University of Southern Queensland

Faculty of Engineering and Surveying

Soil Nitrogen Supply Rates during a Cotton Season

A dissertation submitted by

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ABSTRACT

As a result of the demand for increased yield per hectare production output for cotton farms in Australia, as well as the limitations of expanding paddock areas to meet this demand, an increase of nitrogen - the primary cotton plant is required. This comes at a time when rising input costs, as well as sustainability targets are key factors in determining the type of nitrogen fertiliser applied and other components associated with fertiliser usage such as time and rate of application. By matching a cotton plant's varied nitrogen requirements with peak soil potential nitrogen supply, soil nitrogen losses can be minimised, creating sustainable and more efficient nitrogen use for growers.

The mineral nitrogen supply patterns of Urea, the most commonly used nitrogen fertiliser, as well as ENTEC® Urea, a controlled release fertiliser were investigated. This research was specifically designed to compare the potential net soil mineral nitrogen supply over a period of 60 days for each treatment, as well as the potential greenhouse gas emissions from the soil that included nitrous oxide, carbon dioxide and methane over the same time period.

To compare soil nitrogen mineralisation and nitrogen release patterns and processes between fertiliser treatments, a pot study was conducted under laboratory conditions. The study was based on a 60 day aerobic incubation method with a constant soil temperature (25^{o}C), fertiliser application rate (600 kg/ha N), soil moisture range (>75% field capacity) and soil type (Black Vertosol from an irrigated cotton farm Yargullen, QLD).

Soil extraction (2M KCl), colorimetric and gas chromatography laboratory methods were used to obtain sufficient data for the net soil nitrate and ammonium concentrations on days 0, 3, 7, 14, 30, 45 and 60. Concurrently, soil gaseous emissions in the form of a linear flux over 45 minute sampling periods for days 0-5, 14, 45 and 60 were achieved.

Results indicated that soil applied with ENTEC® Urea provided a delay in the peak net mineral nitrogen supply with a steady increase occurring until day 60, when compared to Urea which peaked in mineral nitrogen supply by day 14. If fertiliser was applied in September at a similar time to planting, net supply from Urea would not coincide with increased nitrogen demands from first boll fill and boll opening phase (50-100 after sowing). ENTEC® Urea however, would meet the nitrogen requirements of cotton plants with a single fertiliser application at season commencement, as well as providing a more sustainable fertiliser option with a 73.3% reduction in nitrous oxide emissions when compared to Urea.

Further investigation could include relating the results to field conditions using a degree day relationship and to compare the mineral nitrogen and gaseous emission results from field experiments.

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CHAPTER 1 INTRODUCTION

Nitrogen use efficiency has become a global agricultural research priority due to the effects of exponential population growth (higher yield demands), reduction of quality farming land, environmental sustainability targets and rising industry expenses. The cotton industry within Australia continuously strives for economic viability, long term productivity, sustainability and efficient farming practices to ensure international competitiveness within the fibre market is maintained. This highlights the need for further research relating to fertiliser usage in cotton farming practices; specifically nitrogen use and application (Roth 2014).

The efficient use of nitrogen in cotton enterprises can be targeted by various strategies, including the use of different nitrogen fertiliser formulations (slow release or controlled release), as well as improving the time of application when plant requirements are known (CottonInfo 2015). The effectiveness of these strategies can vary depending on the characteristics and impacts of the following factors (Ryan 2010):

- Existence of surplus water (water logging or field capacity).
- Soil and soil profile characteristics.
- Slope and topography of land.
- Topography features that may cause run-off variations.
- Proximity to waterways.
- Groundcover and type of crop present.
- Timing and rate of fertiliser application.
- Types of farming practices implemented.

Efficient nitrogen use also encompasses the regulation of soil gaseous emissions to reduce emission intensity of greenhouse gases including nitrous oxide, carbon dioxide and methane. Regulation of fertiliser inputs and knowledge of strategies to reduce losses of nitrogen from the soil-plant system can ensure farming practices do not contribute to global warming and climate change effects.

Research has been conducted that provides growers with recommendations of how much nitrogen a cotton plant will use throughout a growing season, the effects of varying nitrogen application rates, and potential sources and levels of nitrogen losses (including gaseous emissions) (Cotton CRC 2001). Knowledge relating to the estimate of a soil's potential inorganic nitrogen supply over the duration of a cotton season (ammonium (NH_4^+) and nitrate (NO_3^-)) is limited, with current soil mineralisation estimates based on soil organic carbon content. The potential benefits of using a controlled release fertiliser within the cotton industry is also an area requiring further knowledge development.

1.1 Purpose and Aim

The purpose of this research was to quantify rates and amounts of inorganic soil nitrogen supply during a cotton plant growth cycle. This contributes to understanding the potential peak mineral nitrogen supply and loss levels of Urea, a common nitrogen fertiliser, compared to ENTEC® Urea, a controlled release nitrogen fertiliser.

The project aim was to estimate the time of optimum availability of inorganic nitrogen supply from the fertiliser treatments and align this with the seasonal variation of cotton plant demands to determine whether the soil nitrogen supply from application of Urea and ENTEC® Urea matched typical cotton cropping requirements. Determination of gaseous losses (nitrous oxide, carbon dioxide and methane) from both fertiliser types were also identified to estimate potential nitrogen loss and environmental impacts. This is a practical outcome for growers and other professionals associated with the cotton industry.

1.2 Research Objectives

The project objectives and associated experimentation were based on a soil type that is commonly used for irrigated cotton crop enterprises within Australia. This ensured results were applicable for many cotton farms with similar soil characteristics and growing practices.

The following project objectives were identified:

- Conduct an extensive literature review to identify research available that could assist with methodology development to measure and analyse soil inorganic nitrogen supply of Urea and ENTEC® Urea, as well as interpreting the soil mineral nitrogen and gaseous emission results.

- Identify the characteristics of a soil from a typical cotton farm located in Darling Downs, Queensland that invests in cotton as the primary crop.

- Design and conduct an experiment to determine the time of maximum net soil mineral nitrogen supply by measuring the nitrogen supply and release characteristics from a soil amended with two different fertiliser formulations (Urea and ENTEC® Urea).

- Collect soil samples and gas samples at regular intervals during the experimental procedure and analyse using colorimetric methods for soil nitrate and ammonium and gas chromatography for greenhouse gaseous emissions.

- Analyse the data using Excel and Genstat statistical techniques to check for differences between fertiliser treatments as well as differences over time.

- Test the results and validity of two different methods of estimating potentially mineralisable nitrogen in unfertilized soil - anaerobic (7day) versus aerobic (60 day). This was an optional objective.

1.3 Scope

The scope of the investigation was to initiate research within the field of nitrogen fertiliser usage efficiency for the cotton industry and more specifically, soil inorganic nitrogen supply and soil gaseous emissions from two nitrogen fertiliser types. Current research has identified the need to investigate factors other than rate of nitrogen application to assist in increased lint yields, while also maintaining optimal nitrogen use efficiency standards (Smith et al. 2014).

Every attempt was made to ensure the experimental results could be beneficial to farmers and professionals, which is why a typical cotton farm soil was used, as well as a common nitrogen fertiliser (Urea) and an alternative fertiliser option available in Queensland (ENTEC® Urea) used currently in several sugarcane and dairy farm enterprises.

Some limitations of this investigation did exist however due to the potential time and resource constraints. The number of variables investigated for the project objectives were limited to two fertiliser types, one soil type and an investigation of one nitrogen loss pathway (gaseous emissions). One nitrogen mineralisation method was also used. Several other variable limitations also occurred within the methodology of the project, to ensure a reasonable project scope was met; these are identified in further detail within the methodology section.

1.4 Research Outcomes and Benefits

The following expected outcomes were developed in the form of a hypothesis for each objective. These include:

'The controlled release fertiliser will have slower nitrate release when compared to Urea fertiliser, when applied in the same conditions.'

'Urea fertiliser will have a greater total nitrous oxide emissions when compared to a controlled release fertiliser over the same time period and in the same conditions.'

'Urea and controlled release fertiliser will have similar carbon dioxide emissions when the same rate of nitrogen fertiliser is applied, however timing of emissions will vary, reflecting the difference breakdown stages of fertilisers.'

'Urea and controlled release fertiliser will have similar methane production levels in the same soil conditions.'

'The 7 day aerobic method will have a much more stable result for the nitrogen mineralisation rates when compared to the 60 day aerobic method due to the lower risk of nitrogen mineralisation inhibition when no plants are present to use the nitrogen.'

Accompanied with future research investigating other variables that can influence soil inorganic nitrogen supply and losses, the results from this investigation will target fertiliser challenges associated with growing cotton. It will also contribute to an increased understanding of potential farming management strategies to improve the environmental sustainability of cotton enterprises.

This knowledge is important for two primary reasons:

1. To ensure farmers can achieve maximum quality and profitability for cotton enterprises by reducing input costs, maximising yield and ensuring healthy soil development.

2. To reduce the environmental impact of the misuse of fertilisers. The impacts range in severity, from the steady degradation of soils to the contamination of waterways due to leaching and runoff effects as well as greenhouse gas emissions.

1.5 Dissertation Overview

All key areas of the research and experimental processes have been included within this dissertation. Chapter 2 will outline the literature review process by including all of the relevant background knowledge required for the project, as well as critically reviewing sources of research already available.

Chapter 3 will include a detailed description of the methodology applied to undertake experimental research for this project. Soil characterisation, aerobic incubation, gas emission collection and soil and gas analysis procedures will be outlined.

Chapter 4 provides all results obtained throughout the course of the project including soil properties and ammonium, nitrate, nitrous oxide, methane and carbon dioxide data for a control, Urea and ENTEC® Urea treated soil. The raw data preparation and manipulation will also be detailed as well as a review of possible sources of experimental errors and methodology design limitations.

Chapter 5 finalises the findings of this project in a detailed discussion including verification of results with past research findings. Relationships and trends in data will be accounted for as well as recommendations of possible future research options. Chapter 6 relates the project findings to the aim in the form of a concise conclusion.

CHAPTER 2 LITERATURE REVIEW

2.1 Introduction

This literature review provides relevant background information and a critical evaluation of the research available on the topic of soil nitrogen supply and fertiliser usage efficiency in the Australian cotton industry. This includes soil system and structure properties (Black Vertosol), nitrogen use efficiency and associated variables, the characteristics of Urea and ENTEC® Urea fertilisers, cotton plant nitrogen requirements, soil nitrogen supply characteristics, potential soil nitrogen loss pathways and associated factors that may influence losses.

To ensure all information gathered is of high quality and relevance, the literature incorporated in the review is from verifiable sources including journals, conference papers and government and university documents. The research and other information has therefore been reviewed by professionals and potentially cited in further research.

2.2 Black Vertosol Properties

Seventy-five percent of cotton grown in Australia is on Black (or Grey) Vertosols (Roth 2014; Isbell 1996), which is a soil type with a fine surface texture, tending to remain uniform with depth as the dominant clay mineral content Smectite increases (Gray & Murphy 2002). The shrink/expand mechanism of a Black Vertosol forms gilgai and cracks, resulting in the soil having a high water holding capacity (up to 110mm) depending on rainfall conditions (McKenzie et al.2004).

The structure of a Black Vertosol is highly aggregated due to the uneven expansion and contraction of the soil during wet and dry rainfall periods. In a typical wet season, water infiltration into the soil will initially be high, however this rate substantially decreases as swelling occurs. Eventually a

low drainage rate will be reached because of the high clay content. Vertosol expansion characteristics also affect soil aeration which varies between dry and wet periods causing fluctuations of oxygen and nitrogen gases present within the soil (*Vertosols* 2008).

Generally, Black Vertosols are rich in potassium and calcium nutrients and due to the high percentage of clay (approximately 35%), have the potential to retain other nutrient types (low soil leaching) (Jacquier n.d.). Commonly however, deficiencies can occur of nitrogen, phosphorous and zinc; each of which are essential for plant development. The soil maintains neutral pH conditions at the surface and even though lower subsoil is highly alkaline, this is actually beneficial and reduces the effect of the soil gradually becoming acidic over a few growing seasons (*Vertosols* 2008).

Organic carbon content varies depending on land use, however it has been shown that growing a rotational crop of legumes, as well as reduced tillage can substantially improve the presence of organic carbon, as well as nitrogen content (McKenzie et al. 2004). Clay soils such as Black Vertosols typically have between 1.5 – 2.0% organic carbon content compared to values of 0.75 – 1.5% for sandy loam soils (Pattison, Moody & Bagshaw 2010).

Forty percent of Australian Vertosols are black, which is indicative of poor drainage, but also high agricultural potential. The soil generally transitions from being extremely dry and hard, to wet and sticky, which limits the time frame during which seeding, cultivation and other agricultural practices can be applied. The high water holding capacity of these soils however, means crop survival during dry periods is more likely, and crops may also be grown after the rainy season has actually ended (fitting in more than one growing season). It must be noted however, that in this clayey soil, substantial early season rainfall is necessary for moisture to be available during the crop establishment phase and drainage is required otherwise waterlogging is likely to occur (*Vertosols* 2008).

Black Vertosols can have the following limitations to agricultural production:

- Outside of the optimal moisture content of soil, tillage may cause formation of clods or an uneven plant bed.
- Poor surface soil structure causes low permeability.
- Alkalinity, sodicity and salinity at subsoil levels which can potentially restrict depth of root penetration.
- Vertosols generally form in low-lying areas, which may be prone to flooding during wet seasons.
- Formation of crust on soil surface from tillage practices.

To manage these limitations, it is recommended that farmers apply a management strategy that may contain several of the following practices:

- Reduce tillage, retain crop stubble after harvest (prevents rain drops from causing soil aggregates to breakdown and dispersed particles setting hard) and apply gypsum (dependant on current soil gypsum levels) to improve soil structure, infiltration and reduce occurrence of hard soil crust forming and runoff.
- Ensure waterways are included in paddock design in case of flooding in surrounding areas to reduce waterlogging and possible topsoil erosion.

(Vertosols 2008)

2.3 Nitrogen Fertilisers

2.3.1 Introduction

Atmospheric nitrogen can be fixed both biologically (in nature) or industrially, with both processes contributing to the continuous cycling of nitrogen throughout the biosphere. Biological nitrogen fixation can occur from lightning discharges (between 1-60 kg/ha) or more commonly from

symbiotic (*Rhizobium)* or free-living, autotrophic micro-organisms which use nitrogen accompanied with light radiation or carbon dioxide as an energy source in either aerobic or anaerobic conditions (Price 2006).

Biologically fixated nitrogen is limited to the capacity of micro-organisms, making it insufficient for intensive agriculture enterprises and for ensuring maximum cropping yields. Industrial fixation has enabled several types of nitrogen fertilisers to be developed, meeting cropping requirements where required. Nitrogen fertilisers created through industrial processes are synthetic and based on the Haber-Bosch process forming anhydrous ammonia (82% nitrogen).

The ammonia formation involves combining hydrogen and nitrogen gas to create a catalytic reaction to form ammonia gas, removing carbon dioxide and carbon monoxide impurities and finally, compressing the gas to produce ammonia (liquefied gas). For safer transport, storage and application options, anhydrous ammonia has been combined with other waste products of the Haber-Bosch process to develop solid forms of nitrogen fertiliser (i.e. urea) (Tisdale, Nelson and Beattie 1984; Pesek, Stanford & Case 1971).

2.3.2 Urea Fertiliser

Global population growth and the reduction in quality agricultural land has resulted in an intensification of agricultural practices, increasing the demand for yield per hectare in all crop types, including cotton. Global demand for Urea, the most common nitrogen fertiliser, was estimated at 149.10 million tonnes in 2014 (Heffer & Prud'homme 2014). The Australian agricultural industry in 2010 consumed 1238 thousand tonnes of Urea, however the climatic trends of droughts and seasonal flooding greatly impacts the yearly demand of all nitrogen fertilisers (Ryan 2010).

Urea is developed from the combination of ammonia and carbon dioxide (see chemical reaction formula below) and exists in a water soluble granular form with 46% total nitrogen composition.

$$CO_2 + 2NH_3 \rightarrow CO(NH_2)_2 + H_2O$$

The industrial processes required to formulate Urea involve combining liquid ammonia and retrieved carbon dioxide gas (ammonia production waste product) to form Urea and ammonium carbonate, which is then heated to separate the carbonate and form water and Urea as by-products (Tisdale, Nelson and Beattie 1984; Pesek, Stanford & Case 1971).

When applied to the soil as a fertiliser, Urea is already in inorganic form, however not a form suitable for plant uptake. Two reactions need to occur before the fertiliser reaches a plant available inorganic stage of ammonium. These reactions form the process of Urea hydrolysis and are outlined below:

1. $CO(NH_2)_2 + 2H_2O \rightarrow$ Urease Enzyme $\rightarrow (NH_4)_2CO_3$

2. $(NH_4)_2CO_3 + 2H^+ \rightarrow 2NH_4^+ + CO_2 + H_2O$

(Bundy n.d.)

The first reaction states that when Urea is combined with two molecules of water, in the presence of a soil enzyme urease, one molecule of ammonium carbonate will form. The second reaction involves the conversion of ammonium carbonate (when combined with hydrogen ions) to two molecules of plant available ammonium, carbon dioxide gas and water.

Urea hydrolysis is a fast process, therefore if the correct conditions for volatilisation are present, a high likelihood exists of ammonia gas forming instead of inorganic ammonium (nitrogen loss pathway) (Gardinier et al. 2013). This occurs as a reduction in the adsorption of ammonium molecules to soil and humus colloids occurs and the alkalinity of the soil

increases (as the second reaction uses hydrogen ions) (Schlegel, Nelson & Sommers 1987). Refer to Section 2.6.3 for suitable fertiliser management practices to reduce volatilisation losses.

The enzyme urease is a key component to the rate of Urea hydrolysis, and occurs in two forms within the soil medium. Intracellular urease enzymes are present within cells of soil micro-organisms which initiate the Urea hydrolysis reaction when Urea molecules are ingested. A smaller percentage of urease enzymes exist in extracellular form outside of disrupted cells adsorbed to clay colloids and organic matter (Lloyd & Sheaffe 1973).

A method to determine the total soil enzyme activity can be conducted by measuring the carbon dioxide gas fluctuations in a soil incubation method (Guettes, Dott & Eisentraeger 2002). The release of carbon dioxide reflects the second stage of Urea hydrolysis. The temperature, moisture content and enzyme population of the soil are the main factors that influence the enzyme activity, with cold and extremely hot conditions as well as drier soils reducing the rate of activity (Price 2006).

2.3.3 ENTEC® Urea

Controlled release fertilisers (also known as enhanced efficiency fertilisers) have been developed in recent times as an alternative nitrogen fertiliser to urea. ENTEC® urea (46% nitrogen) aims to reduce the total potential nitrate losses (through leaching and gaseous emissions) by containing a nitrification inhibiting agent to slow down the nitrification process.

When soils are at optimal temperature and moisture conditions for bacterial activity, *Nitrosomonas* (bacteria which use ammonium as an energy source) will convert large amounts of ammonium to nitrate to ensure energy levels are maintained. This may occur at rates and time

periods that do not match a crop's requirement, resulting in a soil nitrate excess (Incitec Pivot Fertilisers n.d.).

The active inhibitor in ENTEC® Urea is 3, 4-dimethylpyrazole phosphate (DMPP) produced by a German manufacturer BASF AG. Due to the benefits of slowing the nitrification process when nitrogen fertilisers have been applied, research has been developing in this field over the last several years. Figure 2.1 below outlines the general inorganic nitrogen supply benefits of ENTEC® Urea when compared to traditional nitrogen fertiliser application programs in Australian agriculture (Incitec Pivot Fertilisers n.d.).

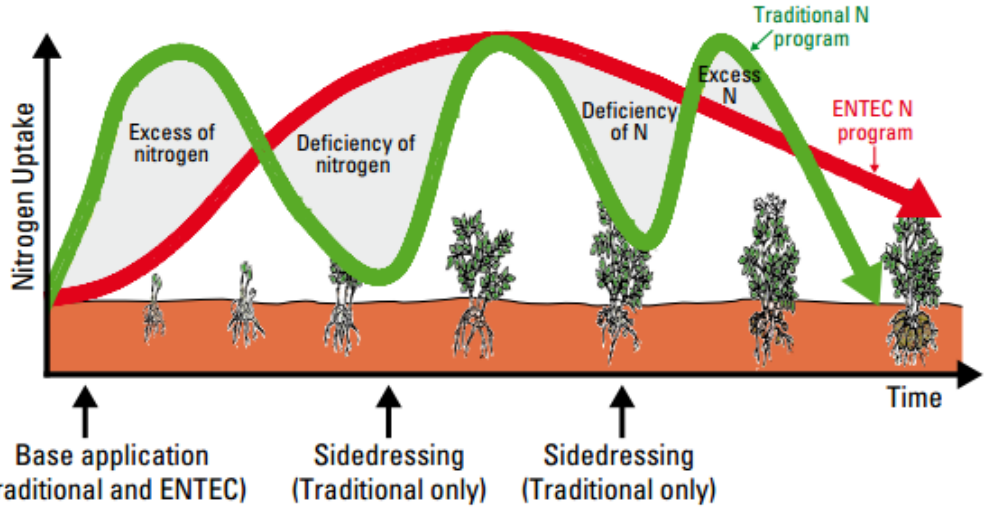


Figure 2.1: Incitec Pivot Fertilisers (n.d.) representation of ENTEC® Urea nitrogen supply improvements compared to traditional nitrogen fertiliser programs

Chen et al. (2008) identified the need to research the use of enhanced efficiency fertilisers specifically within Australian agriculture industries, as the majority of available research was overseas related. In most Australian agriculture systems (cereals, horticulture, cotton etc.) nitrogen inefficiency due to gas losses such as volatilisation, nitrification and denitrification were identified as the main loss pathways.

Nitrification inhibitors used in nitrogen fertilisers within the cotton industry have proven to be effective to reduce the major nitrogen loss effect of denitrification (Rochester & Constable 2000). While nitrate concentrations are maintained at a low level when the nitrification inhibitor is active, a spike in nitrate concentrations is observed when the inhibitor declines in effectiveness. When low nitrogen losses are present within the cotton crop, the effectiveness of applying nitrification inhibitor fertilisers is also reduced, therefore not supporting this type of fertiliser application (Rochester, Constable & Saffigna 1996).

Globally, several studies have been conducted relating to different agricultural industry types that support the use of ENTEC® Urea fertiliser or a fertiliser with the same active nitrification inhibitor (DMPP). Pasda, Hahndel & Zerulla (2001) found that the use of ENTEC® Urea improved the yield of wheat, rice, maize and several horticultural crops, particularly in areas with higher soil moisture content. ENTEC® Urea fertiliser was also found to be a more efficient fertiliser compared to other nitrification inhibitor fertilisers with a lower rate required to achieve the same yield production.

According to the product website (ENTEC Fertilisers 2015), the nitrification inhibitor (DMPP) within ENTEC® Urea varies in the length of time 50% of stabilised nitrogen takes to be nitrified, depending on the average soil temperature. For temperatures between 19-30^{o}C, less than four weeks is the usual time. Global studies have also been completed to determine the potential nitrous oxide emission reduction when using nitrification inhibitors. DMPP was found to decrease the cumulative nitrous oxide emissions in pasture crops such as clover (Macadam et al. 2003) and winter wheat (Linzmeier, Gutser & Schmidhalter 2001).

A study has found however that no real benefit was gained from a cost ratio perception (taking into consideration the higher expense of controlled release fertilisers when applied to cabbage crops (Rodrigues et al. 2010). Harris et al. (2014) also found that application of fertiliser with DMPP did

not minimise the nitrification rate substantially enough to become a viable option for reducing nitrous oxide emissions and increasing fertiliser usage efficiency in high rainfall wheat cropping environments.

The Australia Government recently funded (finalised mid-2012) a large research program for the agriculture industry of Australia which encompassed the effect and level of nitrous oxide gas emissions from various industries. The $4.7 million project included the cotton industry and use of nitrification inhibiting fertilisers as an alternative nitrogen fertiliser supply.

Key findings included:

- Using ENTEC® Urea in sugar cane farming reduced nitrous oxide emissions by 34% and 60% in maize experiment (Kingaroy).
- No specific soil parameter was found to be of primary importance to directly improve the inhibitor fertiliser efficiency – this is a research gap.
- DayCent was a reliable enough model to simulate further data for nitrous oxide emissions.

2.4 Plant – Nitrogen Interactions

Cotton plants prefer to use nitrate forms of inorganic nitrogen from the soil (from 0-50cm topsoil) for growth and can store nitrogen within leaves to assist in later plant developments if soil nitrogen supply becomes depleted. Most inorganic nitrogen is removed from the soil between 50 and 110 days after planting (Cotton CRC 2001) with the peak requirements occurring at peak bloom of first boll (displayed in Figure 2.2 below) and 200kg/ha average total nutrient uptake for a cotton season (Rochester et al. 2012) (refer Figure 2.3).

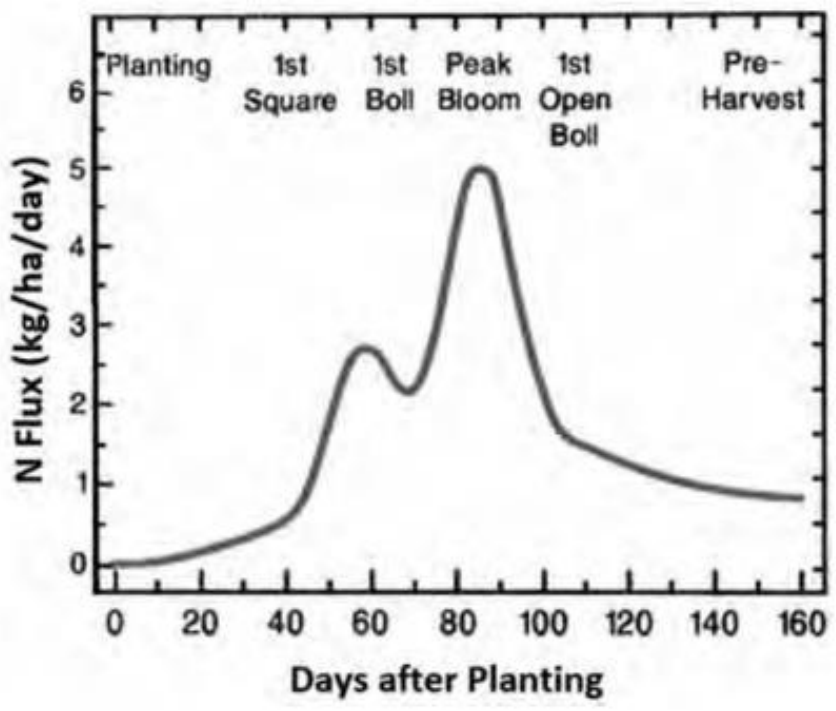


Figure 2.2: Cotton plant nitrogen requirements during a season expressed as nitrogen flux (CottonInfo 2015)

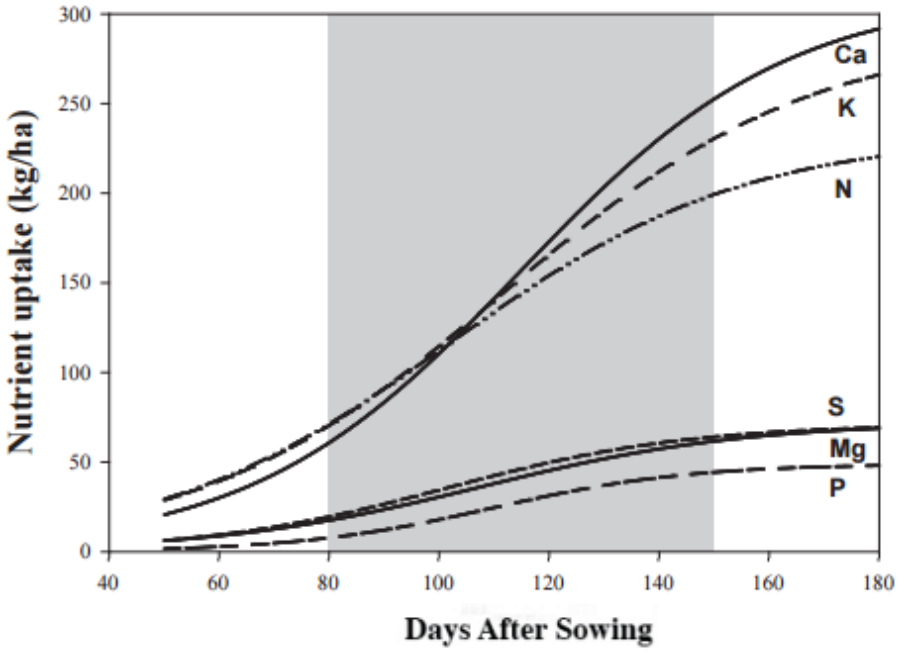


Figure 2.3: Cumulative nitrogen uptake over cotton season duration (Rochester et al. 2012)

An increase in soil mineral nitrogen is a result of mineralisation and nitrification processes occurring within the soil system. A decrease in mineral nitrogen is therefore represented by individually, or a combination of denitrification, immobilisation, leaching and volatilisation (Rochester, Constable & MacLeod 1991). The initial soil nitrate concentrations at the commencement of a cotton season (September) can provide a general indicator of the required nitrogen fertiliser application rate with low concentrations requiring a higher nitrogen fertiliser rate of application (Cotton CRC 2001).

Once applied to the soil, nitrogen fertiliser is circulated between the soil, plant and atmosphere in multiple nitrogen forms, representing the nitrogen cycle. This circulation has been simplified into forms directly related to the fertiliser application as displayed in Figure 2.4.

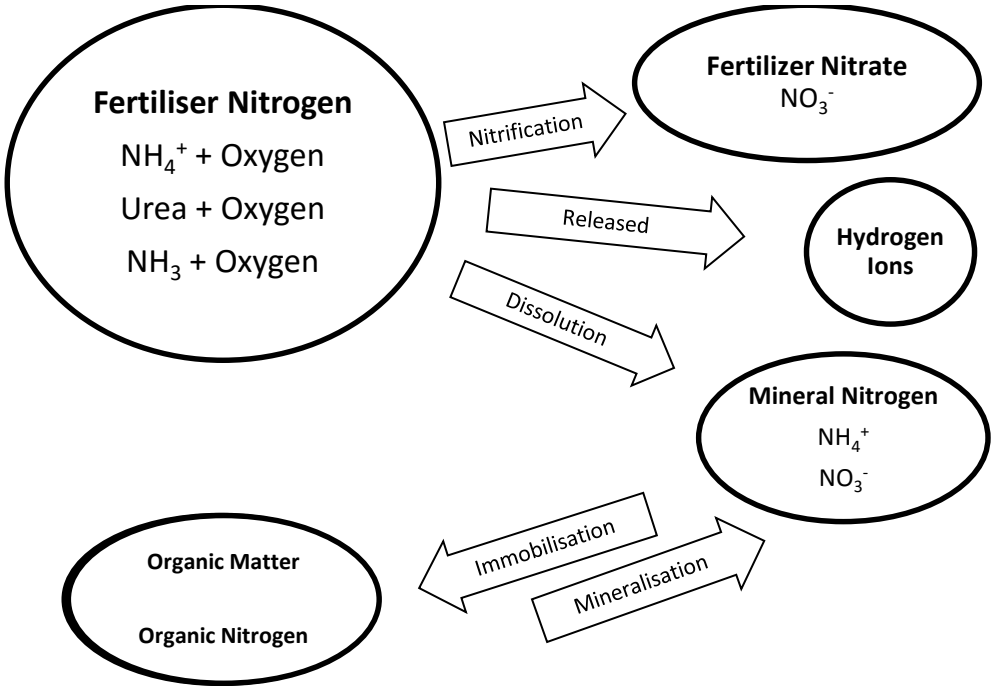


Figure 2.4: Circulation of nitrogen forms within soil (ed. Price 2006, p.26)

Over the duration of a cotton season, the cotton plant sources a combination of both soil nitrogen and fertiliser nitrogen applied at varying stages. Depending on the lint yield, the ratio of soil nitrogen and fertiliser nitrogen required by the plant differs, with higher yields generally requiring a higher fertiliser nitrogen source (refer Figure 2.5).

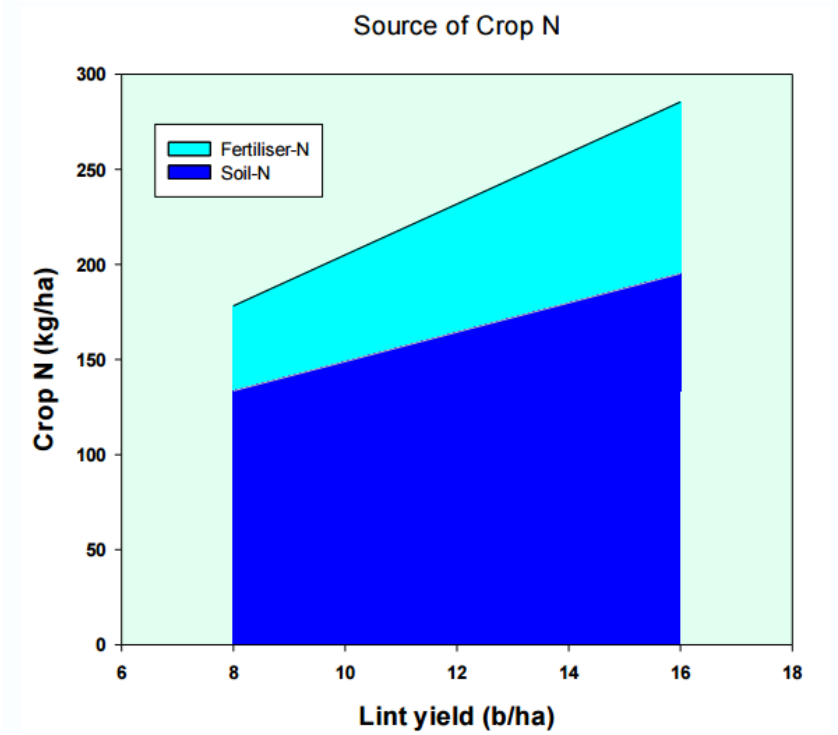


Figure 2.5: Soil and fertiliser N variation with lint yield (Rochester & Gordon 2014)

2.4.1 Mineralisation

The transformation of organic nitrogen to the inorganic nitrogen form ammonium is referred to as mineralisation and is driven by micro-organism decomposition activity within the soil. Mineralisation is a form of net mobilisation and occurs when organic matter within the soil contains high organic nitrogen content. This ensures microbes do not require inorganic nitrogen forms as an alternative energy source to perform decomposition (Singer & Munns 2006).

Factors that affect the rate of mineralisation include population numbers of decomposer organisms, amount of organic matter present, presence of oxygen, pH, salinity and climatic conditions such as temperature and moisture content (Chadwick et al. 2000). As microbes release excess nitrogen from the mineralisation process, high soil in inorganic nitrogen concentrations can develop, resulting in a higher possibility of adverse environmental effects (leaching, gaseous emissions).

If favourable conditions such as warm temperatures and a moist soil are present the ammonium is rapidly converted into nitrate through nitrification (Price 2006). Nitrogen release from organic sources can also be linked with the thermal time (degree days) (Antille et.al. 2014) which uses the temperature of soil at a certain depth over a defined period of time.

2.4.2 Immobilisation

The process of immobilisation is the reverse of nitrogen mineralisation, with micro-organisms using ammonium and nitrate as an energy source and converting them back into organic forms of nitrogen. This is a temporary loss for plant available nitrogen when organic matter does not contain sufficient organic nitrogen supply for decomposer microbes. The nitrogen cycle does however ensure immobilised nitrogen can re-undergo mineralisation to be either used by plants or lost to the environment (Singer & Munns 2006).

Immobilisation is directly affected by the input of natural carbon sources such as crop stubble and some compost compositions. Research has also indicated that while ammonium is the preferred immobilised form, soil pH may influence this conversion. Rochester, Constable and MacLeod (1992) found that in alkaline and neutral soils immobilisation favoured nitrate, however acidic soils favoured ammonium immobilisation. Other conditions that favour the immobilisation process includes low soil temperature as

mineralisation micro-organisms become less active and a higher soil water content (Rochester, Constable and MacLeod 1991).

Micro-organisms require a C:N (carbon:nitrogen) balance to be maintained within the soil, therefore creating the opportunity for nitrogen fertilisers to be applied to the soil when organic matter such as stubble is applied for breakdown. This will generally trigger immobilisation, creating nitrogen stores within the soil for future cropping, however needs to be considered when measuring soil mineral nitrogen at the commencement of a growing season (Smith et al. 2014).

2.5 Nitrogen Use Efficiency

Nitrogen use efficiency (NUE) is a standard measure used within the cotton industry to determine the yield effectiveness of varying nitrogen application rates, timing and methodology. The cotton industry research goal is to maintain maximum yield, with minimal input costs and environmental impact, to achieve substantial profit margins (Cotton Research & Development Corporation 2014). The following expression demonstrates this:

$$\frac{Total\ \ lint\ yield\ (\frac{kg}{ha})}{Total\ nitrogen\ applied\ during\ season\ (\frac{kg}{ha})}$$

Through research trials conducted around nitrogen fertiliser use efficiency, it has been accepted that the lint yield of cotton does not directly depend on the nitrogen applied, but other factors such as soil characteristics, climate and farm management practices (Rochester 2013). 2013 NUE field analyses determined that 74% of 147 irrigated cotton sites were achieving low nitrogen efficiency rates, which can lead to a high expenditure on nitrogen fertiliser input costs and low yield return (Smith et al. 2014). Nitrogen fertiliser inefficiency has been identified as having the potential to cost growers up to $60/ha (Chen & Freney et al. 2008).

Alternative nitrogen use efficiency measures exist and can be based on the nitrogen percentage of cotton seed or yield and actual crop nitrogen ratio. While these measures do provide an estimate for fertiliser application efficiency, the soil nitrogen potential as an individual factor is not considered (Rochester & Gordon 2014). A typical nitrogen use efficiency curve for cotton is displayed in Figure 2.6. Rochester (2014) defines the economic optimum rate of nitrogen to be when $1 of nitrogen added to the crop, returns $1 in lint yield. According to Figure 1, the economic optimum rate of nitrogen application is when the yield/nitrogen ratio is between 13 and 18.

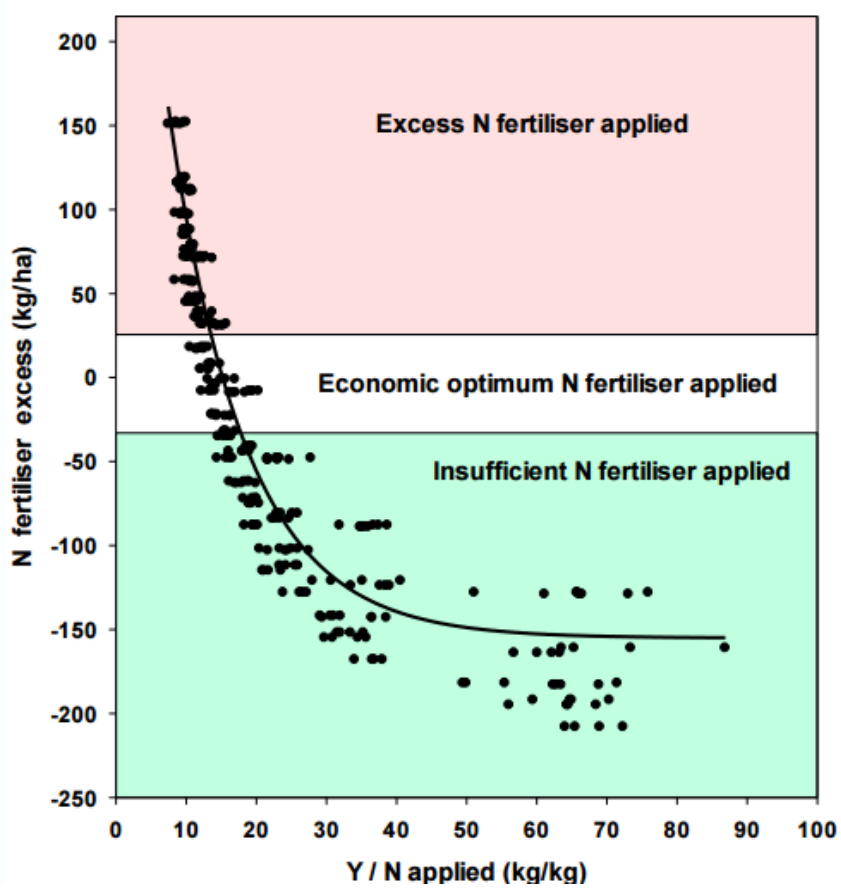


Figure 2.6: Nitrogen use efficiency curve (Rochester & Gordon 2014)

A cotton growers survey conducted in 2013 (Roth 2014) determined the average nutrition practices of Australian cotton growers. The average

nitrogen applied to irrigated cotton, which makes up 95% of the total cotton grown was 243kg N/ha, including both pre-season and in-season applications (gas and solid nitrogen). This value was the highest nitrogen application of all the previous years of data available. A high variation between cotton nutrition input and yield per hectare still exists however, creating the common practice of farmers applying a higher than necessary nitrogen fertiliser amount to ensure the yield is achieved. A method like this however, does not enable nitrogen use efficiency to be optimised and therefore sustainability of the farm is likely to decrease (Smith & Bell 2015).

The concept of nitrogen use efficiency (NUE) targets the reduction of leaching, denitrification, volatilisation and nitrification to ensure available nitrogen is used by plants, environmental impacts are reduced and maximum lint yield occurs (Smith et al. 2014). It is evident that several factors impact the severity level of the nitrogen loss pathways and include:

- Climatic conditions (temperature, rainfall, wind strengths etc.)
- Soil characteristics (texture, pH)
- Fertilizer application practices (split application, fertiliser type, rate of application)
- Other farm management practices (tillage, irrigation, drainage, farm traffic)

The following farm management practices have been researched to increase nitrogen fertiliser use efficiency within cotton farming. When implemented, this an increase cotton farming sustainability by reducing greenhouse gas emissions and losses of ammonium and nitrate compounds.

- Determine the natural sources and amounts of nitrogen available (pre-existing organic and inorganic nitrogen within the soil).
- Gain an understanding of the typical rainfall and temperature patterns that are likely to occur during the crop cycle.

- Match fertiliser type with farm management practices such as water application type and volumes, type of soil and plant requirements.
- Optimise the rate, method (apply as deep as possible in the soil) and timing of fertiliser application.
- Plant cotton following a fallow season to increase the nitrogen use efficiency measure, when compared to back-to-back planting (Smith et al. 2014).
- Match the nitrogen supply (from fertiliser and soil) to the crop demand during crop cycles.
- Maintain plant health during the season with pest and disease control to ensure plant nitrogen uptake occurs.
- Introduce crop rotation techniques such as growing legume crops during fallow seasons to naturally replenish nitrogen stocks within the soil (i.e lucerne).
- Return stubble to the soil after harvest to increase natural nitrogen supply. This enables a balance to be achieved between the denitrification and immobilisation microorganisms with a slowly decomposing material being converted into organic nitrogen sources (Rochester & Constable 2000; Johnson et al. 2005).
- Employ a reduced or no tillage strategy to minimise gaseous emissions and increase the water storage capacity of soil.
- Manage irrigation strategies to reduce waterlogging potential.

(Mosier, Syers & Freney 2004; Roth 2014, Kitchen & Goulding 2001)

2.6 Nitrogen Loss Pathways

The effects of nitrogen losses, particularly gaseous emissions, vary in severity. Generally atmospheric pollution of the troposphere and stratosphere affecting the ozone layer is the most common, however eutrophication of lakes, acidification of soil and an overall reduction in biodiversity are common effects also (Chen et al. 2008).

2.6.1 Denitrification & Nitrous Oxide Emissions

Water logging as a result of excessive irrigation or rainfall, soil compaction and poor drainage can create anaerobic environments within the soil where microorganisms use available nitrates as an energy source (Smith et al. 2014). This is termed denitrification and results in gaseous nitrogen emissions and a permanent loss of in plant available nitrogen. Several types of nitrogen compounds are formed during the denitrification process (Figure 2.7).

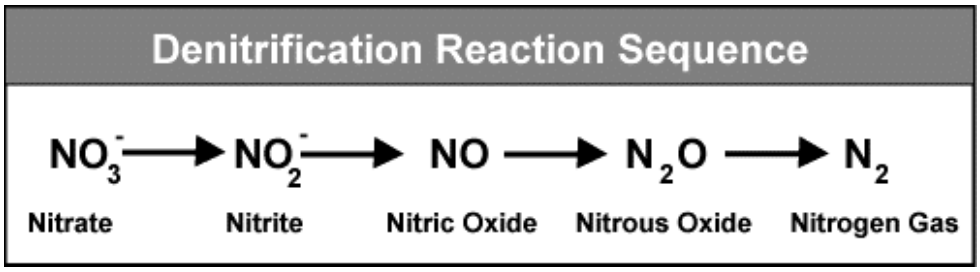


Figure 2.7: Denitrification process from nitrate to nitrogen gas emissions (McFarlane 2005)

Denitrification accounts for 50% of nitrogen losses within cotton industries (Freney et al. 1993 as cited by Rochester & Constable 2000) and on average, agricultural enterprises generate approximately 10kg nitrous oxide per hectare per year (Price 2006).

Nitrous oxide has the most detrimental environmental impact with a greenhouse impact 295 times greater than carbon dioxide (IPPC 2001 as cited by Rochester 2003), when compared to the effect of nitrogen gas (N_2) production which is 80% of the atmospheric composition. Nitrous oxide gas also exists within the atmosphere for an estimated 120 years, increasing the potential effect of global warming (Singer & Munns 2006). Generally a soil temperature between 2^{o}C and 60^{o}C, high soluble organic carbon content and a neutral to alkaline pH increases the occurrence of denitrification processes and therefore gaseous emissions (Price 2006).

Soil Moisture Content

The percentage of water-filled pore space within a soil medium was found to play a key role in the ratio of nitrous oxide and nitrogen gas emitted from the soil (Granli & Bockman 1994, as cited by Aarons et al. 2006). Nitrous oxide emissions are more likely to occur between 60 and 80 percent water-filled pore space, with emission of fully converted nitrogen gas occurring between 70% and 100% water-filled pore space (refer Figure 2.8).

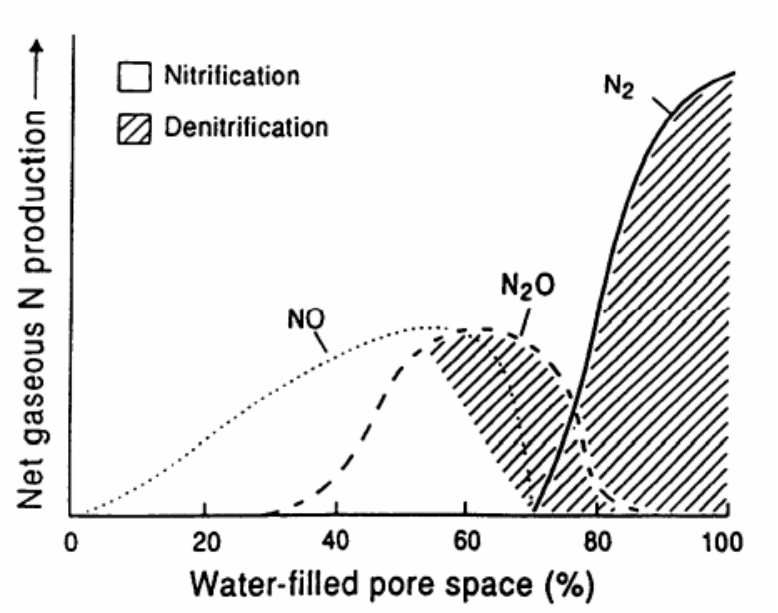


Figure 2.8: Effect of pore space on denitrification gas emissions (Rosen 1996)

Soil pH

Rochester (2003) collated previous research to identify the soil pH impacts on gaseous emissions from the soil. Figure 2.9 displays the results and relationship between soil pH and the ratio of nitrous oxide and nitrogen gas molecules emitted. It should also be noted that while alkaline soils may only emit smaller amounts of nitrogen gas, generally these soil types require a larger nitrogen fertiliser application due to other characteristics (poor fertility), which may therefore increase the overall nitrous oxide emission level in the long term (Rochester 2003).

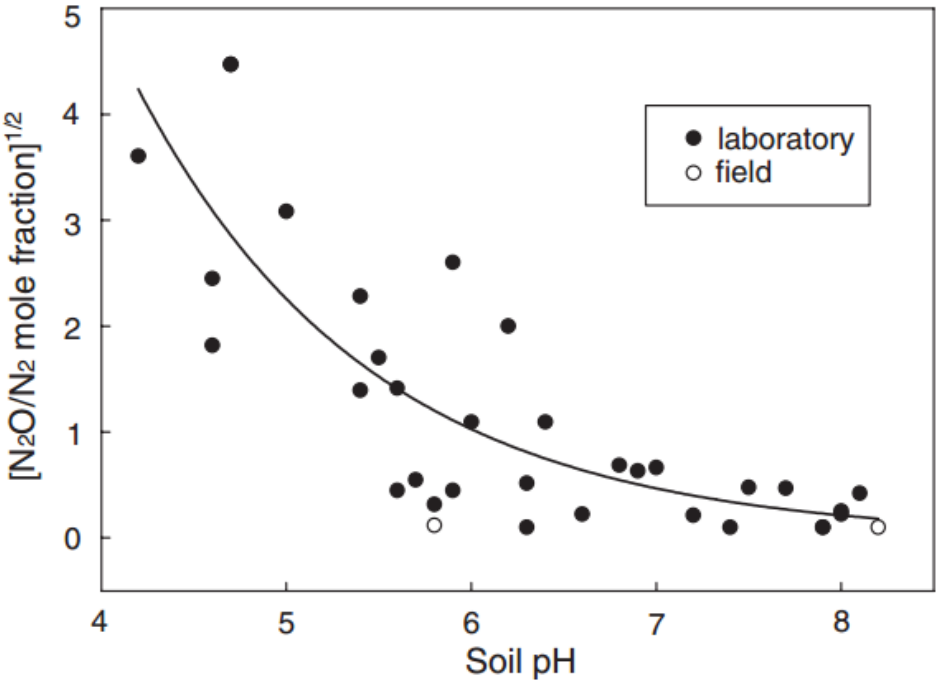


Figure 2.9: Effect of soil pH on the ratio of nitrous oxide and nitrogen gas molecules emitted (Rochester 2003)

Denitrification management practices are based on ensuring soil aeration is achieved (optimum tillage and controlled traffic farming) as clay soils such as Black Vertosols are extremely susceptible to compaction and waterlogging in the right circumstances (Daniells 1989). These practices however, also need to consider the variability of emissions between furrows and mounds in an irrigated cotton paddock, as well as optimum nitrogen application rates. Furrows generally exhibit a higher loss rate when irrigation with fertiliser is applied, or nitrate runoff with irrigation waters occurs and research has found that when more than 200kg N/ha is applied, nitrous oxide emissions could not be managed efficiently (Macdonald et al. 2014).

Scheer (2013) found that a more frequent irrigation schedule that coincides with high soil mineral nitrogen levels increases the nitrous oxide emissions from soil. In instances of high water applications however, the

nitrous oxide emission level was reduced as the cotton yield increased, supporting the need to use irrigation schedule strategies to minimise denitrification processes.

By targeting a sustainable future which incorporates reduction of greenhouse gas emissions, the Australian Government has placed some emphasis on fertiliser use efficiency in irrigated cotton by developing an irrigated cotton calculator. The calculator is included within the *Carbon Credits Determination 2015* and provides a basis of values that represent typical greenhouse gas emissions including both nitrous oxide and carbon dioxide from urea throughout the cotton production process (Australian Government n.d.). The nitrous oxide emissions for applied nitrogen fertiliser are generally accepted to be 1.25 +/- 1.0% of fertiliser N applied (Follett 2001).

The calculator equations are based on the fertiliser rate and nitrous oxide emission graph in Figure 2.10.

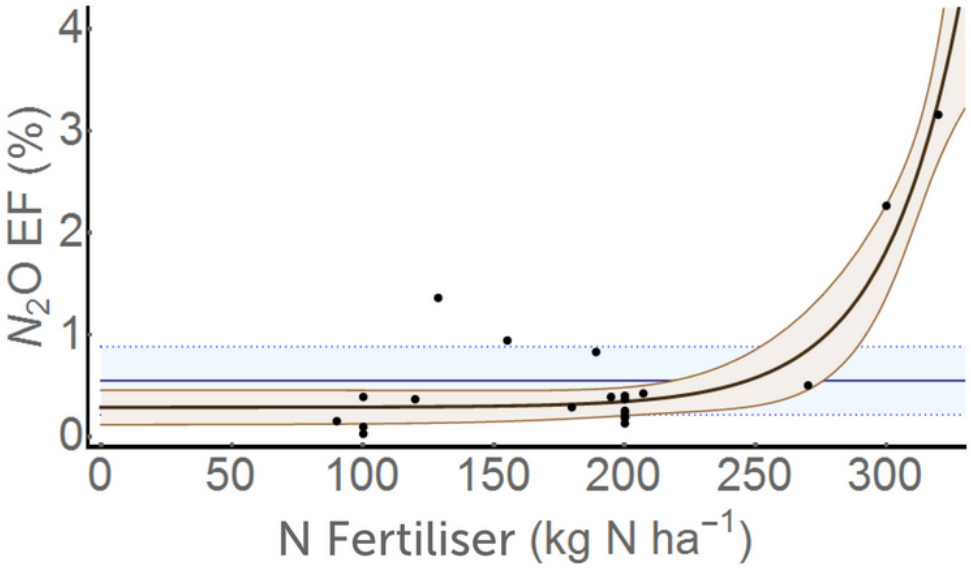


Figure 2.10: Australian Government (n.d.) nitrous oxide emission calculator for cotton enterprises

2.6.2 Leaching

Leaching as a nitrogen loss pathway refers to the movement of nitrate molecules down through the soil profile away from the access of plant roots and into soil drainage zones causing water contamination. Nitrate, is a water soluble ionic compound with negative charge and therefore extremely susceptible to leaching when soil moisture levels are excessive (soil consistently reaches field capacity) (Follett 2001). Dry conditions also have an effect on the leaching process, with nitrate being drawn to the soil surface by capillary action making it more susceptible to becoming a waterway contaminant through runoff. Ammonium, unlike nitrate, is not susceptible to leaching processes due to its positive ionic charge. Clay particles within the soil have an overall negative charge, which results in ammonium molecules being adsorbed to the clay colloids by strong ionic bonds (Price 2006).

Leaching within the cotton industry is a minor form of loss due to the nitrate being present in surface levels of the soil (30cm) which is readily accessible by cotton root structures. This however is highly dependent on the type of fertiliser application and irrigation strategies (Rochester, Constable & MacLeod 1991; Smith et al. 2014). Godin (1999 as cited by Follett 2001) found that unless anaerobic conditions were satisfied excess amounts of nitrate will leach in heavy-textured soils in irrigated crops.

2.6.3 Volatilisation

Both denitrification and leaching have been identified as the two main causes for between 50 and 100kg per hectare of nitrogen losses within the cotton industry (Rochester 2003). The remaining two alternative nitrogen loss pathways are volatilisation and nitrification. Volatilisation refers to the conversion of ammonium to ammonia gas with the correct soil and climatic conditions. A high soil pH (greater than 7), high temperatures, stubble cover and windy conditions and surface fertiliser applications can cause

ammonia gas to develop and be released to the atmosphere as a form of pollution (Johnson et al. 2005; Smith et al. 2014; Follett 2001).

An alkaline soil contains a high proportion of hydroxide ions (OH^-) compared to the acidifying hydrogen ions (H^+). In this instance, ammonium ions react with hydroxide ions forming water and ammonia gas by-products. Soil pH and high soil ammonium concentrations are therefore key drivers of the volatilisation process (Price 2006). The clay particle attractions of ammonium ions also influences the rate of ammonia gas formation, particularly when nitrogen fertiliser is surface applied, causing inefficiency of the clay particle and ammonium adsorption (ammonium ion numbers become greater than surface area of clay available for bonding) (Price 2006). Soils with low cation exchange capacity also contribute to greater volatilisation losses (Follett 2001).

By understanding the drivers of volatilisation, key farming management strategies to reduce potential ammonium losses include applying nitrogen fertiliser in cooler conditions with low wind conditions, apply fertiliser within the soil medium (minimise surface application), or when surface application is necessary applying prior to irrigation or rainfall events.

2.6.4 Nitrification

Nitrification is a rapid conversion of ammonium into nitrate by nitrifying bacteria which use the nitrate and intermediate formation of nitrite as energy sources. The inorganic plant available nitrogen form of nitrate is preferred by cotton plants, over the alternate option of ammonium. This biased ratio therefore makes the nitrification process integral for cotton plant's continuous health and development (Cotton CRC 2001).

To understand the nitrification process in details, two chemical conversions exist during the nitrification process which are outlined by the two reactions below. The two reactions are initiated by *Nitrosomonas* and *Nitrobacter* soil bacteria respectively.

1. $2NH_4^+ + 3O_2 \rightarrow 2NO_2^- + 2H_2O + 4H^+$

2. $2NO_2^- + O_2 \rightarrow 2NO_3^-$

(IPNI 2015)

The first reaction combines two molecules of ammonium with three molecules of oxygen to result in the formation of an intermediate form of nitrogen – nitrite. This initial reaction is important as it outlines the ability of the nitrification process to cause soil acidification (release of hydrogen ions). It also can create nitrous oxide (N_2O) gas emissions if the nitrite decomposes within the soil under acidic soil conditions before the second reaction proceeds (IPNI 2015).

The second reaction converts the two molecules of nitrite, together with a molecule of oxygen into the plant available form of nitrate. Nitrite is toxic to plants therefore it is vital that this secondary conversion occurs (IPNI 2015).

This oxidisation conversions of nitrification optimally occur in a well aerated soil medium with sufficient moisture and warm temperature conditions (15^{o}C – 38^{o}C) that favour biological activity. Rainfall and irrigation events can therefore become constraints to the nitrification process with a reduction in pore space and oxygen supply. Research has indicated that a pore space greater than 60% that is filled with water is nitrification restrictive and field capacity provides an optimal soil moisture level (Price 2006, IFNI 2015). Acidic and alkaline soils can also prevent the activity of the nitrification bacteria as well as high salinity levels.

Nitrification technically isn't a loss of nitrogen from the soil medium, however it is a key process that can potentially increase the risk of losses from the root zone region of a crop due to the susceptibility of nitrate to leaching and denitrification permanent losses. For this reason, the management of nitrate formation and uptake by plants is important and

should be considered when determining best nitrogen farm management practices. These practices include split fertiliser applications to restrict excessive nitrate formation or the use of a controlled release fertiliser.

2.7 Carbon Dioxide and Methane Emissions

Carbon dioxide is classified as a greenhouse gas with one source of soil production from the urea hydrolysis reaction as a by-product. Due to the potential atmospheric warming effects from accumulation of carbon dioxide within the atmosphere, the carbon dioxide emissions from cotton enterprises is included within the Irrigated Cotton Calculator developed by the Australian Government (n.d.). For every atom of nitrogen applied to soil, half an atom of carbon dioxide is released, equating to 0.733 tonnes of carbon dioxide per tonne of urea (Carbon Credits Determination 2015).

Another source of carbon dioxide emissions from the soil medium is from respiration (aerobic conditions) and fermentation (anaerobic conditions) reactions that occur when microorganisms decompose organic matter. The two reactions based on the presence or absence of oxygen are outlined below.

1. Carbohydrate + O_2 → CO_2 + H_2O + energy
2. Carbohydrate → CO_2 + acid or alcohol + energy

Not all carbon dioxide gas produced however is released from the soil medium, with compaction and soil moisture content reducing soil diffusion capabilities (Singer & Munns 2006).

Sistani et al. (2011) identified that differences of methane and carbon dioxide emissions from a variety of fertiliser types including urea was greatly impacted by temperature and moisture conditions.

Methane as a greenhouse gas has an affect that is approximately 20-30 times greater than carbon dioxide in the atmosphere. Production from the

soil is driven by the presence of anaerobic soil conditions as methanogenic bacteria digest organic matter. Methanotrophic bacteria however oxidate methane molecules, and when present in the soil, create a methane sink rather than source to reduce the presence of methane in the atmosphere. (Serrano-Silva et al. 2014).

Soil methanotrophic activity increases as field capacity is reached, however is least effective on soils when moisture content exceeds field capacity. Methanogenesis (production of methane gas) is at an optimum level when soil temperatures are between 30 and 40 degrees Celsius, as well as in heavy clay soils that retain a higher moisture content (Le Mer & Roger 2001).

2.8 Nitrogen Supply Modelling and Prediction Systems

Without needing several years of data to be physically measured, a study by CSIRO (Horton & Corkrey 2011) developed a model linking soil temperature (5-100cm deep) to rainfall and air temperature values of the area. The Bureau of Meteorology's *Climate Data Online* service stores daily temperature and rainfall data for several weather stations around Australia. Nitrogen release from organic sources can be linked with the thermal time (degree days) (Antille et.al. 2014) which uses the temperature of soil at a certain depth over a defined period of time.

Griffin & Honeycutt (2000) successfully matched the nitrate supply and ammonium decrease from animal manures using degree day predictions in a soil incubation experiment using three different incubation temperatures. Honeycutt & Potaro (1990) were also successful in analysing the soil nitrogen mineralisation trends from plant residue applications and the relationship between this and degree day units, with Honeycutt, Zibilske & Clapham (1988) suggesting that prediction of net

nitrogen mineralisation from degree day units could assist in the linkage of laboratory and field studies.

A model exists called NCSOIL which is used to model the nitrogen and carbon transformation in soil (Haskett et.al 1986). The model has relevance to this project because it assumes a constant temperature and moisture content, which is similar to the laboratory conditions used in the experimental process. The program does however have a restricted access, as well as being developed based on international climatic and soil data.

A more relevant model was developed by CSIRO, Department of Fisheries and Forestries (Queensland Government) and The University of Queensland called APSIM (Agricultural Production Systems sIMulator. The model seeks to analyse the complete plant-soil-water system, with one of the components being soil inorganic plant nitrogen supply. The model includes data that relates to Australian and Queensland climatic conditions and soil properties as well as for cotton cropping. The cotton module was incorporated based on a previous model (OZCOT) (Keating et al. 2003).

Within the Australian cotton industry, farmers have access to CottonAssist which is a decision support program designed to assist with cotton enterprise management, (www.cottassist.com.au/Default.aspx). The program was designed by CSIRO and supported by CRDC and the former Cotton Catchment Communities CRC Ltd. Soil and fertiliser management through a subset model NutriLOGIC is incorporated to monitor on-farm conditions, as well as CottBASE to provide results on hypothetical scenario inputs to determine the effects of different climate and soil conditions, as well as farm management practices.

2.9 Summary

After a thorough review on the background knowledge and research conducted surrounding soil inorganic nitrogen supply and gas emissions, it was evident that knowledge gaps exist for the Australian cotton industry, as well as other agricultural industries such as sugarcane and grains. A lack of information relating to specific data for cotton nutrition, mineral nitrogen supply from soil and the effect of nitrogen fertiliser types was apparent.

CHAPTER 3 METHODOLOGY

3.1 Introduction

The project methodology primarily focussed on stages of preparation and design to produce experimental data that could be analysed to address the research aims and objectives (Figure 3.1).

This chapter will outline each of the methodology stages in detail, which are described below.

- *Soil collection, preparation and characterisation:*
  Identified a suitable soil type to use during experimental procedures. Designed methods to collect, transport and store the soil samples, as well as methods to identify key soil properties and prepare the soil for further experimentation.

- *Sample collection:*
  Developed two experimental procedures to collect numerous soil and gas samples. This project used laboratory based procedures which provided some constraints and limitations, also outlined in detail within this section.

- *Sample analysis:*
  Experimental techniques and laboratory instruments were used to analyse samples from the sample collection stage to determine ammonium, nitrate and greenhouse gas emission concentrations. Results will be outlined in Chapter 4 – Results and Discussion.

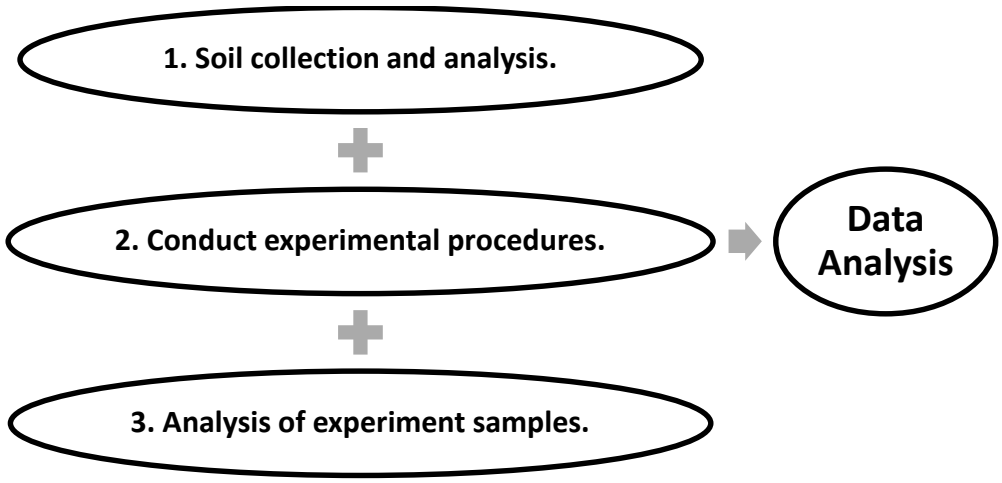


Figure 3.1: Project methodology stages

3.2 Soil Collection

3.2.1 Soil Type Selection Criteria

The soil types in Australia are highly varied, each presenting a different set of properties and characteristics that influence the level of biological activity within the soil and how it functions as a biological medium for plant growth and development. The cotton industry is no exception to the high variation of soils that form the basis of cotton crops.

With Southern Queensland and Northern New South Wales being the hub of cotton farming within Australia, one of the most common soil types within this large region was identified to be a Black Vertosol (commonly known as the fertile black soil of the Darling Downs) (NSW Agriculture 1998). This is a heavy, clay soil type with high fertility and other unique properties, particularly in the wetting and drying phases with the display of shrink/expansion mechanisms (refer Chapter 2 – Literature Review).

To ensure data obtained during the experimental stages reflected Black Vertosol used in a cotton enterprise, the criteria for the soil history and field location were defined. Specific criteria and variations of the criteria for Black Vertosol sample collection included:

- A history of fallow-cotton rotation or similar.
- A history of cotton-winter crop rotation or similar.
- A history of irrigated cotton cropping - 95% of Australia's cotton industry is irrigated with only 5% dryland (CottonInfo 2015).
- A history of farm management practices to ensure sustainable cropping and continuous soil development. These practices could include, but are not limited to controlled traffic farming (CTF), tillage (a biotechnology requirement of cotton farming for pest management), zero tillage (after a winter crop) and subsequent plantation into previous crop stubble, use of organic matter as a soil additive (compost/manure), suitable irrigation practices and regular soil testing.
- No trace of disease or pest infiltration that would prevent the development of a healthy crop (cotton or other variety).
- Suitable soil structure that included a sufficient depth of topsoil and substructure.

The timing of the soil collection was also a critical component to selecting the correct soil sample for experimental use. Ideally the soil was to have a low initial concentration of mineral nitrogen (ammonium and nitrate), as well as having enough organic matter to ensure that biological activity was generated when nitrogen fertilisers and moisture were added to the soil. The low initial mineral nitrogen concentration requirement was to ensure that during the data collection phase, smaller changes in the data could be more effectively identified and analysed.

The property location was the final key factor in choosing a soil collection location. Ideally, a cotton field on the Darling Downs within a suitable distance of Toowoomba was preferred, to ensure some soil samples remained at a low temperature during transportation. With moist soil taken directly from the field, it was important to ensure that the samples were refrigerated straight away to avoid moisture evaporation, continuation of biological activity and a possible alteration of the soil properties at the time

of collection (McKenzie, Coughlan & Cresswell 2002). Prior approval enabling research to be undertaken on the property was also required, to streamline the sample collection process and possibly have access to other experimental data that may have been collected from the same location.

Using these criteria and specifications as a guideline, as well as project timelines, a cotton farm situated at the small locality of Yargullen was selected as the soil sample collection point for this research investigation.

3.2.2 Background of Selected Location and Soil Type

Yargullen is a small area near Jondaryan, Darling Downs, Queensland, situated on a Black Vertosol foundation (latitude: -27.50431°, longitude: 151.64705°). The area is mainly comprised of smaller paddock allotments, each with a variation of crop types. Figure 3.2 displays the relative distance of Yargullen from Toowoomba, with Figure 3.3 displaying local roads at Yargullen.

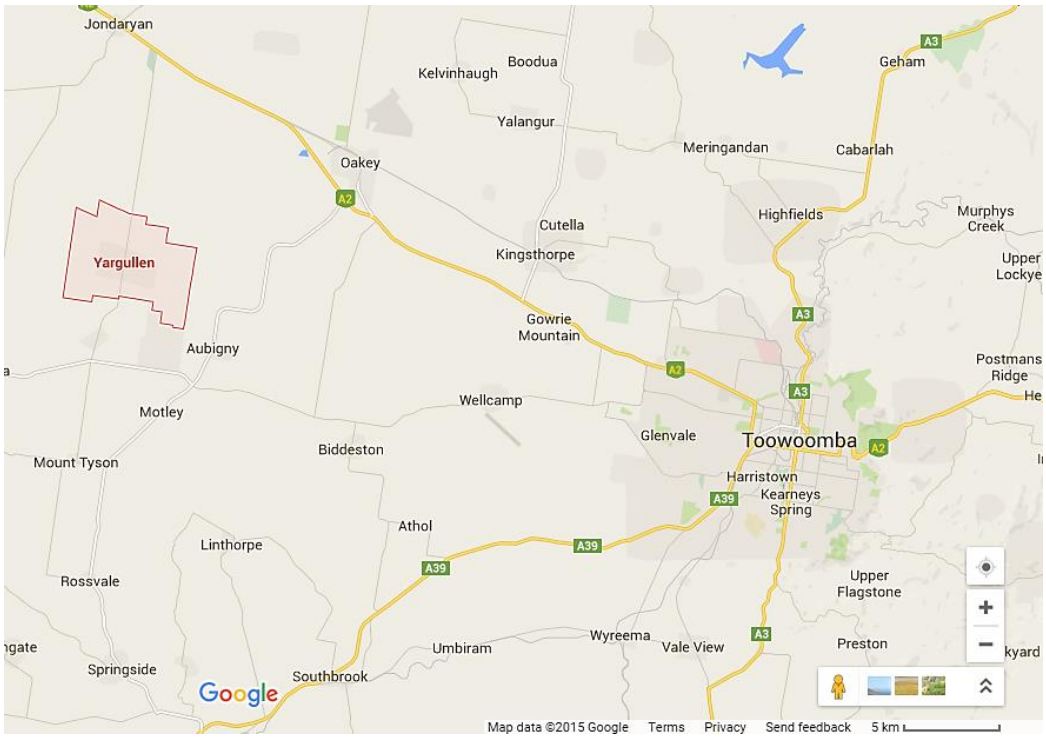


Figure 3.2: Locality of Yargullen with respect to Toowoomba (Google Maps 2015)

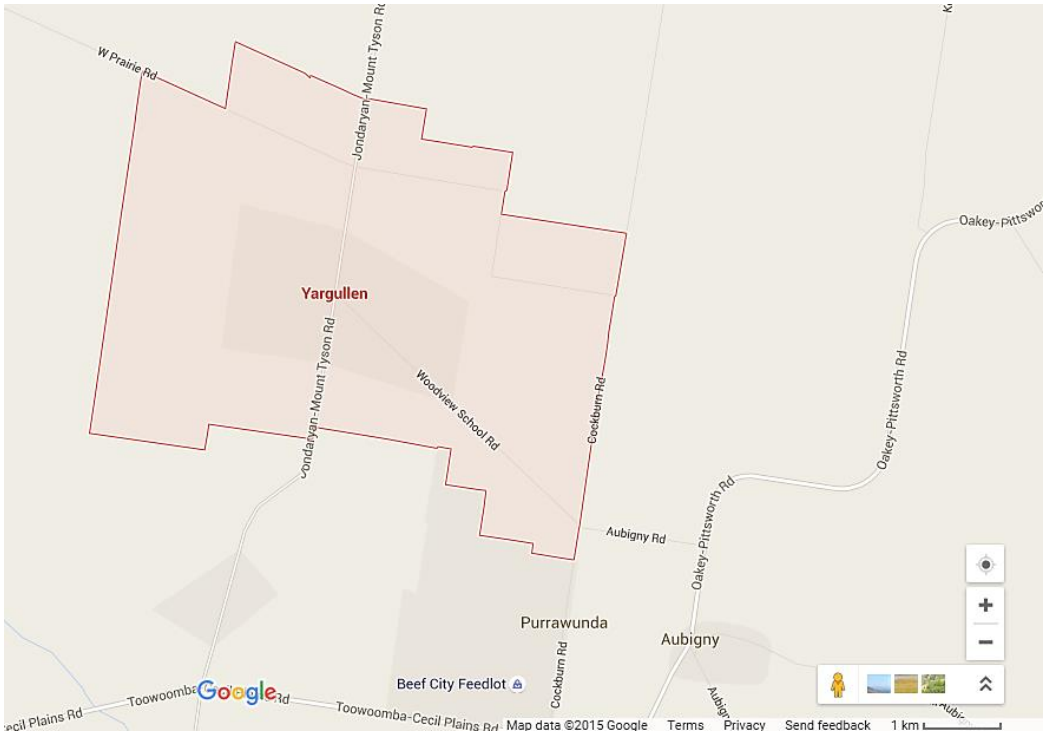


Figure 3.3: Local roads in Yargullen area (Google Maps 2015)

A soil sample collection site was selected on the cotton farm that represented ‘average’ field conditions (McKenzie, Coughlan & Cresswell 2002). This site included the outer edge of two furrows within the centre of the paddock. The specific furrow location was selected due to the constraints of road accessibility, equipment required for collection and to ensure disturbance to the crop was kept to a minimum level. See Figure 3.4 for a photograph looking west at the site on the day of collection (19 January 2015).

Figure 3.4: Paddock and furrow used for soil sample collection 19 January 2015

With the cotton farm located a short distance from Toowoomba, travel time was not an issue and ensured field moist samples could be collected and transported in the required cool conditions. The selected paddock was part of a back-to-back cotton season planting scheme, with cotton grown at the beginning and end of each year. An interim, unirrigated winter cereal crop and the use of furrow design to enable sufficient furrow irrigation to occur during the cotton season, were also part of the crop rotation plan. At the time of soil collection, the second cotton crop for the 2014 year (previous crop 2013/2014) was halfway through a crop cycle, planted on the 21st October 2014 with the cotton variety Bollgard II ®.

The Bollgard II ® cotton variety was developed for improved pest management purposes, therefore most growing characteristics are very similar to other varieties. The majority of cotton varieties are susceptible to protein variation when waterlogging, temperature, light intensity, nutrition and other factors that may result in excessive stress are not at optimal levels to promote healthy plant development. Planting density is recommended at 10-15 seeds per metre (for at least an 85% germination rate) and on average, the variety has a similar season length to both INGARD and conventional cotton varieties – 167 days (Monsanto 2011).

Pre-plantation and seasonal paddock processes occurred at the Yargullen site, to instigate healthy crop and soil development and to assist in maximising yield. The processes included:

- Combined soil preparation before planting, which included tillage (subsoil and disc), soil levelling, furrow moulding and fertiliser application. These processes occurred within the same operation.

- Application rate of 180kg nitrogen per hectare using a urea blend with MAP (mono-ammonium phosphate) on 17th September 2014, prior to planting.

- A second mid-season fertiliser application on 25 January 2015 of 135 L per hectare UAN (urea ammonium nitrogen – 35% nitrogen by weight) via fertigation methods. 10L per hectare of UAN was also applied using a foliar spray method.

- Irrigation schedule for the season (dependant on rainfall events) included an irrigation application in early October prior to planting, second application around Christmas 2014, third application mid-late January 2015 and a final irrigation application in mid-February.

- No CTF (controlled traffic farming) techniques have been incorporated within the farming practices.

The geological history of the cotton farm sat Yargullen impacts greatly on soil type, characteristics and overall performance when used for agricultural purposes. The locality of Yargullen is surrounded by three main rock types including sand plain (Czs), channel and flood plain alluvium of gravel, sand, silt and clay (Qa) and predominantly mafic, volcanic rocks (Czb) (refer Figure 3.5).

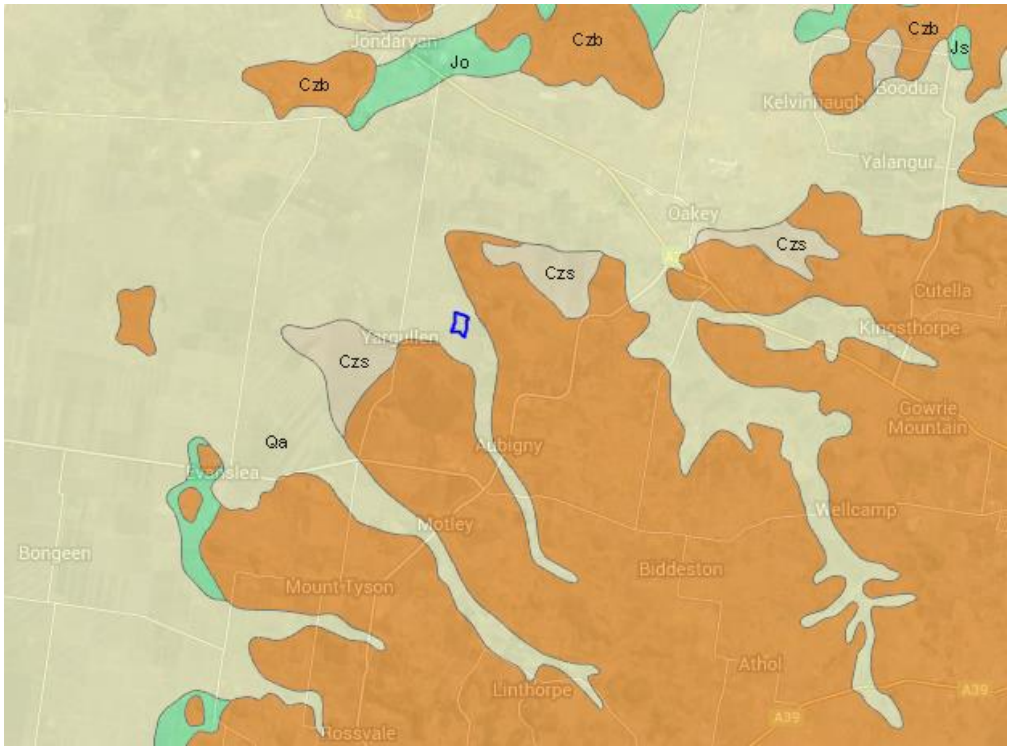


Figure 3.5: Geological formations surrounding Yargullen - the blue square represents the location of cotton paddock selected for soil collection (Geoscience Australia 2015).

While the soil quality within an area can change over time due to farming practices and other major events such as flooding and urbanisation, generally the identified base soil type remains the same, in terms of decades. Using a study conducted in 1999 by Harris, Biggs and Coutts, the general soil type of the Yargullen area was identified as having an associated soil type of ‘moderately deep (50-100cm), soft, granular dark clay on soft calcareous material’ with dominant plant species of Queensland Bluegrass. With only an associated soil type being defined, this means that several other dominant soil types may also be present in the area. The associated soil type for Yargullen was found to have originated from older alluvial plains resulting in broad level plains of basaltic alluvium and predominant had a soil type of black, self-mulching cracking clays (refer Figure 3.6 and Figure 3.7) (Harris, Biggs & Coutts 1999).

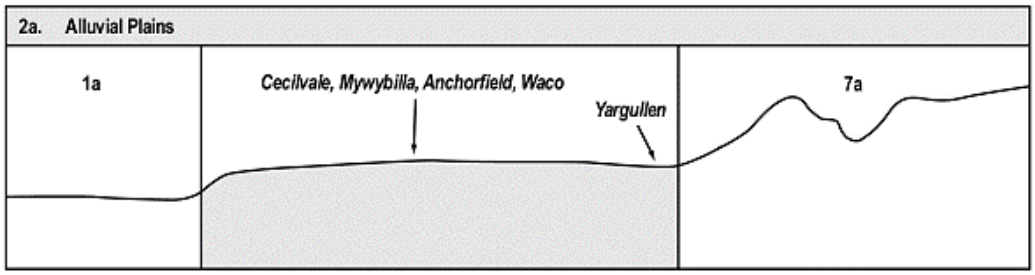


Figure 3.6: Yargullen associated soil type location on typical alluvial plain (Harris, Biggs & Coutts 1999)

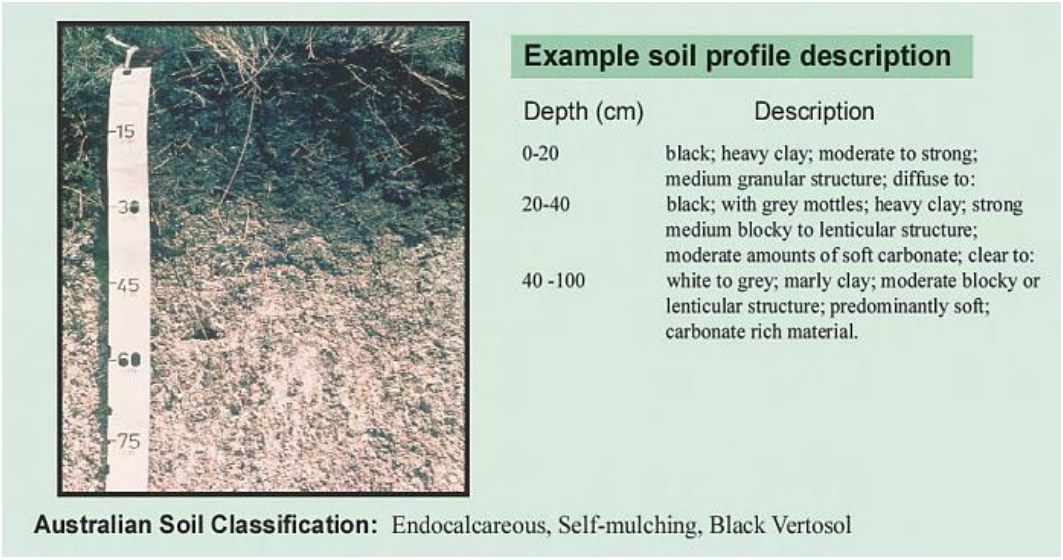


Figure 3.7: Yargullen soil type classification and structural description (Harris, Biggs & Coutts 1999)

3.2.3 Soil Collection – Bulk Samples

For the purpose of maintaining the integrity of experimental data for this investigation, it was necessary to develop soil collection strategies to ensure variability in soil properties ensuing from collecting, transporting and storing the soil samples were minimised. All methods used during the three main processes of soil sample collection (collection, transportation and storage), were developed using existing methods from *Soil Physical Measurement and Interpretation for Land Evaluation* (McKenzie, Coughlan & Cresswell 2002).

The soil collection process had two primary aims:

1. Collect bulk soil samples to be air-dried that represented the ‘average’ soil properties of the cotton paddock.
2. Collect smaller volumes of soil samples to be used in soil tests that required field moist soil conditions.

Conditions on the day of soil collection are displayed in Table 3.1 below.

Table 3.1: Conditions on day of soil collection, Yargullen, QLD

Condition	Description
Date	19 January 2015
Time of Collection	3:00pm
Air Temperature	36^{o}C
Soil Temperature	29.4^{o}C
Date of Last Irrigation Event	24/25/26 December 2014
Date of Last Fertilisation	17 September 2014
Observations	• Soil had a fairly thick dry surface layer (0-5cm) with some cracking of soil starting to appear. • Lower depths did display moisture presence. • Soil collection occurred during a time period that experience several days of high daily temperatures. • Cotton crop was well progressed in first boll development.

The collection of the bulk soil sample was taken from the top 0-20cm of the soil profile, therefore the project results are only applicable to soil conditions at this depth. Soil was collected from the centre and edges of the furrow (without disturbing cotton plant root systems) by taking multiple soil samples from two side-by-side furrows, at approximately 1m distances along each furrow. The variation in sample location was used to ensure the soil had a greater chance of representing the conditions of the entire cotton paddock, not just one small area of one furrow. This then ensured

the results from experimentation also reflected the ‘average’ paddock response.

Approximately 2cm of the surface topsoil was removed prior to collection as this layer was likely to have no moisture content and not reflect the actual soil structure and conditions. This process was important at the time of collection as several days of heatwave conditions had occurred prior to the collection day, which was reflected by the air temperature (36^{o}C) recorded when collecting samples. Using a shovel to collect the sample and a clean plastic bucket for sample transportation, 20kg of soil was obtained for use in the necessary experimental procedures required for this investigation (Figure 3.8).

Figure 3.8: Collection of 20kg bulk soil sample, Yargullen, 19 January 2015

3.2.4 Soil Collection – Field Moist

The collection procedure of soil samples that were to remain field moist for experimental purposes included two types of collection. The initial technique was to collect three large zip-lock bags of soil from 0-15cm layer which was completed by using a trowel to remove soil and ensuring the zip lock bags were sealed immediately once filled.

The second collection technique required six bulk density ring samples to be obtained, which was completed by using a hammer and plank of wood, to gently and evenly hammer the rings (Vehmeyer tubes) perpendicular to the topsoil layer at six different locations. This method was based on Method B5.1 from *Laboratory Methods of Soil and Plant Analysis* (Heanes 1977). The bulk density rings were carefully removed using a trowel to ensure the soil remained within the rings and excess soil was cut away using a knife. The bulk density rings were then placed into zip-lock bags and sealed. The depth and locations at which the bagged and bulk density ring samples were removed were the same as for the bulk soil sample collection as explained in Section 3.2.3.

For transportation purposes, the three bags of field moist soil samples and six bags of bulk density ring samples were required to remain at 4^{o}C or lower to ensure the moisture at time of collection remained the same. To meet this requirement, an esky with ice was used to transport the samples back to the laboratory, where all were placed into a refrigerator set at 4^{o}C.

The flow diagram below (Figure 3.9) contains images taken during collection of bulk density ring samples.

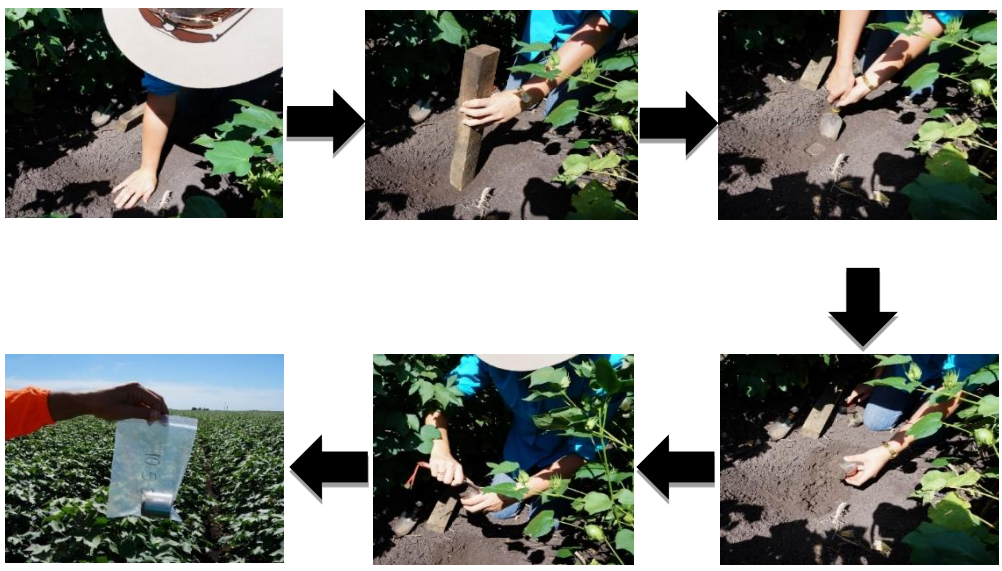


Figure 3.9: Bulk density ring soil sample collection, Yargullen, 19 January 2015

3.2.5 Soil Preparation

To satisfy the research aim and objectives, the preparation of the soil was an important step to ensure any characterisation tests and incubation procedures were performed according to pre-determined methods. Any alteration in preparation methods could have a large impact on experimental results and therefore may introduce a degree of unreliability and inaccuracy when developing project conclusions.

For the purpose of conducting several soil characterisation tests, as well as an aerobic incubation procedure, the bulk soil collected from the field (approximately 20kg), required air-drying once transported back to the laboratory. Using the NCEA's (National Centre for Engineering in Agriculture) Soil and Water laboratory, the 20kg of soil was mixed thoroughly to combine all possible soil layers and spread out in a thin layer on three trays. Large soil aggregates were broken down to improve the efficiency of drying and the trays were then left for six days in hot conditions (less than 40^{o}C to ensure soil properties are not affected) to enable air-drying to occur (McKenzie, Coughlan & Cresswell 2002).

For the purpose of using correctly prepared air-dry soil for experimentation, once the soil sample had accomplished an air-dry state, the soil was sieved through a 2mm sieve (Figure 3.10). All large remaining aggregates were placed in a sealed back and crushed using a mallet, to create particles of uniform size, before sieving continued. To ensure sampling was not biased, the sieved soil was divided into portions when used for further characterisation and experimentation.

Figure 3.10: Sieve process of air-dry soil preparation

The field moist soil was also sieved at 2mm nominal size prior to use. During the sieving process, all visible organic material (i.e. roots) were removed to create a soil sample uniform in size. All soil was used immediately after sieving to ensure soil properties were not altered (i.e. soil moisture content).

3.3 Soil Characterisation

Conducting soil characterisation involved using several different soil analysis methods to determine the initial state of the soil (soil properties) prior to experimentation. Soil properties assist in developing research conclusions as many are factors that can influence the development of different nitrogen compounds and potential nitrogen losses (Rochester and Constable 2000).

Initial soil data required for soil characterisation included soil tests for air-dry, field moist and field capacity moisture contents, initial nitrate, initial ammonium and bulk density.

3.3.1 Air-Dry Soil Moisture Content

An important soil characterisation test is to determine the different soil moisture properties of the soil under investigation. Chapter 2 – Literature Review outlines the typical properties of a Black Vertosol, one of which includes the high moisture capacity potential and shrinking/swelling nature. To ensure results are as accurate as possible, typical literature values were not used for this soil property. The method used to determine the air-dry moisture content was based on gravimetric water content methods to determine total soil water content in different conditions (i.e. air-dry or field moist) from *Soil Sampling, Preparation and Analysis* (Tan 1995).

Air-dry soil moisture refers to the moisture remaining in the micro-pores of the soil when all freely moving water particles have been removed by evaporation, generally when higher temperature and windy conditions exist (McKenzie, Coughlan & Cresswell 2002). A percentage of available moisture may have also been removed by plants when the soil was present in the field. In order to determine the air-dry moisture content, the soil that had undergone the six day air-dry process was used.

Three small samples (between 90-100g) of air-dried soil were weighed into three individual aluminium sample containers, of a pre-determined weight. The soil samples were then placed into an oven at 105^{o}C for four days (Figure 3.11) to ensure all moisture had been removed from the soil. Each sample was then removed and immediately weighed to determine the weight after oven-drying.

Figure 3.11: Oven used for drying soil samples at 105ºC

Using Equation 1 below, the moisture loss for each sample was calculated with the result then inserted into Equation 2 to calculate the air-dry water content percentage of the Black Vertosol sample.

(Equation 1)

$$Moisture\ Loss\ (g) = Weight\ air\ dry\ soil\ (g) - Weight\ oven\ dried\ soil\ (g)$$

(Equation 2)

$$Water\ Content\ (\%) = \left(\frac{Moisture\ Loss\ (g)}{Weight\ Oven\ Dry\ Soil\ (g)} \times 100\right)$$

3.3.2 Bulk Density & Field Moisture

A combined process of determining the bulk density and field moisture of the soil samples was conducted to minimise the number of procedures required throughout this investigation. Bulk density of soil refers to the weight of soil within a given volume and therefore includes pore air

spaces. A higher bulk density (greater than 1.6g/cm^3) is indicative of a soil that is compacted and therefore has the potential to restrict plant growth. The bulk density method was based on Method B5.1 from *Laboratory Methods of Soil and Plant Analysis* (Heanes 1977) using the oven-dried soil moisture content.

Field moisture is the measure of moisture present in the soil at the time of soil sample collection from the field. The process of determining field moisture capacity used the bulk density rings (Vehmeyer tubes) collected from the cotton paddock at Yargullen was similar to that of air-dry moisture. Three bulk density rings containing field moist soil were weighed and placed into the oven at 105^{o}C for 48 hours. Once dried, the rings were removed from the oven and weighed immediately (Figure 3.12). The soil was then removed, individual ring weight measured and the dimensions of each of the bulk density rings measured using a calliper. The bulk density bulk density ring volume was then determined.

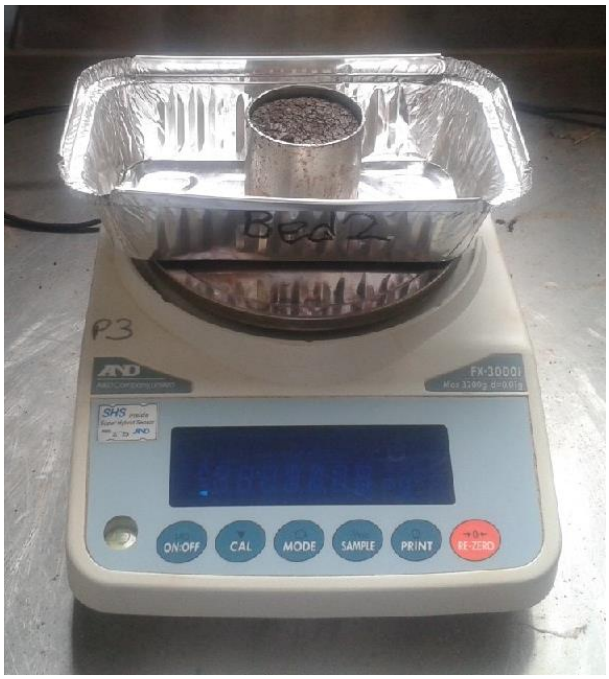


Figure 3.12: Weight of oven-dried bulk density ring samples

Both Equation 1 and 2 from the air-dry moisture calculations were used again to determine the moisture loss from the sample due to the oven drying process and the field moisture capacity (%) of the soil sample. Equation 3 below outlines the bulk density expression used to calculate the bulk density of the soil in field conditions.

(Equation 3)

$$Bulk\ Density\ (g/cm^3) = \frac{Mass\ of\ Oven\ Dry\ Soil\ (g)}{Volume\ of\ Core\ Ring\ (cm^3)}$$

3.3.3 Field Capacity

To conduct a viable incubation study, another soil moisture value required was the determination of the field capacity potential of the Black Vertosol samples. Field capacity refers to the maximum soil moisture percentage that a soil contains after excess runoff has ceased, usually occurring a few days after a rainfall or irrigation event (McKenzie, Coughlan & Cresswell 2002). Prior field work conducted in a concurrent study from the same location provided results that enabled field moisture to be determined (refer section 4.1.3).

Due to the soil moisture values only requiring to be within a 75% or greater range during the incubation period, the field capacity value was an estimate only. By gathering soil moisture data prior to and after an irrigation event and graphing the data points, linear regression was used to determine an accurate estimate of the field capacity value at 48hrs after irrigation.

3.4 Experimental Design

3.4.1 Aerobic Incubation

Aerobic incubation is a laboratory based method that ensures samples are subjected to oxygenated (aerobic) conditions. This method was suitable for this investigation as it enabled simulated field conditions to be achieved in a more controllable format. Conducting an entirely laboratory based method does however reflect some of the limitations of the project results, as the experimental conditions do not completely reflect a soil located in a paddock which is subjected to multiple forms of variability.

The aerobic incubation procedure developed for this investigation was based on methodology undertaken in Antille et al. (2014). Griffin (2008) identified aerobic incubation as a suitable laboratory based technique to quantify mineral nitrogen soil supply, by reviewing results and set conditions of experimentation from multiple sources. All were able to control the laboratory standard to a satisfactory level and obtain quality and reliable data for further analysis.

Before practical experimentation was able to proceed for this investigation, it was important to define the controlled characteristics that would be used throughout the aerobic incubation procedure. This included defining the incubator temperature, soil moisture range to be maintained throughout incubation period, determination of air-dry equivalent weight of soil samples, rate of fertiliser application and finally the length of incubation (days).

Incubator Temperature Setting

The level of soil microbiological activity is directly affected by the variations of temperature, making it a crucial experimental constant to set. To ensure that variables were kept within reasonable limits so that results could be analysed accordingly, one temperature was selected for the duration of the incubation process. The temperature selected represented an average

soil temperature at 20cm depth for the Black Vertosol sample, to reflect conditions of a cotton crop throughout a growing season.

The cotton cropping period is generally between September and May, depending on climatology of the farming enterprise location. The optimum soil temperature for healthy cotton plant growth is between a minimum of 15^{o}C and 20^{o}C and maximum of 27^{o}C and 35^{o}C (NSW Agriculture 1998). An average temperature of 25^{o}C was therefore selected as the set incubation temperature for this investigation.

Air-Dry Soil Equivalent

By calculating the air-dry moisture percentage of Black Vertosol (refer section 4.1.1), Equation 4 below was used determine the air-dry soil equivalent (when compared to oven-dry). Oven dry soil has no moisture content in its weight, only the mass of actual soil particles, whereas air dry soil still contains moisture that cannot be removed by air evaporation. Air-dry soil equivalent formed an important component of soil extraction methods and conversion of concentration units discussed later in this chapter.

(Equation 4)

$$Weight\ moist\ soil\ required\ (g) = \frac{Oven\ dry\ weight\ (g)\ \times (100 + percentage\ water(\%))}{100}$$

Length of Incubation and Sampling Days

Taking into consideration time constraints throughout the year and laboratory access, the aerobic incubation was set to a 60 day period, containing seven sampling days to remove soil samples from pots for further analysis (ammonium and nitrate concentrations). This was at least half the length of a typical cotton growing season and estimated as enough time to compare soil mineral nitrogen supply from Urea and ENTEC® Urea. The seven sampling days included days 0, 3, 7, 14, 30, 45

& 60, with the smaller initial intervals designed to record the smaller increases in ammonium and nitrate concentrations.

Soil Volume in Pots

To ensure the aerobic conditions were maintained, the soil remaining after the entire incubation period had concluded was set at no less than 150g oven-dry equivalent based on the size of the plastic incubation pots selected for use (Figure 3.13)

Figure 3.13: Small plastic pot used during aerobic incubation procedure

The aerobic experiment required that 4g of oven dry equivalent soil was removed per sampling for extraction purposes, to maintain a 1:10 soil to solute ratio for extraction and to fit the 50mL plastic sampling vials used for sample collection (Rayment & Lyons 2011).

An approximate value of total soil removed from pot due to sampling was therefore 28g, if seven sampling days were used. A further 20g of soil each sampling day was removed to verify soil moisture at time of sampling by oven-drying techniques. This equated to approximately 170g of soil removed in total during the incubation experiment. 300g of air-dry soil was therefore used in each pot for the incubation experiment.

Rate of Fertiliser Application

To fulfil the project objectives, the two fertilisers to be used in the aerobic incubation procedure were Urea and ENTEC® Urea (controlled release fertiliser). Urea has the chemical formula of CH_4N_2O with a molecular weight of 60.056 g/mol and a nitrogen content of 46.646% per mole. The derivation of this can be ascertained from the molecular weight (g/mol) breakdown listed below:

- C = 12.0115
- H_4 = (4 x 1.0079) = 4.0316
- N_2 = (2 x 14.0067) = 28.0134
- O = 15.9994

ENTEC® Urea has the same nitrogen content as standard Urea fertiliser (46.646%) (ENTEC Fertilisers 2015). The fertiliser rate of application selected for this investigation was based on the mass of applied nitrogen per hectare of Black Vertosol. To ensure that enough fertiliser was present within the soil to gain valid results, the application rate was set at approximately twice a cotton farmer's usual application rate, which is usually between 150 and 230 kg nitrogen per hectare (Cotton CDC 2001). The fertiliser application rate was kept constant for both Urea and ENTEC® Urea to ensure a direct comparison between fertiliser types could occur.

With the soil samples collected for this research project taken from 0-20cm soil depth (and then homogenised), the fertiliser application per volume was determined based on the bulk density of soil (refer section 4.1.2 - the bulk density of the soil was calculated to be on average 1 g/cm^3). Assuming an application rate of 600kg/ha nitrogen and depth of soil sampling taken to 20cm, the weight of soil in a hectare was calculated as 2 x 10^6 kg. The fertiliser mass to apply to the soil used in the aerobic incubation procedure was then determined based on the oven-dry equivalent soil to be used.

With 300g of air-dry soil added to the incubation pots for the aerobic experiment, the mass of nitrogen required to satisfy 600kg/ha application rate was calculated as 90mg per pot.

With Urea and ENTEC® Urea both having a 46% nitrogen content, the total mass of fertiliser required for a 600kg/ha nitrogen application was 195.76mg.

Soil Moisture Level during Incubation

To reflect the soil condition in the field during a cotton season, as well as ensuring sufficient microbiological activity for the entire incubation procedure, the soil moisture range to maintain the incubation pots at was set at 75% or greater field capacity. The moisture range ensured the number of days required to re-weigh and add moisture to the samples was kept at a minimum over the incubation period. Distilled water was used as the water to be added so no nitrate, ammonium or other interfering compounds such as chlorine were added to the soil system of each pot.

Using Excel, a spreadsheet was developed to assist in monitoring the moisture required for each soil sample before and after each sampling day occurred - an example of an excerpt is provided in Appendix B. It was important to consider the different volumes of soil present in the pot after small samples were removed for further analysis, as the moisture required to maintain the soil at field capacity was reduced.

3.4.2 Soil Greenhouse Gas Emissions

To expand the environmental component of the research project to sustainability issues associated with different fertiliser usage, greenhouse gas emissions from a soil amended with Urea or ENTEC® Urea fertiliser were analysed. In order to match the gas emissions with the soil inorganic plant available nitrogen levels throughout the same time period, the same

pots containing the soil samples used for the aerobic incubation procedure were used. This ensured the same soil base and conditions were maintained for both ammonium and nitrate sampling, as well as analysis of gas emissions.

A laboratory designed method to measure emissions based on the static chamber gas emission method by Collier et al. (2014) was used to identify gas emission concentrations per fertiliser treatment type. To observe small changes in emissions from the incubation process in the early stages, gas samples were taken on days 0, 1, 2, 3, 4, 5, 17, 46 and 60. Unfortunately, external constraints resulted in the day 30 results for gas emissions being missed. To analyse the results effectively and determine the gas emission flux for each fertiliser treatment, gas samples were taken from selected pots on each sampling day at 0, 15, 30 and 45 minute intervals. Pots selected for gas emission sample collection included two control pots, three Urea amended pots and three ENTEC® Urea amended pots. This enabled sufficient verification of data (ability to form averages and identify outliers) to occur.

3.5 Experimental Procedures

3.5.1 Aerobic Incubation Samples

Once the constant variables had been defined, the actual aerobic incubation experimental method was conducted. To ensure sufficient data was obtained to correctly analyse and develop conclusions, four repetitions of each treatment type (Urea and ENTEC® Urea) were used. The aerobic incubation commenced on 25 March 2015 with:

- 4 x control samples (no fertiliser application).
- 4 x soil samples applied with Urea fertiliser.
- 4 x soil samples applied with ENTEC® Urea fertiliser.

Each of the twelve soil samples were placed into round plastic containers (pots), which fitted suitably into the incubator (Figure 3.14). Matching plastic lids with six small holes to maintain aerobic conditions were placed on the pots when in the incubator (Figure 3.15). This was necessary as the incubator had a constant fan operating inside, creating highly evaporative conditions; unsuitable for maintaining soil moisture of 75% or greater. Lids with holes were used to reduce the amount of soil surface subjected to wind, as well as the inclusion of a tray of water in the base of the incubator to assist with a reduced evaporation rate.

Figure 3.14: Layout of aerobic incubation pots containing fertiliser treatments within incubator

Figure 3.15: Lids placed on incubation pots to maintain aerobic conditions.

A consistent mass of 300g air-dry soil was added to each pot and a pre-weighed fertiliser application (600kg/ha) mixed in, with the exception of control pots (Figure 3.16). Each pot was weighed and the Excel template for monitoring soil moisture capacity used to determine the required moisture to be added to reach initial field capacity. To ensure the soil was evenly mixed on day 0 of incubation, the required water mass was added to the pot, contents emptied onto a tray and mixed, and then gently packed back into the pot at an even density to reduce compaction.

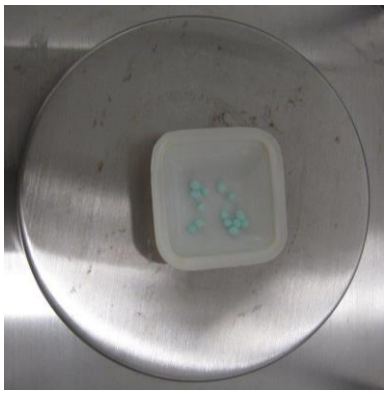


Figure 3.16: Mass of ENTEC® Urea granules equivalent to 600kg/ha nitrogen application

Over the period of 60 days, moisture addition to the pots was required every 2-3 days, which involved removing each pot from the incubator, weighing the pot, calculating required moisture addition to bring soil moisture to field capacity level (Excel template) and adding the water using a hand-held water bottle with fine spout (Figure 3.17). This method ensured no surface soil was disturbed, minimal compaction occurred and moisture was evenly distributed over the sample.

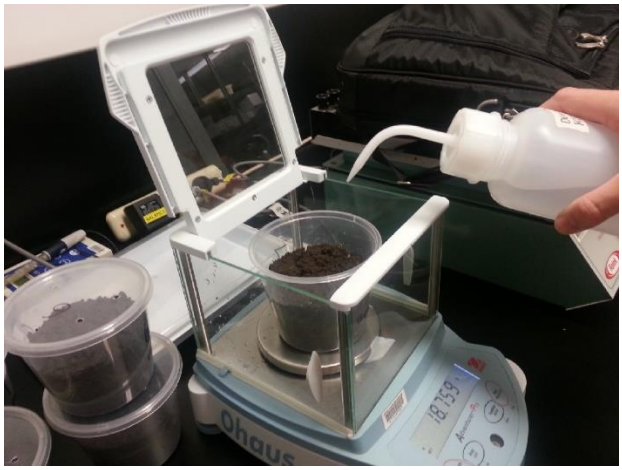


Figure 3.17: Addition of moisture to pot to maintain >75% field capacity

A multi-step process was used on the sampling days (0, 3, 7, 14, 30, 45, 60) to effectively remove required soil samples from each pot. To ensure maximum consistency was achieved, each sampling day, moisture was added to pots on the previous day to reach field capacity. This also ensured the soil was at a reasonable consistency to maintain ease of handling when removing the soil sample.

The steps for taking soil samples included removing the pot from the incubator, emptying contents into a plastic bowl, mixing thoroughly with a spoon and removing the required soil (based on method for soil extraction) to be added into a labelled 50mL plastic vial (Figure 3.18).

Figure 3.18: Soil sample mixing and removal for further analysis

The soil was then carefully placed back into the pot, ensuring no large air-gaps existed within the soil structure, the pot with soil reweighed and the required water added to ensure the soil reached field capacity (based on the new volume of soil that remained after sample taken). The pot was then returned to incubator and carefully maintained until the next sampling day was reached.

It is important to note that during the removal of each sample particularly during the initial three days of sampling, extreme care was taken to not remove fertiliser granules, to ensure the constant fertiliser rate was maintained in each pot. The laboratory where the removal of samples occurred was also air-conditioned and set at a constant 25^{o}C. During each sampling phase, approximately 20g of soil was removed and oven-dried to confirm the moisture level of the soil at sampling time that was calculated using the Excel template.

3.5.2 Gas Emission Samples

Prior to removing gas samples from the incubation pots, crimped lid vials used for collecting the gas samples were vacuumed (to approximately 250 micron) to remove all air using a vacuum pump set-up (Figure 3.19).

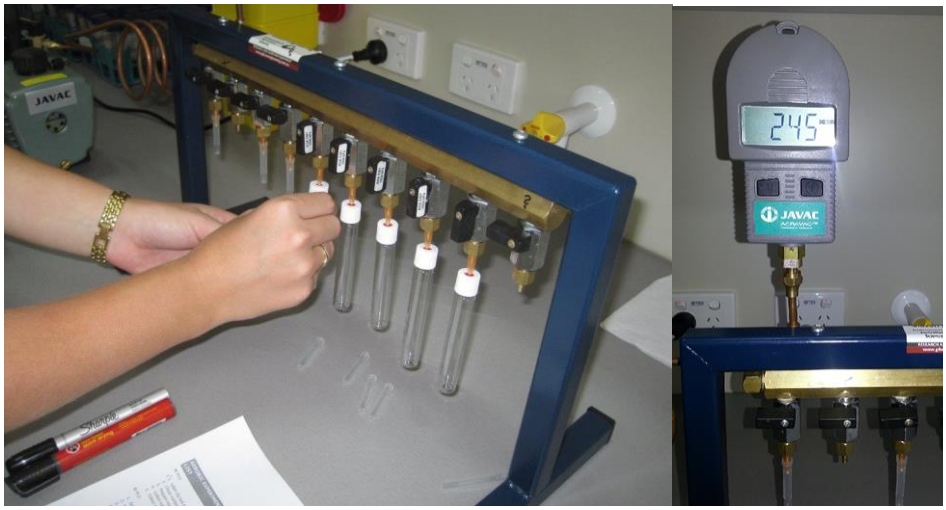


Figure 3.19: Vacuum apparatus set-up to vacuum gas sample vials prior to use (NCEA Laboratory)

Each incubation pot was designated a matching labelled lid that had two septicles (rubber seals) inserted. The septicles were designed so that when the lid was placed on the respective pot, a needle could be inserted and removed from the chamber created without letting any gas enter or escape.

The 0 min gas sample removal commenced once a pot was removed from the incubator and sealed with the correct lid. A 25mL syringe with a needle attached to the end was immediately inserted carefully (Figure 3.20) and air mixed inside the pot by pumping the syringe five times. 25mL of gas was then withdrawn slowly, and 5mL expelled to remove a 20mL total gas sample. A timer was used to ensure gas samples were removed at exactly 15, 30 and 45 minute intervals from each pot.

Figure 3.20: Insertion of syringe needle into pot lid septicles to remove gas sample

Once the gas sample had been removed from the pot, the syringe was carefully inserted into a labelled vial, and all contents expelled slowly (Figure 3.21). All vials were stored in room temperature conditions until gas analysis was conducted.

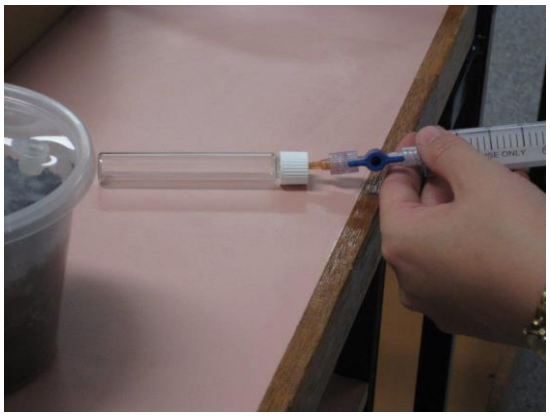


Figure 3.21: Transfer of collected gas sample in syringe to gas sample vial (pre-vacuumed)

To complete the gas sample collection phase, the headspace measurement of each pot was recorded each day of sampling, measured from the surface of the soil to the top edge of the pot. When used in conjunction with the formula for volume of a cylinder, this forms the total volume of gas chamber used and is directly related to the concentration of the gas per 20mL sample.

3.6 Analysis of Samples

3.6.1 2M KCl Extraction Procedure

A two molar potassium chloride (2M KCl) extraction procedure based on Method 7C2a from Rayment and Lyons (2011) was used to extract ammonium and nitrate ions from soil samples removed from the incubation pots. A solution of 2M KCl was made by dissolving 149.1g of potassium chloride salt in 1L of distilled water with 40mL added to each vial containing the soil sample removed from the incubation pots every sampling day, to ensure a soil -solution ratio of 1:10 was achieved.

Once the KCl was added, the vials were mixed for an hour using a clock-face mixer (Figure 3.22) and then placed in a centrifuge machine and

centrifuged at 2500rpm for 3 minutes to separate the solids from solution (Figure 3.23). This process enabled the filtering of the filtrate through 2µm filter paper to occur much more efficiently and effectively , with filtrate stored in clean 50mL, labelled vials in refrigerated conditions (<4^{o}C) until further analysis could occur Figure 3.24.

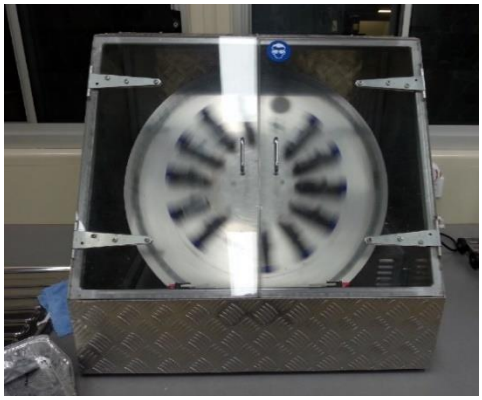


Figure 3.22: Clock-face mixing machine to mix soil sample with KCl extraction solution

Figure 3.23: Centrifuge machine used to separate soil solids from extraction liquid prior to filtration

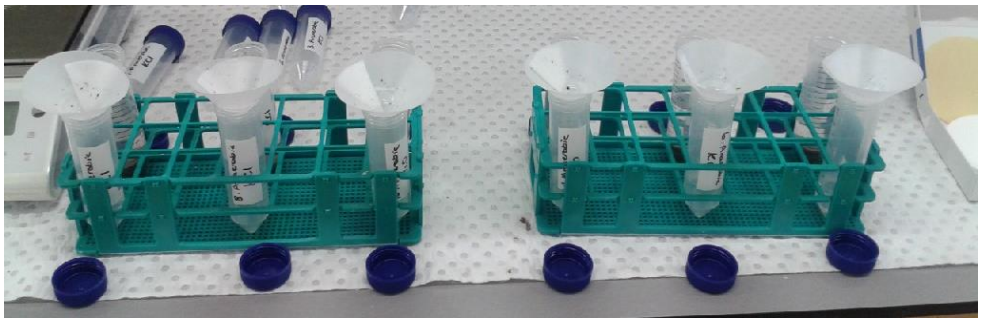


Figure 3.24: Filtration of soil extraction and storage in 50mL plastic vials

3.6.2 Colorimetric Analysis

Due to the logistical constraints of this project, it was decided that a portable spectrophotometer (DR2700™, Hach Company USA/Germany) based on visible light wavelengths (400-900nm) would be used to analyse the ammonium and nitrate concentrations of each of the soil filtrate samples taken throughout the incubation 60 day period.

The spectrophotometer used a known wavelength for specific ammonium and nitrate analysis methods to gain results that reflected the amount of light absorbed when passed through a solution of unknown concentration. Different ions within a solution (such as nitrate or ammonium) have different characteristics when light absorption and reflectance are considered. The Hach Spectrophotometer provided an output of concentration, absorbance (amount of light sample absorbed) and transmittance (amount of light sample transmitted) for each sample tested. The concentration was an instrument calculated figure based on the raw data values for absorbance and transmittance that were recorded. This enables concentration to therefore be manually calculated after the testing has been conducted as long as either absorbance or reflectivity were recorded.

The Hach DR2700 Spectrophotometer tests for ammonium and nitrate used the pre-set wavelengths of 425nm and 500nm respectively with a rated accuracy of ± 1.5nm. These values fall on the upper, short wave boundary of the light spectrum (Hach 2010). For both tests, 1cm round, glass sample vials were used.

The instrument utilises a split beam, optics method of measuring chemical concentrations within solutions, which is stated as having a greater accuracy than more conventional single beam optics. A half mirror is used to split the beam of light of specific wavelength (depending on type of chemical test used), with one beam passing through the sample and the other beam measuring a reference element (refer Figure 3.25). Both beam

measurements of absorbance are then compared to account for any variation in changes of light that passes through the sample, resulting in highly accurate results (Hach n.d.). Absorbance results are accurate to 0.005 at 0.0 - 0.5 absorbance range and 1% for 0.5 - 2.0 absorbance values (Hach Company 2013).

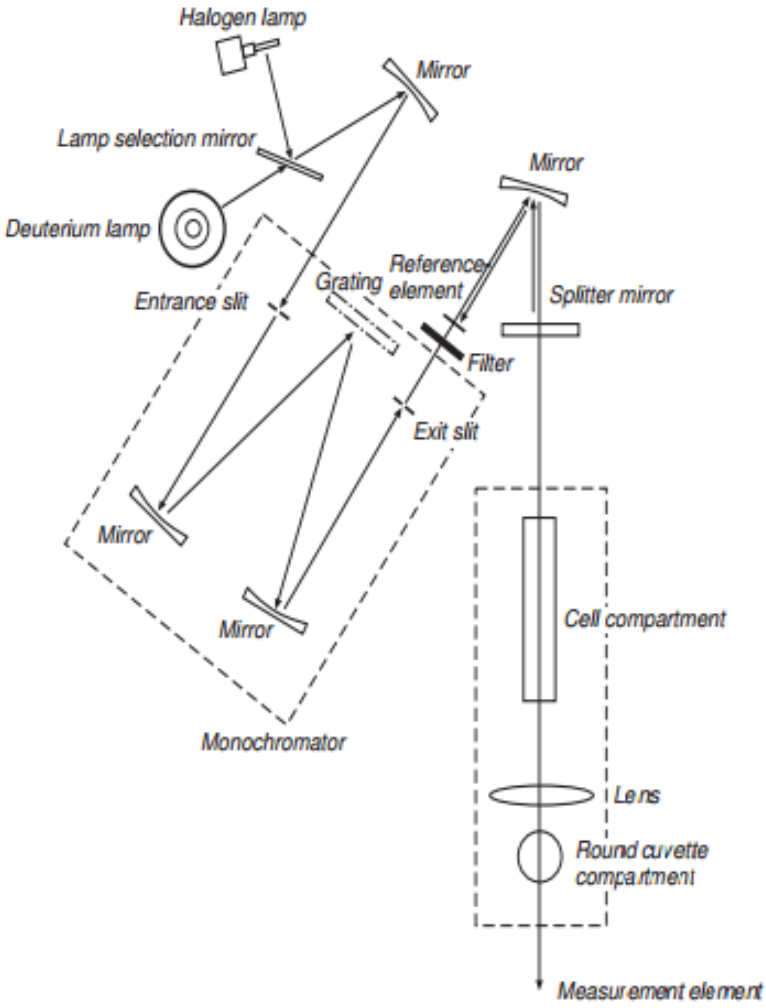


Figure 3.25: Hach DR2700 Spectrophotometer split beam measuring mechanism (Hach n.d)

Calibration of Results

Calibration of the absorbance data was achieved using standard concentration and quality control concentration data for both the ammonium and nitrate methods. The calibration curve was developed on

standards that had the same 2M KCl matrix as the samples. Equation 5 below was used to determine concentration and volume requirements for standard solution preparation.

(Equation 5)

$$C_1V_1 = C_2V_2$$

Where C_1 = concentration of stock solution, C_2 = concentration of new solution, V_1 = volume of stock solution needed to make new solution and V_2 = final volume of new solution. This expression was successful as long as all units of volume and concentration were the same for both sides of the equation.

A 500mg/L ammonium chloride standard was made by oven drying 0.3819g of ammonium chloride salt in the oven for four hours at 105^{o}C (Rayment and Lyons 2011). After four hours, the salt was then dissolved in 500mL of 2M KCl solution using a 500mL volumetric flask and stored in a labelled Winchester bottle to use for preparing standard solutions. A 2M KCl solute was used as the soil extractions were based on this and not a water background.

The procedure for making a 500mg/L potassium nitrate standard was similar, with 1.8046g of potassium nitrate salt oven dried for four hours at 105^{o}C and then dissolved in 500mL of 2M KCl and stored in a labelled Winchester bottle for further use. Storage of both 500mg/L ammonium and nitrate standards was in a refrigerator at 4^{o}C for the duration of the project (Rayment and Lyons 2011).

Using the stock standards of ammonium chloride and potassium nitrate, four smaller concentration standards were made to generate a standard curve and perform quality control checks throughout the duration of the ammonium and nitrate Hach methods. The standard concentrations selected were based on the measurement range capabilities of each method for the spectrophotometer.

The ammonium concentration range capabilities for the Hach DR2700™ Spectrophotometer using Method 8038 (USEPA Nessler Method) was between 0.02 and 2.5 mg/L. The four standard solution concentrations chosen and methods for preparation are outlined in Table 3.2 below. Automatic pipettes and 100mL volumetric flasks were used to prepare the standards.

Table 3.2: Ammonium chloride standard solution development

Standard Solution Concentration (mg/L)	Preparation Method
0.5	Combined 0.25 mL of 500 mg/L ammonium chloride solution with 99.75 mL 2M KCl.
1.0	Combined 0.5 mL of 500 mg/L ammonium chloride solution with 99.5 mL 2M KCl.
1.5	Combined 0.75 mL of 500 mg/L ammonium chloride solution with 99.25 mL 2M KCl.
2.0	Combined 1.0 mL of 500 mg/L ammonium chloride solution with 99 mL 2M KCl.

The nitrate concentration range capabilities for the Hach DR2700™ Spectrophotometer using Method 8039 (Cadmium Reduction Method) was between 0.3 and 30.0 mg/L. The four standard solution concentrations chosen and methods for preparation are outlined in Table 3.3 below.

Table 3.3: Potassium nitrate standard solution development

Standard Solution Concentration (mg/L)	Preparation Method
1.0	Combined 0.2 mL of 500 mg/L potassium nitrate solution with 99.8 mL 2M KCl.
5.0	Combined 1.0 mL of 500 mg/L potassium nitrate solution with 99 mL 2M KCl.
10.0	Combined 2.0 mL of 500 mg/L potassium nitrate solution with 98 mL 2M KCl.
20.0	Combined 4.0 mL of 500 mg/L potassium nitrate solution with 96 mL 2M KCl.

After preparing the required standards, at the commencement of each testing period for both ammonium and nitrate, the Hach was zeroed using a blank cell of 2M KCl that had been treated with the required reagent. The four standard solutions for each test were then measured, results recorded and measurement of the soil filtrate samples then commenced. After each batch of 12 samples had been tested, two standards were then re-measured for quality control checks and a 2M KCl blank tested to check for any background variation of zero. This process was used for both ammonium and nitrate testing.

Dilutions and Glassware Preparation

Due to the limitations of the accurate measuring ranges of each nitrate and ammonium method for the Hach Spectrophotometer and the potential high concentration of each filtrate sample to be tested, a process of dilution was required for the ammonium testing. This required the addition of a known volume of 2M KCl to a known volume of sample filtrate. A dilution factor was then calculated, before testing for concentration proceeded. The dilution factor did not remain constant for all of the samples to be tested, which is a reflection in the variation of ammonium concentrations that were developed over the duration of the 60 day incubation period. The nitrate samples were all within the Hach measurement range and did not require dilution.

The potential error of results from all testing was reduced by ensuring all glassware was acid washed in a 10% hydrochloric acid bath prior to use. When removed from the acid bath, the glassware was rinsed thoroughly with distilled water and placed in a drying oven at 40^{o}C until dry.

Ammonium-N Method

The Hach DR2700 Spectrophotometer method for measurement of ammonium-nitrogen concentration was based on the USEPA Nessler Method. This method is accepted for wastewater analysis, with the Nessler component adapted from the *Standard Methods for the Examination of Water and Wastewater 4500-NH_3 B & C* (Hach n.d).

The ammonium method required the use of three reagents:

1. Mineral stabiliser - complexes hardness in sample.
2. Polyvinyl dispersing agent – aids colour formation when Nessler reagent added.
3. Nessler reagent – reacts with ammonia and other amines to form yellow colour that is proportional to ammonium concentration.

Risk assessments were developed prior to commencing any chemical handling due to the hazardous nature of the reagents and waste products that formed (primarily the presence of mercury in the Nessler reagent). All waste products were disposed of in a heavy metal waste bottle to meet safe disposal standards.

To perform the ammonium method, 25mL of sample solution was prepared within a stoppered measuring cylinder (dilution performed if necessary) and three drops of polyvinyl dispersing agent, three drops of mineral stabiliser and 1mL of Nessler reagent added. After the addition of each reagent, the solution was gently mixed by inverting the measuring cylinder a total of six times. After the final mixing of Nessler reagent ended, a reaction time of one minute proceeded, during which time 10mL of the sample was added to a Hach sample vial. After the conclusion of the one minute period, the sample concentration was measured using the pre-set Hach program - 380 N Ammonia Ness.

Nitrate-N Method

The Hach DR2700 Spectrophotometer method for measurement of nitrate-nitrogen concentration was based on the Cadmium Reduction Method. This method is based on the reduction of nitrate to nitrite with the addition of a cadmium reagent. The colour change is more readily measurable when in nitrite form rather than nitrate (Margeson, Suggs & Midgett 1980).

The cadmium reagent was present in the form of a Hach formulated NitraVer^{5} powder pillow sachet. When added to the filtrate sample, the cadmium reduced nitrate to nitrite, which then reacted with sulfanilic acid to form diazonium salt. An amber colour formed when the salt combined with gentisic acid which was measurable with a 500nm wavelength (Hach n.d.).

To perform the method to create the cadmium reaction, one powder pillow sachet suitable for a 10mL sample was added to 10mL of soil sample filtrate in a Hach sample vial and vigorously mixed with a vortex machine for one minute. At the end of the one minute period, a reaction occurred over a period of five minutes. After the five minute period had ceased, the sample concentration was tested using the pre-set Hach program – 355N Nitrate HR PP.

Both methodologies used for the ammonium and nitrate measurements were lengthy processes, derived to ensure each sample was tested individually, using the same reagents, as well as the same time steps. The reactions created by performing the Hach methods were time dependant therefore, extreme care was taken to ensure every sample tested was subjected to the same mixing and timing stages, reducing the potential margin of error.

3.6.3 Gas Chromatography

A gas chromatograph (GC-2014, Shimadzu, Japan) was used to measure concentrations of nitrous oxide, carbon dioxide and methane in each sample. An automated process was used that enabled vials to be preloaded in a defined order and tested automatically without manual loading.

The gas chromatograph was operated by Dr Alla Marchuk at the NCEA, once all vials were pre-loaded into the automated sampler in a pre-defined order. Results were obtained in units of ppm, with standards and quality control checks used according to the instrument operation.

3.7 Data Analysis

The potential soil inorganic nitrogen supply refers to the fluctuations in ammonium and nitrate concentrations within the soil over the aerobic incubation of 60 days. The concentration was applicable to a soil depth of 20cm; the depth soil collection occurred during in-field collection stage. Values that were measured using colorimetric analysis returned results that were based on mg/L concentration in individual ammonium and nitrate nitrogen units (NH_4^+ – N and NO_3^- – N).

3.7.1 Inorganic Nitrogen Raw Data Analysis

Before a complete analysis of the trends, relationships and values of the data collected from the aerobic incubation procedure could be achieved, calibration and conversion of the raw data was required. This was conducted using Microsoft Excel software. Genstat software (VSN International, 2014) was then used to conduct statistical analyses of the data using two way ANOVA's (Rochester & Constable 2000; Chadwick et al. 2000; Rochester, Constable & Macleod 1992).

The Beer-Lambert law implied that a directly proportional relationship exists between the absorbance of a solution and its associated concentration ((Equation 6).

(Equation 6)

$$A = \varepsilon\, b\, c$$

Where *A* = absorbance of solution, ε = molar absorptivity (light absorbed per unit concentration), *b* = path length of sample (path length of cuvette in which sample contained in cm) and *c* = concentration of compound in solution (mg/L).

A linear regression was fitted to the scatter graph of absorbance versus concentration, using the data from testing prepared standard solutions (ammonium and nitrate). Refer to Appendix C for the calibration graphs and regression equations for ammonium and nitrate data. The slope of the linear regression represented the molar absorptivity value (The University of Newcastle, n.d.), with the complete linear expression used to perform calibration of both the ammonium and nitrate data. Using the add-in Data Analysis tool within Excel, a regression analysis was performed to determine the residual, coefficient of determination (R^2 value) and p value statistics for the calibration data.

For both ammonium and nitrate, the regression graphs were well distributed, with a coefficient of determination value that represented a moderate, but acceptable fit. The p values for the y-intercept (actual concentration axis) were used to determine if the linear trend line could be forced through the origin (0, 0) (when $p > 0.05$) or if the proposed y-intercept from the regression should be used ($p < 0.05$).

After calibrating the data in mg/L, an outlier analysis was conducted using the pivot table function and pivot chart generator in Excel. No significant outliers were observed in the ammonium and nitrate data for each sampling day when the results from four repetitions of each treatment pot were compared.

Raw Data Unit Conversion

To interpret the concentrations of ammonium and nitrate within the incubated soil (mg/kg), conversion from the extract concentration mg/L was required. The conversion was conducted using the volume of 2M KCl added during the extraction phase (40mL), as well as the actual oven-dry equivalent soil removed from the pots and moisture present in the sample each sampling day.

Further conversion from mg/kg into kg/ha was then performed to gain an understating of the larger scale net inorganic nitrate and ammonium concentrations within the soil over the 60 day period. This utilised the bulk density measurement, depth of soil removed from the field (20cm) and necessary area conversions.

Results Interpretation

The concentration of both ammonium and nitrate within the soil each sampling day represented the net mineral nitrogen supply. The net supply was a descriptor used for the combination of inorganic nitrogen formation and inorganic nitrogen losses that occurred in the time period between sampling days. Figure 3.26 represents this concept using a balance model, with the instantaneous (per sampling day) net inorganic nitrogen concentration dependant on the ratio of inorganic nitrogen gains (a form of input) to inorganic nitrogen losses (a form of output).

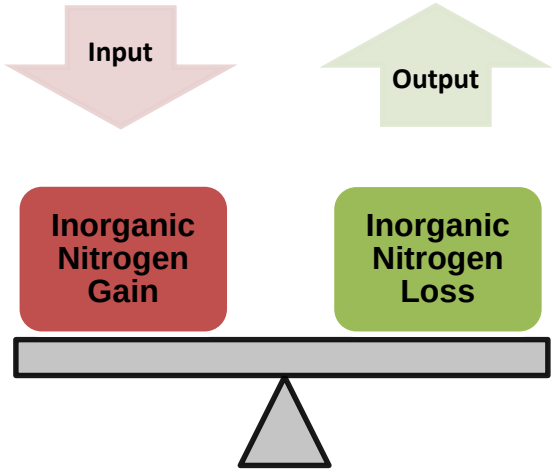


Figure 3.26: Net instantaneous inorganic nitrogen concentration status

The types of inorganic nitrogen gains and losses within the soil are based on the nitrogen cycle interactions which transform ammonium and nitrate compounds. The net inorganic concentration of ammonium was a result of several gains and losses, which are outlined in Figure 3.27 and Figure 3.28. The diagrams only outline the potential gains and losses that arose from the experimental procedure conducted for this investigation and are therefore only a partial balance.

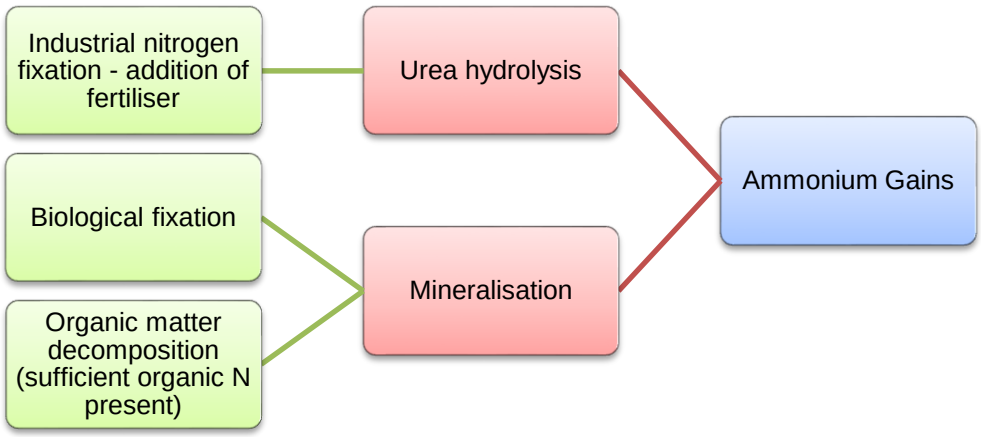


Figure 3.27: Potential sources of ammonium gains in soil medium

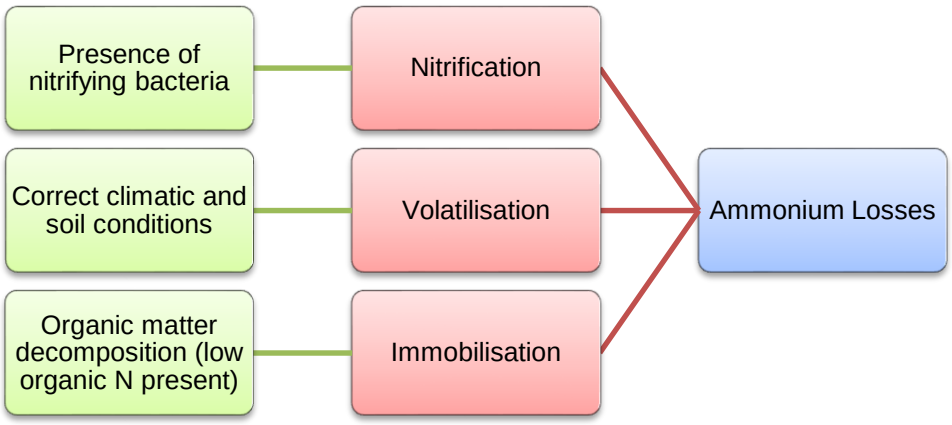


Figure 3.28: Potential sources of ammonium losses in soil medium

Similar to the potential soil ammonium supply, the net inorganic concentration of nitrate was also a result of several gains and losses, which are outlined in Figure 3.29 and Figure 3.30. Again, the diagrams only outline the potential gains and losses that arose from the experimental procedure. For instance, due to the experimental design of using pots during aerobic incubation, leaching losses were negligible.

Figure 3.29: Potential sources of nitrate gains in soil medium

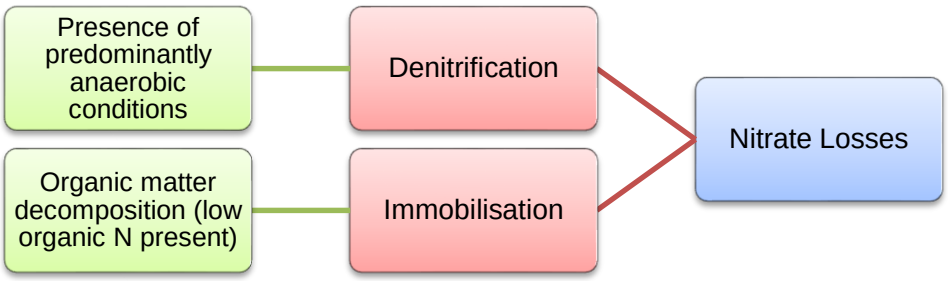


Figure 3.30: Potential sources of nitrate losses in soil medium

3.7.2 Gas Emission Raw Data Analysis

The manipulation of the soil gaseous emission data sets was similar to the ammonium and nitrate raw data analysis. The gas chromatograph provided results that required calibration, which was based on the standard and quality check results provided. Direct measurement of the gas concentration of each sample resulted in an ‘area’ value, which was then converted into concentration (ppm) based on the pre-set calibration within the instrument.

Using the area measurements for each standard and quality check concentration tested throughout the gas sample analysis process, calibration curves for each gas type were developed. A linear regression was fitted to the data to determine the associated equation of the line of best fit (refer Appendix D). To decide whether the y-intercept could be forced through the origin (0, 0) for the calibration curves, a regression and residual analysis was performed. Nitrous oxide and methane calibration data each had a background reading present which made the difference of y-intercept and zero significant ($p < 0.05$). The carbon dioxide calibration data had a y-intercept that wasn’t classified as significantly different from 0 ($p > 0.05$), therefore the origin was used.

Once the data was calibrated, an analysis to determine the data outliers occurred. All values that were equal to zero, or very close to zero (<0.5) were removed, particularly if the error was present between each greenhouse gas measurement. A zero value for gas concentration most likely resulted from a leaky vial or syringe during gas sampling stages.

Raw Data Unit Conversion

Interpretation of gaseous emissions was most effectively achieved when presented in the form of flux measurements. For this investigation, the flux was determined by assuming a linear increase of gaseous emission over each sampling period of 0 - 45min for each soil pot and sampling day. An Excel template was used to convert instantaneous gas emissions into ppb

(parts per billion) and calculate the y-intercept, slope and regression of a linear best line of fit to determine the flux in µg m^{-2} h^{-1}.

Results Interpretation

Nitrous oxide, methane and carbon dioxide gas emission data was gathered over the duration of the aerobic incubation period to quantify net gaseous emissions from soil with no fertiliser treatment and soil treated with Urea and ENTEC® Urea. Results were provided in the form of N_2O - N, CH_4 - C and CO_2 - C, which was the form used throughout the results and discussion sections.

The greenhouse gas emission data represents the total gas released from the pore spaces within the soil into the headspace of the pot and does not therefore reflect total gas formation (some gas remains within the soil). Nitrous oxide, methane and carbon dioxide each have varying formation, release and absorption interactions between the soil medium and the surrounding atmosphere. These are outlined in Table 3.4 below.

Table 3.4: Greenhouse gas emission formation and soil-atmosphere interaction characteristics

Greenhouse Gas Type	Formation	Soil-Atmosphere Interaction
Nitrous Oxide	• During nitrification (decomposition of nitrite). • During denitrification (anaerobic soil, >60% pore filled water).	Released from soil.
Methane	• Digestion of organic matter in anaerobic conditions.	Release and absorbed by soil.
Carbon Dioxide	• Urea hydrolysis reaction. • Organic matter decomposition in aerobic and anaerobic conditions.	Released from soil

3.7.3 Statistical Analysis

Statistical analyses were conducted on all data obtained for ammonium, nitrate, nitrous oxide, methane and carbon dioxide measurements to determine if any significant difference between treatments, incubation day and measured results existed. Acknowledgments to Dr Alice Melland and Dr Diogenes Antille for processing the data through GenStat.

A two way ANOVA between each fertiliser treatment per incubation day for ammonium, nitrate, nitrous oxide, methane and carbon dioxide was performed. A significance level of 0.05 (LSD 5%) was used for each individual two way ANOVA analysis (Payne 2012). See Appendix E for GenStat reports of the analysis of variance for each dataset.

CHAPTER 4 RESULTS

This chapter includes the results from all methodology stages conducted throughout the duration of this investigation. Data that are extensive in nature will be included as a separate appendix for ease of readability. Initial interpretations of results will also occur to outline any trends and relationships, with all information included within this section forming a prelude for Chapter 5 – Discussion.

4.1 Soil Properties

4.1.1 Air-Dry Moisture and Field Moisture

Based on the method from *Soil Sampling Preparation and Analysis* (Tan 1995), gravimetric soil moisture contents for air-dry (refer Table 4.1) and field moisture (refer Table 4.2) were measured for the Black Vertosol soil obtained from Yargullen, QLD.

Table 4.1: Air-Dry moisture measurements for Black Vertosol

Sample No.	Weight Tin (g)	Weight Moist Soil (g)	Weight Oven Dry Soil (g)	Moisture Loss (g)	Air-Dry Moisture Content (%)
1	7.00	85.39	78.54	6.85	8.72
2	6.82	103.08	93.28	9.8	10.50
3	6.88	84.18	77.33	6.85	8.86

The average air-dry soil moisture content was calculated to be 9.36% with a standard deviation of 0.989%.

Table 4.2: Field moisture measurements for Black Vertosol

Sample No.	Weight Tin (g)	Weight Moist Soil (g)	Weight Oven Dry Soil (g)	Moisture Loss (g)	Field Moisture Content (%)
1	6.87	121.55	88.57	32.98	37.24
2	6.52	128.38	93.29	35.09	37.61
3	6.44	121.49	88.64	32.85	37.06

The average field moisture was calculated as 37.3% with a standard deviation of 0.28 %.

4.1.2 Bulk Density

The method for determining the oven-dry bulk density, based on Method B5.1 from *Laboratory Methods of Soil and Plant Analysis* yielded the results displayed in Table 4.3. The bulk density rings each had a dimension of 47mm diameter and 52mm depth.

Table 4.3: Bulk density measurement results for Black Vertosol

Sample No.	Volume Bulk Density Ring (cm^3)	Weight Oven Dry Soil (g)	Bulk Density (g/cm^3)
1	90.217	88.57	0.98
2	90.217	93.29	1.03
3	90.217	88.64	0.98

Conducting a statistical analysis of results provided an average bulk density value of 0.996 g/cm^3 and a standard deviation of 0.029 g/cm^3. With three repetitions, this value was classified as reliable and accurate, however a slight variation may have existed in the value of sample number three, as a medium sized root was extracted from the soil after removal from the bulk density ring.

With a low bulk density value, the Black Vertosol was defined as a soil with sufficient structure and pore space to create conditions suitable for

healthy plant development and growth. A bulk density of approximately 1 g/cm^3 is indicative of a typical heavy clay soil type with a surface layer high in organic matter (Tan 1995).

A summary of the air-dry, field moist and bulk density results is displayed in Table 4.4.

Table 4.4: Summary of soil moisture and bulk density results for Black Vertosol

Sample No.	Air-Dry Moisture Content (%)	Field Moisture Content (%)	Bulk Density (g/cm^3)
1	8.72	37.24	0.98
2	10.50	37.61	1.03
3	8.86	37.06	0.98
Average	9.36	37.3	0.996
Standard Deviation	0.989	0.28	0.029

4.1.3 Field Capacity

From a concurrent study at Yargullen, QLD, Figure 4.1 below outlined the moisture content of the Black Vertosol up to 100 hours after an irrigation event.

Using the linear interpolation equation at 48 hours, the moisture content (%) at approximate field capacity, assuming this occurred 48 hours after an irrigation event, was calculated to be 40.40%.

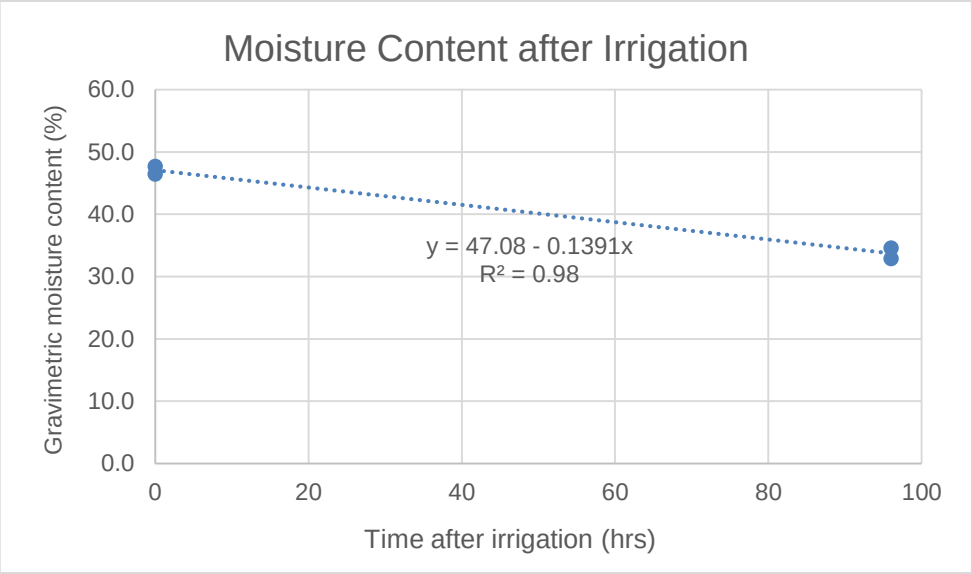


Figure 4.1: Soil gravimetric moisture content after irrigation event, Yargullen (D Antille 2015, pers. comm.)

4.1.4 Initial Ammonium, Nitrate and Phosphorus

The experimental method was designed to gather data pertaining to the initial ammonium and nitrate concentrations for the control, Urea and ENTEC Urea fertiliser applications. Air-dry soil was sent to an external laboratory to gain initial ammonium and nitrate concentration of the control soil. This enabled verification of the experimental results obtained from this investigation to occur.

The initial ammonium and nitrate concentrations were determined by using Method 7C2 from *Soil Chemical Methods – Australasia* (Rayment & Lyons 2011) at the Agricultural Chemistry Laboratory (Ipswich). A 2M KCl extraction and colorimetric methods were used, similar to the experimental design procedure for this investigation.

The initial phosphorus concentration of the soil was not entirely relevant to this investigation however, knowing the value did provide some background information on the nutrition level of the soil. Initial soil phosphorus concentration was determined at the Agricultural Chemistry Laboratory (Ipswich) using Method 9B2 from *Soil Chemical Methods –*

Australasia (Rayment & Lyons 2011). The method was based on a 0.5M $NaHCO_3$ extraction at pH 8.5, with colorimetric methods used also to gain quantifiable results.

Table 4.3 provides a summary of the initial soil characteristics obtained from the external laboratory based on air-dry soil moisture content.

Table 4.5: Initial soil characteristic measurements externally sourced

Characteristic	Value
Ammonium (NH_4 – N) (mg/kg)	3.5
Nitrate (NO_3 – N) (mg/kg)	<0.5
Colwell Phosphorus (mg/kg)	51.4

4.1.5 Potentially Mineralisable Nitrogen

A potential mineralisable nitrogen test was also conducted externally at the Agricultural Chemistry Laboratory, Ipswich, with method 7D1 from *Chemical Methods – Australasia* (Rayment & Lyons 2011) used. The method incorporates the use of hot KCl colorimetric analysis to determine an estimate of the total mineral nitrogen (mg/kg) that can potentially be formed based on the soil organic carbon and other soil properties at time of testing.

The Black Vertosol soil collected from Yargullen, with no fertiliser addition and having undergone air-drying, returned a potential mineralisable nitrogen result of 4.75 mg/kg.

4.2 Individual Treatment Results

The following section includes the data and associated graphs obtained and developed for each fertiliser treatment type (control, Urea and ENTEC® Urea). The individual trends were briefly analysed for each treatment using the ammonium, nitrate, nitrous oxide, carbon dioxide and methane concentrations and fluxes respectively. This enabled an understanding of the net inorganic nitrogen supply and loss characteristics that occurred for each soil treatment over the 60 day incubation period to be developed. Refer to Appendix F for ammonium and nitrate raw data and Appendix G for greenhouse gas emission flux raw data.

4.2.1 Control Samples

Using the four repetitions of control application pots (no fertiliser added), Table 4.6 represents the mean and standard deviation of the measured ammonium and nitrate concentrations.

Table 4.6: Mean and standard deviation of control treatment ammonium and nitrate concentrations (mg/kg) measured during 60 day incubation period.

Incubation Day	Ammonium Concentration (mg N/kg)		Nitrate Concentration (mg N/kg)	
	Mean	Standard Deviation (±)	Mean	Standard Deviation (±)
0	39.8	0.78	97.5	7.11
3	41.4	1.79	67.8	8.82
7	39.1	0.22	75.9	14.81
14	45.6	2.39	82.9	26.55
30	34.4	0.85	87.7	26.61
45	33.8	0.59	105.6	11.29
60	31.5	1.94	65.1	11.25

Figure 4.2 displays the interaction between soil net ammonium and nitrate supply, with the associated error bars calculated from standard deviation values. The location of error bars also indicated the day samples were taken. The measured ammonium concentrations had a minimal associated error with an average standard deviation of ± 3.2%, however nitrate concentrations displayed a much greater variability at ± 18.59% average.

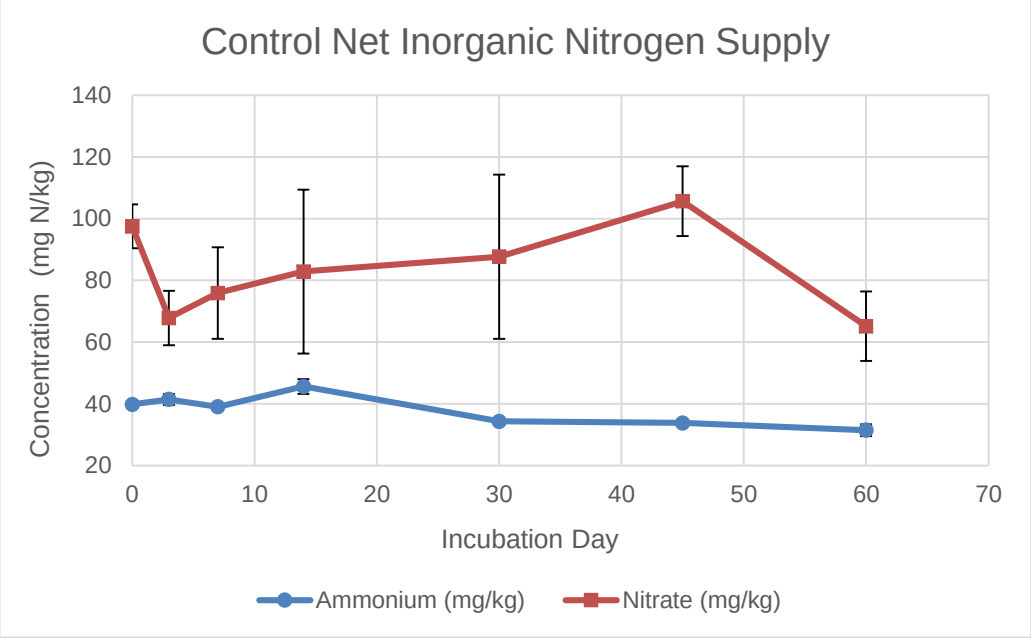


Figure 4.2: Control treatment - ammonium and nitrate net concentration during 60 day aerobic incubation based on average results.

The control results for net inorganic nitrogen concentration fluctuated inconsistently during the 60 day incubation period with total net mineral soil nitrogen supply marginal. This was to be expected, as the ammonium concentration was not increased from the addition of fertiliser, which may have also impacted the biological activity (such as organic matter decomposition) and interaction between gains and losses for ammonium and nitrate.

A peak concentration of 45.6mg/kg of ammonium was observed on day 14, with peak nitrate supply occurring on day 45 at 105.6 mg/kg. Increases

in nitrate resulted from nitrification, with ammonium increases resulting from mineralisation of organic nitrogen to inorganic nitrogen form.

At the conclusion of the 60 day period, the control soil had been depleted of 8.3 mg/kg ammonium and 32.4 mg/kg nitrate when compared to the initial soil concentration on day 0. The reduction in potential soil inorganic nitrogen supply was primarily attributed to the immobilisation of ammonium and nitrate as aerobic conditions were assumed.

Table 4.7 outlines the mean and standard deviation of the calculated flux for nitrous oxide, methane and carbon dioxide emissions measured at specific intervals throughout the duration of the 60 day incubation period.

Table 4.7: Mean and standard deviation of control treatment nitrous oxide, methane and carbon dioxide emissions flux (μg m^{-2} h^{-1})

Incubation Day	**Nitrous Oxide Flux (μg m^{-2} h^{-1})**		**Methane Flux (μg m^{-2} h^{-1})**		**Carbon Dioxide Flux (μg m^{-2} h^{-1})**	
	Mean	Standard Deviation (±)	Mean	Standard Deviation (±)	Mean	Standard Deviation (±)
0	0	0	0.7	0.06	-1068	135.1
1	4.8	0.85	1.0	0.06	2259	152.0
2	0.9	0.14	-0.3	0.39	-3368	859.1
3	2.1	0.46	0.8	0.28	488	91.1
4	3.4	0.92	-0.6	0.10	1313	257.1
5	0.6	1.41	-0.3	0.51	-1996	768.0
14	2.4	0.16	-0.9	0.30	-3011	663.1
45	5.2	2.56	1.3	0.04	-74	246.6
60	1.0	2.75	-0.8	0.37	-546	613.2

Figure 4.3 displays a comparison of soil net nitrous oxide, methane and carbon dioxide emission fluxes for each sample taken during the incubation period, with the associated error bars, calculated from standard deviation values.

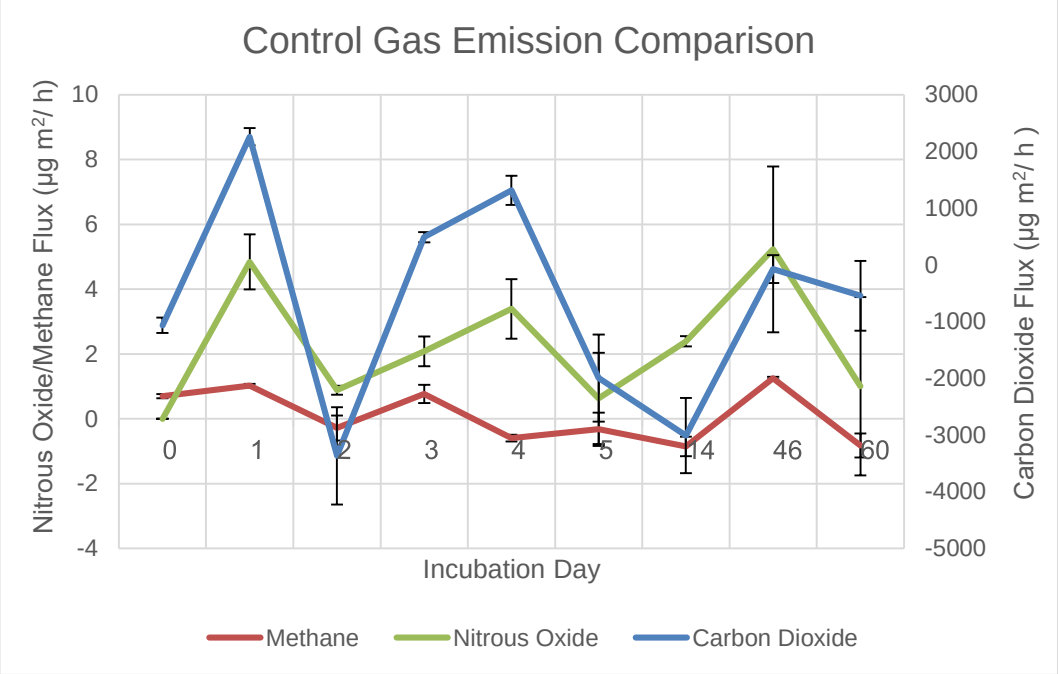


Figure 4.3: Control treatment nitrous oxide, methane and carbon dioxide emission flux during 60 day aerobic incubation showing incubation day means ± standard deviations

The nitrous oxide, methane and carbon dioxide emissions from the control soil provided an indication of the level of activity of several nitrogen cycle soil processes. Methane production represents the presence of anaerobic soil conditions. The experimental design was based on aerobic conditions, therefore methane flux emissions over the duration of the 60 incubation period was minimal. The methane emissions that were present on day 1, 3 and 46 may have been due to pore spaces within the soil having anaerobic conditions from a lack of oxygen supply. Some negative flux values were also present on days 2, 4, 5, 14 and 60, indicating that methane was absorbed by the soil medium during the 45 minute measuring period.

Carbon dioxide and nitrous oxide emissions showed a relationship with increasing and decreasing trends exhibited over the entire 60 day period. Peak carbon dioxide emission occurred on day 1 at 2259 μg m^{-2} h^{-1}. Days 4 and 46 also exhibited high carbon dioxide emissions. These emissions were a result of organic matter decomposition only, as the control had no fertiliser addition to initiate carbon dioxide release from urea hydrolysis reactions.

The trend for nitrous oxide emission was similar, with peak emission occurring on day 1 at 4.84 μg m^{-2} h^{-1}, with days 4 and 46 also exhibiting increased emissions. Day 0 emission for nitrous oxide was assumed as negligible as measurements were taken when moisture had first been added to air-dry soil, therefore biological activity had not fully activated.

The peak nitrous oxide emissions can be primarily attributed to the nitrous oxide formation during the nitrification process rather than denitrification which required anaerobic conditions. Some denitrification may have occurred however, if small anaerobic areas developed within the soil from an excess of microbial activity causing a reduction in available oxygen.

4.2.2 Urea Fertiliser Treatment

Using the four repetitions of Urea fertiliser application pots Table 4.8 represents the mean and standard deviation of net measured ammonium and nitrate concentrations.

Table 4.8: Mean and standard deviation of Urea treatment ammonium and nitrate concentrations (mg/kg) measured during 60 day incubation period.

Incubation Day	Ammonium Concentration (mg N/kg)		Nitrate Concentration (mg N/kg)	
	Mean	Standard Deviation (±)	Mean	Standard Deviation (±)
0	50.9	2.08	73.0	23.46
3	245.6	13.65	252.6	19.81
7	325.5	8.78	277.8	11.51
14	269.8	5.68	471.1	31.83
30	190.9	8.44	448.4	11.42
45	57.2	3.71	423.7	34.81
60	39.3	0.96	309.7	25.61

Figure 4.4 displays the interaction between soil net ammonium and nitrate supply for Urea fertiliser treatment, with the associated error bars calculated from standard deviation values. The location of error bars also indicated the day samples were taken. The measured ammonium concentrations had a minimal associated error with an average standard deviation of ± 3.97%, with nitrate concentrations displaying an average standard deviation of ± 9.98%.

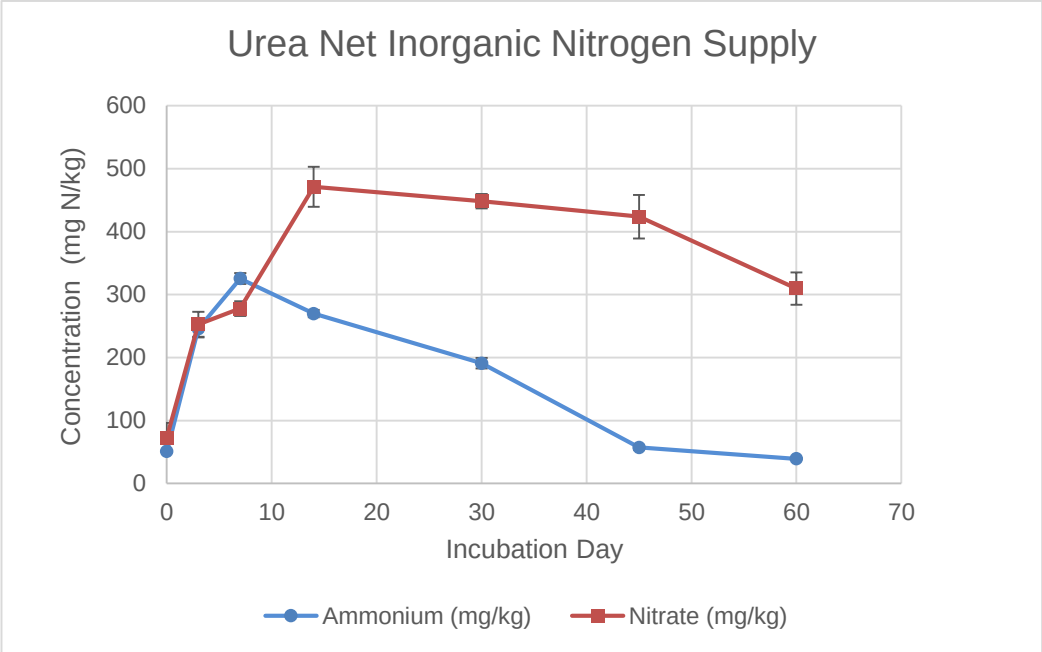


Figure 4.4: Urea treatment - ammonium and nitrate net concentration during 60 day aerobic incubation based on average results.

Ammonium and nitrate net soil concentrations for soil amended with Urea fertiliser exhibited a distinct relationship over the 60 day incubation period. A peak net ammonium concentration of 325.5 mg/kg was observed during the early incubation stages on day 7, indicating that the Urea fertiliser was undergoing hydrolysis reaction in the soil, creating ammonium supply. Beyond day 7, the net ammonium concentration steadily diminished, indicating that nitrification and possibly immobilisation occurred at a greater rate than urea fertiliser hydrolysis and mineralisation of organic nitrogen. Between day 45 and day 60, a net soil ammonium concentration of 39.3 mg/kg is observed, which is at a level similar to the net soil concentrations present in the control soil. Over the 60 day duration, a total of 274.6 mg/kg was net ammonium gain, with 285.3 mg/kg as net ammonium loss.

The peak net nitrate concentration occurred on day 14 at 471.1 mg/kg, indicating that nitrification of the ammonium supplied from Urea fertiliser hydrolysis and organic nitrogen mineralisation had occurred. Beyond day

14, net nitrate concentration within the soil steadily declined, indicating that nitrate losses from immobilisation and possibly denitrification if pore spaces of anaerobic conditions were present within the soil, were greater than the rate of nitrification.

At the conclusion of the 60 day period, the Urea amended soil had a net loss of 11.68 mg/kg ammonium and net gain of 236.7 mg/kg of nitrate when compared with the initial soil concentrations on day 0. Nitrate therefore was still present within the soil to be immobilised and subjected to further losses. Total net release over the 60 day period was 398.1 mg/kg, with net nitrate loss of 161.4 mg/kg.

Table 4.9 outlines the mean and standard deviation of the calculated fluxes for nitrous oxide, methane and carbon dioxide emissions measured at specific intervals throughout the duration of the 60 day incubation period for Urea treated soil.

Table 4.9: Mean and standard deviation of Urea treatment nitrous oxide, methane and carbon dioxide emissions flux (μg m^{-2} h^{-1})

Incubation Day	Nitrous Oxide Flux (μg m^{-2} h^{-1})		Methane Flux (μg m^{-2} h^{-1})		Carbon Dioxide Flux (μg m^{-2} h^{-1})	
	Mean	Standard Deviation (±)	Mean	Standard Deviation (±)	Mean	Standard Deviation (±)
0	0	0	1.0	0.35	870	432.5
1	11.5	0.46	0.7	0.29	3287	134.7
2	8.8	0.83	-0.1	0.07	-1061	605.5
3	11.5	1.17	0.4	0.45	1483	282.7
4	22.3	1.90	0.2	0.78	3295	960.9
5	13.4	1.28	-1.0	0.41	-1709	560.2
14	3.1	1.03	-0.1	0.23	-722	310.3
45	8.1	1.40	1.0	0.81	-1068	139.2
60	3.9	2.30	-0.8	0.22	-2863	1221.5

Figure 4.5 displays a comparison of net nitrous oxide, methane and carbon dioxide emission fluxes for each sample taken during the incubation period for a soil amended with Urea fertiliser. Associated error bars were calculated from standard deviation values.

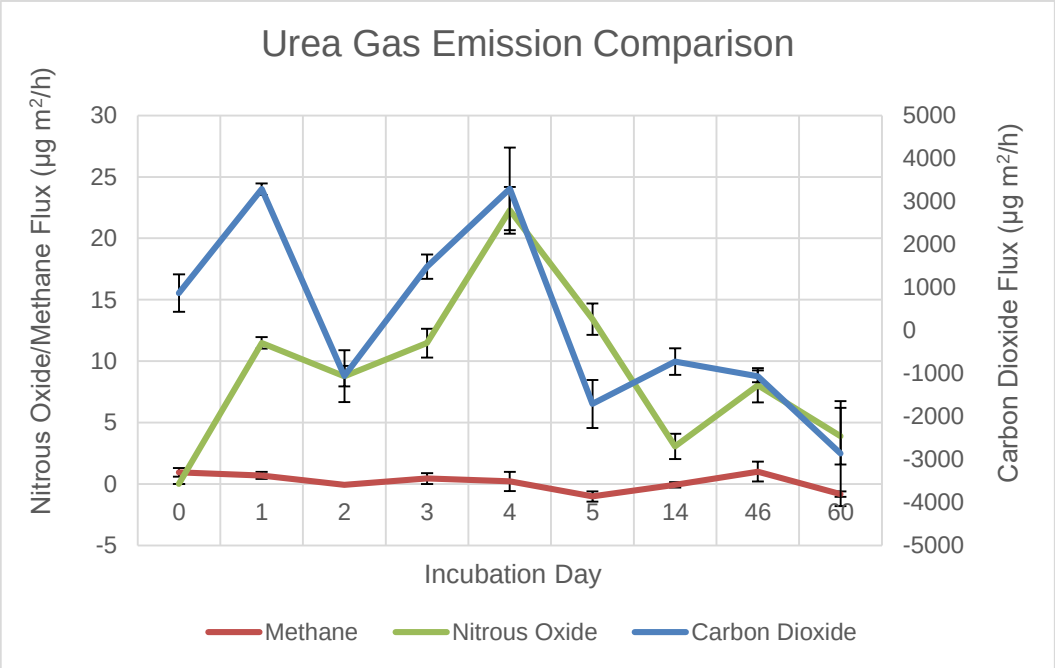


Figure 4.5: Urea treatment nitrous oxide, methane and carbon dioxide emission flux during 60 day aerobic incubation showing incubation day means ± standard deviations

Similar to the control treatment results, methane production for Urea amended soil was minimal over the 60 day incubation period. Slight variations such as on day 5 and 46 are extremely minimal and may have resulted from small pore spaces of the soil being under anaerobic conditions.

Two similar peaks of carbon dioxide emissions were observed on day 1 (3287 µg m^{-2} h^{-1}) and day 4 (3295 µg m^{-2} h^{-1}). These can be attributed to Urea hydrolysis reactions occurring within the soil, as well as a degree of organic carbon decomposition. From day 4, a reduction of carbon dioxide emissions were observed, indicating that Urea had completely hydrolysed by approximately day 4.

The trends for nitrous oxide emission levels were similar to carbon dioxide, however one clear nitrous oxide peak was observed on day 4 at 22.29 µg m^{-2} h^{-1}. Fertiliser applications always result in a spike in nitrous oxide emissions in the first few days (Granli & Bockman 1994). Increases in nitrous oxide emissions were primarily attributed to nitrification processes

whereby nitrite is oxidised to nitrous oxide before nitrate is formed. Some degree of emissions however may have arisen from denitrification if some anaerobic conditions were present within the soil (>60% pore space). From day 4 to day 60, nitrous oxide emissions were relatively low, indicating minimal denitrification and nitrous oxide from nitrification processes had occurred.

4.2.3 ENTEC® Urea Fertiliser Treatment

Using the four repetitions of ENTEC® Urea fertiliser application pots the following Table 4.10 represents the mean and standard deviation of measured ammonium and nitrate concentrations.

Table 4.10: Mean and standard deviation of ENTEC® Urea treatment ammonium and nitrate concentrations (mg/kg) measured during 60 day incubation period.

Incubation Day	Ammonium Concentration (mg N/kg)		Nitrate Concentration (mg N/kg)	
	Mean	Standard Deviation (±)	Mean	Standard Deviation (±)
0	55.2	1.64	41.0	11.36
3	204.2	24.32	158.8	6.61
7	236.9	10.47	118.8	18.03
14	26.9	3.11	157.6	15.17
30	45.4	1.50	294.3	25.31
45	41.5	1.60	373.8	41.17
60	35.0	0.23	379.1	21.18

Figure 4.6 displays the interaction between soil net ammonium and nitrate supply for ENTEC® Urea fertiliser treatment, with the associated error bars calculated from standard deviation values. The measured ammonium concentrations had a small associated error with an average standard deviation of ± 5.52%, with nitrate concentrations displaying an average standard deviation of ± 11.7%.

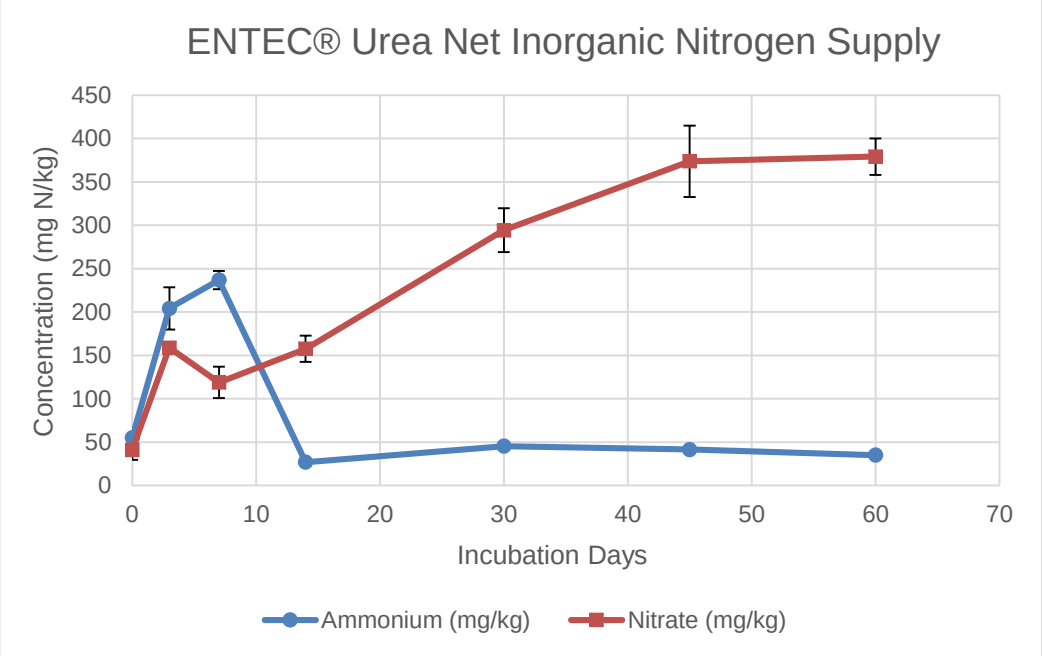


Figure 4.6: ENTEC® Urea treatment - ammonium and nitrate net concentration during 60 day aerobic incubation based on average results.

With a fertiliser amendment of ENTEC® Urea, the peak net ammonium concentration of 236.9 mg/kg was observed during the early incubation stages on day 7, indicating that the ENTEC® Urea fertiliser was undergoing hydrolysis reaction in the soil, creating ammonium supply.

Between day 7 and day 14, the net ammonium concentration rapidly diminished, indicating that primarily immobilisation (and a degree of nitrification) occurred at a significantly greater rate than the urea hydrolysis reaction and mineralisation of organic nitrogen. With a low nitrate to ammonium concentration ratio present in the soil, immobilising microbiology would have used the ammonium concentrations rather than

minimal nitrate concentration. Between day 14 and day 60, the soil was at an ammonium supply and loss equilibrium with an average net soil ammonium concentration of 37.215 mg/kg observed in this period. This was at a level similar to the net soil ammonium concentrations present in the control soil. Total ammonium gain over the 60 day period was 200.15 mg/kg, with total net loss of 220.31 mg/kg.

Between day 7 and day 45 a steady increase in soil nitrate supply was observed, indicating that nitrification processes occurred at a greater rate than potential losses from denitrification and immobilisation. This is also indicative of the expected behaviour of the nitrification inhibitor within the ENTEC® Urea fertiliser, which delays the onset of nitrification. A peak net nitrate concentration was observed on day 45 at 373.8 mg/kg, with this peak concentration maintained in the period between day 45 and day 60. The absolute peak in nitrate supply however may have occurred in the period beyond day 60 as net nitrate supply had not started diminishing, which wasn't investigated in this research.

At the conclusion of the 60 day period, the ENTEC® Urea amended soil had a net loss of 20.15 mg/kg ammonium and net gain of 338.1 mg/kg of nitrate when compared with the initial soil concentrations on day 0. Nitrate therefore was still present within the soil at a very high concentration level. Over the entire 60 day period, total net gains of nitrate was 378.13 mg/kg, with total net losses of 40 mg/kg.

Table 4.11 outlines the mean and standard deviation of the calculated fluxes for nitrous oxide, methane and carbon dioxide emissions measured at specific intervals throughout the duration of the 60 day incubation period for ENTEC® Urea treated soil.

Table 4.11: Mean and standard deviation of ENTEC® Urea treatment nitrous oxide, methane and carbon dioxide emissions flux (μg m^{-2} h^{-1})

Incubation Day	Nitrous Oxide Flux (μg m^{-2} h^{-1})		Methane Flux (μg m^{-2} h^{-1})		Carbon Dioxide Flux (μg m^{-2} h^{-1})	
	Mean	Standard Deviation (±)	Mean	Standard Deviation (±)	Mean	Standard Deviation (±)
0	0	0	0.7	0.14	-110	208.8
1	6.7	2.39	1.6	0.34	3102	372.0
2	6.2	0.48	0.0	0.33	-1061	516.5
3	2.7	0.49	0.8	0.80	2124	436.1
4	5.7	1.03	-0.0	0.29	2938	193.3
5	1.1	0.27	1.0	0.61	-494	913.0
14	4.0	0.67	1.1	0.75	-128	272.0
45	9.6	1.03	-0.6	0.17	355	220.7
60	2.9	1.22	-0.4	0.65	-1450	1123.0

Figure 4.7 displays a comparison of net nitrous oxide, methane and carbon dioxide emission fluxes for each sample taken during the incubation period for a soil amended with ENTEC® Urea fertiliser. Associated error bars were calculated from standard deviation values.

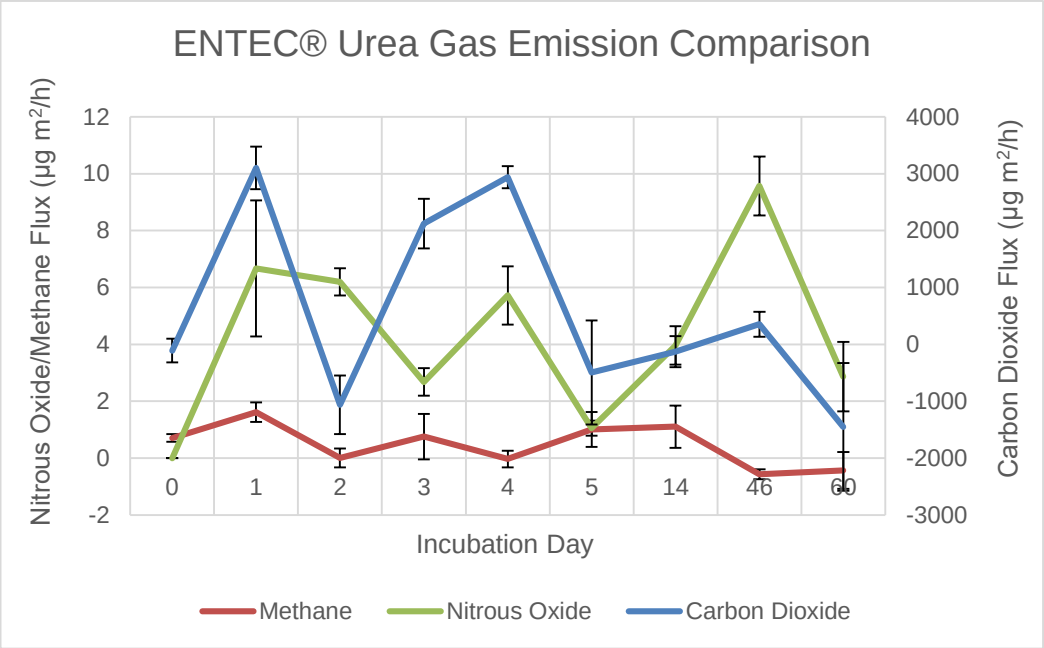


Figure 4.7: ENTEC® Urea treatment nitrous oxide, methane and carbon dioxide emission flux during 60 day aerobic incubation showing incubation day means ± standard deviations

Some methane emission production did occur during the 60 day incubation procedure for soil amended with ENTEC® Urea fertiliser (days 1, 3, 5 and 14). The emission levels were still minimal, however the slight increase in methane production may be attributed to ENTEC® Urea pots being subjected to wetter and therefore more anaerobic conditions (refer to section 4.2.2 on varying moisture contents of pots based on location in incubator during experimental phase).

Carbon dioxide emission peaks were observed on both on day 1 (3102 µg m^{-2} h^{-1}) and day 4 (2938 µg m^{-2} h^{-1}) which was similar to Urea fertiliser amended soil, except level of emission was slightly lower than ENTEC® Urea. Urea hydrolysis reactions were therefore occurring within the soil in the first few days after application as well as organic carbon decomposition. From day 4, a reduction of carbon dioxide emissions were observed, indicating that Urea had completely hydrolysed by approximately day 4.

The trends for nitrous oxide emission levels were slightly varied however, with peaks in emissions on day 1-2, 4 and 46. The highest peak occurred on day 46 at 9.57 μg m^{-2} h^{-1}. This is an indication that nitrification occurred at a later stage in the incubation period, which is the expected behaviour of the nitrification inhibitor present in the fertiliser. Again, the increases in nitrous oxide emissions were primarily attributed to nitrification processes whereby nitrite is oxidised to nitrous oxide before nitrate is formed. Some degree of emissions however may have arisen from denitrification from pore space anaerobic conditions.

4.3 Fertiliser Treatment Comparison

4.3.1 Net Ammonium and Nitrate Supply

The initial two way ANOVA output from GenStat provided variance results that indicated relationships between the following independent variable combinations and ammonium and nitrate concentrations were significant.

- Fertiliser treatment
- Incubation day
- Fertiliser treatment interaction with incubation day

The level of significance was reported at 0.1% ($p < .001$). For ammonium and nitrate soil concentrations, 98.5% and 91.5 % of variance was accounted for respectively by the linear regression modelling.

The ammonium concentration model produced residuals that were not random, therefore a $\log_e$ transformation of the data occurred to enable ANOVA normality assumptions to be met. The nitrate concentration model met ANOVA assumptions and therefore did not require transformation. See Appendix E for the GenStat output residual plots.

To compare the effectiveness of each fertiliser treatment the net ammonium and nitrate concentrations of the soil at each sampling stage

for the control, Urea and ENTEC® Urea treatments were graphed (Figure 4.8 and Figure 4.9). The standard deviation was used from the previous Section 4.2. Standard error could be used, however the only difference was that the error displayed for day 0 for both Urea and ENTEC® Urea was slightly greater (three repetitions used instead of four).

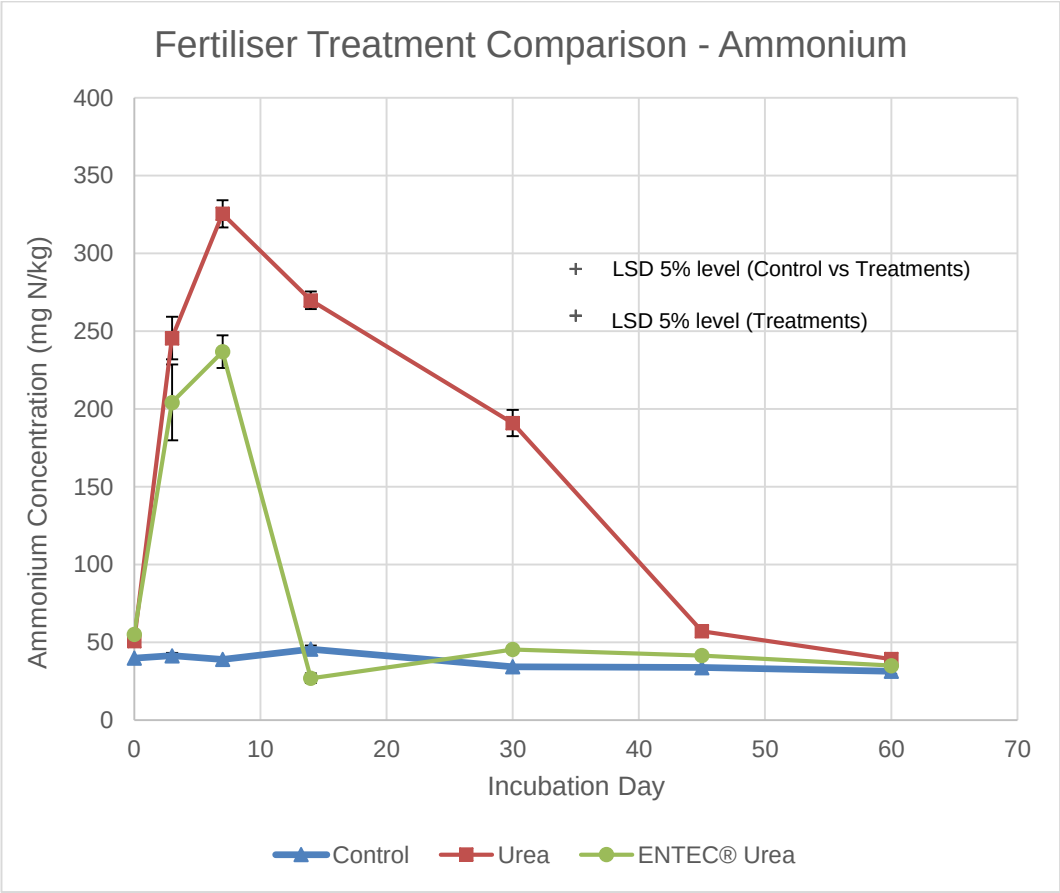


Figure 4.8: Comparison of net ammonium concentration of control, Urea and ENTEC® Urea treatments over 60 day incubation period

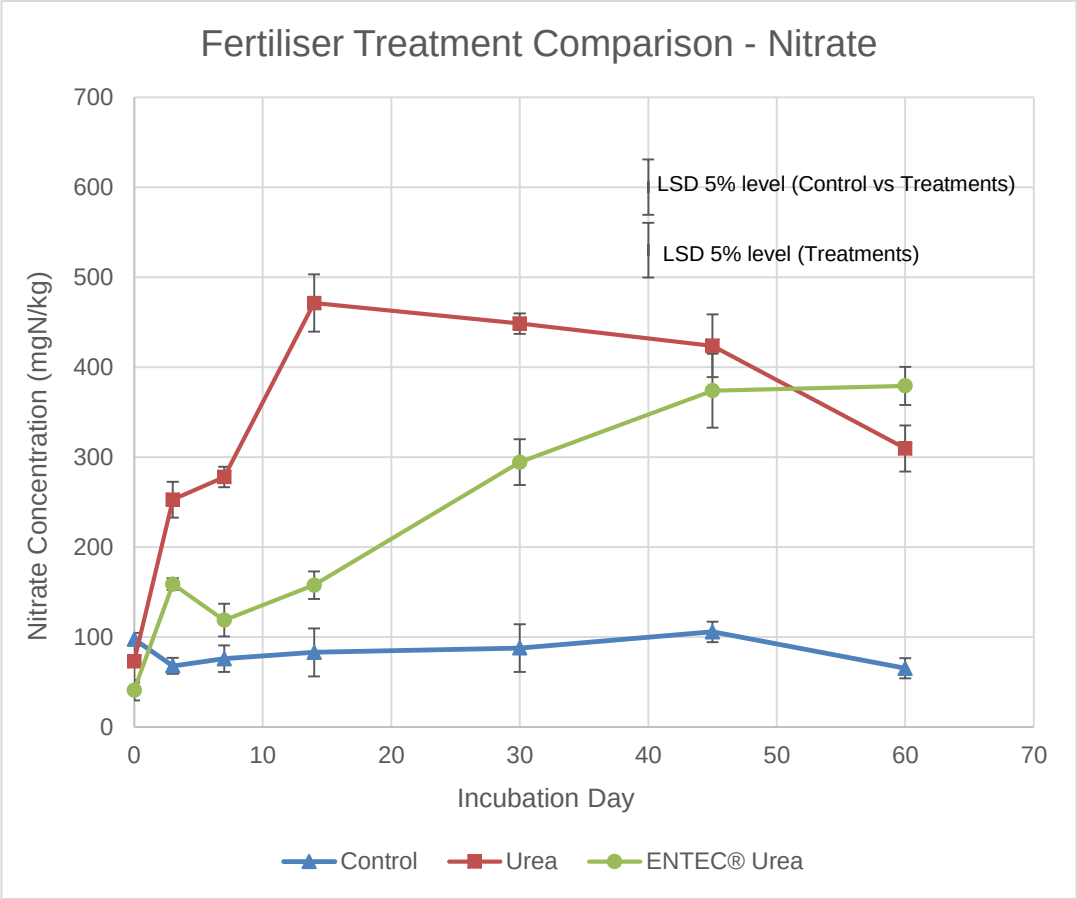


Figure 4.9: Comparison of net nitrate concentration of control, Urea and ENTEC® Urea treatments over 60 day incubation period

ANOVA statistics provided a form verification of the difference between treatment behaviour and the associated statistical significance within the Black Vertosol. Comparisons were made to a 5% level of least significant difference of fertiliser treatments per incubation day when compared to the control concentration on day 0 (LSD bars are situated on each graph for ease of comparison).

A clear difference in Figure 4.8 is observed between the net ammonium supply and loss characteristics for each fertiliser type. The control displayed minimal ammonium and nitrate net production and loss activity and was significantly lower ($p < 0.05$) than both fertiliser treatments on all days except day 14 (greater than ENTEC® Urea) and day 60 (same as ENTEC® Urea). The control soil was likely in equilibrium, with ammonium produced from mineralisation of organic nitrogen and nitrate produced

from nitrification both automatically immobilised to provide decomposer organisms with an energy supply. An alternative explanations was that low initial concentration of ammonium have not provided enough energy for extensive microbiological activity and population growth.

On day 0, Urea and ENTEC® Urea displayed similar concentrations ($p > 0.05$) of initial ammonium concentration, but were significantly higher than the control as a result of rapid urea hydrolysis commencing from when the fertiliser was initially added. The Urea fertiliser application resulted in significantly higher ($p < 0.05$) ammonium concentrations compared to ENTEC® Urea and the control on all incubation days, with the exception of day 0 and 60 when the concentrations were the same ($p > 0.05$).

The peak ammonium supply for both Urea and ENTEC® Urea occurred on day 7, however Urea had a significantly ($p < 0.05$) higher concentration (88.64 mg/kg greater). The nitrate concentration on day 7 for Urea was also significantly greater ($p < 0.05$) than ENTEC® Urea (Figure 4.9) which may account for the peak net ammonium difference. Urea treated soil, with high ammonium and nitrate concentrations present, would have equivalent amounts of both mineral nitrogen immobilised. ENTEC® Urea however had a very low nitrate concentration compared to ammonium, therefore more ammonium was likely to be immobilised, reducing the net supply peak.

Peak net nitrate supply from Urea and ENTEC® Urea were significantly different ($p < 0.05$) and greater than the control for all days except day 0 ($p > 0.05$) and day 7 when ENTEC® Urea had the same concentration. Urea consistently supplied greater concentrations and was significantly different ($p < 0.05$) to ENTEC® Urea for all days except towards the latter stages of the incubation period.

On day 45, Urea and ENTEC® Urea were the same and by day 60 ($p > 0.05$), ENTEC® Urea soil net nitrate concentration was significantly greater than Urea ($p < 0.05$). Soil amended with Urea however, supplied

peak nitrate concentration on day 14 compared to day 45 for ENTEC® Urea. The difference was due to the presence of the nitrification inhibitor in ENTEC® Urea, with mineralisation and nitrification of immobilised ammonium occurring at a greater rate than nitrification and denitrification nitrous oxide losses and immobilisation. After day 14, Urea nitrate supply from nitrification was at a slower rate than potential inorganic nitrogen losses.

Table 4.12 outlines the cumulative ammonium and nitrate gains for the control, Urea and ENTEC® Urea fertiliser treatments in mg N/kg.

Table 4.12: Cumulative net ammonium and nitrate concentration for all treatment types over 60 days.

Day	**Ammonium Concentration (mg N/kg)**			**Nitrate Concentration (mg N /kg)**		
	Control	Urea	ENTEC® Urea	Control	Urea	ENTEC® Urea
0	40	51	55	390	219	123
3	81	296	259	661	1230	758
7	120	622	496	965	2341	1233
14	166	892	523	1297	4225	1863
30	200	1083	569	1647	6019	3041
45	234	1140	610	2070	7714	4536
60	266	1179	645	2330	8952	6052
Total Gain Compared to Control		914	379		6622	3722

The cumulative ammonium concentration data is represented graphically in Figure 4.10 with a clear difference between all three treatments. Urea supplied the greatest quantity of mineral nitrogen in the form of ammonium over the 60 day period (914 mg/kg). It is assumed however, that ENTEC® Urea ammonium (total 60 day gain of 379 mg/kg) was immobilised more rapidly than the ammonium which formed from standard Urea.

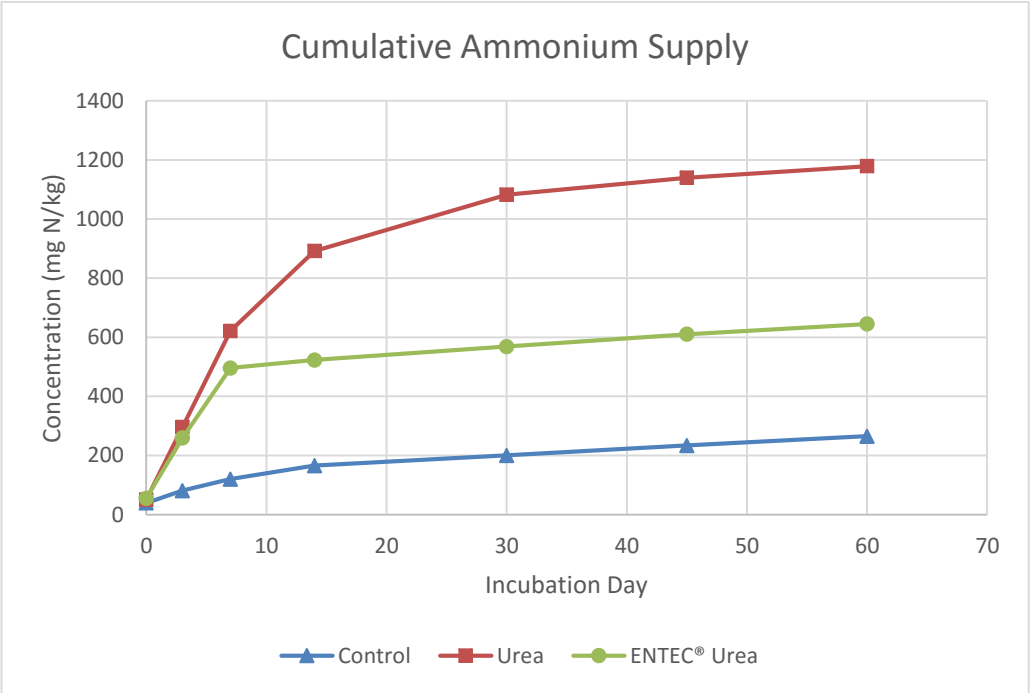


Figure 4.10: Cumulative net ammonium supply (mg/kg) over 60 incubation period for each treatment

Similar mineral nitrogen supply trends were observed for cumulative nitrate supply over the 60 day period for the control, Urea and ENTEC® Urea treatments also (Figure 4.11). Urea had the greatest cumulative nitrate supply (6622 mg/kg) however a longer incubation period would be required to obtain the complete supply characteristics of ENTEC® Urea as peak supply was still occurring at the 60 day mark (3722 mg/kg).

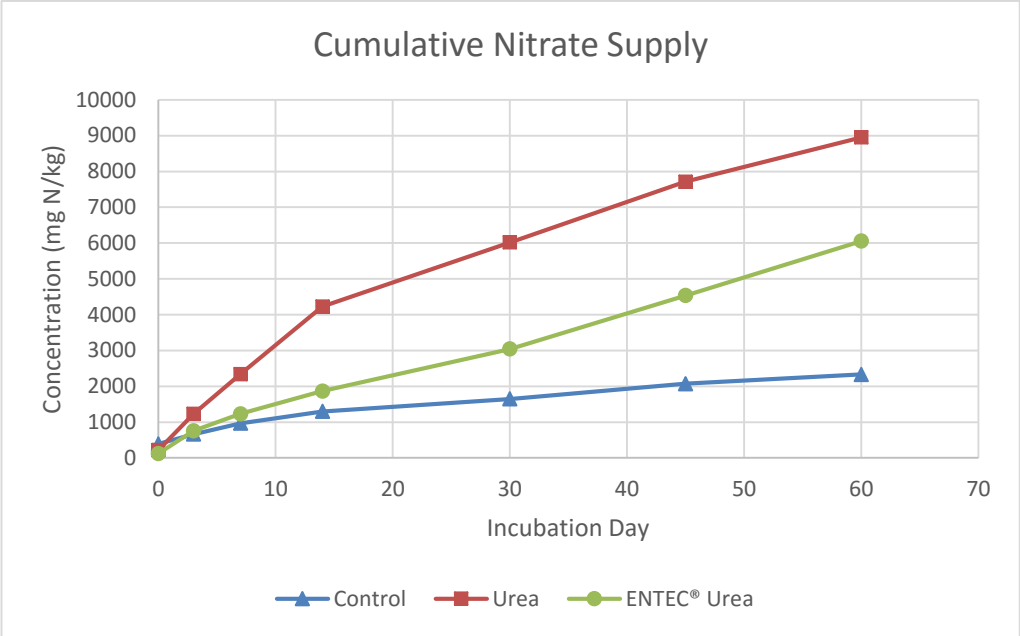


Figure 4.11: Cumulative net nitrate supply (mg/kg) over 60 incubation period for each treatment

As an added guideline to interpret the mineral nitrogen supply trends of Urea and ENTEC® Urea, Table 4.13 outlines the percentage increases and decrease for the individual net gains and losses between sampling days. The day of peak mineral nitrogen concentration within the soil was assumed 100%, with positive values representing net mineral nitrogen gain and negative percentage values representing net losses of mineral nitrogen.

Table 4.13: Percentage of net ammonium and nitrate concentration supplied, per sampling day, per fertiliser treatment type in relation to initial day 0 concentration (Assumed zero for a zero percent increase/decrease).

Day	Ammonium Concentration (%)			Nitrate Concentration (%)		
	Control	Urea	ENTEC® Urea	Control	Urea	ENTEC® Urea
0	0.0	0.0	0.0	0.0	0.0	0.0
3	27.4	70.9	82.0	-365.2	45.1	34.8
7	-13.1	100	100	-265.9	51.4	23.0
14	100	79.7	-15.6	-180.2	100	34.5
30	-94.6	51.0	-5.4	-120.9	94.3	74.9
45	-103.9	2.3	-7.5	100	88.1	98.4
60	-143.8	-4.3	-11.1	-398.5	59.4	100

4.3.2 Net Soil Gaseous Emissions

Graphs outlining the emission fluxes of nitrous oxide, methane and carbon dioxide to compare fertiliser treatments have been included on the following pages, as well as cumulative flux graphs. Applicable error bars were based on standard deviation as per the previous section. For LSD purposes, two repetitions for control treatment were used, with three repetitions for Urea and ENTEC® Urea treated soil.

Nitrous Oxide

For nitrous oxide emission flux, the initial two way ANOVA output from GenStat provided variance results that indicated that relationships were significant at a level of 0.1% significance ($p < .001$). The significant variance existed between fertiliser treatment, incubation day and the fertiliser treatment interaction with incubation day. The linear regression modelling for nitrous oxide accounted for any missing values that were justifiably removed as outliers.

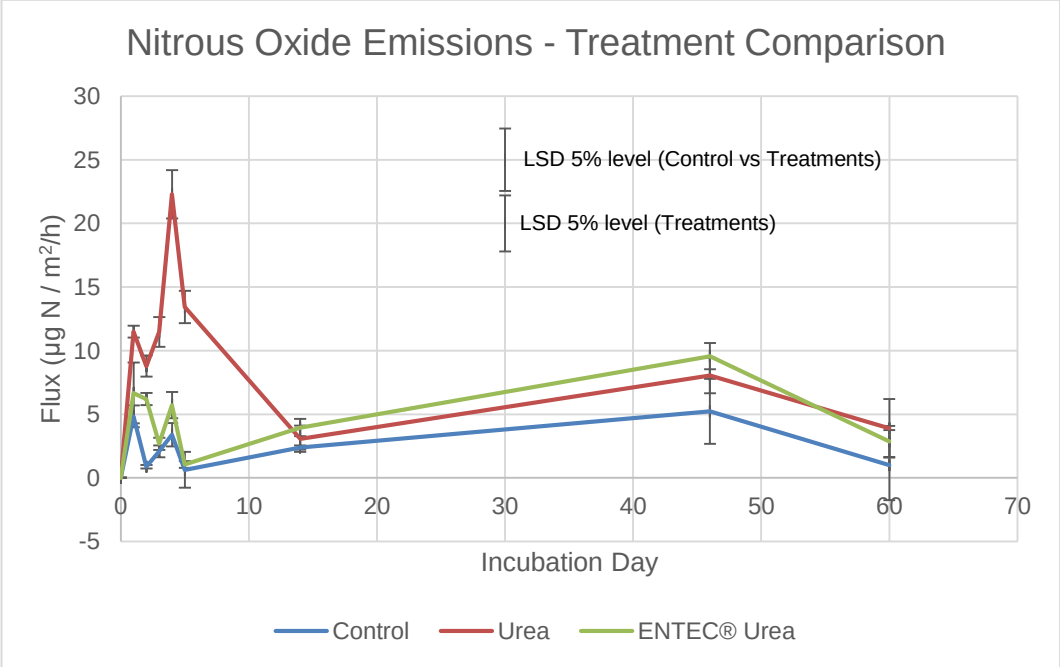


Figure 4.12: Nitrous oxide emission fluxes for control, Urea and ENTEC® Urea treatments over 60 day incubation period

As is evident from Figure 4.12 and ANOVA analysis using a 95% confidence interval, no significant difference ($p < 0.05$) in nitrous oxide emissions existed between the control and ENTEC® Urea treated soils. The exception was day 2 when a significant difference was present ($p < 0.05$). The nitrate produced from nitrification processes for ENTEC® Urea over the incubation period was therefore remaining in nitrate form until immobilised by decomposer organisms.

When a comparison of the Urea fertiliser treatment was made however, a significant difference ($p < 0.05$) existed between Urea and both control and ENTEC® Urea treatments on days 3, 4 and 5. This period was identified as greatest concern for potential environmental pollution as well as fertiliser inefficiency. It also corresponds with the peak Urea ammonium and nitrate formation from net ammonium and nitrate concentration measurements. No significant difference ($p > 0.05$) existed however between Urea and ENTEC® Urea on day 2, with all three treatments being statistically similar on day 1, 17, 46 and 60. The later the time period after application therefore, a lower chance of nitrous oxide emissions exists because nitrate has a higher chance of being taken up by a growing crop.

Figure 4.13 displays the cumulative flux for each fertiliser treatment. The total nitrous oxide emissions for control, Urea and ENTEC® Urea treatments were 20.44, 82.48 and 38.72 μg N m^{-2} h^{-1} respectively. The significantly greater emission level of Urea resulted in the fertiliser type being a less sustainable option, assuming the relative results would be similar under field conditions.

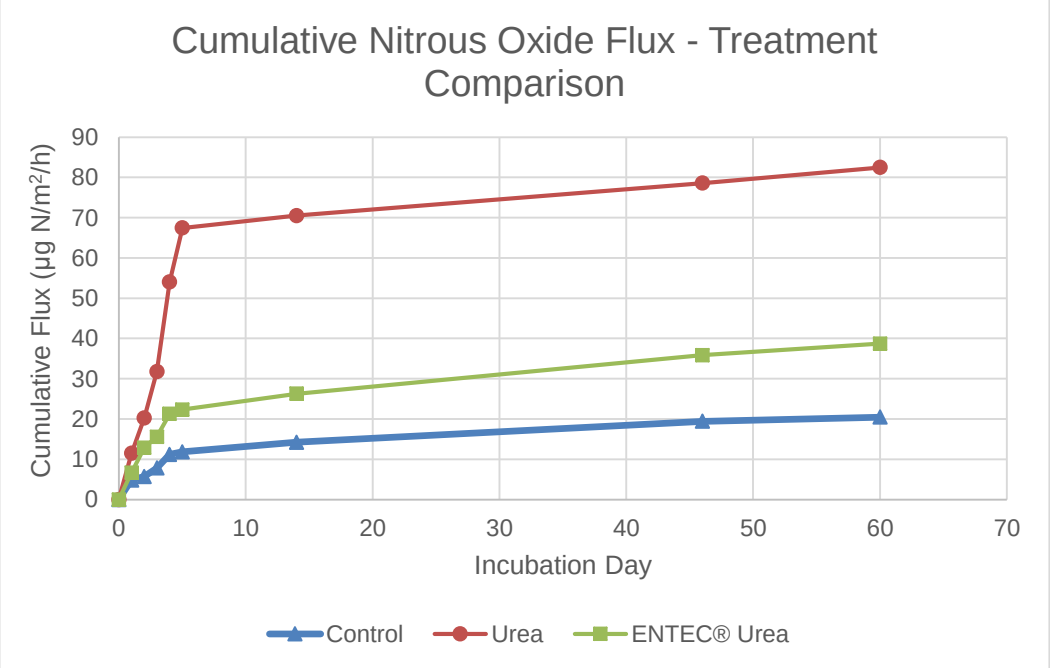


Figure 4.13: Cumulative nitrous oxide flux for control, Urea and ENTEC® Urea treatments over 60 day incubation period

Methane

The ANOVA results when methane emissions were analysed indicated that a significant relationship existed between incubation day and methane emissions at a 95% level of significance ($p < 0.05$). No significant relationship existed between fertiliser treatment and methane emissions ($p > 0.05$) and the interaction of fertiliser treatment and incubation day with methane emissions ($p > 0.05$). 84.45% of the variance was accounted for by the model.

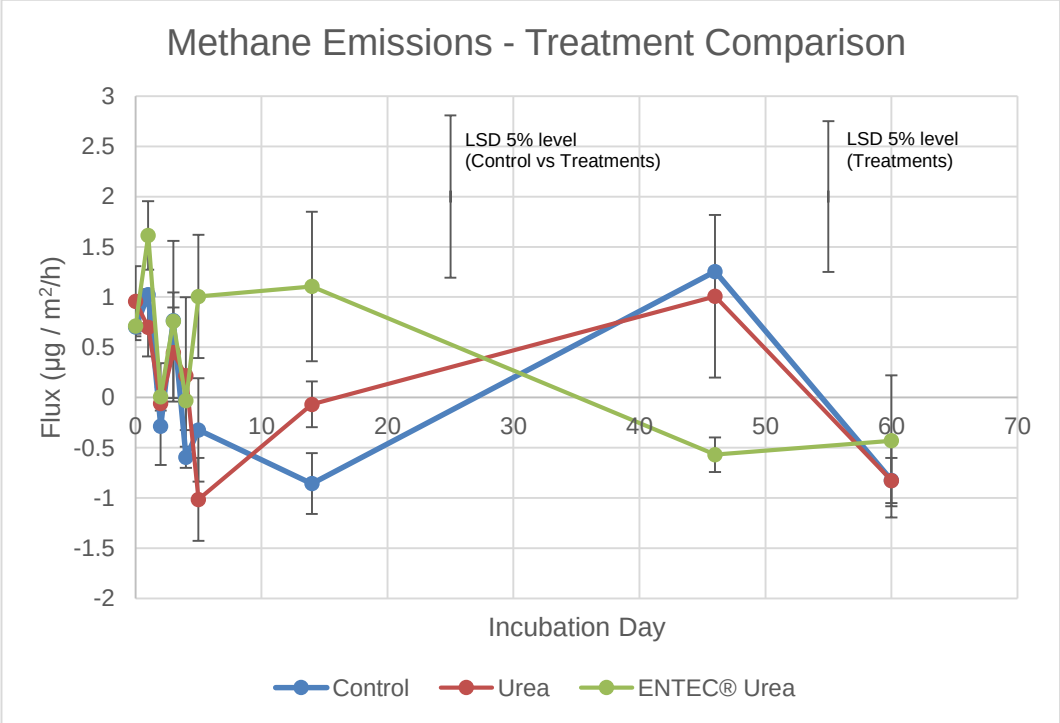


Figure 4.14: Methane emission fluxes for control, Urea and ENTEC® Urea treatments over 60 day incubation period

As is evident from Figure 4.14, the methane emission levels were relatively low for each treatment type, although a slight variation in emission and absorption patterns existed between incubation days. Days 0, 1, 3 and 46 exhibited largely positive and highly variable fluxes, with a

significant difference existing between all other days during incubation ($p < 0.05$) which exhibited smaller and more negative fluxes.

Based on the aerobic experimental design, methanogenesis processes would be expected to remain low as aerobic conditions were maintained. Figure 4.13 displays the cumulative methane flux for each fertiliser treatment. The total methane emissions for control, Urea and ENTEC® Urea treatments were 0.859, 1.35 and 4.16 µg N m^{-2} h^{-1} respectively. Based on the statistical analysis however, these values are highly variable and no significant difference at the 95% confidence level and therefore environmental impact exists between fertiliser treatment types. For a more accurate comparison, a further filtering of results and fluxes when analysing the data would be required.

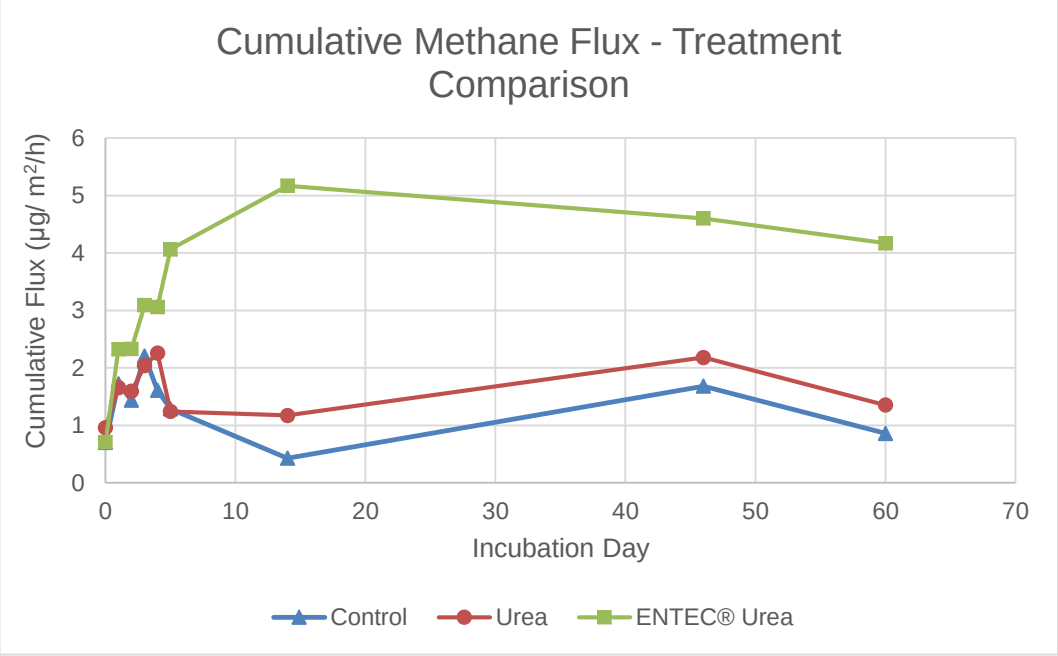


Figure 4.15: Cumulative nitrous oxide flux for control, Urea and ENTEC® Urea treatments over 60 day incubation period

Carbon Dioxide

Carbon dioxide flux ANOVA results indicated that relationships were significant for incubation day and carbon dioxide emissions ($p < 0.05$) as well as fertiliser treatment and carbon dioxide emissions ($p < 0.05$). A significant relationship did not exist however between the interaction of fertiliser treatment and sampling day with carbon dioxide emissions ($p > 0.05$). 96.5% of the variance was accounted for by the model.

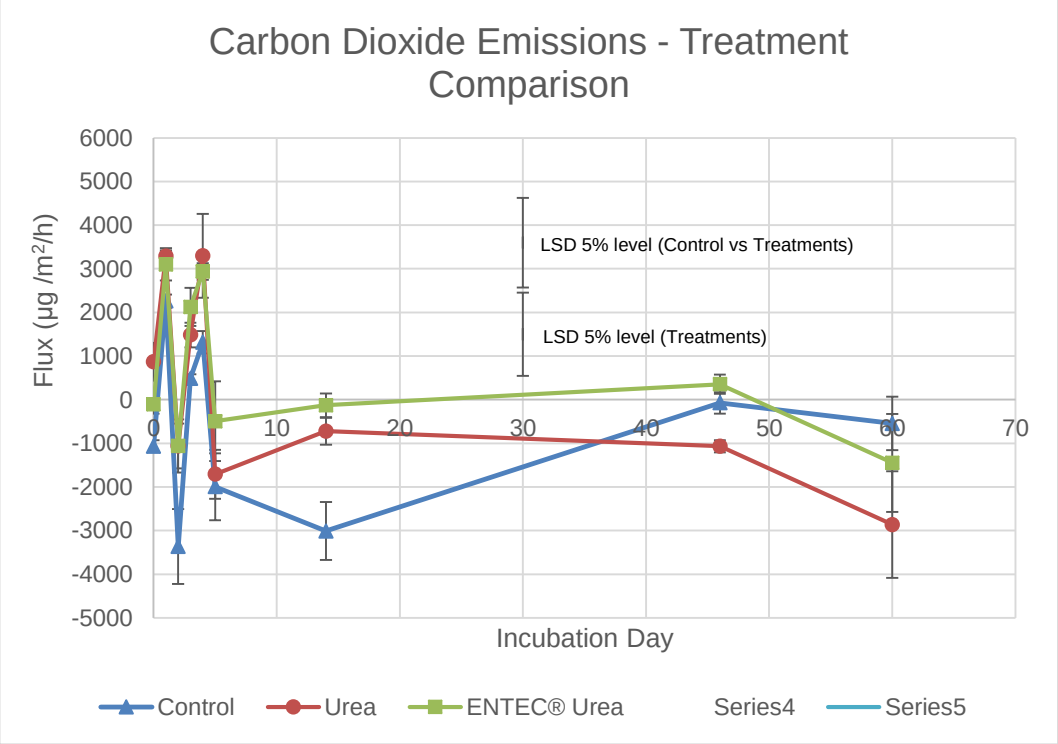


Figure 4.16: Carbon dioxide emission fluxes for control, Urea and ENTEC® Urea treatments over 60 day incubation period

As is evident from Figure 4.16, carbon dioxide emissions from each fertiliser type were highly variable over the 60 day incubation period, particularly in the first 10 days. At a 95% confidence interval, carbon dioxide emissions were found to be significantly different ($p < 0.05$) between the control and both Urea and ENTEC® Urea, which were considered similar ($p > 0.05$) over the entire duration of 60 days. This

presents a possibility of greater environmental pollution risk when applying nitrogen fertiliser.

Differences in incubation day emissions were also present, similar to methane emissions. Days 1, 3 and 4 were highly variable with positive emissions and considered significantly different ($p < 0.05$) to all other incubation days which provided typically negative flux results.

Carbon dioxide emissions are a result of organic matter decomposition in both aerobic and anaerobic conditions, as well as an indication of the urea hydrolysis reaction occurring, which accounts for higher emissions during this initial days after treatment for Urea and ENTEC® Urea.

Figure 4.17 displays the cumulative carbon dioxide flux for each fertiliser treatment. The total carbon dioxide emissions for control, Urea and ENTEC® Urea treatments were -6003, 1511 and 5276 µg N m^{-2} h^{-1} respectively. For a more accurate comparison, a further filtering of results and fluxes when analysing the data would be required.

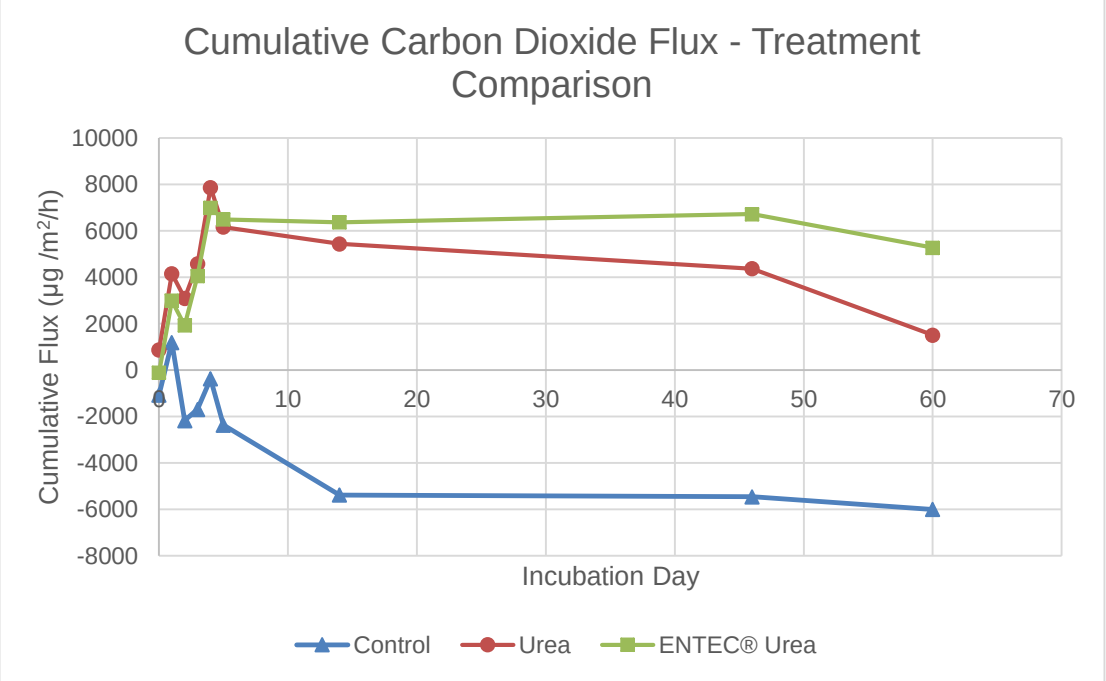


Figure 4.17: Cumulative carbon dioxide flux for control, Urea and ENTEC® Urea treatments over 60 day incubation period

4.4 Review of Research Design and Limitations

The research design of each experimental stage conducted throughout this investigation was developed based on the scope of the research and available time and resources.

Every effort was made to ensure results were accurate and reflected the aim of the experimentation procedures, however there were some limitations of results, with both the precision and accuracy, as well as the breadth of topic investigation. This section provides a review of each methodology component of this investigation with regards to design and limitations.

4.4.1 General Sources of Error

General sources of error includes mainly potential human error, which can cause a slight variation in experimental results. Other minor error sources include the calibration of electronic equipment. General sources of error throughout this investigation and all associated practical work included:

- Calibration accuracy of balances (three types were used for a variety of weight measurements during experimentation).
- Calibration accuracy of automatic pipettes.
- Human measurement during use of measuring cylinder intervals, as well as volumetric flasks.
- Small variations during Hach methods – timing and mixing of solutions.
- Instrument accuracy and precision limitations.

4.4.2 Aerobic Incubation

The aerobic incubation period over 60 days was designed to be ergonomically efficient, however extension of the incubation period to 100 days or longer may have provided results that enabled analysis of soil inorganic nitrogen supply and gas emissions to be completely analysed.

For instance, the peak in net soil inorganic nitrate supply of ENTEC is not visible in the 60 day incubation data as the supply is still increasing.

A constant temperature of 25^{o}C was set for the aerobic incubation experiment to provide sufficient activation of microbiological activity, however to reflect field conditions more thoroughly, a day constant and night constant temperature setting could be used if the incubator was equipped for this.

The field capacity moisture constant used was based on experimental data and may not have reflected actual field capacity of the soil. The potential exists therefore for the soil sampled during incubation to be somewhat drier or somewhat wetter than field capacity which would affect the interpretation of gaseous emissions. This is applicable for nitrous oxide in particular as the emission level is dependent on water filled pore space.

The incubator used for the aerobic incubation contained an internal fan that constantly operated. To ensure evaporation rates from the pots were reduced, the effect of the constant fan operation was included within the experimental design (lids on pots with small holes). A slight variation in soil moisture content, based on the positioning of the pots within the incubator was observed and could be verified by soil moisture loss as each pot remained in the same position in the incubator throughout the entire incubation period.

A variation in soil moisture content can result in variable microbiological activity within the soil, affecting the nitrogen cycle components such as gaseous emissions, nitrification and mineralisation. The figure below contains a comparison of the average soil moisture content for each treatment type within the incubator per sampling day. The moisture in all pots was increased to field capacity the day before sampling, with control pots located along the front of incubator (closest to door), urea in the centre and ENTEC® Urea at the rear.

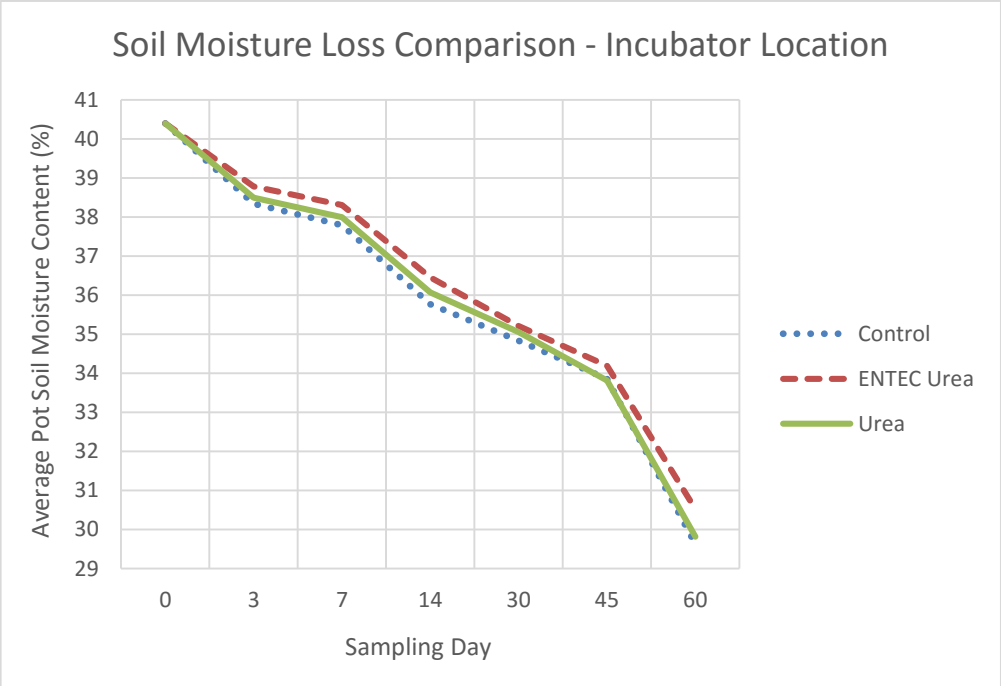


Figure 4.18: Moisture loss from soil based on location in incubator

As is evident from Figure 4.18, the moisture loss that can be attributed to the constant fan operation within the incubator is different based on the location of pots. The control pots located closest to the door experienced a moisture loss consistently lower than Urea and ENTEC® Urea, with ENTEC® Urea maintaining a higher moisture content throughout the incubation period.

4.4.3 Soil Sample Removal and Extraction Process

During the removal of soil samples from aerobic incubation pots, the mixing procedure had some degree of soil loss, which was unable to be reduced completely to zero.

The level gaseous emissions from the soil are dependent on soil moisture and compaction. The re-packing of soil into the pots after each sample was removed, may have introduced variable compaction conditions, with a higher degree of compaction restricting gaseous release to the atmosphere.

4.4.4 Spectrophotometer Analysis of Samples

Unfortunately due to time and resource constraints, the analysis of soil extraction samples for nitrate and ammonium did not occur straight away after extraction were performed. Samples were stored in a cool environment (4^{o}C) until analysis could proceed however, the degree of variance caused by storage of extracts is unknown.

One of the major sources of error during nitrate and ammonium analysis was identified as chlorine interference. Unfortunately the Nessler reagent and Cadmium Reduction Method which the ammonium and nitrate Hach methods are based on respectively, can produce higher or lower results when excessive (>100mg/L) chlorine amounts are present. The ammonium and nitrate extractions were based on 2M KCl which contained significant chlorine concentrations.

Smaller sources of error may have also been introduced during the acid washing and drying stages of the methodology if any residue or natural nitrate sources were present as well as the preparation of standard solutions and dilutions of samples.

Burnett (1972) conducted a study to review the potential sources and extents of errors that could be associated with measurements of molar absorptivity's (colourimetry analysis). The majority of possible error sources were found to have small effects and included:

- Variations in solution temperature caused differences in density and therefore measured absorbance.
- Vials used for analysis may have varied in thickness and width
- 'Reflection' errors as light is passed from one medium such as air to another medium, which in this investigation was the vial glass.
- Accuracy of the wavelength entering the sample

CHAPTER 5 DISCUSSION

The results from this investigation provided an insight into the net potential mineral nitrogen supply from an application of no fertiliser (control) Urea and ENTEC® Urea over a 60 day period. The relative potential environmental impacts from gaseous emissions (nitrous oxide, methane and carbon dioxide) after fertiliser application were also measured.

5.1 Fertiliser Treatment Effect on Inorganic Nitrogen Supply

In the case of both net soil supply of ammonium and nitrate concentrations, all results were found to be significantly greater (by a factor of at least 2) than what would be expected from a fertiliser application rate of 600 kg N/ha. After a thorough checking of methodology and data analysis calculations, it was assumed this error ensued from the interference of very high chlorine concentrations when using the Hach spectrophotometer ammonium and nitrate analysis methods (Hach Company 2013). The trends and differences of results however were still suitable for discussion purposes.

Urea fertiliser was found to have a significantly higher supply rate over the majority of the 60 day incubation period than the control and ENTEC® Urea. Urea supplied 2.4 times the amount of ammonium than ENTEC® Urea, as well as 1.8 times the amount of nitrate over the 60 day period.

Trends of net ammonium and nitrate supply over the 60 period followed trends recorded in a field trial for irrigated cotton. High ammonium concentrations occurred within the first few days, followed by a spike in nitrate concentration thereafter from nitrification processes (Chen & Freney et al. 2008).

From the 60 day incubation, the control soil displayed a very small mineral nitrogen concentration change, which can be attributed to possible immediate immobilisation of mineral nitrogen that formed. Kliese et al.

(2005) indicates a potentially mineralisable nitrogen value of between 110-270mg N/kg of soil is typical for a Black Vertosol, which was not achieved by this investigation.

Peak net ammonium concentration supply (100%) occurred by day 7 for Urea (274.6 mg N /kg), as well as for ENTEC® Urea (181.7 mg N /kg). This is indicative of the similarities between the urea components of the two fertilisers, with urea hydrolysis occurring at similar rates.

Fifty percent of the net nitrate supply occurred by day 7 for Urea (204.8mg N/kg), and reached peak nitrate supply by day 14 (398.1 mg N/kg). Net nitrate supply from ENTEC® Urea fertiliser however, was delayed until day 60, with approximately 75% supplied by day 30 (253.4 mg N/kg). This is a statistically significant timing difference between the two fertiliser types.

The difference can be attributed to the presence of DMPP as the nitrification inhibitor for ENTEC® Urea. This delayed the nitrification of ammonium sources until later in the incubation period. Cotton plants prefer nitrate supply with peak requirements occurring between 50 and 100 days of planting (Cotton CRC 2001). A single application of urea fertiliser will therefore not supply required mineral nitrogen amounts to cotton plants for the entire duration of the season if applied prior to sowing. A significant amount would be subjective to potential losses as the soil would most likely have an abundance of nitrate. This is an inefficient use of nitrogen fertiliser within the cotton industry.

Assuming that Urea was 100% available over the duration of the incubation, ENTEC® Urea supplied about 40% of potential ammonium and 60% of potential nitrate over the same period, therefore a longer incubation period is required to study total soil nitrogen supply from ENTEC® Urea. As a guideline, ENTEC Fertilisers (2015) report that 50% of the ammonium will be nitrified within four weeks or less when the temperature is between 18^{o}C and 30^{o}C.

ENTEC® Urea presented a more efficient alternative fertiliser option to Urea, with only one application most likely providing sufficient mineral nitrogen supply to plants when demand was greatest. This fertiliser type has a longer availability to the crop than Urea. If applied prior to sowing, the peak nitrate supply (60 days) would fall in the period of high nitrogen plant requirements, between first boll and first open boll.

A study by Chen et al. (2008 as cited by ENTEC Fertilisers 2015) found only 1% of applied ammonium from Urea remained in a Black Vertosol after 14 days at 25^{o}C with peak nitrate supply occurring on day 14 – the same result as this investigation. Ammonium supply from ENTEC® Urea however was delayed over a 60 day period. The Urea peak mineral nitrogen supply results are similar to those observed in this investigation, however the ENTEC® Urea ammonium supply was not a delayed supply as previous research suggests, most likely due to the high rate of application and immobilisation effects.

A peak ammonium supply from ENTEC® Urea that occurs in a similar time frame to Urea is similar to the research of Weiske, Benckiser & Ottow (2001) however, who also analysed the effectiveness of DMPP as a potential fertiliser alternative to reduce nitrous oxide emissions through reduced nitrate supply. Results indicated that ammonium concentration differences when compared to the control was minimal, similar to the results in this investigation (control peak ammonium supply occurred day 14 compared to Urea and ENTEC® Urea on day 7). Merino et al. (2001 as cited by Chen et al. 2008) found that DMPP delayed mineral nitrogen supply for 7-14 days, due to the effect of warmer temperatures.

A study conducted in North China (on a different soil type – ‘cinnamon soil’) concluded that the presence of DMPP as a nitrification inhibitor in Urea reduced both the net mineralisation and nitrification rates by 7.3% and 59.1% respectively in the first 14 days after fertiliser application (Zhang et al. 2012). Results from this investigation concluded a difference of 72.3% total mineralised nitrogen and 65.5% of available nitrate after two

weeks between ENTEC® Urea and Urea. Soil potential nitrate supply differences were therefore similar, however the different percentages in total mineral nitrogen may have existed due to the different soil type, moisture and temperature conditions.

5.2 Fertiliser Treatment Effect on Greenhouse Gas Emissions

5.2.1 Nitrous Oxide

Nitrous oxide emissions are relatively small from the soil when compared to the original nitrogen application rate, with nitrification during aerobic conditions considered the primary contributor to the nitrous oxide emissions in this investigation. Emissions of nitrous oxide have also been reported as a result of denitrification from well aerated soils undergoing rapid transition to moist conditions (Snyder et al. 2009). This occurred each time moisture was added to the pots during incubation to reach field capacity conditions during this research, therefore denitrification may have also contributed minor nitrous oxide amounts.

Soil moisture and total water filled pore capacity is directly related to the types of nitrogen gas that form from nitrification/denitrification processes. A clay soil (such as Black Vertosol) typically has an 80% water filled pore space at field capacity At this water filled pore space, denitrification is considered the primary loss of an equal ratio of nitrous oxide to nitrogen gas. Once the soil dries out however, to a water filled pore space of 70% or lower, nitrification becomes a dominant nitrous oxide loss (Granli & Bockman 1994).

Nitrous oxide fluxes over the 60 day (1440 hours) incubation period were strongly affected by the type of fertiliser applied to the soil. The control soil with no fertiliser application had the lowest emission of 20.44 µg N m^{-2} h^{-1}.

Urea amended soil exhibited the highest emission with a total of 82.48 µg N m^{-2} h^{-1} after 60 days, which is a difference of 62.04 µg N m^{-2} h^{-1} when compared to the control soil (used as a datum). With a fertiliser application of 600kg N /ha, this equated to a total of 0.15% of fertiliser nitrogen released as nitrous oxide emissions. This was a result of the increased mineral nitrogen supply in the earlier stages of the 60 day incubation period.

When compared to Urea fertiliser, ENTEC® Urea displayed results that indicated the controlled release fertiliser was a more sustainable option for the cotton industry. A total nitrous oxide emission of 38.72 µg N m^{-2} h^{-1} occurred after 60 days, which amounted to only 18.28 µg N m^{-2} h^{-1} when compared to the total emission from the control. Therefore, only 0.04% of the initial nitrogen applied in fertiliser form was lost as nitrous oxide emissions. This is approximately a 73.3% reduction and improvement in minimisation of nitrous oxide emissions. Both Urea and ENTEC® Urea fertilisers in this investigation conform to the national guideline of approximately 1.25% emissions per nitrogen unit applied.

Several studies have been conducted which quantify the percentage of nitrous oxide gas emitted when Urea fertiliser was applied to the soil. These include:

- 0.07 – 0.2% (Eichner 1990 as cited by Gramli & Bockman 1994)
- 0.01 – 0.6% (Bouwman 1990 as cited by Gramli & Bockman 1994)
- 0.04 – 0.45% (Bremner & Blackmer 1978)

The results from this investigation for Urea fertiliser fall within ranges from previous research. The variation in nitrous oxide emission between fertiliser types is attributed to the presence of the nitrification inhibitor within ENTEC® Urea (DMPP), slowing the nitrate availability within the soil, therefore reducing the amount of nitrate that is subjected to potential losses at any one time.

Fertilisers with DMPP as the active nitrification inhibitor were found to reduce nitrous oxide emissions by 50% (Snyder et al. 2009; Chen et al. 2008), which was achieved by this investigation.

The variation in nitrous oxide emissions between Urea and ENTEC® Urea fertiliser also potentially exist due to the high nitrogen fertiliser application rate used. Rochester (2003) found that when conducting a laboratory based gas emission experiment, the nitrous oxide emissions may not truly represent what is happening in the field, as the shallow soil can allow for conversion to nitrogen gas to occur.

McSwiney and Robertson (2005), also identified that high variability existed in nitrous oxide emissions, when a greater nitrogen fertiliser application rate was applied than requirements of a continuous maize cropping system. Also, a non-linear relationship existed between nitrous oxide emissions and total inorganic soil nitrogen availability.

The variability in emissions at high concentrations is attributed to the complex interaction between processes that control inorganic nitrogen supply and nitrous oxide emissions changing as soil inorganic nitrogen concentrations increase. If denitrification was the primary process for nitrate loss, emissions of nitric oxide and nitrogen would be greater than nitrous oxide, which is similar if nitrification (nitric oxide emissions) was the main loss pathway. At higher concentrations also, microbial immobilisation may also become a restriction to inorganic nitrogen supply.

5.2.2 Carbon Dioxide

Carbon dioxide in agricultural applications is highly cycled throughout the plant, soil and atmosphere system, with net emission from the soil being relatively low compared to fossil fuel use in farming systems (Snyder et al. 2009).

Carbon dioxide emissions from the soil in this investigation were different for all fertiliser treatments and the control. The control flux of carbon dioxide resulted in a total negative flux for the 60 incubation period of -6003 µg C m^{-2} h^{-1}. The soil therefore absorbed more carbon dioxide as a form of carbon sequestration, rather than emitting it as a form of greenhouse gas emission. Another potential reason was the present of negative flux values when applying the assumption that gas concentration increased linearly over the 45 minute sampling period.

Urea fertiliser produced a lower carbon dioxide emission (1511 µg C m^{-2} h^{-1}) when compared to ENTEC® Urea over 60 days (5276 µg C m^{-2} h^{-1}). ENTEC® Urea therefore produced 28.6% greater carbon dioxide emissions than standard Urea. This difference can only be accounted for by an increased organic carbon decomposition. The use of ENTEC® Urea therefore is not a suitable fertiliser alternative to reduce carbon dioxide emissions from soil (further verification of this is required).

Emissions of carbon dioxide that resulted from urea hydrolysis and organic carbon decomposition may have influenced the degree of nitrification within the soil, with carbon dioxide concentration restricting biological activity and therefore nitrification rate (Keeney et al. 1985 as cited by Granli & Bockman 1994). This effect of this however was not quantified in this investigation.

Increases in carbon dioxide concentrations also results in an increase in nitrous dioxide emissions (Keeney et al. 1985 as cited by Granli & Bockman 1994), which accounts for the relationships observed between results measured for both gas types for both fertiliser treatments over the 60 day period.

Weiske, Benckiser & Ottow (2001) discovered that nitrification inhibitors such as DMPP reduced the carbon dioxide emission from soils, however this was unable to be verified by other published data at the time. Results

from this investigation suggest the opposite – that carbon dioxide emissions are increased with the additions of nitrification inhibitor fertiliser.

5.2.3 Methane

Methane emissions are negligible unless anaerobic conditions are present, therefore the soil in this investigation will have a higher potential of forming a sink for absorbing methane gases rather than emitting them (Snyder et al. 2009). Some research has indicated however that a small amount of methane production is possible in soils with an aerobic environment (Serrano-Silva et al. 2014).

Delgado and Mosier (1996) reported that nitrification inhibitors can potentially inhibit CH_4 oxidation in soils, with research by Xu & Inubushi (2004) indicating that urea fertiliser can also reduce the effectiveness of the soil as a methane sink.

Results from this investigation indicated no significant methane emissions existed between the application of control, Urea and ENTEC® Urea. Total emissions for each treatment type were 0.859, 1.35 and 4.16 µg C m^{-2} h^{-1}. ENTEC® Urea however did have a 32.5% greater methane output than standard Urea. The use of ENTEC® Urea to reduce greenhouse gas emissions of methane therefore would not generate any improvements in effectiveness, in aerobic conditions. The effect of the controlled release fertiliser compared to urea fertiliser however, in anaerobic conditions ensuing from sustained rainfall events and irrigation applications would be a potential source of future research.

The slight methane emissions from the soils tested under aerobic conditions may have resulted from the formation of methane under oxidative conditions, if ascorbic acid, ferrihydrite and hydrogen peroxide were present. This concept however is a new area of research with interaction and reasons for methane production under aerobic conditions

still being investigated (Althoff et al. 2010 as cited by Serrano-Silva et al. 2014).

5.3 Future Recommendations

Some future recommendations of further work to compliment the research findings within this investigation include:

- Analysis of the short and long term cost-benefit ratios of using ENTEC® Urea as an alternative nitrogen fertiliser to Urea.
- Determination of the effect of carbon dioxide concentrations within the soil on nitrification rates when ENTEC® Urea and Urea are applied.
- The effect of diurnal temperature fluctuations on net mineral soil nitrogen supply and gaseous emissions within the same laboratory aerobic incubation procedure.
- A field trial or pot experiment with plants incorporating irrigated cotton to determine to total effect of removal of soil mineral nitrogen throughout a cotton season, on net mineral nitrogen supply rates, as well as gaseous emissions. Crops take up nitrate and ammonium and provide organic matter residue to the soil which would minimise immobilisation effects.
- Replication and verification of mineral nitrogen results using modelling programs such as APSIM.
- Comparison of potential mineralisable nitrogen methods (an optional objective within this research investigation) using a 7 day anaerobic procedure.

The results obtained from this investigation are limited to the constraints applied during the laboratory experimentation. Therefore relating the data to the degree days of crops would be beneficial.

CHAPTER 6 CONCLUSION

The aim of this research investigation was to compare the potential mineral nitrogen supply rates of Urea and ENTEC® Urea fertiliser in a cotton based soil type (Black Vertosol). The effect of application of both fertiliser types on greenhouse gas emissions including nitrous oxide, methane and carbon dioxide during a 60 day period was also investigated. It was envisaged that by gathering sufficient data, conclusions could be made that provided justification on a more efficient and environmentally sustainable nitrogen fertiliser option to use within the cotton industry. Justifications were based on matching potential ammonium and nitrate soil supply with the demands of a cotton plant and levels of gaseous emissions.

A Black Vertosol was selected for use in this investigation from a cotton paddock located at Yargullen, QLD. When soil characterisation tests were performed (moisture characteristics, pH and texture), it was evident that the soil type would be a good representation of a soil that was used in the majority of Australian irrigated cotton enterprises.

Results indicated that Urea and ENTEC® Urea soil treatments resulted in vastly different soil inorganic nitrogen supply rates over a 60 day period. Urea achieved peak net ammonium supply on day 7, with peak nitrate and total mineral nitrogen supply occurring on day 14. This large influx of inorganic soil nitrogen created a net supply that would exceed the intake requirements of a cotton plant at this stage of the growth cycle (if sown at a similar time to fertiliser application). Nitrous oxide emissions of Urea fertiliser also exceeded the emissions of ENTEC® Urea by 73.3% over a 60 day aerobic incubation period. Methane emissions were negligible with carbon dioxide emissions also negligible and lower than ENTEC® Urea.

The application of ENTEC® Urea however, presented a more efficient nitrogen fertiliser option, with a delay in net nitrate supply from the soil. 75% of net nitrate supply occurred by day 30, with a peak in supply still

occurring at the conclusion of 60 days. The delay in supply, matches the higher demands of a cotton plant between first boll and boll opening stages (50-100 days after planting). This is justification that use of the controlled release fertiliser may reduce the number of nitrogen applications to one per cotton season if applied before planting occurs.

Negligible methane production occurred with the use of ENTEC® Urea, however a small increase in carbon dioxide emissions was observed when compared to Urea. Further investigation is required into the potential increase in carbon dioxide emissions when a nitrification inhibitor fertiliser is used. Nitrous oxide emissions from the application of ENTEC® Urea however, were significantly reduced, with the slower nitrification rates creating minimal potential for denitrification or loss of nitrous oxide from the nitrification stage. This is a sustainable option for cotton farming, to reduce the overall contribution to detrimental greenhouse gas concentrations within the atmosphere.

LIST OF REFERENCES

Aarons, SR, Melland, AR, Eckard, RJ & Gibson, DM 2006, *The effect of shallow groundwater activity on nitrous oxide emission from dairy pasture*, International Conference on Hydrology and Ecology, Department of Primary Industries, Victoria.

Antille, DL, Sakrabani, R and Godwin, RJ 2014, 'Nitrogen Release Characteristics from Biosolids-Derived Organomineral Fertilizers', *Communications in Soil Science and Plant Analysis*, vol. 45, no. 12, July, pp. 1687 – 1698.

Australian Government 2013, 'Activity Tables', *Australian Greenhouse Gas Emissions System*, Department of Environment, accessed 8 September 2015, <http://ageis.climatechange.gov.au/QueryAppendixTable.aspx>

Australian Government n.d., *Setting up and running an irrigated cotton project,* Clean Energy Regulator, Accessed 17 October 2015, <http://www.cleanenergyregulator.gov.au/ERF/Pages/Forms%20and%20resources/Methods/Resources%20for%20agricultural%20methods/Guide%20reducing%20greenhouse%20gas%20emissions%20from%20fertiliser%20in%20irrigated%20cotton/Setting-up-and-running-an-irrigated-cotton-project.aspx>

Benbi, DK & Khosa, MK 2014 'Effect of Temperature, Moisture and Chemical Composition of Organic Substrates on C Mineralization in Soils', *Communications in Soil Science and Plant Analysis*, vol.45, no. 21, pp. 2734-2753

Bremner, JM & Blackmer, AM 1978, 'Nitrous Oxide: Emission from soild during nitrification of fertilizer nitrogen', *Science*, vol. 199, no. 1978, pp. 295-296, accessed 25 October 2015, Scopus Preview

Bundy, LG n.d., *Managing Urea-Containing Fertilizers*, Presentation Slides, Department of Soil Science, University of Wisconsin, accessed 10 October 2015, <http://www.soils.wisc.edu/ManagingUrea>

Burnett, RW 1972, 'Accurate Measurement of Molar Absorptivities', *Journal of Research of the National Bureau of Standards – A. Physics and Chemistry*, vol. 76A, no. 5, pp.483 – 489, accessed 13 October 2015

Byrnes, B 1990, ‘Environmental effects of N fertilizer use – An Overview’, *Fertilizer Research*, vol. 26, no. 1, pp. 209-215, accessed 14 September 2015, Springer Link Online Database

Carbon Credits Determination 2015, *Equations included in the Irrigated Cotton Calculator under the Carbon Credits Determination 2015,* Australian Government, accessed 17 October 2015, <http://www.environment.gov.au/system/files/pages/aaa6edbb-e4d7-4690-a8c1-e951db165bc0/files/irrigated-cotton-calculator-equations.pdf>

Chadwick, DR, John, F, Pain, BF, Chambers, BJ and Williams, J 2000, ‘Plant Uptake of Nitrogen from the Organic Nitrogen Fraction of Animal Manures: A Laboratory Experiment’, *Journal of Agricultural Science*, Vol. 134, No. 2, March, pp. 159-168

Chen, D, Freney, JR, Rochester, I, Constable, GA, Mosier, AR & Chalk, PM 2008, ‘Evaluation of polyolefin coated urea (Meister) as a fertilizer for irrigated cotton’, *Nutrient Cycling in Agroecosystems*, vol. 81, pp. 245-254, accessed 25 October 2015, Springer Link Online Database

Chen, D, Suter, H, Islam, A, Edis, R, Freney, JR and Walker, CN 2008, ’Prospects of improving efficiency of fertiliser nitrogen in Australian agriculture: a review of enhanced efficiency fertilisers’, *Australian Journal of Soil Research*, vol. 46, no. 4, pp. 289-301, viewed 5 September 2015, CSIRO Publishing, Soil Research Journals Database

Collier, SM, Ruark, MD, Oates, LG, Jokela, WE & Dell, CJ 2014, ‘Measurement of Greenhouse Gas Flux from Agricultural Soils Using Static Chambers’, *Journal of Visualised Experiments*, vol. 90, accessed 25 October 2015, Jove Online Database

Cotton CRC 2001, *NutriPAK*, ed. I Rochester, Australian Cotton Cooperative Research Centre, accessed 10 October 2015, <http://www.cottoninfo.com.au/sites/default/files/documents/NUTRIpak.pdf>

Cotton Research & Development Corporation 2014, *World Leaders in Cotton*, Achievements in Australian cotton research, development and extension 2008-2013, Australian Government

CottonInfo 2015, *Australian Cotton Production Manual 2015*, Cotton Research and Development Corporation, Greenmount Press, accessed 10 October 2015, <http://www.cottoninfo.com.au/sites/default/files/documents/Cotton%20Production%20Manual%202015_LR_0.pdf>

Daniells, IG 1989, ‘Degradation and Restoration of Soil Structure in a Cracking Grey Clay used for Cotton Production’, *Australian Journal of Soil Research*, vol. 27, no. 2, pp. 455-469, accessed 13 September 2015, CSIRO Soil Research Journal Database

ENTEC Fertilisers 2015, *What is Entec®?*, Incitec Pivot Fertilisers Ltd, accessed 12 October 2015, <http://entecfertilisers.com.au/ENTEC%20Fertilisers/What%20is%20ENTEC>

Follett, RF 2001, ‘Chapter 2. Nitrogen Transformation and Transport Processes’, *Nitrogen in the Environment: Sources, Problems and Management*, Elsevier Sciences B.V., The Netherlands

Freney, JR, Chen, DL, Mosier, AR, Rochester, IJ, Constable, GA & Chalk, PM 1993, ‘Use of nitrification inhibitors to increase fertilizer nitrogen recovery and lint yield in irrigated cotton’, *Fertilizer Research*, vol. 34, no. 1, pp. 37 – 44

Gardinier, A, Ketterings, Q, Verbeten, B & Hunter, M 2013, *Urea Fertilizer*, Agronomy Fact Sheet Series, College of Agriculture and Life Sciences, Cornell University Cooperative Extension, accessed 10 October 2015, < http://nmsp.cals.cornell.edu/publications/factsheets/factsheet80.pdf>

Geoscience Australia 2015, *Australian and Region Surface Geology*, Commonwealth of Australia, accessed 27 October 2015, <http://www.ga.gov.au/interactive-maps/#/theme/minerals/map/geology>

Granli, T & Bockman, OC 1994, ‘Nitrous Oxide from Agriculture’, *Norwegian Journal of Agricultural Sciences*, Supplement No. 12, Agricultural University of Norway – Advisory Service

Gray, J & Murphy, B 2002, *Predicting Soil Distribution*, Joint Dept. of Land & Water Conservation (DLWC) & Aust. Society for Soil Science Technical Poster, DLWC, The Australian Soil Club, viewed 14 April 2014, <http://www.soil.org.au/soil_types.htm>

Griffin, T 2008, *Nitrogen Availability (Chapter 15)*, Nitrogen in Agriculture Systems, Schepers, JS & Raun, WR (eds.), Agronomy Monograph 49, accessed 27 October 2015, Google Books

Griffin, TS & Honeycutt, CW, ‘Using growing degree days to predict nitrogen availability from livestock manures’, *Soil Science Society America Journal*, vol. 64, pp. 1876-1882

Guettes, R, Dott, W & Eisentraeger, A 2002, 'Determination of urease activity in soils by carbon dioxide release for ecotoxicological evaluation of contaminated soils', *Ecotoxicology*, vol. 11, no. 5, pp. 357-64, National Centre for Biotechnology Information Database, accessed 10 October 2015

Hach 2010, *DR 2700™Spectrophotometer*, Data Sheet, U.S.A, accessed 18 October 2015, < http://www.hach.com/dr-2700-portable-spectrophotometer-with-lithium-ion-battery/product-downloads?id=7640439008>

Hach Company 2013, *Hach DR2700 User Manual*, 4th edn., Germany, accessed 18 October 2015, <http://www.hach.com/dr-2700-portable-spectrophotometer-with-lithium-ion-battery/product-downloads?id=7640439008>

Hach n.d., *Split Beam vs Single Beam Optics*, Technical Note, Hach Company World Headquarters, USA, accessed 18 October 2015, <http://www.hach.com/dr-2700-portable-spectrophotometer-with-lithium-ion-battery/product-downloads?id=7640439008>

Hao, N, Trang, T & Tinh, T 2004, *Net N mineralisation in acid sulfate soils amended with different sources of organic matter, lime and urea,* Australian Journal of Soil Research, Vol. 42, pps 685-691, CSIRO Publishing

Harris, PS, Biggs, AJW & Coutts, AJ (eds.) 1999, *Central Darling Downs Land Management Manual – Field Manual*, Department of Natural Resources, Queensland, DNRQ990102, accessed 5 October 2015, <https://publications.qld.gov.au/storage/f/2014-11-19T06%3A43%3A54.223Z/mcd-dnrq990102-central-darling-downs-field-manual.pdf>

Harris, R, Armstrong, R, Belyaeva, O, Fogarty, K & Wallace, A 2014, *The contribution of nitrogen (N) fertiliser to nitrous oxide (N_2O) emissions and wheat productivity in a high rainfall cropping environment*, 2014 Soil Science Conference Proceedings, accessed 25 October 2015, <http://www.soilscience2014.com/proceedings/Harris.pdf>

Haskett, J, Molina, J & Clapp, C 1986 *Nitrogen Carbon Transformation in Soil (NCSOIL) – User's Guide*, Department of Soil Science, University of Minnesota, Soil Series 122, Accessed 10 August 2015, < https://passel.unl.edu/UserFiles/Image/siteImages/1066162614.pdf>

Heanes, D 1977, *Laboratory Methods of Soil and Plant Analysis*, 2nd edn., Soil Conservation Branch Report S5/77, Department of Agriculture Fisheries and Forestries, South Australia

Heffer, P & Prud'homme, M 2014, *Fertilizer Outlook 2014-2018*, International Fertilizer Industry Association, 82nd IFA Annual Conference, Sydney, May 2014, accessed 10 October 2015, < http://www.fertilizer.org/imis20/images/Library_Downloads/2014_ifa_sydney_summary.pdf?WebsiteKey=411e9724-4bda-422f-abfc-8152ed74f306&=404%3bhttp%3a%2f%2fwww.fertilizer.org%3a80%2fen%2fimages%2fLibrary_Downloads%2f2014_ifa_sydney_summary.pdf>

Henzel, T 2007, *Australian Agriculture – Its History and Challenges*, CSIRO Publishing, Victoria, Australia, pp. 205-224, https://books.google.com.au/books

Honeycutt, CW & Potaro, LJ 1990, 'Field evaluation of heat units for predicting crop residue carbon and nitrogen mineralisation', *Plant and Soil*, vol. 125, pp. 213-220

Honeycutt, CW, Zibilske, LM & Clapham, WM 1988, 'Heat units for describing carbon mineralization and predicting net nitrogen mineralization', *Soil Science Society America Journal*, vol. 52, pp. 1346-1350

Horton, B and Corkrey, R 2011, 'A Weighted Coefficient Model for Estimation of Australian Daily Soil Temperature at Depths of 5cm to 100cm based on Air Temperature and Rainfall', *Soil Research*, vol. 49, pp. 305-314

IPNI 2015a, *Nitrogen Notes Fact Sheets – Nitrification*, International Plant Nutrition Institute, California Department of Food and Agriculture, Western Plant Health Association, no. 4, accessed 10 October 2015, <http://www.ipni.net/publication/nitrogen-en.nsf/book/7F7F448C4D064A5985257C13004C83A3/$FILE/NitrogenNotes-EN-04.pdf>

IPPC 2001, *Climate change 2001: A Scientific Basis*, Intergovernmental Panel on Climate Change, Editors: (JT Houghton, Y Ding, DJ Griggs, M Noguer, PJ van der Linden, X Dai, CA Johnson, K Maskell), Cambridge University Press, Cambridge, UK

Isbell, RF 1996, *The Australian Soils Classification*, CSIRO Publishing, Australia

Jacquier, D n.d., *Interactive Key to the Australian Soil Classification*, CSIRO Land and Water, viewed 8 September 2015, <http://www.clw.csiro.au/aclep/asc_re_on_line/ve/vertsols.htm>

Johnson, C, Albrecht, G, Ketterings, Q, Beckman, J & Stockin, K 2005, *Nitrogen Basics – The Nitrogen Cycle*, Agronomy Fact Sheet Series No. 2, Department of Crop and Soil Sciences, Cornell University Cooperative Extension, accessed 13 September 2015, < http://onondaga.cce.cornell.edu/resources/nitrogen-basics-the-nitrogen-cycle>

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

Kitchen & Goulding 2001, 'Chapter 13. On-Farm technologies and practices to improve nitrogen use efficiency', *Nitrogen in the Environment: Sources, Problems and Management*, Elsevier Sciences B.V., The Netherlands

Kliese, YJ, Dalal, RC, Strong, WM & Menzies, NW 2005, 'Piggery pond sludge as a nitrogen source for crops. 1. Mineral N supply estimated from laboratory incubations and field application of stockpiled and wet sludge', *Australian Journal of Agricultural Research*, vol 56, pp. 245-255, accessed 29 October 2015, CSIRO Publishing

Le Mer, J & Roger, P 2001, 'Production, oxidation, emission and consumption of methane by soils: A review', *European Journal of Soil Biology*, vol. 37, no. 1, pp. 25-50, accessed 17 October 2015, Science Direct Online Database

Linzmeier, W, Gutser, R & Schmidhalter, U 2001, 'Nitrous oxide emission from soil and from a nitrogen-15-labelled fertiliser with the new nitrification inhibitor 3, 4-dimethylpyrazole phosphate (DMPP)', *Biology and Fertility of Soils*, vol. 34, pp. 103-108, accessed 12 October 2015, Science Direct Online Database

Lloyd, AB & Sheaffe, MJ 1973, 'Urease Activity in Soils', *Plant and Soil*, vol. 39, no. 1, pp. 71-80, accessed 10 October 2015, Springer Link Database

Macadam, XMB, Prado, A, Merino, P, Estavillo, JM, Pinto, M & Gonzalez-Murua, C 2003, 'Dicyandiamide and 3, 4-diemthylpyrazole phosphate decrease N_2O emissions from grassland but dicyanidiamide produces deleterious effects in clover', *Journal of Plant Physiology*, vol. 160, pp. 1517-1523, accessed 12 October 2015, Science Direct Online Database

Macdonald, B, Rochester, I, Ringrose-Voase, A, Nadelko, T & Chang, Y 2014, *Nitrogen Losses from Soil and Irrigation Water*, 17th Australian Cotton Conference, Queensland, Australia, August 2015

Margeson, JH, Suggs, JC & Midgett, MR 1980, 'Reduction of Nitrate to Nitrite with Cadmium', *Analytical Chemistry*, vol. 52, no. 12, pp. 1955-1957, American Chemical Society, accessed 18 October 2015, ACS Publications

McFarlane, S 2005, *Carbon and Nitrogen Cycling in Groundwater*, USGS (U.S. Geological Survey National Research Program Central Branch), accessed 17 October 2015, <http://wwwbrr.cr.usgs.gov/projects/EC_biogeochemistry/Cape.htm>

McKenzie, N, Coughlan, K & Cresswell, H 2002, *Soil Physical Measurement and Interpretation for Land Evaluation*, CSIRO Publishing, Collingwood, Australia

McKenzie, N, Jacquier, D, Isbell, R & Brown, K 2004, *Australian Soils and Landscapes – An illustrated compendium,* CSIRO Publishing, Collingwood, Victoria

McSwiney, CP & Robertson, GP 2005, 'Nonlinear response of N_2O flux to incremental fertilizer addition in a continuous maize (*Zea mays L.)* cropping system', *Global Change Biology*, vol. 11, pp. 1712-1719, accessed 25 October 2015, Wiley Online Library

Monsanto 2011, *Bollgard II®Cotton: Technical Manual*, accessed 4 October 2015, < http://www.monsanto.com/global/au/products/documents/bollgard-ii-technical-manual.pdf>

Mosier, AR, Syers, JK & Freney, JR 2004, *Agriculture and the Nitrogen Cycle: Assessing the Impacts of Fertiliser Use on Food Production and the Environment*, Scientific Committee on Problems of the Environment (SCOPE), United States of America,

NSW Agriculture 1998, *SOILpak Cotton Growers*, NSW Department of Primary Industries, NSW Government, accessed 27 October 2015, <http://www.dpi.nsw.gov.au/agriculture/resources/soils/guides/soilpak/cotton>

Pasda, G, Hahndel, R & Zerulla, W 2001, 'Effect of fertilizers with the new nitrification inhibitor DMPP (3, 4-dimethylpyrazole phosphate) on yield and quality of agricultural and horticultural crops', *Biology and Fertility of Soils,* vol. 34, no. 2, pp. 85-97, accessed 12 October 2015, SpringerLink Online Database

Pattison, T, Moody, P & Bagshaw, J 2010, *Soil Health for Vegetable Production in Australia*, Department of Environment and Resource Management & Department of Employment, Economic Development and Innovation, Queensland Government, accessed 26 October 2015, < https://www.daf.qld.gov.au/__data/assets/pdf_file/0008/69812/Soil-health-vegetable-production.pdf>

Payne, R 2012, *A Guide to Regression, Nonlinear and Generalized Linear Models in Genstat*®, 15th edn., VSN International, UK

Pesek, J, Stanford, G & Case, NL 1971, 'Nitrogen Production and Use', in RA Olsen (ed.), *Fertiliser Technology and Use*, 2nd edn, Soil Science Society of America, Wisconsin

Price, G (ed.) 2006, *Australian Soil Fertility Manual*, 3rd edn., Fertilizer Industry Federation of Australia Inc., Chapter 3, pp. 23-37, CSIRO Publishing, Collingwood

Rayment, G & Lyons, D 2011, *Soil Chemical Methods – Australasia*, CSIRO Publishing, Collingwood, Victoria, pp. 134 – 146

Rochester, I & Gordon, K 2014, *Nitrogen Use Efficiency*, CSIRO Narrabri, Presentation at 17th Australian Cotton Conference, Queensland, Australia, August 2014

Rochester, I 2003, 'Estimating nitrous oxide emissions from flood-irrigated alkaline grey clays', *Australian Journal of Soil Research*, vol. 41, no. 2, pp. 197-206, accessed 14 September 2015, CSIRO Soil Research Journal Database

Rochester, I 2013, *Developing N-efficient cotton systems that produce low GHG emissions ad promote healthy soil*, Final Report, Cotton Research and Development Corporation, Narrabri, NSW, Australia

Rochester, I 2014, *Growing High-Yielding Nitrogen-Efficient Cotton*, CSIRO Agriculture Flagship, 17th Australian Cotton Conference, Queensland, Australia, August 2015

Rochester, I, Constable, G & Saffigna, P 1996, 'Effective nitrification inhibitors may improve fertilizer recovery in irrigated cotton', *Biology and Fertility of Soils*, vol. 23, no. 1, pp. 1-6, accessed 13 September 2015, Springer Link Database

Rochester, IJ & Constable GA 2000, 'Denitrification and immobilisation in flood-irrigated alkaline grey clays as affected by nitrification inhibitors, wheat straw, and soil texture', *Australian Journal of Soil Research*, vol. 38, pp. 633-642, CSIRO Publishing, accessed 13 September 2015, CSIRO Soil Research Journals Database

Rochester, IJ, Constable GA, MacLeod, DA, 1991, 'Mineral nitrogen dynamics in a fallow grey clay', *Australian Journal of Experimental Agriculture*, vol. 31, no. 2, pp. 237-244, accessed 13 September 2015, CSIRO Animal Production Science Journal Database

Rochester, IJ, Constable, GA & MacLeod, DA 1992, 'Preferential Nitrate Immobilization in Alkaline Soils', *Australian Journal of Soil Research*, vol. 30, no. 5, pp. 737 – 749, accessed 13 September 2015, CSIRO Soil Research Journal Database

Rochester, IJ, Constable, GA, Oosterhuis, DM & Errington, M 2012, 'Nutritional Requirements of Cotton during Flowering and Fruiting', *Flowering and Fruiting in Cotton*, No. 8, The Cotton Foundation Reference Book Series, Tennessee, USA, accessed 17 October 2015, <https://www.cotton.org/foundation/upload/FLOWERING-AND-FRUITING-IN-COTTON.pdf>

Rodrigues, MA, Santos, H, Ruivo, S & Arrobas, M 2010, 'Slow-release N fertilisers are not an alternative to urea for fertilisation of autumn-grown tall cabbage', *European Journal of Agronomy*, vol. 32, no. 2, pp. 137-143, accessed 12 October 2015, Science Direct Online Database

Rosen, A 1996, *Denitrification by Rhizobium meliloti*, Department of Microbiology, Swedish University of Agricultural Sciences, Sweden, accessed 17 October 2015, <http://www.vaxteko.nu/html/sll/slu/rapport_mikrobiologi/RMB65/RMB65.HTM>

Roth, G 2014, *Australian Grown Cotton Sustainability Report 2014*, Cotton Australia & Cotton Research and Development Corporation, Australian

Government, accessed 13 September 2015, <http://cottonaustralia.com.au/uploads/publications/Sustainability_report_201114.PDF>

Roth, I 2013, *A Snapshot of Grower Nutrition Practices from 2013 Cotton Practices Survey*, 17th Australian Cotton Conference 2014 Presentation, Queensland, accessed 13 September 2015

Ryan, T 2010, *The Australian Fertiliser Industry – Values and Issues*, Australia Fertiliser Industry Conference Paper, Meta Economics Consulting Group, accessed 17 October 2015, <http://www.fertilizer.org.au/files/pdf/publications/Australian%20Fertilizer%20Industry%20Value%20and%20Issues%20August%202010.pdf>

Scheer, C, Grace, PR, Rowlings, DW & Payero, J 2013, 'Soil N_2O and CO_2 emissions from cotton in Australia under varying irrigation management', *Nutrient Cycling in Agroecosystems*, vol. 95, no. 1, pp. 43-56, accessed 14 September 2015, Springer Link Online Database

Schlegel, AJ, Nelson, DW & Sommers, LE 1987, 'Use of urease inhibitors and urea fertilizers on winter wheat', *Nutrient Cycling in Agroecosystems*, vol. 11, no. , pp. 97-111, accessed 10 October 2015, Springer Link Database

Serrano-Silva, N, Sarria-Guzman, Y, Dendooven, L & Luna-Guido, M 2014, 'Methanogenesis and Methanotrophy in Soil: A Review', *Pedosphere*, vol. 24, no. 3, pp. 291-307, accessed 25 October 2015, Science Direct Online Database

Singer, MJ & Munns, DN 2006, *Soils – An Introduction*, 6th edn., Pearson Prentice Hall, United States of America

Sistani, KR, Jn-Baptiste, M, Lovanh, N & Cook, KL 2011, 'Atmospheric emissions of nitrous oxide, methane and carbon dioxide from different nitrogen fertilisers', *Journal of Environmental Quality*, vol. 40, United States, Abstract accessed 17 October 2015, USQ Library

Smith, J & Bell, M 2015, *Response of irrigated cotton to applied nitrogen*, 2nd Australian Cotton Research Conference, University of Southern Queensland, Toowoomba, September 2015

Smith, J, Devlin, A, Thomas, A, Hunter, G, O'Keeffe, K, Dickinson, S, Clift, S & Waters, W 2014, *Nitrogen Fertiliser Use Efficiency Across the Regions*, CottonInfo Team, 17th Australian Cotton Conference, Queensland, Australia, August 2014

Snyder, CS, Bruulsema, TW, Jensen, T & Fixen, PE 2009, 'Review of greenhouse gas emissions from crop production systems and fertilizer management effects', *Agriculture, Ecosystems and Environment*, vol. 133, no. 3-4, pp. 247-266, accessed 25 October 2015, Science Direct Online Database

Tan, KH 1995, *Soil Sampling, Preparation and Analysis*, Marcel Dekker INC., New York

The University of Newcastle n.d., *Analysis of Nitrate Ion Concentration in Water*, Poster Educational Resource, accessed 13 October 2015, < https://www.newcastle.edu.au/__data/assets/pdf_file/0004/193963/UV-Vis-poster.pdf>

Tisdale, SL, Nelson, WL & Beaton, JD 1984, 'Soil and Fertiliser Nitrogen', Chapter 5*, Soil Fertility and Fertilizers*, 4th edn, Macmillan Publishing Company, New York

UN Industrial Development Organisation 1998, *Fertilizer Manual*, Chapter 2: The role of fertilizers in agriculture, International Fertilizer Development Centre, Kluwer Academic Publishers, The Netherlands, accessed 20 September 2015, Google Books

Vertosols 2008, Victorian Resources Online – Wimmera, Department of Environment and Primary Industries, Victoria Government, Viewed 14 April 2014,<http://vro.depi.vic.gov.au/dpi/vro/wimregn.nsf/pages/natres_soil_vertosols>

Virmani, S, Sahrawat, K & Burford, J 1982, *Physical and Chemical Properties of Vertisols and their Management*, Vertisols and Rice Soils of the Tropics, viewed 14 April 2014, <http://oar.icrisat.org/4038/1/0048.pdf>

VSN International 2014, Genstat 17th Edition, UK

Weiske, A, Benckiser, G & Ottow, JCG 2001, 'Effect of the new nitrification inhibitor DMPP in comparison to DCD on nitrous oxide (N2O) emissions and methane (CH4) oxidation during 3 years of repeated applications in field experiments', *Nutrient Cycling in Agroecosystems*, vol. 60, pp. 57-64, accessed 8 September 2015, Springer Link

Xu, X & Inubushi, K 2004, 'Effects of N source and methane concentrations on methane uptake potential of a typical coniferous forest and its adjacent orchard soil', *Biology of Fertile Soils*, vol. 40, pp. 215-221, accessed 25 October 2015, Springer Link Online Database

Zhang, Y, Xu, H, Xia, ZW & Guo, YL 2012, ‘Effects of nitrification inhibitors DCD and DMPP on cinnamon soil’s gross nitrogen mineralization and nitrification rates’, *The Journal of Applied Ecology*, vol. 23, no. 1, pp. 166-172, accessed 25 October 2015, PubMed Online Database

APPENDICES

Appendix A – Project Specification

University of Southern Queensland

FACULTY OF ENGINEERING AND SURVEYING

ENG4110/4111 Research Project

STUDENT: Constance Coverdale

TITLE: Nitrogen Supply Rates during a Cotton Season

SUPERVISORS: Dr Alice Melland & Dr Diogenes Antille (Research Fellows NCEA)

SPONSOR: USQ and Cotton Australia

PROJECT AIM: To estimate the quantities of mineral nitrogen supply from Urea and ENTEC® Urea by determining the potential and actual soil N supply and greenhouse gas emission losses using a Black Vertosol.

OBJECTIVES:

The research project will have several objectives, including:

- Estimate the nitrogen supply and release characteristics from a soil amended with nitrogen fertiliser and from this, determine the total mineral nitrogen supply.
- Test the differences in nitrogen supply and release characteristics from a soil after application of two different nitrogen fertilizers (Urea and controlled release - ENTEC® Urea).

- Determine the differences in greenhouse gas emissions (nitrous oxide, methane and carbon dioxide) from Urea and ENTEC® Urea applications.
- Conduct a 60 day aerobic incubation to obtain soil and gas samples for ammonium, nitrate and greenhouse gas emission data.
- (Option depending on time) Compare the results of two different methods of plant nitrogen mineralisation estimation (anaerobic-7day vs aerobic – 90 day).

It is envisaged that the result of this research project should provide an accurate estimate as to when the maximum availability of nitrogen to plants from the nitrogen fertiliser occurs; taking the soil nitrogen mineralisation rate and other soil properties into consideration. The time of maximum availability of nitrogen can then be compared against the seasonal variation of crop demand to determine whether the nitrogen availability matches typical cotton cropping requirements.

PROGRAMME: (Issue C, 10 May 2015)

1. Obtain soil samples from a cotton paddock and identify initial soil properties such as field capacity, texture, pH, total soil carbon content, bulk density, moisture contents (air-dry & field moist), soil organic matter content and initial nitrogen levels.

2. Investigate the literature to determine the possible methods that exist to conduct a nitrogen mineralisation experiment in laboratory conditions, where removal of nitrogen for plant use does not occur.
3. Conduct research (literature review) to determine the possible effects of factors that can cause varying nitrogen mineralisation rates and gaseous emissions.

4. Sample the soil and determine the mineralized nitrogen level at 0, 7, 15, 30 and 60 day intervals. Ensure that at least four repetitions occur to get averaged results.

5. Take gas samples and analyses gaseous emissions of CO_2, N_2O and CH_4 over time from aerobically incubated soils.

6. Use statistical analysis software (Genstat & Excel) to analyse results and determine differences between fertiliser application types (Urea and ENTEC Urea).

7. (Optional) Conduct a soil incubation experiment for 7 days (anaerobic conditions) and/or 60 days (aerobic) with two fertilizer types and a control. Ensure the temperature and moisture levels remain at a constant level based on researched values.

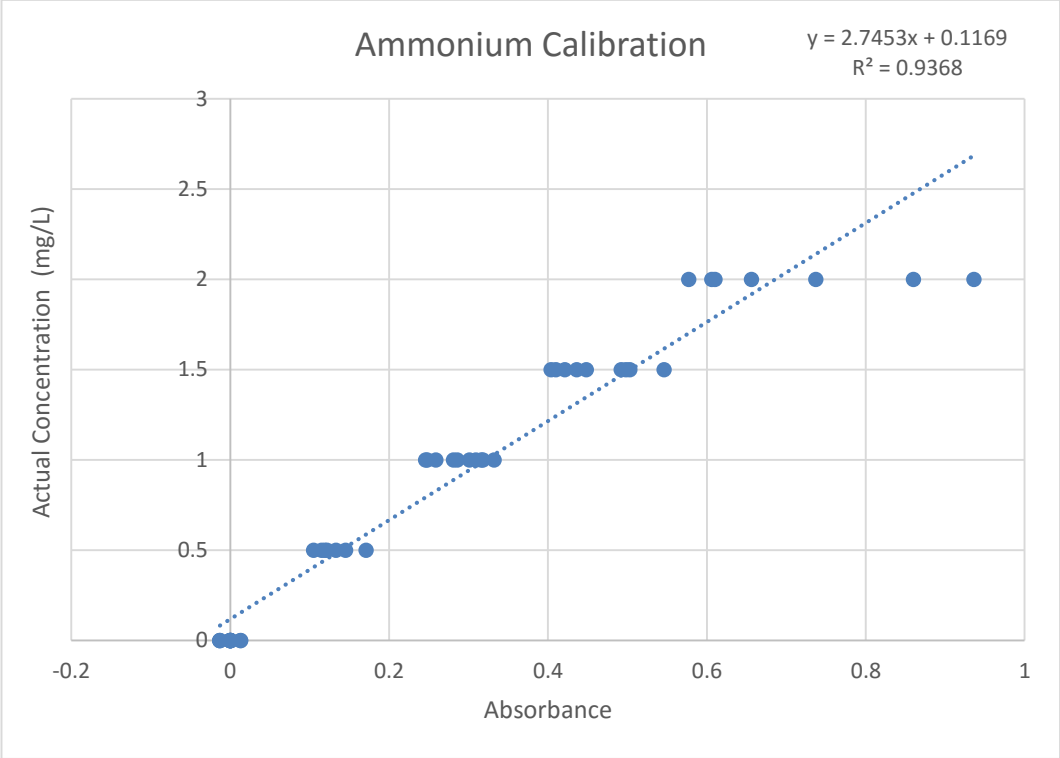
AGREED:

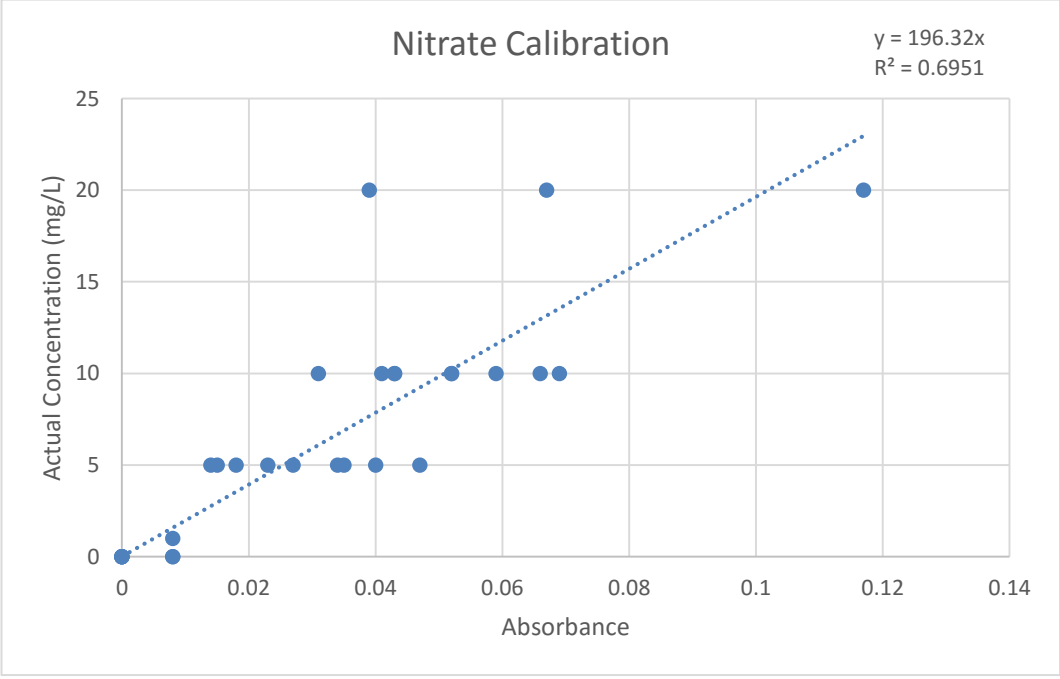
_____ (STUDENT) _____ (SUPERVISORS)

Appendix B – Pot Moisture Maintenance Template (Example)

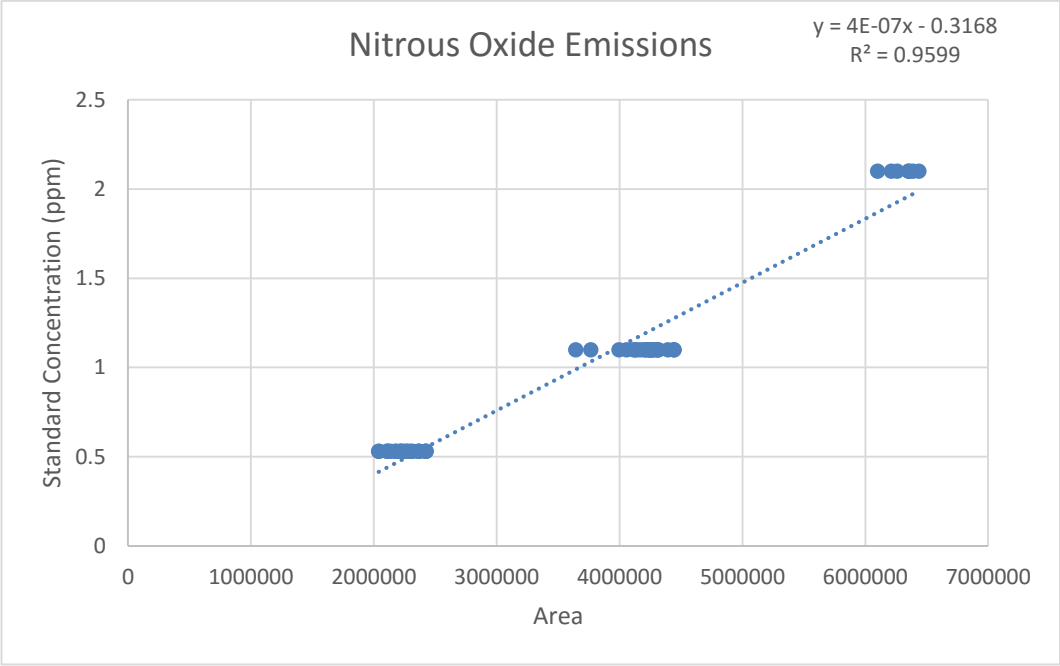
Pot No.	Treatment	Weight Pot and Soil for Field Capacity (g)	Weight Pot and Soil for 75% Field Capacity (g)	Weight Pot and Soil Before Sampling (g)	Field Capacity of Wet Soil (%)	Soil Moisture Content Acceptable? (1=Yes, 0=No)	Moisture Percentage of Incubated Soil (%)	Mass Soil Sample to be Removed (4g oven-dry equiv.) (g)	Mass Soil Removed for Moisture Test (g)	Weight Pot and Soil After Sampling (g)	Weight Wet Soil (g)	Water to be Added to Reach Field Capacity (g)	Total Weight Water at Field Capacity (g)	Weight Pot and Soil at Field Capacity (g)	Weight of Soil and Pot at 75% Field Capacity (g)
1	Control	403.88	376.41	398.86	95.43	1	38.56	5.54	19.44	374	351.79	4.69	144.03	379	342.56
2	Control	403.67	376.20	397.11	94.03	1	37.99	5.52	19.18	372	350.53	6.13	144.10	379	342.51
3	Control	403.89	376.42	397.84	94.49	1	38.18	5.53	19.99	372	350.22	5.64	143.78	378	342.02
4	Control	403.75	376.28	398.9	95.59	1	38.62	5.54	19.17	374	352.23	4.53	144.14	379	342.68
5	Urea	403.78	376.31	400.1	96.65	1	39.05	5.56	20.38	374	352.17	3.43	143.67	378	341.67
6	Urea	403.84	376.37	397.84	94.54	1	38.20	5.53	19.42	373	350.84	5.60	144.01	378	342.49
7	Urea	403.84	376.37	398.25	94.91	1	38.35	5.53	19.68	373	350.99	5.21	143.92	378	342.27
8	Urea	403.77	376.30	398.28	95.00	1	38.38	5.54	19.66	373	351.10	5.12	143.93	378	342.22
9	ENTEC	403.90	376.43	398.98	95.52	1	38.59	5.54	20.30	373	351.03	4.58	143.68	378	341.80
10	ENTEC	403.96	376.49	400.37	96.73	1	39.08	5.56	19.42	375	353.22	3.35	144.06	379	342.72
11	ENTEC	403.81	376.34	400.2	96.72	1	39.08	5.56	19.76	375	352.86	3.37	143.93	378	342.26
12	ENTEC	403.83	376.36	398.35	95.01	1	38.39	5.54	20.09	373	350.68	5.11	143.75	378	341.89

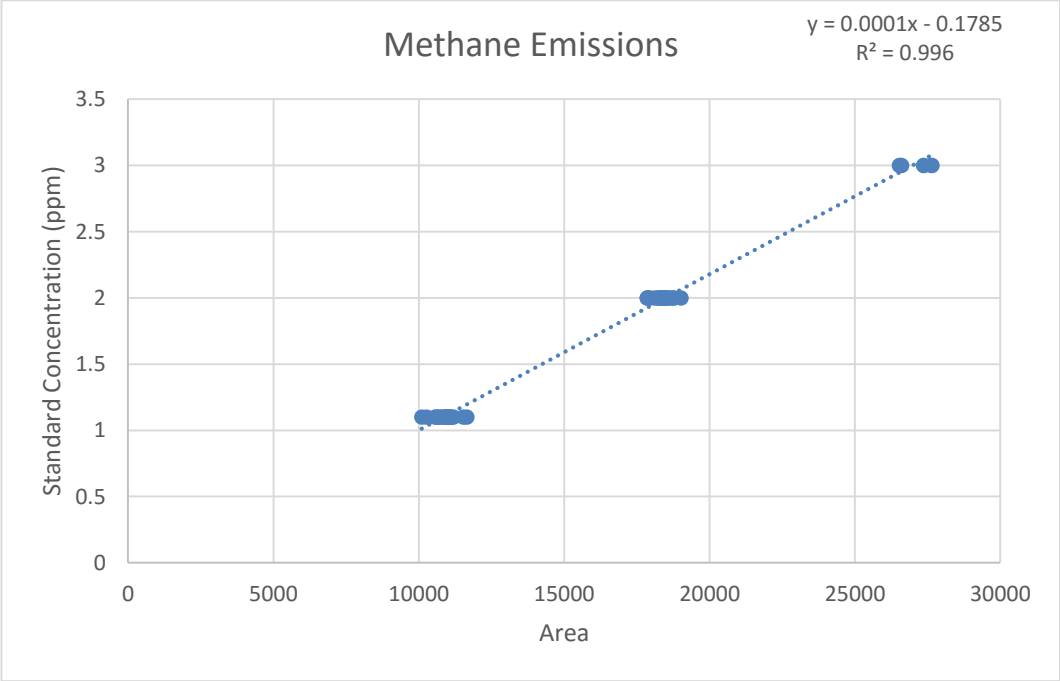
Appendix C – Mineral Nitrogen Calibration

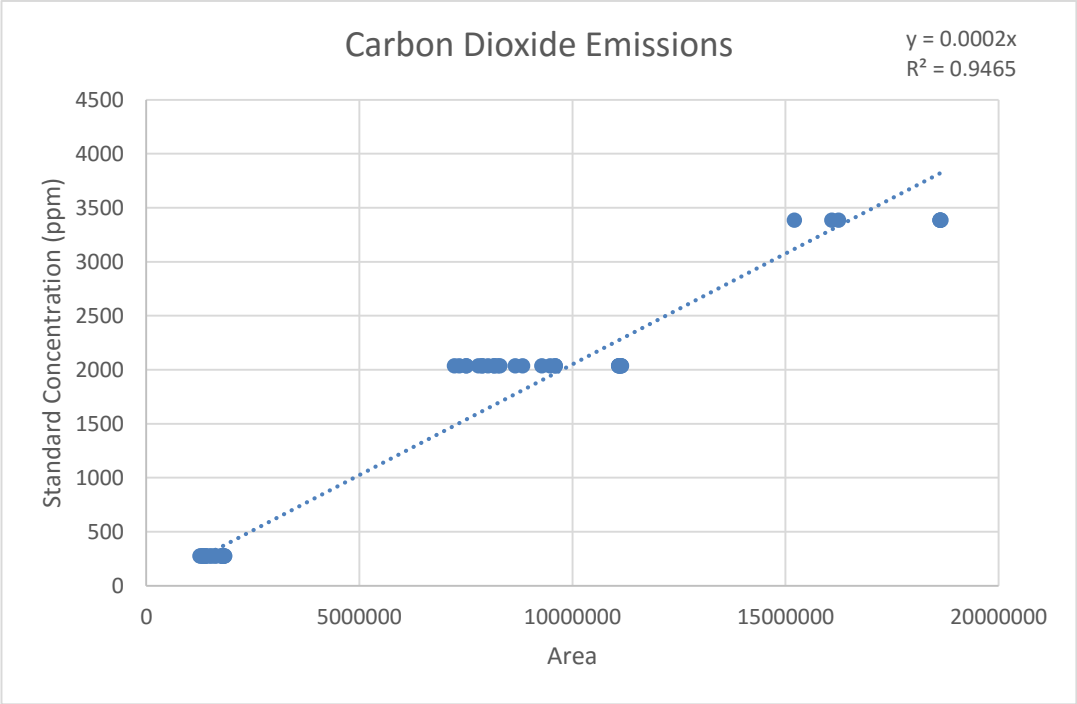




Appendix D – Gas Emission Calibrations






Carbon Dioxide Emissions
y = 0.0002x
R² = 0.9465
Standard Concentration (ppm)
4500
4000
3500
3000
2500
2000
1500
1000
500
0
0
5000000
10000000
15000000
20000000
Area

Appendix E – Genstat ANOVA Outputs

Ammonium

Accumulated analysis of variance

Change	d.f.	s.s.	m.s.	v.r.	F pr.
+ Application_Type	2	20.86940	10.43470	981.13	<.001
+ Incubation_Day	6	20.13011	3.35502	315.46	<.001
+ Application_Type.Incubation_Day	12	16.97200	1.41433	132.98	<.001
Residual	61	0.64876	0.01064		
Total	81	58.62028	0.72371		

Nitrate

Accumulated analysis of variance

Change	d.f.	s.s.	m.s.	v.r.	F pr.
+ Application_Type	2	854230.	427115.	231.34	<.001
+ Incubation_Day	6	398711.	66452.	35.99	<.001
+ Application_Type.Incubation_Day	12	397245.	33104.	17.93	<.001
Residual	61	112623.	1846.		
Total	81	1762808.	21763.		

Nitrous Oxide

Analysis of variance

Variate: Flux_g_N2O_N_m_2_h_1

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Treatment	2		661.811	330.906	47.38	<.001
Day	7		429.928	61.418	8.79	<.001
Treatment.Day	14		541.474	38.677	5.54	<.001
Residual	35	(5)	244.454	6.984		
Total	58	(5)	1779.486			

Methane

Analysis of variance

Variate: FluxCH4_C

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	2	1.9244	0.9622	1.16	0.324
Sampling_Day	8	19.7902	2.4738	2.97	0.009
Treatment.Sampling_Day	16	17.3816	1.0863	1.31	0.236
Residual	45	37.4557	0.8323		
Total	71	76.5519			

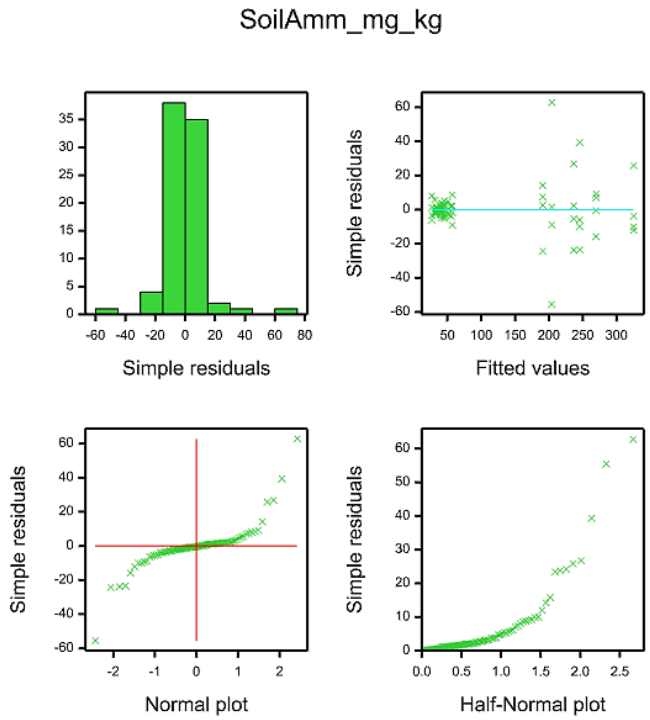
Carbon Dioxide

Analysis of variance

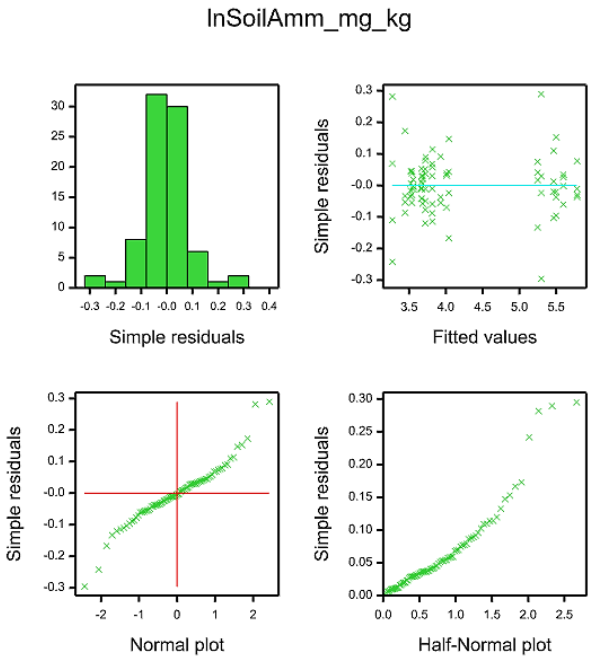
Variate: FluxCO2_C

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	2	17078098.	8539049.	6.36	0.004
Sampling_Day	8	213359783.	26669973.	19.87	<.001
Treatment.Sampling_Day	16	29149647.	1821853.	1.36	0.207
Residual	45	60398692.	1342193.		
Total	71	319986219.			

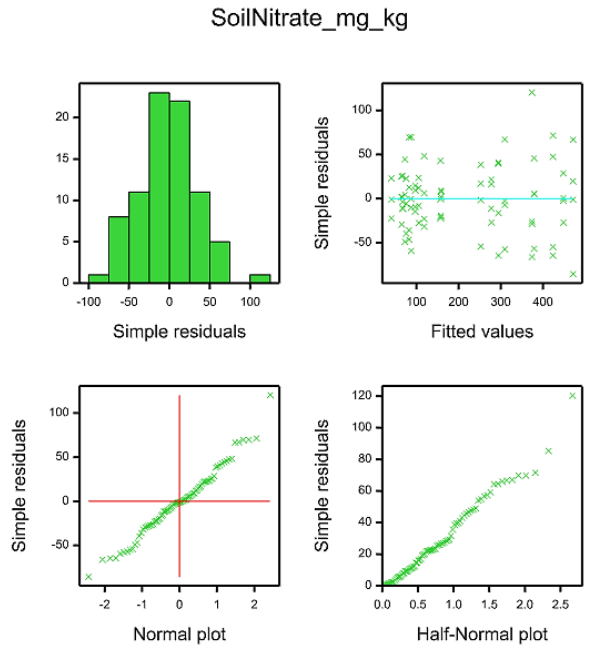
Ammonium residual output – did not meet ANOVA model assumptions.



Ammonium residual output log_e transformation – met ANOVA model assumptions.



Nitrate residual output met ANOVA model assumptions.



Appendix F – Mineral Nitrogen Raw Data

Ammonium Concentration:

Incubation Day	Application Type	Pot Number	Ammonium Concentration (mg/L)	Ammonium Concentration (mg/kg)	Ammonium Concentration (kg/ha)
0	Control	1	2.99	40.63	80.94
0	Control	2	2.76	37.58	74.86
0	Control	3	3.02	41.07	81.81
0	Control	4	2.94	40.07	79.83
0	Urea	5	3.53	48.11	95.83
0	Urea	6	4.09	55.70	110.96
0	Urea	7	3.60	48.98	97.56
0	Entec	9	3.78	51.41	102.40
0	Entec	10	4.21	57.26	114.06
0	Entec	11	4.18	56.89	113.32
3	Control	1	2.69	36.64	72.99
3	Control	2	2.91	40.85	81.37
3	Control	3	3.18	43.50	86.65
3	Control	4	3.23	44.72	89.09
3	Urea	5	16.17	222.11	442.45
3	Urea	6	17.06	239.59	477.26
3	Urea	7	20.49	284.92	567.56
3	Urea	8	16.99	235.57	469.25
3	Entec	9	19.19	267.05	531.96
3	Entec	10	10.68	148.80	296.41
3	Entec	11	13.84	195.35	389.13
3	Entec	12	15.14	205.76	409.87
7	Control	1	2.75	39.47	78.62
7	Control	2	2.77	39.46	78.61
7	Control	3	2.69	38.62	76.92
7	Control	4	2.72	38.77	77.24
7	Urea	5	22.07	315.52	628.51
7	Urea	6	22.07	313.46	624.41
7	Urea	7	22.55	321.67	640.77
7	Urea	8	25.09	351.31	699.82
7	Entec	9	16.17	231.53	461.22
7	Entec	10	16.85	239.15	476.38
7	Entec	11	18.23	263.64	525.18

7	Entec	12	15.28	213.09	424.47
14	Control	1	2.85	40.56	80.80
14	Control	2	3.00	42.85	85.35
14	Control	3	3.22	48.19	95.99
14	Control	4	3.39	50.96	101.52
14	Urea	5	19.05	269.21	536.27
14	Urea	6	17.61	254.00	505.97
14	Urea	7	19.05	279.10	555.97
14	Urea	8	19.05	276.94	551.67
14	Entec	9	1.42	20.73	41.29
14	Entec	10	2.40	35.00	69.71
14	Entec	11	1.93	28.30	56.38
14	Entec	12	1.63	23.66	47.13
30	Control	1	2.27	32.49	64.72
30	Control	2	2.36	33.32	66.38
30	Control	3	2.56	35.66	71.04
30	Control	4	2.48	35.92	71.56
30	Urea	5	11.57	166.65	331.96
30	Urea	6	13.63	198.34	395.08
30	Urea	7	13.63	193.51	385.48
30	Urea	8	13.90	205.17	408.70
30	Entec	9	3.30	48.54	96.70
30	Entec	10	3.35	46.72	93.06
30	Entec	11	3.04	44.88	89.39
30	Entec	12	2.98	41.53	82.72
45	Control	1	2.40	34.03	67.79
45	Control	2	2.49	35.36	70.45
45	Control	3	2.22	33.25	66.23
45	Control	4	2.18	32.61	64.97
45	Urea	5	3.26	48.11	95.84
45	Urea	6	3.77	55.51	110.58
45	Urea	7	4.04	59.36	118.24
45	Urea	8	4.61	65.87	131.21
45	Entec	9	2.76	39.41	78.50
45	Entec	10	2.93	42.95	85.56
45	Entec	11	2.59	38.32	76.34
45	Entec	12	3.12	45.27	90.18
60	Control	1	2.21	28.74	57.24
60	Control	2	2.38	29.70	59.16

60	Control	3	2.38	30.30	60.36
60	Control	4	2.96	37.25	74.19
60	Urea	5	2.98	37.60	74.90
60	Urea	6	2.98	37.65	74.99
60	Urea	7	3.22	41.36	82.39
60	Urea	8	3.20	40.38	80.44
60	Entec	9	2.78	35.68	71.07
60	Entec	10	2.74	34.60	68.91
60	Entec	11	2.76	34.95	69.63
60	Entec	12	2.76	34.88	69.48

Nitrate Concentration:

Incubation Day	Application Type	Pot Number	Nitrate Concentration (mg/L)	Nitrate Concentration (mg/kg)	Nitrate Concentration (kg/ha)
0	Control	1	6.48	88.16	175.62
0	Control	2	6.09	82.82	164.98
0	Control	3	7.85	106.87	212.88
0	Control	4	8.25	112.21	223.52
0	Urea	5	5.69	77.48	154.34
0	Urea	6	8.64	117.55	234.16
0	Urea	7	1.77	24.04	47.90
0	Entec	9	1.37	18.70	37.25
0	Entec	10	4.71	64.12	127.73
0	Entec	11	2.94	40.07	79.83
3	Control	1	4.71	64.22	127.92
3	Control	2	6.67	93.76	186.77
3	Control	3	4.12	56.35	112.25
3	Control	4	4.12	57.03	113.60
3	Urea	5	19.63	269.69	537.23
3	Urea	6	14.14	198.50	395.41
3	Urea	7	18.06	251.12	500.23
3	Urea	8	21.01	291.22	580.11
3	Entec	9	11.98	166.67	332.00
3	Entec	10	10.01	139.52	277.93
3	Entec	11	11.39	160.77	320.26
3	Entec	12	12.37	168.10	334.85
7	Control	1	6.87	98.68	196.58

7	Control	2	2.55	36.35	72.41
7	Control	3	6.87	98.53	196.27
7	Control	4	4.91	70.07	139.57
7	Urea	5	18.65	266.62	531.11
7	Urea	6	17.67	250.94	499.87
7	Urea	7	21.01	299.63	596.86
7	Urea	8	21.01	294.12	585.89
7	Entec	9	6.09	87.15	173.60
7	Entec	10	11.78	167.13	332.92
7	Entec	11	8.64	124.94	248.88
7	Entec	12	6.87	95.85	190.92
14	Control	1	2.55	36.28	72.28
14	Control	2	6.67	95.35	189.93
14	Control	3	10.21	152.60	303.98
14	Control	4	3.14	47.24	94.10
14	Urea	5	27.29	385.62	768.15
14	Urea	6	32.59	470.06	936.35
14	Urea	7	36.71	537.83	1071.36
14	Urea	8	33.77	490.87	977.81
14	Entec	9	9.42	137.37	273.65
14	Entec	10	13.74	200.69	399.77
14	Entec	11	9.23	135.33	269.57
14	Entec	12	10.80	156.96	312.65
30	Control	1	10.99	157.53	313.80
30	Control	2	5.50	77.50	154.38
30	Control	3	6.28	87.34	173.99
30	Control	4	1.96	28.41	56.59
30	Urea	5	29.25	421.32	839.27
30	Urea	6	32.79	477.10	950.39
30	Urea	7	31.41	445.99	888.42
30	Urea	8	30.43	449.03	894.47
30	Entec	9	22.77	335.30	667.92
30	Entec	10	16.49	229.84	457.83
30	Entec	11	18.85	278.13	554.04
30	Entec	12	23.95	334.12	665.57
45	Control	1	5.50	78.08	155.54
45	Control	2	6.87	97.52	194.26
45	Control	3	8.64	129.27	257.50
45	Control	4	7.85	117.70	234.45

45	Urea	5	33.57	495.29	986.61
45	Urea	6	32.00	471.31	938.86
45	Urea	7	25.13	368.87	734.79
45	Urea	8	25.13	359.33	715.78
45	Entec	9	24.34	347.93	693.08
45	Entec	10	23.56	345.24	687.71
45	Entec	11	33.37	494.21	984.47
45	Entec	12	21.20	307.80	613.15
60	Control	1	2.75	35.77	71.25
60	Control	2	7.26	90.58	180.43
60	Control	3	5.30	67.43	134.33
60	Control	4	5.30	66.73	132.94
60	Urea	5	24.34	307.01	611.56
60	Urea	6	29.84	376.82	750.63
60	Urea	7	19.63	252.22	502.41
60	Urea	8	23.95	302.57	602.72
60	Entec	9	30.04	384.85	766.62
60	Entec	10	30.43	384.37	765.67
60	Entec	11	33.57	424.90	846.41
60	Entec	12	25.52	322.35	642.12

Appendix G – Gas Emission Raw Data

Nitrous Oxide Emission Flux

Pot	Date Samples Taken	Fertiliser Treatment	Incubation Day	Flux (μg N_2O-N m^{-2} h^{-1})
1	28-Mar-15	Control	0	0
2	28-Mar-15	Control	0	0
5	28-Mar-15	Urea	0	0
6	28-Mar-15	Urea	0	0
7	28-Mar-15	Urea	0	0
9	28-Mar-15	Entec	0	0
10	28-Mar-15	Entec	0	0
11	28-Mar-15	Entec	0	0
1	29-Mar-15	Control	1	6.04
2	29-Mar-15	Control	1	3.64
5	29-Mar-15	Urea	1	10.61
6	29-Mar-15	Urea	1	11.42
7	29-Mar-15	Urea	1	12.44
9	29-Mar-15	Entec	1	11.03
10	29-Mar-15	Entec	1	7.44
11	29-Mar-15	Entec	1	1.55
1	30-Mar-15	Control	2	1.07
2	30-Mar-15	Control	2	0.68
5	30-Mar-15	Urea	2	9.49
6	30-Mar-15	Urea	2	6.89
7	30-Mar-15	Urea	2	9.97
9	30-Mar-15	Entec	2	5.29
10	30-Mar-15	Entec	2	7.20
11	30-Mar-15	Entec	2	6.10
1	31-Mar-15	Control	3	2.72
2	31-Mar-15	Control	3	1.43
5	31-Mar-15	Urea	3	10.26
6	31-Mar-15	Urea	3	14.16
7	31-Mar-15	Urea	3	9.97
9	31-Mar-15	Entec	3	
10	31-Mar-15	Entec	3	1.99
11	31-Mar-15	Entec	3	3.37
1	1-Apr-15	Control	4	2.09
2	1-Apr-15	Control	4	4.69

5	1-Apr-15	Urea	4	21.95
6	1-Apr-15	Urea	4	26.25
7	1-Apr-15	Urea	4	18.68
9	1-Apr-15	Entec	4	3.91
10	1-Apr-15	Entec	4	7.95
11	1-Apr-15	Entec	4	5.29
1	2-Apr-15	Control	5	
2	2-Apr-15	Control	5	0.63
5	2-Apr-15	Urea	5	13.07
6	2-Apr-15	Urea	5	16.15
7	2-Apr-15	Urea	5	11.07
9	2-Apr-15	Entec	5	0.67
10	2-Apr-15	Entec	5	
11	2-Apr-15	Entec	5	1.42
1	14-Apr-15	Control	17	2.17
2	14-Apr-15	Control	17	2.61
5	14-Apr-15	Urea	17	4.64
6	14-Apr-15	Urea	17	0.74
7	14-Apr-15	Urea	17	3.85
9	14-Apr-15	Entec	17	2.55
10	14-Apr-15	Entec	17	5.24
11	14-Apr-15	Entec	17	4.10
1	13-May-15	Control	46	8.84
2	13-May-15	Control	46	1.61
5	13-May-15	Urea	46	11.27
6	13-May-15	Urea	46	6.53
7	13-May-15	Urea	46	6.34
9	13-May-15	Entec	46	11.41
10	13-May-15	Entec	46	7.34
11	13-May-15	Entec	46	9.96
1	27-May-15	Control	60	-2.90
2	27-May-15	Control	60	4.89
5	27-May-15	Urea	60	
6	27-May-15	Urea	60	0.65
7	27-May-15	Urea	60	7.15
9	27-May-15	Entec	60	4.60
10	27-May-15	Entec	60	
11	27-May-15	Entec	60	1.14

Methane Emission Flux

Pot	Date Samples Taken	Fertiliser Treatment	Incubation Day	Flux (μg CH_4 - C m^{-2} h^{-1})
1	28-Mar-15	Control	0	0.609
2	28-Mar-15	Control	0	0.792
5	28-Mar-15	Urea	0	1.186
6	28-Mar-15	Urea	0	1.512
7	28-Mar-15	Urea	0	0.173
9	28-Mar-15	Entec	0	0.453
10	28-Mar-15	Entec	0	0.673
11	28-Mar-15	Entec	0	1.004
1	29-Mar-15	Control	1	1.101
2	29-Mar-15	Control	1	0.944
5	29-Mar-15	Urea	1	1.339
6	29-Mar-15	Urea	1	0.543
7	29-Mar-15	Urea	1	0.214
9	29-Mar-15	Entec	1	1.731
10	29-Mar-15	Entec	1	2.230
11	29-Mar-15	Entec	1	0.880
1	30-Mar-15	Control	2	-0.833
2	30-Mar-15	Control	2	0.264
5	30-Mar-15	Urea	2	0.070
6	30-Mar-15	Urea	2	-0.208
7	30-Mar-15	Urea	2	-0.045
9	30-Mar-15	Entec	2	0.773
10	30-Mar-15	Entec	2	-0.446
11	30-Mar-15	Entec	2	-0.301
1	31-Mar-15	Control	3	1.164
2	31-Mar-15	Control	3	0.370
5	31-Mar-15	Urea	3	0.057
6	31-Mar-15	Urea	3	1.479
7	31-Mar-15	Urea	3	-0.199
9	31-Mar-15	Entec	3	2.176
10	31-Mar-15	Entec	3	-0.977
11	31-Mar-15	Entec	3	1.077
1	1-Apr-15	Control	4	-0.742
2	1-Apr-15	Control	4	-0.448
5	1-Apr-15	Urea	4	0.153
6	1-Apr-15	Urea	4	1.813

7	1-Apr-15	Urea	4	-1.318
9	1-Apr-15	Entec	4	-0.411
10	1-Apr-15	Entec	4	-0.326
11	1-Apr-15	Entec	4	0.642
1	2-Apr-15	Control	5	-1.052
2	2-Apr-15	Control	5	0.405
5	2-Apr-15	Urea	5	-0.092
6	2-Apr-15	Urea	5	-1.275
7	2-Apr-15	Urea	5	-1.678
9	2-Apr-15	Entec	5	0.006
10	2-Apr-15	Entec	5	2.374
11	2-Apr-15	Entec	5	0.637
1	14-Apr-15	Control	17	-1.283
2	14-Apr-15	Control	17	-0.430
5	14-Apr-15	Urea	17	-0.585
6	14-Apr-15	Urea	17	0.100
7	14-Apr-15	Urea	17	0.278
9	14-Apr-15	Entec	17	1.036
10	14-Apr-15	Entec	17	-0.349
11	14-Apr-15	Entec	17	2.629
1	13-May-15	Control	46	1.306
2	13-May-15	Control	46	1.202
5	13-May-15	Urea	46	-0.019
6	13-May-15	Urea	46	0.170
7	13-May-15	Urea	46	2.872
9	13-May-15	Entec	46	-0.592
10	13-May-15	Entec	46	-0.215
11	13-May-15	Entec	46	-0.901
1	27-May-15	Control	60	-0.300
2	27-May-15	Control	60	-1.347
5	27-May-15	Urea	60	-1.343
6	27-May-15	Urea	60	-0.566
7	27-May-15	Urea	60	-0.572
9	27-May-15	Entec	60	-1.303
10	27-May-15	Entec	60	-1.056
11	27-May-15	Entec	60	1.066

Carbon Dioxide Emission Flux

Pot	Date Samples Taken	Fertiliser Treatment	Incubation Day	Flux (μg CO_2 - C m^{-2} h^{-1})
1	28-Mar-15	Control	0	-1258.618
2	28-Mar-15	Control	0	-876.450
5	28-Mar-15	Urea	0	132.792
6	28-Mar-15	Urea	0	655.023
7	28-Mar-15	Urea	0	1822.310
9	28-Mar-15	Entec	0	-417.956
10	28-Mar-15	Entec	0	-276.543
11	28-Mar-15	Entec	0	365.353
1	29-Mar-15	Control	1	2044.093
2	29-Mar-15	Control	1	2473.913
5	29-Mar-15	Urea	1	3582.813
6	29-Mar-15	Urea	1	3221.221
7	29-Mar-15	Urea	1	3056.162
9	29-Mar-15	Entec	1	3748.763
10	29-Mar-15	Entec	1	3268.025
11	29-Mar-15	Entec	1	2288.770
1	30-Mar-15	Control	2	-4582.868
2	30-Mar-15	Control	2	-2152.954
5	30-Mar-15	Urea	2	-2427.150
6	30-Mar-15	Urea	2	-637.926
7	30-Mar-15	Urea	2	-118.913
9	30-Mar-15	Entec	2	-1670.780
10	30-Mar-15	Entec	2	131.347
11	30-Mar-15	Entec	2	-1644.704
1	31-Mar-15	Control	3	359.127
2	31-Mar-15	Control	3	616.824
5	31-Mar-15	Urea	3	1276.286
6	31-Mar-15	Urea	3	2122.099
7	31-Mar-15	Urea	3	1049.502
9	31-Mar-15	Entec	3	2958.946
10	31-Mar-15	Entec	3	1218.939
11	31-Mar-15	Entec	3	2195.480
1	1-Apr-15	Control	4	949.864
2	1-Apr-15	Control	4	1676.982
5	1-Apr-15	Urea	4	4807.961
6	1-Apr-15	Urea	4	1132.918

7	1-Apr-15	Urea	4	3944.548
9	1-Apr-15	Entec	4	3278.114
10	1-Apr-15	Entec	4	3018.640
11	1-Apr-15	Entec	4	2517.821
1	2-Apr-15	Control	5	-909.855
2	2-Apr-15	Control	5	-3082.102
5	2-Apr-15	Urea	5	-951.367
6	2-Apr-15	Urea	5	-1179.799
7	2-Apr-15	Urea	5	-2995.893
9	2-Apr-15	Entec	5	1370.927
10	2-Apr-15	Entec	5	-2278.229
11	2-Apr-15	Entec	5	-574.996
1	14-Apr-15	Control	17	-3948.950
2	14-Apr-15	Control	17	-2073.532
5	14-Apr-15	Urea	17	-751.666
6	14-Apr-15	Urea	17	-1327.749
7	14-Apr-15	Urea	17	-87.530
9	14-Apr-15	Entec	17	115.649
10	14-Apr-15	Entec	17	252.243
11	14-Apr-15	Entec	17	-750.860
1	13-May-15	Control	46	-422.926
2	13-May-15	Control	46	274.382
5	13-May-15	Urea	46	-902.563
6	13-May-15	Urea	46	-1388.896
7	13-May-15	Urea	46	-911.443
9	13-May-15	Entec	46	127.968
10	13-May-15	Entec	46	72.420
11	13-May-15	Entec	46	863.271
1	27-May-15	Control	60	321.126
2	27-May-15	Control	60	-1413.197
5	27-May-15	Urea	60	-114.978
6	27-May-15	Urea	60	-3686.654
7	27-May-15	Urea	60	-4787.886
9	27-May-15	Entec	60	-3754.770
10	27-May-15	Entec	60	-1326.742
11	27-May-15	Entec	60	732.158