. spring) by limiting N supply. The potential N supply from soil that can contribute to pasture growth is not well understood such that growers are not able to account for this supply when making fertiliser decisions. Knowledge of the supply of N from mineralisation will help the industry to make better decisions about fertiliser use.

The variation in N utilisation leads to times of high and low risk for N to be lost to the environment. Reducing the risk of N loss can be achieved by dropping N rates or using enhanced efficiency fertilisers (EEFs) which target different N loss pathways. However the risk of lost production with reduced inputs is often too high for change to occur, and the benefits from use of EEFs has been variable. Knowledge of the outcomes from these management strategies under HRZ dairy systems will better enable the industry in making fertiliser decisions.

## Research objectives

The research undertaken had the following objectives for HRZ pastures of southern Australia;

1. Measure and understand the seasonal responses of irrigated and dryland pastures to N
2. Quantify the amount of mineralisation that occurs monthly, seasonally and annually and identify the key drivers of mineralisation
3. Quantify the fate of N applied as fertiliser and the contributions of fertiliser and soil mineralised N to pasture N uptake
4. Quantify the impact of urease and nitrification inhibitors on N dynamics and pasture productivity
5. Estimate the losses of N including via denitrification and leaching
6. Enable growers to better understand N cycling to make better fertiliser decisions
7. Test the suitability of using remote sensing approaches when making N fertiliser decisions



### **Key recommendations for the industry**

The key recommendations for industry are to consider the seasonal variation in N responses and apply N based on expected dry matter response, rather than using the current standard practice of a set rate following each grazing. This decision should be made taking into account the soil moisture, growing conditions as influenced by climate (i.e. season), potential reserves of mineral N available in the soil, and potential loss pathways for N. Consideration of these factors is relevant to both irrigated and dryland production systems. This will reduce the risk of N contamination that can arise from over-fertilisation, as well as ensuring maximum pasture growth is achieved at key growth periods. Particular difficulties exist for growers in making decisions on the shoulders of the season – autumn and late spring – most noticeably, but not restricted to, in dryland production systems. Further investment in understanding the interaction between N and soil moisture reserves, including to below the main root zone, at these points through the year will facilitate better decision making by growers.

### **Key messages for industry primary producers**

Primary producers can utilise N fertiliser more efficiently to ensure fertiliser is not wasted, growth opportunities are not wasted, and a better economic outcome is achievable. Considering pasture production in light of past, current and future climatic conditions and, for irrigated systems, the irrigation plan, and the impact on pasture growth, soil mineralisation, and losses will lead to a more efficient system. Under irrigation, to optimise pasture growth, soil moisture levels should be maintained to depth at the edges of the season to widen the productive seasons. Under dryland systems, decisions around fertiliser benefits also need to consider moisture but will continue to have greater uncertainty than systems with irrigation.

Savings in N fertiliser inputs can be made where mineralisation leads to a build-up of mineral N which is available for pasture uptake, such as at the end of summer under dryland systems, or in spring. Making predictions of the N available and that will become available should consider the soil (C content), past and future rainfall and temperatures, and the past rate of N that has been applied.

## 2 Method and project locations

The project involved an extensive field experiment to measure cycling of N in HRZ dairy pastures of southwest Victoria under dryland and irrigated conditions. This work was supported by additional laboratory experiments designed to investigate mechanisms of gaseous emissions and mineralisation. A mineralisation calculator was developed using the data collected during the course of the experimental work.

### Field Experiments

The research was conducted at two locations under perennial ryegrass dominant pasture located in the HRZ of Victoria. At one location, 5048 Great Ocean Road, Mepunga West (Allansford), SW Victoria (38°25'05" S, 142°38'24"E), two sites were established in September 2016 (irrigated and dryland). At the second location, 401 Cooriemungle Rd, Cooriemungle, SW Victoria (38°53'40" S, 143°07'12"E) one dryland site was established in late-2018 to measure the 2019 autumn response. Data (climate, soil moisture and temperature, soil mineral N, herbage dry matter production and N rate response, pasture N content, impact of enhanced efficiency fertilisers, <sup>15</sup>N fate of urea and urine, N<sub>2</sub>O emissions) was collected at the sites following establishment covering one dryland growing period (2017) and 2.5 years of irrigated pasture production (November 2016 to June 2019) at the Allansford site, and an autumn break (April-June 2019) at the Cooriemungle site.

A randomised block design of nine treatments replicated five times and applied to 3 x 3 m plots was established at the Allansford sites. Nitrogen responses and agronomic nitrogen utilisation efficiency (NUE) were investigated for granular urea surface broadcast at 0, 20, 40 (regional standard rate), 60 and 80 kg N/ha following harvest to simulate grazing (21 to 56 days depending on the season). In addition, the impact of enhanced efficiency fertilisers (EEFs) using either NBPT (applied as Green Urea NV™) or DMPP (applied as Urea with ENTEC®) depending on season, was investigated at rates of 10, 20 and 40 kg N/ha. Pasture harvest were taken at 3 leaf stage manually using a mower, and dry matter production determined after drying at 60°C until constant weight, and N uptake determined from pasture biomass and N content. Nitrogen dynamics in the pasture soils were investigated through regular soil sampling following fertilisation, and through deep soil cores collected during the experimental period. Soil moisture and temperature were monitored continuously at 5 cm depth on selected treatments, and deep soil moisture and temperature measurements were collected every 10 cm to a depth of 80 cm at three locations across each trial site.

Agronomic N utilisation efficiency of fertiliser (NUE) for each harvest [harvested product per unit applied N] was determined as;  $NUE = (\text{dry matter production for the fertilised treatment} - \text{dry matter production for the unfertilised treatment}) / \text{kg of N applied}$ .

Seasonal average biomass and NUE were determined using data across the three years. The seasons are defined as harvests that occurred in the following months; Summer: December, January, February, Autumn: March, April, May, Winter: June, July, August, Spring: September, October, November.

The fate of urea fertiliser applied was determined using <sup>15</sup>N microplots (23.7 cm diameter, 25 cm depth), which were installed at Allansford on April 4<sup>th</sup>, 2017 and September 9<sup>th</sup>, 2017 on the dryland and irrigated sites. The fate of urine was studied using <sup>15</sup>N-enriched urine using the same size microplots which were installed at the Allansford dryland and irrigated sites on 6<sup>th</sup> June 2017, 14<sup>th</sup>

October 2017, 23<sup>rd</sup> November 2017, and 9<sup>th</sup> February 2018 (irrigated only due to pasture death on the dryland site over the dry summer in 2017/2018).

In 2018/2019, <sup>15</sup>N microplot cores from six different sites, representing key dairy soils in the region, were collected in triplicate, and placed at the irrigated site at Allansford to determine the fate of fertiliser N applied in spring (23<sup>rd</sup> October 2018, N rate 20 kg N/ha) and autumn (16<sup>th</sup> April 2019, N rate 40 kg N/ha).

Losses of N from the pasture system as N<sub>2</sub>O emissions were measured using the static chamber method for 21 days following fertilisation at i) the beginning of spring 2018, ii) the beginning of autumn 2018, and iii) the beginning of autumn 2019.

Hyperspectral imagery was collected using drones on December 18<sup>th</sup>, 2017 (irrigated and dryland sites) and February 19<sup>th</sup>, 2019 (irrigated site) to assess this technology for making predictions of pasture biomass and N content. Hand-held VNIR spectra and chlorophyll (Soil Plant Analysis Development (SPAD) meter) measures was also assessed at four different times covering different growth periods (i.e. one growth cycle) in May-June 2018 and in January-February 2019.

### **Laboratory Experiments**

Laboratory experiments were conducted to support the field experiments by investigating soil moisture and temperature conditions on N loss as N<sub>2</sub>O and N<sub>2</sub>, and the mineralisation of soil N.

#### *1. Nitrous oxide (N<sub>2</sub>O) and dinitrogen (N<sub>2</sub>) soil gaseous losses*

Soil samples (0-10cm) collected from the buffer zone of the Allansford irrigated site were incubated (replicated 4 times) with <sup>15</sup>N labelled potassium nitrate (K<sup>15</sup>NO<sub>3</sub>, 99 atom%) at 3 different moisture contents; 60%, 80%, and 100% water filled pore space (WFPS). Gas samples were collected over 10 days and were analysed for N<sub>2</sub>O using gas chromatography (GC Agilent 7890), and <sup>15</sup>N<sub>2</sub>O and <sup>15</sup>N<sub>2</sub> using gas chromatography coupled with isotope ratio mass spectrometer (GC-IRMS). After 9 days, the samples were analysed for mineral N by extraction with KCL (1:5 soil:solution)

#### *2. N mineralisation*

Mineralisation rates were determined for pasture soils collected from the region in two different seasons – summer (January 2019) and autumn (April/May 2019), under three moisture conditions and three different temperatures, to understand the key drivers of mineralisation.

### **Mineralisation calculator**

The mineralisation calculator was developed based on an existing model (The Water and Nitrogen Management Model (WNMM)) using data collected from the Allansford field sites.

### **Industry locations and regions where research findings are applicable**

The field work was undertaken in south-west Victoria in the HRZ. Three experimental sites were established at two locations, on commercial farms. Two sites (irrigated and dry land) were established at 5048 Great Ocean Road, Mepunga West (38°25'05" S, 142°38'24"E) (location 1), and the third (dryland) at 401 Cooriemungle Rd, Corriemungle (38°53'9866" S, 143°05'5220"E) (location 2). The soils at the 2 locations differed in their physical and chemical properties (see Technical report). The climate of the region is temperate, with long-term average annual precipitation of 743 mm, 70% of which falls during April-October, and mean annual monthly minimum and maximum temperature of 9.6°C and

17.9°C respectively (Commonwealth Bureau of Meteorology). At all sites, there is a history of long-term pasture (>20 years) mainly consisting of perennial ryegrass (*Lolium perenne*).

Additional soils were collected from 5 further farms in the region with the aim of understanding N dynamics in different soils using <sup>15</sup>N techniques (see Technical report). The work with these additional soils used <sup>15</sup>N microplots with fertiliser applied in spring 2018 and autumn 2019.

The findings from the research trials are relevant to regions with a similar climate as climate is a key driver of pasture growth and N cycling in pasture soils, which would include two of the major regions of dairy production in Victoria – the Western District and West Gippsland. The knowledge gained in the research work regarding dry matter production, seasonal variation in N responses, and NUE is relevant to other regions where there are clear seasonal differences, and pasture species are dominated by ryegrass (e.g. northern Victoria). The mineralisation calculator developed used data collected from the location at Allansford, and as such it has been developed for use in the climatic region of validation. Extrapolation of the findings to other dairy zones (e.g. northern Victoria, west Gippsland) would require further calibration of the model, which is possible utilising available and published datasets.

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Research Site Type	Name	Location	Coordinates	Active Site Period	Experimental treatments
Field	Allansford irrigated site	5048 Great Ocean Road, Mepunga West, near Allansford, SW Victoria	38°25'05" S, 142°38'24" E	Sept 2016-July 2019	<p>Nitrogen (N) response and agronomic nitrogen use efficiency were investigated in response to two fertilization strategies with and without addition of (i) the urease inhibitor N-(n-butyl) thiophosphorictriamide (NBPT) and (ii) nitrification inhibitor 3,4-Dimethylpyrazole phosphate (DMPP). The nitrogen response was studied from application of urea at 0, 20, 40, 60 and 80 kg N /ha. The response of inhibited urea was studied from application of urea at 0, 10, 20 and 40 kg N /ha.</p> <p>In addition to this, the nitrogen availability from urine patches was studied from application of synthetic urine at 1000 kg N ha<sup>-1</sup>.</p>
Field	Allansford dryland site	5048 Great Ocean Road, Mepunga West, near Allansford, SW Victoria	38°25'05" S, 142°38'24" E	Sept 2016 – Dec 2018	Same as above
Field	Cooriemungle dryland site	Cooriemungle Rd, Mepunga West, near Timboon, SW Victoria	38°53'40" S, 143°07'12" E	Feb 2019 – July 2019	The nitrogen response and agronomic nitrogen utilisation efficiency were studied from application of urea at 0, 20, 40, 60 and 80 kg N/ha.

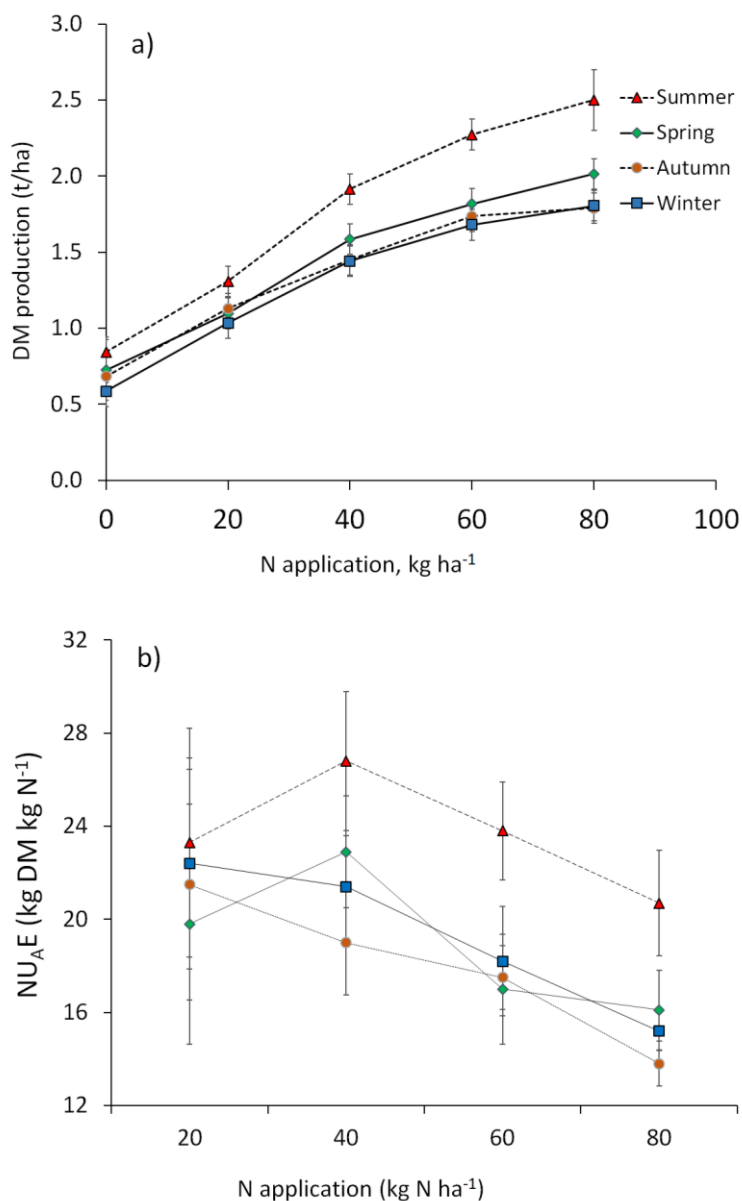
### 3 Project Outcomes

The following summarises the key Project Outcomes which are presented in more detail in the Technical Report (Section 8)

#### 1. Field experiments in rainfed and irrigated dairy pastures of southwest Victoria.

##### i) Seasonal variation in Dry matter production and NUE

The field trials at Allansford showed a seasonal variation in biomass production in response to N, and in nitrogen use efficiency (NUE) defined in this report as nitrogen utilisation efficiency ( $NU_{AE}$ ), as indicated below (Figure A).



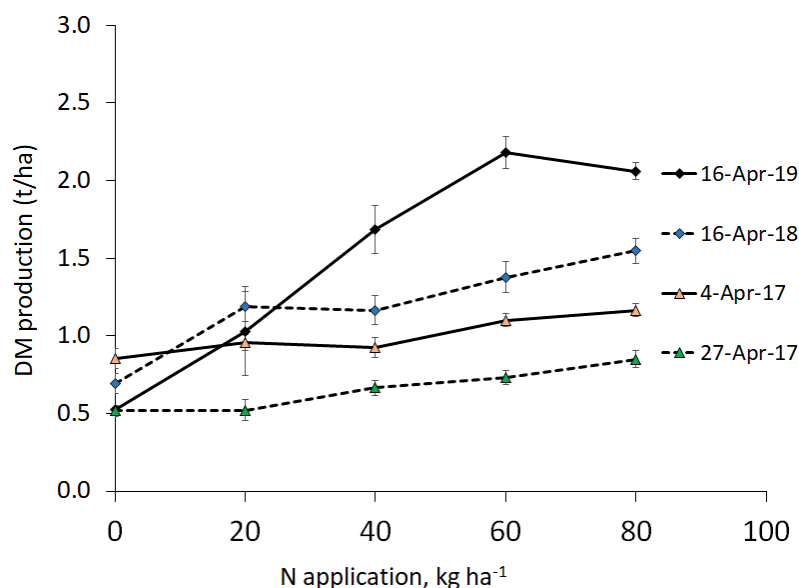
**Figure A. Seasonal pasture dry matter production (a) and Agronomic nitrogen utilisation efficiency ( $NU_{AE}$ ) (b) for the Allansford irrigated site averaged across all years.**

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A key recommendation from this work to improve NUE is for fertiliser inputs to be applied in relation to the expected biomass production rather than at the current practice of a blanket rate following each grazing event.

## 2. Water and Nitrogen Management

Annual variations in N responses at the autumn break (April) were observed on the irrigated site and found to coincide with soil moisture available through the deeper (> 20 cm depth) part of the soil profile (Figure B).



**Figure B. Autumn break (April) pasture dry matter production for the Allansford irrigated site.**

A recommendation is to lengthen the irrigation time at the shoulders of the autumn and spring growing seasons, either by stopping later (autumn) or starting earlier (spring) as this will provide an opportunity to increase dry matter production and home-grown feed. However, implications on winter soil moisture and site condition needs to be considered in this management decision.

## 3. Recovery of fertiliser N and supply of N from Soil Reserves

Pasture N uptake was found to be largely from the soil reserves with >70% of pasture N sourced from the soil and < 30% from the applied fertiliser (Table A). Nitrogen fertiliser recovered in the plant was < 30% of the applied N after one harvest event, and after 6-12 months an additional 10-15% of the applied N was utilised by the pasture. This result highlights that the fertiliser applied to pasture can be immediately utilised by the plant, or immobilised into the organic pool. Once immobilised, the N is subsequently released via re-mineralisation and can be utilised by pasture into the future. Under low N input systems, almost all of the applied N (>95%) was found in the plant and soil after 1 year. In our system a single dose of fertiliser was applied, but with repeated fertiliser additions at each harvest it is possible the recovery from this first application would be less over a year. But with increased N inputs, the recovery decreased as N was lost through one or more of the following pathways; volatilisation, leaching, or denitrification.

**Table A. Source of N taken up by the plant in the first month following fertilisation**

	N rate kg/ha	Biomass (t/ha)		N removed by pasture (Kg/ha)		<sup>15</sup> N recovery %		N derived from fertiliser (%)		N derived from soil (%)	
		Autumn	Spring	Autumn	Spring	Autumn	Spring	Autumn	Spring	Autumn	Spring
Irrigated	20	1.0±0.1	2.3±0.6	40±4.5	36±7.5	24.3±2.8	22.5±3.3	11	13	89	87
	40	1.2±0.1	2.4±0.3	46±4.2	39±3.7	26.2±2.4	22.2±2.5	21	23	79	77
Dryland	20	1.1±0.2	3.0±0.3	41±5.9	50±1.8	23.5±3.0	30.1±4.8	10	12	90	88
	40	0.9±0.1	2.7±0.8	33±3.7	47±12.5	20.6±2.6	21.2±5.1	23	18	77	82

N supply from fertiliser is required to build the organic matter reserves and maintain organic matter, but excessive supply will lead to N loss. The recommendation is to consider the impact of N applied as fertiliser over the longer-term. The nitrogen budgets determined for the 2.5 years of experimentation (irrigated) showed that higher rates of N input were leading to a cumulative build-up of excess N, which is expected to be even more when pasture returns and urine and manure deposition are factored into the equation, further recommending that high N rates are avoided.

Seasonal and annual soil mineral N supplied through mineralisation was determined and is discussed below (see mineralisation calculator)

#### 4. Use of remote sensing to predict N content and biomass of pasture

Remote sensing indexes were identified that could predict with reasonable reliability the pasture biomass and N content. Remote sensing measurement of pasture before canopy closure was identified as the most effective time for making predictions. Climate conditions somewhat hindered the use of the drone-based remote sensing approaches in the southwest Victoria region.

## **2. N<sub>2</sub> and N<sub>2</sub>O losses from pastures**

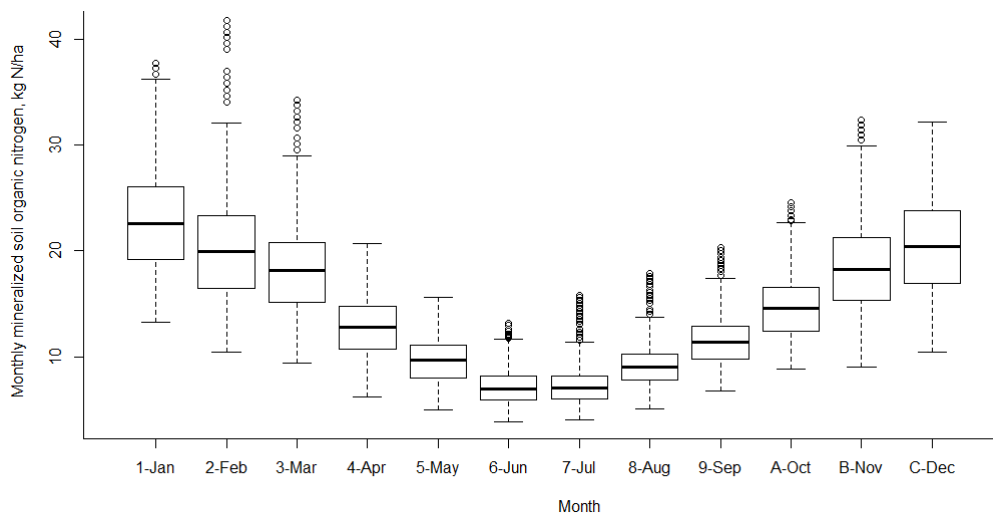
Nitrous oxide (N<sub>2</sub>O) emissions from the field site were small compared to the total N budget. Based on the laboratory experiments, as soil becomes wetter (i.e. to saturation), the losses of N<sub>2</sub> will increase, and the ratio of N<sub>2</sub>:N<sub>2</sub>O will also increase. Under the waterlogged conditions that occur over the winter-time, N<sub>2</sub> loss from denitrification could be a substantial loss pathway of N.

## **3. Mineralisation calculator**

A mineralisation calculator was developed and showed the seasonal fluctuations in mineralisation that occur (Figure C). The pattern of mineralisation was similar for the irrigated and dryland systems, but under the dryland systems, a greater amount of N is predicted to be available at the start of autumn (autumn break) than in the irrigated system due to lack of pasture growth and N utilisation over summer. For an average year, around 200 kg N was mineralised annually in the pasture soils.



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**Figure C: Predicted annual dynamics of mineralization of soil organic nitrogen for the irrigated system at Allansford.**

The mineralization calculator provides advisors with a good understanding of the drivers of mineralization which will help with decision making. Long-term N rate and future (autumn) temperatures were identified as key drivers of mineral N available for plant growth over autumn (including that available prior to autumn and that mineralized over autumn). The recommendation to industry is to consider the soil available N across the seasons based on an understanding of the key drivers, and to change N inputs accordingly to optimize NUE.

#### 4. Engagement with Industry

Workshops and field days held during the project have provided an opportunity to engage with industry to refine various components of the project (e.g. mineralization calculator development) as well as keep the industry informed of current findings from the research trials. Positive feedback has been received from these sessions, and these interactions are instrumental in achieving valuable applied research outcomes.

### 3.1 Project level achievements

Provide a description of project achievements against the final KPIs and outputs of the research project. As these final KPI have been worded to conclude the body of long-term investigation, please ensure the final findings are clearly articulated and linkage to impact upon current and future industry knowledge and practice is explained.

KPI no. and description	KPI Due Date	Relevant CRDC FRP Milestone Number/s.	Final outcome of the body to reserarch concluded by this KPI
KPI 7.5 – Provide brief commentary on the technical reference groups established, field days held and the outcome of the dairy knowledge exchange workshops (Output 6(j)).	30 November 2019	3.2	<input checked="" type="checkbox"/> Achieved <input type="checkbox"/> Partially achieved <input type="checkbox"/> Not achieved <p>Workshops (2) and field days (2) have been held as outlined in KPI 5.7 and 5.10 in Partner Research Milestone 6 Report. Further workshops (after June 2019) held have been related to the mineralization calculator as reported in KPI 7.7. The workshops have provided a good opportunity to obtain feedback on the research and to refine the interpretation of the collected data.</p>
KPI 7.6 – Provide an update on the N experiments on irrigated and rain-fed dairy farms in south west Victoria (Output 6(k)).	30 November 2019	1.7	<input checked="" type="checkbox"/> Achieved <input type="checkbox"/> Partially achieved <input type="checkbox"/> Not achieved <p>All field trials have been completed (June 2019).</p> <p>From May 2019 to June 2019 final harvests and soil samples were collected from all sites. <sup>15</sup>N microplots installed using 7 different soils at the Allansford irrigation site on 23<sup>rd</sup> October, 2018 and 16<sup>th</sup> April, 2019 were removed on 20<sup>th</sup> June, 2019, with samples processed and analysed to determine fertiliser recovery and the source of N taken up by the plant.</p> <p>Field trials were conducted over ~ 2.5 years at the Allansford irrigation site (November 2016 to June 2019), and 1 year at the dryland site (November 2016 to November 2017). Field trials were conducted at Coorriemungle from February to June 2019 to obtain additional dryland autumn break data.</p>

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			<p>Drivers of mineralization have been identified through prediction modelling as being primarily climate (moisture and temperature) and also N fertilisation. The future temperature is most correlated with predicted mineralisation during the autumn months for both the irrigated and dryland systems, while the mineral N that builds up over summer and is available at the autumn break is most correlated with the previous 3 months of rainfall in the dryland system, and with N rate in the irrigated system. The rate of N applied had more impact on predictions of N available via mineralisation in the irrigated systems than in the dryland due to the continuous production and high inputs under irrigation.</p>
<p>KPI 7.7 – Provide brief commentary on the development of the mineralisation calculator and the dairy workshop held to demonstrate it (Output 6(m)).</p>	<p>30 November 2019</p>	<p>3.2</p>	<p><input checked="" type="checkbox"/> Achieved  <input type="checkbox"/> Partially achieved  <input type="checkbox"/> Not achieved</p> <p>The mineralization calculator is being developed and refined. The workshop held on 23<sup>rd</sup> May presented the first form of the mineralization calculator to a group of industry representatives. Based on feedback from the 1<sup>st</sup> workshop (May 23<sup>rd</sup> 2019) and other considerations, the mineralization calculator is being refined.</p> <p>Further workshops have been held (28<sup>th</sup> August, 6<sup>th</sup> November, 2019) to refine the mineralization calculator with invited participants (3 advisors), based on feedback from the 1<sup>st</sup> workshop and other sources. Participants at the workshops have been given the opportunity to test the calculator to assess the user friendliness and usefulness. Further work on the calculator interface and delivery, plus identification of responses to extreme climate years developed from this</p>

## 3.2 Contribution to MPfN program objectives

**Activity 5:** The project has identified that N fertilisation in the HRZ of south-west Victoria needs to consider the seasonal variation in N response, rather than following the current standard practice of a set rate after each grazing event. By modifying the rate of N applied seasonally, growers will be able to make better use of N in times of high pasture growth (e.g. spring) and reduce the risk of environmental impacts during period of lower productivity (e.g. autumn). N recovery in pasture from applied fertiliser was low (<30%) one month after fertiliser was applied, with the remaining N being either immobilised or lost. An additional 15% of the applied fertiliser was released from the organic pool via re-mineralisation over a 12 month period. The immobilised N is expected to slowly re-mineralise over time. Infact when N rates were low (U10, U20) almost all of the applied N (> 95%) could be accounted for in this system (plant-soil-roots) with minimal loss through ammonia volatilisation, leaching or denitrification. However at higher N rates N was lost from the system (e.g. U80; 15-41% loss). Reducing N rates will improve NUE and reduce environmental losses, but needs to be balanced with pasture productivity requirements and considering the cost of feed.

The project also identified that water availability in the profile is a key driver of production at the shoulders of the season. Management of deep soil moisture will enable better utilisation of applied N and hence increase NUE at these times.

**Activity 6:** Mineralisation contributions to pasture growth were found to be substantial, with around 200 kg N/ha estimated to be mineralised annually from the irrigated and dryland pastures under fertilisation. Mineralisation and accumulation of N in the soil profile occurred at all times of the year but was higher through summer and spring. At the autumn break, the soil profile contained between 49 and 90 kg N/ha in the irrigated and dryland systems (2017 data), with the greater accumulation in the dryland system occurring due to a lack of summer pasture growth and N uptake. Mineralised N provided a substantial amount of N for plant uptake with >70% of the N taken up by pasture plants one month after fertilisation coming from the soil.

The mineralisation calculator identified that the key drivers for mineral N in the profile at the autumn break were N rate, and for the dryland system the previous rainfall and temperature. The key drivers of the amount of N that mineralises in the month after the autumn break were N rate, future temperature and to a lesser extent soil organic matter content and past rainfall, under both dryland and irrigated systems.

**Activity 4:** Examination of enhanced efficiency fertilisers to match expected loss pathways was carried out at the Allansford field sites. Current enhanced efficiency fertiliser appear to provide limited benefits in terms of productivity in the HRZ pasture systems. However this may be partly due to the management conditions. For example, in terms of the urease inhibitor, irrigation limits ammonia loss, and in autumn on the dryland system a good supply of N from the soil may limit any biomass benefit from the urease inhibitor, particularly at the low rates of application where

we also expect low rates of ammonia loss. A positive impact of DMPP during one winter season shows that the nitrification inhibitor can enable reduced N inputs and boost NUE, however often N savings are not translated into biomass.

### **3.3 Demonstrable more profit from nitrogen**

The results from the N fertilizer response trials of irrigated and dryland pasture conducted as part of this project demonstrate that adjusting the timing and rate of N fertilizer applied to dairy pastures depending on the time of the year, the growing conditions and the value of the forage at that time can improve the efficiency and therefore the economics of N fertilizer use. This is in contrast with the increasingly common practice of applying the same rate of N fertilizer at the same time interval across the growing season in a simple “recipe” approach on many dairy farms. For example in 2017, the Nitrogen Use Efficiency (NUE) of dryland pasture with applications of 60 kg N/ha was 3, 10, 17, 23, 25, 10 and -3 kg DM/ kg of N for harvests on the 27<sup>th</sup> April, 6<sup>th</sup> June, 1<sup>st</sup> August, 13<sup>th</sup> September, 14<sup>th</sup> October, 1<sup>st</sup> November and 22<sup>nd</sup> November respectively. Clearly, the low NUE’s for the 27<sup>th</sup> April and 22<sup>nd</sup> November harvests are unlikely to be economic and are most probably due to inadequate soil moisture levels to support good pasture growth. In contrast the good winter NUE’s are likely to be a cost effective way of boosting winter feed whilst the high spring NUE’s demonstrate that N fertilizer can be used to maximize spring pasture production. The results also demonstrate declining NUE’s with increasingly heavy rates of N application; for example the spring 2017 NUE’s on irrigated pasture were 31, 25, 18 and 16 kg DM/kg of N for applications of 20, 40, 60 and 80 kg N/ha respectively.

The field trials results indicate that the routine use of currently available Enhanced Efficiency Fertilizers (EEF’s) (a combination of urease and nitrification inhibitors applied depending on season and predicted loss pathway) cannot be justified, at least in the district where the trials were conducted. The only significant yield advantage to their use over conventional urea fertilizer was in the first winter with the use of DMPP (a nitrification inhibitor) applied to the irrigated pasture, which occurred at the low 20 kg N/ha application rate. These results indicate that the additional expense of applying either the urease or nitrification inhibitors as EEF’s cannot be justified at this time under irrigation where ammonia losses will have been low ,and on our dryland site where there were high background mineral N levels.

Soil N mineralization studies conducted as part of the project demonstrated that substantial and useful amounts of soil N are mineralized and are converted to plant available forms each year. It is estimated that around 200 kg N/ha is mineralized annually, with slight variation between years and between the dryland and irrigated systems. These amounts of mineralized N should be taken into account when determining N requirements of the pasture and should be able to replace some of the applied fertilizer N. For example, < 30% of applied fertilizer N is taken up by the pasture after the first growth cycle, and <50% after one year, and of the nitrogen taken up by the plant, >70% of this is derived from the soil. However, uncertainty around the actual uptake of mineral N versus available mineral N in the soil still exists.

## 4 Collaboration

The major collaboration established during the project was with the Melbourne School of Engineering (MSE) at the University of Melbourne - Professor Dongryeol Ryu and Manish Patel (PhD student). Dongryeol and Manish undertook studies of the use of hyperspectral and other imagery, including an investigation of the potential to use satellite imagery, on making predictions of pasture N and biomass, and using this to make predictions of N use.

The collaboration here has led to the presentation of a number of conference papers by the PhD student as outlined in the section 7.1.

This collaboration is likely to continue and is supported by the new appointment of Professor Pablo Zarco-Tejada at the University in the Faculty of Veterinary and Agricultural Sciences and MSE.

Collaboration on the sensor technologies was further strengthened by connections made with the University of Manchester, UK, through an internal University seed funding program. Use of field based nutrient sensors was also investigated through collaborations with the Faculty of Science at the University of Melbourne (Prof. Spas Kolev).

Utilisation of sensor technologies will be the future approach required for a more accessible and fast assessment to inform farm management. While field based investigations provide detailed information on agronomic responses and nutrient cycling, and enable mechanistic studies to be undertaken, this approach is time consuming.

## 5 Extension and adoption activities

### 5.1 Extension of the research to the end-user

- All project extension activities, field days, workshops and targeted dairy consultant workshops were well attended and received by the dairy advisor community. These dairy advisors are very influential in determining nitrogen fertilizer use and practices on farm in the dairy industry. This combined with the number of dairy farmer clients that they deal with means that by targeting these advisors, the project has been able to effect greater awareness and uptake of the recommendations of this project across the industry.
- Other extension activities include additional field days held outside the investigation region (Gippsland and Gellibrand), and conferences (Fertiliser Industry Australia conference, Agronomy Conference, Soil Science Australia Conference, Soil Organic Matter Conference, World Soils Congress, ASA-CSSA-SSSA conference (US), AGU Fall Meeting (US)).
- Findings from the project have been used to revise the “Best Management Practices for nitrogen (N) fertiliser use on dairy pastures” guidelines.

### 5.2 Recommendations to industry on adoption of the research outcomes.

The Australian dairy and fertilizer industries have a firm and ongoing commitment to improving the responsible, efficient and effective use of nitrogenous fertilizers in dairying. This is driven by a number of factors including the objective of reducing the environmental footprint of dairying; reducing the losses of nutrients to the environment; maintaining the social licence of the dairy industry; improving the efficiency of dairy production systems; and improving the profitability of dairy farming.

To these ends both the dairy and fertilizer industries have a number of established and respected extension and education programs on the use of fertilizers, including nitrogen fertilizers, on dairy farms and in the broader dairy industry. It is recommended that the results and recommendations arising from this current project, and the two other dairy projects in the “More Profit from Nitrogen program” be, where possible, incorporated into and included in these existing dairy fertilizer extension and education programs. This would entail updating the current content of these programs, the writing of specific materials such as technical notes and/or video clips for inclusion in these programs. Some suggested suitable programs for the inclusion of these updates include:

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- The “Dairying for Tomorrow” website ([www.dairyingfortomorrow.com.au](http://www.dairyingfortomorrow.com.au)). This site already has a section giving details on the three current “More Profit from Nitrogen” projects. This will service both farmer and advisor audiences.
- The “Fert\$mart” Dairy Soils and Fertilizer Manual and farmer courses. “Fert\$mart” is the dairy industry’s central repository of soil and fertilizer knowledge and best practice information and is held and maintained by Dairy Australia. The manual can be accessed on the “Dairying for Tomorrow” website. Similarly, “Fert\$mart” farmer courses run by the Regional Develop Programs of Dairy Australia will be an important vehicle in extending the results and recommendations to the farmer level.
- Updating the BMPs for the Dairy Industry (Best Management Practices for nitrogen (N) fertiliser use on dairy pastures. Knowledge gained in this project has been incorporated into the BMPs and will be developed in two guides, to be available on the Dairy Australia website and as a pocketbook (distributed through the Dairy Australia networks).
- The “Fertcare” program. “Fertcare” is a joint venture between Fertilizer Australia and the Australian Fertilizer Services Association. It offers a range of training and accreditation courses to people working in the fertilizer industry - in particular the “Fertcare C” – accredited advisors qualification designed to train those giving detailed fertilizer advice. A Dairy Farm Nutrient Management module to train advisors in developing dairy farm nutrient management plans has recently been developed. It is suggested that “Fertcare C” would be a suitable vehicle to extend recommendations to train advisors.



# 6 Lessons learnt

## 6.1 Research level

The project has utilised experimental work at the field and lab scales to address the issue of N efficiency in HRZ dairy pastures of southern Australia. The information collected during this study enabled us to:

- Understand the drivers of seasonal pasture productivity, N uptake and NUE from urea fertilised dairy pastures under dryland and irrigated production systems;
- Quantify N dynamics in and through the soil and plant pools, and estimate losses from the system using <sup>15</sup>N mass balance methodology on a range of soil types from the HRZ in south-west of Victoria;
- Quantify gaseous N<sub>2</sub>O losses in the field and estimate N<sub>2</sub> losses based on laboratory experiments, and identify controlling factors;
- Propose improved N fertiliser management approaches based on better predictions of N mineralisation.

The seasonal variations seen in pasture productivity and NUE indicate a change in N fertiliser management is required (see section 6.2 below). Use of the <sup>15</sup>N fertiliser approach has enabled a clear identification of the role of soil-N in pasture nutrition, which is much higher than imagined at commencement of the project. The long-term impacts of a single fertiliser event should be considered when thinking about pasture N nutrition. Contributions of N to the soil organic matter pool, and subsequent release may provide an opportunity to target fertilisation to times of low loss, and to ensure more efficient N fertiliser use.

The outcomes from the mineralisation calculator showed that mineralisation can occur even under periods where there is little soil moisture (summer) if there are regular, albeit they may be small, inputs of water to the system.

The field experimental program worked well in many aspects through development of a good working relationship between the commercial growers and the research team, facilitated by Mr Graeme Ward. Climatic and management (e.g. irrigator) factors were difficult to manage and led to some difficulties collecting data from dryland production systems at the core site. Establishment of earlier irrigation intervention on the dryland site may have prevented this, as while irrigation was applied, the extreme conditions over the 2017/18 summer led to loss of pasture, and future conditions were inadequate for pasture re-establishment. Having the ability to access a satellite site addressed this problem and enabled another autumn break measurement to be collected. Having a number of field site options was beneficial to manage this risk.

Interaction with industry groups was critical for getting feedback on the project direction and the development of the mineralisation calculator. Commencing this engagement early in the program

enabled the research to ensure there was a good focus on providing meaningful industry outcomes. Linking in with extension opportunities (e.g. with AgVic provider breakfast, WestVic Dairy field days) enabled access to a greater audience for discussion on the project. Interaction with invited consultants to refine the mineralisation calculator has led to increasing awareness from the research modeller about what industry needs from this tool, which will lead to development of a more robust tool.

## 6.2 Industry level

The results of the field trials conducted as part of this project confirm that the use of N fertilizer is a particularly cost effective and practical method of growing additional forage on both dryland and irrigated perennial pasture on southern Australian dairy farms. They further confirm that to obtain optimum economic returns to applied N fertilizer and to reduce the risk of excessive losses of N to the environment and the associated detrimental environmental effects, the rate and timing of N fertilizer applications will vary across the growing season. As such, the same simple, fixed “recipe” for N fertilizer applications for the entire growing season does not lead to the safest and most efficient use of N fertilizer.

Heavy applications of N fertilizer (e.g. 60 & 80 kg N/ha) per application resulted in significant declines in the Nitrogen Use Efficiencies (NUE) (kg DM/kg N applied) with increasingly lower percentages of applied N being recovered in the herbage leaving a greater proportion at risk of being lost to the environment compared to applications of 40 – 50 kg N/ha.

The project results highlighted that for dryland pastures, the times of the year with the greatest uncertainty and risk for N fertilizer applications are the “shoulder” periods - after the autumn break and late spring - when soil moisture levels are low, restricting plant growth and likely N responses. It is recommended that further research is warranted on improved prediction/decision making tools for N fertilizer applications and the fate of N applied at these times, particularly around the relationship between N uptake, pasture growth and profile soil moisture.

The use of Enhanced Efficiency Fertilizers (EEF's) compared with conventional urea fertilizer had little effect on improving the pasture dry matter (DM) yields. The use of DMPP (a nitrification inhibitor) coated urea only produced a significant increase in pasture DM yield in one winter, and then only on the very low 20 kg N/ha application rate. As such there is little evidence from this project that the use of current EEF fertilizers on perennial pastures will improve production and NUE's under the conditions and the environment of the trial site. However, it should be noted that the response to the urease inhibitor coated urea (targeting ammonia loss) under dryland was limited to one year only where high background N may have limited a biomass response. Under irrigated systems we do not expect a response to the urease inhibitor if the irrigation is timed to follow fertilisation.

Several new technologies to assist in improving the prediction of optimum pasture N requirements were investigated as part of this project. The hyperspectral imaging of pasture showed some promise, demonstrating that the technology can work. However weather

conditions at the trial site, specifically variable and intermittent sunlight intensity due to cloudiness made the measurements problematic. This technology shows potential, but requires further development before it could be used with confidence by the dairy industry. The computer based soil N mineralization calculator, designed to estimate the total amount of mineral N in the soil and the rate of mineralization each month that potentially could offset the amount of fertilizer N, was further developed and tested with a group of dairy farm consultants. The calculator provides confidence for advisors and growers to predict N supply from soil, and is currently useable in the local climate region. Further work would be required to expand the region where it could be confidently used.

The project has also improved our knowledge and understanding of the dynamics of fertilizer and soil N in dryland and irrigated perennial dairy pastures in southern Victoria. This includes the finding that the plant uptake of N directly from applied N fertilizer is quite low with the bulk coming from soil N reserves (60-94%), although a greater percentage coming from fertilizer as fertilizer application rate is increased. Similarly, the project has generated important data on the rate of soil N mineralization, and thus mineral N available for plant uptake for each month of the year. Whilst these findings will not be directly adopted by the dairy industry on-farm, they have increased our knowledge and understanding of the dynamics and behaviour of N and fertilizer in these pastures for use in future research, and by advisors.

### **6.3 Service Provider Level**

Most of the learnings for the dairy industry in general as listed in section 6.2 above are equally applicable to the dairy service provider community. In particular, the findings of this project have demonstrated that pasture responses to N fertilizer applications, both in terms of DM yield responses and NUEs are quite variable across the year and between dryland and irrigation pastures. As a result advisors need to consider these differences at different times of the growing season in determining an economically optimum N fertilizer program. This is in contrast to recommending a constant “recipe” program across the growing season. This will include taking into account amounts of mineral (plant available) N in the soil made available from mineralization, particularly over summer and autumn in dryland pastures.

The project results also demonstrate that some caution should be applied to recommending N fertilizer application rates greater than current usual industry practice of 40-50 kg N/ha per application due to reduced NUE (affecting economics of the application) and the large amounts of N not taken up by the pasture and potentially available for loss to the environment. Likewise caution should be used if considering a drop in N rate as pasture responses to low application rates of 20 kg N/ha per application were found to be quite variable and inconsistent.

Our field trials demonstrated that highly productive pastures are removing large amounts of N as well as P, K, S and Mo, and that deficiencies in any of these other essential plant nutrients could reduce pasture responses to N. It can be difficult to distinguish N deficiency from deficiencies in

the other nutrients, particularly S, as the plant symptoms are similar, and service providers should be aware of total pasture nutrient needs.

## **6.4 Primary Producer Level**

As for the general industry and service provider learnings described above, a key recommendation at the dairy farmer level is that the use of a constant, regular “recipe” for N fertilizer applications across the whole growing season can often be inefficient and not optimize the economic and productivity benefits of N fertilizer applications to pasture. Nitrogen fertilizer responses to applications during autumn on dryland pastures were often found to be low, most likely due to inadequate soil moisture levels and possibly also due to the accumulation of mineral N from soil mineralization. Similarly pasture responses in late spring were found to be unreliable/inconsistent due to low and/or falling soil moisture levels on dryland pastures. These findings suggest that farmer monitoring/awareness of soil profile moisture levels, together with the outlook for follow-up rainfall at these times are important in N fertilizer application decision making. The results also demonstrate that an awareness of likely DM yield responses to N fertilizer and the economic value of these responses at different times of the growing season is important in optimizing economic returns of N fertilizer use. For example, the NUE of winter applications, although often not high, demonstrates that grown pasture can be more cost effective than alternative feed sources. During spring on dryland pastures and spring-summer on irrigated pastures, the high NUEs at these times demonstrate that higher rates of N fertilizer can be used to optimize DM production. As discussed for the service providers (section 6.3), the importance of ensuring that deficiencies of other nutrients do not limit N responses should be emphasised.

# 7 Appendix - additional project information

## 7.1 Project material and intellectual property

### 7.1.1 Journal Papers published

No journal papers have been published at the time of this report

### 7.1.2 Journal Papers in preparation and review

Belyaeva O, Ward G, Wijesinghle T, Chen D, Suter H (in preparation) Nitrogen use efficiency and nitrous oxide emissions from the Australian high rainfall zone pasture.

Pandey A, Belyaeva O, Wijesinghle T, Chen D, Suter H (in preparation) Effects of soil moisture on N<sub>2</sub> and N<sub>2</sub>O emissions from dairy pasture.

Manish Kumar Patel, Dongryeol Ryu, Andrew Western, Helen Suter and Iain M Young (in preparation) Evaluation of Remote Sensing Indices for Canopy Nitrogen Concentration Estimation in an Irrigated Pasture Crop under Varying Growth Stages and Seasons.

### 7.1.3 Conference Papers

#### Peer Reviewed conference papers

Belyaeva O, Suter H, Ward G, Chen D (2019) Field calibration of the capacitance soil moisture probes for Brown Sodosol. *19<sup>th</sup> Australian Agronomy conference: Cells to satellites*, August 2019, Wagga-Wagga, Australia.

#### Conference presentations / posters

Belyaeva O, Ward G, Chen D, Suter H (2018) Impact of urease and nitrification inhibitors on ryegrass productivity in the high rainfall zone of southern Australia. *21<sup>st</sup> World Congress of Soil Science (WCSS)*, Rio-De-Janeiro, Brazil, August 2018

H.C. Suter, O. Belyaeva and Graeme Ward (2018) Predicting nitrogen supply from mineralization in temperate dairy pastures, *National Soil Science Conference*, Canberra, 18-23 November

Patel M, Ryu D, Western AW, Suter H, Young I (2018) Planar Domain based Remote Sensing of Canopy-Level Nitrogen in Ryegrass and Phenological Effect. *3rd UAS4RS Conference*, Melbourne, Australia, December 2018

Helen Suter, Oxana Belyaeva, Graeme Ward, Yong Li (2018) Nitrogen supply from soil mineralisation in dairy pastures, *Australian Fertiliser Industry Conference*, Canberra, 10-11 October, 2018

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Alexis Pang, Manish Patel and Helen Suter (2018) More profit from nitrogen using remote and proximal sensing technologies: integrating across scales and sensors, *Australian Fertiliser Industry Conference*, Canberra, 10-11 October, 2018

Alexis Pang, Manish Patel and Helen Suter (2018) More Profit from Nitrogen using Remote and Proximal Sensing Technologies, *Australian Fertiliser Industry Conference*, Canberra, 10-11 October, 2018

Tord Ranheim Sveen, Deli Chen, and Helen Suter (2019) Nitrogen mineralization related to light-fraction and hot-water extractable carbon in pasture and cropping soils, *Soil Organic Matter Conference: In a stressed world*, Canberra, 6-11 October, 2019

Helen Suter, Oxana Belyaeva and Graeme Ward (2019) Utilising Soil Nitrogen to Enhance Nutrient Use Efficiency in Temperature Dairy Pastures, *ASA-CSSA-ASSA International Annual Meeting: Embracing the digital environment*, San Antonio, Texas, USA, 10-13 November

Helen Suter, Oxana Belyaeva, Graeme Ward, Yong Li (2019) Improving dairy farm nitrogen efficiency using advanced technologies, *Australian Fertilizer Industry Conference*, Gold Coast, 5-6 September

Dongryeol Ryu, Manish Kumar Patel, Andrew W Western, Helen Suter and Iain Young (2019) Hyperspectral Remote Sensing of Canopy Nitrogen Concentration in Ryegrass using a Partial Least Square Regression Model, *AGU Fall Meeting*, San Francisco, USA, 7-11 December

### 7.1.4 Intellectual property

IP generated during the program includes the mineralisation calculator developed by Yong Li as part of the research project.

## 7.2 Equipment and assets

List of all equipment or assets created or acquired during the period covered by the project.

Item purchased	Date of purchase	Purchase price (GST exclusive)
No items of capital of >\$10,000 purchased		

## 7.3 Media and communications material

Communication and extension activities have been provided in the MPfN database.

## 7.4 Resource outputs for industry

The following has been produced during the course of the project

Rural R&D for Profit Program Final Report

More Profit from Nitrogen Program

<b>Title</b>	<b>Author</b>	<b>Date finalized</b>	<b>Host platform for ongoing use</b>
Mineralisation calculator	Yong Li	30 <sup>th</sup> May, 2020	To be available on line

# 8 Appendix – Project technical report

## **1. Project Highlights**

### **1.1 Summary of key activities for the project**

The major activities within the project are:

- Field experiments at two sites running from November 2016 to November 2017 (Allansford dryland), November 2016 to July 2019 (Allansford Irrigated) and April 2019 to June 2019 (Corriemungle dryland) including long-term measurements of climate, pasture biomass, soil mineral N and moisture (surface and profile).
- Investigation of the fate of applied fertiliser N and the source of plant N using <sup>15</sup>N microplots at 4 different application times (April 2017, November 2017, October 2018 and April 2019)
- Development of a mineralisation calculator
- Identification of the key drivers of mineralisation
- Estimating losses of N as gaseous emissions of N<sub>2</sub> and N<sub>2</sub>O using targeted field measurements and laboratory incubations
- Communication and extension activities including workshops / field days (9) and conferences (10)
- Publication in peer reviewed conference proceedings (1)
- Collaboration with Melbourne School of Engineering (UM), School of Chemistry (UM) and Manchester University (UK).

### **2. Workshop and Field days (KPI 7.5 Output 6j and 6m)**

The following workshops and field days have been held and presented to report on project findings as part of the research program.

- Fert\$mart presentation, Terang (23/02/2017)
- Field Day, Improving Nitrogen Fertilizer Use on Dairy Farms (21/03/2017)
- Field Day: Latest Developments in Nitrogen Fertilizer Use From Local Trials (23/02/2018)
- Gellibrand Sustainable Dairy Project (11/10/2018)
- GippsDairy Field Day (01/02/2019)
- Naringal Field day (22/05/2019)
- Service Provider Update (23/05/2019)
- Mineralisation workshop (28/08/2019)
- Australian Fertiliser Industry Conference Field day (06/09/2019)
- Mineralisation workshop (06/11/2019)



### **3. Field experiments (KPI 7.6 Output 6 (k))**

#### **3.1 Methodology**

##### **3.1.1. Site Details and Experimental Design: Allansford**

The site was located at 5048 Great Ocean Road, Mepunga West, SW Victoria (38°25'05" S, 142°38'24" E). Dryland and irrigated field experimental sites were established on perennial ryegrass dominant pasture. Data collected at the site (climate, soil moisture and temperature, soil mineral N, herbage dry matter production and N rate response, pasture N content, impact of enhanced efficiency fertilisers, <sup>15</sup>N fate of urea and urine) covered 1 dryland growing period (April – November 2017), and 2.5 years of irrigated pasture production (November 2016-June 2019). Climate data was collected from the sites (dryland and irrigated) from commencement until 6<sup>th</sup> July, 2019.

The treatments established at Allansford on both dryland and irrigated areas were;

- 1-5: N response : Urea at 0 (C), 20 (U20), 40 (U40), 60 (U60) and 80 (U80) kg N/ha
- 6-8: EEF: Green UreaNV™ or Urea with ENTEC® (Table 1) at 10 (EEF10), 20 (EEF20) and 40 (EEF40) kg N/ha
- 9: Urine: synthetic at 1000 kg N/ha

Fertiliser was applied immediately following pasture harvest to simulate fertilisation after grazing. Urine patches were applied to the soil 3 times in the dryland site (6<sup>th</sup> June 2017, 14<sup>th</sup> October 2017, 23<sup>rd</sup> November 2017) and 4 times in the irrigated site (as for dryland plus 9<sup>th</sup> February 2018), with equivalent of 1000 kg N/ha applied over ~ 0.35m<sup>2</sup> area each time to a different quarter of each urine treatment plot. The treatment schedule is shown in Table A1 (Appendix).

Pasture herbage dry matter (kg dry matter per hectare) was collected by manual mowing after ryegrass reached the 3-leaf stage. Pasture dry matter yield was measured from an area of 1.5 m<sup>2</sup> on all plots. Pasture harvest wet weight was recorded, and a subsample of approximately 0.2 kg of fresh forage was oven-dried at 70°C until constant weight to determine pasture dry matter (DM). Remaining plant residues were removed from all plots and discarded (non-return). Soil mineral N was determined from samples (0-10 cm) collected using a 2.5 cm internal diameter corer, originally at each harvest date and then 2 weeks after fertiliser application to trace the mineral N dynamics from the fertiliser (2 weeks post fertiliser collection commenced after the 1<sup>st</sup> August 2017 harvest). On each occasion 5 soil cores (0-10 cm) were collected randomly from each plot and combined to create one composite sample. Samples were oven dried at 40°C until constant weight and then passed through a 2 mm sieve for chemical analysis. Soil moisture and temperature fluctuations were determined using capacitance probes installed in triplicate on each site, which were removed on 6<sup>th</sup> July 2019. Additional surface soil moisture measurements were done using 0-6 cm theta probes installed into selected treatments. Deep soil cores were collected at 5 times (3<sup>rd</sup> April 2017, 2<sup>nd</sup> August 2017, 7<sup>th</sup> November 2017, 12<sup>th</sup> December 2018 and 4<sup>th</sup> June 2019) to calibrate the capacitance probes and determine soil bulk density.

Soil profile N was determined at commencement of the experiment on the buffer (non-fertilised) zone on 4<sup>th</sup> April 2017 on the dryland and irrigated sites. Soil profile N was then determined from deep soil cores to 60 cm depth collected in each replicate plot of 5 selected treatments (Control, U40, EEF40, U80, Urine on 23<sup>rd</sup> November 2017, 18<sup>th</sup> May 2018 and 17<sup>th</sup> April 2019 on the irrigated site, and on 23<sup>rd</sup> November 2017 on the dry land site).

*Dryland site:* Data collection on the dryland site occurred only in 2017 due to the hot dry 2017-2018 summer causing pasture death, and a lack of effective pasture re-establishment and vigour in 2018. A total of 8 fertiliser events were placed onto the dryland site (Nov-16 to Nov-17), with between 80 (EEF10) and 640 (U80) kg N/ha applied in total (Table A1). Three <sup>15</sup>N microplots were established on 16<sup>th</sup> April 2019 on areas with adequate ryegrass growth, with <sup>15</sup>N urea applied at 40 kg N/ha to assess the fate of fertiliser N applied in autumn, and were excavated on 20<sup>th</sup> of June 2019.

*Irrigated site:* Data collection continued on the irrigated pasture site until 6<sup>th</sup> June 2019. A total of 31 fertiliser events were placed onto the irrigated site (Nov-16 to Jun-19), with between 310 (EEF10) and 2,480 (U80) kg N/ha applied in total (Table A1).

<sup>15</sup>N microplots (23.7 cm diameter, 25 cm depth) were installed on site at Allansford to investigate the fate of urea applied on April 4<sup>th</sup> 2017 and September 9<sup>th</sup> 2017 on both the dryland and irrigated sites. All <sup>15</sup>N fertiliser plots were removed from the sites on May 1<sup>st</sup> 2018.

The fate of urine-N was examined using <sup>15</sup>N using the same size microplots which were installed at Allansford on the irrigated and dryland sites on 6<sup>th</sup> June 2017, and removed on 11 September 2018; 14<sup>th</sup> October 2017, and removed on 23<sup>rd</sup> November 2018; 23<sup>rd</sup> November 2017, and removed on 12<sup>th</sup> December 2018; and 9<sup>th</sup> February 2018 (irrigated site only due to dry conditions leading to pasture death on the dryland site) and removed on 21<sup>st</sup> February 2019.

In 2018/2019, cores from six different soils were collected as <sup>15</sup>N microplots in triplicate, to include at the irrigated site at Allansford to determine the fate of fertiliser N applied in spring (23<sup>rd</sup> October 2018, N rate 20 kg N/ha) and autumn (16<sup>th</sup> April 2019, N rate 40 kg N/ha).

Losses of N from the pasture system as N<sub>2</sub>O emissions were measured using the static chamber method for 21 days following fertilisation at i) the beginning of spring 2018, ii) the beginning of autumn 2018, and iii) the beginning of autumn 2019.

### 3.1.2. Site Details and Experimental Design: Cooriemungle

An additional experimental site was established at 401 Cooriemungle Road, Cooriemungle (38.5367°, 143.059°) on 1<sup>st</sup> February 2019, following removal of the site from grazing in November 2018, and was maintained until 6<sup>th</sup> of July 2019, when the last harvest was completed. There were five treatments applied to plots of 3 m x 3 m, replicated five times and arranged in a randomised block design. The treatments imposed were urea at 0, 20, 40, 60 and 80 kg N/ha.

Capacitance probes and a weather station were established on site from 12<sup>th</sup> February 2019 to 6<sup>th</sup> June 2019. A total of 2 fertiliser events were applied onto this dryland site (16<sup>th</sup> April 2019 and 3<sup>rd</sup> June 2019) with between 40 (U20) and 160 (U80) kg N/ha applied in total.

Deep soil cores were taken from 5 locations across the site to determine the basic soil properties, N and soil moisture contents up to 60 cm depths on 16<sup>th</sup> of April 2019 prior to fertiliser application. Soil surface mineral N was also monitored from 0-10 cm soil samples collected using a 2.5 cm internal diameter corer 2 weeks after fertiliser application. On each occasion 5 soil cores were randomly collected to a depth of 10 cm, from all treatments and combined within each plot. Samples were oven dried at 40°C until constant weight and then passed through a 2 mm sieve for chemical analysis.

Pasture herbage dry matter (kg dry matter per hectare) was determined from manually mown harvests when pasture reached the 3-leaf stage. Grass yield was measured from a 1.5 m<sup>2</sup> area on all treatment and control plots. The wet weight of the harvested vegetation from each plot was recorded and a subsample of approximately 0.2 kg of fresh pasture was oven-dried at 70°C to determine pasture dry matter (DM). Remaining plant residues were discarded and not returned to the plots.

Three <sup>15</sup>N microplots were established at the site on 16<sup>th</sup> April 2019 with <sup>15</sup>N urea applied at 40 kg N/ha, and were excavated on 20<sup>th</sup> June 2019.

### 3.1.3. <sup>15</sup>N microplots to study the influence of soil types on the fate of N and source of N utilised by pastures.

In addition to the microplots established at Allansford and Coorimungle as described above, in 2018/2019, <sup>15</sup>N microplot cores (23.7 cm diameter, 25 cm depth) from six different soils were collected in triplicate, and established at the irrigated site at Allansford to determine the fate of fertiliser N applied in spring (2<sup>nd</sup> October 2018, N rate 20 kg N/ha) and autumn (16<sup>th</sup> April 2019, N rate 40 kg N/ha). These cores were established alongside three <sup>15</sup>N microplots from the Allansford irrigated site. Cores were established by cleaning the base of the cores and covering this with a permeable mesh, then placing the cores onto a drainage base (sand and gravel) to prevent saturation of the base of the cores. Cores were placed under irrigation to eliminate the risk of pasture death due to lack of water after the October application. Details of the soil properties for the seven soils are provided in Table 1. These soils represented the variety found in the western Victoria dairy region. The first set of microplots were excavated on 19<sup>th</sup> March 2019 and the last set were excavated on June 20<sup>th</sup>, 2019.

**Table 1. Properties of the seven <sup>15</sup>N microplot soils established at the Allansford irrigated site**

Soil	Depth (cm)	pH <sub>w</sub>	pH <sub>CaCl2</sub>	Clay	Silt %	Sand	CEC cmol(+)/kg	Org C %	P* mg/kg	K* mg/kg	S
Coorimungle-1	0-10	5.7	5	34	14	53	11.3	5.5	63	210	19
	10-20	5.7	4.7	38	19	43	9.71	3.4	35	160	10
Coorimungle-2	0-10	5.1	4.2	11	5	85	6.93	7.4	23	100	12
	10-20	5.4	4.5	13	9	78	4.52	3.1	21	62	6
Naringal-1	0-10	5.4	4.7	9	16	75	23.6	11	18	200	44
	10-20	5.3	4.4	12	24	64	12.4	6.5	7	110	27
Naringal-2	0-10	6.4	5.3	4	1	95	11.8	3.5	17	130	14
	10-20	5.9	4.5	4	1	95	8.58	2.9	9	43	7
Panmure	0-10	5.9	5	15	5	80	9.95	4	27	190	8
	10-20	6	4.9	15	6	79	6.61	3.3	14	110	7
Allansford – site 3	0-10	7.1	6.4	9	5	86	12.5	3.2	69	160	34
	10-20	7.4	6.6	7	7	85	11.2	2.4	21	110	24
Allansford -irrigated	0-10	6.3	6.7	4	11	84	13.9	-	115	158	19
	10-20	6.3	6.6	6	12	82	11.3	-	45	71	10

\*Olsen P, Ammonium acetate K.

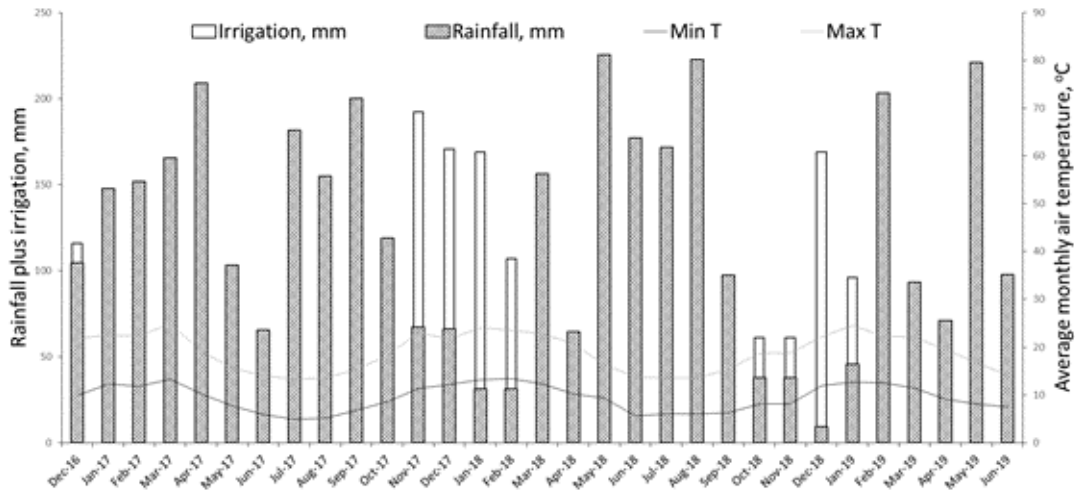
## 3.2 Results

### 3.2.1. Climatic and soil conditions

#### *Allansford*

The climate data for the Allansford sites are shown in Figure 1. Monthly air temperature fluctuations were typical of seasonal conditions in this region. The daily air temperature varied from -0.7°C (July 2017) to + 42°C (January 2019). During the trial, rainfall was lower than the long term mean which is 743 mm (Commonwealth Bureau of Meteorology) ([www.bom.gov.au/climate](http://www.bom.gov.au/climate)). A total of 601 mm of rain fell on the dryland site in the first year of the trial (Dec 2016 – Nov 2017), 475 mm in the second year (Dec 2017-Nov 2018) and 216 mm in the last eight months of the trial from Dec 2018 to July 2019. Irrigation additions in the irrigated site increased the amount of water received to 1490 mm in the first year (Dec 2016 – Nov 2017), 1349 mm in the second year (Dec 2017-Nov 2018) and 815 mm in the last eight months of the trial from Dec 2018 to July 2019.

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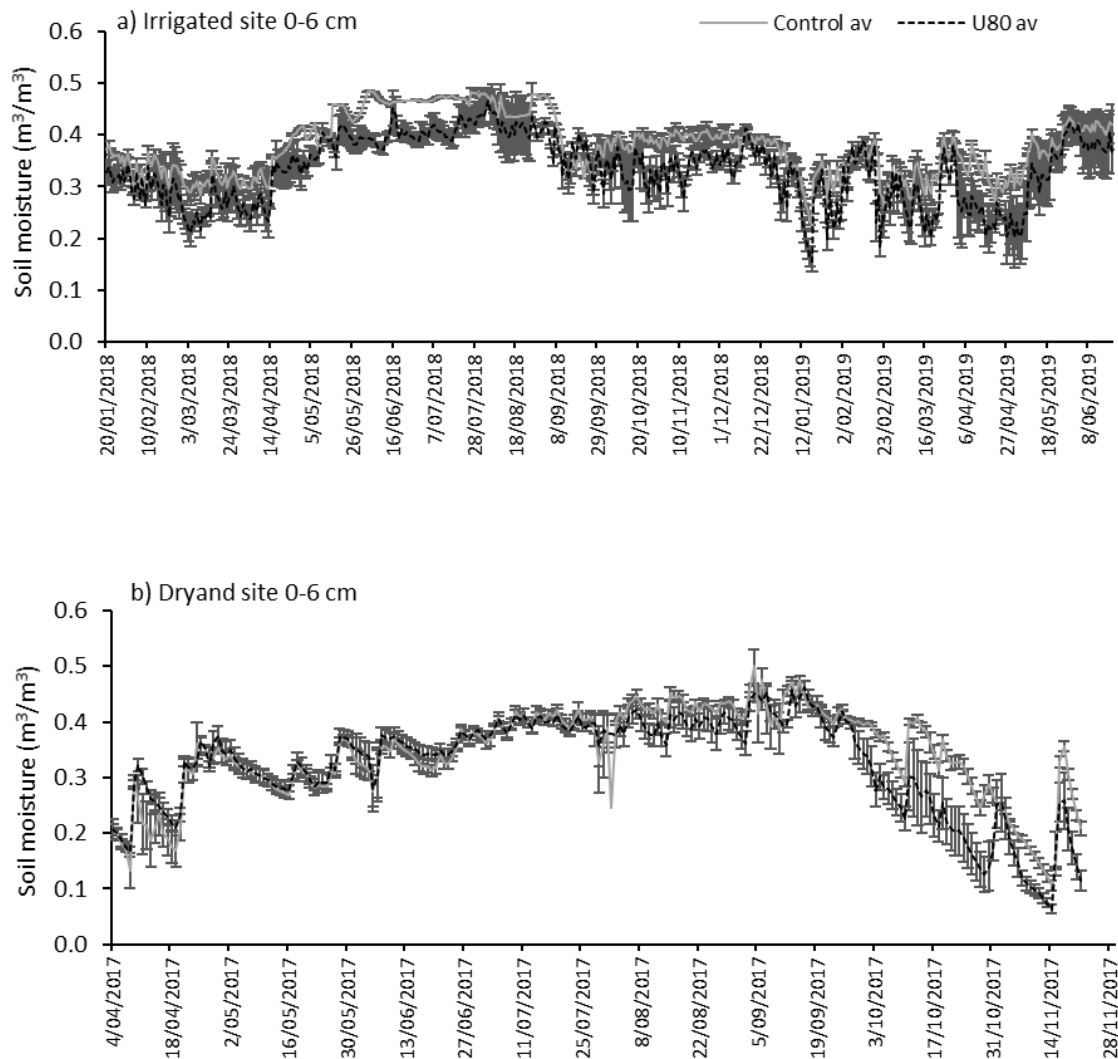


**Figure 1. Monthly rainfall (irrigation and dry land sites) plus irrigation (irrigation site) and minimum and maximum air temperature at the Allansford sites (Dec 2016 to June 2019)**

Profile soil moisture (volumetric water content) fluctuated in response to rainfall and irrigation inputs, and on the irrigated site ranged from 0.11 m<sup>3</sup>/m<sup>3</sup> to 0.80 m<sup>3</sup>/m<sup>3</sup> (10 cm), 0.08 m<sup>3</sup>/m<sup>3</sup> to 0.63 m<sup>3</sup>/m<sup>3</sup> (20 cm), 0.05 m<sup>3</sup>/m<sup>3</sup> to 0.51 m<sup>3</sup>/m<sup>3</sup> (30 cm), and below 30 cm the moisture content was above 0.19 at all times and showed much less variability (e.g. 0.19 m<sup>3</sup>/m<sup>3</sup> to 0.66 m<sup>3</sup>/m<sup>3</sup> (40 cm), 0.25 m<sup>3</sup>/m<sup>3</sup> to 0.63 m<sup>3</sup>/m<sup>3</sup> (50 cm) due to the higher clay content. On the dryland site the soil was much drier through the profile with moisture content ranging from 0.07 m<sup>3</sup>/m<sup>3</sup> to 0.66 m<sup>3</sup>/m<sup>3</sup> (10 cm), 0.07 m<sup>3</sup>/m<sup>3</sup> to 0.54 m<sup>3</sup>/m<sup>3</sup> (20 cm), 0.05 m<sup>3</sup>/m<sup>3</sup> to 0.47 m<sup>3</sup>/m<sup>3</sup> (30 cm), 0.05 m<sup>3</sup>/m<sup>3</sup> to 0.30 m<sup>3</sup>/m<sup>3</sup> (40 cm), 0.15 m<sup>3</sup>/m<sup>3</sup> to 0.29 m<sup>3</sup>/m<sup>3</sup> (50 cm). The dry 2017-2018 summer is reflected in the soil moisture on the dryland site (Figure A1, Appendix 1).

In the top 0-10 cm layer the volumetric soil moisture content ( $\theta_v$ ) (Figure 2) ranged from 0.11 m<sup>3</sup>/m<sup>3</sup> to 0.50 m<sup>3</sup>/m<sup>3</sup> with short-term peaks coinciding with rainfall or irrigation events. More moisture was removed on both the dryland and irrigated sites from the U80 treatments to support increased biomass production (59.1 t/ha from the U80 plots and 20.1 t/ha from the control plots on the irrigated site and 14.9 t/ha from the U80 and 9.0 t/ha from the control plots on the dry land). This trend was especially pronounced on the dry land site (Figure 2b) where moisture was not influenced by irrigation.

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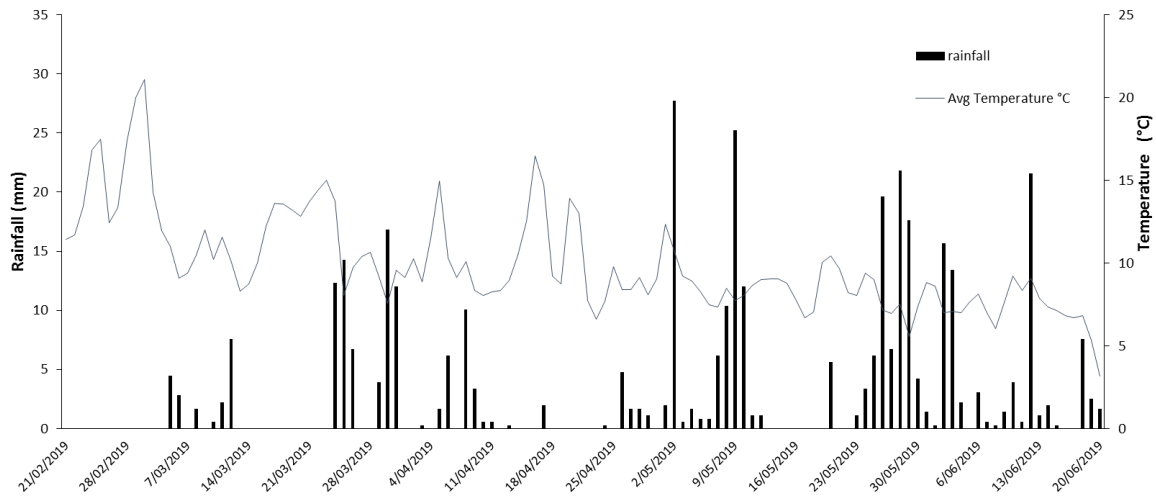


**Figure 2. Surface soil moisture (0-6 cm) for the (a) irrigation and (b) dryland Allansford sites. Note different x axes scales for a) and b).**

*Coorimungle*

A total of 268 mm of rain fell on the Coorimungle dryland site during the study from 24<sup>th</sup> February to 6<sup>th</sup> July 2019 (Figure 3) which is within of the 50-year long-term mean of 262 mm for this time of year. The long-term average annual rainfall for the Coorimungle site is 956.3 mm (Commonwealth Bureau of Meteorology) ([www.bom.gov.au/climate](http://www.bom.gov.au/climate)). The maximum daily rainfall was 19.8 mm recorded on 5<sup>th</sup> May 2019. Average daily air temperature fluctuations ranged from -1.3 °C in July to 31.7°C in April (Figure 3).

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**Figure 3. Rainfall and daily average temperature measured at the Cooriemungle site.**

### 3.2.2. Pasture herbage dry matter production and agronomic efficiency

#### *Allansford*

The pasture dry matter yield is shown in Table 2. In 2017-2019, at each harvest, 0.6 t/ha to 1.1 t/ha of pasture DM was produced without fertiliser addition (control plots), using background soil N reserves. At the irrigated site, the pasture productivity from the control treatments was higher in the first year of study (2017), about 0.9 t/harvest on average, but declined after this to 0.5 t/ha per harvest in the 2018. This suggests that the available soil N reserves are being utilised., although 2018 may have been a less productive year considering the biomass production from the fertilised plots.

With increased N application rate DM production increased and the highest values were obtained in the U80 treatment (up to 2.2 DM t/ha/harvest) at all sites. However, despite the increase, generally no significant difference was found between U60 and U80 treatments.

Application of NBPT coated urea did not significantly change DM production compared to the equivalent urea treatment. Urea coated with DMPP at a rate of 20 kg N/ha increased DM production significantly in winter 2017 only. There was no effect of applying DMPP coated urea at a rate of 40 kg N ha<sup>-1</sup> on ryegrass yield.

#### *Cooriemungle*

On the Cooriemungle dry land site, the  $NU_{AE}$  measured in the May and June 2019 harvests ranged from 5 kg DM/kg N applied (U40) to 11 kg DM/kg N applied (U20) for urea. At the Allansford dryland site, the  $NU_{AE}$  in May-June 2017 ranged from 8 kg DM/kg (U80) to 11 kg DM/kg N applied (U20). At the Allansford irrigated site, the  $NU_{AE}$  for a comparable time period in 2017, 2018 and

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**Table 2. Pasture dry matter production ( $\pm$ SE) summed over the year, growing season or until the end of the experiment, and associated agronomic N utilisation efficiency ( $\pm$  SE) at the Allansford and Cooriemungle sites. Where the period covers less than a year, details of seasons included are provided.**

	C	U20	U40	U60	U80	EEF10	EEF20	EEF40
Site/number of harvests	Total pasture dry matter production, t/ha							
Apr-Nov 2017 – Dryland Allansford /8 (autumn, winter, spring)	9.0 $\pm$ 0.1	11.3 $\pm$ 0.2	13.0 $\pm$ 0.2	13.9 $\pm$ 0.1	14.9 $\pm$ 0.1	10.2 $\pm$ 0.1	10.6 $\pm$ 0.1	13.5 $\pm$ 0.1
Jan-Dec 2017 – Irrigated Allansford/12	10.8 $\pm$ 1.2	14.6 $\pm$ 0.1	19.9 $\pm$ 0.1	22.4 $\pm$ 0.1	24.3 $\pm$ 0.1	12.4 $\pm$ 0.1	16.6 $\pm$ 0.1	19.6 $\pm$ 0.1
Jan-Dec 2018 – Irrigated Allansford/12	7.0 $\pm$ 0.1	13.0 $\pm$ 0.1	17.9 $\pm$ 0.1	21.8 $\pm$ 0.1	23.5 $\pm$ 0.2	10.7 $\pm$ 0.1	12.7 $\pm$ 0.1	19.6 $\pm$ 0.1
Jan-Jun 2019- Irrigated Allansford/7 (summer, autumn and winter)	4.1 $\pm$ 0.1	7.6 $\pm$ 0.1	11.5 $\pm$ 0.1	14.9 $\pm$ 0.1	14.8 $\pm$ 0.1	7.8 $\pm$ 0.1	9.9 $\pm$ 0.1	13.3 $\pm$ 0.1
Apr-Jun 2019 -Dry land Cooriemungle /2 (autumn only)	1.3 $\pm$ 0.6	1.4 $\pm$ 0.1	1.7 $\pm$ 0.1	2.2 $\pm$ 0.1	2.4 $\pm$ 0.1	-	-	-
	Average agronomic N utilisation efficiency, total kg DM / total kg N applied							
Apr-Nov 2017 – Dryland Allansford /8 (autumn, winter, spring)	-	18 $\pm$ 6.9	16 $\pm$ 3.7	13 $\pm$ 2.0	11 $\pm$ 1.6	22 $\pm$ 10.0	13 $\pm$ 6.3	17 $\pm$ 3.3
Jan-Dec 2017 – Irrigated Allansford/12	-	16 $\pm$ 4.9	19 $\pm$ 2.0	16 $\pm$ 1.9	14 $\pm$ 1.0	14 $\pm$ 8.0	24 $\pm$ 4.9	19 $\pm$ 2.0
Jan-Dec 2018 – Irrigated Allansford/12	-	25 $\pm$ 4.1	23 $\pm$ 2.3	21 $\pm$ 2.1	17 $\pm$ 2.1	31 $\pm$ 9.5	24 $\pm$ 3.4	27 $\pm$ 2.8
Jan-Jun 2019- Irrigated Allansford/7 (summer, autumn and winter)	-	28 $\pm$ 6.1	29 $\pm$ 3.3	26 $\pm$ 2.2	21 $\pm$ 1.8	35 $\pm$ 10.7	33 $\pm$ 5.4	33 $\pm$ 3.1
Apr-Jun 2019 -Dry land Cooriemungle /2 (autumn only)	-	11 $\pm$ 4.6	5 $\pm$ 3.0	10 $\pm$ 7.5	7 $\pm$ 5.7	-	-	-



2019 was between 7 kg DM/kg N applied to 28 kg DM/kg N applied (U20) and between 11 kg DM/kg N applied to 14 kg DM/kg (U80).

On the Allansford dryland site, the annual  $NU_{AE}$  (total pasture DM production divided by the total N applied), ranged from 11 kg DM/kg N applied (80) to 18 kg DM/kg N applied (U20) across the standard urea treatments, and reached 22 kg DM/kg N applied for the EEF applied at 10 kg N/ha (Table 2). The annual  $NU_{AE}$  is based on inputs and pasture growth that occurred only during the growing season, and for the remainder of the year no N was applied or pasture harvested.

On the Allansford irrigated site the average annual agronomic efficiency ranged from 21 kg DM/kg N applied (U80) to 28 kg DM/kg N applied (U20) across the standard urea treatments and reached 35 kg DM/kg N applied for the EEF applied at 10 kg N/ha. Slightly higher agronomic efficiency was observed in the irrigated site in 2017 compared to 2018 due to higher pasture DM production in response to climate (Table 2).

Across all sites, the  $NU_{AE}$  tended to be lowest at the highest rates of urea (U60 and U80) and highest at the lower rates of urea (EEF10, EEF20 and U20). Both dryland sites had lower  $NU_{AE}$  values compared to the irrigated site, which can be due to the higher profile (0-60 cm) initial mineral N content of 92 kg N /ha and 165 kg N /ha in the Allansford (4<sup>th</sup> April 2017) and Coorimungle (16<sup>th</sup> April 2019) dryland sites respectively, compared to the irrigated Allansford site (49 kg N/ha) (4<sup>th</sup> April 2017).

The  $NU_{AE}$  of the EEF products was comparable to straight urea at the same N rate on both the irrigated and dryland systems.

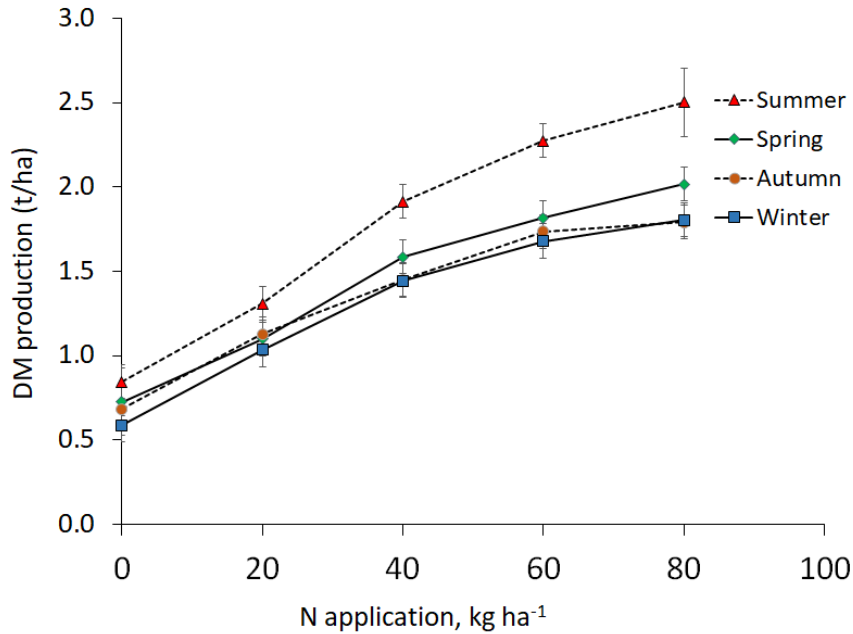
#### *Seasonal changes in DM yield and agronomic efficiency*

The averaged seasonal N responses curves for the Allansford irrigated site are shown in Figure 4. The responses follow growing season conditions with good responses in summer and spring (warm temperatures and plenty of rainfall under the irrigated system, with high solar radiation), and slightly lower responses in autumn and winter (cooler temperatures, lower solar radiation). The higher growth response in spring and summer coincides with a short growth cycle (around 21-28 days to 3-leaf stage) while in autumn, the typical growth cycle is around 28 days and in winter the growth cycle extends to 56 days.

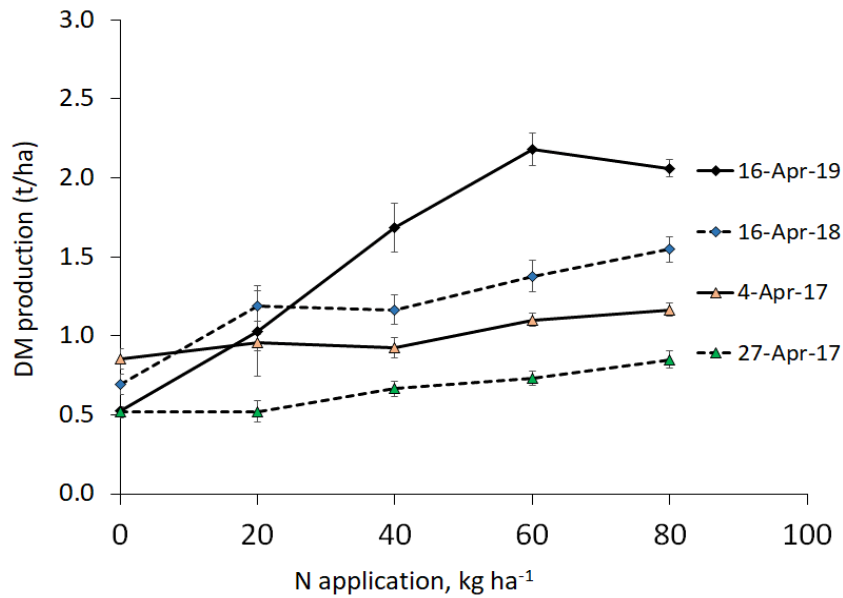
We observed a large inter-annual variation in the pasture response to applied N at the autumn break (April harvests) with flat responses in 2017 and 2018 and a good response in 2019 (Figure 5). Looking at the soil profile water, the soil water content at 0-10 cm and 10-20 cm is slightly lower in 2019 than in 2018, so it appears that the water content at depths below 20 cm, which is higher in 2019 than the other years, is driving this response (Figure 6). There was a higher input of water in January, February and March 2019 than in 2018 which appears to have sustained the deeper profile soil moisture (Figure 1). This finding has implications for water management. Typically, irrigation is turned off around March in this region to prevent the soils becoming too

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wet over winter. Based on the 2019 autumn response, consideration should be made of the benefits of extending the irrigation into autumn, balancing this with potential impacts from excess water in the profile over winter, and the value of feed at this time of year. Climate forecasting is required to help farmers make this decision.

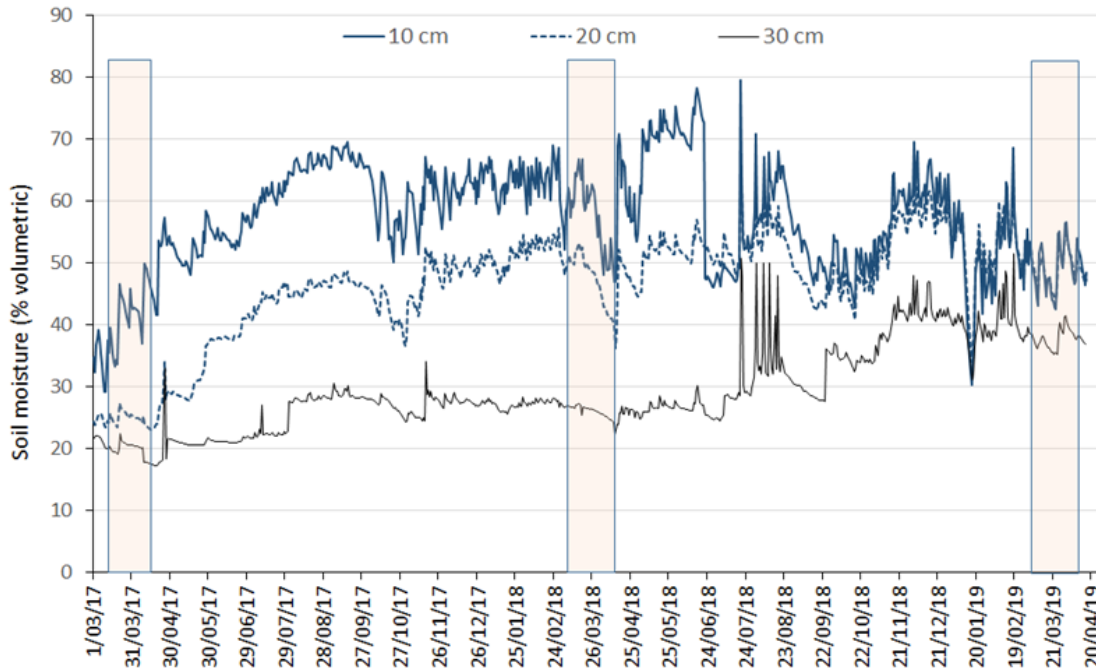


**Figure 4. Averaged seasonal dry matter response to N application at the Allansford irrigated site (2017-2019).**



**Figure 5. Variation in autumn response to fertiliser addition at the Allansford irrigated site during autumn 2017, 2018 and 2019.**

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**Figure 6. Soil volumetric water content over 30 cm depth (at 10, 20, 30 cm) leading up to the autumn break harvests (indicated by coloured column) at the Allansford irrigated site in 2017, 2018 and 2019.**

Average seasonal DM production was determined using two methods. First, the DM production for each harvest within each season of each year was added together to give a seasonal dry matter production. Values for each season were then averaged for the experimental period (2.5 years for the Allansford irrigated site, 1 year for the Allansford dryland site). Second, an average grazing rotation DM production per season was calculated by averaging the DM for each harvest within a season for all years of measurement. This meant that in some seasons the average was based on four harvests and in others on only two. In winter there were two harvest, each of between 33 to 41 days long. In spring, summer and autumn there were four harvests, each of 20-30 days long. As before, the data was determined from all years of data available (2.5 years for the Allansford irrigated site, 1 year for the Allansford dryland site). The seasonal DM production shown in Table 3 was calculated from harvests as follows: Spring: from 1<sup>st</sup> September to 30<sup>th</sup> November 2017 and 2018, which includes 8 harvests in total (four per year); Summer: from 1<sup>st</sup> December to 28<sup>th</sup> February 2017, 2018 and 2019, which includes 10 harvests in total (three to four per year); Autumn: from 1<sup>st</sup> March to 31<sup>st</sup> May 2017, 2018 and 2019, which includes 9 harvests in total (three per year); Winter: from 1<sup>st</sup> June to 31<sup>st</sup> August 2017 and 2018, and from 1<sup>st</sup> June to 31<sup>st</sup> of June 2019, which includes 5 harvests in total (two per year in 2017, 2018 and one in 2019).

The highest DM production tended to be obtained in spring-summer on the irrigated site (Table 4). In summer, the pasture productivity reached 2.5 tonnes at 80 kg N/ha, whereas in autumn - winter the maximum productivity was 1.8 t/ha. The plateau effect after the U60 rate was especially pronounced over the autumn (Table 3).

**Table 3. Average seasonal DM production (t/ha) ( $\pm$ SE), and average seasonal growth cycle DM production (t/ha) ( $\pm$ SE) at the Allansford irrigated site.**

	Spring		Summer		Autumn		Winter	
	Seasonal	Growth cycle	Seasonal	Growth cycle	Seasonal	Growth cycle	Seasonal	Growth cycle
Control	2.8 $\pm$ 0.2	0.7 $\pm$ 0.2	2.8 $\pm$ 0.1	0.8 $\pm$ 0.1	2.1 $\pm$ 0.04	0.7 $\pm$ 0.04	1.0 $\pm$ 0.1	0.5 $\pm$ 0.1
U20	4.3 $\pm$ 0.1	1.1 $\pm$ 0.1	4.4 $\pm$ 0.1	1.3 $\pm$ 0.1	3.4 $\pm$ 0.1	1.1 $\pm$ 0.1	2.0 $\pm$ 0.1	1.0 $\pm$ 0.1
U40	6.3 $\pm$ 0.1	1.6 $\pm$ 0.1	6.4 $\pm$ 0.1	1.9 $\pm$ 0.1	4.3 $\pm$ 0.1	1.4 $\pm$ 0.1	2.8 $\pm$ 0.1	1.4 $\pm$ 0.1
U60	7.2 $\pm$ 0.1	1.8 $\pm$ 0.1	7.6 $\pm$ 0.1	2.3 $\pm$ 0.1	5.2 $\pm$ 0.1	1.7 $\pm$ 0.1	3.3 $\pm$ 0.1	1.6 $\pm$ 0.1
U80	8.0 $\pm$ 0.1	2.0 $\pm$ 0.1	8.3 $\pm$ 0.6	2.5 $\pm$ 0.2	5.4 $\pm$ 0.1	1.8 $\pm$ 0.1	3.6 $\pm$ 0.1	1.8 $\pm$ 0.1
EEF10	3.6 $\pm$ 0.1	0.9 $\pm$ 0.1	3.5 $\pm$ 0.1	1.1 $\pm$ 0.1	2.9 $\pm$ 0.1	1.0 $\pm$ 0.1	1.7 $\pm$ 0.1	0.9 $\pm$ 0.1
EEF20	4.6 $\pm$ 0.1	1.2 $\pm$ 0.1	4.5 $\pm$ 0.1	1.4 $\pm$ 0.1	3.6 $\pm$ 0.1	1.3 $\pm$ 0.1	2.3 $\pm$ 0.1	1.2 $\pm$ 0.1
EEF 40	6.5 $\pm$ 0.1	1.6 $\pm$ 0.1	6.7 $\pm$ 0.1	2.0 $\pm$ 0.1	4.6 $\pm$ 0.1	1.5 $\pm$ 0.1	2.9 $\pm$ 0.1	1.5 $\pm$ 0.1

Seasonal  $NU_{AE}$  decreased linearly with increasing urea application in autumn and winter (Figure 7a), from 22 and 23 kg DW  $kgN^{-1}$  (U20) to 14 to 16 kg DW  $kgN^{-1}$  (U80) respectively. In spring and summer,  $NU_{AE}$  increased linearly with increased N fertiliser rate up to 40 kg N  $ha^{-1}$  (additional 19 to 30 kg DW  $ha^{-1}$  per additional kg of N applied). When the higher rates of urea were applied (60 kg N  $ha^{-1}$  and 80 kg N  $ha^{-1}$ ), the additional DM produced per extra unit of N applied decreased to 10 to 18 kg DW  $ha^{-1}$  per additional kg of N applied, leading to a decline in  $NU_{AE}$  (Figure 7a).

Using inhibitors as a fertiliser mitigation strategy tended to increase the  $NU_{AE}$  in all seasons when either of the inhibitors (NBTPT and DMPP) were used, although this was not significant except for the case of DMPP use in winter when urea was applied at a low rate (20 kg N  $ha^{-1}$ ) and led to 10 kg more DM per unit N applied compared to granular urea (Figure 7b). However there is a high degree of variability in the DM response to EEFs (large standard error bars) due to variations in climate and edaphic factors across the three years which would affect N loss and pasture response, so caution should be exercised when considering the benefits of EEFs.

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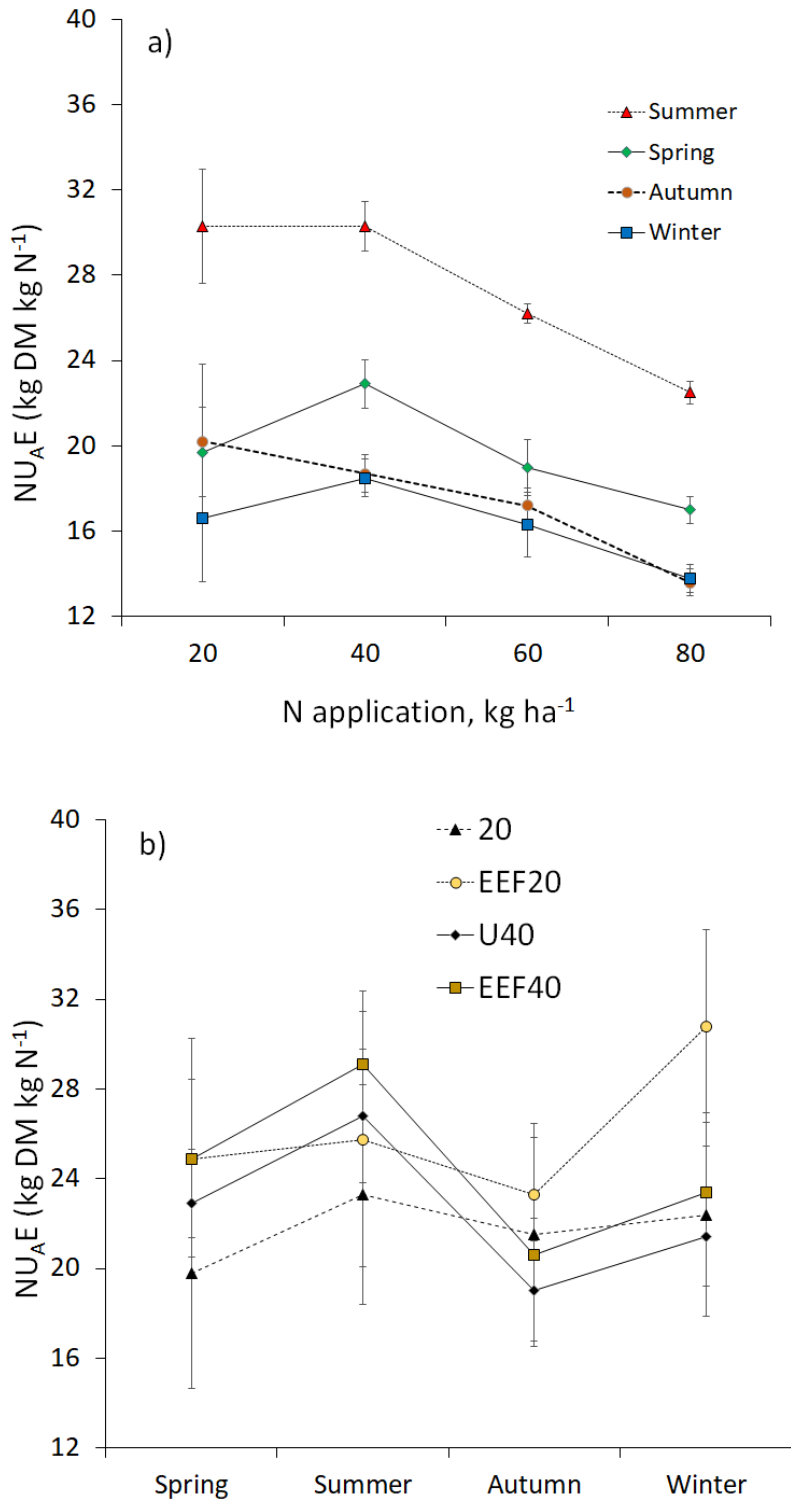


Figure 7. Agronomic efficiency of N fertiliser (NU<sub>A</sub>E) as affected by a) season, and (b) use of EEFs.

Note: y- axis starts at 12

### 3.2.3. Nitrogen budget

#### *Allansford*

The N balance was determined for each year from the cumulative N input (N applied per fertilisation event multiplied by the number of fertilisation events) and the cumulative N removed in the pasture (pasture DM production per harvest multiplied by the N concentration of the pasture, and summed for all pasture harvest over the year). In viewing this data it should be noted that the trial design (cut and removal) means that there are less N returns in pasture biomass than would occur in a grazing system. In addition, no manure or urine additions are applied to these sites, with the exception of the urine treatment plots. Under this cut and removal system we would expect the net-N removal to be greater than in a grazed pasture.

The N balance for the Allansford irrigated site (Table 4) shows that N plant uptake exceeded N supplied through fertilisation, except for the U80 and urine treatments. Throughout the study (33 months), 681 kg N was removed in biomass from the control treatment, which is supplied through mineralisation of soil organic N. Over the experimental period, there was a negative N balance for the lower rates of urea, resulting in a net-removal of 457 kg N/ha (U20), 322 kg N/ha (U40) and 70 kg N/ha (U60). For each year the N balance varied reflecting differences in pasture growth. The largest total N taken by the pasture was 2319 kg N/ha under U80, which was 321 kg N/ha less than the total N received by this treatment. The N balance was also negative under the EEFs, which were applied only at the lower rates, and varied between a net removal of 399 kg N/ha (EEF40), 501 (EEF20) kg N/ha and 570 (EEF10) kg N/ha.

The N balance for the Allansford dryland site showed more treatments with an N surplus from fertilisation compared to the irrigated site (U60, U80 and urine) (Table 5). This is likely due to the reduced pasture growth in this dryland system due to moisture limitations, which leads to reduced N uptake.

Pasture biomass from the urine patch treatment plots at Allansford were taken from strips as for the other sites, which represented 1.5 m<sup>2</sup>. These strips included pasture from directly under a urine patch and from un-fertilised areas or areas that had previously received a urine application (out of three applications in the dryland and four applications in the irrigated system), with the urine patch applied to each of the four quarters of the treatment plot during the course of the experiment. Therefore they incorporate three different types of N application rates (urine, 'old' urine, and no-N) and should be viewed in light of a field situation, where urine patches are randomly deposited across a field. From these plots under irrigation, 739 kg N/ha (representing 18% of the applied N in the irrigated system) was taken up by plants throughout the experiment. Under the dryland system 302 kg N/ha (or 15% of the applied N to November 2017) was taken up by the plants. The N applied in a urine patch is far in excess of the N requirements of the pasture plants, and the applied N poses a high leaching risk. This was observed at our site with the urine N moving down the soil profile below 40 cm depth (see Figure 12) and therefore no longer being accessible to the plant roots.

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**Table 4. Cumulative N applied, cumulative N removed in pasture (kg N/ha) ( $\pm$ SE), and net balance during the years 2016 (Nov-Dec), 2017 (Jan-Dec), 2018 (Jan to Dec) and 2019 (Jan-June) at the Allansford irrigated site.**

Treatment	2016			2017			2018			2019		
	Applied	Removed	Balance	Applied	Removed	Balance	Applied	Removed	Balance	Applied	Removed	Balance
Control		48 $\pm$ 6.2	-48		336 $\pm$ 21.26	-336		183 $\pm$ 15.03	-183		114 $\pm$ 8.07	-114
U20	40	56 $\pm$ 4.9	-16	240	447 $\pm$ 8.66	-207	240	378 $\pm$ 14.45	-138	140	220 $\pm$ 13.69	-80
U40	80	84 $\pm$ 2.3	-4	480	642 $\pm$ 16.94	-162	480	564 $\pm$ 14.27	-84	280	354 $\pm$ 9.45	-74
U60	120	94 $\pm$ 3.1	26	720	771 $\pm$ 16.81	-51	720	723 $\pm$ 10.71	-3	420	475 $\pm$ 7.71	-55
U80	160	119 $\pm$ 5.6	41	960	837 $\pm$ 19.61	123	960	849 $\pm$ 43.01	111	560	534 $\pm$ 11.96	26
EEF10	20	46 $\pm$ 4.9	-26	120	371 $\pm$ 8.34	-251	120	300 $\pm$ 17.39	-180	70	183 $\pm$ 8.68	-113
EEF20	40	62 $\pm$ 5.4	-22	240	508 $\pm$ 10.8	-268	240	359 $\pm$ 16.05	-119	140	232 $\pm$ 13.74	-92
EEF40	80	87 $\pm$ 11.9	-7	480	634 $\pm$ 15.72	-154	480	607 $\pm$ 18.69	-127	280	391 $\pm$ 13.76	-111
Urine	n/a*	-	-	3048	337 $\pm$ 15.59	2711	1016	274 $\pm$ 14.88	742	n/a	-	-

\* Urine was not applied in 2016 and 2019

**Table 5. Cumulative N applied, cumulative N removed in pasture (kg N/ha) ( $\pm$ SE), and net balance during the years 2016 (Nov-Dec) and 2017 (Jan-Dec) at the Allansford dryland site**

Treatment	2016			2017		
	Applied	Removed	Balance	Applied	Removed	Balance
Control		46 $\pm$ 6.9	-46		316 $\pm$ 19.6	-316
U20	40	55 $\pm$ 4.0	-15	220	401 $\pm$ 20.1	-181
U40	80	76 $\pm$ 4.5	4	440	478 $\pm$ 15.6	-38
U60	120	88 $\pm$ 4.6	32	660	524 $\pm$ 36.8	136
U80	160	89 $\pm$ 4.2	71	880	597 $\pm$ 24.8	283
EEF10	20	50 $\pm$ 4.7	-30	110	365 $\pm$ 22.0	-255
EEF20	40	56 $\pm$ 4.2	-16	220	334 $\pm$ 50.2	-114
EEF40	80	71 $\pm$ 4.4	9	440	491 $\pm$ 23.6	-51
Urine	n/a*	42 $\pm$ 2.9	-42	3048	316 $\pm$ 24.0	2732

n/a\*\* Urine was not applied in 2016

The findings show the annual balance, but seasonally differences exist. In autumn for example, when there is often a poorer response to pasture growth, N supplied in fertiliser is not being removed by the pasture and is being added to the organic N pool via immobilisation, creating a positive balance. In spring when growth conditions are ideal, high pasture growth means that the pasture utilises more than is applied, creating a negative balance. A greater understanding of this seasonal balance can help growers modify their fertiliser program. However, as stated above this experimental design does not account for pasture returns, urine and manure inputs all of which would build the soil mineral and organic N pools and therefore would reduce the 'mining' of soil N that might be considered to occur from the balance presented in Tables 4 and 5.

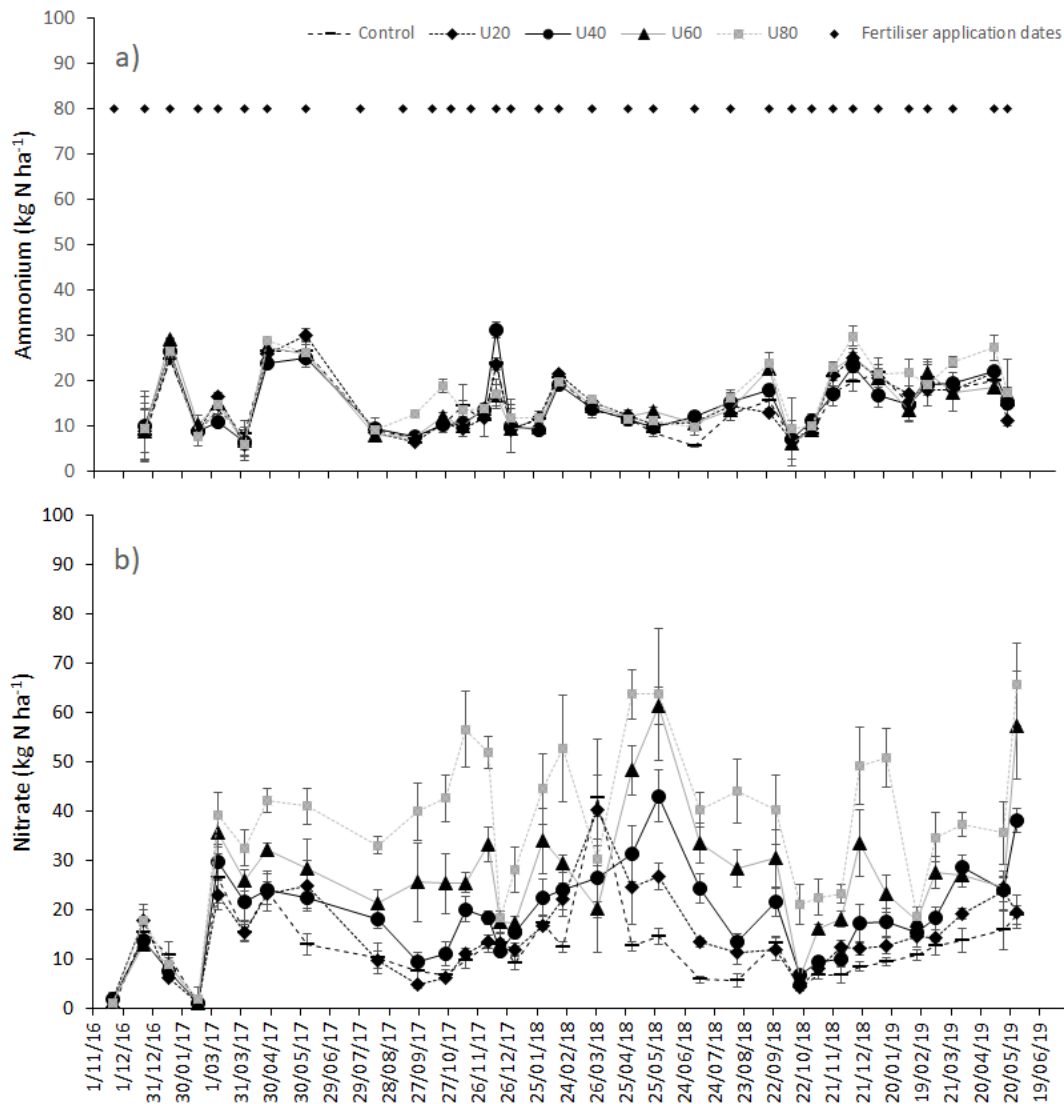
#### 3.2.4. Soil mineral nitrogen

Temporal variations in  $\text{NH}_4^+$ -N and  $\text{NO}_3^-$ -N in the top 10 cm of soil at the Allansford irrigated site are shown in Figure 8. This dynamic nature of mineral N is typical in pasture soils. In most cases, variations of  $\text{NH}_4^+$  in the top 10 cm of soil did not differ between treatments (Figure 8a) and there was no significant difference in average  $\text{NH}_4^+$  between treatments over the trial period. The average concentration of  $\text{NO}_3^-$  significantly differed between treatments, with the exception of the control and U20 treatments, with statistically higher average  $\text{NO}_3^-$  over the trial period as N rate increased. The average  $\text{NO}_3^-$  was 12.8 $\pm$ 9.5 kg N ha<sup>-1</sup> (C), 15.2 $\pm$ 9.5 kg N ha<sup>-1</sup> (U20), 19.1 $\pm$ 10.8 kg N ha<sup>-1</sup> (U40), 26.2 $\pm$ 15.3 kg N ha<sup>-1</sup> (U60) and 36.4 $\pm$ 19.5 kg N ha<sup>-1</sup> (U80). Treatment effects were more pronounced at certain times of the year and in different years (Figure 8b).

On the dryland site, the average concentration of  $\text{NO}_3^-$  significantly differed between treatments, with the exception of the control and U20 treatments, as for the irrigated site, with statistically higher average  $\text{NO}_3^-$  over the trial period as N rate increased. The average  $\text{NO}_3^-$  was 10.6 $\pm$ 1.4 kg N ha<sup>-1</sup> (C), 10.4 $\pm$ 1.0 kg N ha<sup>-1</sup> (U20), 16.0 $\pm$ 1.4 kg N ha<sup>-1</sup> (U40), 23.4 $\pm$ 2.0 kg N ha<sup>-1</sup> (U60) and 38.3 $\pm$ 3.1 kg N ha<sup>-1</sup> (U80). Treatment effects were seen throughout the growing season (Figure 9). Application of EEFs did not affect mineral N levels (data not shown).



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**Figure 8. Soil ammonium (a) and nitrate (b) (0-10 cm) at the Allansford irrigated site for the five fertiliser application rates**

On the Coorimungle dryland site, soil NH<sub>4</sub><sup>+</sup> and NO<sub>3</sub><sup>-</sup> levels changed marginally over the short time period of sampling (Figure 10). There was a significant treatment effect on NO<sub>3</sub><sup>-</sup>, with higher average NO<sub>3</sub><sup>-</sup> (31.3 and 35.5 kg NO<sub>3</sub><sup>-</sup>-N ha<sup>-1</sup>) with 60 and 80 kg N ha<sup>-1</sup> applied respectively compared to when 20 or 0 kg N ha<sup>-1</sup> was applied (18.3 and 21.81 kg NO<sub>3</sub><sup>-</sup>-N ha<sup>-1</sup> respectively) (Figure 10). The average NO<sub>3</sub><sup>-</sup> levels when 40 kg N ha<sup>-1</sup> was applied were significantly higher (30.3 kg NO<sub>3</sub><sup>-</sup>-N ha<sup>-1</sup>) than the control but not significantly different to all other treatments.

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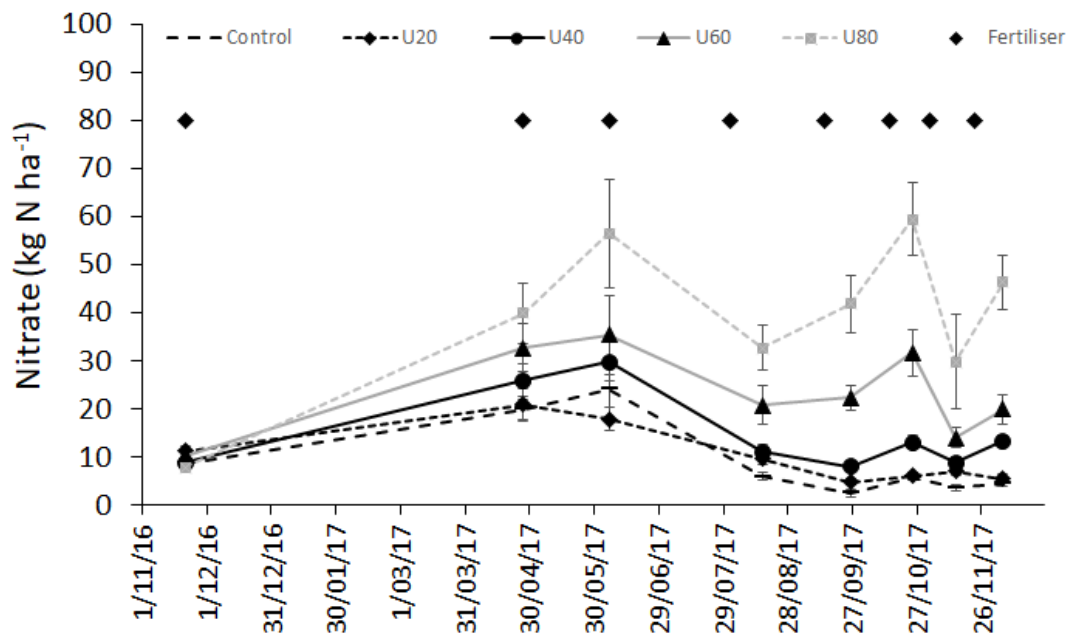


Figure 9. Soil nitrate (0-10 cm) at the Allansford dryland site for the five fertiliser application rates

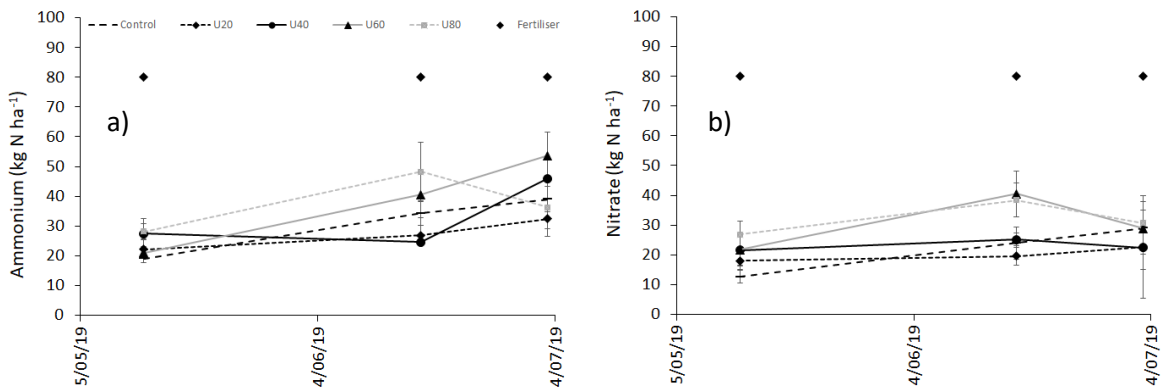


Figure 10. Soil ammonium (a) and nitrate (b) (0-10 cm) at the Cooriemungle dry land site for the five fertiliser application rates.

3.2.5. Soil profile mineral N

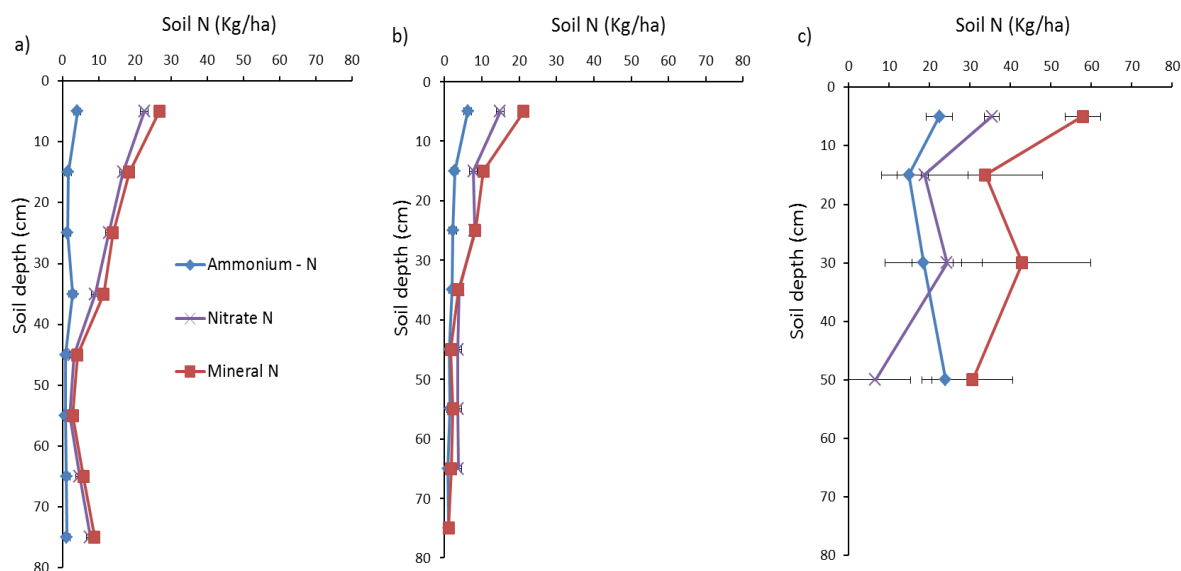
Baseline

On the 4<sup>th</sup> April 2017, soil mineral N ( $\text{NO}_3^-$  and  $\text{NH}_4^+$ ) were measured throughout the soil profile to determine the baseline profile mineral N on the irrigated (Figure 11a) and dryland (Figure 11b) Allansford sites. On the 4<sup>th</sup> April, 2017 at the Allansford irrigated site, 50 kg/ha of soil mineral N was stored in the top 80 cm of the profile of which 78% was  $\text{NO}_3^-$  (Figure 11a). At the dryland site the stored profile mineral N (92 kg/ha) was nearly double that at the irrigated site (50 kg N/ha) and 84% was  $\text{NO}_3^-$  (Figure 11b). The majority of the mineral N, up to 70%, was found in the top 35 cm at both sites.

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On the Cooriemungle dryland site, the baseline mineral N in the top 60 cm of the profile was 166 kg N/ha measured on 16<sup>th</sup> April 2019 (Figure 11c) of which 52% was NO<sub>3</sub><sup>-</sup>.

There was substantially more mineral N found on the two drylands sites compared to the irrigated site, which is due to an accumulation of nitrogen from mineralisation over the four summer months when pasture is not growing (December-March). At the irrigated site, continuous pasture growth leads to less buildup of soil mineral N as any mineralised N is utilised by the growing pasture.



**Figure 11. Baseline distribution of soil nitrate, soil ammonium and mineral N on the irrigated (a) and dryland (b) Allansford sites on 4<sup>th</sup> April 2017 and (c) Cooriemungle dry land site on 16<sup>th</sup> April 2019**

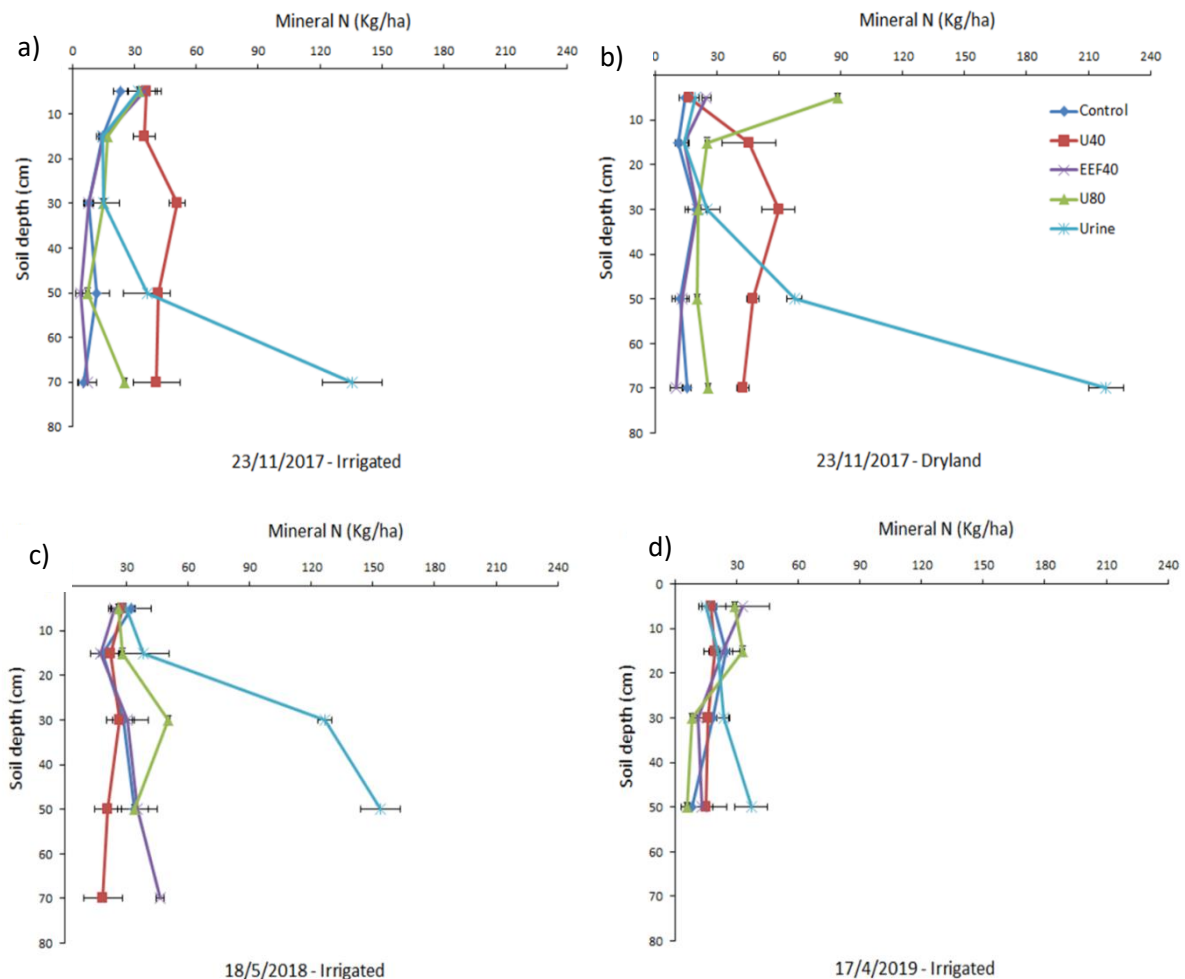
#### *Soil profile mineral N in response to fertilisation*

In November 2017, six months after the initial profile N sampling occurred, and 40 days after a treatment fertiliser addition (14<sup>th</sup> October), the soil profile mineral N in the Allansford dryland site showed that the control and EE40 treatments had a similar amount of mineral N as seen at commencement of the study (Figure 11b) but the U40, U80 and urine treatments showed elevated mineral N (Figure 12b). Application of high rates of N (U80) lead to elevated levels in the topsoil, whereas higher mineral N at 20-50 cm depth was observed for the U40 treatment, and similar mineral N throughout the profile for the EE40 and control treatments (Figure 12b). Under the urine patch treatment, there was a high amount of N (as NO<sub>3</sub><sup>-</sup>) (up to 90%) at > 50 cm depth in the soil. This occurred 10 days after the urine was applied to the treatment plots and indicates that leaching loss from urine patches is a major concern for this system.

On the Allansford irrigated site the mineral N in November 2017 was similar to the dryland site when compared to the baseline levels (Figure 11a). Under the irrigated system there was generally less mineral N than observed in the dryland site which reflects the continuous uptake of nutrients under irrigation (Figure 12a and 12b). Most noticeably the high concentration of N under the U80 treatment observed on the dryland system was not seen under irrigation, and the bulge of mineral N in the U40

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treatment was much smaller than seen in the dryland system. As for the dryland site, under the urine patch treatment there was a high amount of N (as  $\text{NO}_3^-$ ) (up to 90%) at depth in the soil, 10 days after the urine was applied to the treatment plots. Under the irrigated systems less mineral N as found under the urine patch which likely indicates that the  $\text{NO}_3^-$  was leached beyond the depth of measurement (70 cm). The hydraulic conductivity measurements from the site indicated that the unsaturated hydraulic conductivity in the surface was similar for both sites ( $43\text{-}46 \text{ mm hr}^{-1}$ ) but at depth the hydraulic conductivity was greater on the irrigated system than on the dryland ( $33\text{-}50 \text{ mm hr}^{-1}$  from  $> 25 \text{ cm}$  depth, compared to  $12\text{-}16 \text{ mm hr}^{-1}$  respectively). The saturated hydraulic conductivity was also greater at depth under the irrigated system than the dryland system ( $44\text{-}65 \text{ mm hr}^{-1}$  compared to  $22 \text{ mm hr}^{-1}$  respectively). In addition, the irrigated site received much greater water input ( $233 \text{ mm}$ ) than the dryland site ( $30 \text{ mm}$ ) between October 14<sup>th</sup> (when urine was applied) to November 23<sup>rd</sup> which would have increased the risk of movement of excess  $\text{NO}_3^-$  down the profile to depths  $> 70 \text{ cm}$ .



**Figure 12. Distribution of soil mineral N in the soil profile at the Allansford site after 6-months on the irrigated site (a) and the dryland site (b), and on the irrigated site after 12- months (c) and after 24 months (d).**

In May 2018, 32 days after the previous fertiliser addition (16<sup>th</sup> April), soil mineral N levels under the irrigated system were slightly higher than the baseline levels for all treatments, and slightly higher than in November 2017 for the control and EEF40 but lower than for the U40 treatment, but there

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was little difference between the fertiliser treatments (Figure 12c). This may indicate mineralisation occurring at this time producing a similar level of N regardless of N inputs. The mineral N under the urine patch treatment again showed a high amount of N (as  $\text{NO}_3^-$ ) had moved to below 20 cm depth in the soil, and was still there 98 days after the urine was applied to the treatment plots (9<sup>th</sup> February).

On the 17<sup>th</sup> April 2019, two years after the baseline profile mineral N was measured, the concentration of mineral N appears largely unchanged to those measured at the beginning of the experiment on April 4<sup>th</sup> 2017. This suggests that the annual inputs of N as fertiliser match the removal in pasture and the added N is cycling through the soil organic matter pool, being immobilised and then re-mineralised.

Slightly elevated mineral N was found at depth under the urine patch even though 432 days had passed since the last application of urine. This suggests that the urine mineral N (mainly  $\text{NO}_3^-$ ) has slowly leached from the profile over time as pasture is unlikely to source significant amounts of N from 50 cm depth. The urine additions therefore represent an important source of leached nitrate under diary pasture systems, and pose a risk for off-site contamination.

### 3.2.6. Nitrogen recovery ( $^{15}\text{N}$ )

Plant recovery of applied fertiliser over a period of between 8 and 12 months was between 35 and 48% of applied N on both the dryland and irrigated pastures, with 70-98% recovered in the first two harvests (Table 6). The pattern of uptake differed slightly between autumn and spring with greater uptake in the second growth cycle in spring due to the good growing conditions. Over the remaining month, progressively less N was removed by the pasture with < 1% recovery after 7 harvest events.

**Table 6. Cumulative and harvest event fertiliser ( $^{15}\text{N}$ ) recovery (%) in pasture from urea applied on April 4<sup>th</sup> 2017 and September 13<sup>th</sup> 2017 at the Allansford sites, to May 1<sup>st</sup> 2018.**

Site	N kg/ha	27/04/17	5/06/17	1/08/17	13/09/17	13/10/17	1/11/17	22/11/17	19/12/17 to 1/05/18	Total plant recovery
<b>4<sup>th</sup> April 2017 application</b>										
Irrigated	U10	18.5±1.1	6.8±1.0	2.5±0.4	2.3±0.2	0.9±0.1	2.3±0.2	0.2±0.0	2.5±0.0	35.8±2.9
Irrigated	U20	24.3±2.8	5.4±0.4	2.1±0.0	1.6±0.3	0.5±0.1	2.1±0.1	0.2±0.0	2.3±0.2	38.5±3.0
Irrigated	U40	26.2±2.4	7.4±0.9	2.2±2.2	1.5±1.5	0.6±0.6	1.9±1.9	0.2±0.2	2.3±0.9	42.2±2.3
Dryland	U10	23.4±2.0	6.4±0.5	2.3±0.2	2.2±0.1	1.1±0.3	2.0±0.1	0.0±0.0	0.4±0.2	37.9±1.7
Dryland	U20	23.5±2.4	5.0±2.1	2.6±0.1	1.3±0.2	0.6±0.2	1.9±0.1	0.1±0.0	0.2±0.0	35.1±5.5
Dryland	U40	20.6±2.1	5.3±1.7	2.8±0.3	1.3±0.1	0.3±0.1	2.2±0.2	0.1±0.0	0.1±0.0	32.7±3.5
<b>13<sup>th</sup> September application</b>										
Irrigated	U20	-	-	-	-	22.5±3.3	16.1±1.4	0.9±0.1	3.5±0.0	43.1±2.3
Irrigated	U40	-	-	-	-	22.2±2.5	13.0±1.0	1.1±0.0	3.9±0.1	40.3±1.3
Irrigated	U80	-	-	-	-	28.6±1.9	15.3±0.9	1.2±0.1	3.5±0.1	48.5±0.8
Dryland	U20	-	-	-	-	30.1±4.8	14.1±2.5	0.8±0.1	1.8±0.9	46.7±6.4
Dryland	U40	-	-	-	-	21.1±5.1	12.6±0.8	0.4±0.2	0.3±0.2	34.4±4.5
Dryland	U80	-	-	-	-	28.9±1.9	15.0±1.0	0.6±0.1	0.2±0.1	44.8±1.4

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Between 25% and 77% of the applied N was recovered in the soil over the eight to twelve month period (Table 7), and generally more N was recovered in the soil at lower application rates. Recovery in the roots was minimal and was noticeable lower when the fertiliser was applied in autumn, and after 12 months of growth (1.0%-1.9%) than when the fertiliser was applied in spring and there had been eight months of growth (2.7 to 4.2%).

**Table 7. Fertiliser recovery ( $^{15}\text{N}$ ) (%) in soil (0-40 cm), harvested pasture and plant roots over one year from urea applied on April 4<sup>th</sup> 2017 and September 13<sup>th</sup> 2017 at the Allansford sites. The final harvest for both occurred on 1<sup>st</sup> May**

Treatment	Soil				Roots*			Plants	Total
	0-10 cm	10-20cm	20-40cm	Total	0-10 cm	10-20cm	Total		
<b>Irrigated - 4<sup>th</sup> April 2017 application</b>									
U10	25.0±1.5	23.2±1.1	10.2±0.6	58.4±2.2	1.4±0.3	0.02±0.01	1.4±0.3	35.8±2.9	95.7±2.0
U20	17.4±1.2	13.3±2.6	12.1±0.4	42.7±2.8	1.7±0.3	0.02±0.00	1.7±0.3	38.5±3.0	82.5±5.3
U40	21.0±1.7	8.4±0.9	6.2±0.6	35.5±1.7	1.4±0.4	0.02±0.31	1.5±0.4	42.2±2.3	78.8±1.6
<b>Dryland - 4<sup>th</sup> April 2017 application</b>									
U10	55.8±8.8	13.5±0.8	7.5±1.6	76.8±11.0	1.0±0.4	0.01±0.01	1.0±0.4	35.1±5.5	115.9±11.0
U20	34.3±1.2	10.9±2.1	4.8±1.9	50.0±5.0	1.2±0.5	0.01±0.02	1.2±0.5	32.7±3.5	86.9±10.6
U40	16.8±1.6	6.0±1.2	1.7±0.3	24.5±2.2	1.9±0.9	-	1.9±0.9	35.8±2.9	58.7±2.1
<b>Irrigated - 13<sup>th</sup> September application</b>									
U20	23.1±2.7	8.4±1.7	7.8±0.9	39.3±4.3	2.7±1.2	0.00±0.00	2.7±1.2	43.1±2.3	85.9±3.4
U40	16.4±0.9	5.1±0.8	4.2±1.4	25.7±1.0	2.7±0.3	0.06±0.02	2.8±0.3	40.3±1.3	68.6±1.9
U80	18.0±1.2	3.4±0.3	1.9±0.3	23.3±1.0	4.2±2.0	0.00±0.00	4.2±2.0	48.5±0.8	74.4±0.7
<b>Dryland - 13<sup>th</sup> September application</b>									
U20	32.1±1.5	10.6±0.6	5.3±1.3	48.0±0.3	3.2±0.6	0.03±0.01	3.2±0.6	34.4±4.5	98.6±5.7
U40	26.1±3.0	18.2±2.6	10.4±1.1	54.7±6.5	3.7±0.9	0.06±0.02	3.8±0.9	44.8±1.4	92.0±7.8
U80	21.2±2.1	8.9±1.8	5.1±1.3	35.2±4.1	3.2±1.4	0.00±0.00	3.2±1.4	43.1±2.3	84.8±4.4

\* No roots in 20-40 cm layer

The total recovery of N in the plant and soil was more than 59% with greater recovery at the lower rates of N application. These results show that applying higher rates of N increases the risk of N loss which causes environmental and economic impacts. At lower N rates, where N is not immediately used by the plant it continues to cycle through the organic matter pool for extended periods of time.

The majority of the N taken up by the plant in the first cycle following fertilisation was sourced from the soil (>77%) in both autumn and spring and as the rate of N applied increased, the percentage of N sourced from soil decreased (Table 8). This, along with the data in Tables 6 and 7, shows that a longer term view of NUE may be required as applied N cycles through the organic matter pool.

**Table 8. Source of N taken up by the pasture in the first month following fertilisation**

	N rate kg/ha	Biomass (t/ha)		N removed by pasture (kg/ha)		<sup>15</sup> N recovery %		N derived from fertiliser (%)		N derived from soil (%)	
		Autumn	Spring	Autumn	Spring	Autumn	Spring	Autumn	Spring	Autumn	Spring
Irrigated	20	1.0±0.1	2.3±0.6	40±4.5	36±7.5	24.3±2.8	22.5±3.3	11	13	89	87
	40	1.2±0.1	2.4±0.3	46±4.2	39±3.7	26.7±2.4	22.2±2.5	21	23	79	77
	80	-	2.8±0.3	-	53±4.1	-	28.6±2.0	-	42	-	58
Dryland	20	1.1±0.2	3.0±0.3	41±5.9	50±1.8	23.5±3.0	30.1±4.8	10	12	90	88
	40	0.9±0.1	2.7±0.8	33±3.7	47±12.5	20.6±2.6	21.2±5.1	23	18	77	82
	80	-	3.1±0.5	-	64±7.8	-	28.9±1.9	-	36	-	64

*Recovery of <sup>15</sup>N urine in pasture and in soil*

Total plant recovery of the <sup>15</sup>N-labeled urine-N (in shoots and roots) ranged from 26.5% to 34.8% depending on an application date (Table 9) with most of applied <sup>15</sup>N, up to 99.8%, recovered in shoots. <sup>15</sup>N recovery and % N in pasture both decreased over time due to depletion of inorganic N and changes in biomass production. Initially urine N availability is high but as plant uptake, immobilisation, leaching, denitrification and gaseous loss occur less urine N is available for subsequent plant uptake.

**Table 9. Recovery of <sup>15</sup>N urine (%) (±SE) after a year following application of urine to the Allansford irrigated and dryland sites at four different times.**

Date of application	Site	Soil	Pasture		Total
		0-40 cm	Roots	Shoots	
6/06/2017	Irrigated	19.3±1.6	2.3±1.1	24.0±2.0	46.0±4.0
	Dry land	19.1±0.3	0.5±0.2	29.6±2.0	49.1±1.0
14/10/2017	Irrigated	15.4±2.8	0.9±0.2	29.0±0.8	45.3±1.5
	Dry land	13.1±2.6	0.4±0.4	26.0±0.8	40.4±4.4
23/11/2017	Irrigated	14.7±1.5	1.2±0.1	33.5±1.9	49.8±0.4
	Dry land	14.9±1.4	0.8±0.1	n/a*	n/a*
9/02/2018*	Irrigated	35.7±5.0	0.08±0.0	29.9±2.7	65.7±7.3

\*On the dry land site loss of plants due to drought conditions over 2017/2018 summer meant no pasture as harvested from the 23/11/17 urine application, and no <sup>15</sup>N urine application was made in February 2018.

The recovery of the <sup>15</sup>N-labeled urine – N in soil at the end of each experimental period ranged from 13 to 36% in the 0-40 cm layer. Most of the applied <sup>15</sup>N, up to 65-75% was found in the top 10 cm of the soil profile, 17-29% was detected in the 10 to 20 cm layer, and only 2-9% was found at 20 to 40

cm depth. However based on the deep profile cores collected following urine application (Figure 12) it is highly likely that the urine-N has leached below the depth of sample collection.

The total recovery of the <sup>15</sup>N-labeled urine – N (soil and plant) was between 40 to 66%, although only the February 2018 application gave > 50% recovery. The unaccounted for N is assumed to have been lost predominantly via leaching as we found that one year after urine application, most of urine-N in the soil (up to 82%) was found below 35 cm depth on the Allansford site, and was primarily NO<sub>3</sub><sup>-</sup> (89%). The sandy nature of the topsoil means this soil is not saturated for long periods (See Figure A1) and so these conditions promote nitrification and hence formation of NO<sub>3</sub><sup>-</sup> which increases the risk of leaching loss.

#### *Recovery of <sup>15</sup>N in soils from major dairy soil types in the region*

Application of N to the six dairy soils in April lead to higher biomass (average 1.1 t/ha (range 0.6-2.0) over the first growth cycles) compared to application in October (average 0.8 t/ha (range 0.4-1.7) over the first growth cycles), as well as a greater N concentration in the biomass (3.9% compared to 2.7% in the first growth cycle respectively) (data not shown). This led to higher plant removal of N following the April fertilisation compared to October.

The recovery of N in the system ranged from 56 to 81% across all treatments and application times (Table 10). The averaged total recovery across both application times showed there was significantly higher recovery in the Naringal (50) soil compared to the Naringal (42) soil, but for all other comparisons there was no significant difference in plant N recovery. The Naringal (50) soil has the lowest clay content, and lower C, and mid-range pH of all samples examined. The Naringal (42) had the lowest recovery and highest OC (11%). It is possible that with the high organic carbon content the urease activity would be high in this soil and potentially lead to rapid urea hydrolysis driving NH<sub>3</sub> loss even though the pH is acidic. However there was no clear relationship between pH, OC and N recovery for the six soils.

Significantly more N was recovered in the plant from the April application than the October application, and significantly less recovered in the soil plus roots leading to no difference in the total N recovered in the soil-plant system. This likely reflects better growing conditions that led to greater pasture growth and higher N removal by the plant in April, utilising the readily available fertiliser N. This was also shown by the significantly higher amount of N taken up by the pasture derived from fertiliser in the April application compared to the October application. This indicates that i) where ideal growing conditions occur, fertiliser N is utilised to a greater extent by the plant, and ii) where N is not used by the plant it has the potential to be stored in the soil organic matter pool, if there are no major loss mechanisms.

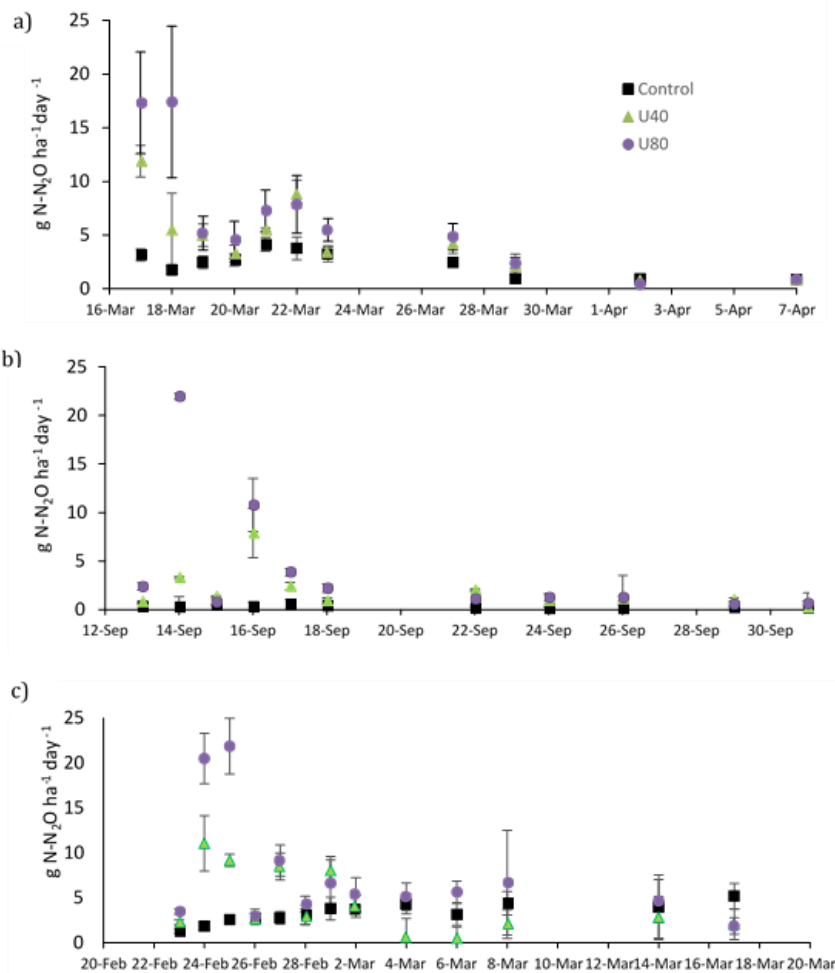


**Table 10. Recovery of  $^{15}\text{N}$  (%) ( $\pm\text{SE}$ ) following application of urea to six soils collected from the region, under irrigation, cumulative biomass and soil (plot removal on 19<sup>th</sup> March 2019 (October application) and 20<sup>th</sup> June 2019 (April application)). Note the time frame of measurement differs for the two application times (147 days for the October application, 65 days for the April application).**

Site (paddock number)	Texture	pH (CaCl <sub>2</sub> )	OC	N recovery (%)					N recovery (%)				
				Plant shoots	Soil+ roots	Total	Ndff	Ndfs	Plant shoots	Soil+ root	Total	Ndff	Ndfs
Application date				23 <sup>rd</sup> October 2018					16 <sup>th</sup> April 2019				
Coorimungle (27)	Silty loam	4.9	5.5	35.8 $\pm$ 2.7	31.4 $\pm$ 1.3	71.8 $\pm$ 2.0	15.6 $\pm$ 2.3	84.4 $\pm$ 2.3	33.7 $\pm$ 1.7	33.6 $\pm$ 3.7	69.5 $\pm$ 5.0	32.9 $\pm$ 4.9	67.1 $\pm$ 4.9
Coorimungle (81)	Loamy sand	4.3	7.4	32.5 $\pm$ 2.1	27.3 $\pm$ 1.6	62.1 $\pm$ 3.9	20.1 $\pm$ 1.9	79.9 $\pm$ 1.9	42.8 $\pm$ 2.4	23.1 $\pm$ 2.7	69.8 $\pm$ 0.9	23.9 $\pm$ 1.5	76.1 $\pm$ 1.5
Naringal (42)	Sandy clay	4.8	11.0	30.3 $\pm$ 3.1	27.2 $\pm$ 2.8	59.6 $\pm$ 6.0	20.3 $\pm$ 1.3	79.7 $\pm$ 1.3	34.8 $\pm$ 3.8	19.8 $\pm$ 3.0	57.5 $\pm$ 4.1	23.0 $\pm$ 2.0	77.0 $\pm$ 2.0
Naringal (50)	Sand	5.3	3.5	29.4 $\pm$ 4.6	32.0 $\pm$ 2.0	65.1 $\pm$ 5.2	19.9 $\pm$ 1.8	80.1 $\pm$ 1.8	38.7 $\pm$ 1.2	31.9 $\pm$ 2.3	73.5 $\pm$ 2.1	22.5 $\pm$ 3.2	77.5 $\pm$ 3.2
Panmure (10)	Loamy sand	5.0	4.0	31.2 $\pm$ 1.7	31.4 $\pm$ 3.3	67.4 $\pm$ 1.4	23.3 $\pm$ 2.0	76.7 $\pm$ 2.0	39.2 $\pm$ 1.4	26.9 $\pm$ 2.4	68.3 $\pm$ 1.6	31.1 $\pm$ 2.5	68.9 $\pm$ 2.5
Allansford-3	Loamy sand	6.5	3.2	28.8 $\pm$ 0.7	36.5 $\pm$ 3.8	71.2 $\pm$ 5.1	34.8 $\pm$ 0.7	65.2 $\pm$ 0.7	31.5 $\pm$ 0.7	25.9 $\pm$ 2.5	59.9 $\pm$ 1.6	26.3 $\pm$ 0.8	73.7 $\pm$ 0.8

### 3.2.7. Nitrous oxide emissions

The majority of the N<sub>2</sub>O emission (up to 89%) occurred during the first week following fertilisation, with peak emissions between the day 2 and day 4 at all sample dates (Figure 13). N<sub>2</sub>O emissions from the control treatment were smallest and ranged from 3.8 to 42.9 g N<sub>2</sub>O-N ha<sup>-1</sup> per fertilization event (21-day phase) in the spring and the autumn respectively (Figure 13). Over the three measurements periods (16<sup>th</sup> March to 11<sup>th</sup> April 2018, 12<sup>th</sup> September to 1<sup>st</sup> October 2018 and 23<sup>rd</sup> February to 17<sup>th</sup> March 2019), N<sub>2</sub>O emission fluxes ranged from 0.1 g N<sub>2</sub>O-N ha<sup>-1</sup> per day to 21.9 g N<sub>2</sub>O-N ha<sup>-1</sup> per day across all treatments. Increasing the rate of N applied increased N<sub>2</sub>O emissions, and there was a noticeable seasonal effect (Figure 13a, b and c). N<sub>2</sub>O emissions from the U40 treatment were 1.3-6.2 times higher than the control, and from the U80 treatment were between 2.3 and 12.6 times greater than from the control.



**Figure 13. Daily N<sub>2</sub>O emissions after N fertiliser application on a) 22<sup>nd</sup> of February 2018, b) 12<sup>th</sup> of September 2018 and c) 23<sup>rd</sup> of February 2019**

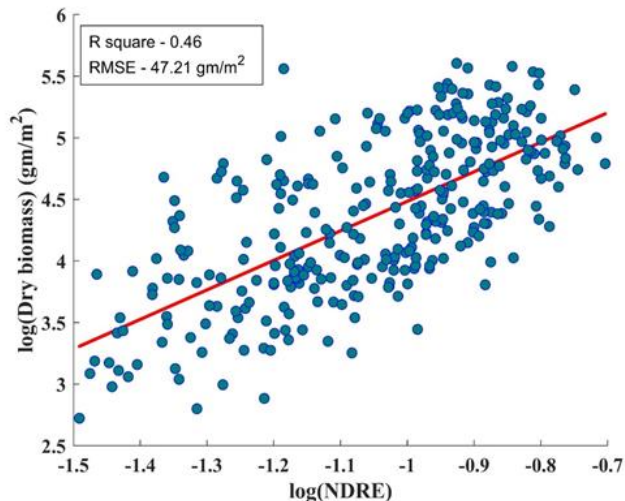
Emissions were lower for the early spring period (Figure 13b) ranging from 12% to 59% of those emitted at the autumn period (Figure 13a and 13c) over a 21-day period. Emissions of N<sub>2</sub>O are influenced by N inputs, soil moisture and temperature. Under the irrigated system the water filled

pore space (WFPS) was between 61 and 89% which favours nitrification and denitrification, and soil moisture is very similar over the three time periods examined (Figure 5). Therefore the difference is most likely due to higher soil temperatures during autumn (average 21°C) compared to early spring (average 8°C) (Figure A1).

### 3.3 Utilisation of new technologies

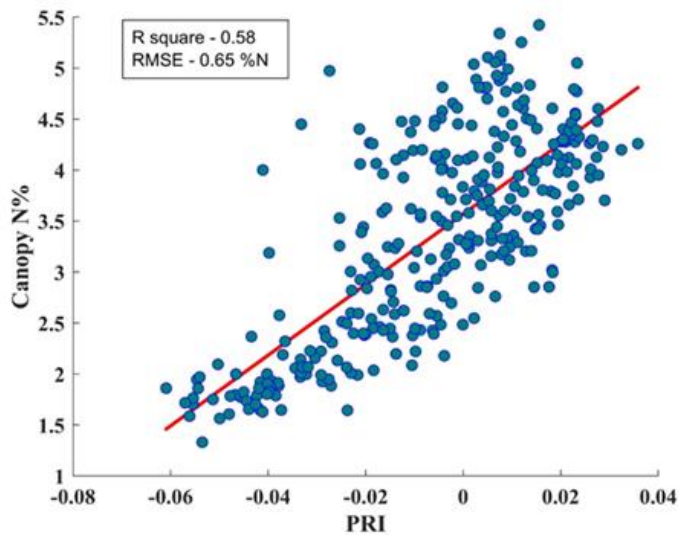
Drone footage with hyperspectral imagery was collected from the irrigated site on December 18<sup>th</sup>, 2017 and February 19<sup>th</sup>, 2019 to cover 2 different periods. In 2017 appropriate band widths for best prediction of soil moisture and pasture dry matter were identified. Validation samples were collected from the field site at the same time. The suitability of using a hand-held VNIR spectra and chlorophyll measures was also assessed across the project. This hand-held unit was used to determine changes in spectra as the pasture grows with measurements being taken for one growth cycle in May-June 2018 (17<sup>th</sup> May, 25<sup>th</sup> May, 4<sup>th</sup> June and 18<sup>th</sup> June 2018) and in January-February 2019. Chlorophyll measures using a Soil Plant Analysis Development (SPAD) meter were collected for the irrigated site on June 2018, to compare to data previously collected in Dec-2017. This work is part of collaboration with the Melbourne School of Engineering (MSE) at the University of Melbourne. Data presented in this report represent a snapshot of data that is part of the PhD of Mr Manish Patel (supervised by Dongryeol Ryu, Andrew Western and Iain Young).

Based on the data collected during winter 2018 and summer 2019 using the hand-held VNIR spectrometer, the Normalized Difference Red Edge (NDRE) was identified as the best predictor of above ground pasture biomass production (Figure 14). The Photochemical Reflectance Index (PRI) was identified as the best predictor of ryegrass canopy nitrogen concentration for the winter 2018 and summer 2019 data collection times (Figure 15).



**Figure 14. Correlation of Normalized Difference Red Edge (NDRE) and dry biomass of ryegrass in winter 2018 and summer 2019 (graph supplied by Mr Manish Patel, MSE).**

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**Figure 15. Correlation of Photochemical Reflectance Index (PRI) and ryegrass N content in winter 2018 and summer 2019 (graph supplied by Mr Manish Patel, MSE).**

During the measurements using the hand-held NVIR it was determined that measurements had the greatest predictive ability when collected at an intermediate growth stage, before canopy closure occurs. It was also identified that there was some seasonal effect in the prediction of canopy nitrogen which would require further calibration of the models used if extending beyond the seasons examined.

#### **4. Laboratory experiments (KPI 5.6 Output 6 (I))**

##### **4.1 Mineralisation in different soils – key drivers**

This component of the work was completed as part of a Masters project conducted by Mr Tord Ranheim Sveen.

###### **4.1.1 Methodology**

The experimental design comprised a 2-factorial experiment with Moisture (air dry (AD), field capacity (FC), 150% FC) and temperature (10, 20, 30°C). The soils were incubated aerobically and extracted after 14 (t14) and 28 (t28) days. Tests for differences in means of one variable (i.e. mineralisation rates, organic carbon) between landuse systems were performed with Welch's t-test assuming unequal variances. The impact and interactions of landuse, moisture and temperature on mineralisation rates was computed with a three-way ANOVA. Differences between treatment levels of the temperature and moisture factors were tested using Tukey's single-step multiple comparison method reporting adjusted p-values.

Soils used in dairy pasture systems were collected from farms in the region between Port Campbell and Warrnambool in south-western Victoria. This region is dominated by sedimentary

plains on exposed limestone toward the coast, and by volcanic plains formed by basalt of varying thickness further inland and to the east (Robinson et al. 2003). Soils are predominantly yellow duplex soils with poor drainage characteristics and heavy-textured and acid subsoils (Maher & Martin 1987). The area is considered an important dairy region, and accounts for approximately 23% of the national Australian milk production (Dairy Australia 2019). The pasture invariably used was perennial ryegrass (*Lolium perenne*), generally grazed in a 21-28 day rotation pattern.

Soils were sampled down to 10 cm depth along a single transect. The transect samples were then pooled into a bulk sample and transported to the laboratory facilities where they were kept in refrigerated conditions until further processing. In the laboratory, the fresh bulk samples were sieved to 4 mm, and subsamples of these were subsequently used to determine the gravimetric moisture content ( $\theta_g$ ), and field capacity (FC).

Gravimetric moisture ( $\theta_g$ ) was determined on duplicates on dry weight basis according to Black (1965), in which subsamples of fresh moist soil is dried in an oven on 105°C for 24 hours. FC was determined through a Heins' apparatus (Battacharya & Michael 2010). Briefly, a subsample of soil was mounted onto a porous plate and thoroughly soaked for 12 hours, whereafter a -10 kPa matric potential was established and the soil was drained until reaching a new moisture equilibrium corresponding to FC. The obtained FC values ranged between 28-60 % moisture by weight.

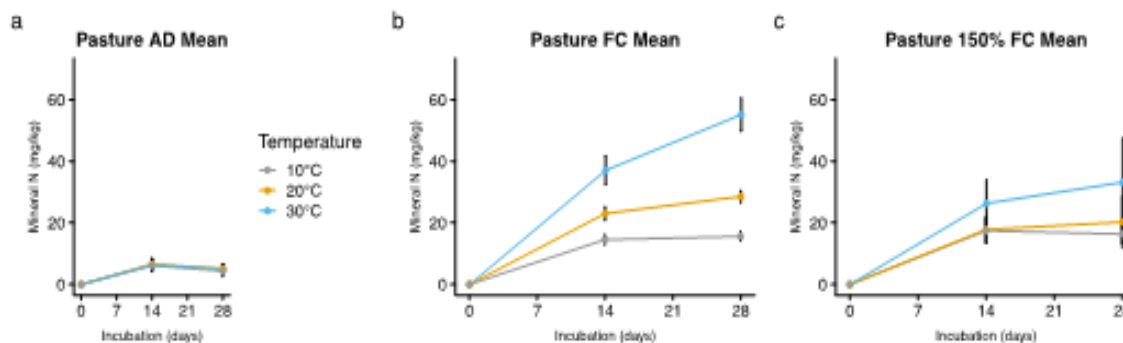
An additional subsample of around 300 g was taken from each fresh bulk of soil and oven-dried at 40°C for 5 days, representing air-dried (AD) soil (Bolland & Allen 1996). Moisture contents of soils at kept at AD conditions ranged between 1.7 - 3.9 % moisture by weight.

Soils were weighed into 30 ml transparent vials, using 3 g (dry-weight equivalent) of fresh moist or air-dried soil and in triplicates for every soil, moisture and temperature treatment level. The moisture content was then adjusted to FC or 150% FC by adding the corresponding amount of purified (RO) water to the soil surface through pipetting. The vials were then covered with Parafilm® to allow for gas exchange, and incubated at adequate temperature level. Moisture content was monitored and adjusted two times a week by adding more RO-water if necessary. After the incubation period, samples were extracted with 2 M KCl in a 10:1 extractant ratio (Carter et al. 2007); placed on a head-over-head shaker for 1 hour, centrifuged at 3000 rpm for 5 minutes, and gravitationally filtered using Whatman® No. 42 filter papers. The resulting extractant solutions were kept frozen at -20°C until analysed for mineral nitrogen using a Skalar San+ segmented-flow (SFA) analyser by the TrACEES platform services.

#### 4.1.2 Results and Discussion

Results from the incubation done on samples collected in January an average mineralisation rate of 0.66 mg N kg<sup>-1</sup> day<sup>-1</sup> across the dairy pasture soils. Temperature and moisture significantly affected mineralisation, with moisture being the main limiting factor (Figures 16a to 16c).

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**Figure 16. Average mineralised nitrogen after 14 and 28 days of incubation at 10, 20 and 30°C across all pasture soils for soil that was air dried (AD) (a), at field capacity (FC) (b) and at 150% FC (c) of samples collected in January 2019.**

Soils wetted to field capacity (FC) moisture content mineralised significantly more nitrogen than any other moisture levels, with clear differences to be discerned also between the temperature levels (Table 12, Figure 16b). Soils wetted to 150% of field capacity showed greater variability in mineralising nitrogen (Figure 16c) with less pronounced effects of temperature differences. Two possible hypotheses could account for this; the first one being that the approximately waterlogged conditions at 150% FC in combination with higher incubation temperatures lead to ascending levels of denitrification (Maag & Vinther 1996). A second possible explanation is that the soils at 150% FC in combination with higher temperatures lead to patterns of transient waterlogging, which drastically reduce mineralisation (Wang et al. 2001, Tete et al. 2015).

**Table 11. Mineralised nitrogen for pasture and cropping soils collected in January and after the autumn, April/May. Figures are mean N  $\pm$ SD (mg/Kg) of 10 and 6 soils for pasture and cropping systems, respectively.**

Treatment	January 2019	April – May 2019
10°C, Air dry	5.0 $\pm$ 5.6	-0.28 $\pm$ 7.8
20°C, Air dry	14.9 $\pm$ 4.6	4.9 $\pm$ 8.7
30°C, Air dry	16.5 $\pm$ 9.7	5.2 $\pm$ 8.4
10°C, FC	5.6 $\pm$ 4.7	0.35 $\pm$ 6.8
20°C, FC	29.0 $\pm$ 6.2	12.7 $\pm$ 7.5
30°C, FC	19.6 $\pm$ 27.6	15.3 $\pm$ 8.9
10°C, 150% FC	5.5 $\pm$ 4.7	0.32 $\pm$ 5.0
20°C, 150% FC	57.0 $\pm$ 16.7	42.4 $\pm$ 16.4
30°C, 150% FC	32.5 $\pm$ 47.8	32.1 $\pm$ 25.6

Preliminary data on the amount of mineralised N at the different sampling times suggests higher

mineralisation rates from soils collected in January compared to the ones collected after the autumn break in April/May (Table 11). A plausible explanation for this is the higher levels of particulate organic matter left in the soil in January which is not decomposing due to limited moisture.

The findings from the laboratory incubation support the identified key predictors of mineralisation identified in the mineralisation calculator modelling.

## **4.2 N<sub>2</sub>:N<sub>2</sub>O**

### 4.2.1. Methodology

#### ***Soil sampling and experiment setup:***

Soil samples (0-10cm) were collected from the buffer zone of the irrigated site, located at 5048 Great Ocean Road, Mepunga West, near Allansford, SW Victoria (38°25'05" S, 142°38'24" E), Australia. Soil samples were air dried, sieved to < 4 mm. Before the incubation, gravimetric water content was determined after drying the soil at 105°C for 24 hours.

A 3 × 2 factorial incubation experiment with four replicates was set to monitor N<sub>2</sub> and N<sub>2</sub>O emissions, where water filled pore space (WFPS; 60, 80 and 100 %) and <sup>15</sup>N enrichment (2 and 5 %) were the first and the second factor, respectively. Exactly 150 g airdried soil was packed in 500 mL glass jars and water was added to achieve three different levels of WFPS, 60 %, 80 %, and 100 %. The vials were pre-incubated for seven days at 20 °C, while maintaining the soil moisture in the jars by weighing them daily, to stabilize microbial activity in the soil. The incubation temperature used during the experiment represents the average soil temperature in the field. After seven days of pre-incubation, K<sup>15</sup>NO<sub>3</sub> (99 atom % <sup>15</sup>N) solution was added to the soil in such a way that each of the WFPS treatment receive both 2 and 5 atom % <sup>15</sup>N enrichment of the soil N. The solution was injected into the soil evenly using a syringe with 10 cm long needle to ensure even distribution of the substrate. Extra vials were prepared in the same way as above, but without N amendment, to monitor the change in mineral N concentrations and background N<sub>2</sub>O production.

#### ***Gas sampling:***

Gas sampling was started immediately after the addition of <sup>15</sup>N (day 0) and continued through day 1, day 2, day 4, day 6 and day 9. On each sampling day, a 3 mL gas sample was collected twice from inside each incubation jar using a syringe and injected into two separate pre-evacuated exetainer vials (Labco). These samples were considered as time 0 sample. Thereafter the jars were closed with airtight lids equipped with a gas sampling port and a 3 mL gas sample was collected twice from each jar at 6, 12 and 24 hours after the lid closure and injected into two separate pre-evacuated exetainer vials. Incubation jars were left open except during the 24 h gas sampling period to maintain aerobic condition and allow the headspace atmosphere to equilibrate. One of the two vials collected at each time point was used for N<sub>2</sub>O analysis using gas chromatography (GC Agilent 7890), and the second vial was used for <sup>15</sup>N<sub>2</sub> and <sup>15</sup>N<sub>2</sub>O (<sup>15</sup>N<sub>2</sub>O was analysed only on

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the 24<sup>th</sup> hour vial from each sampling day) analysis using gas chromatography coupled with isotope ratio mass spectrometer (GC-IRMS). At the end of the incubation, 20 g of soil from each incubation jar was extracted with 100 ml of 2 M KCl solution. Extracted solutions were analysed for  $\text{NH}_4^+$  and  $\text{NO}_3^-$  with a segmented flow analyser (Skalar San<sup>++</sup>, Skalar, Holland). The extract was also used to estimate  $^{15}\text{N}$  enrichment of  $\text{NO}_3^-$  pool.

**Calculation of  $\text{N}_2$  and  $\text{N}_2\text{O}$  production:**

The  $\delta^{15}\text{N}$  of  $\text{N}_2$  in the gas sample was calculated using the following equation:

$$\delta^{15}\text{N} - \text{N}_2 = \delta^{15}\text{N}_2 + 2 \times \delta^{30}\text{N}_2$$

where,  $\delta^{29}\text{N}_2$  and  $\delta^{30}\text{N}_2$  are the excess production of  $^{29}\text{N}_2$  and  $^{30}\text{N}_2$  in the gas vials calculated from 29/28 and 30/28 (Thamdrup & Dalsgaard 2000). 29/28 and 30/28 were determined from the  $m/z$  28, 29, and 30 peak areas obtained from the IRMS.  $^{15}\text{N}_2$  fluxes were then calculated using the linear increment of  $\delta^{15}\text{N}-\text{N}_2$  in the headspace with time while taking into account the incubation temperature and the volume of the incubation jar. Total denitrification derived  $\text{N}_2$  flux ( $^{14}\text{N}_2 + ^{15}\text{N}_2$ ) was determined by dividing the  $^{15}\text{N}_2$  fluxes by the  $^{15}\text{N}$  enrichment of the  $\text{N}_2\text{O}$  pool at 24 h on each sampling day (Yang et al. 2014). Net  $\text{N}_2\text{O}$  fluxes were determined from the gas chromatography.  $\delta^{15}\text{N}$  in  $\text{N}_2\text{O}$  was calculated as described by Yang et al. (2014):

$$\delta^{15}\text{N}-\text{N}_2\text{O} = 100 \times ({}^{45}\text{R} + 2 \times {}^{46}\text{R} - {}^{17}\text{R} - 2 \times {}^{18}\text{R}) / (2 + 2 \times {}^{45}\text{R} + 2 \times {}^{46}\text{R})$$

where  ${}^{45}\text{R} = 45/44$  and  ${}^{46}\text{R} = 46/44$  determined from the  $m/z$  44, 45, and 46 peaks obtained from the IRMS and  ${}^{17}\text{R} = 3.8861 \times 10^{-4}$  and  ${}^{18}\text{R} = 2.0947 \times 10^{-3}$ . The fluxes of  $\text{N}_2$  and  $\text{N}_2\text{O}$  were finally presented in a per hectare basis, considering the bulk density of the top 10 cm soil.

**4.2.2 Results and Discussion**

We observed higher  $\text{N}_2$  and  $\text{N}_2\text{O}$  emissions at 100% water filled pore space (WFPS) compared to the other moisture levels (Figures 18 and 19). There were no detectable  $\text{N}_2$  and very low  $\text{N}_2\text{O}$  production with 60% WFPS. Soil moisture influences the partitioning of soil  $\text{NO}_3^-$  between  $\text{N}_2$  and  $\text{N}_2\text{O}$ , where higher  $\text{N}_2$  compared to  $\text{N}_2\text{O}$  is expected at higher moisture levels due to a stronger anaerobic condition developed in soils. We observed similar levels of  $\text{N}_2$  flux between 80% and 100% WFPS on day 0, but the  $\text{N}_2$  flux from 80% WFPS declined considerably on the following days, whereas it remained high in 100% WFPS, leading to significantly higher cumulative  $\text{N}_2$  emissions from 100% WFPS (18-21 kg N ha<sup>-1</sup>) compared to 80% WFPS (4-5 kg N ha<sup>-1</sup>). There was no significant difference in cumulative  $\text{N}_2$  emissions between the two N enrichment treatments (2 and 5 atom%  $^{15}\text{N}$ ) within a WFPS level.  $\text{N}_2\text{O}$  production was below 2 g ha<sup>-1</sup> day<sup>-1</sup> at 60% WFPS throughout the experiment (Figure 19). Production of  $\text{N}_2\text{O}$  peaked on day 1 and 2 at 80% and 100% WFPS, respectively, and declined thereafter. There was no significant difference in cumulative  $\text{N}_2\text{O}$  emissions between the two N enrichment levels within a WFPS level, except at 100% WFPS. More than 1 kg N ha<sup>-1</sup> was lost as  $\text{N}_2\text{O}-\text{N}$  from the 5 atom%  $^{15}\text{N}$  enrichment treatment at 100% WFPS.



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While N<sub>2</sub> production was still high, N<sub>2</sub>O production started declining rapidly after day 2 in 100% WFPS. This is due to the favourable conditions for complete denitrification created by stronger

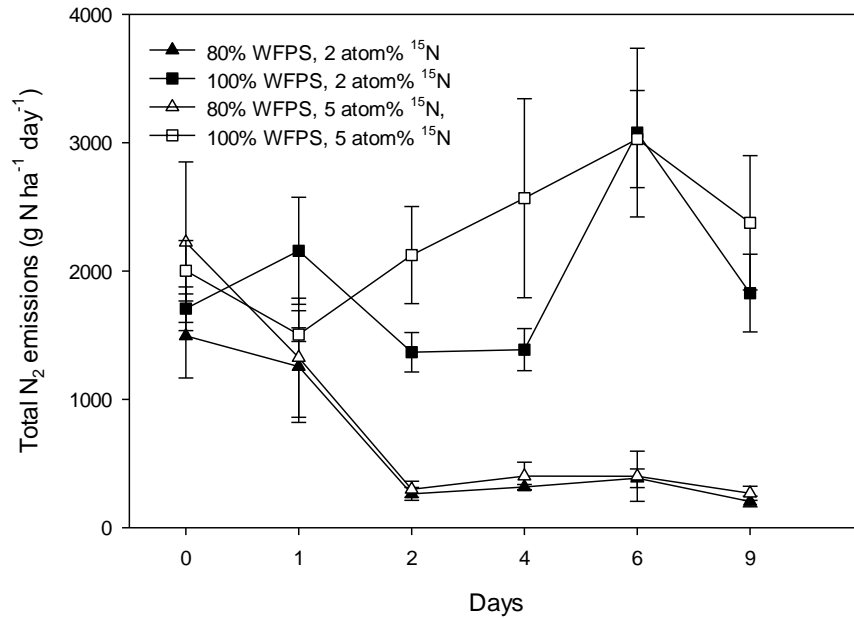


Figure 18. N<sub>2</sub>-N flux (g N ha<sup>-1</sup> day<sup>-1</sup>) in 80% and 100% water filled pore space (WFPS) with 2 and 5 atom% <sup>15</sup>N enrichment. N<sub>2</sub> production was not detected in 60% WFPS. Error bars represent ±1 standard error.

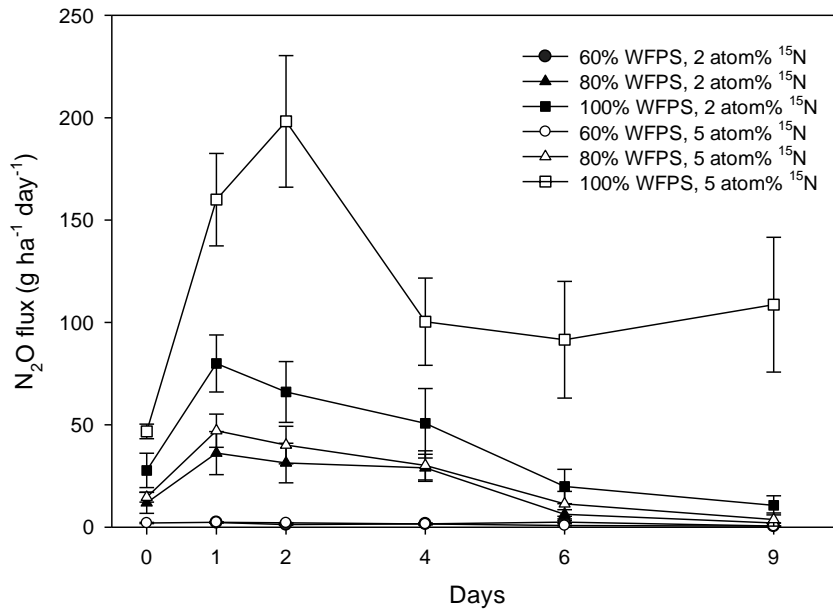
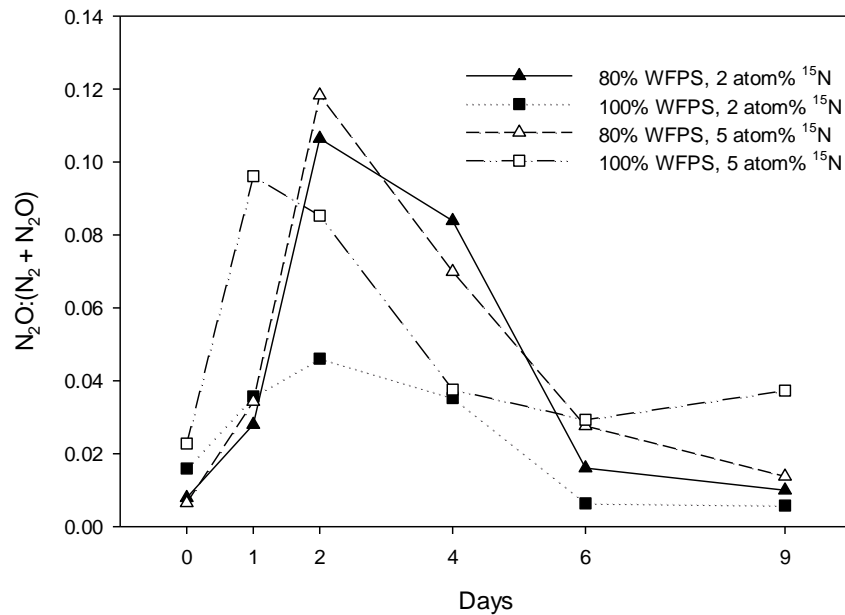


Figure 19. N<sub>2</sub>O-N flux (g ha<sup>-1</sup> day<sup>-1</sup>) in 60%, 80% and 100% water filled pore space (WFPS) with 2 and 5 atom% <sup>15</sup>N enrichment. Error bars represent ±1 standard error.

anaerobic conditions in soil as a result of consumption of trapped oxygen by the soil microbes. Despite the decline, the  $\text{N}_2\text{O}$  flux was still at high levels ( $>92 \text{ g N}_2\text{O-N ha}^{-1} \text{ day}^{-1}$ ) after 2 days with 5 atom%  $^{15}\text{N}$  enrichment at 100% WFPS because of the high  $\text{NO}_3^-$  concentration ( $> 60 \text{ mg NO}_3^- \text{-N kg}^{-1} \text{ soil}$ ) in the soil inhibiting  $\text{N}_2\text{O}$  reduction to  $\text{N}_2$ .

Although  $\text{N}_2$  was the main denitrification product in both the 80 and 100% WFPS, the ratio of  $\text{N}_2\text{O}:(\text{N}_2+\text{N}_2\text{O})$  was higher at 80% WFPS compared to 100% WFPS after day 1, except with 5 atom%  $^{15}\text{N}$  enrichment at 100% WFPS (Figure 20). This indicates that compared to 100% WFPS, 80% WFPS favours  $\text{N}_2\text{O}$  over  $\text{N}_2$  production when the soil  $\text{NO}_3^-$  concentration is below  $40 \text{ mg kg}^{-1} \text{ soil}$ . Higher  $\text{N}_2$  compared to  $\text{N}_2\text{O}$  production in 80% WFPS can be explained by the possibility of large numbers of anaerobic microsites accompanied by high organic carbon content in the soil.



**Figure 20. The ratio of  $\text{N}_2\text{O}:(\text{N}_2+\text{N}_2\text{O})$  produced at 80% and 100% WFPS with 2 and 5 atom%  $^{15}\text{N}$  enrichment.**

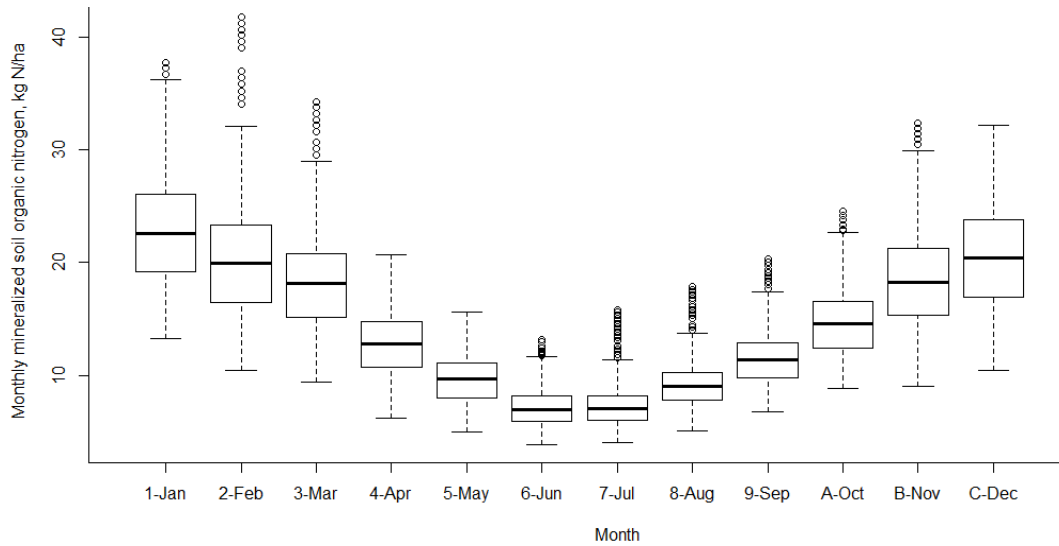
### **5. Mineralisation calculator (KPI 7.6 Output 6 (k))**

The mineralisation calculator is provided as an excel file as part of this report.

The mineralisation calculator was developed based on data collected from the Allansford dryland and irrigated sites. Using stepwise regression, the key predictors of mineralisation have been identified by running the mineralisation model for a 20-year period using climate data from the Bureau of Meteorology. The annual dynamics of mineralisation of the different soil organic nitrogen pools (passive, active, microbial biomass and plant residues) over this time period have been predicted (Figure 21). The predictions for the dryland and irrigated sites follow a similar

pattern although mineralisation in the dryland site over summer is less than under irrigation due to lower soil water content.

Annual mineralisation predicted from the calculator is around 200 kg N/ha, with marginally higher mineralisation in the dryland system.



**Figure 21: Annual dynamics of mineralization of soil organic nitrogen for the irrigated system at Allansford.**

Currently the calculator is provided in excel, and discussion with industry groups have been held on 22<sup>nd</sup> May, 28<sup>th</sup> August and 6<sup>th</sup> November 2019 to gain an understanding of its usefulness and limitations from the industry point of view, and to refine the model, including the development of a user-friendly interface.

The model has defined limits of climate, N rate and soil organic carbon, and can predict the mineral N available in the soil at a particular time, and the N that could be mineralised over a defined period into the future. Testing of the model has been around the autumn break period, with predictions of soil profile mineral N available at the end of March, and mineralised N provided through April and May.

Scenarios have been run to look at the mineralisation in different years (Figure 22) and to identify the key drivers of mineralisation. Initially a factor analysis was performed to identify which factors had a positive or negative correlation with soil mineral N availability (Figure 23). The key predictors were then determined using a Structural Equation modelling (SEM) approach (Figure 24). Under the irrigated system the key driver of mineralised N (a combination of mineral N available at the end of March and of mineralizable N that can be released over April and May) were the future temperature (correlation coefficient of 0.37) (i.e. the temperature during the months of April and May) and the N rate (correlation coefficient of 0.22). Both N rate and future temperature were positively related to the potential mineral N that could be available for pasture

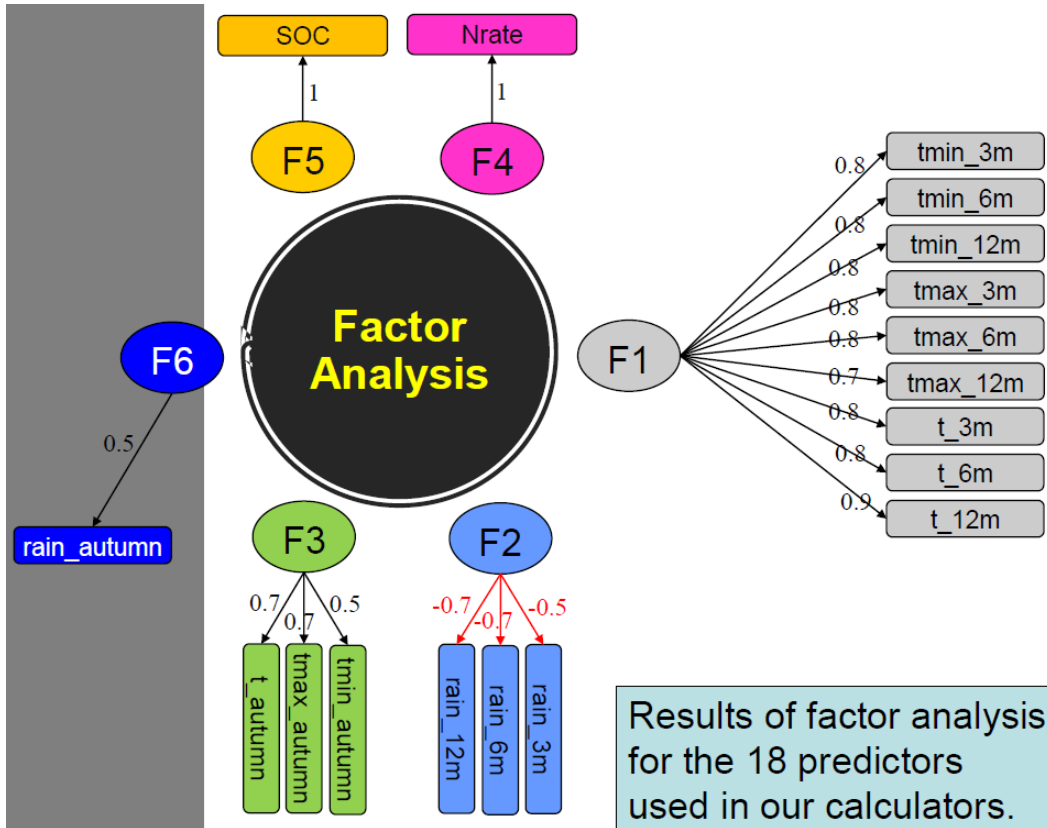
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growth, as was the soil organic carbon and the past temperature. Considering only mineralisation over April and May, the future temperature was highly correlated (0.76) and the most important driver, followed by the N rate (0.42) and soil organic carbon content (0.14) (data not shown). Considering mineral N available in the profile prior to autumn, N rate was the most highly correlated factor (0.12) (data not shown).

A calculator to estimate the mineralization of soil organic nitrogen at Autumn (April & May)						
Irrigated pasture						
MODEL	PREDICTOR	VALUE	VALUE 2001	VALUE 2008	VALUE 2013	VALUE 2017
SOIL	SOC (0-10 cm)	4	4	4	4	4
N rate	Fertilizer N rate (kg N/ha) long-term	40	40	40	40	40
Temperat	Maximum - minimum air temperature (last 3 months, average)	12.5	12.7	12.6	13.0	11.5
	Maximum - minimum air temperature (last 6 months, average)	12	12.6	12.5	12.8	11.4
	Maximum - minimum air temperature (last 12 months, average)	10.5	10.5	10.6	10.6	9.8
	Maximum - air temperature (last 3 months, average)	24	24.2	23.0	24.8	23.2
	Maximum - air temperature (last 6 months, average)	22	23.6	22.7	23.9	22.4
	Maximum - air temperature (last 12 months, average)	19	19.3	19.0	19.5	18.7
	Average air temperature (last 3 months)	18.0	16.1	16.7	17.4	18.1
	Average air temperature (last 6 months)	16.3	17.2	16.4	17.5	16.7
	Average air temperature (last 12 months)	13.8	14.0	13.7	14.2	13.8
Rainfall	Rainfall (last 3 months)	85	85	133	70	284
<b>Total mineral N and mineralised N (April+May) kg N/ha</b>		<b>62.0</b>	<b>34.3</b>	<b>54.9</b>	<b>45.3</b>	<b>55.6</b>
N removal by pasture						
	April		39	kg N/ha		
	May		45	kg N/ha		

Figure 22: Example image of the mineralization calculator interface in excel showing the scenarios presented at the mineralization calculator workshop on May 22<sup>nd</sup>, 2019.

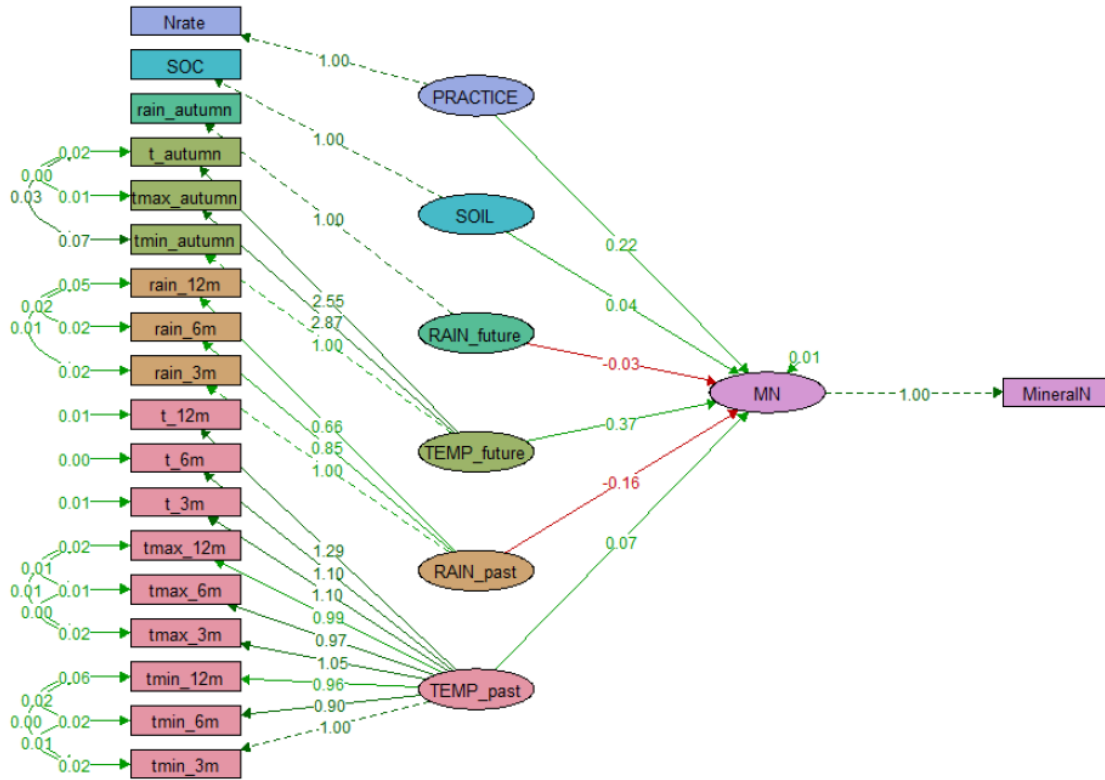
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**Figure 23. Identification of key predictors of mineralized N available for pasture to utilize during the autumn break period. Factors are F1- t: past ambient temperature including the time of measurement (3, 6 or 12 months) and whether average, minimum or maximum, rain: F2 - rainfall in the past 3, 6 or 12 months, F3- autumn temperature (min, max, average) (future), F4- Nrate: rate of N applied, F5 - SOC: soil organic carbon, F6- autumn rains (future).**

For the dryland site, the SEM approach showed a similar pattern to the irrigated site with the key drivers being future temperature, then N rate, and to a lesser extent past temperature and soil organic carbon content. In both the irrigated and dryland systems, the past rainfall was negatively correlated with mineral N available at the end of March, but this was more noticeable on the dryland site because additional rainfall would lead to pasture growth which would remove N from the soil. Under the irrigated system pasture productivity is not limited by water to the same extent at this time of year.

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**Figure 24. Key predictors of mineralized N available for pasture to utilize under the irrigated system during the autumn break period as determined using a structural Equation Modeling (SEM) approach.**

The calculator is an easy to use tool which has enabled advisors to gain an understanding of the key drivers of mineralisation under pasture systems in the HRZ of south-west Victoria. The calculator is available for use in excel format but may require some further refinement.

## 6. Conclusions

The project has identified key factors driving NUE in southern Australian HRZ dairy pastures. Clear seasonal N responses and NUE indicate that N fertilisation should be considered in light of predicted pasture growth and N uptake, and N loss risk rather than a blanket application per grazing cycle. Less than 30% of applied N is recovered by the pasture one grazing cycle after fertilisation, with the remainder being immobilised and subsequently released over time, or lost. Under low N input systems (e.g. 20 kg N/ha), almost all of the N is accounted for in the plant and soil. However, applying higher rates of N (e.g. 80 kg N/ha) leads to N losses which have a negative environmental and economic impact. Increasing N rate leads to increased N loss. Management of soil water deep in the profile appears to be a critical component to boost pasture production and NUE on the season shoulders (early autumn and late spring).

Mineralisation contributions were around 200 kg N/ha annually under dryland and irrigated systems, with seasonal variation in the amount of mineralisation related to climatic conditions.

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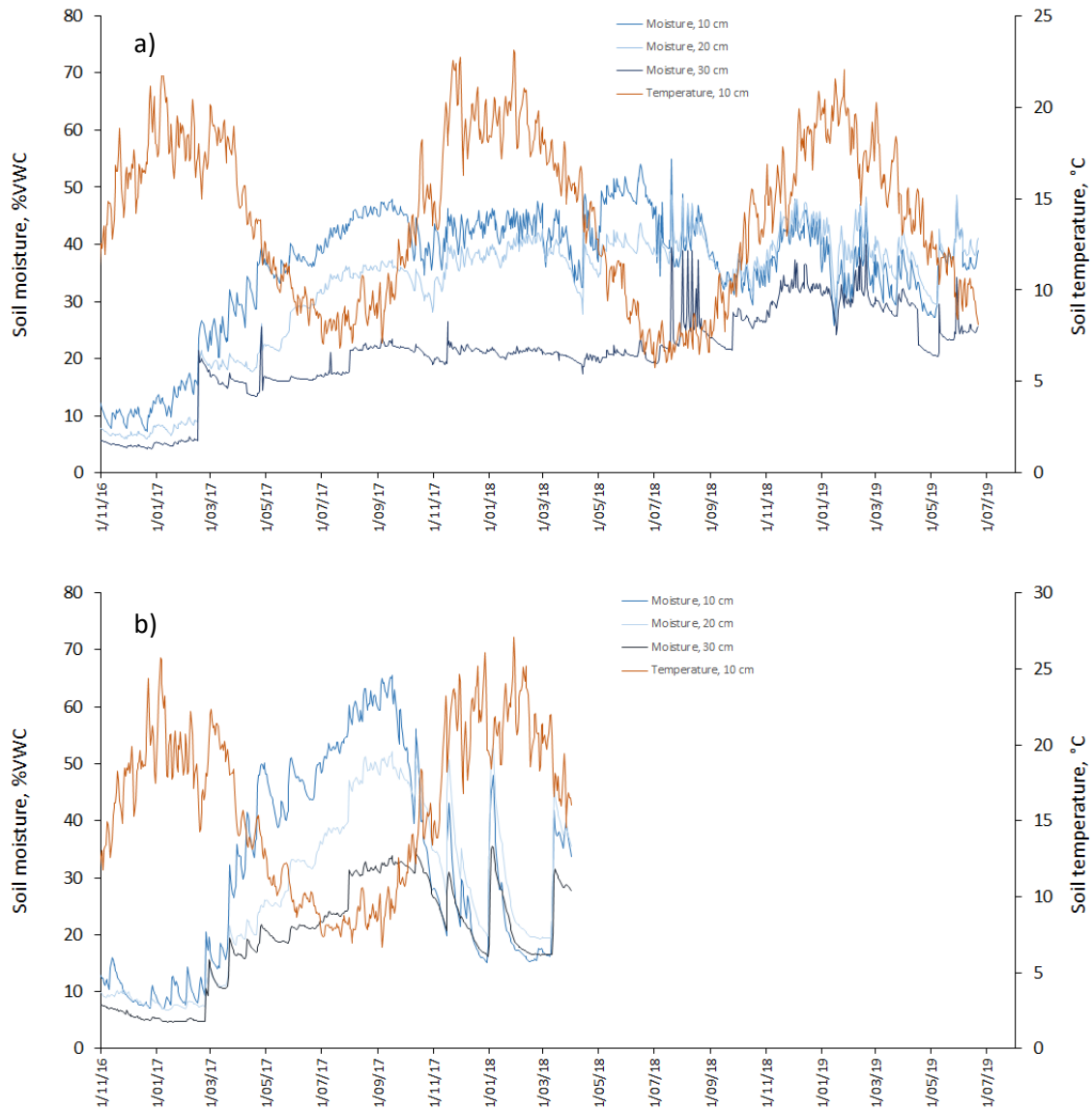
The key drivers of mineralised available N in autumn were N rate and predicted future temperature.

Remote sensing approaches show promise as a new tool for assessing pasture N and biomass, but require further work, particularly around their useability in the climate experienced at the field sites.

The benefits to industry from this project are that improvements to NUE have been shown to be possible using a different approach to fertiliser management that accounts for seasonal variability, and reconsideration of irrigation strategies. Understanding the cycling of N through the organic matter pool through the development of the mineralisation calculator and identification of the key drivers of mineral N availability from soil, will assist growers to have greater confidence in identifying when they can better utilise soil N and reduce fertiliser N inputs.

Rethinking management of N and water at the shoulder periods of the seasons provides a great opportunity to improve NUE in these HRZ pasture systems. Further investigation of how much water is required and when this can be extended to would be beneficial for expanding the growing season and producing more feed.

APPENDIX 1



**Figure A1. Soil temperature at 10 cm depth and soil volumetric moisture at 10, 20 and 30 cm depths on the irrigated (a) and dryland (b) sites. Note: the dryland data only covers the time of measurement.**



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**Table A1. Schedule of fertilisation events at the Allansford sites.**

Fertiliser application dates	26/10/16	21/11/16	22/12/16	17/01/17	15/02/17	8/03/17	4/04/17	27/04/17	6/06/17	1/08/17	14/09/17	14/10/17	2/11/17	23/11/17	19/12/17	19/01/18	9/02/18	14/03/18	16/04/18	17/05/18	19/06/18	2/08/18	11/09/18	2/10/18	23/10/18	13/11/18	11/12/18	4/01/19	31/01/19	21/02/19	19/03/19	16/04/19	14/05/19	
Days b/w fertiliser		26	31	26	29	21	27	23	40	56	44	30	19	21	26	31	21	33	33	31	33	43	40	21	21	21	28	24	27	21	26	28	28	
Dryland	✓	-	-	-	-	✓	✓	✓	✓	✓	✓	✓	✓	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
Irrigated	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
EEF*	GU	GU	GU	GU	GU	GU	GU	GU	EU	EU	EU	EU	EU	GU	GU	GU	GU	GU	GU	GU	EU	EU	GU	GU	GU	GU	GU	GU	GU	GU	GU	GU	GU	
<sup>15</sup> N fertiliser (multiple rates)**	-	-	-	-	-	-	✓	-	-	-	✓	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
Urine and <sup>15</sup> N urine**	-	-	-	-	-	-	-	✓	-	-	✓	-	✓	-	-	✓	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
<sup>15</sup> N fertiliser** (single rate, 5 soils)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	✓	-

\*EEF: Enhanced efficiency fertiliser program. GU: Green urea (urea with NBPT, urease inhibitor); EU: Urea with ENTEC (DMPP, nitrification inhibitor). \*\*Both dryland and irrigated. <sup>15</sup>N fertiliser applied at 20 and 40 kg N/ha plus 80 kg N/ha in spring, at 10% atom enrichment. Urine and <sup>15</sup>N urine (5% atom enrichment) applied at 1000 kg N/ha. Where <sup>15</sup>N single rate, 5 soils) is applied to the soils collected from the region and established on the irrigated site.