Aqueous Nitrogen Dynamics in Irrigated Cropping Systems: Improving precision agriculture and environmental performance for the Australian cotton industry

by

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Candidate's Declaration

This thesis is submitted as a traditional thesis with the inclusion of one co-authored manuscript in accordance with the Australian National University policies and procedures. I declare that the research presented in this thesis represents original work that I carried out during my candidature at the Australian National University, except for contributions to multi-author papers incorporated in the thesis where my contributions are specified in the Statement of Contribution.

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Abstract

The Australian cotton industry is a prime example of precision agriculture in action, having achieved significant efficiency gains in yield (kg lint ha^{-1}) and water use (kg lint ML^{-1}) over the past 50 years. Unfortunately, nitrogen fertiliser use efficiency (NFUE) has not experienced the same gains over this period, and has instead declined. Australian irrigated cotton production requires high nitrogen (N) inputs to maintain its high yields. N application rates (kg N ha^{-1}) have increased over recent decades due to a range of factors, including low fertiliser costs and grower risk appetites. Average yields have also increased over this period; however, they have not been proportional to the rise in N applications, resulting in steadily declining NFUE. While significant research describing N dynamics in Australian cotton systems already exists, there remain many research gaps to be filled.

This thesis aims to address four research gaps to provide additional management levers for the Australian cotton industry to improve NFUE. The four topics explored herein are: (1) the uniformity of aqueous N application (fertigation or water-run); (2) the mechanisms driving surface runoff N losses in furrow flood irrigation; (3) the reaction rates and residence times of aqueous N; and (4) the degree of plant access to different soil N molecules. A series of field, laboratory, and glasshouse experiments were used to address these questions. Three field experiments measuring fertigation application efficacies were conducted on private farms in the Riverina, New South Wales (NSW) over the 2016-17 summer season. Another field experiment was performed at the Australian Cotton Research Institute (ACRI) in Narrabri, NSW over the 2017-18 season, measuring N runoff variations in alternate furrow irrigation configurations. Two laboratory experiments were performed at the Commonwealth Scientific and Industrial Research Organisation (CSIRO) Black Mountain site in Canberra, Australian Capital Territory, measuring the reaction rates and residence times of dissolved urea in soil-water systems. Finally, a glasshouse experiment was performed at CSIRO Black Mountain in January 2019, assessing the N uptake capabilities and preferences of three cotton (*Gossypium hirsutum* L.) varieties using ^{15}N-^{13}C-stable isotope analysis. The specific research questions these experiments aimed to answer are detailed in the paragraphs below.

Chapter 2 aims to answer the question: "*How effective are current Australian irrigated cotton fertigation practices at delivering consistent N to crops, and what management levers can be identified to improve outcomes?"*. Aqueous N application is increasingly popular in Australian irrigated cotton production systems, despite limited data describing its efficacy. Significant research gaps exist around application homogeneity, aqueous residence times, and attributable runoff losses. These unknown factors represent a swathe of unutilised management levers that growers could use to improve NFUE. This chapter uses data from three field experiments performed on private farms to assess the application consistency of common fertigation techniques. It was found that the distribution of aqueous N within fields was uniform when supplied consistently, but that achieving consistent supply was difficult. Poor urea tank management, inconsistent irrigation channel network mixing, and the colourless nature of dissolved N were all factors contributing to poor fertigation results.

Chapter 3 aims to answer the question: *"How does N surface runoff vary spatially and temporally at sub-field and intra-irrigation scales respectively, and can this high resolution be used to identify specific mechanistic drivers of N runoff?".* N surface runoff is well quantified spatially at a field-scale and temporally at an inter-irrigation scale. This is useful for identifying macro trends such as the majority of runoff losses occur at the beginning of the season; however, analyses at higher spatial and temporal resolutions are required to identify the mechanisms driving these losses. This chapter is built around a field experiment performed at the ACRI in 2017-18, and includes data from four additional unpublished studies performed by other researchers to support the observed trends. All studies were performed in alternate furrow flood irrigation systems, whereby irrigation water is supplied via syphon

to every second furrow. It was shown that in these systems as irrigation water passes through the hill from the irrigated-furrow to the non-irrigated-furrow (alternate-furrow), it leaches significant soluble material from the soil. Alternate-furrows discharged the majority of field-derived N (87.5 ± 4.4% compared to 12.5 ± 4.4% for irrigated-furrows), which was predominantly composed of nitrate (NO_3^-; 58.3 ± 7.5%) and dissolved organic nitrogen (DON; 31.9 ± 7.6%). Field-derived N runoff during the first irrigation event of the season was equivalent to 6.1 ± 0.4% of applied pre-season fertiliser, with whole of season losses equivalent to 12.1 ± 0.7%. Runoff N losses would likely be reduced considerably by replacing alternate-furrows with an extra-wide bed/hill.

Chapter 4 aims to answer the question: *"What is the residence time of dissolved N in irrigation water, and how does it vary across the farm environment?"*. Urea is the most commonly water-run nitrogenous fertiliser in Australian cotton systems. Solid (prilled) urea is a chemically stable compound, but once dissolved in irrigation water it quickly mineralises via the enzyme urease, and can be lost to the environment if not immobilised. Despite the widespread uptake of water-running urea, there is limited information available to growers describing N residence times in irrigation waters. This chapter reports the findings of two laboratory experiments designed to generate a queryable dataset for predicting urea mineralisation rates in irrigation waters. After 24 hours, 32.5 ± 3.0% and 41.0 ± 0.8% of the urea had mineralised in the 15°C and 35°C treatments respectively, increasing to 87.7 ± 0.6% and 94.3 ± 0.2% after seven days. Urea mineralisation was positively correlated with both soil exposure and temperature. For growers, this means that mineralisation will be most rapid when urea-laden water is travelling down furrows, as high soil interaction and water temperatures produce high urease exposure and activity.

Chapter 5 aims to answer the question: "*Which soil N species can commercial cotton (G. hirsutum) directly take up, and what preferences does it exhibit when given a choice?"* Bulk plant uptake of applied fertiliser N (fertiliser recovery) is well characterised in Australia and internationally. While current research demonstrates that cotton obtains the majority of its N from the soil, it does not identify the chemical speciation of the N taken up. This represents a research gap and potential opportunity to improve fertiliser recovery in Australian cotton systems. This chapter uses a ^{15}N-^{13}C-stable isotope glasshouse experiment to assess the N uptake capabilities and preferences of commercial cotton. Three varieties of *G. hirsutum* were grown from seed to a 2-4 leaf stage in clean sand rhizotubes to allow full control over their nutrition: Sicot 746B3F, a genetically modified (GM) current commercial cultivar; Sicala V2, an obsolete non-GM commercial cultivar; and Tx III, a Guatemalan landrace accession that represents the native origins of the commercial *G. hirsutum* varieties. Labelled fertiliser compounds ^{15}N-NO_3^-, ^{15}N-ammonium (NH_4^+), ^{15}N-^{13}C-urea (CH_4N_2O) and ^{15}N-^{13}C-alanine ($C_3H_7NO_2$) were supplied to the seedlings concurrently, with all four N species rapidly taken up. After 180 minutes, the percentage of added NO_3^-—N, NH_4^+—N, urea—N and alanine—N taken up was 25.1 ± 1.9%, 16.6 ± 1.9%, 22.2 ± 1.9% and 14.3 ± 1.9% respectively. Consistent uptake ratios of alanine—^{13}C and —^{15}N indicate that up to 33% of the absorbed alanine—N was taken up in the form of whole alanine molecules. Direct plant access of soluble organic N suggests that increasing soil organic matter (SOM) would not necessarily reduce plant-available N.

Improving NFUE represents a triple bottom line opportunity for the Australian cotton industry. Economically, it will save growers the cost of wasted fertiliser, and reduced yields from over-application. Environmentally, it will reduce N_2O greenhouse gas emissions, increase soil carbon stocks, and reduce N deep drainage. And socially, it can help to grow Australian cotton's reputation as the most resource-efficient in the world, and build its public profile and brand recognition. This thesis aims to improve precision agricultural practices and environmental performance for the Australian cotton industry by providing new information and management tools to increase NFUE.

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List of Abbreviations

^{13}C	Heavy stable isotope of carbon containing seven neutrons
^{15}N	Heavy stable isotope of nitrogen containing eight neutrons
%NDFT	Percentage Nitrogen Derived from Transfer
ACT	Australian Capital Territory
AlaDC	Alanine Decarboxylase
ANU	Australian National University
BSIA	Bulk Stable Isotope Analysis
C	Carbon
CO_2	Carbon Dioxide
CRDC	Cotton Research and Development Corporation
CSD	Cotton Seed Distributors
CSIRO	Commonwealth Scientific and Industrial Research Organisation
CSIRO DAP	Commonwealth Scientific and Industrial Research Organisation Data Access Portal
DON	Dissolved Organic Nitrogen
EC	Electrical Conductivity
FDRN	Field-Derived Runoff Nitrogen
GHG	Greenhouse Gas
GM	Genetically Modified
Ha	Hectare (10,000 m^2) – non-SI unit in standard use
IRMS	Isotope-Ratio Mass Spectrometry
LMW	Low Molecular Weight
N	Nitrogen
N_2	Dinitrogen Gas

N_2O	Nitrous Oxide
NFUE	Nitrogen Fertiliser Use Efficiency
NH_3	Ammonia – also referred to as 'Anhydrous Ammonia' in farming contexts
NH_4^+	Ammonium
NO_3^-	Nitrate
N rate	Nitrogen Fertiliser Application Rate
NSW	New South Wales
NSW DPI	New South Wales Department of Primary Industries
P	Phosphorous
QLD	Queensland
SOM	Soil Organic Matter
S	Sulfur
TDN	Total Dissolved Nitrogen
TRN	Total Runoff Nitrogen
USA	United States of America
WUE	Water Use Efficiency

Additional Material Generated During this Project

Published Manuscripts

Latimer, J.O., Macdonald, B.C.T., Schwenke, G.D., Nachimuthu, G., Baird, J.C. (2020), *Alternate furrow flood irrigation removes significant nitrogen from the field through lateral leaching*. Soil and Tillage Research [Under review with Soil and Tillage Research]

Macdonald, B.C.T., **Latimer, J.O.**, Schwenke, G.D., Nachimuthu, G., Baird, J.C., (2018), *The current status of nitrogen fertiliser use efficiency and future research directions for the Australian cotton industry*. Journal of Cotton Research 1 (1), 10.1186/s42397-018-0015-9

Published Data Sets

Latimer (2020b), *High resolution nitrogen runoff chemical data from alternate furrow flood irrigated cotton field. v1.* CSIRO. Data Collection. csiro:45108 License: Creative Commons Attribution 4.0 International Licence, Persistent Link: https://data.csiro.au/collections/collection/CIcsiro:45108

Latimer (2020a), *Chemical data: leaching of soil nitrogen by progressing irrigation front* DOI:[Currently being processed] CSIRO (Ed.1). Data Access Portal

Latimer and Farrell (2020), *Chemical isotope data: nitrogen uptake preferences and capabilities of ^{15}N-^{13}C-labelled nitrate, ammonium, urea and alanine by three varieties of Gossypium hirsutum*, DOI: [Currently being processed] CSIRO (Ed.1). Data Access Portal

Latimer and Macdonald (2020), *Chemical data: urea mineralisation rates at three temperatures in a closed soil and water system*, DOI: [Currently being processed] CSIRO (Ed.1). Data Access Portal

Industry Communication

Spotlight magazine article (*Summer 2017-18 edition*): Is your urea tank supplying the nitrogen you think it is?

Spotlight magazine article (*Winter 2018 edition*): How quickly does urea mineralise when water-running urea?

Spotlight magazine article (*Spring 2020 edition*): When it comes to choosing nitrogen, cotton goes organic.

Spotlight magazine article (*Summer 2020-21 edition*): Urea mineralisation in water and soil.

Grower Tour Publication: Pitfalls in water-running urea.

Chapter 1 – Introduction

1.1 – Foundations

The Australian cotton industry's nitrogen fertiliser use efficiency (NFUE) has been in decline for many years (Macdonald et al., 2018; Welsh et al., 2015). This is contrary to other production metrics such as yield per hectare (kg ha^{-1}) and water use efficiency (WUE; ML kg^{-1}) that have steadily increased over the same period (Constable, 2004; Roth, 2014a). Poor NFUE can lead to adverse economic, social and environmental outcomes for individual growers and the wider industry. While aqueous nitrogen (N) application is increasingly common in the Australian cotton industry, there is limited data available describing its efficacy. This represents a key knowledge gap that if filled could significantly improve the industry's production efficiency. The Australian cotton industry can improve its NFUE by applying a similar precision agriculture approach to N management as has helped it achieve so many other advancements in production efficiency. This would improve the industry's triple bottom line, yielding economic, environmental and social benefits to all growers. This thesis will use field and laboratory experiments to explore the application, distribution and transformation of aqueous N in irrigated cotton systems. Through these generated data, improved management levers will be identified that growers can use to improve application efficiency and by extensions production efficiency.

1.2 – Precision Agriculture and the Australian Cotton Industry

The term 'precision agriculture' has typically been used to refer to agricultural management practices that respond to within-field spatial or temporal variability (Stafford, 2000; Zhang et al., 2002). While many suggest this is a relatively new phenomenon, the practice of responding to in-field variability has been appreciated for millennia (Stafford, 2000), rendering the term somewhat superfluous. In this thesis, I use the term precision agriculture more in line with Gebbers and Adamchuk (2010) to refer broadly to the targeted application of technology and management with the aim of maximising resource use efficiency in agricultural production systems. My definition implicitly includes the assumption that spatial and temporal variability must be controlled if resource use efficiencies are to be maximised. The primary motivation for pursuing increases in production efficiency can vary depending on the stakeholders driving change, and can be in nature:

1. ***Economic***: Reducing fertiliser waste (Chen et al., 2008), water usage (Chen et al., 2020; Roth et al., 2013; Zahoor et al., 2019), or labour requirements (Vandeplas et al., 2010)
2. ***Environmental:*** Reducing greenhouse gas (GHG) emissions (Reay et al., 2012), groundwater contamination (Zhang et al., 1996), or nutrient runoff into waterways (Carpenter et al., 1998)
3. ***Social:*** Feeding more people (Kummu et al., 2012), improving community relations and social licences to operate (Moffat et al., 2016), or securing strategic national security interests (Kukal and Irmak, 2020; Lambert and Hashim, 2017)

While there is often just one cited reason for management practice change, advances in resource use efficiency will affect all three categories. This is because our society, economies and environments are intrinsically linked, and sustainable management of farming systems must engage with all three. The 'three pillars of sustainability' is a common framework for describing the interconnectivity between the environmental, economic and social aspects of our world (Fig. 1.1A; Barbier (1987); Purvis et al. (2018). The symbolism of pillars is useful for encouraging thought about the broader effects of management decisions; however, the imagery implies that the aspects are independent, when in reality they are subsets of one another. A more accurate analogy would be that of nested concentric circles to communicate that there is no economy without society, and no society without an environment (Fig. 1.1B).

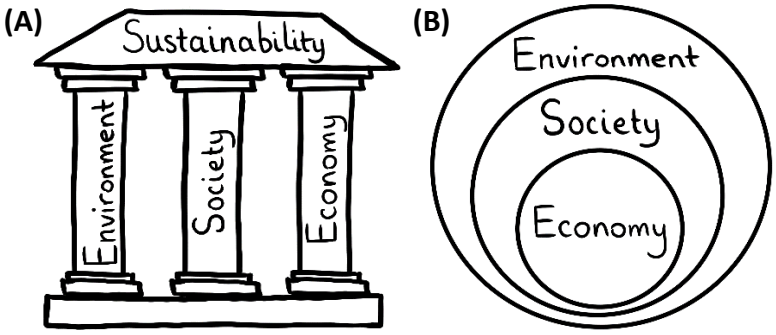


Figure 1.1. *Competing visual representations of the connection between the economy, society and environment.* ***(A)*** *Original depiction of the three pillars of sustainability first described by Barbier (1987).* ***(B)*** *More recent depiction of the three pillars of sustainability illustrating that the three aspects are not independent, equally important concepts.*

Regardless of the specific imagery employed, if agricultural enterprises wish to prosper then all stakeholders need to consider carefully the social, environmental and economic ramifications of management decisions. In the more business-centric vernacular introduced by Elkington (1998), improving resource use efficiency will raise the triple bottom line of any organisation, leading to better environmental, economic and social outcomes.

Resource use efficiency in agriculture – whether relating to the use of water, energy or nutrients – is typically defined in terms of final crop yield (Bai et al., 2020; Cassman et al., 1998; Hsiao et al., 2007; Meul et al., 2007). Efficiency gains are achievable through increasing yields for the same resource use, or by decreasing the resources used to achieve the same yield (Eq. 1.1).

$$Resource\ Use\ Efficiency = \frac{Crop\ Yield}{Resource\ Used}$$

Equation 1.1. *General equation for calculating resource use efficiency.*

The suite of technologies available for improving agricultural resource use efficiency has increased significantly since the first Agricultural Revolution (circa 10,000 BCE). For the majority of agrarian human history, yield gains have been achieved incrementally through selective breeding, manure application, and the adoption of irrigation. This changed during the Industrial Revolution with widespread mechanisation and the adoption of synthetic fertilisers that significantly increased agricultural yields and signalled the beginning of modern industrial agriculture (Ang et al., 2010). The next rapid increase in agricultural production was achieved through advances in crop genomics and the introduction of high yielding varieties during the Green Revolution of the 1950s and 1960s (Lynch, 2007), which eventually developed into direct genetic modification of crops in the 1980s and 1990s (Raman, 2017). The advent of the Information Age has introduced new tools for improving agricultural resource use efficiency while simultaneously transforming previous advances in precision agriculture. With widely available on- and off-farm computing, mechanisation has become automation, genomics has become bioinformatics, and aerial field surveys that were once achieved by looking out the window of a light aircraft are now remotely captured using multiple spectra with analytics performed before the drone has even landed (Farrell et al., 2018).

In order to achieve production gains from advances in precision agriculture, many aspects of the farming system need to be optimised simultaneously (Watt et al., 2006). This concept is most succinctly illustrated by the Sprengel-Liebig Law of the Minimum (Ploeg et al., 1999), often visualised as a water-filled barrel with staves of varying heights (Fig. 1.2). The principle states that growth – represented by the height of water within the barrel – is determined not by the total resources available but by the scarcest resource, or lowest stave. A good example of this is the British Agricultural Revolution of the 17th and 18th centuries, when crop yields were rapidly increased by concurrently addressing a range of metaphorical staves, including: the introduction of leguminous crop rotations

to increase plant-available N, improvements to transportation infrastructure and the removal of domestic customs barriers to increase distribution capabilities, continued selective crop breeding to increase varietal yield limits, and plough design improvements to reduce labour requirements for soil cultivation (Overton, 1996). More recently in the 20th century, the introduction of synthetic nitrogenous fertilisers has usurped plant-available N as the limiting factor to growth in many countries with other constraints like potassium availability and soil compaction (Erisman et al., 2008).

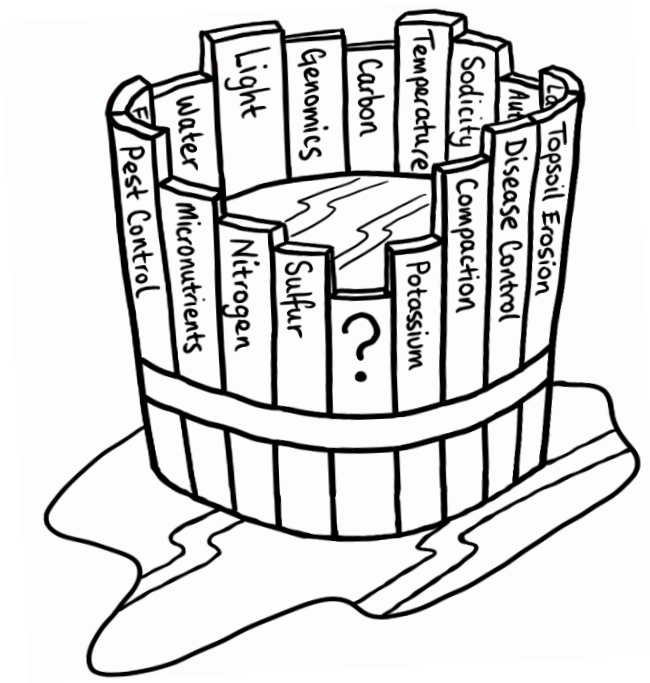


Figure 1.2. *Visual representation of the Sprengel-Liebig Law of the Minimum (also known as the Liebig Law of the Minimum). Just as the capacity of a barrel with staves of unequal length is limited by the shortest stave, so too is a plant's growth limited by the nutrient in shortest supply. Staves are not necessarily independent of one another in this analogy, with co-limitations occurring between some production aspects like soil nutrients (Kirkby et al., 2011).*

The Australian cotton industry is an archetypal example of precision agriculture in action. Average Australian cotton yields are the highest in the world, at 2360 kg lint ha^{-1} (Cotton Australia, 2019; FAO, 2019), which has been achieved through consistent research and optimisation of many components of the farming system concurrently, including:

- ***Genomics and Bioinformatics*** **–** More than 99% of Australia's commercially grown cotton (*Gossypium hirsutum* L.) is genetically modified (GM), which has resulted in a 95% decrease in insecticide use since the introduction of the first GM varieties in 1993 (Cotton Australia, 2020; OGTR, 2018; Roth, 2014a). New varieties of *G. hirsutum* specifically adapted to Australian environmental conditions are continuously being developed in partnership with the Commonwealth Scientific and Industrial Research Organisation (CSIRO) using genes provided by Bayer (previously Monsanto; Liu et al., 2013; Trapero et al., 2016). Recent CSIRO research has produced prototype cultivars with yellow-, red- and purple-coloured lint that have the potential to reduce dye requirements, which are frequently associated with significant environmental and social harm (The World Bank, 2014). Bioinformatics and genomics are also used to describe and optimise soil microbial communities that heavily impact soil properties like cohesion, plant-available water, and nutrient speciation (Shaw, 2005).
- ***Pest and Disease Management*** **–** Pests and diseases represent a core threat to cotton production, and significant research and development investment has been made to improve resilience. The genetic modifications in Australian cotton offer significant pest resistance, but only if strict management practices are adhered to. To that end, growers are required to implement Bollgard resistance management plans, and strongly encouraged to adopt

integrated pest and disease management plans (CRDC, 2019c, 2020b). Ongoing research efforts are directed at managing many tens of disease, insect and weed species, all of which are region-specific to some degree (CRDC, 2019c). As such, biosecurity is taken very seriously in the Australian cotton industry, with growers strictly adhering to quarantine procedures like "Come clean, go clean" (CottonInfo et al., 2020; CRDC, 2018, 2019c; Trapero et al., 2016).

- ***Field Engineering and Management*** **–** Irrigated cotton fields are typically laser-levelled to control water flow speeds and distribution uniformity, with 0.04° (1:1500) and 0.006° (1:10000) slopes common for different surface irrigation configurations (CRDC, 2020b; Roth et al., 2018). Bed configuration is precise, with consistent seed spacing across fields producing consistent plant growth and yields. Most farms practice controlled traffic regimes within fields to limit soil compaction from tractors and pickers (CRDC, 2020b). Soil properties like compaction, mineralogy and sodicity impact yield, and are researched and monitored by the industry (McGee and Loke, 2007; Shaw, 2005).
- ***Automation and Mechanisation*** **–** Irrigation automation is increasingly used to reduce labour requirements, with options developed for all four major irrigation techniques used in Australia: syphon, bankless channel, overhead and drip (CRDC, 2020b; Roth et al., 2018; Smith et al., 2015a). Australian cotton is predominantly harvested with sophisticated spindle pickers (like the John Deere CP690) that include significant computerisation capable of tracking and mapping harvest properties in real time for post- and syn-harvest analytics.
- ***Real-Time Crop Monitoring*** **–** The use of hand-held, fixed, tractor-mounted, and unmanned aerial vehicle sensors is widespread in the Australian cotton industry (CRDC, 2020d; Volkova et al., 2018). Common measurements include soil moisture, canopy temperature, petiole testing, crop emergence, soil temperature, and photosynthetic activity. These data can be collected in real time and inform management decisions such as when to water, re-sow, add fertiliser, harvest, or spray for pests and diseases.
- ***Water Application*** **–** WUE in the Australian cotton industry has steadily increased over the past 50 years (Roth, 2012a) to the point where it is now the most water efficient cotton industry in the world (CRDC, 2010). However, the most common irrigation technique for Australian cotton growers is still furrow-flood irrigation (Nachimuthu et al., 2018), which is a subset of flood irrigation, the least water efficient large-scale watering technique available (CRDC, 2020b). Despite leading the world on WUE and decreasing water use (ML ha^{-1}) by 48% since 1992 (Cotton Australia, 2020), WUE improvements could still be made in the Australian cotton industry with further adoption of drip and overhead irrigation techniques (Smith et al., 2015a; Thorp et al., 2020).
- ***Nutrient Application*** **–** The application and management of nutrients, of which N constitutes the largest component, is the least precise aspect of cotton production in Australia. Nitrogen application typically exceeds industry recommended rates, is commonly applied many months prior to sowing, resulting in large pre-season N losses, and is often applied without taking existing soil N stores into account (CRDC, 2019a; McIntyre et al., 2001; Roth, 2012b). The Australian cotton industry has identified N use efficiency as lagging behind other aspects of its precision agricultural operations, and has in recent years invested in improving nutrient efficiency outcomes (CRDC, 2020e; Macdonald et al., 2018).

1.3 – Cotton in Australia

The Australian cotton industry is young by global standards. It was founded in the 1960s in the Namoi region of New South Wales (NSW), and rapidly expanded through significant immigration from North America (Chamala and Spies, 1999; Shaw, 2012). Throughout its growth, the industry's growers have remained well connected thanks to the presence of the Cotton Research and Development

Corporation (CRDC) – one of the Australian Government's 15 Rural Research and Development Corporations – and Cotton Australia, cotton's legislated industry body (CRDC, 2013, 2016). The industry is highly profitable, which has funded significant research and development through a mandatory CRDC grower levy (Roth, 2014a). The relative homogeneity of the Australian cotton industry has meant that research findings are widely applicable and create excellent returns on investment (CRDC, 2017, 2020b). Research commissioned by the CRDC is efficiently disseminated to growers through programs like CottonInfo (CottonInfo, 2020), and is typically well adopted by farm managers due to the high level of trust CRDC enjoys with growers (CRDC, 2017, 2018, 2019a).

From its origins in the Namoi catchment, cotton production expanded north and south along a semi-arid climate band from Emerald in Queensland (QLD) to the Riverina in NSW (Fig. 1.3). This region has a stable climate with relatively consistent soils and high incident sunlight. Cotton represents a highly profitable use of irrigation water allocations – typically the most significant constraint on agricultural production in Australia (Conaty, 2010) – and as such its expansion has displaced many other farming operations, especially rice, sheep and beef production.

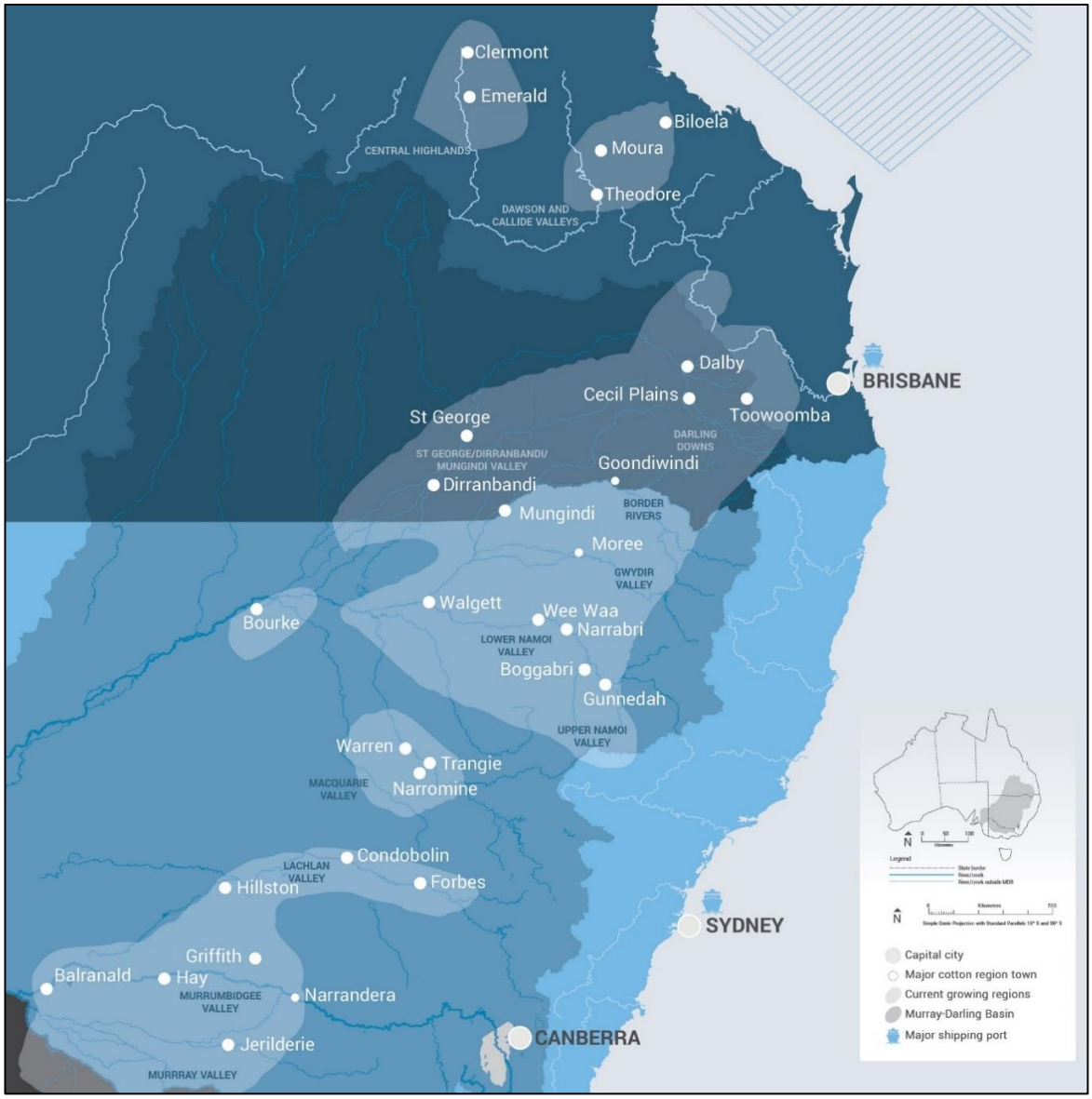


Figure 1.3. *Geographic extent of Australian cotton production. Minor cotton production also occurs in northern Queensland and the Ord River catchment in the Northern Territory. Image from Cotton Australia website.*

Australian cotton is predominantly grown on Vertosols (Hulugalle and Scott, 2008a), which are characterised by expansive clay minerals that confer high shrink-swell and self-mulching properties, as seen in the haplic medium Grey Vertosols of the Australian Cotton Research Institute (ACRI; Isbell, 2016), alternatively classified as fine, thermic, smectitic, Typic Haplusterts (Soil Survey Staff, 2010). Increasingly, cotton production is expanding into relatively lower clay content soils like Chromosols, Dermosols and Sodosols (Hulugalle and Scott, 2008a; McKenzie et al., 2003). The geographic restriction of the industry has simplified extensive optimisation of region-specific agricultural variables like pests and diseases, soil amelioration, and transport chain management, and has contributed to Australia's high and consistent yields. Early in the industry's history, cotton production expanded into the Ord River Irrigation Scheme in the Northern Territory, but was abandoned in early 1970s due to unmanageable pests and disease (Chamala and Spies, 1999). Production has recently restarted in the region, with future success more achievable due to increased knowledge, tools and management levers available to growers.

More than 99.5% of Australian cotton plantings are comprised of GM *G. hirsutum* (also known as Upland Cotton or Mexican cotton). The gene splices for these GM varieties are created by Bayer (previously Monsanto) and incorporated into Australian-optimised *G. hirsutum* cultivars by CSIRO in partnership with CRDC. GM cotton was introduced to Australia in 1993, with new varieties containing additional traits introduced periodically. Commercial Australian cotton currently uses the Bayer's Bollgard®3 gene sequence that contains *Agrobacterium spp.* genes (CP4 EPSP) for glyphosate resistance, and *Bacillus thuringiensis* (Bt) genes for the control of *Helicoverpa spp.* and other bollworms. Since the adoption of GM cotton, insecticide use has decreased by 95% in Australian cotton production (Cotton Australia, 2020; OGTR, 2018).

The majority of Australian cotton is grown in irrigation schemes that reduce the impacts of environmental fluctuations on crop yields, sometimes defined as Irrigation Induced Reduction in Crop Yield Variability (Kukal and Irmak, 2020). Flood irrigation is the most common irrigation technique in Australian cotton production, with 'syphon' and 'bankless channel' the two most common subsets (Nachimuthu et al., 2018). Some rain fed (also known as dryland) cotton is also grown, but its planting extent is heavily dependent on rainfall predictions (Fig. 1.4). Cotton is typically grown in rotation with at least one other crop, with winter wheat the most popular for at least the past 20 years (McIntyre et al., 2001). The industry recommends the inclusion of rotation crops, with legumes especially encouraged to increase soil N without adding fertiliser (CRDC, 2020c; Hulugalle and Scott, 2008b).

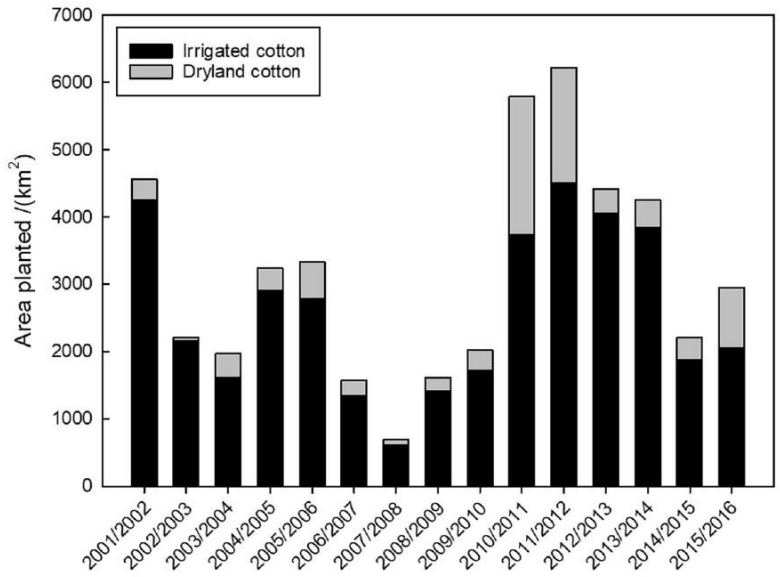


Figure 1.4. *Annual plantings of irrigated and dryland cotton by area. Dryland cotton plantings are heavily dependent on seasonal rainfall predictions, with 2010-12 representing strong consecutive La Niña years (BOM, 2012). Figure from Macdonald et al. (2018).*

Australia has a major international cotton presence in both market share and research contributions. Australia is the third largest exporter of cotton after the United States of America (USA) and India, predominantly servicing markets in China, Indonesia, Vietnam and Bangladesh (ACSA, 2019), and generating AU$1.9 billion in annual export revenue (CRDC, 2020a).

The majority of global cotton research has historically been produced in the USA, with Chinese research publication outputs increasing to comparable levels over the past decade. Australian cotton research standings have improved consistently over the past 50 years, largely due to the presence of the ACRI – formerly the National Cotton Research Centre – and consistent research and development investment through the CSIRO, the NSW Department of Primary Industries (NSW DPI), Cotton Seed Distributors (CSD), and the CRDC. The ACRI is owned by the NSW DPI and jointly operated with CSIRO, and generates the majority of Australia's cotton research output. Research at the ACRI covers a broad range of aspects of the cotton system, which in addition to the areas outlined in Section 1.2 include quality assurance (NSW DPI, 2020), grains and companion crop agronomy (Farrell et al., 2008a; Hulugalle et al., 2002), and climate change adaptation (Broughton, 2015; Broughton et al., 2020; Luo et al., 2016; Luo et al., 2015). The ACRI research has identified that NFUE is one of the Australian cotton industry's lowest metaphorical production staves, and that this needs to be addressed if its precision agricultural outcomes are to be improved (Macdonald et al., 2018).

1.4 – Nitrogen in Agricultural Systems

Agricultural plant growth is constrained by the availability of chemically reactive N more often than any other element. Prior to the 20th century, agricultural yields were largely constrained by the natural N fixation rate of soils, assisted anthropogenically by the cultivation of leguminous crops and the application of manures and other organic nitrogenous fertilisers (Steffen et al., 2005). This paradigm changed at the beginning of the 20th century with the development of the Haber-Bosch process, allowing for the manufacture and application of near-limitless reactive N (Leigh, 1995; Smil, 2000). Few innovations have so fundamentally changed our society as the introduction of synthetic fertilisers, which since their adoption have been integral to the ~360% increase in human population from 1.7 billion to 7.8 billion individuals (Steffen et al., 2005; UNFPA, 2020; United Nations, 1999, 2015).

While synthetic fertilisers have increased global agricultural production significantly, they have also altered global N budgets greatly (Johan et al., 2009; Nash et al., 2017). In 2010, approximately 120 Tg N of reactive N was generated via the Haber-Bosch process, roughly twice as much as the 63 Tg N generated from natural terrestrial sources (Fowler et al., 2013). More than half of the N applied to croplands is lost to the environment (Lassaletta et al., 2014; Shahzad and Ahmad, 2019), and while not all Haber-Bosch-derived N is used for fertiliser production, this still represents an enormous perturbation to the N cycle and potentially the greatest planetary boundary exceedance to date (Fig. 1.5; Johan et al., 2009; Nash et al., 2017; Steffen et al., 2015b). The consequences of excessive anthropogenic reactive N emissions to the environment include eutrophication of surface waters (Alexander et al., 2000; Carpenter et al., 1998; Glibert et al., 2006; Steffen et al., 2015a), nitrate-loading of groundwater (Ju et al., 2006; Skaggs et al., 1994; Tilman et al., 2001; Ward et al., 2018; Zhang et al., 1996), soil nutrient losses and acidification (Miao et al., 2010; Vitousek et al., 1997), reductions in soil microbial biodiversity (Lekberg et al., 2021; Oehl et al., 2004; Vitousek et al., 1997), and GHG emissions (Dalal et al., 2003b; Millar et al., 2010; Reay et al., 2012). Each of these environmental impacts has accompanying financial and social costs. The brunt of the environmental, financial and social costs are frequently borne by the broader community rather than the emitter, and can contribute to intergenerational inequity by eroding environmental capital (Chebai, 2017; FAO, 2011; Steffen et al., 2015b).

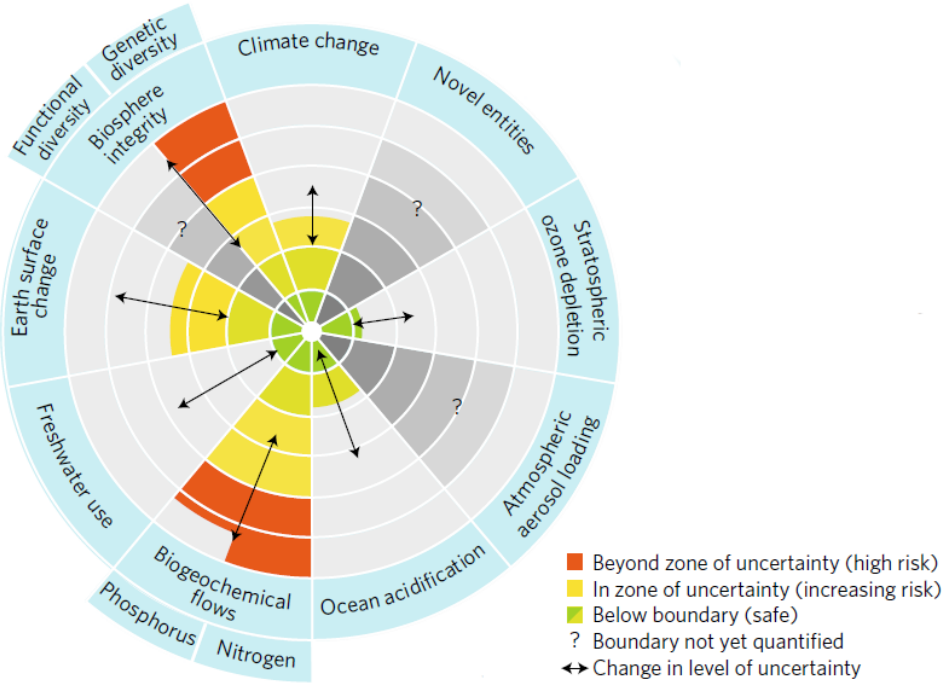


Figure 1.5. *A representation of Earth's planetary boundaries and the degree to which humanity is currently acting within them. Excessive inefficient use of Haber-Bosch-derived reactive N has caused significant perturbation of the global biogeochemical N cycle. Agriculture is the largest source of reactive N emissions – with the majority of Haber-Bosch-derived N used for fertiliser production – and as such will be required to undergo significant management change if further planetary alterations are to be avoided. Figure modified from Nash et al. (2017).*

When N is added to irrigated cropping systems, the fertiliser is supplied to an area of land (reported as kg N ha^{-1}) rather than to individual plants. While much of this N is taken up by the target crop, a large proportion will transition into other parts of the environment. This thesis defines five N sinks that functionally describe the possible destinations into which applied fertiliser N can transition (Fig. 1.6). These are:

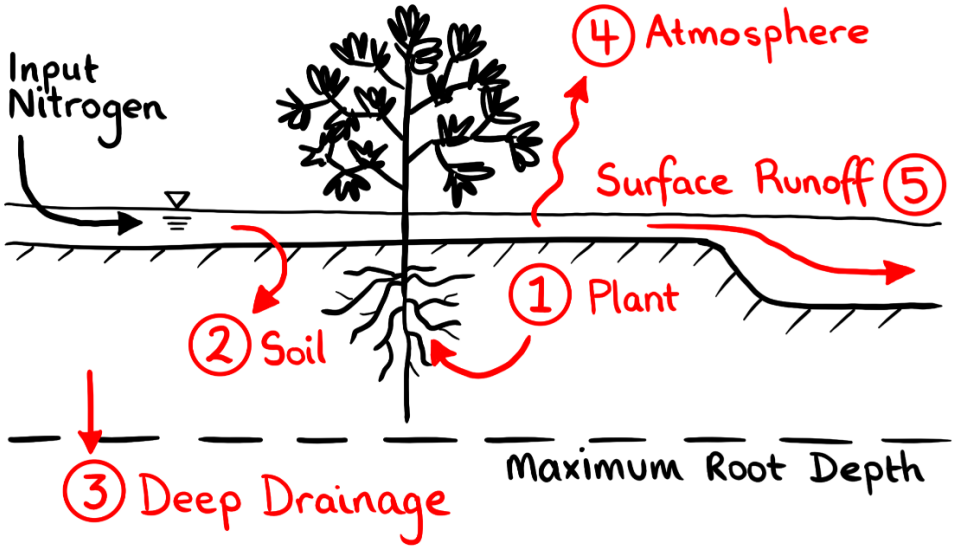


Figure 1.6. *Nitrogen entering a field (either as fertiliser or* in situ *fixation) fluxes into one of five sinks: the plant, the soil, the atmosphere, deep drainage, or surface runoff. Reactive N within a given sink is immobilised only temporarily, and can flux into another sink at any time.*

1. ***The Plant*** – This is the most desirable sink, and the reason for N addition in agricultural contexts. The majority of N taken up by the plant is removed from the system when the crop is harvested. Post-harvest, the incorporation of plant stubbles and residues can reduce the amount of N removed from farming systems if soil ratios of carbon (C), N, phosphorus (P) and sulfur (S) are appropriately balanced (Kirkby et al., 2011).
2. ***The Soil*** – Most of the plant's N is derived from the soil (CRDC, 2020b; Macdonald et al., 2017a). The majority of fertiliser N converted to soil N remains plant-available, and can be taken up later in the season. Sequestration of N into the soil can increase soil C stocks, which can reduce atmospheric GHG emissions (Bond-Lamberty et al., 2020; Janzen, 2006; Minasny et al., 2017). Heterogeneous soil N can also promote diverse and stable soil microbial communities that increase a soil's resilience to disease and drought (Lal, 2015; Polain et al., 2020b).
3. ***Deep Drainage*** – When N is transported below the root zone it can no longer be accessed by plants, and becomes functionally separate to the soil N sink. Nutrients can accumulate at depth to create long term toxicity and salinity problems, especially when they interact with groundwater systems (McLay et al., 2001; Nielsen and Lee, 1987). Of particular concern is nitrate (NO_3^-) leaching into aquifer systems, which can create significant health and eutrophication issues (De Roos et al., 2003; Wu and Sun, 2015).
4. ***The Atmosphere*** – Nitrogen fluxes to the atmosphere are considered losses from agricultural systems. The majority of atmospheric agricultural N emissions are in the form of unreactive dinitrogen (N_2), produced through denitrification (Macdonald et al., 2017a). While N_2 emissions do not cause further environmental hazards and typically represent only economic inefficiencies, reactive N losses of ammonia (NH_3), nitrous oxide (N_2O), and nitric oxides (NO_x) can lead to significant environmental issues including toxicity, pollution, and climate change (Dalal et al., 2003a; Kurvits and Marta, 1998; Tian et al., 2020).
5. ***Surface Runoff*** – Surface runoff derived from irrigation or rainfall can transport N off the field (Pérez-Gutiérrez et al., 2020). If N-rich runoff waters are captured and recirculated back on to fields then the consequences of these fluxes are low. As a rule, the Australian cotton industry captures and recirculates surface runoff water (Nachimuthu and Webb, 2016). However, many agricultural settings do not, allowing surface runoff to freely flow into watercourses and bodies where it can create significant environmental, social and economic damage (Burkart and James, 1999; Mitsch et al., 2001; Thorburn et al., 2011; Tian et al., 2007; Zhao et al., 2012).

1.5 – Nitrogen Use in the Australian Cotton Industry

Australian irrigated cotton production requires high N inputs to maintain its high yields (Macdonald et al., 2018; Rochester and Bange, 2016). The recommended fertiliser application rate (N rate) for irrigated cotton production varies depending on the target yield, which is determined by other constraints like sodicity and compaction, as per the Sprengel-Liebig Law of the Minimum (Fig. 1.2). NFUE is an appropriate metric for calculating target N rates (Eq. 1.2), and growers should be aiming to fall within the industry benchmark NFUE window of 13-18 kg lint per kg fertiliser N applied (Fig. 1.7; CRDC, 2020b). Sustainable cotton yields can be achieved within this NFUE window up to a theoretical maximum yield of 5000 kg lint ha^{-1}, requiring a N rate of 320-420 kg N ha^{-1} (Constable and Bange, 2015; Rochester and Bange, 2016). In order to achieve the industry average yield of 2360 kg lint ha^{-1} while remaining within the optimal NFUE window, 131-182 kg N ha^{-1} of added fertiliser is theoretically required.

$$NFUE_{(kg\ lint/kg\ fertiliser\ N\ applied)} = \frac{Lint\ Production\ (kg\ ha^{-1})}{Fertiliser\ N\ Application\ (kg\ N\ ha^{-1})}$$

Equation 1.2. *Nitrogen Fertiliser Use Efficiency (NFUE) calculation.*

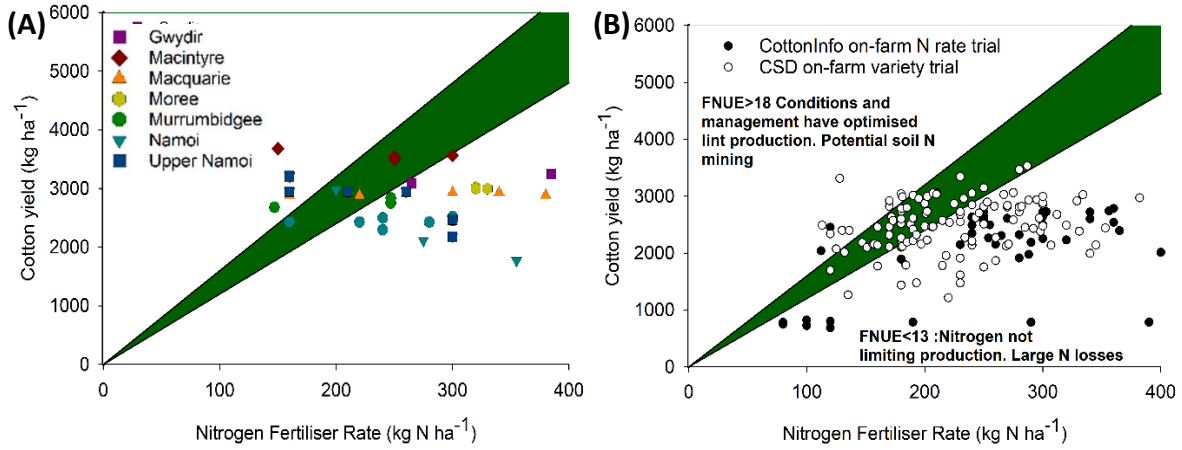


Figure 1.7. *NFUE of assorted Australian cotton production operations. Green wedge represents the optimal NFUE window for Australian irrigated cotton systems.* ***(A)*** *NFUE by cotton growing region.* ***(B)*** *NFUE of specific farming operations. Images from CottonInfo (2012) and Macdonald et al. (2018).*

Nitrogen application (kg ha^{-1}) has increased over recent decades due to a range of factors including low fertiliser costs and low grower risk appetites for under-fertilising (CRDC, 2019a; McIntyre et al., 2001; O'Keeffe, 2020; Roth, 2012b). The average 2018-19 season N rate for irrigated cotton production was 325.1 kg N ha^{-1}, split 182.8 kg N ha^{-1} pre-season and 142.3 kg N ha^{-1} in-season, achieving an average NFUE of 8.8 (CRDC, 2019a). Nitrogen rates vary by region, with the lowest and highest mean application rates for different areas 205.8 kg N ha^{-1} and 443.5 kg N ha^{-1} respectively (CRDC, 2019a). These N application rates far exceed those required for the achieved yields. Across the industry, fertiliser N is not being uniformly converted to lint at rates above 200-240 kg N ha^{-1}, which suggests that factors other than N availability are limiting yield and contributing to the industry's poor NFUE (Macdonald et al., 2018). Growers should be adding fertiliser at rates suitable for achieving realistic yield targets. Anecdotally, many growers are aiming for yields in excess of 3000 kg lint ha^{-1}, despite other production constraints limiting maximum potential yield well below that figure. This misalignment of expectations results in significant fertiliser N over-application and wastage, and poor NFUE. Growers are also encouraged to test pre-season soil N and incorporate it into seasonal N budgets before adding any fertilisers (CRDC, 2020b). However, while 70% of irrigated growers performed 0-30 cm soil tests and 62% performed tests below 30 cm, only 34% of growers said they used soil testing to vary their N application rates (CRDC, 2019a). This indicates that many growers are not applying their collected data., which may be occurring for a range of reasons. Soil testing should reduce grower uncertainty and facilitate decision making, and if quantitative tests are not achieving this outcome then qualitative tests like the 'Soil Your Undies' campaign from Oliver Knox at the University of New England should be considered.

Dryland cotton production systems produce substantially lower yields than their irrigated counterparts, but also require significantly reduced inputs. In the 2018-19 growing season, the mean dryland N rate was 92.5 kg N ha^{-1}, with 31% of the planting extent (by area) receiving no added N fertiliser (CRDC, 2019a). Optimal NFUE rates have not yet been calculated for dryland cotton production systems, but the framework of calculating nutrient use efficiency is still useful. Many dryland systems can achieve NFUE values in excess of 18 kg lint per kg fertiliser N applied (Fig. 1.8; Roth, 2014b). This suggests that not enough fertiliser N is being applied to achieve the generated yields, and that the soil is being mined of nitrogen. It should be noted that the majority of Australian cotton research has been performed at the ACRI on Vertosols in irrigated systems, and as such the

generated optimal NFUE window is not necessarily applicable for soils and climates outside that region. While this is generally acknowledged and reflected in the commonly used agronomical tool NutriLOGIC, as cotton production expands into landscapes further from Narrabri (i.e. the Northern Territory), and climate change affects rainfall and temperature patterns (Dey et al., 2020), these NFUE relationships may describe the on-farm reality less and less.

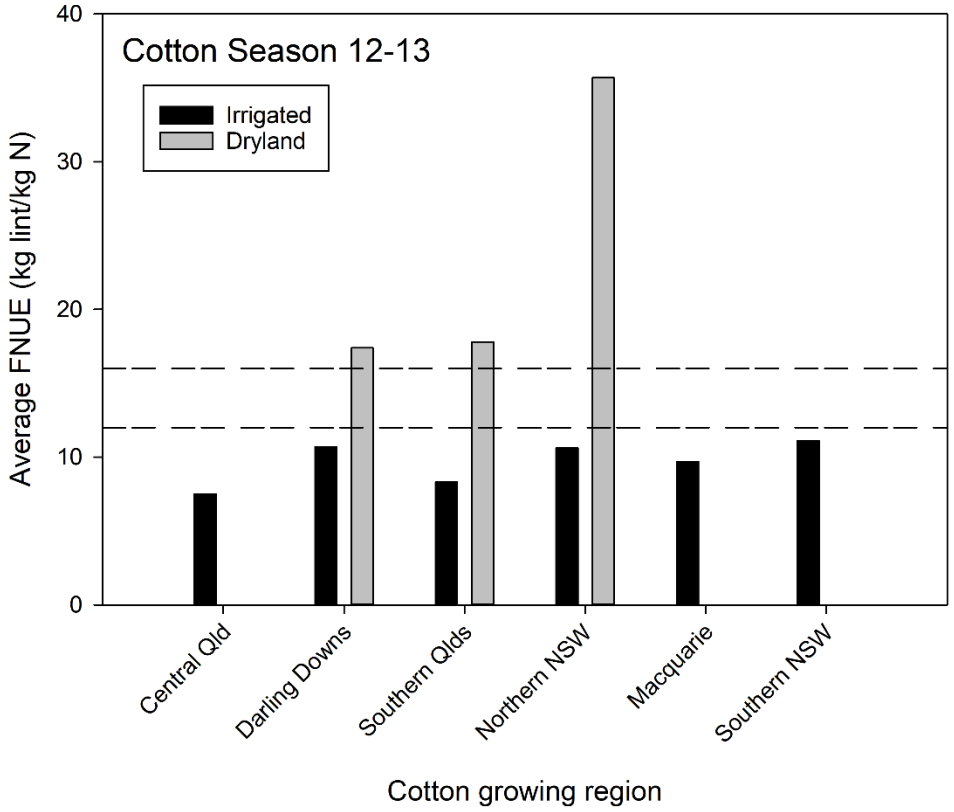


Figure 1.8. *Average NFUE for Australian irrigated and dryland cotton production systems by growing region in the 2012-13 summer season. Dashed line represents optimal NFUE window for Australian cotton production. Irrigated systems typically have low NFUE that generates losses to the environment, while many dryland operations have very high NFUE that indicates soil nutrient mining. Image from Roth (2014b).*

Growers should also carefully consider N application timing, ideally splitting fertilisation between pre-season and in-season events. Significant N losses can occur through denitrification when all of the fertiliser application occurs pre-season, especially when applied months prior to sowing. Similarly, applying fertiliser too late in the season can create significant losses, as fertiliser N is not efficiently converted to yield. In addition to wasting purchased fertiliser, excessive and poorly timed N applications can reduce yields by encouraging “rank growth” and fruit shedding, reducing lint production, hampering defoliation, encouraging insects and disease, and delaying plant maturity (Rochester, 2001).

Rapidly increasing NFUE in the Australian cotton industry will likely prove challenging given current economic incentives. In Australia, urea fertiliser is readily available and inexpensive, with the cost to growers of fertiliser over-application and wastage far exceeded by the potential costs of under-application. This can lead growers with low risk appetites to overapply N fertilisers, especially given the lack of legislative regulation. Globally, governments are increasingly introducing legislation to regulate the use and emission of C, which is changing management practice and improving C use efficiency (Eskander and Fankhauser, 2020). As efforts to improve resource use efficiency and reduce

environmental pollution continue, N regulation is likely to follow. Compounds like NH_3 and urea contain significant embedded chemical and production energy that make their wastage both economically and environmentally deleterious. Legislation governing the use of N would likely provide cotton producers with incentives and frameworks to improve NFUE without significantly affecting the individual enterprises' budgets (Welsh et al., 2015).

The industry recommends growers include other crops in rotation with cotton to improve soil conditions and symbiotic microbial diversity, diversify income streams, manage weeds, pests and disease, reduce fertiliser requirements, and increase cotton yields in subsequent seasons (ACSA, 2019; Hulugalle and Scott, 2008b; Shaw, 2005; Tanveer et al., 2019; Van Deynze et al., 2018; Wasaya et al., 2019). However, cotton production is a highly profitable use of water allocations – which are typically the main constraint for irrigated agricultural production in eastern Australia (Conaty, 2010) – and each time a summer cotton crop is replaced with a rotation crop, growers' revenue is reduced. In the 2018-19 season, 26% of cotton plantings immediately followed a previous cotton crop, suggesting that this is an issue for growers (CRDC, 2019a). Growers are also encouraged to replace fallow periods with cover crops to reduce soil erosion and nutrient volatilisation, improve soil biological parameters, and soak up excess fertiliser N (Bell et al., 2006; CRDC, 2020c; Rochester and Peoples, 2005). Unfortunately, this practice is not widely adopted, with 66% of 2018-19 plantings immediately following a period of fallow (CRDC, 2019a). Ultimately, growers are operating a business with financial motivations, and improved NFUE outcomes often do not align with financial incentives (Powell and Scott, 2012). This is an issue that the industry needs to address if it wishes to improve NFUE.

However, making N management decisions using purely financial metrics can externalise and ignore accompanying environmental and social consequences. In the context of Australian cotton, this management approach can negatively affect long term triple bottom line outcomes for both individual grower enterprises and the broader industry, because the environmental, social and economic outcomes are intrinsically linked (Fig. 1.1). While the environmental costs of N mismanagement are severe and outlined in Section 1.4, poor NFUE can also have social consequences that represent a potential risk to Australian cotton's social licence to operate. The production of cotton in Australia is already a polarising topic domestically, with irrigated cotton production a significant user of water in the over-allocated Murray Darling Basin (Grafton et al., 2020; Gupta and Hughes, 2018; Williams and Grafton, 2019). The extensive adoption of GM technology can also make cotton producers the target of harassment and negative public discourse, with tensions exacerbated by association with historical international tragedies (Elliott, 1860; Micklin, 2007). Globally, agricultural N pollution attracts significant and warranted public criticism, with environments like the Gulf of Mexico and North American Great Lakes experiencing significant degradation due to agricultural runoff (Bhagowati and Ahamad, 2019; Du et al., 2020; Howarth and Paerl, 2008; Munawar and Fitzpatrick, 2019; Schindler et al., 2016; Wilson et al., 2018). Enterprises and industries responsible for environmental pollution are increasingly being held accountable for their actions, both legally and through consumer boycotts. The Australian cotton industry has a lot to lose from negative shifts in public perception, but it also has a lot to gain if it can further demonstrate its environmental credentials. The Australian cotton industry is already a global leader in efficient agricultural production, with demonstrated advances like a 95% drop in pesticide use and 48% drop in water use (ML ha^{-1}) since the early 1990s (Cotton Australia, 2020; OGTR, 2018). Improving NFUE outcomes represents another component of the continually improving precision agriculture and environmental performance narrative that supports the cotton industry's place in the Australian landscape.

Improving NFUE is a strategic opportunity for the Australian cotton industry to raise the height of one of its shortest metaphorical staves. In doing so, the industry would improve its triple bottom line by

reducing economic inefficiencies and emissions to the environment, while also improving cotton's reputation and social licence to operate in Australia.

1.6 – Aqueous Nitrogen Application

Nitrogen can be added to crops in a range of formats and delivery mechanisms. Anhydrous ammonia (NH_3) has historically been a popular fertiliser in the Australian cotton industry due to its high N content (82% N by mass) and cheap cost. However, it is also toxic, requires specialised equipment to use, and is easily lost to the atmosphere if soil injection is not followed by thorough water application. In response to these factors, prilled (solid) urea has become increasingly popular despite its lower N content (46% N by mass), as it is non-toxic, chemically stable as a solid, and can be applied in a variety of ways (CRDC, 2020b). This popularity echoes global sentiments toward urea fertiliser (Glibert et al., 2006).

In Australian irrigated cotton production, the majority of urea fertiliser is applied as a solid, but aqueous applications (also referred to as fertigation or water-run urea) are increasingly common, especially for in-season application (CRDC, 2019a; Roth, 2012b). Anecdotally, reasons driving this increase in urea fertigation popularity include:

- ***Labour saving*** – Allowing the irrigation water to distribute N across a field saves on time spent systematically traversing that field manually applying solid or gaseous N.
- ***Reduced in-field traffic*** – Solid and gaseous N fertiliser applications are performed using heavy machinery that can compact soils, which can decrease yields.
- ***Reduced nitrogen losses*** – Applying N in multiple smaller applications throughout the season results in fewer gaseous losses than applying it all upfront, as the plant is supplied with N at a rate more comparable to its uptake needs.
- ***Changing climates*** – High December rainfall has historically discouraged growers from relying on irrigations during this period to apply N; however, as the December wet period decreases as a result of climate change, growers can plan for fertigation events earlier in the season.

The industry supports aqueous N application; however, despite the numerous perceived benefits associated with aqueous N application, there is little cotton-specific research to support these claims (Buresh and Datta, 1990). A key difficulty in managing aqueous N is its colourless nature once dissolved, meaning that growers cannot visually check where it has been applied. Instead, they must trust that the dissolved N has been transported consistently along with the irrigation water to the target destination. This is different to applying NH_3 or prilled urea, when growers can clearly track where they have and have not applied the N.

Key questions describing aqueous N application that remain unanswered include:

- Are current management practices applying aqueous N homogenously and consistently?
- What management techniques contribute to effective aqueous N application?
- Does in-field aqueous N distribution match irrigation water distribution?
- How long does aqueous N remain in the water column?
- Does significant fertigation N run off the field in tail water?
- Does fertigation improve NFUE?

These unknown factors represent a swathe of management levers that growers could use to improve NFUE for themselves and the broader industry., and will be explored in this thesis.

1.7 – Identifying Further Gaps in the Research Landscape

Significant N research is already available to the Australian cotton industry to use in determining which staves in the metaphorical production barrel are well understood and which are not (Macdonald et al., 2018). Using the N sink framework identified in Section 1.4, here follows a brief account of the available management levers and research gaps describing N that has been applied to the field.

Bulk plant uptake of applied fertiliser N, or 'fertiliser recovery', is well characterised in Australian irrigated cotton systems. Approximately 30% of applied fertiliser N is taken up by the plant during the season (Macdonald et al., 2018). This figure is consistent with a previous Australian study showing 32% recovery (Constable and Rochester, 1988), and is slightly lower than published international figures of 30-35% from China (Yang et al., 2013), and 30-38% and 42-49% from the USA (Fritschi et al., 2004; Navarro-Ainza, 2007). While these works demonstrate total N plant uptake rates, they do not identify the chemical speciation of the N taken up. This represents a research gap and potential opportunity to improve fertiliser recovery in Australian cotton systems by identifying the uptake preferences and capabilities of cotton (*G. hirsutum*) for different N species.

Cotton acquires the majority of its N from the soil, rather than directly from added fertilisers (Macdonald et al., 2017a). For growers, this information should reframe the purpose of fertiliser addition in terms of supplying the soil rather than the plant. However, as previously stated, beyond bulk N uptake quantities no information exists describing which soil N molecules cotton can and does take up. The inorganic N forms of ammonium (NH_4^+) and nitrate (NO_3^-) are well known to be plant-available, but these constitute only a small fraction of the total soil N (Prendergast-Miller et al., 2015). Organic N comprises the largest N pool in most soils (Lehmann and Kleber, 2015), with low molecular weight (LMW) dissolved organic N (DON) molecules like amino acids already shown to be plant-available in other crops (Farrell et al., 2014; Näsholm et al., 2000b). If LMW DON was shown to be nutritionally relevant to cotton production, this would further reinforce the narrative that growers should fertilise to manage their soils rather than their crops.

Deep drainage losses are difficult and expensive to accurately measure, and as such have only been well quantified in Vertosols (smectitic Typic Haplusterts). Helpfully, this is the soil type on which most Australian irrigated cotton is grown (Hulugalle and Scott, 2008a). In these soils, deep drainage occurs through a combination of matrix and bypass flows, with the former transporting more N despite the latter conveying higher volumes of water (Ringrose-Voase and Nadelko, 2013). Deep drainage N losses typically account for 3-5% of applied fertiliser N, comprised mostly of NO_3^- (59%) and DON (40%), with minor NH_4^+ (1%; Macdonald et al., 2017b). Deep drainage rates are a function of irrigation timing, and can be controlled by reducing water over-application. Currently, deep drainage does not contribute a large percentage to total N losses in Australian irrigated cotton systems, and so the costs of identifying new management levers likely outweighs the benefits.

Atmospheric N losses have been well-quantified in Australian irrigated cotton systems (Welsh et al., 2015), with grower management levers clearly identified. Approximately 30% of applied fertiliser N is lost to the atmosphere, predominantly as unreactive denitrified N_2 (Macdonald et al., 2017a). N_2O emissions – a potent GHG with a global warming potential of 298 times that of carbon dioxide (CO_2; IPCC, 2014) – occur exponentially proportional to the applied N rate, and typically constitute 1-3.5% of applied fertiliser N (CRDC, 2020b; Grace et al., 2016). Emissions are largely generated in-field, with irrigation networks contributing 2.4-4% of total farm N_2O emissions (Macdonald et al., 2016). Growers are well informed that fertiliser over-application and waterlogging are factors that increase gaseous N losses (CRDC, 2020b). Improving NFUE will likely be more effectively achieved through optimising other aspects of production than with further research in this space.

Runoff N losses are well quantified spatially at a field-scale and temporally at an inter-irrigation scale (McHugh et al., 2008; Nachimuthu et al., 2018). This is useful for identifying macro trends such as the majority of runoff losses occur at the beginning of the season (Macdonald et al., 2017a; Macdonald et al., 2017b). However, analyses at higher spatial and temporal resolutions are required to identify the mechanisms driving loss, and these experiments are missing from the research landscape. Identifying these mechanisms would provide growers with management levers they can pull to reduce runoff losses and improve NFUE. Additionally, detailing the chemical speciation of aqueous N both on and off the field has relevance for environmental N losses, soil organic matter (SOM) stability, and plant nutrition. Exploring the plant availability of dissolved urea, nitrate and DON – the most common aqueous N molecules in irrigated cotton systems – should provide further information on the potential yield effects of artificially skewing these N pools.

1.8 – Project Scope and Aims

Based on the knowledge gaps identified in Section 1.6 and 1.7, this thesis aims to answer the following research questions:

1. How effective are current Australian irrigated cotton fertigation practices at delivering predictable N to the field, and what management levers can be identified to improve outcomes?
2. How does N runoff vary spatially and temporally at sub-field and intra-irrigation scales respectively? Additionally, can this increased resolution be used to identify specific mechanistic drivers of N runoff?
3. What is the residence time of dissolved N in irrigation water, and how does it vary across the farm environment?
4. Which chemical N species can commercial cotton (*G. hirsutum*) directly take up, and what preferences does it exhibit when given a choice? Furthermore, have genetic engineering and selective breeding practices altered these characteristics from a baseline of the native *G. hirsutum* landrace accession?

1.9 – Thesis Narrative and Structure

This thesis will address the research questions outlined in Section 1.8. Building on the contextual literature grounding of Chapter 1, four experimental chapters will present and discuss new experimental data along a narrative that follows aqueous N through a generalised irrigated cotton system (Fig. 1.9). A concluding chapter will then summarise and provide management and future research recommendations.

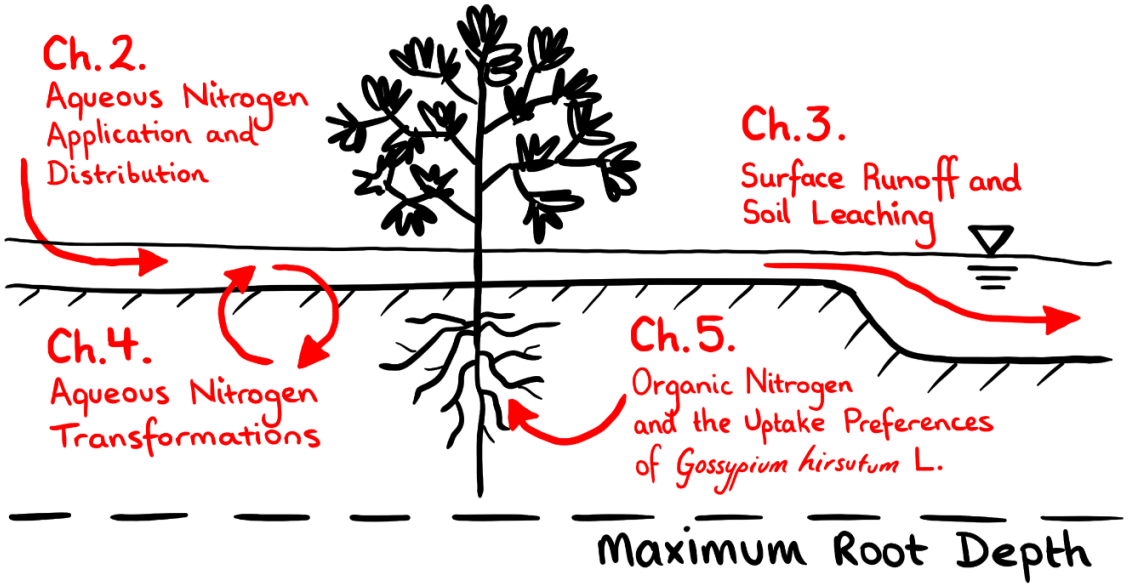


Figure 1.9. *Graphical representation of thesis structure.*

Chapter 2 – *Aqueous Nitrogen Application and Distribution* – This chapter examines current aqueous N application uniformity in flood irrigated cotton systems using data collected from two field studies. Through this data collection, the three management levers required for uniform fertigation are identified.

Chapter 3 – *Surface Runoff and Soil Leaching* – This chapter explores surface runoff N losses at higher spatial and temporal resolutions than is typical of analogous experiments. Data from five alternate furrow flood irrigation field studies are drawn upon in this chapter: one designed and conducted by James Latimer, and four previously conducted by other researchers. The text of this chapter is lifted from a first author journal article currently under review with *Soil and Tillage Research*, *"Alternate furrow flood irrigation removes significant nitrogen from the field through lateral leaching".* James conceived this manuscript, performed all of the analyses, and wrote more than 95% of the text.

Chapter 4 – *Aqueous Nitrogen Transformations* – This chapter reports on the transformation rates of aqueous N in agricultural settings using several laboratory incubation studies. The results of these experiments can be used to predict urea mineralisation rates and residence times in agricultural soil and water systems at different temperatures and locations in the farm system. These data did not previously exist, and will provide farmers in many agricultural disciplines with management levers for minimising environmental N losses and maximising NFUE.

Chapter 5 – *Organic Nitrogen and the Uptake Preferences of G. hirsutum* – This chapter explores the N uptake capabilities and preferences of *G. hirsutum*, globally the most common commercial cotton species. Uptake time series of ^{15}N- and ^{13}C-labelled NO_3^-, NH_4^+, urea and alanine over a three hour interval in cotton seedlings show that *G. hirsutum* can rapidly access a range of N species. To provide comment on the effects of selective breeding and genetic engineering practices, three varieties of *G. hirsutum* were examined: a current GM commercial cultivar, an obsolete non-GM commercial cultivar, and a landrace accession representing the uncultivated origins of the species. Special focus is placed on the role of DON in crop nutrition, and how this relates to plant-available N paradigms.

Chapter 6 – *Conclusions* – This chapter discusses the implications of this thesis for improving precision agriculture and environmental performance outcomes for the Australian cotton industry. Management recommendations are made for the industry to consider that aim to improve NFUE above current practices. Finally, future research recommendations are presented that provide the industry with investment options going forward.

This project included four field experiments, conducted in the Riverina and Namoi regions of NSW. It also included three lab-based and one glasshouse-based experiment, all performed at CSIRO Black Mountain in Canberra, Australian Capital Territory (ACT; Table 1.1).

Table 1.1. *List of experiments conducted during this PhD project.*

Experiment Name	Type	Location	Date	Associated Chapter
Uniformity of fertigation N application distribution: Bankless channel irrigation	Field	Private Farm #1, Riverina, NSW	01/2017	Chapter 2
Uniformity of fertigation N application distribution: Syphon irrigation	Field	Private Farm #2, Riverina, NSW	01/2017	Chapter 2
Soil N leaching by advancing irrigation front in syphon irrigation	Field	Private Farm #2, Riverina, NSW	01/2017	Chapter 2
Surface runoff N losses under alternate furrow irrigation	Field	ACRI, Narrabri, NSW	10/2017-01/2018	Chapter 3
Urea mineralisation incubation: Part 1	Laboratory	CSIRO Black Mountain, Canberra, ACT	06/2016	Chapter 4
Urea mineralisation incubation: Part 2	Laboratory	CSIRO Black Mountain, Canberra, ACT	09/2016	Chapter 4
Urea mineralisation incubation: Part 3	Laboratory	CSIRO Black Mountain, Canberra, ACT	11/2019	Chapter 4
Gossypium hirsutum L. ^{15}N-^{13}C-isotope uptake glasshouse study	Glasshouse	CSIRO Black Mountain, Canberra, ACT	02/2019	Chapter 5

During the project, these experiments generated four published data sets and one academic paper (Table 1.2). James Latimer also contributed to another academic paper reviewing the current status of NFUE research in the Australian cotton industry (Macdonald et al., 2018), and produced several articles for the industry magazine *Spotlight*.

Table 1.2. *List of products produced during this PhD project additional to this thesis.*

Product Type	Product Name	Publication	Reference	Associated Chapter
Journal Article (1st author)	Alternate furrow flood irrigation removes significant nitrogen from the field through lateral leaching	Soil Tillage and Research	Latimer et al. (2020) [Under Review]	Chapter 3
Journal Article (2nd author)	The current status of nitrogen fertiliser use efficiency and future research directions for the Australian cotton industry	Cotton Research	Macdonald et al. (2018)	Chapter 1
Data Set	High resolution nitrogen runoff chemical data from alternate furrow flood irrigated cotton field	CSIRO Data Access Portal (DAP)	Latimer (2020b)	Chapter 3
Data Set	Chemical data: leaching of soil nitrogen by progressing irrigation front	CSIRO DAP	Latimer (2020a)	Chapter 3
Data Set	Chemical isotope data: nitrogen uptake preferences and capabilities of 15N 13C labelled nitrate, ammonium, urea and alanine by three varieties of *Gossypium hirsutum* L.	CSIRO DAP	Latimer and Farrell (2020)	Chapter 5
Data Set	Chemical data: urea mineralisation rates at three temperatures in a closed soil and water system	CSIRO DAP	Latimer and Macdonald (2020)	Chapter 4

Magazine Article	Is your urea tank supplying the nitrogen you think it is?	Spotlight	Spotlight Summer 2017-18	Chapter 2
Magazine Article	How quickly does urea mineralise when water-running urea?	Spotlight	Spotlight Winter 2018	Chapter 4
Magazine Article	When it comes to choosing nitrogen, cotton goes organic.	Spotlight	Spotlight Spring 2020	Chapter 5
Magazine Article	Urea mineralisation in water and soil.	Spotlight	Spotlight Summer 2020-21	Chapter 4
Grower Information Article	Pitfalls in water-running urea.	Nitrogen Information Tour Booklet	CottonInfo (2016)	Chapter 2

Chapter 2 – Aqueous Nitrogen Application and Distribution

2.1 – Introduction

Irrigated cotton comprises the largest share of Australian cotton production by both yield and area planted (Fig. 1.4). Surface (or flood) irrigation is the most common irrigation technique used in Australian irrigated cotton systems, with only minor adoption of overhead and drip infrastructure. Syphon-fed (syphon) is the most common subset of surface irrigation, employing the titular plastic syphons to passively transport water from raised irrigation channels running perpendicular to the field into sloped furrows of gradients ~0.04° (1:1500; Fig 2.1). Syphon configurations are widely adopted because they have relatively low capital cost requirements, are robust and long-lived, and are gravity-fed; however, syphon systems are also labour intensive, which has led to increased adoption of bankless channel (bankless) configurations. Bankless systems are the second most popular irrigation technique used in Australian cotton production (CRDC, 2020b), supplying water to consecutive terraced beds in series (Fig. 2.2). Bankless irrigation systems are gravity-fed similarly to syphon configurations, but require significantly less operational labour at the cost of increased initial capital expenditure. Field slopes within bankless bays are commonly <0.006° (1:10000; CRDC, 2020b).

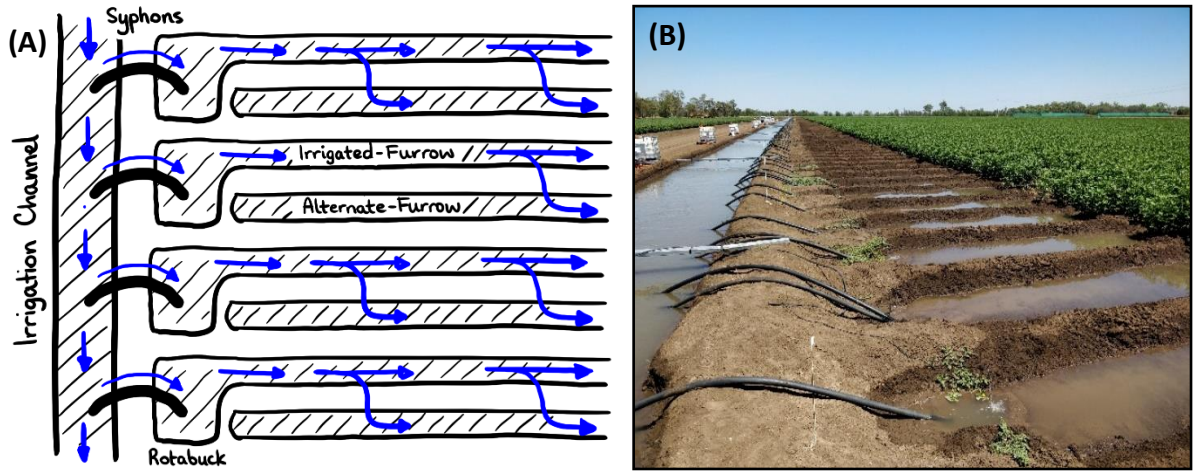


Figure 2.1. *Syphon-fed surface irrigation is the most common irrigation technique used in Australian irrigated cotton production.* ***(A)*** *Aerial schematic of syphons supplying multiple furrows in parallel from raised perpendicular irrigation supply channel.* ***(B)*** *Photo of syphons supplying multiple furrows in parallel from raised perpendicular irrigation supply channel at the ACRI.*

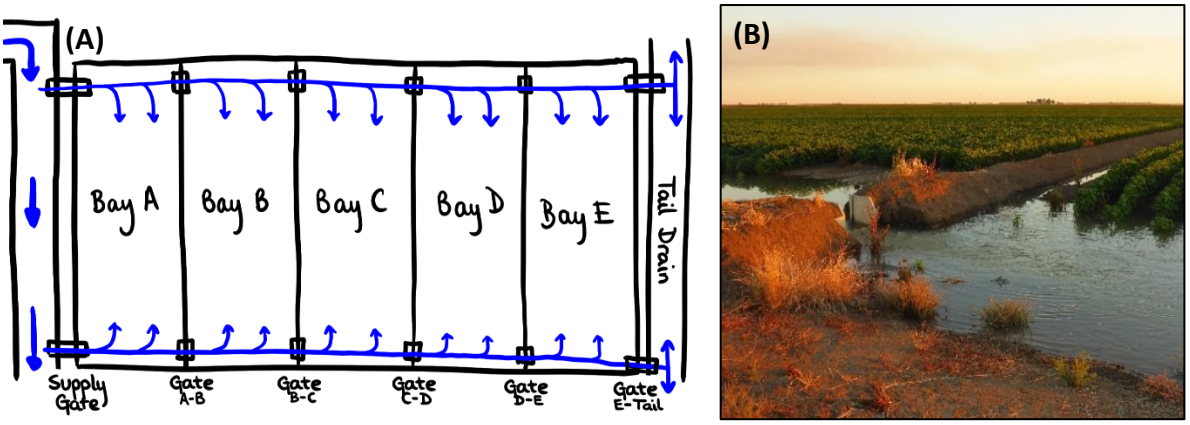


Figure 2.2. *Bankless channel surface irrigation is the second most common irrigation technique used in Australian irrigated cotton production.* ***(A)*** *Aerial schematic of a bankless system supplying terraced bays in series from an irrigation supply channel. Blue arrows denote water flow directions.* ***(B)*** *Photo of water transfer between terraced bays in bankless configuration on a private farm.*

The industry recommends splitting N applications between pre-season and in-season events to reduce environmental losses and maximise crop yields (CRDC, 2020b). There are many ways to add N to a crop: as different compounds – like NH_3, urea (CON_2H_4), ammonium nitrate (NH_4NO_3), or organic compounds like manures – and via different application mechanisms – like broadcasting solid pellets, cutting compounds into the hill, gaseous injection, or dissolved in the irrigation water. Aqueous fertiliser application is an effective way of adding in-season N that reduces labour requirements and minimises in-field traffic compared to other techniques. Fertiliser application uniformity is crucial for achieving consistent high-yielding crops, regardless of how the N is applied. When applying N as a solid or gas, growers can be assured of uniformity by monitoring vehicle movements as they systematically traverse the fields. However, with water-run N the dissolved fertiliser is invisible, so growers can only assume where it has been applied. It is assumed that the dissolved N is transported uniformly to wherever the irrigation water travels, but there is little data to support this.

Fertigation systems typically operate by steadily mixing small volumes of concentrated N solution into large volumes of passing irrigation water throughout the irrigation event. In Australian irrigated cotton systems, aqueous N is most frequently supplied as high concentration urea solution (usually 15-26% N by mass), slowly released into the irrigation supply channel as the irrigation water flows past. While commercial pre-mixed solutions exist (SLTEC, 2016), most Australian irrigated cotton growers mix their own concentrates. For surface irrigation configurations, this commonly involves dissolving several tonnes of prilled urea in a large water tank that slowly discharges the high concentration N solution into the main supply channel (Fig. 2.3). Both syphon and bankless irrigation configurations use networks of irrigation channels to move water around the farm (Macdonald et al., 2016). Due to on-farm logistics, the concentrated N source (urea tank) typically comprises a single application point along the irrigation network that may be anywhere from 1 m to >3 km from the point of field application. In some instances there exists a single pathway for the urea-laden water to travel to the field, while in other instances there is a branching irrigation network with competing flows complicating the mixing profile. When growers employ these fertigation techniques, they are assuming a consistent amount of urea is added to each ML of passing water, and that this is resulting in an effective fertiliser application; however, in practice the colourless nature of dissolved urea makes this easy to mismanage. Once the N-laden water of known concentration is delivered to each section of the field, the crop should be receiving a predictable amount of N. This assertion is predicated on the assumption that the dissolved N is transported throughout the field consistently along with the irrigation water, and is not hindered by environmental processes such as adsorption to soil particles higher up the field. While this is likely true, there is limited available data to support this assumption.

Figure 2.3. *Common aqueous urea delivery setup using a large water tank to dispense high concentration urea—N solution into the main irrigation supply channel. Image from SLTEC (2016).*

This chapter draws on data from three field experiments to examine NFUE in irrigated cotton systems from the context of current application and delivery practices. The primary goal of these experiments was to identify the management levers that growers must address to achieve uniform aqueous N

applications. The assumption that effective N application is occurring using current fertigation practices can be challenged from three aspects: source consistency, irrigation network mixing, and in-field distribution homogeneity (Fig. 2.4). Growers need to manage all three aspects if they wish to achieve consistently uniform aqueous N application. For testing, the first hypothesis was that self-managed on-site urea tank mixing would result in variable N delivery over time. The second hypothesis was that N concentration delivered to different field sections would vary due to inconsistent irrigation network mixing. The final hypothesis was that aqueous N would distribute homogenously in-field from the points of application. The results and discussion section will address each of these hypotheses in turn.

Figure 2.4. *The three aspects of fertigation management required for uniform aqueous N application. All three aspects must be controlled to achieve effective fertigation. While each aspect contains its own management levers, there are common themes throughout.*

2.2 – Materials and Methods

The three field experiments drawn on in this chapter were performed across two private farms in the Riverina region of NSW during January 2017 (summer). Both sites were located in a semi-arid climate, with mean annual rainfall and January daytime temperatures of approximately 410 mm and 33°C respectively (BOM, 2020). The first experiment was performed on a bankless channel configured field situated on Red-Brown Chromosols (Isbell, 2019). This site was used to assess application supply consistency, inter-bay distribution uniformity, and intra-bay distribution uniformity over a 40 hour irrigation (Fig. 2.5). The second and third experiments were performed on a syphon configured farm situated on a Grey Vertosol. This site was used to assess the mixing consistency of the irrigation channel network, the down-furrow water chemistry variations, and the variations in N runoff totals and speciation (Fig. 2.6).

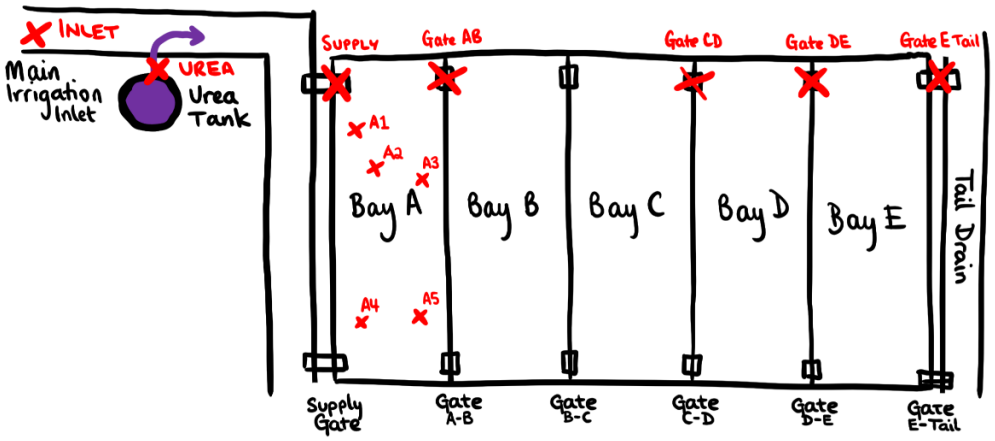


Figure 2.5. *Aerial schematic of bankless channel irrigated cotton field where first field experiment sampling was conducted. Red crosses denote sampling locations. Urea tank sampling occurred at location 'UREA'. Intra-bay distribution assessment sampled at points A1, A2, A3, A4 and A5. Inter-bay distribution assessment utilised all other sampling locations. Irrigation water flowed from Inlet, past the urea tank (Urea), into Bay A through Supply, and then flooded each subsequent bay in series when the intervening gates were manually opened.*

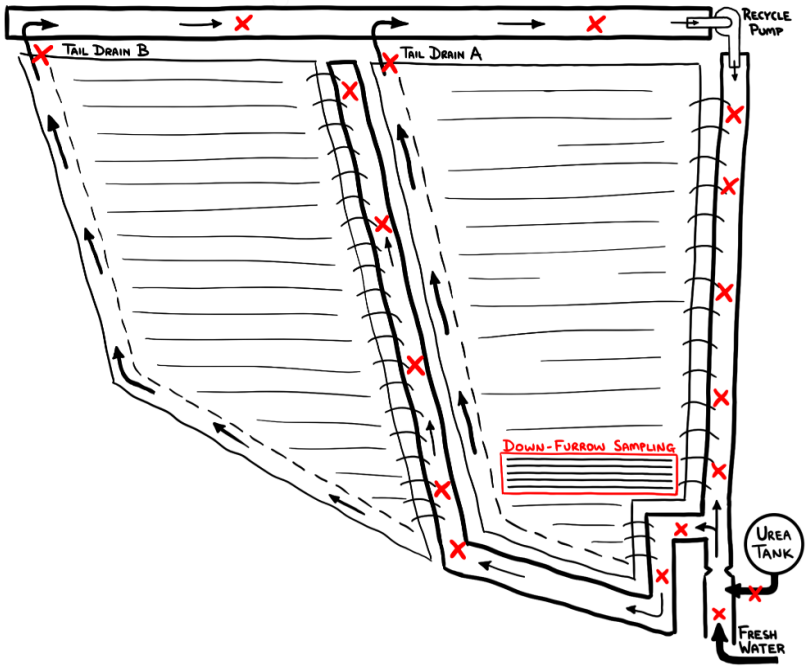


Figure 2.6. *Aerial schematic of syphon configuration irrigated cotton farm where the second and third field experiment sampling was conducted. Red crosses denote irrigation network sampling locations. Red box denotes down-furrow sampling experiment.*

Water samples were collected from the irrigation supply channels manually or via autosamplers comparable to the ISCO 6712C Compact Portable Sampler (Teledyne, Lincoln NE, USA). When sampling the propagating irrigation front within furrows, water samples were collected manually within 5 m of the undisturbed irrigation front. Intra-bay water sampling in the first experiment was performed manually. All collected water samples were greater than 50 ml in volume. Collected samples were filtered through 0.45 µm nylon filters and fixed with phenylmercuric acetate (PMA; 1–5 mg L^{-1}) and/or frozen on site to inhibit microbial activity and prevent N transformation until laboratory analysis. Differences in preservation method were due to variable access to electricity. Electrical conductivity (EC; µS cm^{-1}), pH and temperature measurements were taken *in situ* using a TPS Aqua-CP/A multi-probe (TPS, Springwood QLD, AU). Collected water samples were analysed in triplicate at the CSIRO Black Mountain laboratories using an Alpkem Segmented Flow Analyser (Perstorp Analytical Co., Wilsonville, OR 97070 USA). The concentrations of NO_2^- and NO_3^- were determined according the United States Environmental Protection Agency (USEPA) method 353.2 (USEPA, 1993), NH_4^+ was determined according to USEPA method 350.1 (USEPA, 1983), urea was determined through the USEPA P-dimethylaminobenzaldehyde (PDAB) method (USEPA, 1979), and total N was determined according to USEPA method 353.2 (USEPA, 1993).

The discharge characteristics of a urea tank was measured over the course of a 35 hour irrigation in the first experiment. In this experiment, a 20 kL rainwater tank was filled with approximately 4.6 tonnes of prilled urea (2130 kg urea–N), with the remaining volume made up with irrigation water. The tank was mixed using a diesel pump to circulate the contents from the outlet at the bottom of the tank back into the opening at the top of the tank. Once the tank was mixed – for an amount of time determined by the fuel tank on the pump's generator – the tank was passively discharged into the main irrigation supply channel, driven solely by the tank's head pressure. The urea concertation (g urea-N L^{-1}) and flow rate (L min^{-1}) were recorded irregularly at the tank outlet during the 32 hours over which it was discharging (Fig. 2.7). Urea load (kg urea—N h^{-1}) was calculated from concentration and flow.

2.3 – Results and Discussion

2.3.1 – Source Consistency

The observed urea tank discharge varied significantly over time, and was unlikely to achieve a uniform aqueous N application. The tank was not properly mixed prior to discharge, with considerable undissolved urea still present at the bottom of the tank. This resulted in suspended solid urea being discharged along with the dissolved urea—N during the first five hours of the irrigation, and a decreasingly concentrated solution being discharged after that (Fig. 2.7A). The tank's gravity-fed outflow rate was also inconsistent over time (Fig. 2.7B). As the water level in the tank dropped, so too did the driving head pressure, decreasing the outflow rate from the tank. At two points towards the end of the irrigation, the flow rate spiked as a result of the outflow valve being manually opened wider. The tank's variable urea—N concentration and outflow rate were both skewed in the same direction, compounding their effects and leading to the acceptance of the first hypothesis (Fig. 2.7C). Due to the colourless nature of dissolved urea and the opaque sides of the rainwater tank, the grower was unable to see that the N supply consistency was poor. This left the grower with little chance of accomplishing a uniform aqueous N application, regardless of how successfully they achieved other aspects of fertigation management.

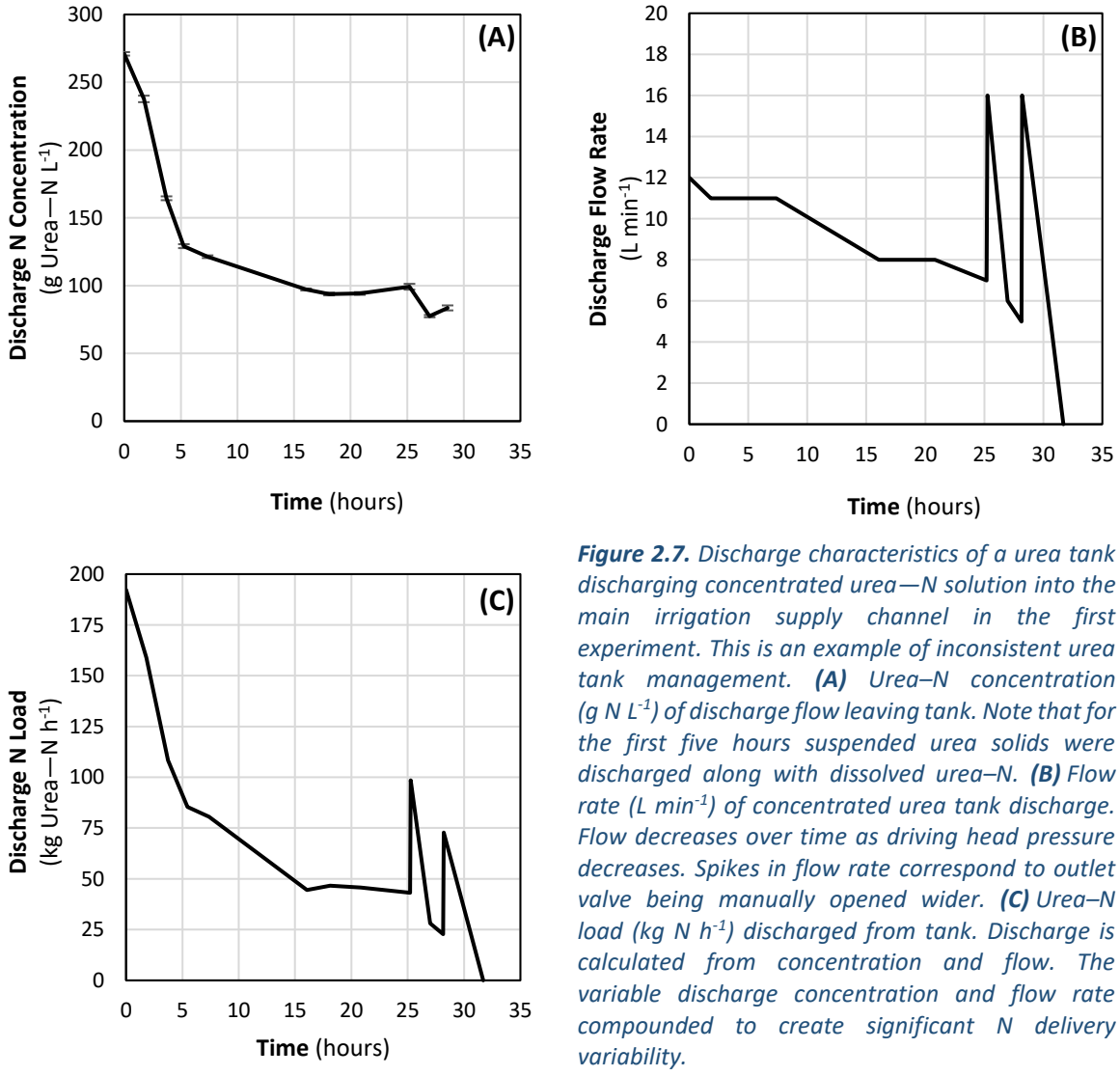
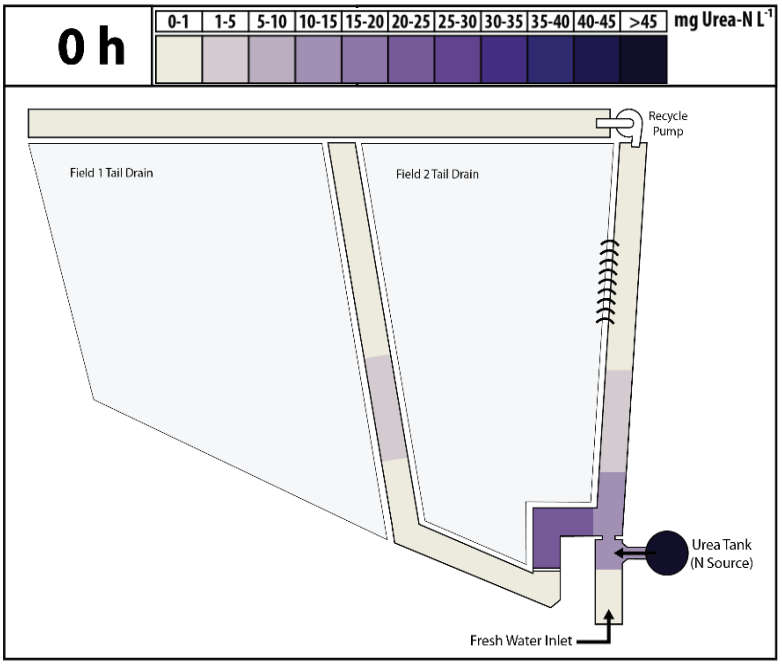


Figure 2.7. *Discharge characteristics of a urea tank discharging concentrated urea—N solution into the main irrigation supply channel in the first experiment. This is an example of inconsistent urea tank management.* ***(A)*** *Urea–N concentration (g N L^{-1}) of discharge flow leaving tank. Note that for the first five hours suspended urea solids were discharged along with dissolved urea–N.* ***(B)*** *Flow rate (L min^{-1}) of concentrated urea tank discharge. Flow decreases over time as driving head pressure decreases. Spikes in flow rate correspond to outlet valve being manually opened wider.* ***(C)*** *Urea–N load (kg N h^{-1}) discharged from tank. Discharge is calculated from concentration and flow. The variable discharge concentration and flow rate compounded to create significant N delivery variability.*



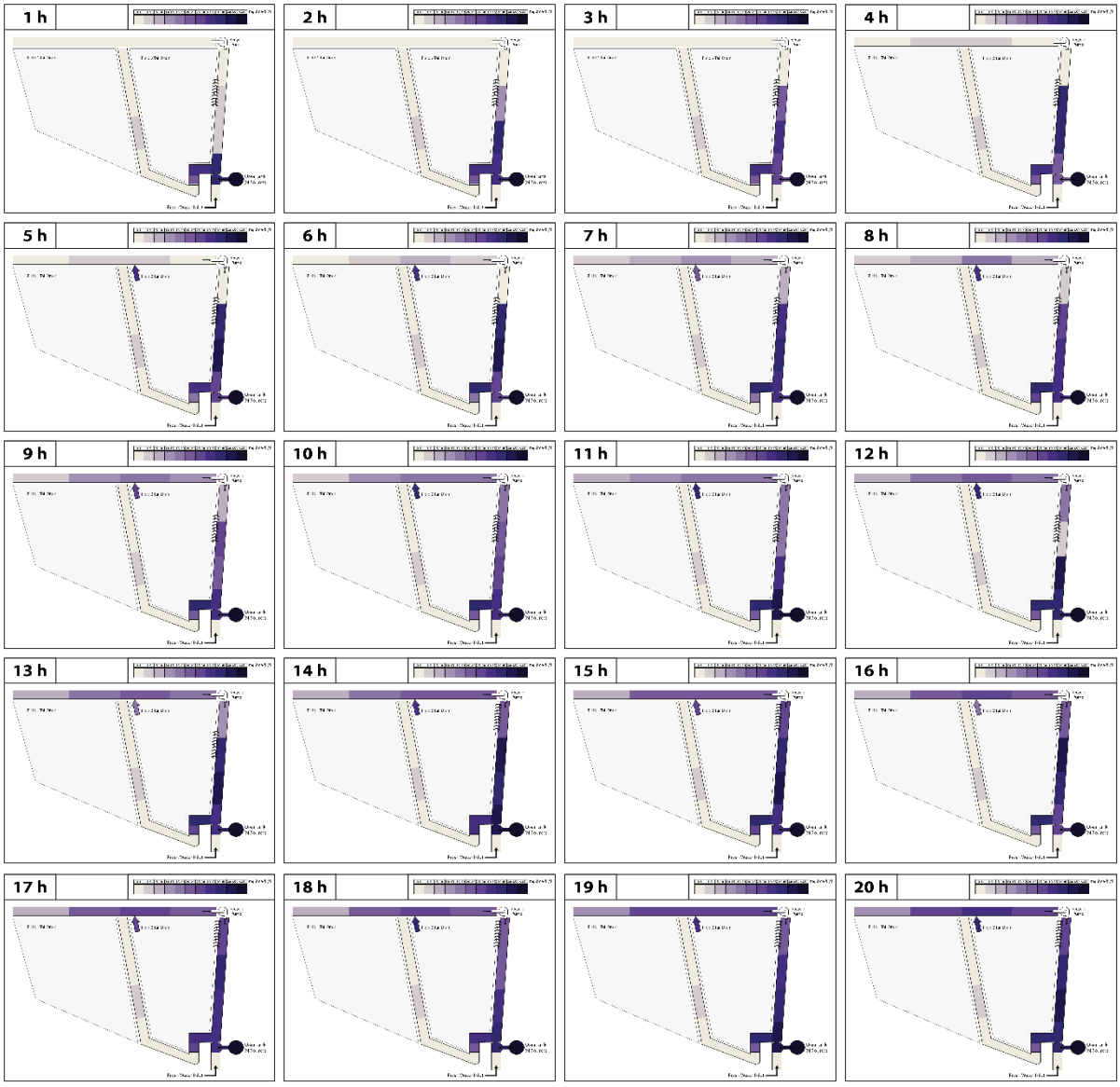


Figure 2.8. *Aqueous urea—N concentration in the irrigation supply channel network on a private farm over the first 20 hours of an irrigation event in the second experiment. Purple saturation denotes urea—N concentration. Information is here presented as still frames at one-hour intervals. Sampling locations shown in Figure 2.6. Figure is excerpt of full data set, showing only urea–N concentration over the first 20 hours. Full dataset includes TDN, urea–N, NO_3^-–N and NH_4^+–N over a 40-hour time series.*

2.3.2 – Irrigation Network Mixing

The second aspect of uniform aqueous N application is achieving consistent irrigation network mixing. The concentrations of total dissolved N (TDN), urea–N, NO_3^-—N and NH_4^+–N were measured hourly in an irrigation supply network over the course of a 40-hour irrigation event. Competing flows between a recirculation pump, the main supply inlet, and branching irrigation channels all contributed to heterogeneous mixing within the irrigation network (Fig. 2.8). The first 3-4 hours of this irrigation saw little aqueous N supplied to the initial block of syphons (servicing approximately 120 furrows, each 1200 m long). Consistent applications occurred from the 10-14 hour mark, once the entire network contained a similar concentration of aqueous N and the urea tank discharge rate had equilibrated with the incoming supply water flow. Because the delivery of aqueous N varied between group of syphons, the second hypothesis was accepted; however, cumulative deposition of N in the field was not measured, raising the question of to what extent this variable delivery matters to crop nutrition. While this should be explored in future experiments, it is plausible that there will be an effect on plant N supply as syphon systems are sensitive to variations in irrigation supply channel N concentration, with large swathes of field easily over- or under-supplied with fertiliser (Fig. 2.9).

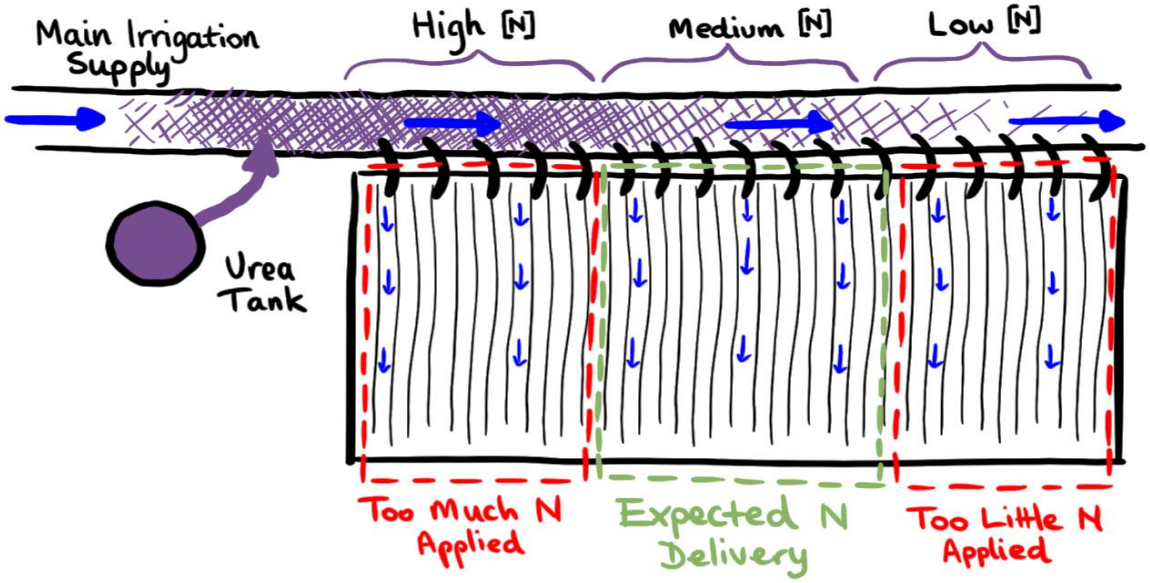


Figure 2.9. *Generalised aerial schematic illustrating how inconsistent irrigation supply channel mixing in syphon irrigation configurations can create significant heterogeneity in aqueous N application. Blue arrows denote water flow direction and purple density represents urea concentration.*

2.3.3 – In-Field Distribution Homogeneity

The third aspect of uniform aqueous N application is achieving in-field distribution homogeneity. For Australian cotton, which is dominated by flood irrigation, homogenous in-field N distribution translates to intra-bay and inter-bay distribution in bankless configurations, and down-furrow distribution in syphon configurations. Intra-bay sampling indicated that supplied fertigation N distributes well within flooded bays (Fig. 2.10). Of the five intra-field sampling locations, four maintained similar N concentrations throughout the flooding phase of the irrigation (zero to nine hours), with all five converging once the bay began draining. Heterogeneous N supply from a poorly managed urea tank (Fig. 2.7) increased the complexity of the N distribution, obscuring experimental results. Despite this, the third hypothesis should be accepted in the case of intra-bay aqueous N distribution.

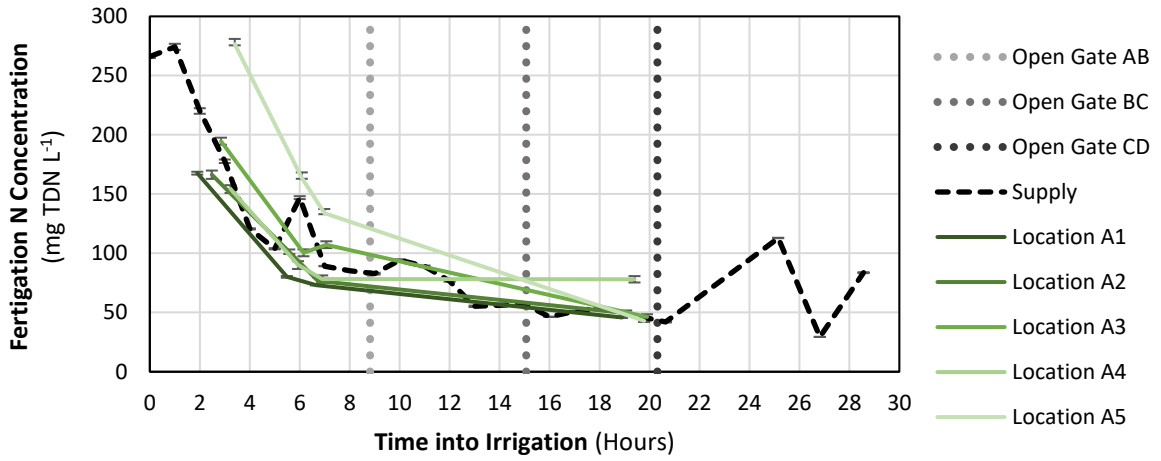


Figure 2.10 *Irrigation water TDN concentration within the first bay of a bankless channel irrigated cotton field during the first experiment. Concentration measured from samples collected manually at five locations within bay over four time intervals. Error bars represent standard error of samples analysed in triplicate. First three sampling times occurred while bay flooded, with final point occurring during drainage. The incoming supply N concentration is also shown for reference. Variable N supply was the result of poor urea tank mixing. Sample locations are detailed in Figure 2.5.*

Inter-bay N distribution similarly conforms with supplied N concentration (Fig. 2.11). This experiment was prompted by questions from growers concerned that different bays may be receiving different amounts of N due to the morphology of the irrigation configuration. Competing theories suggested that leached soil—N may be transported from higher bays down-field, while others suggested that dissolved N might adsorb to soils and not fully progress to the lower bays. The experimental results presented here do not support either of these concerns, instead suggesting that each bay receives N consistent with the source concentration, and that the third hypothesis should also be accepted for inter-bay aqueous N distribution. However, consistent with the intra-bay experiment, the heterogeneous supply of N in this experiment complicated the interpretation of these results, making it difficult to draw definitive conclusions.

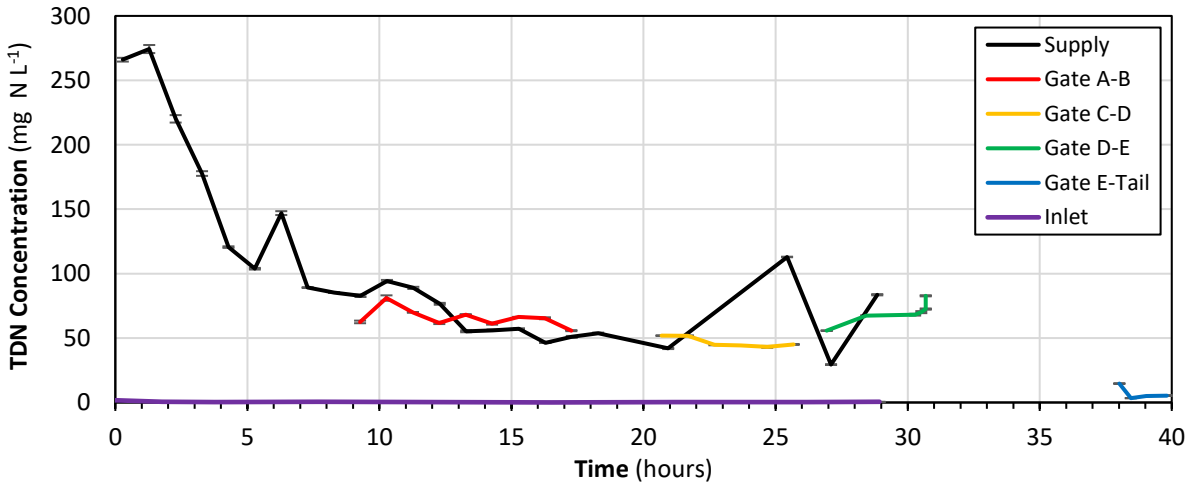


Figure 2.11. *TDN concentration (mg N L^{-1}) in irrigation supply water of bankless irrigation configuration over 40 hour fertigation event during the first experiment. Value reported as sample means ± standard error. Sampling locations are shown in area schematic Figure 2.5. Inter-bay gates were sampled for several hours from when first opened.*

Down-furrow N sampling returned no correlations between distance down furrow (m) and absolute N concentrations (mg N L^{-1}) of TDN, urea, NO_3^- or NH_4^+. Similarly, no correlations were observed between distance down furrow and percentage increases in N concentration on initial value (t = 0), or percentage increases on previous up-field sample (Fig. 2.12). These results suggest that negligible longitudinal N trends exist in these systems, and that the third hypothesis should be accepted for down-furrow aqueous N distribution. A minor relationship between distance down furrow and pH was observed; however, the change in pH was small and possibly the statistically anomalous result of analysing two clustered sets of values. The only firm relationship observed was between distance down furrow and temperature ($p < 0.01$), which was merely highlighting the relationship between ambient temperature and time of day (Fig. 2.13). An inconsistent N source concentration made interpreting results difficult and findings inconclusive, similar to the bankless experiments (Fig. 2.14). This is a common problem associated with experiments performed on commercial farms, and demonstrates why facilities like the ACRI are extremely valuable to the industry.

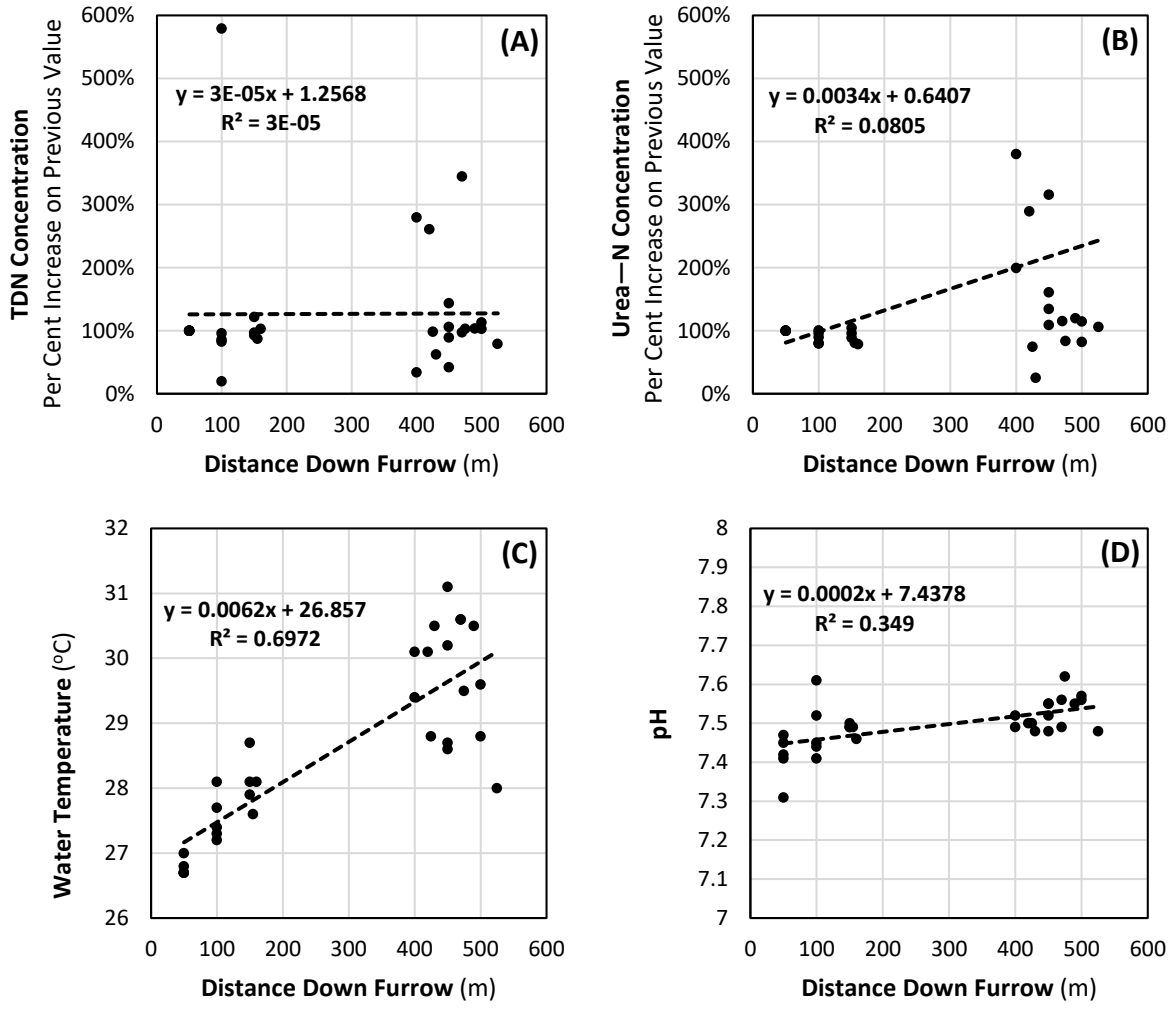


Figure 2.12. *Correlations between distance down furrow (m) and assorted characteristics of the irrigation front in five separate irrigated-furrows (n = 5) in the third experiment. All samples collected within 2 m of the advancing irrigation front.* ***(A)*** *Percentage increase in TDN concentration of sample on previous up-furrow sample vs. distance down furrow. No down-furrow TDN trends ($p < 0.05$) were observed.* ***(B)*** *Per cent increase in urea—N concentration of sample on previous up-furrow sample vs. distance down furrow. No statistically significant down-furrow urea—N trends were observed ($p > 0.05$).* ***(C)*** *Temperature vs. distance down furrow. Temperature correlates with distance because it also correlates with time of day, with ambient temperature increasing throughout sampling period (Fig. 2.13).* ***(D)*** *pH vs. distance down furrow. Negligible change in irrigation front pH was observed over the sample interval. Correlation likely attributable to the two clustered sets of values.*

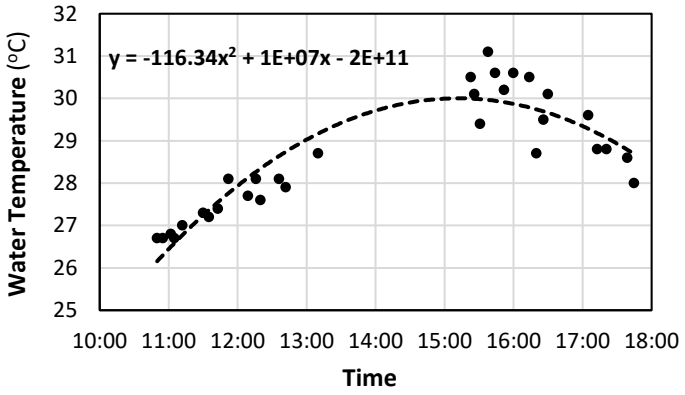


Figure 2.13. *Water temperature of advancing irrigation front in five irrigated-furrows (n = 5) plotted against time of day in the third experiment. High correlation reflects relationship between ambient air temperature, water temperature, and time of day, rather than a soil interaction from travelling down a furrow (Fig. 2.12C).*

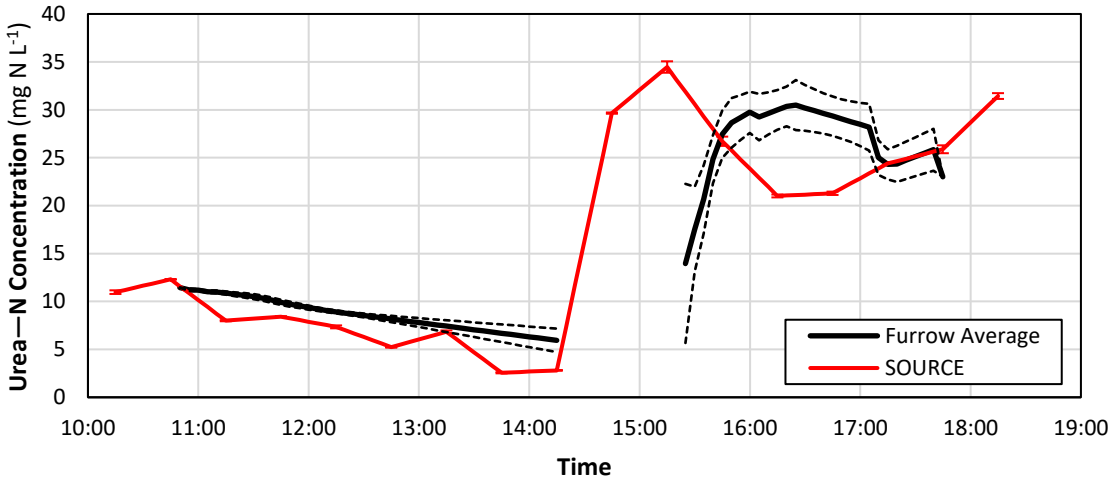


Figure 2.14. *Average irrigation front urea—N concentration (mg N L^{-1}) in the third experiment. Solid line represents average of all measured furrows (n = 5) with dashed lines representing plus or minus standard error. Source was measured at irrigation canal <10 m from syphons feeding the five measured furrows. Error bars represent standard error for triplicate analysis.*

The results of these three experiments suggest that aqueous N distributes well in-field along with the irrigation water, and that the delivered N is largely consistent with the source concentration. Thus, the third hypothesis should be accepted.

2.3.4 – Management Levers: Achieving Uniform Aqueous Nitrogen Applications

In order to achieve uniform aqueous N application, growers need to manage source consistency and irrigation network mixing (Fig. 2.4), with experimental results suggesting that in-field distribution homogeneity is not a concern. The field experiments reported in this chapter have identified several management levers that growers can use to improve their fertigation and NFUE outcomes.

2.3.4.1 – Achieving Source Consistency

Achieving a predictable aqueous N application is impossible if the source consistency is unknown. To operate a urea tank effectively, growers need to ensure that the tank is adequately mixed, and that the tank outflow rate matches that of the main irrigation supply. Tank outflow can be easily regulated using a header tank. This regulator does not need to be expensive to be effective, and can be constructed from a barrel and a toilet float switch (Fig. 2.15). Ensuring that the urea tank is well mixed can be less straightforward to achieve, and will vary depending on water temperature, mix time, and degree of agitation. If growers are unwilling to purchase pre-mixed commercial aqueous urea products, they should attempt to improve their urea tank management processes iteratively. This process could involve consistently recording parameters like mass of added urea, water volume, water

temperature, mixing technique, and mixing time, and then collecting water samples for TDN analysis and comparison against procedures.

Figure 2.15. *Photo of grower-made header tank for regulating the discharge rate from their urea tank. Header tank constructed from half a 44 gallon drum and a valve regulated with an old toilet cistern float switch.*

2.3.4.2 – Achieving Consistent Network Mixing and Nitrogen Delivery

In relatively simple irrigation networks like the example shown in Figures 2.6 and 2.8, achieving homogenous mixing can be challenging but is feasible; however, in large complex irrigation networks like at the ACRI (Fig. 2.16), achieving consistent channel mixing becomes extremely difficult. The best way to minimise the effects of poor channel mixing is to add the concentrated N solution as close to the field as possible, advice consistent with industry recommendations (CRDC, 2020b). In syphon systems, applying N immediately prior to the syphons will lead to the most uniform and predictable results. This can be achieved through mobile delivery modules or by using multiple N sources (Fig. 2.17). Unfortunately, these methods are likely to prove more expensive to operate than the commonly adopted single urea source configurations, and as such their attractiveness will likely vary between growers. If growers are operating a small supply network, they could increase consistency by performing some degree of channel pre-mixing before irrigation begins. For the example shown in Figure 2.8, the grower could have run their recirculation pump for an hour prior to irrigating, which would have provided the first block of syphons with more uniform N supply. As a backup, varying application practices throughout the season can be a way of minimising the effects of uneven fertigation. For example, by varying the order in which sections of crop are irrigated, the consequences of heterogeneous applications if systemic mixing issues are occurring will be less likely to compound over the whole season.

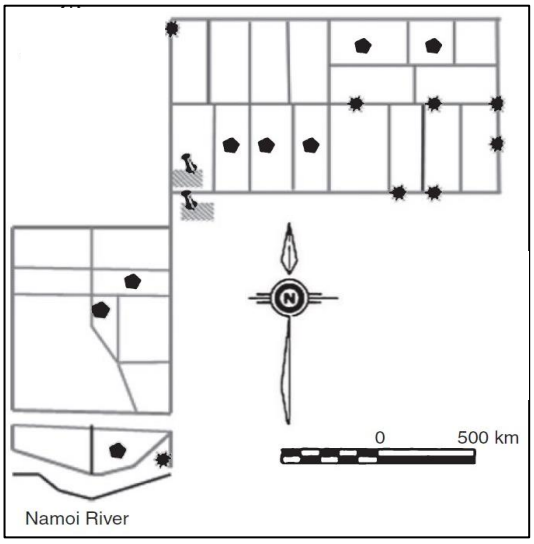
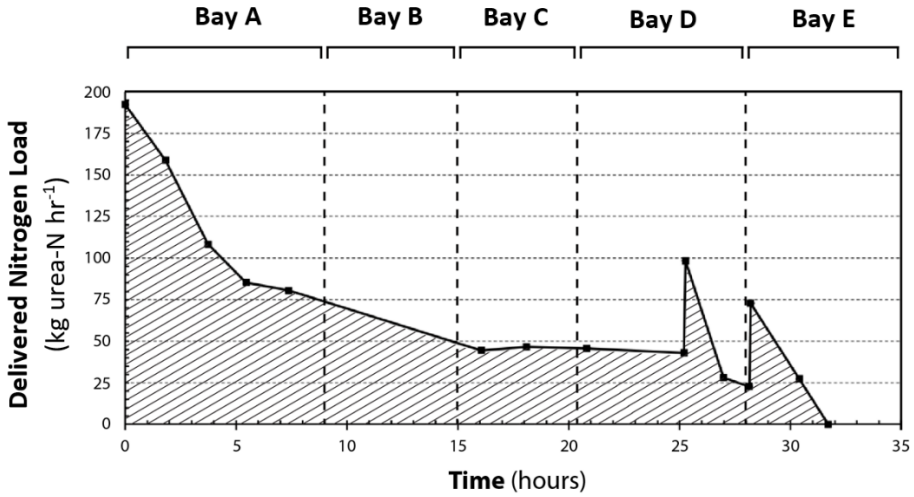


Figure 2.16. *Map of the ACRI irrigation network. The interconnectivity of the supply channels creates competing flow directions and makes achieving consistent aqueous N concentrations in the network ostensibly unachievable. Aqueous N is instead supplied from mobile units immediately adjacent to the application site. Image modified from Macdonald et al. (2016).*

Figure 2.17 *Example of a syphon irrigation system using multiple concentrated N sources to supply targeted fertigation N immediately prior to the field. The close proximity of the fertigation N will help achieve a uniform application. In this example, anhydrous ammonia is the nitrogenous fertiliser used, which necessitates the use of personal protective equipment. Photo taken at the ACRI.*

2.3.4.3 – Calculating Nitrogen Budgets

Once aqueous N is being supplied predictably to fields, growers should record the predicted concentration and length of time that N is being applied to each section of crop, and use this information to refine their seasonal N budgets. Water is the most valuable input in Australian irrigated cotton production, so growers carefully monitor water volumes and where they are applied. When irrigation water contains dissolved N, varying the length of irrigations also varies the amount of supplied N. Supplying consistent N can be difficult when sections of field require different irrigation lengths, as is the case with bankless configurations where bays of uniform size can require different amounts of time to fully flood due to variations in elevation and pre-irrigation soil moisture. Revisiting the urea tank example shown in Figure 2.7, the application uniformity problems already discussed were further exacerbated by variable supply times to each bay (Fig. 2.18). Due to the compounding of high N concentration and longer irrigation timing, Bay A received 49% of the N supplied to all five bays, or approximately 250% of the target N (an equal share of the total applied N). Bays C and E received only 9% and 6% respectively, experiencing shorter irrigation lengths and lower N concentrations.



Delivered N Loads		
Bay	**kg N**	**%**
A	1020	49%
B	381	18%
C	241	9%
D	358	18%
E	130	6%
ALL	**2130**	**100%**

Figure 2.18. *Nitrogen load (kg N h^{-1}) delivered to each of five bays in series in a bankless channel irrigation configuration in the first experiment. Intervals between vertical dashed lines indicate time over which each bay was flooded. Inconsistent N delivery compounded with variable bay irrigation times to deliver an extremely heterogeneous fertigation event. Bay A received approximately 50% of the total N, while Bays C and E received 9% and 6% respectively.*

Both over- and under-application of N can reduce yields and create suboptimal NFUE outcomes (Rochester, 2001). Accurately monitoring and improving aqueous N use will require extra resources that growers may be unable or unwilling to commit. While professional services exist to manage this process, many growers see these services as unjustifiably costly, and instead just add a few extra tonnes of urea to the tank, hoping it will cover any deficits. Given the current low price of urea and lack of legislative incentive to do otherwise, this has proved a valid strategy for many Australian cotton growers. However, the industry context will inevitably change when fertiliser prices eventually increase and legislation governing N use is introduced. When this happens, improving NFUE will be a critical component of growers' financial (and hopefully, triple) bottom line considerations. More immediately, poor N application uniformity can diminish crop yields (Rochester, 2001) and hurt soil health (Singh, 2018), which are very real, current incentives to improve practices. Growers should work to improve the accuracy of their seasonal N budgets now while fertilisers are relatively inexpensive and readily accessible, and while they are not subject to any national or global nutrient regulations. When these conditions inevitably change, operators with robust and efficient N management strategies in place will be more likely to thrive.

2.3.5 – Nitrogen Runoff from Fertigation

Runoff N totals and speciation were also measured during the two syphon irrigation field experiments (Fig. 2.19; 2.20). Tailwater was collected at hourly intervals for the same field in the third and fourth irrigations of the season. Both irrigations were fertigation events, with runoff dominated by urea—N. Significant DON was also present in the first syphon experiment (which took place on the third irrigation of the season), especially in the first half of the irrigation. Little DON was observed in the tailwater of the second syphon experiment, which occurred on the fourth irrigation of the season. The high and declining DON concentration in runoff water suggests that soil—N is being leached by the irrigation water and transported into the tail drain. Addressing this loss of soil nutrition may represent a significant potential improvement to NFUE for the Australian cotton industry, and is the subject of Chapter 3.

Furrow irrigation, the most common irrigation technique employed by Australian irrigated cotton growers, typically generates significant runoff. When fertigating, this runoff will contain dissolved urea—N in the order of 20-40 mg N L^{-1}. Australian irrigated cotton farms typically capture and recirculate runoff on to other fields, which greatly improves WUE. However, it remains unclear how this practice affects NFUE when the water contains significant dissolved N. This answer to this question has significant implications for the Australian cotton industry, and is explored in Chapter 4.

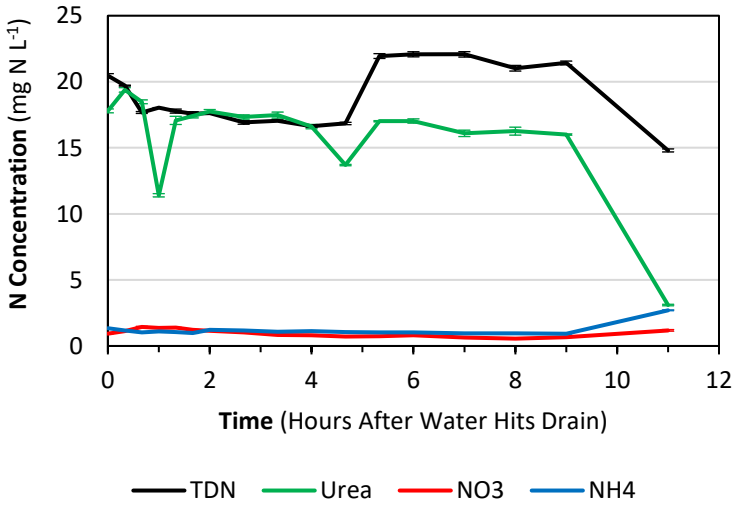


Figure 2.19. *Nitrogen concentration in runoff tailwater from the fourth irrigation of a syphon configuration irrigated cotton field in the third experiment. Irrigation was a fertigation event and supply water contained dissolved urea. TDN, urea—N, NO_3^-—N and NH_4^+—N concentration (mg N L^{-1}). Value reported ± standard error. Input fertigation N (dissolved urea) contributed the majority of runoff N. Field is same site as reported in Fig 2.20.*

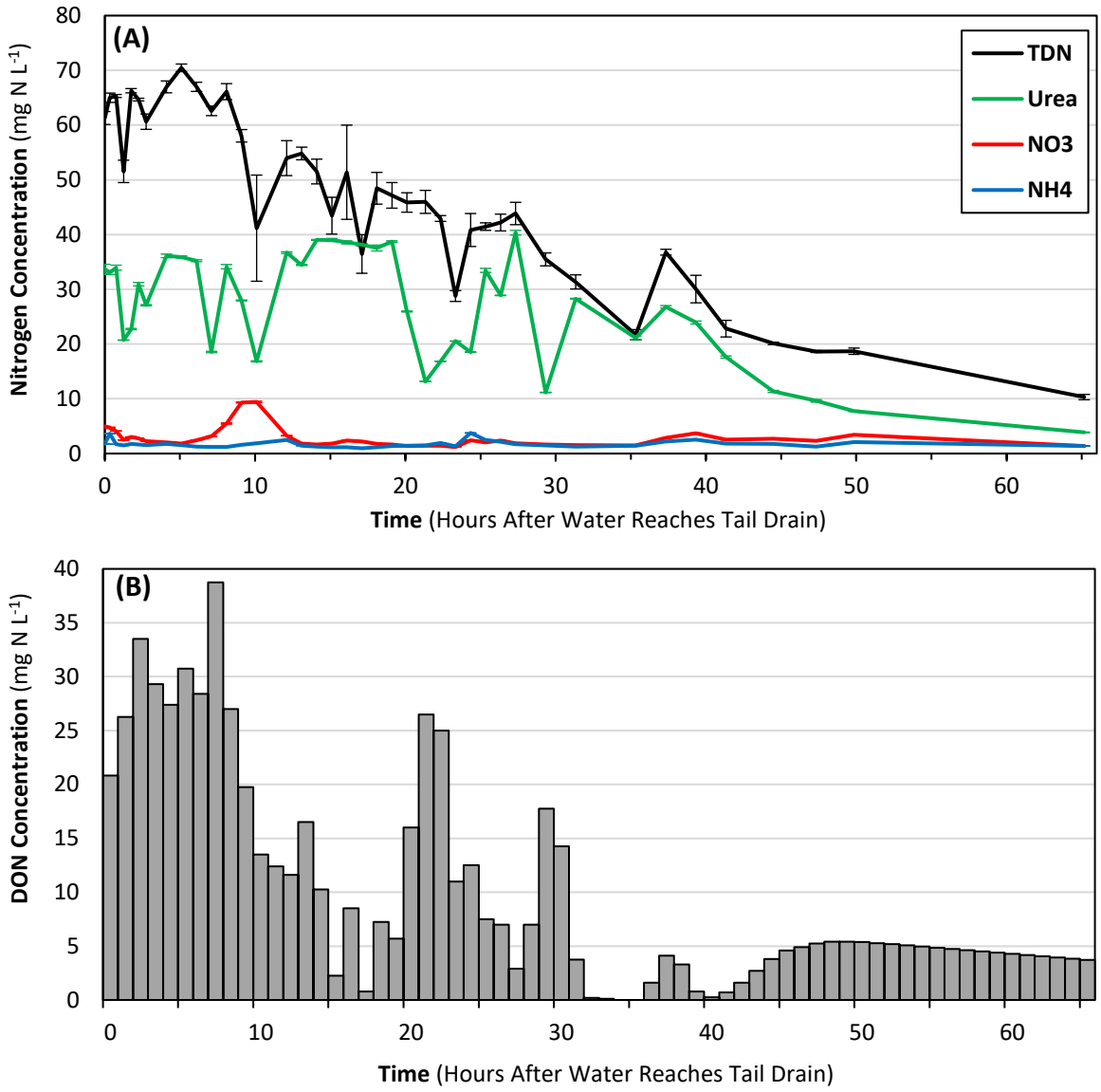


Figure 2.20. *Nitrogen concentration (mg N L^{-1}) in runoff tailwater from the third irrigation of a syphon configuration irrigated cotton field in the second experiment. Irrigation was a fertigation event and supply water contained dissolved urea. Field is same site as reported in Fig 2.19.* ***(A)*** *TDN, urea—N, NO_3^-—N and NH_4^+—N concentration (mg N L^{-1}). Value reported ± standard error. Input fertigation N (dissolved urea) contributed the majority of runoff N.* ***(B)*** *Calculated DON concentration in runoff tailwater. DON values calculated by subtracting measured urea—N, NO_3^-—N and NH_4^+—N concentrations from measured TDN.*

2.4 – Conclusions

Aqueous N application (fertigation or water-run N) is easy to mismanage due to the colourless nature of dissolved N. In order to achieve uniform aqueous N application, growers need to manage source consistency, irrigation network mixing, and in-field distribution homogeneity (Fig. 2.4). Achieving source consistency with a self-managed urea tank requires adequate mixing and a predictable outflow rate (Fig. 2.7). This was not observed in the single irrigation event during which this was measured. The first hypothesis was thus accepted, however this experiment should be viewed as an example of what can happen, rather than the rule. Growers should consider including header tanks in their configurations, which can be cost effective ways to regulate urea tank outflow. Where syphons draw

water from multiple points along an irrigation channel, poor irrigation network mixing can create significant heterogeneity in N applications for (Fig. 2.9). Achieving adequate mixing in complex irrigation networks can be difficult, and concentrated N should always be added as close as practicable to the site of application to maximise NFUE. Consistent mixing and N delivery were not observed, and the second hypothesis was accepted. Experimental results suggest that aqueous N distributes homogenously in-field along with the irrigation water, provided that the supplied N concentration is consistent (Fig. 2.10; 2.11), with the third hypothesis being accepted. Growers should monitor the N supplied to different fields and create budgets in the same manner as they would for water application. Useful metrics to record include flow rate (L min^{-1}), exposure time (hours), and concentration (mg N L^{-1}), with the ultimate goal of calculating load (kg N). Anecdotally, many growers consider water-run urea as a way to provide extra 'insurance' N, and are not too concerned with application accuracy. While this is a viable strategy in the current economic climate, it leads to poor NFUE and reduced yield outcomes. Growers should aim to develop improved N management practices now, partly so as to be prepared for inevitable paradigm shifts. While several management recommendations have be suggested from the findings presented in this chapter, it should be noted that their efficacies have not been tested here, and that these experiments do not capture cumulative effects.

The next chapter explores surface runoff N losses at within-field spatial scales and within-irrigation temporal scales. This represents a higher research resolution than most current studies, and allows for more targeted identification of specific loss pathways and potential management options. Data from five alternate furrow flood irrigation field studies are drawn upon in this chapter: one designed and conducted by James Latimer, and four previously unpublished experiments conducted by other researchers.

Chapter 3 – Surface Runoff and Soil Leaching

The text of this chapter is adapted from the following academic paper:

Alternate Furrow Flood Irrigation Removes Significant Nitrogen from the Field through Lateral Leaching

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3.0 – Abstract

Alternate furrow irrigation is a type of furrow-flood irrigation, and one of the most common water delivery method used in the production of Australian irrigated cotton. In order to maintain high lint yields, these systems require significant N inputs that, if not carefully matched to crop demand, can cause significant environmental impact. Added fertiliser N not assimilated by the plant can exit the field via several routes, including in irrigation runoff. This paper uses data from five field experiments comprising 12 different N rate treatments across three irrigated cotton production regions in NSW, Australia. The primary data set is an intra-irrigation time-course study of N runoff flux, while the other data sets demonstrate inter-irrigation trends. Lateral leaching of the hill by irrigation water passing from the irrigated- to the non-irrigated-furrow (alternate-furrow) drives the generation of N surface runoff, with the majority of runoff occurring at the start of the irrigation season and from the alternate-furrow. During the initial three irrigations of the season, alternate- and irrigated-furrows discharged water with average TDN concentrations of 83.3 ± 14.0 mg TDN L^{-1} and 27.4 ± 6.2 mg TDN L^{-1} respectively, equating to average TDN discharge loads of 7.4 ± 0.9 kg TDN ha^{-1} and 5.6 ± 1.2 kg TDN ha^{-1} per irrigation. From the fourth irrigation onwards, the average TDN discharge concentrations of alternate- and irrigated-furrows converged to 9.8 ± 1.6 mg TDN L^{-1} and 7.3 ± 0.9 mg TDN L^{-1} respectively, yielding average TDN runoff loads of 0.6 ± 0.1 kg TDN ha^{-1} and 0.8 ± 0.1 kg TDN ha^{-1} per irrigation event. Fertigation contributed significantly to total N runoff. Dissolved N in supply water accounted for the majority (63.4 ± 9.5%) of seasonal TDN runoff (kg TDN ha^{-1}) in treatments that included a deliberate fertigation event, disproportionately increasing the N discharged from irrigated-furrows. Of the discharge N sourced from the field, alternate-furrows were responsible for 87.5 ± 4.4%, with irrigated-furrows discharging the remaining 12.5 ± 4.4%. Field-derived TDN runoff during the first irrigation of the season was equivalent to 6.1 ± 0.4% of applied pre-season fertiliser, with 12.1 ± 0.7% discharged over the whole season. For a pre-season fertiliser application of 270 kg N ha^{-1}, this equated to 32.6 ± 1.8 kg N ha^{-1} lost from the field as surface runoff during the season. TDN runoff was dominated by NO_3^- and DON, which respectively comprised 58.3 ± 7.5% and 31.9 ± 7.6% of the TDN discharged during the first irrigation of the season, with the remaining proportion composed of NH_4^+ and urea (CON_2H_4). Efforts to reduce surface runoff N losses during irrigation should focus on the lateral leaching mechanism, with consideration given to the elimination of alternate-furrows via the adoption of extra-wide beds.

3.1 – Introduction

The production of high-yielding irrigated cotton in Australia requires N at rates of >200 kg N ha^{-1} (Rochester, 2011), sourced from SOM mineralisation and fertiliser application. The majority of applied N is in the form of LMW synthetic fertilisers, mainly urea or NH_3, at rates between 100 and 400 kg N ha^{-1} (CRDC, 2019a). Much of this applied fertiliser N is not translated into commercial yield (cotton lint and seed), with significant N losses from the soil-plant system in surface runoff, deep drainage, or atmospheric emissions (Macdonald et al., 2017a). Off-farm N movement from agriculture can cause environmental impacts such as eutrophication in surface waters (Alexander et al., 2000; Carpenter et al., 1998; Steffen et al., 2015a), nitrate-loading of groundwater (Skaggs et al., 1994; Tilman et al., 2001; Ward et al., 2018; Zhang et al., 1996), soil nutrient loss and acidification (Miao et al., 2010; Vitousek et al., 1997), reductions in soil microbial biodiversity (Oehl et al., 2004; Vitousek et al., 1997), and greenhouse gas emissions (Dalal et al., 2003b; Millar et al., 2010; Reay et al., 2012). However, Australian irrigated cotton farms are typically designed with closed irrigation networks, where tail water is returned to on-farm dams or diverted to other fields within the farm (Nachimuthu and Webb, 2016). These closed irrigation networks minimise off-farm nutrient transport via runoff, significantly reducing the environmental damage potential of irrigated cotton systems while also improving their WUE.

Consistent development of plant genomics (Liu et al., 2013), genetic modification (Trapero et al., 2016), precision landscape engineering, aerial telemetry (Volkova et al., 2018), improved agronomy and field management, and automation has seen the Australian cotton industry's lint yield (kg lint ha^{-1}) and WUE (kg lint ML^{-1}) steadily increase over the past 50 years (Constable, 2004; Liu et al., 2013; NSW DPI, 2019; Roth et al., 2013). However, NFUE has not experienced the same consistent improvement over this period, which has prompted the industry to review current practices (Macdonald et al., 2018).

Furrow irrigation is the most common water delivery system used in Australian irrigated cotton production and is used by 82% of growers (CRDC, 2018). Most growers use an alternate furrow irrigation system as it is more water efficient than irrigating every furrow (Golzardi et al., 2017; Kang et al., 2000). In alternate furrow irrigation systems, water is directed down every second furrow (the irrigated-furrow), where it percolates laterally through the hill out into the non-irrigated-furrows (alternate-furrows), eventually exiting the field from all furrows. Due to their respective flow pathways, irrigated-furrows typically discharge significantly more than alternate-furrows; however, this split can vary significantly, with alternate-furrows capable of discharging more than irrigated-furrows where large cracks and break-ins occur through the hill.

This study examines surface runoff N losses in alternate furrow irrigated cotton systems using data from five experiments at three locations in NSW, Australia. Nitrogen loss studies typically aggregate runoff spatially at a field-scale and temporally at an irrigation-event or day-scale (McHugh et al., 2008; Tian et al., 2007; Zhao et al., 2012). While this is helpful for gauging total N losses and seasonal trends, it is insufficient for identifying specific loss mechanisms needed to improve management practice and NFUE outcomes. We hypothesised that the alternate-furrows would contribute more to surface runoff N losses than the irrigated-furrows. Our reasoning was that irrigation water laterally leaching through the hill should leach nutrients from the soil more efficiently than water travelling longitudinally down a single furrow. Our second hypothesis was that N losses would be most severe in the first irrigation and much less so in subsequent irrigations. Here our reasoning was that there would be a greater amount of mobile N initially available at the start of the growing season, and that in combination with the higher N uptake by the plant later in the season, progressive irrigations would remove this mobile N.

3.2 – Materials and Methods

3.2.1 – Data Sources

This paper draws on data from five unpublished field experiments comprising 12 N rate treatments (Table 3.1). Three irrigated cotton production regions in northwest NSW, Australia are represented in these data: the ACRI in Narrabri and private farms in Moree and Gunnedah.

Table 3.1. *List of experiments used. Five experiments were conducted at three locations in northwest NSW, with 12 different N rate treatments. N26 is a commercial aqueous urea solution containing 26% N made by Yara Australia Pty. Ltd. (6 Holt Street, McMahons Point, NSW 2060 Australia).*

			Total Applied N	Pre-Season N Application		In-Season N Application		
Exp.	Date	Location	N Rate (kg N ha^{-1})	N Rate (kg N ha^{-1})	Type & Placement	N Rate (kg N ha^{-1})	Type & Placement	Timing (Irri. no.)
ACRI-1	Summer 2016-17	Narrabri, NSW	0	0	n/a	0	n/a	n/a
			78	78	Urea, banded both sides of plant line	0	n/a	n/a
			182	182	Urea, banded both sides of plant line	0	n/a	n/a
			252	252	Urea, banded both sides of plant line	0	n/a	n/a
ACRI-2	Summer 2017-18	Narrabri, NSW	35	35	Urea, banded both sides of plant line	0	n/a	n/a
Moree	Summer 2014-15	Moree, NSW	240	140	Urea, banded on alternate-furrow side	100	Urea, water-run	2
			310	210	Urea, banded on alternate-furrow side	100	Urea, water-run	2
			370	270	Urea, banded on alternate-furrow side	100	Urea, water-run	2
Gunn-1	Summer 2014-15	Gunnedah, NSW	230	200	NH_3, drilled on alternate-furrow side	30	N26, water-run	3
Gunn-2	Summer 2015-16	Gunnedah, NSW	130	100	NH_3, drilled on alternate-furrow side	30	N26, water-run	3
			230	200	NH_3, drilled on alternate-furrow side	30	N26, water-run	3
			330	300	NH_3, drilled on alternate-furrow side	30	N26, water-run	3

Experiment “ACRI-2” forms the primary data set in this paper (Latimer, 2020b). Its high intra-irrigation temporal resolution enables more targeted identification of specific loss pathways than other studies from comparable systems. The other four data sets are of a lower resolution more typical of contemporary irrigation studies, and are used here to support large scale trends identified in ACRI-2.

3.2.2 – Site Descriptions

Experiments ACRI-1 and ACRI-2 were conducted at ACRI in Narrabri, NSW, Australia (30°12’S 149°35’E). This site is dominated by high shrink-swell, haplic, self-mulching medium Grey Vertosols (Isbell, 2016), alternatively classified as fine, thermic, smectitic, Typic Haplusterts (Soil Survey Staff, 2010). The Moree (29°27’S 149°50’E) and Gunnedah (31°S 150.3°E) sites are both private farms on similar Vertosols. The climate at all three locations is semi-arid with mild winters, hot summers, and average annual rainfalls of 576 mm, 619 mm and 659 mm for Moree, Gunnedah and Narrabri respectively (BOM, 2019). All sites used alternate furrow irrigation with tilled hills approximately 20 cm high at 1 m spacing. Pre-season N fertiliser was banded direct-drilled urea in all experiments except those performed at Gunnedah (Gunn-1 and Gunn-2), which used direct-drilled NH_3 (Table 3.1). All in-season N fertigation events used some form of urea dissolved in the irrigation supply water. Data sets Moree, Gunn-1 and Gunn-2 were generated from N fertiliser rate response experiments, while

the irrigation N loss data we present from the other experiments are from single selected treatments within larger N fertiliser management experiments.

3.2.3 – Water Sampling and Analyses

Water samples were collected from the end of each furrow at least 3–5 m before the tail of the furrow to avoid contamination from tail drain water backing up into the furrows. Collected samples were filtered through 0.45 µm filters, fixed with phenylmercuric acetate (PMA; 1–5 mg L^{-1}) and/or frozen to inhibit microbial activity and prevent N transformation until laboratory analysis. Differences in preservation method were due to variable access to electricity. Analyses were performed at the CSIRO Black Mountain laboratories or NSW DPI Tamworth Agricultural Institute using an Alpkem Segmented Flow Analyser, Alpkem Corporation, Perstorp Analytical Co., Wilsonville, OR 97070 USA. The concentrations of nitrite (NO_2^-) and nitrate (NO_3^-) were determined according the USEPA method 353.2 (USEPA, 1993), NH_4^+ was determined according to USEPA method 350.1 (USEPA, 1983), urea—N was determined through the USEPA PDAB method (USEPA, 1979), and total N was determined according to USEPA method 353.2 (USEPA, 1993). Lab samples were analysed in triplicate. EC (µS cm^{-1}), pH and temperature (°C) measurements were taken *in situ* using a TPS Aqua-CP/A multi-probe.

3.2.4 – Discharge and Load Calculations

Discharge volumes (L ha^{-1} min^{-1}) were used to convert discharge N concentrations (mg N L^{-1}) into discharge loads (kg N ha^{-1} min^{-1}). At ACRI-2, discharge from alternate- and irrigated-furrows was measured separately using portable long-throated Replogle-Bos-Clemmens (RBC) flumes (Clemmens et al., 1984), installed 5 m from the furrow's end prior to each irrigation. Pressure sensors (vanEssen Baro Divers) continuously logged water depth (mm) in the flumes, from which discharge (L ha^{-1} min^{-1}) was calculated. ACRI-1 used semi-permanent flumes that aggregated flows from four furrows (two irrigated and two alternate) and measured discharge using pressure transducers in a similar manner to ACRI-2. Discharge volumes at Moree, Gunn-1 and Gunn-2 were calculated using measured tail drain flows and input irrigation volumes with industry standard irrigation efficiencies of 80% (20% of applied irrigation water runs off the field as tail water; Roth et al., 2013).

Where discharge was not measured separately for the two furrow types, we have assumed the total discharge to be split between irrigated- and alternate-furrows 70% to 30% respectively. To our knowledge this split has not previously been published, likely a product of its highly variable nature. Anecdotal and unpublished data suggest that 70:30 is a credible seasonal average split for alternate furrow irrigation systems on high-clay soils like Vertosols (Foley et al., 2020; Nadelko, 2009). Where large cracks and break-ins through the hill occur, the dominant hydrological flow in the alternate-furrow can shift from matrix-flow to bypass-flow. This is particularly prevalent in the cracking clay Vertosols on which most Australian irrigated cotton is grown (Ringrose-Voase and Nadelko, 2013), and may significantly increase the total nutrient discharge from the field.

Where 'field-derived N' values are reported, the concentration of N in the supply water has been subtracted from that in the discharge water before multiplying by the discharge flow:

$$Field\ Derived\ N = \left([N]_{Discharge\ Water} - [N]_{Supply\ Water}\right) \cdot Flow_{Discharge}$$

Equation 3.1. *Field-derived N runoff calculation.*

We assumed that all remaining discharge N was sourced from the field, either from pre-season fertiliser, soil-mineralised N, or SOM. Final N discharge values for each furrow type are reported on a per hectare basis (kg N ha^{-1}) for comparison between systems of different dimensions. This has been calculated from the discharged N (kg N) measured in each furrow, and the furrow dimensions:

$$N\ Discharge\ (kg\ N \cdot ha^{-1}) = Furrow\ N\ Discharge\ \cdot \frac{1\ ha}{Furrow\ Spacing \times Furrow\ Length} \cdot \frac{1}{2}$$

Equation 3.2. *Runoff N discharge load calculation for each furrow type.*

Where total N discharge from the field is reported, individual N discharge values for alternate- and irrigated-furrows have been summed.

3.3 – Results and Discussion

3.3.1 – Seasonal Nitrogen Losses

Across all treatments and sites with sufficient temporal spread to infer seasonal trends, the majority of N runoff was produced at the beginning of the season, with only minor losses occurring after the third irrigation (Fig. 3.1). This is consistent with our second hypothesis and with other comparable studies (Macdonald et al., 2017a; Macdonald et al., 2017b). Not all N in runoff water is sourced from the field, with N in supply waters contributing significantly to total N runoff (Fig. 3.1). When these inputs are removed, field-derived N losses show that soil nutrient leaching was greatest in the first irrigation, then lower in each successive irrigation event.

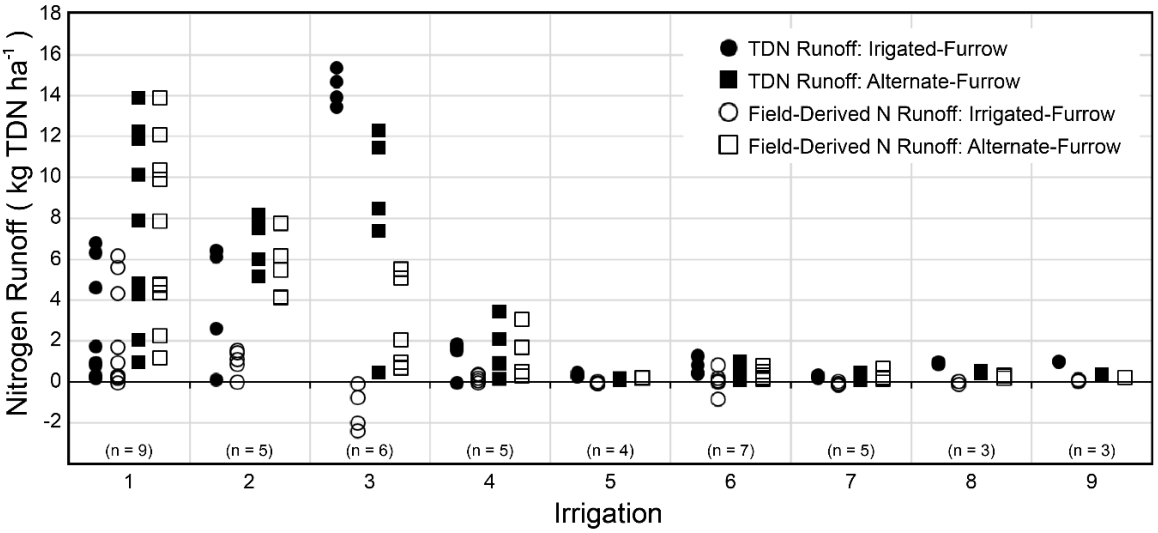

Figure 3.1. *Nitrogen runoff (kg TDN ha^{-1}) for all treatments by irrigation event. Negative field-derived N values occur where more N enters the field in irrigation water than runs off. n equals number of treatments represented in each irrigation.*

3.3.2 – Nitrogen Loss through Lateral Leaching

For the first three irrigations across all treatments, alternate-furrows discharged water with significantly higher field-derived TDN concentration than did irrigated-furrows, with average discharge concentrations of 83.4 ± 14.0 mg TDN L^{-1} and 27.4 ± 6.2 mg TDN L^{-1} respectively. From the fourth irrigation onwards, discharged N concentration was more even between the two furrow types, with average TDN concentration discharges of 9.8 ± 1.6 mg TDN L^{-1} and 7.3 ± 0.9 mg TDN L^{-1} for alternate- and irrigated-furrows respectively. These spatial and temporal variations in discharge water N concentration indicate the mechanism of loss. In alternate furrow irrigation systems, the distinguishing mechanistic difference between water discharged from the two furrow types is that alternate-furrow discharges have first passed through the hill. It is reasonable to assume that this lateral percolation of water through the hill is leaching significant soluble N, and acting as the main pathway for N runoff loss in these systems.

The removal of soluble soil components from the field over time can be observed on a whole-of-season scale (Fig. 3.1), as well as within single irrigation events (Fig. 3.2). In experiment ACRI-2, the EC, pH and TDN concentration of the alternate- and irrigated-furrow discharges converged over time as the hill was depleted of soluble components. By the fourth irrigation, much of the hill's soluble content was removed, resulting in the alternate-furrow water chemistry closely matching that of the irrigated-furrow, which in turn closely resembled that of the supply water.

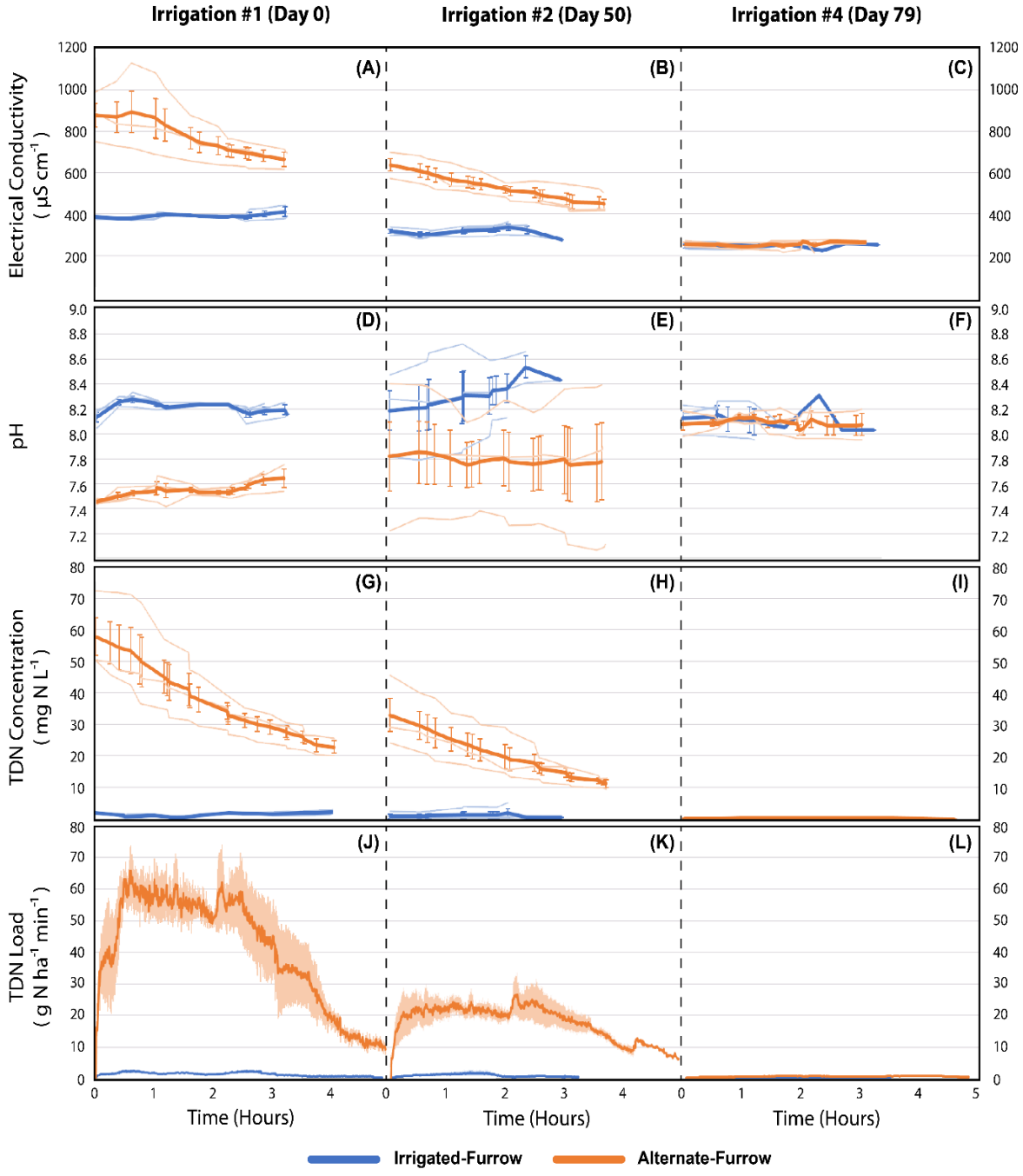

***Figure 3.2**. EC (µS cm^{-1}), pH, TDN concentration (mg N L^{-1}), and TDN load (g N ha^{-1} min^{-1}) in discharge water from alternate- and irrigated-furrows during irrigation events 1, 2 and 4 of experiment ACRI-2. Heavier-weighted lines with error bars represent averages of three replicates with standard error, and lighter-weighted lines represent the individual replicate sites. Day number refers to days after planting. Experiment ACRI-2 applied pre-season fertiliser as banded urea at a rate of 35 kg N ha^{-1} with no in-season N application.*

While alternate-furrows consistently discharged higher N concentrations (mg N L^{-1}) than irrigated-furrows, the final amount of N discharged (g N ha^{-1} min^{-1}) was highly influenced by the relative flow volumes (L min^{-1}) exiting the two furrow types. Where total discharge was sourced 70:30 from irrigated- and alternate-furrows respectively (as has been assumed for experiments ACRI-1, Moree, Gunn-1 and Gunn-2), total N discharged from the field was supressed by the higher N concentration of the alternate-furrows being multiplied by the lower flow volume. However, in reality the relative discharge split between the two furrow types is highly variable and is dependent on a range of soil characteristics. When large cracks and break-ins occur through the hill, the alternate-furrow's flow can increased relative to that of the irrigated-furrow, which increases the flow multiplier on the higher N concentration discharge source. While the increased alternate-furrow flow could theoretically dilute rather than multiply the high N concentration leachate with bypass water, and thus only slightly increase the total N discharged, this was not observed in experiment ACRI-2 in which alternate-furrow discharge was consistently higher than that of irrigated-furrows (Table 3.2). This suggests that when the flow through the hill increases, so too does the amount of leached soil material discharged from the field, despite the shift in dominant hydrological flow from matrix-flow to bypass-flow.

Assuming a 70:30 split in discharge flow between the two furrow types, alternate-furrows discharged more N than irrigated-furrows across the 12 treatments, on average producing 65.9 ± 6.9% and 34.1 ± 6.9% of the TDN discharge respectively (Table 3.2). Of the N runoff discharged from irrigated-furrows, however, the majority was derived from input supply water N, and not from the soil. When comparing field-derived N runoff, alternate-furrows were responsible for the majority, and discharged 87.5 ± 4.4% of the total field-derived N discharged (Table 3.3). While most field-derived N was discharged from the alternate-furrow, both furrow types exhibited the same sequentially decreasing N loss trend (Fig. 3.3), which suggests that some leaching is occurring as a result of water travelling longitudinally along the irrigated-furrow, but that it is a weaker leaching mechanism than the lateral percolation through the hill.

***Table 3.2**. Irrigated- and alternate-furrow N runoff for all treatments by irrigation event. Values are reported as mean kg TDN ha^{-1} ± standard error. Values reported with * were water-run N fertigation events. Additional information can be found in Appendix A.*

	Experiment:	ACRI-1	ACRI-1	ACRI-1	ACRI-1	ACRI-2	Moree	Moree	Moree	Gunn-1	Gunn-2	Gunn-2	Gunn-2
N Rate (kg N ha^{-1}):		**0**	**78**	**182**	**252**	**35**	**240**	**310**	**370**	**250**	**150**	**250**	**350**
#1	Irri-Furrow:	0.2 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	1.8 ± 0.7	0.4 ± 0.2	6.4 ± 0.3	4.6 ± 0.5	6.8 ± 1.0	0.8 ± 0.1	-	-	-
	Alt-Furrow:	2.1 ± 1.2	4.6 ± 0.2	10.2 ± 1.2	11.9 ± 1.2	12.3 ± 1.6	4.9 ± 0.5	4.3 ± 0.6	8.0 ± 1.8	13.9 ± 0.7	-	-	-
#2	Irri-Furrow:	-	-	-	-	0.2 ± 0.1	6.2 ± 0.4*	6.5 ± 0.3*	6.5 ± 0.4*	2.7 ± 0.1	-	-	-
	Alt-Furrow:	-	-	-	-	5.2 ± 0.8	7.5 ± 0.1*	6.0 ± 0.4*	8.1 ± 0.0*	8.2 ± 0.5	-	-	-
#3	Irri-Furrow:	-	-	-	-	-	-	-	-	14.0 ± 0.8*	14.7 ± 0.8*	13.5 ± 0.5*	15.4 ± 0.3*
	Alt-Furrow:	-	-	-	-	-	0.5 ± 0.0	0.5 ± 0.0	-	12.3 ± 0.7*	11.5 ± 0.8*	7.4 ± 0.2*	8.5 ± 0.6*
#4	Irri-Furrow:	-	-	-	-	0.0 ± 0.0	-	-	-	1.7 ± 0.0	1.9 ± 0.0	1.8 ± 0.0	1.6 ± 0.0
	Alt-Furrow:	-	-	-	-	0.2 ± 0.0	-	-	-	3.5 ± 0.5	1.0 ± 0.1	2.1 ± 0.0	0.9 ± 0.2
#5	Irri-Furrow:	-	-	-	-	-	-	-	-	0.4 ± 0.0	0.3 ± 0.0	0.5 ± 0.1	0.3 ± 0.1
	Alt-Furrow:	-	-	-	-	-	-	-	-	0.1 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.1
#6	Irri-Furrow:	-	-	-	-	-	1.4 ± 0.1	0.9 ± 0.1	1.4 ± 0.3	0.5 ± 0.1	0.5 ± 0.1	0.5 ± 0.1	0.5 ± 0.1
	Alt-Furrow:	-	-	-	-	-	1.1 ± 0.4	0.9 ± 0.0	0.5 ± 0.1	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0
#7	Irri-Furrow:	-	-	-	-	-	-	-	-	0.2 ± 0.0	0.3 ± 0.0	0.3 ± 0.1	0.4 ± 0.1
	Alt-Furrow:	-	-	-	-	-	0.5 ± 0.0	-	-	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.2 ± 0.0
#8	Irri-Furrow:	-	-	-	-	-	0.9 ± 0.1	1.0 ± 0.0	1.1 ± 0.0	-	-	-	-
	Alt-Furrow:	-	-	-	-	-	0.6 ± 0.2	0.6 ± 0.0	0.4 ± 0.0	-	-	-	-
#9	Irri-Furrow:	-	-	-	-	-	1.1 ± 0.1	1.0 ± 0.1	1.0 ± 0.1	-	-	-	-
	Alt-Furrow:	-	-	-	-	-	-	-	0.4 ± 0.1	-	-	-	-

***Table 3.3**. Irrigated- and alternate-furrow field-derived N runoff for all treatments by irrigation event. Values are reported as mean kg TDN ha^{-1} ± standard error. Field-derived N runoff is calculated by subtracting input supply water N. Negative values occur where more N enters the field in irrigation water than runs off. Values reported with * were water-run N fertigation events. Additional information can be found in Appendix A.*

	Experiment:	ACRI-1	ACRI-1	ACRI-1	ACRI-1	ACRI-2	Moree	Moree	Moree	Gunn-1	Gunn-2	Gunn-2	Gunn-2
	N Rate (kg N ha^{-1}):	**0**	**78**	**182**	**252**	**35**	**240**	**310**	**370**	**250**	**150**	**250**	**350**
#1	Irri-Furrow:	0.2 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	1.8 ± 0.7	0.0 ± 0.0	5.6 ± 0.5	4.4 ± 0.6	6.2 ± 1.3	0.4 ± 0.1	-	-	-
	Alt-Furrow:	2.1 ± 1.2	4.6 ± 0.2	10.2 ± 1.2	11.9 ± 1.2	9.7 ± 1.4	4.6 ± 0.6	4.2 ± 0.6	7.7 ± 1.9	13.7 ± 0.7	-	-	-
#2	Irri-Furrow:	-	-	-	-	0.1 ± 0.0	0.9 ± 0.4*	1.6 ± 0.3*	1.5 ± 0.4*	1.2 ± 0.1	-	-	-
	Alt-Furrow:	-	-	-	-	3.5 ± 0.1	5.3 ± 0.1*	3.9 ± 0.4*	6.0 ± 0.0*	7.6 ± 0.5	-	-	-
#3	Irri-Furrow:	-	-	-	-	-	-	-	-	-2.3 ± 0.8*	-0.7 ± 0.8*	-1.9 ± 0.5*	0.0 ± 0.4*
	Alt-Furrow:	-	-	-	-	-	0.5 ± 0.0	0.5 ± 0.0	-	5.3 ± 0.7*	4.9 ± 0.8*	0.8 ± 0.2*	1.9 ± 0.6*
#4	Irri-Furrow:	-	-	-	-	0.0 ± 0.0	-	-	-	0.2 ± 0.0	0.4 ± 0.0	0.4 ± 0.1	0.1 ± 0.1
	Alt-Furrow:	-	-	-	-	0.1 ± 0.0	-	-	-	2.9 ± 0.5	0.3 ± 0.1	1.5 ± 0.0	0.3 ± 0.2
#5	Irri-Furrow:	-	-	-	-	-	-	-	-	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.0	0.0 ± 0.1
	Alt-Furrow:	-	-	-	-	-	-	-	-	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.1
#6	Irri-Furrow:	-	-	-	-	-	0.3 ± 0.1	-0.8 ± 0.1	0.9 ± 0.3	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1
	Alt-Furrow:	-	-	-	-	-	0.6 ± 0.4	0.2 ± 0.0	0.3 ± 0.1	0.0 ± 0.0	0.1 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
#7	Irri-Furrow:	-	-	-	-	-	-	-	-	-0.1 ± 0.0	0.0 ± 0.1	0.0 ± 0.1	0.2 ± 0.2
	Alt-Furrow:	-	-	-	-	-	0.5 ± 0.0	-	-	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
#8	Irri-Furrow:	-	-	-	-	-	-0.1 ± 0.1	0.1 ± 0.0	0.1 ± 0.0	-	-	-	-
	Alt-Furrow:	-	-	-	-	-	0.1 ± 0.2	0.2 ± 0.0	0.0 ± 0.0	-	-	-	-
#9	Irri-Furrow:	-	-	-	-	-	0.1 ± 0.1	0.2 ± 0.1	0.1 ± 0.1	-	-	-	-
	Alt-Furrow:	-	-	-	-	-	-	-	0.0 ± 0.1	-	-	-	-

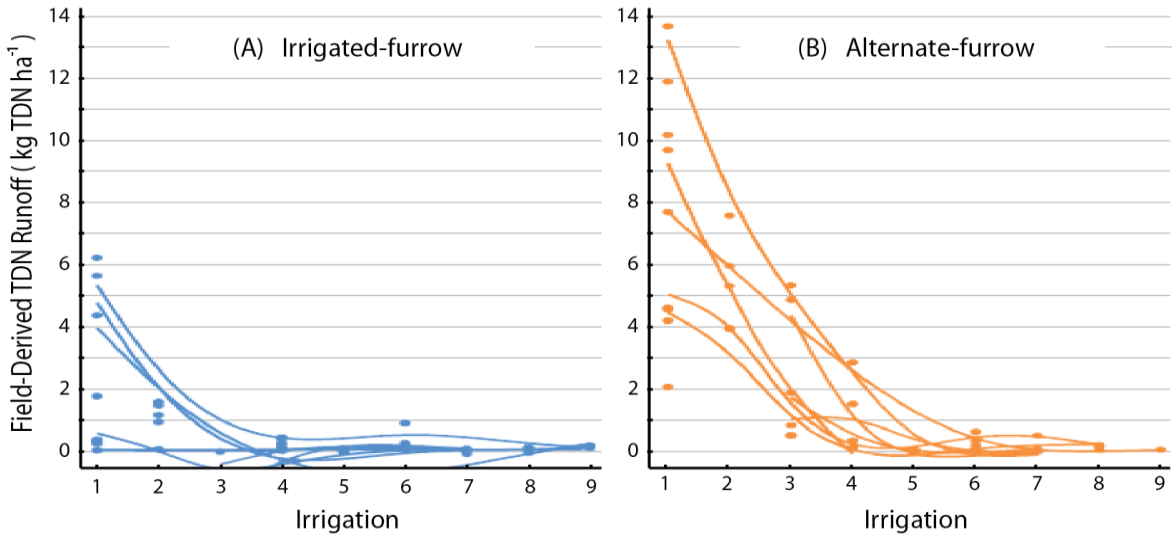

***Figure 3.3.** Field-derived N runoff losses (kg TDN ha^{-1}) by irrigation event for all 12 treatments. Figure is intended to convey general trends and not absolute values or ratios. **(A)** Alternate-furrow N discharge with cubic spline trends; **(B)** Irrigated-furrow N discharge with cubic spline trends.*

While not explicitly measured in this study, heavy rainfall will also affect the movement of solutes in furrow-irrigated cropping systems. Rainfall-induced leaching should affect the two furrow types equally, generating a net hydraulic gradient through the hill that is vertical rather than lateral. Rainfall can transport nutrient enriched soil from the hill into both irrigated- and alternate-furrows, where subsequent irrigations can remove solutes in runoff (Nachimuthu et al., 2018). Despite the potential for rainfall to transport solutes deeper into the soil profile, this is unlikely to reduce long term irrigation-induced leaching, as capillary rise processes should bring the solutes back to the surface over time (Prathapar et al., 1992).

3.3.3 – Nitrogen Speciation

The high TDN runoff losses observed in the early irrigations were dominated by NO_3^- and DON (<0.45 µm; Table 3.4). Negatively charged compounds like NO_3^- and deprotonated LMW organic acids are highly mobile in predominantly negatively charged clay soils such as Vertosols. NO_3^- losses are of particular environmental concern as some may be rapidly converted to N_2O, a potent GHG that has 298 times the global warming potential of CO_2 (IPCC, 2014; Macdonald et al., 2016). However, negatively charged DON also represents a significant mass loss and is frequently overlooked in nutrient accounting practices.

Table 3.4. *Dissolved N speciation of discharge water in the first irrigation of eight treatments ± standard error. n = number of replicates included in calculation. n.d. = no speciation data measured.*

Data Set	TDN Runoff (kg N ha^{-1}) (Measured)	% Ammonium (Measured)	% Nitrate (Measured)	% Urea (Measured)	% Organic N (Calculated)
ACRI-1 (0 N)	2.3 ± 1.2 (n = 9)	14.6 ± 1.6%	44.4 ± 2.9%	0.1 ± 0.0%	41.0 ± 3.8%
ACRI-1 (78 N)	4.9 ± 0.2 (n = 21)	14.7 ± 1.0%	48.1 ± 2.3%	0.2 ± 0.1%	37.0 ± 1.6%
ACRI-1 (182 N)	10.5 ± 1.3 (n = 3)	23.9 ± 7.0%	48.6 ± 10.2%	0.2 ± 0.0%	27.4 ± 7.1%
ACRI-1 (252 N)	13.7 ± 1.9 (n = 6)	12.5 ± 2.8%	73.2 ± 11.6%	0.1 ± 0.1%	14.2 ± 10.2%
ACRI-2 (35 N)	12.6 ± 1.7 (n = 28)	0.6 ± 0.2%	72.9 ± 9.1%	3.0 ± 0.0%	23.3 ± 13.7%
Moree (240 N)	19.0 ± 1.5 (n = 12)	n.d.	70.5 ± 9.0%	1.3 ± 0.6%	28.2 ± 8.6%
Moree (310 N)	15.8 ± 2.1 (n = 12)	n.d.	37.4 ± 6.1%	5.2 ± 2.4%	57.4 ± 6.3%
Moree (370 N)	21.5 ± 2.3 (n = 14)	n.d.	71.3 ± 8.8%	2.3 ± 0.8%	26.4 ± 9.1%
Mean	12.5 ± 1.5 (n = 13)	13.2 ± 2.5%	58.3 ± 7.5%	1.5 ± 0.5%	31.9 ± 7.6%

Leaching from the hill is further characterised by the temporal variations in discharge water N speciation. In experiment ACRI-2, DON makes the largest contribution to TDN discharge during the first irrigation, of which most came out of the alternate-furrow (Fig. 3.4). DON losses were low after the initial flushing, suggesting that the majority of the hill's soluble organic N was removed in and around the first irrigation. The majority of Australian cotton farmers practice crop rotation with wheat where the stubbles are incorporated into the soil, enhancing decomposition of organic matter (Farrell et al., 2008b). This partly decomposed organic matter is likely a major contributor to the observed DON in the first irrigation, with all study sites having a history of wheat rotation followed by stubble incorporation. DON runoff also draws attention to the concurrent loss of organic C from the field (Nachimuthu et al., 2018), linking N losses to the decline of soil C stocks observed in many agricultural systems (Luo et al., 2010; Rabbi et al., 2014). NO_3^- losses were very high in the first two irrigations of ACRI-2, constituting the dominant form of dissolved N loss for both furrow types. Conversely, NH_4^+ losses were low in all three irrigations, which is to be expected as the positively-charged molecule has low mobility in clay soils with high cation exchange capacity.

Urea losses in experiment ACRI-2 were consistently low for both furrow types, and remained largely unchanged across the four irrigations. While these low urea losses are encouraging for broader NFUE, direct fertiliser N losses are influenced by a range of factors, such as N placement location, soil moisture, and timing relative to irrigations (Macdonald et al., 2017a). Low urea concentrations may also be evidence of rapid urea hydrolysis occurring in the system. Because of this, and ACRI-2's atypically low N rate, broader industry-wide generalisations from these data about direct leaching of fertilisers early in the season should be avoided.

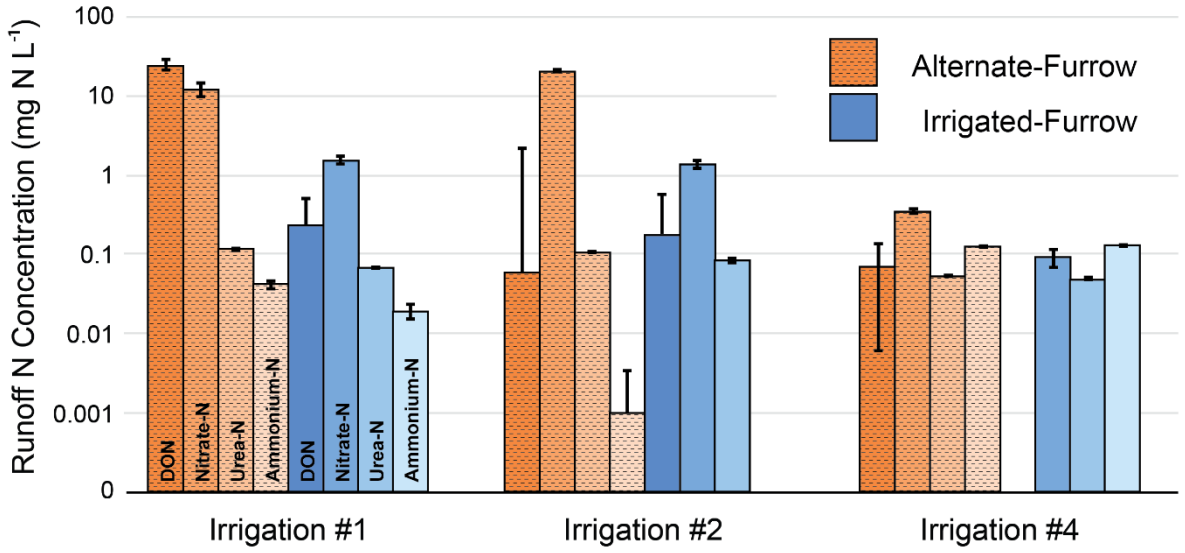

Figure 3.4. *Average concentration of four N species in runoff water for each ACRI-2 irrigation, disaggregated by furrow type. Experiment ACRI-2 does not include any fertigation events.*

3.3.4 – Pre-Season Fertilisation Application Rates

Across the 12 treatments, TDN runoff losses from an irrigation event tended to increase with the rate of fertiliser N addition. This positive relationship was most clearly observed when comparing N runoff during the first irrigation event of the season (Fig. 3.5). ACRI-2 was the only treatment not to closely conform with the collective N runoff median of 6.1 ± 0.4% of applied pre-season fertiliser N in the first irrigation, which was likely a result of the high alternate-furrow discharge flows creating greater N runoff; however, as flow data split by furrow type was only measured for ACRI-2, it is not possible to be sure.

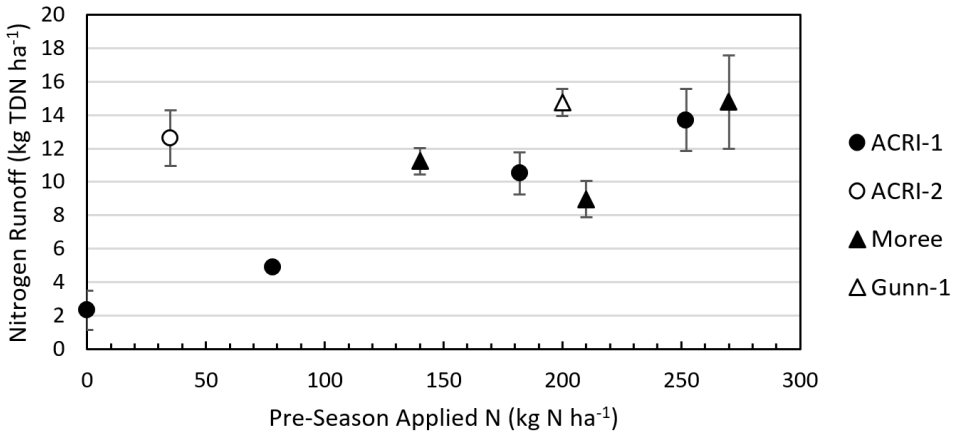

Figure 3.5. *Total N runoff (kg TDN ha^{-1}) from the first irrigation event of experiments. All values ± standard error. Experiment Gunn-2 does not have any first irrigation data.*

If total seasonal runoff N is extrapolated from the first irrigation's runoff, assuming that N leaching follows a sequentially decreasing N loss trend similar to Figure 3.3, then total seasonal runoff losses would equate to approximately 12.1 ± 0.7% of applied fertiliser N. For a pre-season fertiliser application of 270 kg N ha^{-1}, this equates to 32.6 ± 1.8 kg N ha^{-1} lost from the field as surface runoff during the season. These losses are higher than those reported by McHugh et al. (2008), but are consistent with work done by Macdonald et al. (2017a), who demonstrated with ^{15}N-labelled fertilisers

that in comparable cotton production systems approximately 10% of applied (pre-season) fertiliser N is lost through surface runoff across the growing season.

3.3.5 – Fertigation

Fertigation events are a major contributor to total N runoff. Nitrogen runoff during a fertigation event can easily exceed 15 kg N ha^{-1}. For 1000 hectares of cotton and a urea price of $400 per tonne, this equates to $13,000 worth of fertiliser per irrigation event flowing into the tail drain.

Of the seven treatments that included deliberate fertigation, 63.4 ± 9.5% of the total seasonal N runoff was derived from input supply water N (Fig. 3.6).

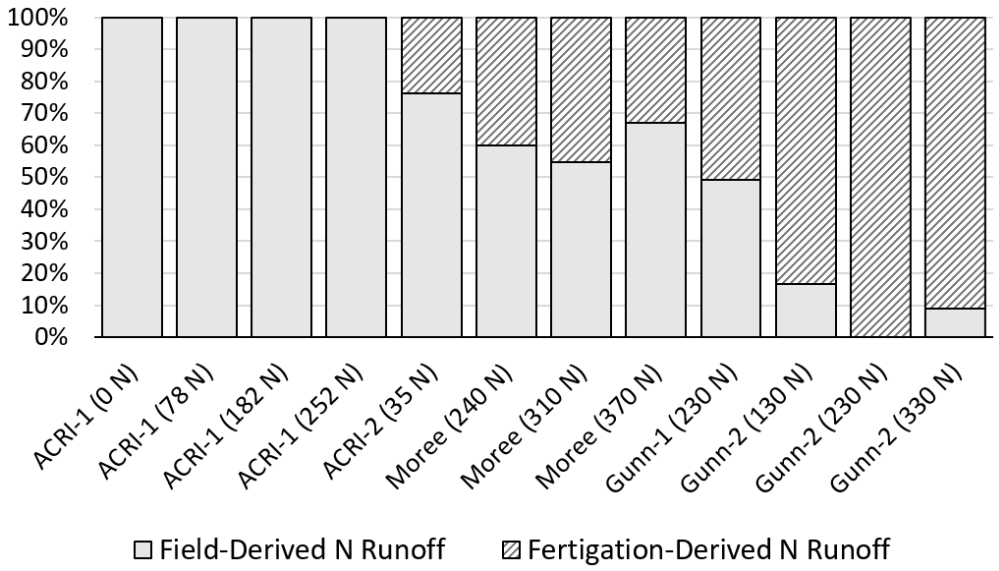

Figure 3.6. *Percentage contribution to total N runoff from field-derived N and fertigation-derived N for each treatment. Moree, Gunn-1 and Gunn-2 treatments contained one fertigation event each. Note that experiment ACRI-2 was not deliberately fertigated, and the input aqueous N was due to recirculated N-rich irrigation water.*

The Australian cotton production guide now recommends splitting N fertiliser application between pre-season and in-season applications to increase lint yield and reduce N losses, especially through denitrification (CRDC, 2019b). Fertigation is an effective way to supply N to the crop mid-season that limits in-field traffic and maximises labour efficiency. However, in alternate furrow irrigation schemes where a significant proportion of applied water exits the field, fertigation may be a wasteful application method. In Australian irrigated cotton production, tail water is captured and recirculated for use on other paddocks, so runoff N is lost from the paddock of application but is not directly discharged into the environment. The final calculation of N lost to the environment will therefore depend on factors including: whether N-rich tail water is recycled back onto the field; how quickly this water is recycled; soil permeability; soil microbial activity; and water temperature. In Australian irrigated cotton, total reactive N emissions from irrigation networks have not been fully quantified but are estimated to be low (Macdonald et al., 2016).

3.3.6 – Modifying Management Practice

Efforts to mitigate N losses through surface runoff in alternate furrow irrigation systems should target the lateral leaching mechanism. Modifying row configuration to remove the alternate-furrow entirely would likely prove the most effective strategy for reducing N runoff. By filling in (or not tilling) the alternate-furrow and creating an extra-wide hill (bed), any irrigation water leaching soluble soil components would be prevented from freely flowing from the field (Fig. 3.7). Extra-wide beds would also reduce the probability of large cracks, break-ins and tunnels occurring through the hill that can

increase total N discharge loads, while retaining the higher WUE of alternate furrow irrigation relative to irrigating every furrow (Kang et al., 2000). Further research should test the efficacy of extra-wide beds for mitigating alternate-furrow N runoff, as this thesis does not explore this topic.

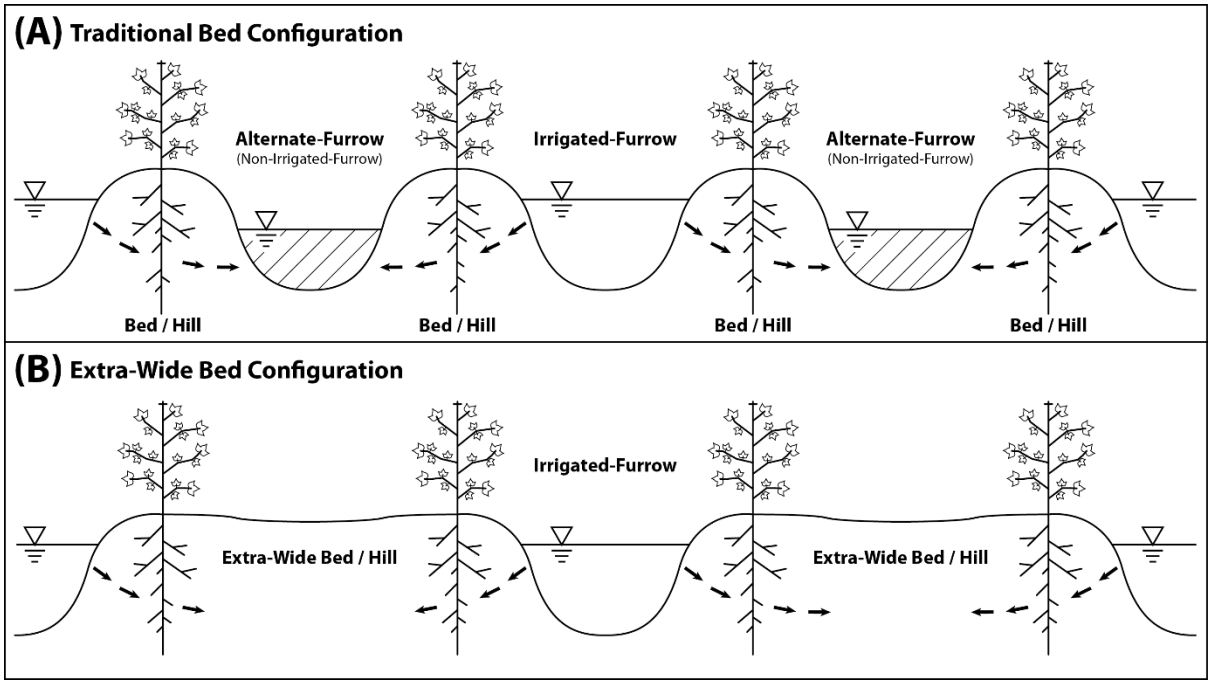

Figure 3.7. *Elevation cross-section of alternate furrow irrigation and extra-wide bed irrigation setups.* ***(A)*** *Traditional bed configuration for alternate furrow irrigation. High-solute irrigation water is able to freely flow out from the alternate-furrow;* ***(B)*** *Proposed extra-wide bed configuration.*

Other potential mitigation strategies include mitigating large cracks and break-ins through the hill, and eliminating the alternate-furrow by directing irrigation water down every furrow simultaneously and symmetrising the hydraulic gradient within the hill. While this may reduce N leaching, the practice of irrigating every furrow is commonly less water efficient (Golzardi et al., 2017; Kang et al., 2000), and is not recommended by the Australian cotton industry. Embracing even greater management change, flood irrigation systems could be replaced with overhead or drip irrigation, which have been shown to reduce N runoff and increase WUE (Antille, 2018; Smith et al., 2015b).

If modifying furrow configuration is infeasible, then reducing pre-season fertiliser application should reduce N runoff losses. However, if this is accompanied by an increase in fertigation use, then total N runoff may well increase. Placing N deeper in the soil profile below the laterally percolating irrigation water may reduce lateral N leaching, but capillary rise processes might negate any perceived advantages, and this should be explored further with modelling or field trials (Prathapar et al., 1992; Siyal et al., 2012). Unfortunately, neither of these strategies address the loss of soil-mineralised N or other valuable soil components that would continue to be leached under the current alternate furrow irrigation conditions. Synthetic N fertilisers are readily accessible for Australian cotton growers, which makes replacing lost soil N an economically viable option. However, this is not guaranteed to remain the case going forward, with price increases or government regulation capable of constraining reactive N application. Another problem is the loss of other elements like potassium, P and S over longer timescales. Natural replenishment of these elements in the soil column can be extremely slow, and replacement fertilisers are less renewable, affordable and available than Haber-Bosch-derived N.

3.4 – Conclusion

Significant surface runoff N is being generated in alternate furrow irrigated cotton systems. Discharge of N and other solutes (EC) decreased over time both within and between irrigations, with most N runoff generated at the beginning of the season, and few losses occurring after the third irrigation. The key mechanism driving these losses is lateral leaching through the hill by irrigation water passing from the irrigated- to the alternate-furrow, with leached nutrients then predominantly discharged from the alternate-furrow. In this study, average alternate- and irrigated-furrow TDN discharge concentrations were 83.3 ± 14.0 mg TDN L^{-1} and 27.4 ± 6.2 mg TDN L^{-1} respectively over the first three irrigations, decreasing to 9.8 ± 1.6 mg TDN L^{-1} and 7.3 ± 0.9 mg TDN L^{-1} respectively averaged over all the subsequent irrigations. Assuming total discharge volume from the field was sourced from irrigated- and alternate-furrows at a ratio of 70:30, these N concentrations equated to average TDN runoff loads during the first three irrigations of 7.4 ± 0.9 kg TDN ha^{-1} per irrigation for alternate-furrows and 5.6 ± 1.2 kg TDN ha^{-1} per irrigation for irrigated-furrows, decreasing to 0.6 ± 0.1 kg TDN ha^{-1} per irrigation and 0.8 ± 0.1 kg TDN ha^{-1} per irrigation respectively from the fourth irrigation onwards. However, much of the TDN discharged from irrigated-furrows was derived from input supply water N and not from the field. While TDN runoff from the field was on average discharged 65.9 ± 6.9% from alternate-furrows and 34.1 ± 6.9% from irrigated-furrows, field-derived N runoff was sourced 87.5 ± 4.4% and 12.5 ± 4.4% from the two furrow types respectively. N discharged from irrigated- and alternate-furrows was highly dependent on their relative flow volumes. Where alternate-furrow discharge flow is increased, as is the case when large cracks or break-ins through the hill occur, TDN concentration is multiplied by a higher flow rate which increases the total N discharged from the field. Fertigation was a major contributor to N runoff, accounting for 63.4 ± 9.5% of TDN runoff in treatments that included a deliberate fertigation event. TDN runoff was dominated by NO_3^- and DON, with average contributions to runoff during the first irrigation of 58.3 ± 7.5% and 31.9 ± 7.6% respectively. Efforts to mitigate N losses through surface runoff in alternate furrow irrigation systems should target the lateral leaching mechanism, with a possible solution in the adoption of extra-wide beds.

The next chapter reports on the transformation rates of aqueous N in soil-water systems for the purposes of predicting urea mineralisation rates and residence times at different temperatures and locations within the farm system. These data did not previously exist, and will provide farmers in many agricultural disciplines with management levers for maximising NFUE.

Chapter 4 – Aqueous Nitrogen Transformations

4.1 – Introduction

Nitrogen can be applied to crops as solids, liquids and gases, but does not become plant-available until it is in an aqueous form (Maathuis, 2009). Aqueously applying N (also referred to as fertigation or water-run N) is increasingly popular within the Australian cotton industry (CRDC, 2019a; Roth, 2012b). Applying fertiliser N aqueously can yield many positives, including minimising labour and in-field traffic, reducing environmental losses through smaller and more regular applications, and being immediately plant-available (CRDC, 2020b). Despite the widespread uptake of water-run urea, there is limited information available to growers describing N residence times in irrigation waters. Not knowing whether dissolved N remains in the water column for hours, days or weeks can make efficient N management difficult. N-rich irrigation water can spend considerable time not on the desired site of application; fertilisers can be dissolved in irrigation channels many kilometres upstream of the target field, and N-rich runoff water can take many hours or days to be recirculated back on to fields.

Urea is the most commonly water-run nitrogenous fertiliser in Australian cotton systems. Solid urea (prilled urea) contains 46% N by mass and is a stable product at room temperature; however, once dissolved in irrigation water, urea—N enters a dynamic system and can be rapidly transformed and lost as a range of different molecules (Fig. 4.1). To simplify management, these molecules are typically aggregated into different chemical N pools based on context-specific functionality. In this thesis, the spectrum of agriculturally-relevant water- and soil-borne nitrogenous molecules are categorised into four pools that commonly dominate these highly-modified agricultural systems:

- ***Inorganic Nitrogen*** – Sometimes called 'mineral N', this pool contains the small charged molecules NO_2^-, NO_3^- and NH_4^+. Nitrogen in this pool is typically short-lived and can be rapidly taken up by plants or microbes, or volatilise and be lost to the atmosphere. NO_3^- especially is easily lost from the system through leaching due to its negative charge.
- ***Dissolved Organic Nitrogen*** **–** The DON pool is comprised of soluble LMW organic molecules like amino acids, humic acids, and short peptide chains. These molecules are commonly negatively charged, making them easily leached from fields under excessive irrigation.
- ***Synthetic Nitrogen*** **–** This pool is composed of fertiliser N, and is not a commonly defined N pool. In agricultural settings like Australian irrigated cotton systems, fertiliser can dominate the soil- and water-N profiles. This creates unique chemical gradients that warrant defining fertiliser N in its own pool. This pool can be comprised of different compounds like ammonium nitrate or NH_3 depending on the context, but in this thesis the synthetic N pool will be composed solely of urea.
- ***Insoluble Organic Nitrogen*** **–** This pool consists of high molecular weight organic compounds like proteins, chitin and decaying microbes. Most of this pool is not directly plant-available, and represents longer term soil N storage and the source of future plant-available mineralised N. Despite its longer residence times, N in this pool is not permanently immobilised and will eventually flux out through microbial decomposition.

In order to improve fertigation knowledge and NFUE in the Australian cotton industry, a greater understanding of the fluxes between these N pools is required. Reaction kinetics can be used here to provide comment on the rates at which different steps in the N cycle progress, and in turn to identify bottlenecks or points of interest within the cycle that can be modified to enhance NFUE. The ammonification (mineralisation) of urea to NH_4^+ is an important stage in the environmental dissemination of urea—N, and the rate at which this occurs is an important determinant in the subsequent quantities of nitrification and denitrification. Accurately describing this bottleneck

reaction under different agricultural conditions will help the Australian cotton industry improve its NFUE through more precise fertigation management.

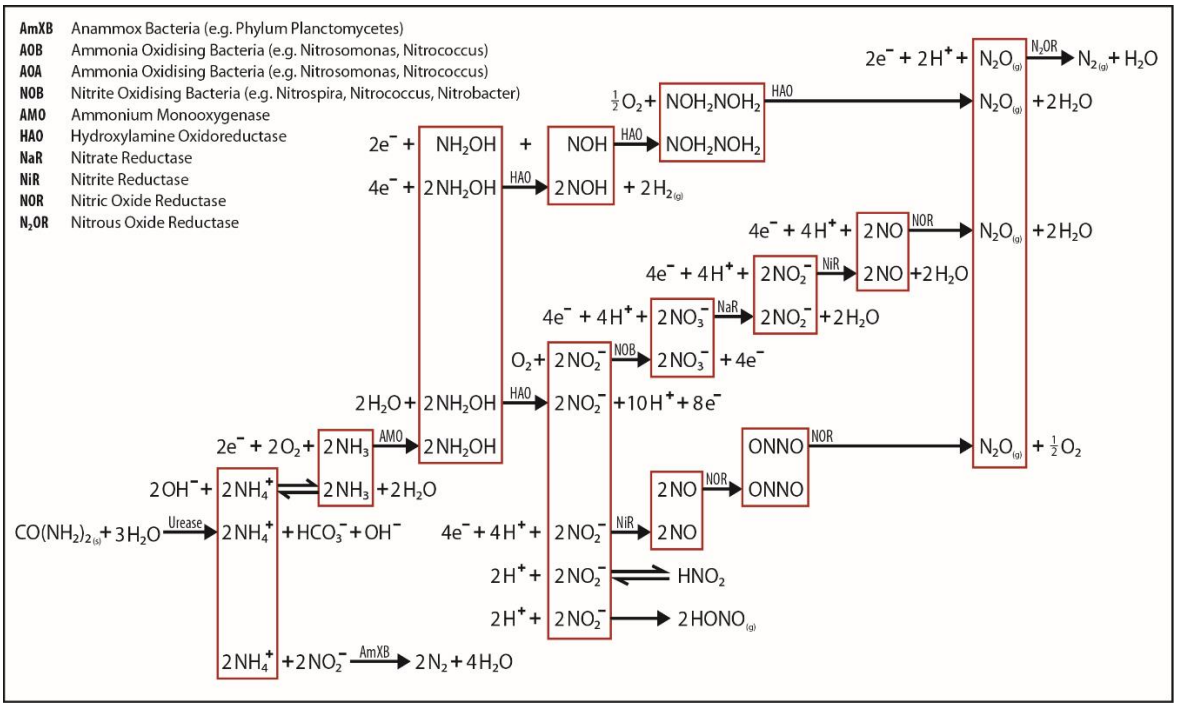

Figure 4.1. *A non-exhaustive representation of the complex reaction pathways available to aqueous urea—N. All reactions are biologically mediated to some degree. The pathway that urea—N takes in a given environment is dependent on factors like oxygen availability, concentration of N and other elements, temperature, and microbial speciation. All N species are aqueous unless otherwise indicated.*

This chapter draws on data from three laboratory experiments to estimate the residence time of dissolved N in irrigation water. Urea mineralisation is biologically mediated by the enzyme urease, suggesting that factors affecting enzyme activity will increase mineralisation and decrease N residence times. Based on this assertion, the four hypotheses of these experiments were:

1. Urea mineralisation rate (mg urea—N min^{-1}) is unaffected by urea concentration, and presents as a zero order reaction.
2. Living soil microbes are the major driver of urea mineralisation in irrigation systems.
3. Temperature positively correlates with urea mineralisation rate.

These experiments also incorporated an additional aim of creating a queryable dataset from which to create predictive models for N residence times in irrigation water. The management levers produced by these models have the potential to be considerable assets for growers adopting fertigation practices.

4.2 – Materials and Methods

The first two experiments were performed in mid-2016 at CSIRO Black Mountain laboratories in Canberra, ACT. In the first, three concentrations of urea (0.1, 1.0, 10.0 g N L^{-1}) were dissolved in Murrumbidgee River water and incubated at two different temperatures (10, 20°C) for 0-24 hours in triplicate (n = 3; Table 4.1). For the second incubation, urea (250 mg urea—N L^{-1}) was dissolved in five types of water of varying microbial content, combined in three soil treatments (no soil, soil, sterilised soil), and incubated at 15°C for 1-120 hours (Table 4.2). The soil used in this incubation was a high shrink-swell, haplic, self-mulching medium Grey Vertosol (Isbell, 2016) collected from the ACRI.

The first and second incubation experiments both followed the same experimental procedure. Samples were mixed in 50 ml centrifuge tubes, vortex agitated for 10 seconds to ensure mixing, and then incubated. Each tube was destructively sampled after the designated time interval. Upon removal from the incubation chambers, samples were centrifuged at 2500 rpm for 5 minutes, with the aqueous layer decanted and filtered through a 0.45 µm filters. Urea—N, NH_4^+—N and NO_3^-—N and TDN were measured using an Alpkem Segmented Flow Analyser, Alpkem Corporation, Perstorp Analytical Co., Wilsonville, OR 97070 USA. The concentrations of NO_2^- and NO_3^- were determined according the USEPA method 353.2 (USEPA, 1993), NH_4^+ was determined according to USEPA method 350.1 (USEPA, 1983), urea was determined through the USEPA PDAB method (USEPA, 1979), and total N was determined according to USEPA method 353.2 (USEPA, 1993).

Table 4.1. *Experimental treatments for the first incubation experiment. Incubation was performed in triplicate (n = 3), generating 54 samples.*

Treatment Variable	Number of Treatments	Treatment Values
Urea—N Concentration	3	100, 1000, 10000 mg N L^{-1}
Temperature	2	10, 20°C
Time	3	0, 3, 24 hours

Table 4.2. *Experimental treatments for the second incubation experiment. Incubation was not replicated (n = 1), generating 75 samples.*

Treatment	No.	Treatments Values	Descriptions
Water	5	MilliQ Deionised Water	Contained no appreciable biological material.
		Canberra Tap Water	Contained very little biological material, consistent with ACT water quality guidelines.
		ACRI Irrigation Water	Bore water collected from the ACRI. Indicative of irrigation water commonly used in cotton production in the Namoi catchment, northern NSW.
		Murrumbidgee River Water	Indicative of irrigation water commonly used in cotton production in the Riverina catchment, southern NSW.
		Lake Burley Griffin Water	Contained significant algae and other aquatic organisms.
Soil	3	No Soil	Treatment contained only urea and water.
		Soil	Treatment mixed ACRI Vertosol with water treatments in a 1:2 ratio.
		Sterilised Soil	ACRI Vertosol was sterilised using an autoclave prior to incubation to kill all microbes. It was then combined with water treatments in 1:2 ratio.
Time	5	1, 50, 70, 100, 120 hours	

The third incubation experiment was performed in late 2019, also at CSIRO Black Mountain laboratories. This experiment contained only a single urea concentration, soil and water treatment, instead expanding the temperature treatments, temporal resolution, and replication (n = 5; Table 4.3). Urea was dissolved in MilliQ deionised water (chosen as the single water treatment to maximise compatibility with future supplementary experiments) at a single concentration of 25 mg urea—N L^{-1}. The lower urea—N concentration was chosen based on field observations (Fig. 2.8). This urea solution was then combined with fresh samples of Grey Vertosol collected from the same site as used in experiment 2. The soil contained 22% water by mass, and was combined with the urea solution in a

ratio of 1:2 by wet mass. Eight incubation intervals of 0, 1, 2, 4, 8, 18, 72 and 168 hours (nominally) were chosen to best describe the N transformations within and between irrigations. Three temperature treatments of 15, 25 and 35°C were selected based on observations made during previous field experiments (Fig. 4.2). Samples were lightly orbitally agitated throughout the incubations to simulate *in situ* hydrological flows. Similarly to the two previous experiments, incubations were performed in sealed 50 ml centrifuge tubes to prevent cross-contamination between samples.

Table 4.3. *Experimental treatments for the third incubation experiment. Incubation was performed in quintuplicate (n = 5), generating 120 samples.*

Treatment Variable	Number of Treatments	Treatment Values
Urea—N Concentration	1	25 mg urea—N L^{-1}
Water	1	Deionised MilliQ
Soil	1	Narrabri Grey Vertosol
Temperature	3	15, 25, 35°C
Time	8	0, 1, 2, 4, 8, 18, 72, 168 hours

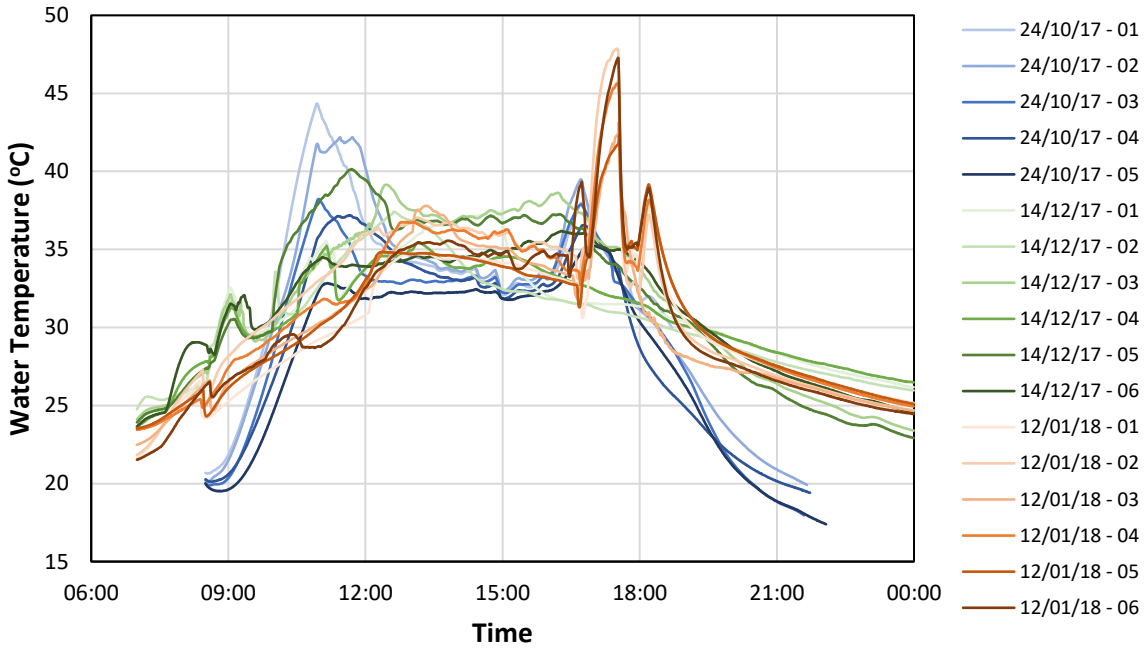

Figure 4.2. *In-furrow water temperature time series measured during irrigation events at the ACRI over the 2017-18 summer growing season (from the same experiments as reported in Chapter 3). Average summer day and night water temperatures are ~35°C and ~20-25°C respectively. Temperature spikes at 11:00 and 17:00 are largely the result of slow flows allowing pooled water to heat up in the sun. Water temperatures in irrigation channel networks and storage dams will be significantly lower than these in-furrow temperatures.*

Incubation vessels were destructively sampled after the elapsed incubation times. Samples were centrifuged at 2500 rpm for 10 minutes at 0°C to arrest the incubations, after which the aliquots of the aqueous component were filtered through 0.45 µm filters and transferred to microplate wells. The soil and surplus aqueous components were frozen for later extraction and analysis. Analyses conducted on the primary aliquots used a microplate reader in accordance with the following methods: urea—N analysis as per Greenan et al. (2008), NH_4^+—N and NO_3^-—N analyses as per Hernández-López and Vargas-Albores (2003), and soil urease activity as per Sinsabaugh et al. (2000).

After the initial incubation experiment, the frozen soil samples were extracted using 0.5 mol L^{-1} K_2SO_4 solution, after which the extracts were refrozen. These frozen extracts were sent to CSIRO Adelaide, South Australia, along with the frozen surplus aqueous components for further N speciation analysis.

All data were combined and published in the CSIRO DAP (Latimer and Macdonald, 2020).

4.3 – Results and Discussion

4.3.1 – Reaction Kinetics

Within treatments, the rate of urea mineralisation was independent of urea—N concentration, indicating zero order reaction kinetics (Fig. 4.3). This is consistent with expectations and the first hypothesis, as many enzymes exhibit zero order kinetics once saturated with substrate (Laidler and Hoare, 1949; Moyo et al., 1989).

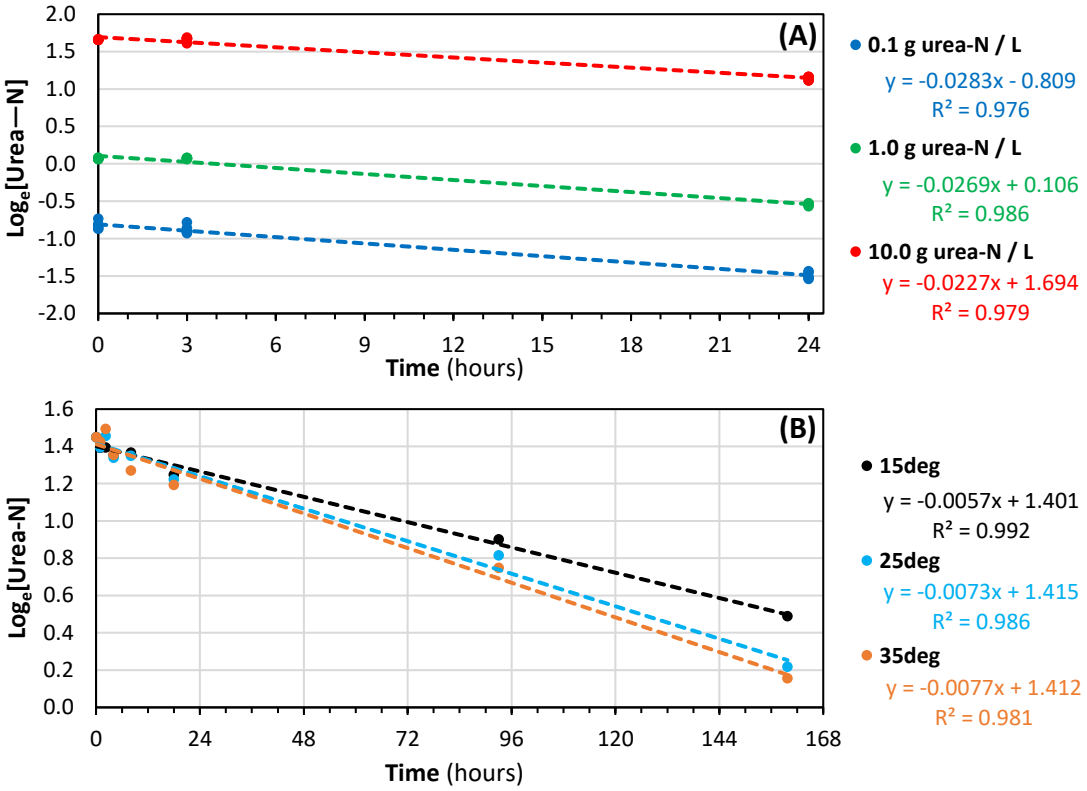

Figure 4.3. *Natural log of urea—N concentration (g urea—N L^{-1}) plotted against time for the first and third incubation studies. High linear correlations ($r^2 > 0.97$) within treatments indicate zero order reaction kinetics.* ***(A)*** *First incubation experiment. Treatments varied by initial urea—N concentration. Natural log of sample values plotted (n = 18 per concentration treatment). Data combines 10°C and 20°C treatments.* ***(B)*** *Third incubation experiment. Treatments varied by temperature. Natural log of treatment means (n = 5) plotted.*

4.3.2 – Microbial Effects

Urea mineralisation was affected by both water type and soil interaction (Fig. 4.4). The second incubation experiment demonstrated the importance of biologically active soil on urea mineralisation. The water-only treatments experience little mineralisation, suggesting that the majority of urease-containing microbes reside in the soil rather than the water column. The sterilised soil treatment exhibited some mineralisation in the initial stages of the incubation. The short-lived nature of the mineralisation may be attributable to extracellular urease enzymes exuded from lysing microbes, explaining why the mineralisation was not sustained; however, these mechanisms were not explored in these experiments. These findings indicate that living soil microbes are a major driver of

urea mineralisation in agricultural systems, and that the second hypothesis should be accepted. In contrast to the results of experiment 2, rapid urea mineralisation was observed in experiment one, which contained no soil (Fig. 4.3A). While the inherent variability of biological samples makes direct comparisons between incubations tenuous, the incongruity in the results is noteworthy and should be explored further in future studies. Of the five water treatments included in the second incubation, those with higher microbial contents showed greater urea mineralisation; however, the effect was small and overshadowed by the importance of soil interaction.

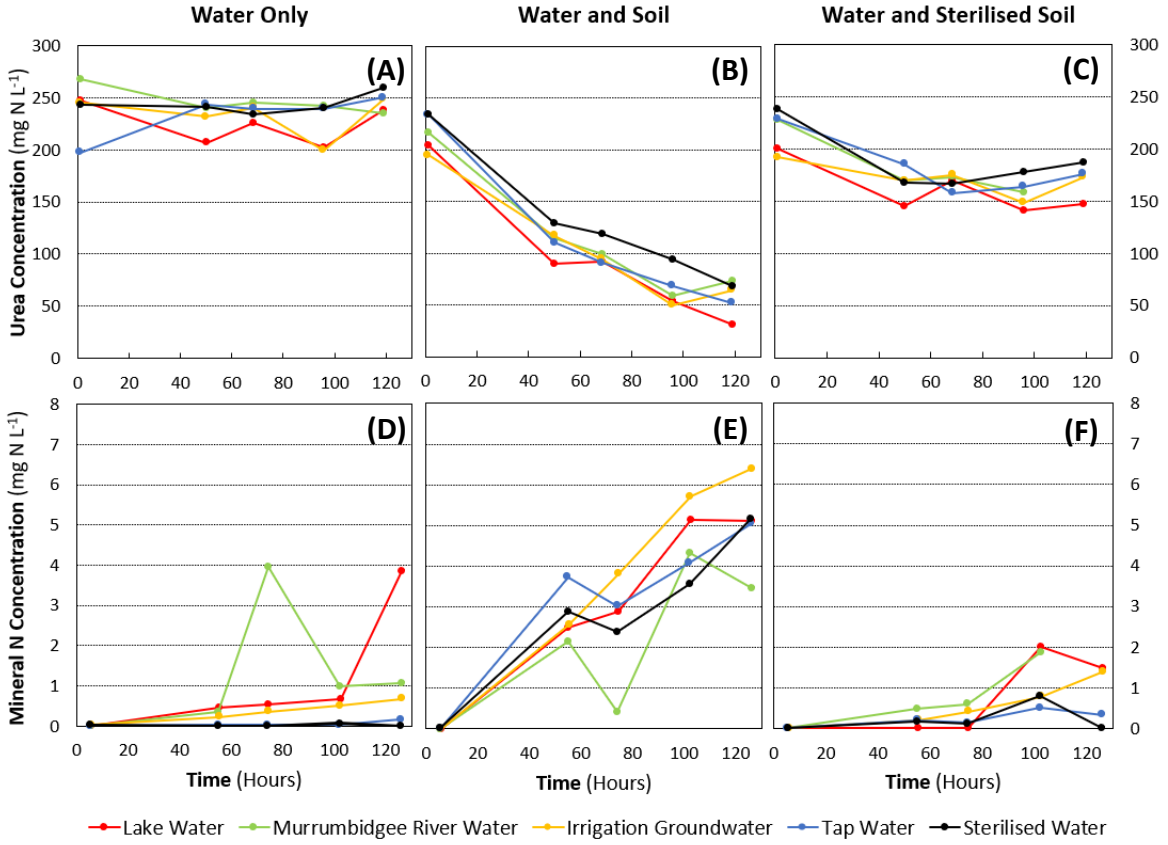

Figure 4.4. *Time series of aqueous N concentration (mg N L^{-1}) in the second incubation experiment. Mineral N is the sum of NH_4^+—N and $NO3^-$—N. Experiment contained no replication (n = 1).* ***(A)*** *Urea—N concentration in water-only system.* ***(B)*** *Urea—N concentration in 1:2 soil and water system.* ***(C)*** *Urea—N concentration in 1:2 sterilised soil and water system.* ***(D)*** *Mineral N concentration in water-only system.* ***(E)*** *Mineral N concentration in 1:2 soil and water system.* ***(F)*** *Mineral N concentration in 1:2 sterilised soil and water system.*

4.3.3 – Temperature Effects

Contrary to standard temperature enzyme activity relationships (Krajewska, 2016; Sahrawat, 1983), the first incubation experiment observed a negative correlation between temperature and urea mineralisation, with 1.8 ± 0.5% more urea mineralised after 24 hours in the 10°C treatment than the 20°C treatment (Fig. 4.5). The observed effect was small and unlikely to be replicated in larger studies. Indeed, the third incubation experiment that included more replication exhibited the predicted strong positive correlation between urea mineralisation and temperature (Fig. 4.6), suggesting that the negative correlation observed in the first experiment should be dismissed as anomalous and that the third hypothesis can be accepted. Urea concentration decreased by 32.5 ± 3.0% and 41.0 ± 0.8% after 24 hours in the 15°C and 35°C treatments respectively, with 87.7 ± 0.6% and 94.3 ± 0.2% mineralised after 7 days (Table 4.4). Of the inorganic products of mineralisation, NO_3^- concentration in the water column was more affected by temperature than NH_4^+.

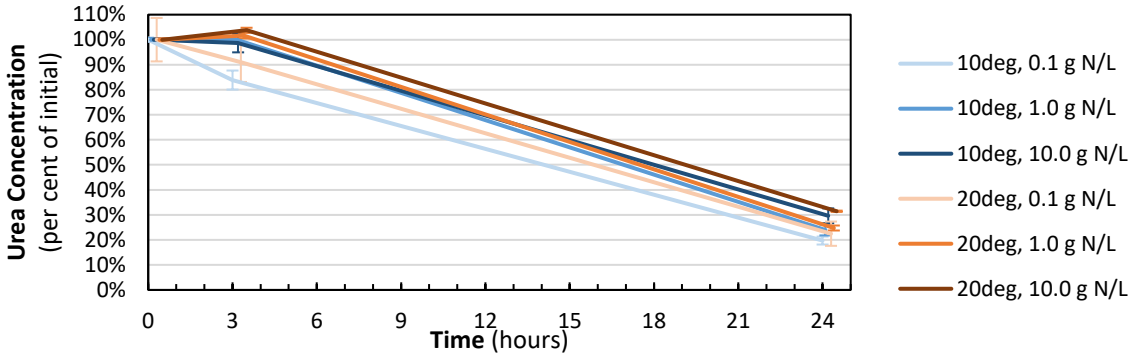

Figure 4.5. *Urea concentration as a percentage of initial concentration in first incubation experiment. Values reported as treatment means ± standard error (n = 3). The small negative correlation between temperature and urea mineralisation is contrary to general enzyme activity relationships and is likely anomalous.*

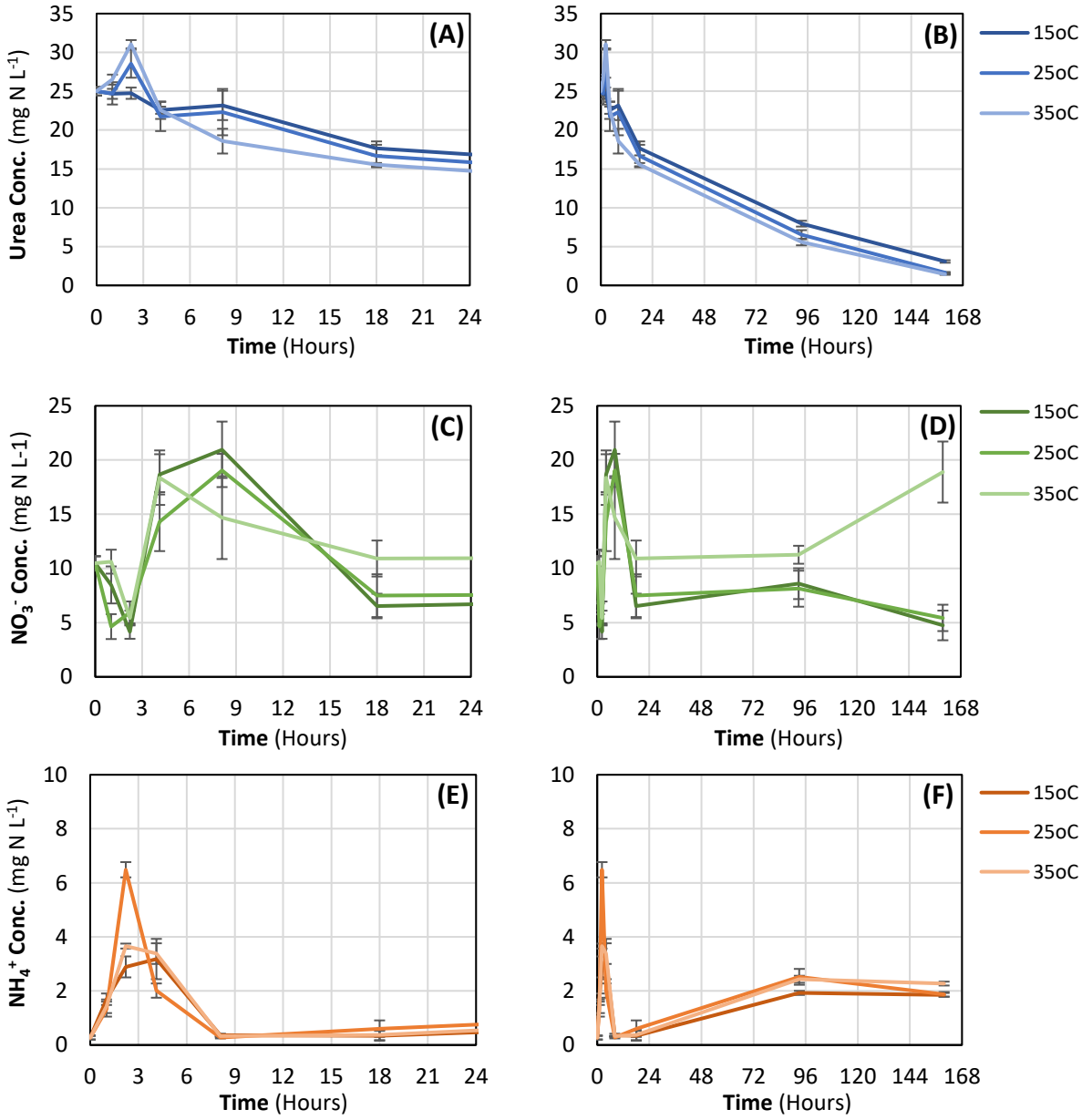

Figure 4.6. *Concentration of N species in water column of third incubation study, by temperature treatment. Two different time scales are shown to convey reaction characteristics at different temporal resolutions. Values are reported as treatment means ± standard error (n = 5).* ***(A)*** *Urea—N, 24 hours.* ***(B)*** *Urea—N, seven days.* ***(C)*** *NO_3^-—N, 24 hours.* ***(D)*** *NO_3^-—N, seven days.* ***(E)*** *NH_4^+—N, 24 hours.* ***(F)*** *NH_4^+—N, seven days.*

Table 4.4. *Nitrogen in soil and water column remaining after 24 hours and seven days (per cent of initial ± standard error). Values disaggregated by temperature treatment.*

	15°C	25°C	35°C
Remaining urea after 24 hours:	67.5 ± 3.0%	63.4 ± 4.9%	59.0 ± 0.8%
Remaining water-borne—N after 24 hours:	61.9 ± 6.6%	58.4 ± 6.6%	72.5 ± 4.1%
Remaining soil- and water-borne—N after 24 hours:	66.2 ± 6.3%	64.5 ± 6.6%	76.4 ± 4.1%
Remaining urea after 7 days:	12.3 ± 0.6%	6.6 ± 0.3%	5.7 ± 0.2%
Remaining water-borne—N after 7 days:	19.2 ± 3.2%	19.8 ± 2.6%	39.4 ± 5.4%
Remaining soil- and water-borne—N after 7 days:	41.8 ± 4.0%	40.0 ± 4.0%	58.1 ± 5.6%

4.3.4 – Soil Nitrogen Dynamics

Significant soil—N fluxed into the water column upon mixing at the beginning of the third incubation (Fig. 4.7). Prior to mixing with the soil, the water component contained only urea—N at 25 mg N L^{-1}. The influx was dominated by NO_3^- and DON (Fig. 4.8). Soil NH_4^+ was the only N species to increase in concentration over the incubation, constituting 30.6 ± 0.6% to 41.0 ± 1.5% of the remaining N in the soil and water after seven days (Table 4.5). This accumulation of NH_4^+ may signal the development of anoxic conditions, a theory further supported by the increased DON mineralisation observed with increasing temperature (Fig. 4.8B; 4.8F; Haynes et al., 1986). Soil DON also technically increased over the incubation; however, the water column's DON was soil-derived, and when both DON pools are combined DON declined significantly.

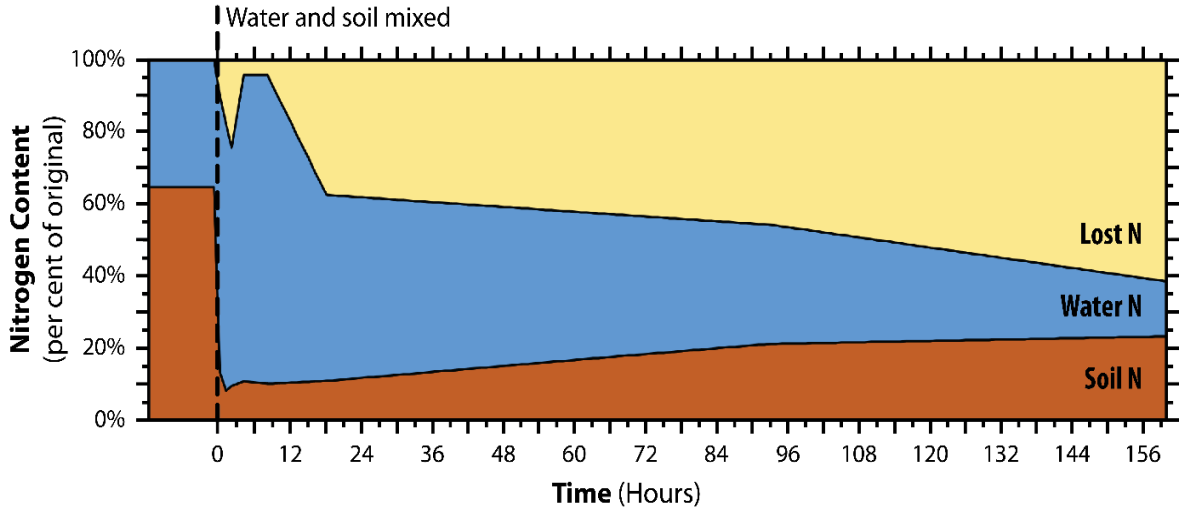

Figure 4.7. *Depiction of soil—N flux into the water column during the third incubation experiment. The effect is analogous to irrigation water passing over the field and transporting soil—N into the tail drain. This effect is particularly severe when irrigation water travels through soil rather than over it, as is the case in alternate furrow irrigation configurations (see Chapter 3). 'Lost N' can include N emitted to the atmosphere and taken up by microbes.*

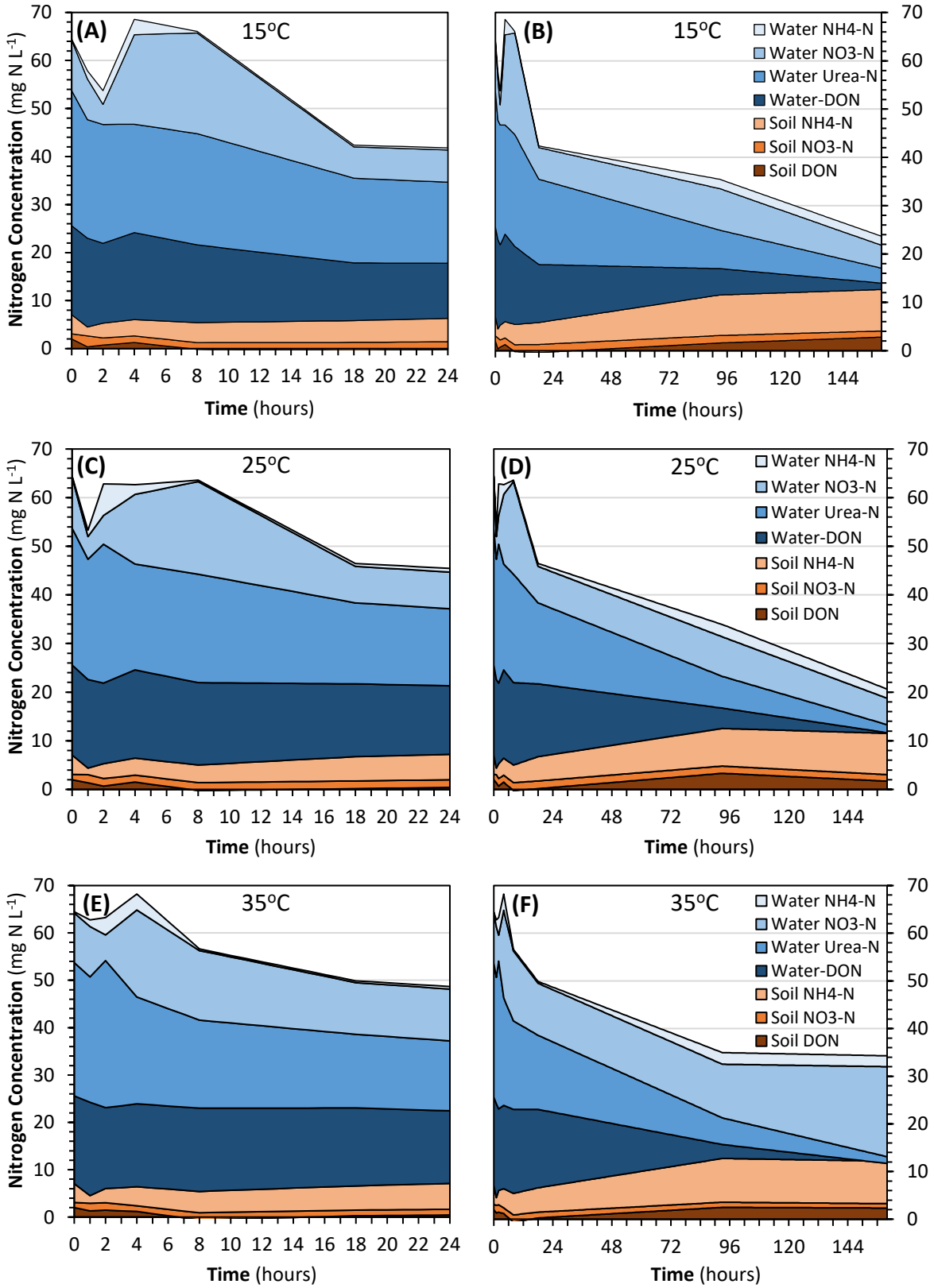

Figure 4.8. *Speciated N concentration in soil and water columns.* ***(A)*** *15°C treatment, 24 hour window.* ***(B)*** *15°C treatment, seven day window.* ***(C)*** *25°C treatment, 24 hour window.* ***(D)*** *25°C treatment, seven day window.* ***(E)*** *35°C treatment, 24 hour window.* ***(F)*** *35°C treatment, seven day window.*

Table 4.5. *Speciation of remaining soluble soil- and water-borne—N after seven days. Values reported as "per cent of combined soil and water N" ± standard error. Values disaggregated by temperature treatment. Remaining N is dominated by NO_3^- in the water column and NH_4^+ in the soil.*

	15°C	25°C	35°C
Water NH_4^+—N:	6.7 ± 0.2%	7.0 ± 0.3%	5.9 ± 0.2%
Water NO_3^-—N:	17.0 ± 4.9%	20.3 ± 4.5%	48.6 ± 7.3%
Water Urea—N:	11.0 ± 0.6%	6.2 ± 0.3%	3.7 ± 0.2%
Water DON:	4.8 ± 0.9%	9.0 ± 0.5%	0.0 ± 0.3%
Soil NH_4^+—N:	41.0 ± 1.5%	42.4 ± 1.3%	30.6 ± 0.6%
Soil NO_3^-—N:	6.0 ± 0.2%	6.7 ± 0.2%	3.2 ± 0.1%
Soil DON:	13.5 ± 1.2%	8.5 ± 2.7%	8.0 ± 1.1%

Because the incubations took place in sealed vessels, both the soil—N and atmospheric—N had the opportunity to reincorporate into the water column. In a farm environment, fluxes to the soil column would only remain agriculturally relevant if they occurred within the field bounds; N fluxes into the soil column that occur in irrigation networks and other off-field locations will likely be lost to the atmosphere before they can become plant-available once more. Similarly, gaseous N emissions from a real farm would be permanently lost to the atmosphere, and not build up in an enclosed environment. It is possible that the sealed nature of the incubation chambers suppressed the production of gaseous N (NH_3, N_2O, N_2) relative to a farm environment due to limited oxygen supply and increased local partial pressures as per Le Chatelier's principle. These perturbations were predicted to be negligible; however, this remains a major assumption in the transferability of these data to farming systems. In the event that gaseous N production rates were suppressed in this experiment, it would mean that on-farm atmospheric losses would be even higher than predicted here.

4.3.5 – Gaseous Nitrogen Inferences

Gaseous N components were not directly measured in these experiments. However, due to the sealed nature of the incubation chambers, credible inferences can be made as to the likely prevalence of various nitrogenous gases through time. Initial gaseous N production was likely dominated by NH_3, as the volatilisation product of the initial mineralisation reaction (Fig. 4.9). Inert N_2 then likely became the dominant gaseous N product once sufficient NO_3^- had formed through nitrification to subsequently denitrify. This progression of reactions can be observed in the incubation results (Fig. 4.10). Generation of NH_4^+ through mineralisation begins immediately after mixing, with nitrification commencing several hours after that. The rate of nitrification appears to exceed mineralisation within four to five hours, after which NH_4^+ concentration falls. Denitrification exceeds nitrification from approximately eight hours into the incubation, after which significant N losses are observed (Fig. 4.8). After seven days, N_2 is likely the dominant gaseous N product.

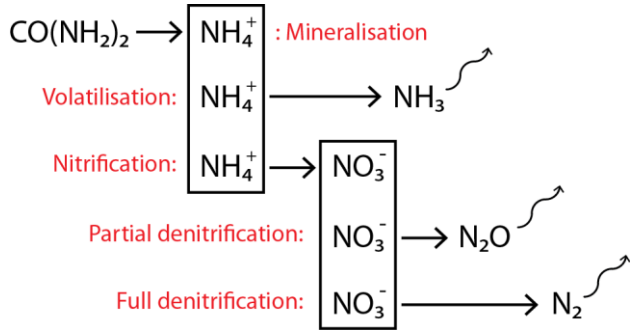

Figure 4.9. *Simplified reaction series depicting possible gaseous loss pathways for urea—N. N_2O emissions are the most damaging N emissions, as the molecule is a potent GHG with a warming potential of 298 CO_2-e. Chemically unreactive N_2 represents the least environmentally damaging emission, and is the ultimate fate of all reactive N.*

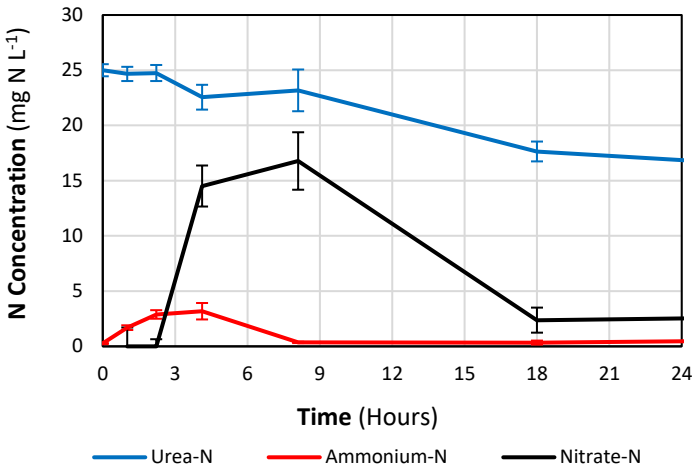

Figure 4.10. *Nitrogen concentration of water column in excess of initial influx from soil—N in 15°C treatment. Values reported as treatment means ± standard error. Relative temporal sequencing of time series illustrates reaction series shown in Figure 4.9, depicting immediate production of NH_4^+—N from urea—N, followed by significant NO_3^-—N production after a short lag. NO_3^-—N concentration falls after approximately eight hours when the rate of denitrification exceeds that of nitrification.*

4.3.6 – Nitrogen Modelling

The data generated from these incubation experiments can be used to create quantitative predictive management tools for irrigators. These additional management levers will facilitate fertigation optimisation to reduce N losses that can be both financially and environmentally costly. These data could exist as stand-alone applications for predicting urea mineralisation given a range of user-defined parameters (e.g. water EC, pH or temperature, or soil parameters such as clay content or microbial activity), or be incorporated into existing decision-making tools like myBMP or the Australian Cotton Production Manual. An example of a generalised reaction curve depicting urea mineralisation rates at different temperatures is shown in Figure 4.11. The urea mineralisation reaction curve is well described by an exponential function (Eq. 4.1).

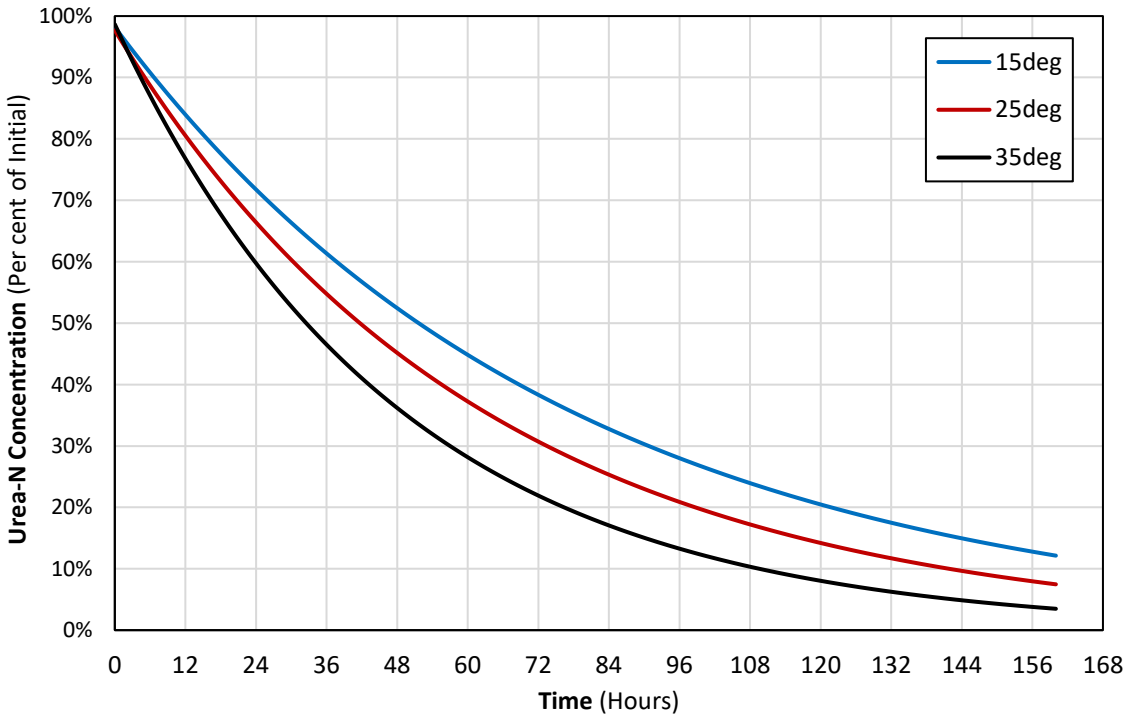

Figure 4.11. *Generalised urea mineralisation lookup chart for easy grower reference. Chart reports urea—N time series as a percentage of initial urea concentration. Transformation rates are indicative of in-field scenarios where N-rich irrigation water experiences high soil contact. Trends generated from third incubation experiment with initial urea concentration of 25 mg urea—N L^{-1}. Mineralisation rates could differ at different initial urea concentrations, soil types, and water sources.*

$$[Urea - N] = a \cdot e^{-b \times time}$$ ***Equation 4.1.*** *Generalised urea mineralisation rate in irrigation water.*

Accurately modelling the concentrations of NO_3^- and NH_4^+ in the water column is less straightforward than for urea, but can still be moderately approximated by a gamma distribution (Eq. 4.2). This distribution yields fair approximations of aqueous NO_3^- and NH_4^+ concentration in the first 24 hours after urea dissolution for the three temperature treatments (Fig. 4.12). Similar to Figure 4.11, the generalised curves shown in Figure 4.12 can be used to create grower reference material to assist in aqueous N management and NFUE improvement.

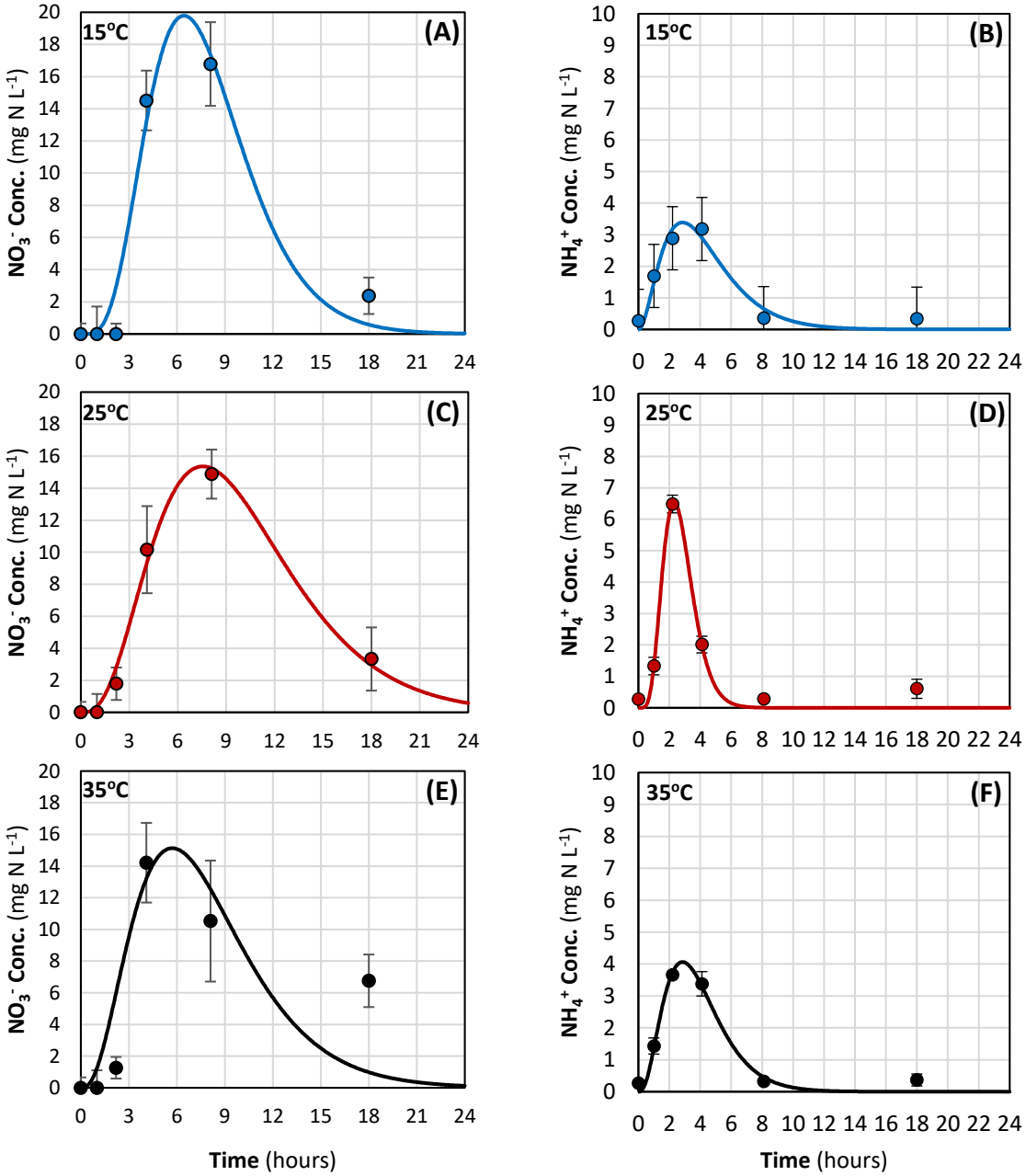

Figure 4.12. *Generalised models for NO_3^- and NH_4^+ concentration in the water column given an initial total N concentration of 25 mg urea-N L^{-1} at three different temperatures. Continuous line represents gamma distribution model (Eq. 4.2), points represent measured values ± standard error.* ***(A)*** *NO_3^-—N concentration at 15°C over 24 hours.* ***(B)*** *NH_4^+—N concentration at 15°C over 12 hours.* ***(C)*** *NO_3^-—N concentration at 25°C over 24 hours.* ***(D)*** *NH_4^+—N concentration at 25°C over 12 hours.* ***(E)*** *NO_3^-—N concentration at 35°C over 24 hours.* ***(F)*** *NH_4^+—N concentration at 35°C over 12 hours.*

$$[N] = \frac{\beta^{\alpha} \cdot time^{\alpha-1} \cdot e^{-\alpha \cdot time}}{\Gamma(\alpha)}$$

Equation 4.2. *Gamma distribution equation, where α and β are positive real numbers. Equation can be used to approximate the concentrations of NO_3^- and NH_4^+ in the water column during the first 12-24 hours after urea dissolution.*

4.3.7 – Implications for Management Practice

Urea mineralisation is a crucial gateway reaction in the dispersal of urea—N to the broader environment, and is entirely controlled by the availability of the enzyme urease (Callahan et al., 2005; Cartes et al., 2009; Tabatabai and Bremner, 1972). Urease presents as much more strongly associated with microbes in the soil than in the water (Fig. 4.4), making the degree of soil exposure a key determinant of the urea mineralisation rate. Different soils support different microbial communities that may mineralise urea at different rates. It is likely that the Grey Vertosols on which these experiments were performed sit at the higher end of microbial activity when compared to other Australian soils – due to greater water availability (Polain et al., 2018) and the protection clays provide to organic matter (Wang et al., 2003) – and so will exhibit higher urea mineralisation rates. However, agricultural systems on other continents with more organic-rich soils will likely experience higher mineralisation rates again than those observed here (Polain et al., 2020a). Temperature is another key factor affecting urea mineralisation, with the rate positively correlated with temperature within the examined window (Fig. 4.6; 4.11). This is consistent with the general temperature-rate interactions observed in most biologically mediated reactions. The urease enzyme can withstand considerable heat, with its activity continuing to increase in line with temperature until approximately 60°C (Sahrawat, 1983). Based on previously published urease activity increases, it is likely that urea mineralisation would occur considerably more rapidly than was observed in this experiment in water temperatures of 40-50°C (Moyo et al., 1989).

For growers, the combined effects of temperature and soil exposure on urea mineralisation rate mean that dissolved urea fertiliser will mineralise at different rates at different locations around the farm. When advancing down furrows, fertigation water can reach high temperatures (Fig. 4.2) and experience high soil exposure, resulting in high mineralisation rates. Conversely, urea-laden water in storage dams will experience much lower temperatures and soil exposures, significantly diminishing urea mineralisation rates. Irrigation channel networks will likely exhibit urea mineralisation rates between those of furrows and storage dams, as the water temperature and soil exposure are typically also between the two. Theoretically, aqueous urea fertiliser in a storage dam or large irrigation supply channel will be slow to mineralise and subsequently denitrify, giving growers ample time to recirculate the dissolved N back on to fields. While this may prove true in some circumstances, by the time urea fertigation runoff recirculates back into the network it has already undergone significant mineralisation, with the dissolved N profile now dominated by NO_3^- and NH_4^+. This inorganic N can continue to denitrify in the deeper and colder waters without the requirement for significant soil exposure, and can be lost to the atmosphere before it is reapplied. It is therefore important that growers recirculate N-rich tailwaters back on to fields as soon as possible, minimising the time N spends off-field. TDN in the soil and water dropped significantly from four to eight hours after dissolution, depending on the temperature treatment. This likely coincides with an increase in denitrification, and growers should ensure that N-rich irrigation water is on the field after this time to immobilise the N and minimise gaseous emissions. This incubation also highlighted the rapid flux of soil—N into the water column upon mixing, especially of negatively charged NO_3^- and DON (Fig. 4.7). If the soil—N scavenging water remains within the field boundary, this N mobilisation is not a problem. If, however, this N-enhanced water runs off the field or drains below the maximum root depth, it will remove nutrients from the field and create sub-optimal environmental and economic outcomes. This topic is covered in more detail in Chapter 3.

4.5 – Conclusions

Within treatments, urea mineralisation demonstrated zero order kinetics in all experiments (Fig. 4.3), allowing acceptance of the first hypothesis. Urea mineralisation was significantly higher in soil treatments than no-soil treatments, with sterilised soil treatments showing only minor unsustained mineralisation (Fig. 4.4). This suggests that living soil microbes are the driving force behind urea mineralisation in irrigation environments, and that the second hypothesis should be accepted. Water treatments with higher microbial content also appeared positively correlated with urea mineralisation; however, this effect was much weaker than the effect of living soil, and is not a major determining factor in urea mineralisation in irrigation environments. The mineralisation rate was positively correlated with temperature, with 6.6 ± 0.8% more urea mineralised in the third experiment after seven days in the 35°C treatment than the 15°C treatment. These results were contradicted by the first incubation experiment that showed a negative correlation with temperature. However, given the higher replication of the third experiment, and the prevailing relationship between temperature and enzyme activity in other contexts, the negative correlation should be rejected and the third hypothesis should be accepted.

Urea mineralisation (ammonification) is biologically driven by urease, with greater exposure to the enzyme and increased temperature-induced activity yielding greater mineralisation. For growers, this means urea fertiliser will mineralise at different rates at different locations around the farm. In the field, urea-laden irrigation water will experience elevated temperatures (>35°C) and high soil exposure, resulting in elevated mineralisation. Conversely, urea-laden water in storage dams will experience much lower temperatures and degree of soil exposure, significantly diminishing urea mineralisation rates. Irrigation channel networks will likely exhibit urea mineralisation rates between those of furrows and storage dams. However, runoff water will likely contain significant post-mineralisation inorganic N that will continue to denitrify and volatilise without the need for significant soil interaction. Growers should therefore attempt to recirculate N-rich runoff as quickly as possible. In the third incubation, TDN losses from the soil and water columns increased significantly after four to eight hours, likely the result of increasing denitrification. Growers should ensure that N-rich irrigation water is on the field after this time to maximise immobilisation and minimise gaseous emissions.

Based on the experimental results presented in this chapter, predictive models describing the concentration of urea, NO_3^- and NH_4^+ in the water column have been constructed to help growers better manage fertigation practice. The concentration of urea over seven days can be well generalised with negative exponential equations, while the concentrations of NO_3^- and NH_4^+ can be generalised over the first 24 hours with gamma distributions. Further experiments would increase the functionality and applicability of these data. Repeating the experiment with different starting urea—N concentrations and soil types, and expanding the temperature range, would increase the validity of applying these findings to agricultural settings outside Australian irrigated cotton production on Vertosols. Regardless, these data should still provide farmers in many agricultural disciplines with additional management levers for maximising NFUE.

The next chapter explores the N uptake capabilities and preferences of the most common commercial cotton species, *G. hirsutum*. Bulk fertiliser N recovery has been previously well characterised in *G. hirsutum*; however, the N speciation of this uptake remains unknown. This research gap is explored through a glasshouse experiment, and represents a potential opportunity to improve fertiliser recovery in Australian cotton systems. Special focus is placed on the role of DON in crop nutrition, and how this relates to plant-available N paradigms.

Chapter 5 – Organic Nitrogen and the Uptake Preferences of *Gossypium hirsutum* L.

5.1 – Introduction

Chemically reactive N exists in the environment in a range of molecular forms and weights. The molecular masses of nitrogenous molecules can range from 18-62 g mol^{-1} for LMW inorganic aqueous and gaseous molecules like NO_3^- and N_2O, 75-3000 g mol^{-1} for DON molecules like humic and amino acids, and 10^2-10^6 g mol^{-1} for macro-biomolecules like phospholipids and proteins (Perminova et al., 2003). Nitrogen continually fluxes between these different compounds, with no molecule providing permanent immobilisation (Lehmann and Kleber, 2015). To simplify N management, these molecules are typically aggregated into different pools based on context-specific functionality. As outlined in Section 4.1, this thesis categorises the spectrum of agriculturally-relevant water- and soil-borne nitrogenous molecules into four pools: inorganic N, DON, synthetic N, and insoluble organic N. The size of these pools can vary considerably throughout the year (Fig. 3.2; Farrell et al., 2011), and Australian cotton growers are strongly encouraged to monitor them, especially prior to planting and fertiliser application (CRDC, 2020b).

Soil N and C are intrinsically linked through SOM, with the terms soil organic N and soil organic C referring to the same molecules in most cases (Fig. 5.1). SOM typically constitutes the largest N pool in healthy soils, and contains more C than the atmosphere and global vegetation combined (Bond-Lamberty et al., 2020; Lehmann and Kleber, 2015). Globally, soils have lost approximately half of their C stocks since the adoption of soil cultivation techniques (Paustian et al., 2000), and have contributed more than 50 x 10^{15} grams of C to the atmosphere (Paustian et al., 1997). This is also true in Australia, where European agricultural practices like soil cultivation and stubble burning have decreased soil C stocks significantly over the past century (Luo et al., 2010).

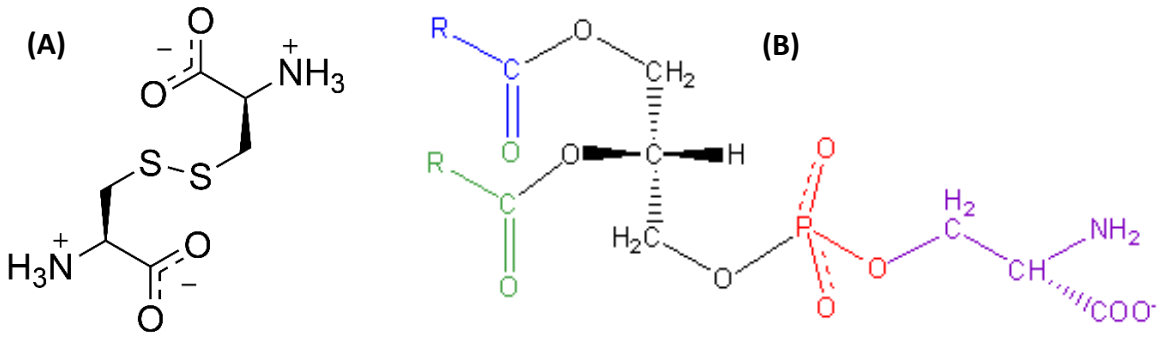

Figure 5.1. *Examples of organic molecules commonly found in soils. SOM links C, N, P and S. Increasing SOM stores requires that all of these elements be present (Kirkby et al., 2011).* ***(A)*** *The dipeptide cystine is a dimer of two amino acids and has a chemical formula of $C_6H_{12}N_2O_4S_2$.* ***(B)*** *Phosphatidylserine is an example of a phospholipid, one of the most common biological molecules. Images sourced from their respective Wikipedia entries.*

SOM is frequently overlooked when considering crop nutrition, with the focus often exclusively directed at inorganic N (NO_3^- and NH_4^+). Since the mid-19th century and the dismissal of the humus theory of plant nutrition, the dominant agricultural paradigm has held that plants only take up N from the soil in the inorganic forms of NH_4^+ and NO_3^-. This limited view of plant nutrition pervaded to the end of the 20th century, likely due to the ease with which inorganic N can be measured, the fact that it is definitely plant-available, and an increased understanding of the role microbes play in soil N cycling combined with an over-extrapolation of plants' reliance on them for nutrition (Schimel and Bennett, 2004). In addition, the rise of Haber-Bosch-derived fertilisers at the beginning of the

20th century shifted the focus away from organic soil amendments and fixation as the primary means of increasing plant-available N. With productivity gains so easily achieved by synthetic fertilisers, the research community also shifted its focus toward optimising this new tool.

Research demonstrating the importance of organic N in plant nutrition is not new (Vantsis and Bond, 1951; Virtanen and Linkola, 1946; Waksman, 1932). However, it was not until the discoveries that organic soil N can be the principal N pool for some plants (Kielland, 1994), and that LMW organic N molecules can be taken up intact by plants (Näsholm et al., 1998; Näsholm et al., 2000b) that the mainstream view of terrestrial N dynamics shifted to include direct plant uptake of SOM from the soil. The uptake of soil organic N has been documented in many plant species, but has not yet been observed in commercial cotton (*Gossypium hirsutum* L.).

The most common modern technique for measuring N uptake by plants is stable isotope geochemistry (Hoefs, 2015; Sharp, 2017). For nitrogenous fertilisers, whether organic or inorganic, this involves using ^{15}N-labelled compounds. These lab-created compounds have artificially skewed isotope ratios that can be tracked through systems by comparing the isotope fractionations of samples to known natural abundances using mass spectrometry (IAEA, 1983). In the case of N analyses, atmospheric N_2 is the most common reference standard, which has an average ^{14}N and ^{15}N composition of 99.634% and 0.366% respectively (Chalk et al., 2014). Intact uptake of organic N molecules is typically demonstrated with bulk stable isotope analysis (BSIA) using dual labelled ^{15}N and ^{13}C compounds (Chalk et al., 2014). However, as is frequently the case with studies of this nature, the validity of this methodology has been the subject of debate (Näsholm et al., 2009a; Rasmussen and Kuzyakov, 2009). Dion et al. (2018) have since demonstrated intact uptake of both D- and L-alanine isomers in cucumbers (*Cucumis sativus*) using a different technique, position-specific labelling and compound-specific stable isotope analysis. This further supports the uptake finding of BSIA studies, and lends further weight to the modern model of direct plant uptake of organic N.

This chapter discusses the findings of a glasshouse experiment designed to quantify the N uptake capabilities and preferences of commercial cotton. Australian cotton plantings are almost exclusively comprised of *G. hirsutum* (also known as upland cotton or Mexican cotton), which is derived from South America and makes up 90% of the global market. No previous experiments have demonstrated *G. hirsutum*'s ability to take up organic N, or its preference for one N species over another. In this experiment, four N species (NO_3^-, NH_4^+, urea and alanine) were concurrently added to three *G. hirsutum* varieties. The first experimental hypothesis was that *G. hirsutum* would not exhibit a N uptake preference between the different N species. The second hypothesis was that alanine—N and alanine—C uptake would correlate, indicating that alanine is taken up whole by *G. hirsutum*. The third hypothesis was that urea—N and urea—C uptake would correlate, indicating that urea is taken up whole by *G. hirsutum*. The fourth experimental hypothesis was that the three *G. hirsutum* varieties would not exhibit different N uptake behaviours. This experiment was not designed to emulate natural conditions, but rather to assess the plant's capabilities and preferences.

5.2 – Materials and Methods

5.2.1 – Experimental Design

Three varieties of *G. hirsutum* were grown to a two- to four-leaf stage in a randomised block design with 10 replicates. The three varieties used were: Sicot 746B3F, a GM current commercial cultivar accounting for more than half (54%) of the 2017-18 Australian summer plantings; Sicala V2, an obsolete non-GM commercial cultivar released in 1994; and Tx III, a Guatemalan landrace accession that represents the native origins of the commercial *G. hirsutum* varieties. Plants were grown in 300 mm deep low-density polyethylene (LDPE) pots, consistent with the rhizotube design used by Hill

and Jones (2018). Tubes were open-bottomed, allowing drainage and preventing waterlogging. Pasteurised and washed sand of <1 mm particle size was used as the growing medium to enable complete control over plant-available N pools. Plants were watered using a nutrient solution mixture that constituted 100% of the plants' soil-derived nutrient supply (Table 5.1). The plants were grown in glasshouses at the CSIRO Black Mountain site in Canberra, ACT (35°16'S 149°7'E) in January (summer) 2018. The high solar radiation experienced by Canberra in summer required the use of 70% shade cloth, as sand temperatures exceeded 60°C when exposed to direct sunlight.

Table 5.1. *Nutrient solution prepared based on the Hoaglands solution and recommendations from Oliver Knox, University of New England. Mass and mol per cent are calculated excluding oxygen and hydrogen. Solution was pH balanced to 7 ± 0.05 and EC kept below 1000 µS cm^{-1} to reduce the chance of acidification or salinisation.*

Element	Mass Per Cent	Mol Per Cent	Compounds
N	25.17%	44.29%	KNO_3 , $Ca(NO_3)_2$, FeNaEDTA
P	3.61%	2.88%	KH_2PO_4
K	27.38%	17.25%	KNO_3
Ca	23.38%	14.38%	$Ca(NO_3)_2$
C	2.80%	5.75%	FeNaEDTA
S	7.48%	5.75%	$MgSO_4$
Mg	5.67%	5.75%	$MgSO_4$
Na	1.08%	1.16%	FeNaEDTA , $Na_2MoO_4{\cdot}2H_2O$
Cl	0.85%	0.59%	$MnCl_2{\cdot}4H_2O$, $ZnCl_2$, $CuCl_2{\cdot}2H_2O$
B	0.584%	1.330%	H_3BO_3
Fe	1.303%	0.575%	FeNaEDTA
Mn	0.586%	0.263%	$MnCl_2{\cdot}4H_2O$
Zn	0.062%	0.023%	$ZnCl_2$
Cu	0.022%	0.008%	$CuCl_2{\cdot}2H_2O$
Mo	0.012%	0.003%	$Na_2MoO_4{\cdot}2H_2O$

All plants were dosed with a N solution containing a mixture of NO_3^-, NH_4^+, urea and alanine. One of the four N compounds was isotopically-labelled in each treatment, with a fifth control treatment containing no labelling (Table 5.2). The four N compounds were supplied in the same concentration to every seedling, proportionally to the three bioavailable N pools found in intensive agricultural environments: ~33% inorganic N (NO_3^- and NH_4^+), ~33% DON (alanine), and ~33% synthetic N (urea).

Table 5.2. *Nitrogen* treatments for each of the three G. hirsutum varieties included in this study. *The total N concentration of all solutions was 2.95 mmol N L^{-1}, with approximately 1 mmol N L^{-1} apportioned to each of the three plant-available N pools outlined in this study: mineral N, synthetic N and DON.*

	N Treatment	Nitrate	Ammonium	Urea	Alanine
Plant-Available N Pool:		Mineral N	Mineral N	Synthetic N	LMW DON
Nitrogen Isotope Purity:		99.8% ^{15}N	99.5% ^{15}N	99% ^{15}N	99% ^{15}N
Carbon Isotope Purity:		-	-	98% ^{13}C	99% ^{13}C
Concentration (mmol N L^{-1}):		0.61	0.35	1.00	1.00
Concentration (mmol C L^{-1}):		-	-	0.50	3.00
	0	Unlabelled	Unlabelled	Unlabelled	Unlabelled
	1	^{15}N-Labelled	Unlabelled	Unlabelled	Unlabelled
	2	Unlabelled	^{15}N-Labelled	Unlabelled	Unlabelled
	3	Unlabelled	Unlabelled	^{15}N-^{13}C-Labelled	Unlabelled
	4	Unlabelled	Unlabelled	Unlabelled	^{15}N-^{13}C-Labelled

5.2.2 – Plant Dosing and Processing

Once plants reached a two- to four-leaf stage, the rhizotube pots were injected with 2 ml of solution at three points around the root base, with special care taken to avoid injecting into any roots (Fig. 5.2).

Plants were then placed in direct sunlight for their allocated uptake period (5, 60 and 180 minutes). After the treatment times had elapsed, plants were carefully extracted from their pots, thoroughly washed of substrate, bagged, and placed on dry ice ($CO_{2\ (s)}$) to halt metabolism. Plants were transferred to ovens to air dry (at 40°C) completely at the end of each day. Dried samples were weighed and ground to a fine powder using a ball-mill. Ground samples were analysed for $\delta^{13}C$ and $\delta^{15}N$ using isotope ratio mass spectrometry (IRMS) at the Research School of Biology at the ANU, Canberra, ACT. In total, 450 seedlings were analysed, comprising 10 replicates each of 45 unique treatments: five N treatments, three *G. hirsutum* varieties, and three isotope exposure times.

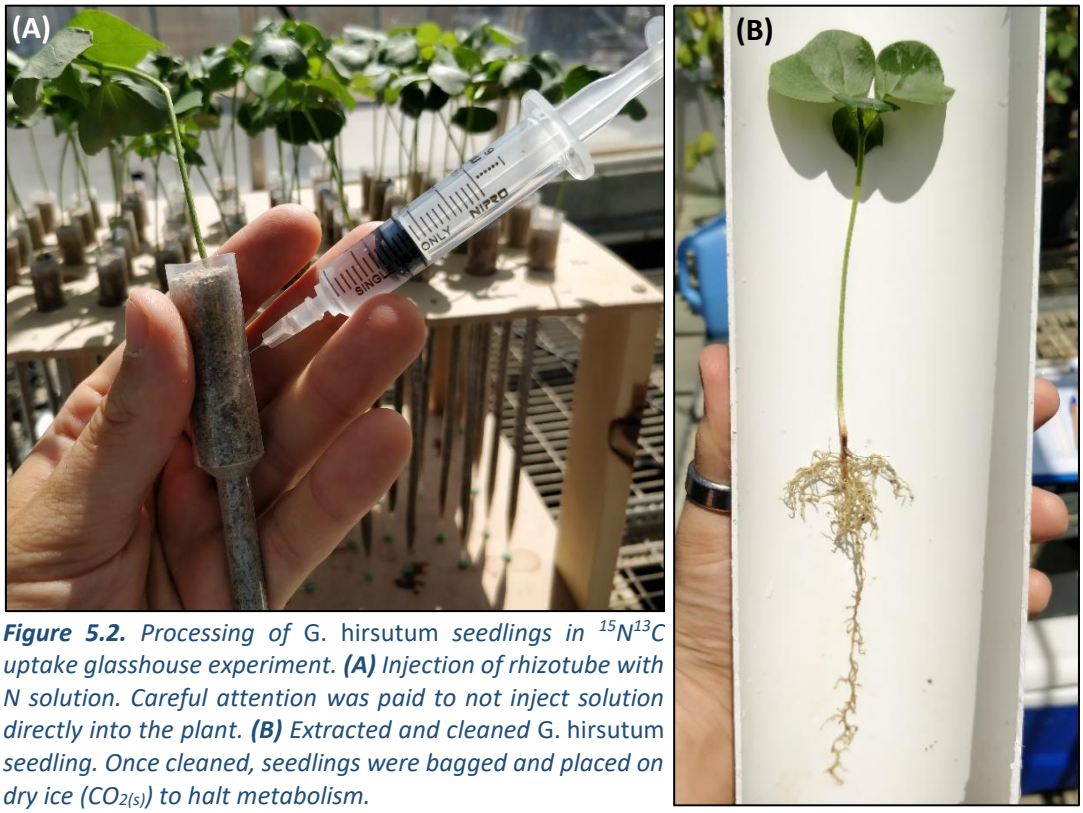

Figure 5.2. *Processing of* G. hirsutum *seedlings in* $^{15}N^{13}C$ *uptake glasshouse experiment.* ***(A)*** *Injection of rhizotube with N solution. Careful attention was paid to not inject solution directly into the plant.* ***(B)*** *Extracted and cleaned* G. hirsutum *seedling. Once cleaned, seedlings were bagged and placed on dry ice* ($CO_{2(s)}$) *to halt metabolism.*

5.2.3 – Data Processing

Understanding and processing isotope data can be challenging, with few clearly written guides available for, or methodological communications pitched at, beginners. This may be a product of the stable isotope research community generally consisting of experienced academics, with mass spectrometers expensive to own and operate and commonly inaccessible to junior researchers (this experiment cost more than half of this PhD's research budget). In response to this apparent gap in the publicly available documentation of stable isotope processing, there follows here a brief workflow guide explaining how isotope data is processed and interpreted.

When using IRMS data, isotope values can be expressed in relative units, called delta values (δ) and reported as 'per mil' (parts per thousand), or they can be reported in absolute units as Mass Fractions (X; Chalk et al., 2014). Delta values are reported with reference to a standard that could be a natural reference standard like atmospheric N_2 or the Vienna Pee Dee Belemnite (VPDB), or to the experimental control. Delta values are most appropriate when reporting small deviations in isotopic ratios, like the natural fractionation processes that happen in C3 plants like *G. hirsutum* (Chalk et al., 2015). Absolute Mass Fractions or Atomic Percentages are appropriate when reporting large deviations in isotopic fractionation, such as when using isotope labelled compounds to deliberately skew ratios.

In this experiment, data was received from the IRMS as relative $\delta^{15}N$ and $\delta^{13}C$ per mil values; however, due to the large shifts in isotopic ratios derived from the artificially labelled fertilisers, absolute units would be a more appropriate way to report the findings. Percentage fertiliser uptake was chosen as the most informative final reporting metric, but these data could be presented in several formats. The process of transposing relative $\delta^{15}N$ and $\delta^{13}C$ values to absolute percentage uptake values is described in Figure 5.3 and Equations 5.1, 5.2, 5.3 and 5.4. All equations listed below were used for both N and C calculations, with the ^{15}N and ^{14}N equation pronumerals substituted with ^{13}C and ^{12}C respectively in the C calculations.

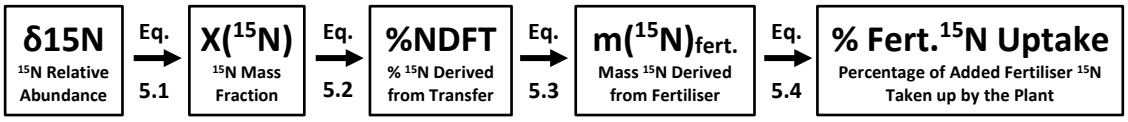

Figure 5.3. *Four step calculation of absolute value percentage fertiliser ^{15}N uptake from IRMS output of relative δ values.*

$$X(^{15}N)_{sample} = \frac{R(^{15}N)_{standard} \cdot (\frac{\delta^{15}N}{1000} + 1)}{1 + (R(^{15}N)_{standard} \cdot (\frac{\delta^{15}N}{1000} + 1))}$$

Equation 5.1. *Calculation of mass fraction (X) from relative isotopic enrichment (δ). $R(^{13}C)_{standard} = 0.011178$ and $R(^{15}N)_{standard} = 0.00367647$.*

$$\%NDFT = \frac{\%\ ^{15}N\ Atom\ Excess_{sample}}{\%\ ^{15}N\ Atom\ Excess_{donor}} \times 100\% = \frac{X(^{15}N)_{sample} - X(^{15}N)_{variety\ control}}{X(^{15}N)_{label} - X(^{15}N)_{variety\ control}} \times 100\%$$

Equation 5.2. *Calculation of percentage N derived from transfer (%NDFT) from mass fractions (X) of sample, isotope label, and cultivar variety control. $X(^{15}N)_{label} = \%^{15}N\ Purity_{label}$ and $X(^{15}N)_{variety\ control} = (\sum X(^{15}N)_{control}) \div n$, where n = number of replicates (always 10 in this experiment).*

$$m(Fert.^{15}N)_{taken\ up} = \%NDFT \times m(Plant)_{dry} \times Plant\ \%N$$

Equation 5.3. *Calculation of the mass of fertiliser ^{15}N taken up by the plant using %NDFT, plant dry mass (grams), and plant percentage N.*

$$\%\ Fert.^{15}N\ Uptake = \frac{m(Fert.^{15}N)_{taken\ up}}{m(Fert.^{15}N)_{added}} = \frac{m(Fert.^{15}N)_{taken\ up}}{[Label - N] \times \%Purity \times V_{fert.sol.} \times M_r(^{15}N)}$$

Equation 5.4. *Calculation of percentage fertiliser ^{15}N uptake by plant using molar mass (M_r) of ^{15}N (15 mg $mmol^{-1}$), percentage purity (%Purity) of isotope labels (98.0-99.8%), N concentration (mmol L^{-1}), and volume (L).*

5.2.4 – Data *Analysis*

The majority of statistical analyses were performed in R (R Core Team, 2020) using code written by James Latimer and Mark Farrell. Additional to base R, several packages were used including *lmerTest* for constructing linear mixed effect models (Kuznetsova et al., 2017), *emmeans* for calculating estimated marginal (least-squares) means (Lenth, 2020), *lubridate* for date-time format modification (Grolemund and Wickham, 2011), *ggsci* for figure creation (Xiao, 2018), and *tidyverse* for a range of

additional applications (Wickham et al., 2019). Some analyses were also performed using the statistical software package JMP (Jones and Sall, 2011). Based on the high replication of this experiment (n = 10), estimated marginal means are reported instead of sample means.

5.3 – Results

5.3.1 – Growing Phase

The washed and pasteurised sand used as the growing medium was analysed using an Elementar VarioMAX CNS elemental analyser to ensure that total N and total C concentrations were negligible prior to planting (Table 5.2).

Table 5.2. *Total C and total N in washed and pasteurised sand growing medium prior to planting (n = 5). Negligible soil nutrition ensured that the supplied nutrient solutions were the sole source of root nutrition.*

Parameter	Total Nitrogen	Total Carbon
Mean Value:	0.00313 wt. %	0.02992 wt.%
Standard Deviation:	0.00038	0.00265
Standard Error:	0.00017	0.00118

The three *G. hirsutum* varieties did not exhibit uniform germination characteristics (Fig. 5.4). The two commercial cultivars – Sicot 746B3F and Sicala V2 – showed similar rapid germination three to five days after planting, a product of generational selective breeding. The landrace accession Tx displayed much slower germination characteristics, but still reached a comparably high germination rate after three weeks. Upon conclusion of the experiment, the Sicot, Sicala and Tx varieties achieved germination rates of 88%, 97% and 86% respectively.

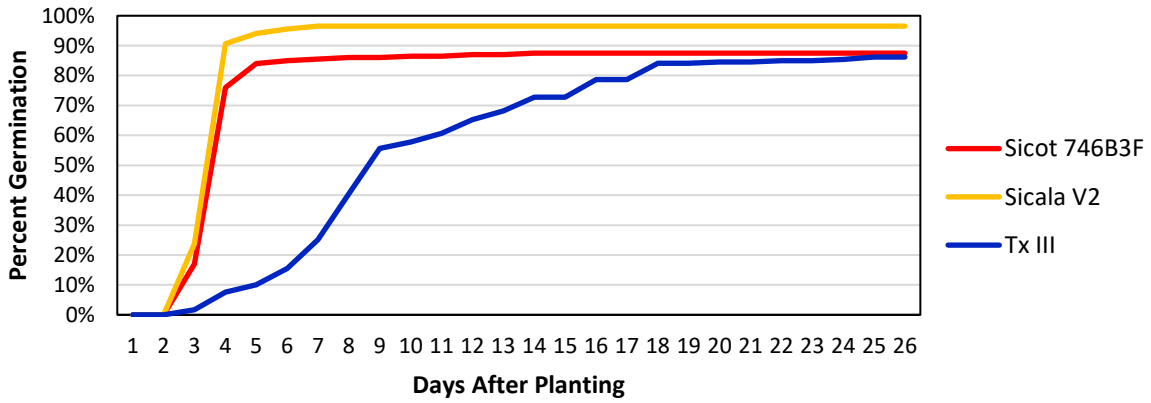

Figure 5.4. *Germination percentage for each of the three* G. hirsutum *varieties: Sicot 746B3F, a GM current commercial cultivar; Sicala V2, an obsolete non-GM commercial cultivar; and Tx III, a Guatemalan landrace accession. n = 720 seeds.*

5.3.2 – Nitrogen Uptake

When added concurrently, NO_3^-, NH_4^+, urea (CH_4N_2O) and alanine ($C_3H_7NO_2$) were all rapidly utilised by the three *G. hirsutum* varieties (Fig. 5.5). After 180 minutes, the average plant uptake for each N species was between 14.3 ± 1.9% and 25.1 ± 1.9% of added fertiliser N (Fig. 5.6; Table 5.3). Neither total nor speciated N uptake were statistically different between the three *G. hirsutum* varieties ($p = 0.20$; $p = 0.47$). Differences in total N uptake from the different N species were significant ($p < 0.001$). After 180 minutes, the uptake of NH_4^+–N and alanine—N were not statistically different ($p = 0.77$). Neither were NH_4^+–N and urea-N ($p = 0.11$). The uptake of alanine—N and urea—N represented the only uptake difference between the varieties, and were statistically different in the

landrace accession Tx III ($p < 0.05$) but not the two commercial cultivars ($p = 0.58$). Uptake of NO_3^-–N and alanine—N, and NO_3^-–N and NH_4^+–N were both statistically different across all varieties ($p < 0.01$), while NO_3^-–N and urea—N were not ($p = 0.63$). All three *G. hirsutum* varieties showed a small preference for inorganic N (NO_3^- and NH_4^+) over organic N (alanine and urea), with respective mean uptakes of added N 20.9 ± 0.04% and 18.3 ± 0.04% for the two N pools.

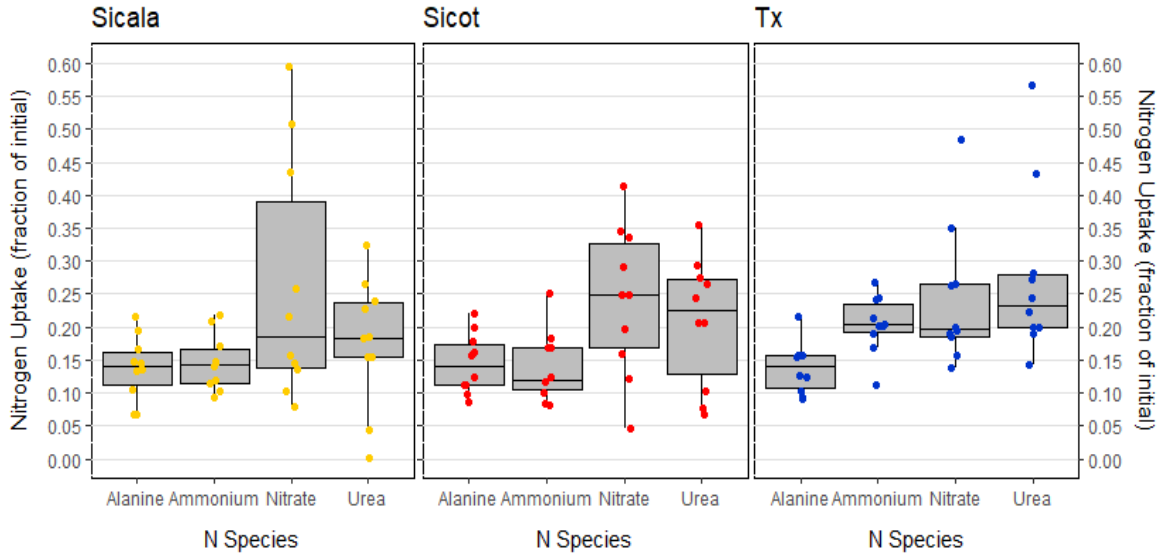

Figure 5.5. *Speciated N uptake after 180 minutes (nominal) for each* G. hirsutum *variety: Sicot 746B3F, Sicala V2 and Tx III. The four N species NO_3^-, NH_4^+, urea (CH_4N_2O) and alanine ($C_3H_7NO_2$) were added concurrently.*

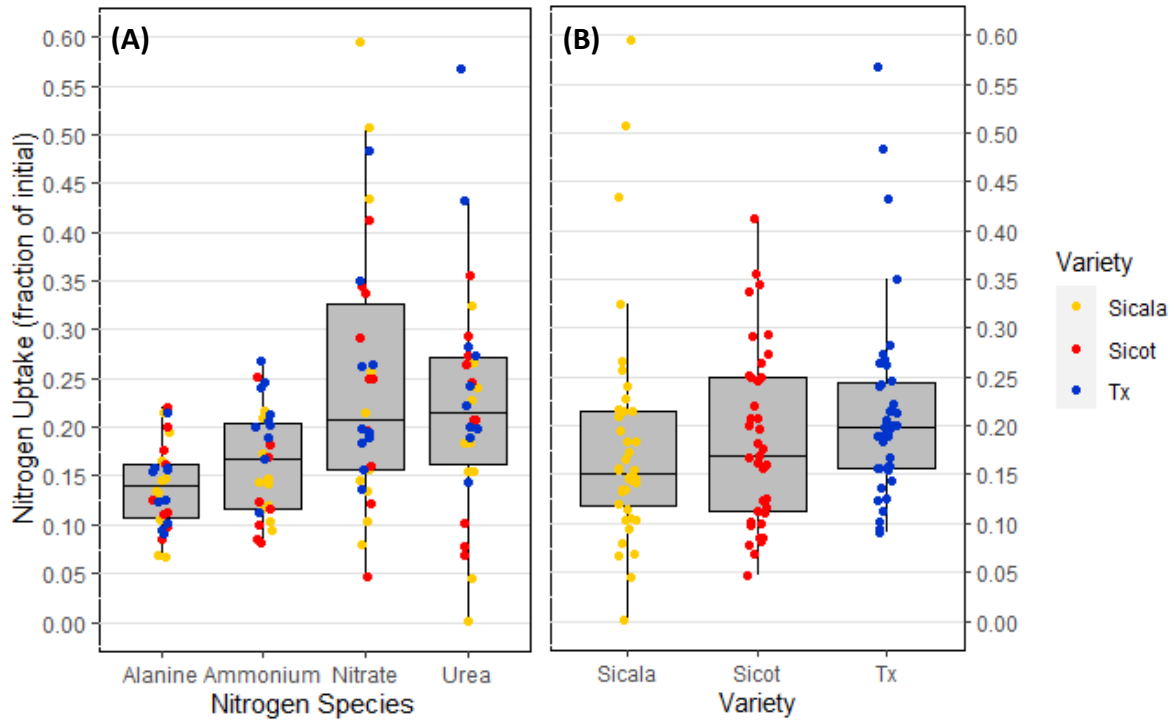

Figure 5.6. *Nitrogen uptake after 180 minutes (nominal).* ***(A)*** *Speciated average N uptake for all varieties. Speciated N uptake was not statistically different between the three* G. hirsutum *varieties.* ***(B)*** *Total N uptake for each* G. hirsutum *variety. Nitrogen uptake was not statistically different between the three varieties.*

Table 5.3. G. hirsutum *estimated marginal mean uptake of N species after 180 minutes of exposure. Values reported as "per cent of added" ± standard error.*

Nitrogen Species	Mean Uptake (per cent of added)
Alanine	14.3 ± 1.9%
Ammonium	16.6 ± 1.9%
Nitrate	25.1 ± 1.9%
Urea	22.2 ± 1.9%
Inorganic N (NO_3^- + NH_4^+)	20.9 ± 3.8%
Organic N (alanine + urea)	18.3 ± 0.38%

Uptake of all N species presented as linear over the 180 minute experiment window, suggesting zero order kinetics over this timeframe. Supplied N was taken up at rates of 4.6-8.1% of added fertiliser N per hour, equating to 0.28-2.11 mg N $hour^{-1}$ (Table 5.4).

Table 5.4. *Linear N uptake rate of each fertiliser species for average of all three* G. hirsutum *varieties (n = 90).*

Nitrogen Species	Uptake Rate (% uptake per hour)	Uptake Rate (Hours for 100% Uptake)	Fit (r^2)
Alanine	4.6%	21.7	0.997
Ammonium	5.4%	18.6	0.997
Nitrate	8.1%	12.3	0.994
Urea	7.1%	14.1	0.973

5.3.3 – Carbon Uptake

Alanine—C was taken up linearly over the 180 minute window by all *G. hirsutum* varieties, similarly to alanine—N (Fig. 5.7). There were no statistically significant differences in alanine—C or alanine—N uptake between the three *G. hirsutum* varieties (p = 0.73). C and N were taken up in a consistent ratio of approximately 0.32:1 (Fig. 5.8).

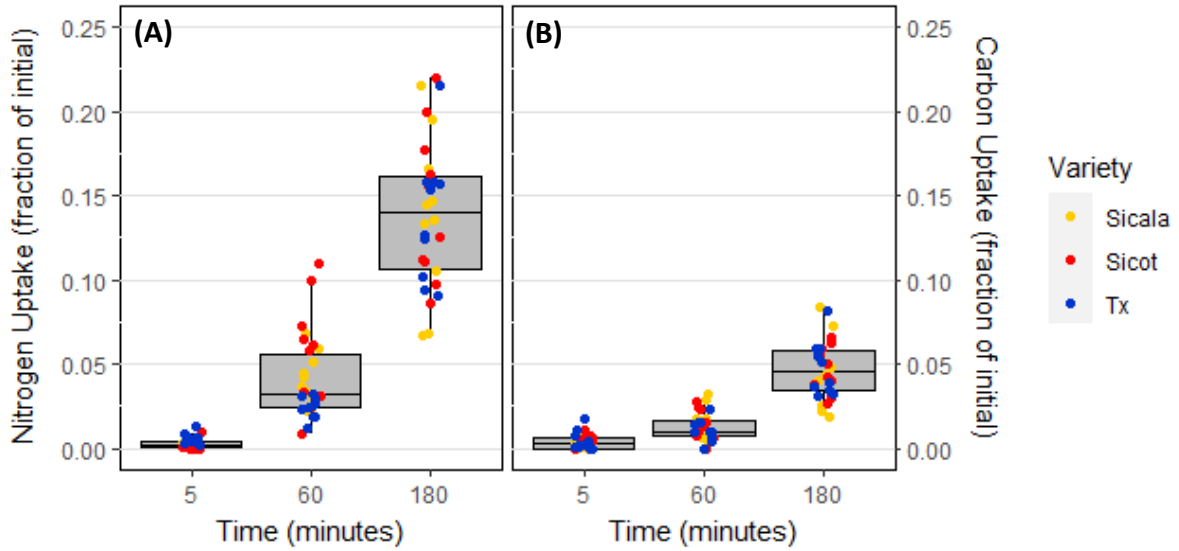

Figure 5.7*. Average alanine uptake of all* G. hirsutum *varieties over the three sampling intervals.* ***(A)*** *Alanine—N uptake.* ***(B)*** *Alanine—C uptake.*

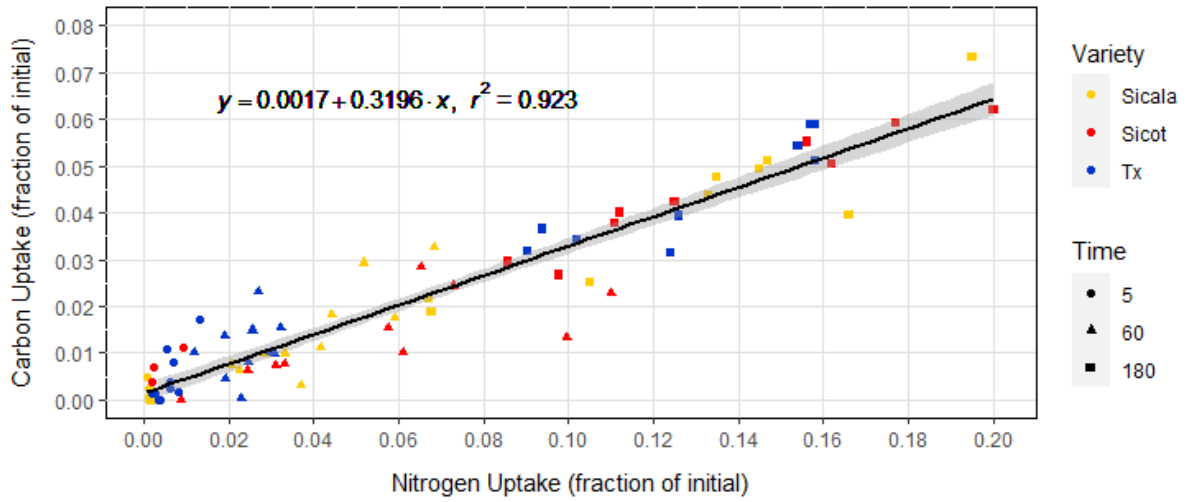

Figure 5.8. *Alanine—C uptake against alanine—N uptake plotted as fraction of initial concentration. Equation describes the linear regression that is plotted in black with 95% confidence interval in grey. Data point shapes denote isotope exposure times in minutes. The uptake of alanine—C and alanine—N are highly correlated (r^2 = 0.92, p < 0.001).*

The uptake of urea—C is the only measured element not to experience consistent linear take-up over the 180 minute experiment window (Fig. 5.9). Over the full 180 minutes, no correlation was found between urea—C uptake and variety or urea—N uptake (p = 0.96; Fig. 5.10). Internal plant urea—C concentration rose over the first 60 minutes, and then fell back to almost zero by the end of the 180 minutes.

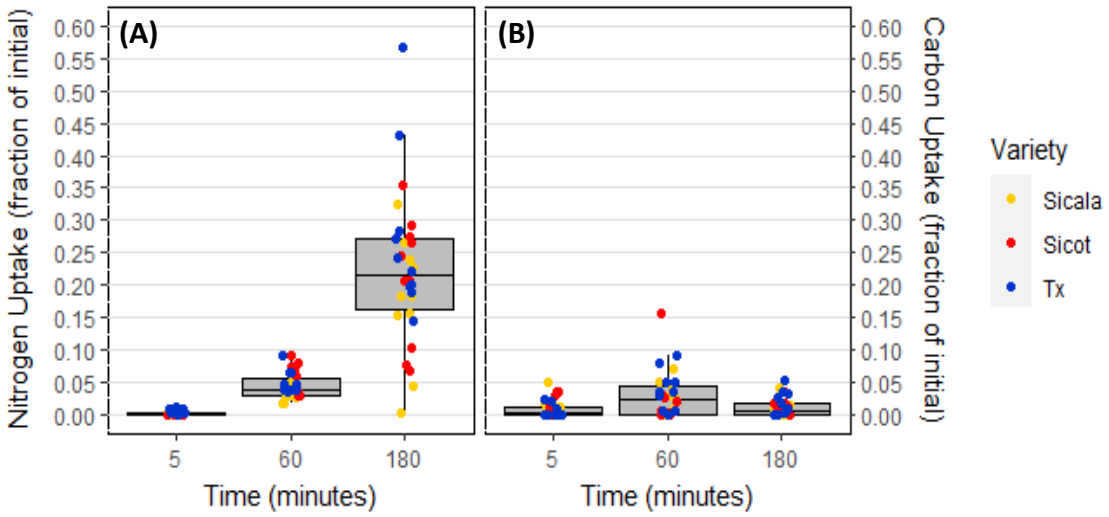

Figure 5.9. *Average urea uptake of all* G. hirsutum *varieties over the three sampling intervals.* ***(A)*** *Urea—N uptake.* ***(B)*** *Urea—C uptake, which does not increase consistently over time.*

The full data set from this experiment is published and accessible on the CSIRO DAP as Latimer and Farrell (2020).

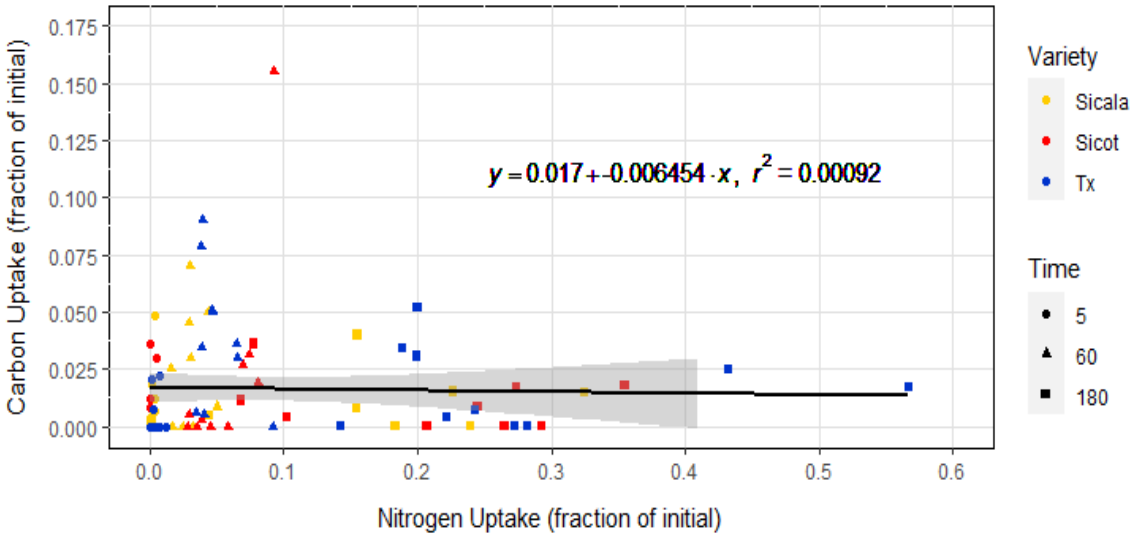

Figure 5.10. *Urea—C uptake against urea—N uptake plotted as fraction of initial concentration. Equation describes the linear regression that is plotted in black with 95% confidence interval in grey. Data point shapes denote isotope exposure times in minutes. Urea-N and urea-C uptake do not correlate over the whole 180 minute experimental window ($r^2 < 0.001$, $p = 0.96$).*

5.4 – Discussion

5.4.1 – Direct Plant Access of Soil Organic Nitrogen

In recent years, studies have shown that a wide variety of plants can take up organic N, including: trees (Gruffman et al., 2014; Gruffman et al., 2013; Leberecht et al., 2016; Li et al., 2016; Liu et al., 2017; Liu et al., 2018; Wei et al., 2013; Wei et al., 2014), grasses and sedges (Jiang et al., 2015; Moore et al., 2018; Robinson et al., 2011; Vinall et al., 2012; Xu et al., 2004), mosses and lichens (Dahlman et al., 2004; Moore et al., 2018; Song et al., 2016), fruits (Dion et al., 2018; Zhou et al., 2016), and broadacre crops including wheat, maize, chicory and lupine (Moran-Zuloaga et al., 2015; Näsholm et al., 2000a; Saia et al., 2013). The experiment presented in this chapter expands the list of plants known to take up organic N to include commercial cotton (*G. hirsutum*), a high-value, high-input pillar crops.

This experiment shows that LMW soil organic N can be a source of nutrition for commercial cotton (*G. hirsutum*). Building on these results, it is not unreasonable to assume that LMW SOM comprises a meaningful portion of Australian irrigated cotton's N uptake, given that cotton obtains the majority of its N from the soil (Macdonald et al., 2017a) and that organic N is a major soil N pool in most agricultural soils (Farrell et al., 2011; Prendergast-Miller et al., 2015). However, as this experiment was not designed to emulate real-world conditions, further soil-based glasshouse and *in situ* experiments will be required to comment further on this. This experiment also demonstrates that *G. hirsutum* does not exhibit any extreme uptake preferences between the four N species added. All four N species were taken up concurrently rather than sequentially, suggesting that cotton is not very selective about the form in which it receives N. All three *G. hirsutum* varieties exhibited a small preference for inorganic N (NO_3^- and NH_4^+) over organic N (alanine and urea), with respective mean uptakes of the two N pools 20.9 ± 0.04% and 18.3 ± 0.04% of added N. These research findings are consistent with those observed in Australian sugarcane (*Saccharum officinarum* L.), which is produced in high-input systems comparable to Australian irrigated cotton. Similarly to the results discussed in this chapter, sugarcane has been shown to readily take up soil amino acids, which can constitute a significant source of N for commercial crops (Robinson et al., 2011; Vinall et al., 2012).

The *G. hirsutum* plants used in this experiment were grown from seed in washed and pasteurised river sand. While this may not have completely eliminated mycorrhizal fungi from the growing environment, it will most likely have significantly diminished their presence and role in plant N uptake. This experiment did not test for mycorrhizal activity, but it is a credible assumption that the *G. hirsutum* seedlings took up the majority of the alanine—N and urea—N without the aid of symbiotic mycorrhizal fungi (Adamczyk et al., 2008; Chapin III et al., 1993; Kielland, 1994).

5.4.2 – Organic Carbon Uptake Mechanism

Similarly to alanine—N, alanine—C was taken up linearly over the 180 minute window by all *G. hirsutum* varieties. The consistent internal seedling ^{13}C:^{15}N ratio of 0.32:1 suggests that approximately 32% of the alanine—N taken up was in the form of whole alanine molecules. Many other studies have already demonstrated the intact uptake of the LMW DON molecules alanine (Hill and Jones, 2018), arginine (Gruffman et al., 2014), glycine (Gallet-Budynek et al., 2009), acetate (Moran-Zuloaga et al., 2015) and trialanine (Farrell et al., 2014), as well as plant consumption of whole proteins and microbes (Adamczyk et al., 2008; Hill et al., 2013; Paungfoo-Lonhienne et al., 2010), but never before in *G. hirsutum*. While it is possible that the alanine—C taken up was not in the form of intact alanine, functionally this is indistinct from intact alanine uptake (Fig. 5.11). The majority of alanine—N taken up was not associated with alanine—C, suggesting that extracellular deamination is the dominant alanine—N uptake pathway. It is also possible that all absorbed alanine—N was as whole alanine molecules, and that approximately 68% of the alanine—C was subsequently expelled post-intracellular deamination. Regardless, these results suggest that at least some alanine was taken up whole.

In the early stages of growth, much N taken up from the soil is typically transported to the leaves to create proteins like Ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco; Feller et al., 2008; Perchlik and Tegeder, 2018). Taking up intact amino acids and transporting them whole may provide a biochemical energy advantage over taking up inorganic N and assembling amino acids from it. This should be explored further, with previous studies already confirming that high organic N uptake can alter plant internal N distribution (Vinall et al., 2012).

Urea—C uptake did not follow the same linear trend observed in the other N and C species, with internal plant urea—C concentration increasing over the first 60 minutes and subsequently falling to almost zero by 180 minutes. This was true for all three *G. hirsutum* varieties, with no significant differences between them ($p = 0.34$). One possible mechanistic explanation for this behaviour would be the intact uptake of some urea, followed by intracellular ammonification and subsequent expulsion of the urea—C as CO_2. This mechanism can be loosely described by a polynomial model with peak urea–^{13}C content occurring approximately 90 minutes after dosing (Fig. 5.12). During the first 60 minutes, urea—C and urea—N uptakes do correlate ($p = 0.013$), suggesting that the intact uptake theory has some merit; however, upon further interrogation, the relationship appears tenuous at best (Fig. 5.13). While the dominant urea—N uptake pathway appears to involve extracellular ammonification, there is likely additional complexity and this should be explored further in future experiments.

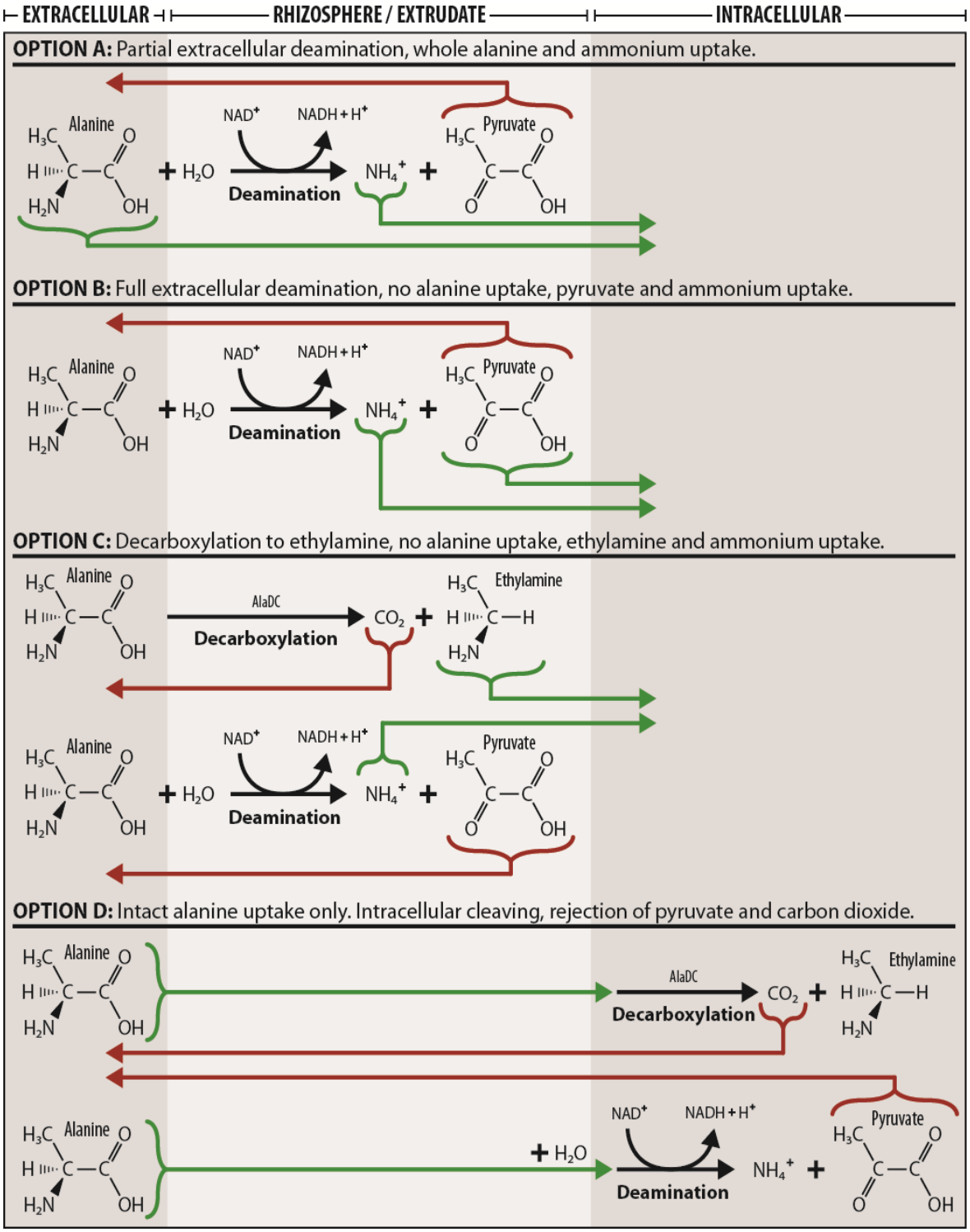

***Figure 5.11**. Four potential alanine—N and –C uptake scenarios. Experimental results suggest the majority of alanine—N is taken up as an amine functional group, potentially NH_4^+, which would likely mean extracellular deamination. Alanine—C uptake suggests that up to 32% of alanine—N was taken up as whole alanine molecules. Actual uptake behaviour may be a combination, or none, of these options. AlaDC is an abbreviation of the enzyme alanine decarboxylase.*

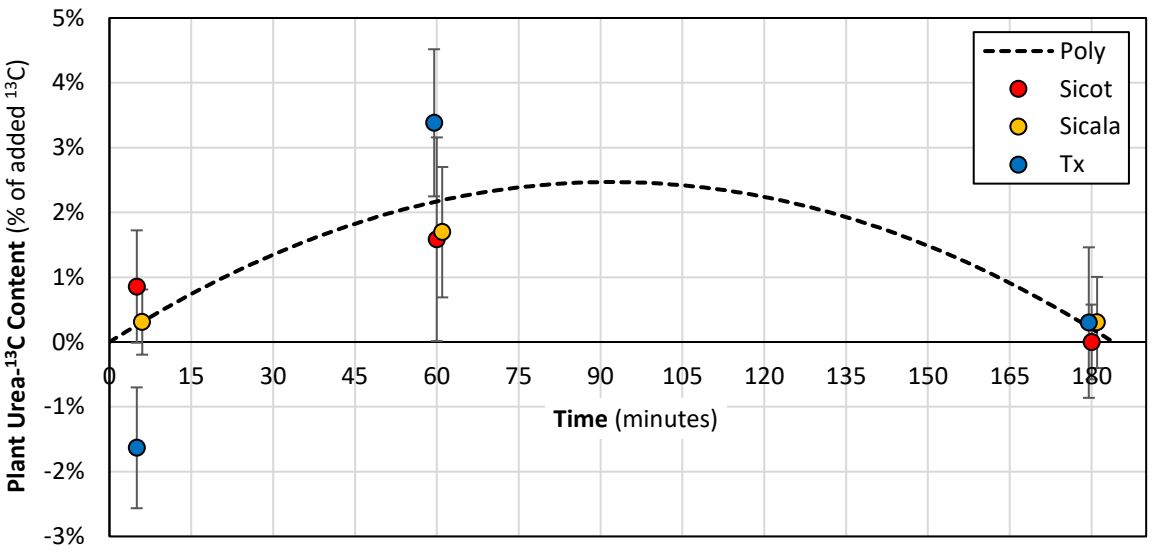

Figure 5.12. *Average internal urea–^{13}C concentration at time intervals 5, 60 and 180 minutes for each* G. hirsutum *variety as a per cent of added urea—C. Sample means plotted with standard error. A potential urea—C uptake curve is shown in black, and is loosely described by the quadratic function Urea–$^{13}C = -2.92 \cdot 10^{-6} \cdot x^2 + 5.37 \cdot 10^{-4} x$. Datapoints have been horizontally offset in figure to show error bars more clearly, with actual points existing at concurrent time intervals.*

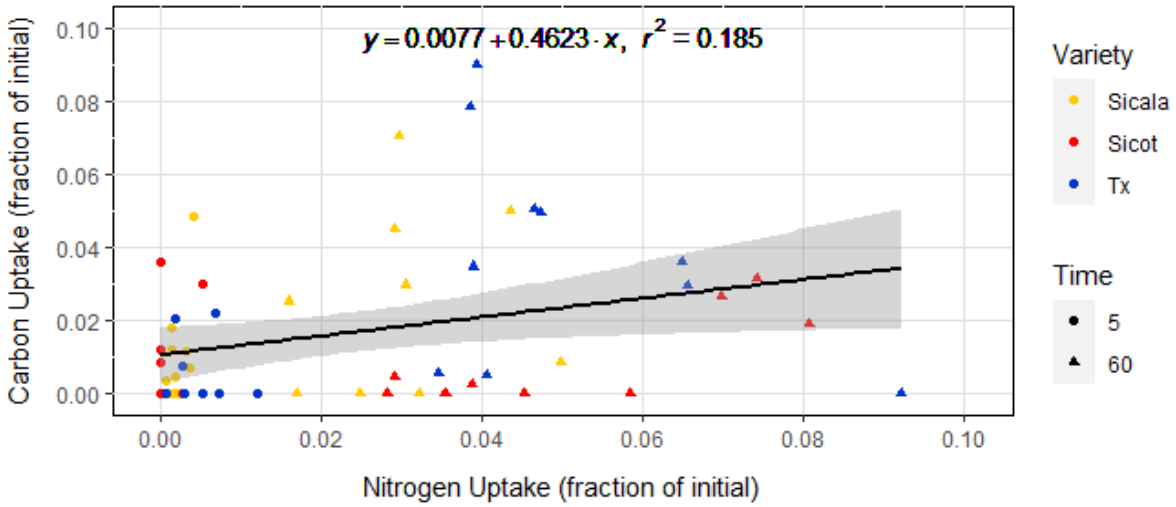

Figure 5.13. *Urea—C uptake against urea—N uptake over the first 60 minutes plotted as fraction of initial concentration. Equation describes the linear regression that is plotted in black with 95% confidence interval in grey. Data point shapes denote isotope exposure times in minutes. Urea—N and urea—C uptake correlate during the first 60 minutes of the experimental window ($p < 0.0125$); however, the relationship appears tenuous ($r^2 = 0.185$).*

5.4.3 – Management Implications

Increasing SOM in Australian cotton systems would provide many tangible environmental, social and financial benefits to the industry (Coutts et al., 2017). These include: improving soil physical properties like aggregate stability, bulk density, and water holding capacity (Ahmed et al., 2019; Li et al., 2020); improving soil chemical properties like acid neutralising capacity and cation exchange capacity (Murphy, 2015); increasing soil microbial functionality and diversity (Guggenberger et al., 1999; Ozlu et al., 2019); and sequestering atmospheric C (Minasny et al., 2017). Likely flow-on effects from the improvement of these factors include yield increases, improved resilience during drought conditions,

and improved brand recognition and industry perception. When SOM forms (immobilises), it does so from inorganic N in the soil. If one subscribed to the model of NO_3^- and NH_4^+ constituting 100% of plant N uptake, then this shrinking of the inorganic N pool would suggest a reduction in plant-available N that would be counterproductive to maximising yields. However, this experiment and the existing body of organic N uptake literature suggests that some of this SOM would remain plant-available, especially the LMW DON fraction (Chapin III et al., 1993; Kielland, 1994; Näsholm et al., 2009b). This indicates that growers could potentially achieve all of the benefits associated with increasing SOM without suffering the yield penalty of reducing plant-available N.

The wider adoption of organic amendments as fertilisers should be considered by the industry (Ma et al., 2020). The economic efficiency of this policy would be improved by utilising existing waste streams either from within the Australian cotton industry – like turning gin trash into biochar or fertilisers (Ghosh et al., 2011; Lee, 2020) – or from other industries, like using livestock or human waste streams (CRDC, 2019d; Magwaza et al., 2020; McCabe et al., 2020). The supplementation of synthetic fertilisers with organic amendments is already commonplace in Australian cotton production systems, with 30% of growers using some kind of manure in the 2018-19 season (CRDC, 2019a). This suggests that many growers are already considering the organic complexity and health of their soils, and would be receptive to more targeted industry engagement in this area.

Outside Australian cotton, many agricultural enterprises are moving away from the industrial farming model of monocultures with heavy synthetic fertiliser reliance, as demonstrated by the steady rise of organic farming (Oehl et al., 2004; Reganold and Wachter, 2016; Tuomisto et al., 2012) and conservation agriculture (Bhan and Behera, 2014; Busari et al., 2015; Hobbs, 2007; Kertész and Madarász, 2014). Interest in these new farming paradigms is not typically a response to new organic N plant uptake research, but rather to factors like land degradation and declining yields (Luo et al., 2010) or social and economic pressures from changing consumer demographics and preferences (Lea and Worsley, 2005; Lea and Worsley, 2008; Lockie et al., 2004; Malek et al., 2019). Regardless of the motivations, these alternative farming practices commonly result in improved soil health outcomes (Mahajan et al., 2019; Ozlu et al., 2019; Sarker et al., 2018). The Australian cotton industry is likely incompatible with many aspects of these alternative systems, but much could be learned and adopted from their SOM management practices. If the Australian cotton industry is to appreciably and sustainably increase SOM, it will also need to adopt more deliberate P and S management strategies to complement its N and C policies (Kirkby et al., 2011).

5.5 – Conclusions

This experiment has shown that *G. hirsutum* can rapidly and concurrently access the DON, inorganic N and synthetic N soil chemical pools. While all N species were taken up, total N uptake from each N species was different ($p < 0.001$), thus rejecting the first hypothesis. *G. hirsutum* exhibited a small preference for inorganic N (NO_3^- and NH_4^+) over organic N (alanine and urea), with respective mean uptake of 20.9 ± 0.04% and 18.3 ± 0.04% of added N for the two pools. Urea—N and NO_3^-—N were taken up more than alanine—N and NH_4^+—N. The consistent uptake ratio of alanine—C to alanine—N of 0.32:1 indicates that some alanine is likely taken up intact by the plant ($p < 0.001$, $r^2 = 0.923$). The second hypothesis can therefore be accepted. Urea—C uptake did not follow the same linear trend as was exhibited by the other measured chemical species. Over 180 minutes, no correlation was found between urea—C uptake and urea—N uptake ($p = 0.92$), indicating that urea undergoes extracellular ammonification, and necessitating the rejection of the third hypothesis. Urea—C and urea—N uptake did correlate over the first 60 minutes ($p = 0.013$), following a potential polynomial trend, but this relationship is tenuous, and requires more data before it should be given

too much weight. Total and speciated N uptake were not statistically different between the three *G. hirsutum* varieties ($p = 0.53$), leading the fourth hypothesis to be accepted.

Increasing SOM in Australian cotton systems represents a triple bottom line opportunity for the industry, and would provide many tangible environmental, social and financial benefits. Growers have the potential to improve soil parameters, sequester atmospheric C, improve drought resilience, and boost the industry's reputation without necessarily decreasing plant-available N. The Australian cotton industry should consider adopting more targeted SOM policies, and encourage growers to consider SOM when budgeting crop N requirements. Organic waste streams from within or outside the industry may provide cost-effective organic fertilisers for the industry, and this should be explored further. This experiment was not designed to emulate natural conditions, but rather to assess the plant's capabilities and preferences. The logical next step from this work would be to replicate this experiment in natural systems.

Chapter 6 – Conclusions

6.1 – Improving Precision Agriculture and Environmental Performance

The Australian cotton industry is an archetypal example of precision agriculture in action, having achieved significant efficiency gains in yield (kg lint ha^{-1}) and water use (kg lint ML^{-1}) over the past 50 years through sustained research and development investment. NFUE has not experienced the same gains over this period, which the industry has identified and is taking steps to improve. This thesis constitutes a component of the Australian cotton industry's progress towards improving NFUE. Improving NFUE can create tangible benefits for the industry and its grower constituents. Economically, it can save growers the cost of wasted fertiliser, and reduce yield discounts that can arise from N over-application. Environmentally, it can reduce N_2O GHG emissions, increase soil C stocks, and reduce N accumulation at depth. And socially, it can help to grow Australian cotton's reputation as a global leader in efficient agricultural production, and build its public profile and brand recognition.

Four research questions were determined to assess and address some of the deficiencies of current N management in the Australian cotton industry. These were:

1. How effective are current Australian irrigated cotton fertigation practices at delivering consistent N to the field, and what management levers can be identified to improve outcomes?
2. How does N runoff vary spatially and temporally at sub-field and intra-irrigation scales respectively? Additionally, can this increased resolution be used to identify specific mechanistic drivers of N runoff?
3. What is the residence time of dissolved N in irrigation water, and how does it vary across the farm environment?
4. Which chemical N species can commercial cotton (*G. hirsutum*) directly take up, and what preferences does it exhibit when given a choice? Furthermore, have genetic engineering and selective breeding practices altered these characteristics from a baseline of the native *G. hirsutum* landrace accession?

The questions were answered in sequential chapters with a strong focus placed on relating findings to on-farm management practices.

6.2 – Research Findings

Chapter 2 addressed the first research question, exploring how effectively current Australian irrigated cotton fertigation practices deliver N to crops, and what management levers can be identified to improve outcomes. Aqueous N application is increasingly popular in Australian irrigated cotton production systems, despite limited data describing its efficacy. The chapter posits that in-field aqueous N distribution is typically uniform when N supply is consistent, but that achieving this consistency is difficult. Poor urea tank management, inconsistent irrigation channel network mixing, and the colourless nature of dissolved N are all factors contributing to poor fertigation results. When applying aqueous N, growers need to calculate nutrient budgets similarly to water budgets; flow rate, exposure time, and N concentration are all important metrics to monitor and record if accurate N accounting is to be accomplished. Currently low urea prices and an absence of legislative restrictions has fostered poor N management among many Australian cotton growers. The industry context will inevitably change when fertiliser prices eventually increase and legislation governing N use is introduced. When this happens, improving NFUE will be a critical component of growers' financial (and hopefully, triple) bottom line considerations. More immediately, poor N application uniformity

can diminish crop yields, increase GHG emissions, and negatively affect soil health, which are very real, current incentives for practice improvement.

Chapter 3 addresses the second research question, exploring spatial and temporal N runoff at sub-field and intra-irrigation scales. Significant surface runoff N is being generated in alternate furrow irrigated cotton systems. This N runoff is typically captured and recirculated in Australian irrigated cotton systems, minimising the potential for eutrophication of proximal watercourses; however, it can still volatilise or become deep drainage during recirculation, representing a loss of soil nutrients and wasted fertiliser. The experimental results presented in this thesis show that discharged N and other solutes decrease over time both within and between irrigations, with most N runoff generated at the beginning of the season, and few losses occurring after the third irrigation. While some N is leached by water passing longitudinally down a furrow, the key mechanism driving these losses is lateral leaching through the hill by irrigation water passing from the irrigated- to the alternate-furrow. Leached nutrients are then predominantly discharged from the alternate-furrow. Efforts to mitigate these losses should target the lateral leaching mechanism, with a possible solution in the adoption of extra-wide beds. By eliminating the alternate-furrow, potentially leached soil material is unable to freely flow from the field, and will be largely retained in the soil profile. Fertigation was also shown to be a major contributor to N runoff in these systems.

Chapter 4 addresses the third research question, exploring the residence times of dissolved N in irrigation water. Urea is the most commonly water-run nitrogenous fertiliser in Australian cotton systems. This chapter demonstrates that urea mineralises quickly when dissolved in irrigation water, and that the rate of urea mineralisation is influenced by soil exposure, temperature, and initial urea concentration. Translating to the farm environment, this suggests that mineralisation will be most rapid when urea-laden water is travelling down furrows, as high soil interaction and water temperatures produce high urease exposure and activity. Conversely, storage dams and irrigation networks should experience lower urea mineralisation rates due to the deeper waters reducing temperature and soil interaction. Experimental results showed that significant mineralisation occurs within the first 24 hours, with little urea remaining in the water column after seven days. TDN in the water column begins to fall approximately four to eight hours after dissolution, marked with an increase in denitrification. Urea concentration in irrigation water can be well generalised over seven days by a mathematical model, allowing growers to estimate aqueous N concentrations in irrigation water at different temperatures. NO_3^- and NH_4^+ concentrations were also reliably generalisable, providing growers with predictive tools for them as well. Hopefully these data and predictive tools will help the Australian cotton industry improve NFUE when fertigating, and be applicable to many agricultural contexts outside this industry.

Chapter 5 addresses the fourth research question, exploring the N uptake capabilities and preferences of cotton (*G. hirsutum*). Cotton receives the majority of its N from the soil, rather than directly from added fertilisers, with the prevailing wisdom suggesting this to be solely in the inorganic forms of NO_3^- and NH_4^+. This chapter demonstrates that *G. hirsutum* can directly take up LMW DON in addition to inorganic N. *G. hirsutum* was observed concurrently taking up alanine—N, urea—N, NO_3^-—N and NH_4^+—N, revealing no exclusionary hierarchical preference between the different N sources. Within the four tested N species, a minor preference for NO_3^-—N and urea—N was observed. Alanine—C was also taken up by the experiment plants proportionally to alanine—N, suggesting that some alanine is taken up as whole molecules. The results presented in this chapter suggest that increasing soil organic N at the expense of more chemically reactive inorganic N may not negatively affect plant-available N. This would mean that the benefits of increasing SOM such as improved aggregate stability, water

holding capacity, cation exchange capacity, and atmospheric C sequestering could be achieved without a yield penalty.

6.3 – Management Recommendations

This thesis aimed to provide new information and management tools to improve precision agricultural practices and environmental performance in the Australian cotton industry. Several recommendations for management practice change have been made based on the experimental findings presented in this thesis, and are listed here:

- When fertigating, growers should carefully consider their operational processes to maximise application homogeneity. If using urea tanks, growers should ensure the tank is properly mixed before discharging, and that the discharge rate is consistent (or at least predictable) by using flow regulators like header tanks.
- Concentrated N should be added to the irrigation water as close to the site of application as possible to mitigate the effects of heterogeneous network mixing. If N is supplied to irrigation channels an appreciable distance from the site of in-field application, consideration should be given to the channel's mixing profile.
- Once growers are delivering a consistent N concentration to the field, they should calculate nutrient budgets by recording flow rate, exposure time, and concentration. These will facilitate more accuracy in achieving N rate targets, and improve NFUE.
- Growers who use alternate furrow irrigation (the majority of Australian cotton growers) should consider adopting extra-wide beds to replace the existing alternate-furrows. This switch would require minimal management change (same watering and controlled traffic regimes, machinery spacing, etc.) while largely eliminating the loss pathway, and could save 10-15% of applied pre-season fertiliser from being transported into the tail drain.
- When fertigating, growers should try to be economical with water application, ideally minimising tailwater runoff, deep drainage, and water-logging. Input fertigation N can constitute a significant proportion of a season's total N runoff, often discharging during a single irrigation event. Nitrogen runoff during a fertigation event can easily exceed 15 kg N ha^{-1}. For 1000 hectares of cotton and a urea price of $400 per tonne, this equates to $13,000 worth of fertiliser per irrigation event flowing into the tail drain.
- Growers should attempt to recirculate captured N-rich runoff as soon as possible, especially when fertigating. After 24 hours, 28-40% of dissolved urea fertiliser can mineralise, increasing to 88-96% after seven days.
- Total N losses from the soil and water columns increase significantly from four to eight hours after urea fertiliser dissolution, likely the result of increasing denitrification. Growers should ensure that N-rich irrigation water is on the field after this time to minimise gaseous emissions.
- Growers should consider building up their SOM stores. In order to produce sustained SOM increases, soil C, N, P and S all require deliberate management.

6.4 – Future Research Recommendations

There is significant potential for new research to build upon the findings presented in this thesis. Recommendations for continued research in this field include:

- ***Developing a method to track aqueous urea—N or TDN in real time.*** Core difficulties of fertigation management are that dissolved N is colourless, and that existing real-time N monitoring tools do not measure urea—N. Ideally, continuous data-loggers would provide real-time TDN concentrations for the entire irrigation network. As a proxy for direct N

measurement, a short-lived biodegradable dye could be combined with the urea concentrate to provide growers with a sufficient visual aid to help gauge mixing.

- ***Assess the efficacy of adopting extra-wide beds to reduce alternate-furrow N runoff.*** Replacing the alternate-furrow with an extra-wide bed presents as an effective potential solution for eliminating the major N runoff loss pathway without requiring significant management change (such as converting to drip irrigation). However, no field experiments or modelling studies currently exist to support these hypothesised advantages.
- ***Expand the range of treatments included in urea mineralisation incubations.*** Including different soil types and initial urea—N concentrations, as well as expanding the range of temperatures, would provide increased applicability of the data presented in this thesis.
- ***Test urea mineralisation rates in the field.*** The quantitative predictive tools presented in this thesis should be useful for predicting fertiliser residence times in irrigation water. However, until they are tested with *in situ* field measurements, they remain theoretical analogues only.
- ***Test the N uptake preferences and capabilities of cotton in soil.*** The experiment presented in this thesis demonstrated which N species *G. hirsutum* has the potential to take up. A different experiment performed in soil is needed to demonstrate which N species *G. hirsutum* does take up under farm conditions.
- ***Test the N uptake preferences and capabilities of cotton at later growth stages.*** The N requirements of *G. hirsutum* vary throughout its growth cycle, and it is possible that its preference for different N species does too. The experiment presented in this thesis was performed on small seedlings, whose N uptake characteristics may not be representative of the plant's entire growth cycle. Additionally, including some Australian native *Gossypium* species (like *Gossypium australe*) in these experiments could yield valuable results in the ongoing adaption of commercial cotton to the Australian climate.
- ***Assess the viability of converting gin trash into organic amendments.*** Australian cotton growers would benefit from increased SOM cultivation, which could be encouraged with the application of targeted organic amendments. Cotton ginning produces significant and predictable organic waste streams that have the potential to be converted into organic fertilisers and biochars for use within the industry. These waste streams could also create new revenue streams though conversion into activated C or other products to be sold outside the industry. Exploration of gin trash's viability as a potential commodity could be explored with some desktop studies and batch laboratory experiments, the outcomes of which would inform future decisions on the suitability of scaled up trials.
- ***Optimal NFUE rates should be further refined for dryland systems and irrigated production outside the Namoi catchment.*** Australian cotton is grown over an increasingly disparate range of landscapes and climates. Development and extension effort should be put into ensuring research from the ACRI and other historical cotton growing regions is translated into target values for different soils and climates. This will be useful for optimising cotton's further geographic expansion, as well as preparing for climate change's effects on rainfall and temperature patterns.

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APPENDIX A – Surface Runoff Nitrogen Table

***Table A1**. Irrigated- and alternate-furrow N runoff for all treatments by irrigation event. Values are reported as mean kg TDN ha^{-1} ± standard error. Data is split into total runoff N (TRN) and field-derived runoff N (FDRN). FDRN is calculated by subtracting input supply water N. Negative FDRN values occur where more N enters the field in irrigation water than runs off. Values reported with * were water-run N fertigation events. Table data relates to Chapter 4, and is published in Latimer et al. (2020).*

			ACRI-1				ACRI-2	Moree			Gunn-1	Gunn-2		
	N Rate (kg N ha^{-1}):		0	78	182	252	35	240	310	370	250	150	250	350
#1	Irrigated-Furrow	TRN:	0.25 ± 0.01 (n = 9)	0.28 ± 0.01 (n = 21)	0.34 ± 0.02 (n = 6)	1.79 ± 0.68 (n = 6)	0.36 ± 0.20 (n = 3)	6.37 ± 0.32 (n = 6)	4.65 ± 0.53 (n = 5)	6.83 ± 1.02 (n = 5)	0.87 ± 0.10 (n = 12)	-	-	-
		FDRN:	0.23 ± 0.01 (n = 9)	0.26 ± 0.01 (n = 21)	0.32 ± 0.02 (n = 6)	1.77 ± 0.68 (n = 6)	0.02 ± 0.01 (n = 3)	5.65 ± 0.53 (n = 6)	4.36 ± 0.56 (n = 5)	6.22 ± 1.31 (n = 5)	0.37 ± 0.10 (n = 12)			
	Alternate-Furrow	TRN:	2.07 ± 1.16 (n = 8)	4.62 ± 0.16 (n = 21)	10.18 ± 1.24 (n = 6)	11.90 ± 1.19 (n = 6)	12.26 ± 1.64 (n = 3)	4.86 ± 0.48 (n = 6)	4.32 ± 0.57 (n = 7)	7.95 ± 1.77 (n = 9)	13.89 ± 0.71 (n = 12)	-	-	-
		FDRN:	20.6 ± 1.16 (n = 8)	4.61 ± 0.16 (n = 21)	10.17 ± 1.24 (n = 6)	11.89 ± 1.19 (n = 6)	9.69 ± 1.40 (n = 3)	4.55 ± 0.57 (n = 6)	4.19 ± 0.58 (n = 7)	7.69 ± 1.90 (n = 9)	13.67 ± 0.71 (n = 12)			
#2	Irrigated-Furrow	TRN:	-	-	-	-	0.17 ± 0.06 (n = 3)	6.17 ± 0.37* (n = 3)	6.50 ± 0.33* (n = 3)	6.50 ± 0.36* (n = 3)	2.67 ± 0.14 (n = 8)	-	-	-
		FDRN:					0.05 ± 0.03 (n = 3)	0.94 ± 0.37* (n = 3)	1.59 ± 0.33* (n = 3)	1.47 ± 0.36* (n = 3)	1.16 ± 0.14 (n = 8)			
	Alternate-Furrow	TRN:	-	-	-	-	5.18 ± 0.77 (n = 3)	7.55 ± 0.11* (n = 3)	6.03 ± 0.37* (n = 3)	8.09 ± 0.00* (n = 1)	8.22 ± 0.55 (n = 7)	-	-	-
		FDRN:					3.54 ± 0.08 (n = 3)	5.31 ± 0.11* (n = 3)	3.93 ± 0.37* (n = 3)	5.95 ± 0.00* (n = 1)	7.57 ± 0.55 (n = 7)			
#3	Irrigated-Furrow	TRN:	-	-	-	-	-	-	-	-	14.00 ± 0.76* (n = 9)	14.75 ± 0.79* (n = 6)	13.49 ± 0.51* (n = 6)	15.41 ± 0.35* (n = 6)
		FDRN:									-2.33 ± 0.76* (n = 9)	-0.69 ± 0.84* (n = 6)	-1.95 ± 0.57* (n = 6)	-0.02 ± 0.40* (n = 6)
	Alternate-Furrow	TRN:	-	-	-	-	-	0.51 ± 0.04 (n = 3)	0.49 ± 0.02 (n = 3)	-	12.33 ± 0.71* (n = 8)	11.49 ± 0.75* (n =	7.43 ± 0.22* (n = 6)	8.49 ± 0.56* (n = 6)
		FDRN:						0.51 ± 0.04 (n = 3)	0.49 ± 0.02 (n = 3)		5.33 ± 0.71* (n = 8)	4.88 ± 0.78* (n = 6)	0.82 ± 0.25* (n = 6)	1.88 ± 0.58* (n = 6)
#4	Irrigated-Furrow	TRN:	-	-	-	-	0.02 ± 0.00 (n = 3)	-	-	-	1.70 ± 0.04 (n = 9)	1.90 ± 0.00 (n = 2)	1.85 ± 0.00 (n = 4)	1.59 ± 0.01 (n = 3)
		FDRN:					0.01 ± 0.00 (n = 3)				0.24 ± 0.04 (n = 9)	0.44 ± 0.05 (n = 2)	0.39 ± 0.06 (n = 4)	0.13 ± 0.05 (n = 3)
	Alternate-Furrow	TRN:	-	-	-	-	0.16 ± 0.02 (n = 3)	-	-	-	3.48 ± 0.49 (n = 9)	0.95 ± 0.08 (n = 3)	2.14 ± 0.00 (n = 4)	0.94 ± 0.15 (n = 3)
		FDRN:					0.11 ± 0.02 (n = 3)				2.85 ± 0.49 (n = 9)	0.33 ± 0.10 (n = 3)	1.51 ± 0.02 (n = 4)	0.32 ± 0.17 (n = 3)
#5	Irrigated-Furrow	TRN:	-	-	-	-	-	-	-	-	0.36 ± 0.03 (n = 12)	0.32 ± 0.04 (n = 3)	0.53 ± 0.06 (n = 3)	0.33 ± 0.07 (n = 3)
		FDRN:									0.01 ± 0.03 (n = 12)	-0.02 ± 0.04 (n = 3)	0.09 ± 0.05 (n = 3)	-0.02 ± 0.05 (n = 3)
	Alternate-Furrow	TRN:	-	-	-	-	-	-	-	-	0.15 ± 0.01 (n = 11)	0.19 ± 0.01 (n = 3)	0.21 ± 0.04 (n = 3)	0.17 ± 0.08 (n = 2)
		FDRN:									-0.01 ± 0.01 (n = 11)	0.02 ± 0.01 (n = 3)	0.03 ± 0.03 (n = 3)	0.01 ± 0.05 (n = 2)
#6	Irrigated-Furrow	TRN:	-	-	-	-	-	1.40 ± 0.07 (n = 3)	0.89 ± 0.09 (n = 3)	1.37 ± 0.32 (n = 3)	0.51 ± 0.05 (n = 10)	0.48 ± 0.07 (n = 5)	0.46 ± 0.10 (n = 5)	0.50 ± 0.10 (n = 4)
		FDRN:						0.25 ± 0.07 (n = 3)	-0.79 ± 0.09 (n = 3)	0.90 ± 0.32 (n = 3)	0.12 ± 0.05 (n = 10)	0.09 ± 0.11 (n = 5)	0.06 ± 0.14 (n = 5)	0.10 ± 0.14 (n = 4)
	Alternate-Furrow	TRN:	-	-	-	-	-	1.07 ± 0.40 (n = 3)	0.90 ± 0.02 (n = 2)	0.53 ± 0.05 (n = 3)	0.21 ± 0.01 (n = 11)	0.22 ± 0.03 (n = 5)	0.15 ± 0.02 (n = 5)	0.18 ± 0.01 (n = 5)
		FDRN:						0.61 ± 0.40 (n = 3)	0.18 ± 0.02 (n = 2)	0.33 ± 0.05 (n = 3)	0.04 ± 0.01 (n = 11)	0.05 ± 0.04 (n = 5)	-0.02 ± 0.04 (n = 5)	0.01 ± 0.03 (n = 5)
#7	Irrigated-Furrow	TRN:	-	-	-	-	-	-	-	-	0.24 ± 0.01 (n = 12)	0.31 ± 0.04 (n = 4)	0.31 ± 0.08 (n = 5)	0.41 ± 0.15 (n = 4)
		FDRN:									-0.10 ± 0.01 (n = 12)	-0.02 ± 0.05 (n = 4)	-0.02 ± 0.10 (n = 5)	0.07 ± 0.17 (n = 4)
	Alternate-Furrow	TRN:	-	-	-	-	-	0.49 ± 0.00 (n = 1)	-	-	0.12 ± 0.00 (n = 12)	0.15 ± 0.02 (n = 3)	0.13 ± 0.01 (n = 5)	0.19 ± 0.01 (n = 3)
		FDRN:						0.49 ± 0.00 (n = 1)			-0.02 ± 0.00 (n = 12)	0.00 ± 0.03 (n = 3)	-0.02 ± 0.02 (n = 5)	0.05 ± 0.02 (n = 3)
#8	Irrigated-Furrow	TRN:	-	-	-	-	-	0.93 ± 0.09 (n = 3)	0.97 ± 0.04 (n = 3)	1.05 ± 0.04 (n = 3)	-	-	-	-
		FDRN:						-0.05 ± 0.09 (n = 3)	0.10 ± 0.04 (n = 3)	0.08 ± 0.04 (n = 3)				
	Alternate-Furrow	TRN:	-	-	-	-	-	0.56 ± 0.16 (n = 2)	0.56 ± 0.02 (n = 3)	0.44 ± 0.04 (n = 3)	-	-	-	-
		FDRN:						0.14 ± 0.16 (n = 2)	0.18 ± 0.02 (n = 3)	0.02 ± 0.04 (n = 3)				
#9	Irrigated-Furrow	TRN:	-	-	-	-	-	1.10 ± 0.05 (n = 3)	1.05 ± 0.07 (n = 3)	1.02 ± 0.07 (n = 2)	-	-	-	-
		FDRN:						0.10 ± 0.05 (n = 3)	0.18 ± 0.07 (n = 3)	0.15 ± 0.07 (n = 2)				
	Alternate-Furrow	TRN:	-	-	-	-	-	-	-	0.41 ± 0.05 (n = 4)	-	-	-	-
		FDRN:								0.03 ± 0.05 (n = 4)				