Immobilization of Nitrogen Fertilizer in Residue-Retained Mediterranean Semi-Arid Cropping Systems

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Table of contents

List of tables

List of figures

Abstract

Nitrogen (N) is the most limiting nutrient for crop production. Synthetic N fertilizer is required to produce enough food to feed the current global population, and human reliance on N fertilizer will increase with future population growth. Production of N fertilizer requires fossil fuels, and once applied to croplands can be lost from the soil-plant system with environmentally harmful offsite effects. Therefore, more efficient use of fertilizer N which seeks to minimise losses will be critical for sustainable achievement of global food production goals. This thesis focused on efficiency of fertilizer N applied to dryland crops in the semi-arid Mediterranean cropping systems of south-eastern Australia. Most grain growers in this region retain crop residues to protect soils from wind and water erosion. Residues with low carbon (C) to N (C:N) ratio such as those of wheat (*Triticum aestivum*) cause N-immobilization by soil microorganisms which can negatively affect crop growth, particularly if it occurs at times of high crop N demand. A simulation experiment quantified sources of N inefficiency across a rainfall gradient within these cropping systems and predicted N immobilization to be the largest source of inefficiency at N application rates currently used by growers. A pot experiment and two field experiments were used to investigate management factors to reduce immobilization and increase crop access to N in the presence of retained crop residues. A comparison of crop species (wheat vs. canola, *Brassica napus*) found that canola could accumulate more N, but that was due to longer life cycle rather than suppression of immobilizing soil organisms. Timing of N application (sowing vs. mid-season during early stem-elongation) interacted with species; applications at sowing increased N uptake in canola and mid-season increased N uptake in wheat. In-crop mid-row banding improved crop recovery of ^{15}N-labelled fertilizer in both species compared to broadcast application.

Statement of authorship

This thesis includes work by the author that has been published as described in the text.

Except where reference is made in the text of the thesis, this thesis contains no other material published elsewhere or extracted in whole or in part from a thesis or any other degree or diploma.

No other person's work has been used without due acknowledgment in the main text of the thesis.

This thesis has not been submitted for the award of any degree or diploma in any other tertiary institution.

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Chapter 1. Literature Review. Immobilization of nitrogen fertilizer in residue-retained Mediterranean semi-arid cropping systems

1.1 Introduction

Nitrogen (N) is an essential macro-nutrient for plant growth and a key agricultural input that has been increasingly used in food production since the invention of the Haber-Bosch process to reduce atmospheric N_2 gas to reactive forms of N (Lemaire et al., 2018a) which is usable by plants. Nitrogen is required for plant function and is a key component of amino acids, which form the building blocks of plant proteins and enzymes (Novoa and Loomis, 1981). It consists of 1-5% of total plant dry material and contributes up to 80% of total nutrients absorbed by plant roots (Mengel and Kirkby, 2012). Among all nutrients, N is the most important growth-limiting factor. The N demand for agricultural production of plants is increasing because of increasing human population (Erisman et al., 2013). As a consequence, agriculture now has a great influence on the global N cycle (Erisman et al., 2011).

Most of the N in surface soil is present as organic N and the inorganic (mineral N) in the soil is a small fraction of the total soil N (Hofman et al., 2001). The principal forms of mineral N in the soil which are usable by plants are ammonium (NH_4^+), and nitrate (NO_3^-). Within the soil, N cycles continuously from organic to inorganic forms and vice versa. Nitrogen mineralization is the production of inorganic N from organic N and N immobilization is the incorporation of inorganic N into organic forms. Soil microorganisms are responsible for both of these processes (Jamir et al., 2019). Nitrogen cycling can be modified by several factors affecting soil biological activity which influence N transformation rates (Hassink, 1992).

Nitrogen availability to crop plants can be lower because of immobilization and possible environmental losses including volatilization, denitrification and leaching. Nitrogen leaching occurs from cropping soils when water drains through the soil beyond the root zone carrying dissolved NO_3^- with it (Aulakh and Malhi, 2005). This is influenced by the amount and pattern of rainfall relative to evapotranspiration, soil texture and the extent of the crop rooting zone. Excessive N fertilization causes accumulation of mineral N in soil, largely in the form of NO_3^-. High nitrification potential and low NO_3^- immobilization are responsible for NO_3^- accumulation in agricultural soils (Zhang et al., 2013). Nitrate leaching from agricultural land is both economically and environmentally undesirable (Quan et al., 2016) and the associated contamination of ground and surface water is a major environmental concern in many countries (Yu et al., 2019).

Denitrification is an anaerobic respiratory process performed by many bacterial species (van Spanning et al. 2007). This process describes the consecutive reduction of NO_3^- to nitrite (NO_2^-), nitric oxide (NO),

nitrous oxide (N_2O) and nitrogen (N_2) gaseous forms (Van Spanning et al., 2005) which represents a loss of N from the soil-plant system. Application of N fertilizer can promote denitrification due to increasing soil NO_3^- concentrations. It is the major biological process that returns fixed N to the atmosphere (Philippot et al., 2009) and the major source of atmospheric N_2O, an important greenhouse gas that also consumes stratospheric ozone (Robertson and Groffman, 2007). Heavy rainfall after mineralization can provide conditions that favour denitrification and it is favoured by heavy textured soils and at warmer temperatures conducive to bacterial growth (Pu et al., 2001).

Ammonia volatilization is a chemical process that occurs at the soil surface when ammonium (NH_4^+) is converted to ammonia gas (NH_3) at high pH. Soil pH surrounding fertilizer granules is increased by the hydrolysis of urea. This process increases the risk of volatilization (Chien et al., 2009). The formation of gaseous NH_3 in soil with lack of plant growth and water can lead to considerable losses of N from agricultural land (Sigunga et al., 2002). Surface application of non-nitrate based nitrogen fertilizer increases the risk of ammonia volatilization (Rowlings et al., 2016). As most of the cropping areas in southern Australia are non-irrigated, farmers are dependent on rainfall to move surface-applied fertilizers into the soil where these are safe from being volatilized (Fenn and Miyamoto, 1981). Field experiments have shown that when urea is applied to Australian cropping systems during winter, volatilization losses are low (~5% of fertilizer applied) (Turner et al., 2012; Schwenke et al., 2014).

Nitrogen immobilization is defined as the transformation of inorganic N forms such as NO_3^-, nitrite, ammonia and NH_4^+ into organic forms which are not available to plants (Zagal and Persson, 1994). Many factors affect N mineralization and immobilization rates, and these include soil water content, crop residue amount and type, temperature, soil organic matter, soil nutrient availability and soil microbial C and N (Schmidt et al., 1999). Microbes are responsible for breaking down organic compounds into inorganic forms which provides microbes with a C and N source for respiration and growth. Cereal crop residues contain more C than N and have high C:N ratio (typically 80:1). Thus, they provide a good source of energy (C) for soil microorganisms, but when microorganisms start decomposing residues, they require mineral N to sustain growth. The N contained in high C:N ratio residue is not sufficient to meet demand, and microbes will immobilize mineral N from the soil. This can include mineral N derived from applied N fertilizer. This immobilization can lead to crop N deficiency, especially early in the growing season when plants are not big enough to compete with microbes, and this has been observed to depress crop yield in some circumstances (Beri et al., 1995; Ichir and Ismaili, 2002). As a result of the simultaneous immobilization and mineralization processes, it is important to make a difference between gross and net mineralization and immobilization. Net N mineralization is the balance between the gross mineralization and immobilization

which is the total amount of soluble N produced by microorganisms or consumed respectively (Robertson and Groffman, 2007). Inorganic N in the soil increases when gross mineralization exceeds gross immobilization and decrease when gross immobilization exceeds gross mineralization.

Residue with high C:N ratio increases the competition between soil microbes and crops for inorganic N. It is generally accepted that residues with high C:N ratio such as cereals (~80:1) decompose more slowly than the residues with low C:N ratio (Parr and Papendick, 1978; Moritsuka et al., 2004). Microbial lifecycles are not as long as crops, thus throughout the lifecycle of a crop there are numerous concurrent cycles of N immobilization and mineralization or "turn-over". The temporal advantage of plants over microorganisms in attaining N is due to differences in life/death cycle rates, and the capacity of plants to capitalise on mineralized nutrients (Kuzyakov and Xu, 2013). N mineralization is the process by which organic N in humus or plant residues is converted to plant available inorganic forms. Mineral N accumulates in the soil only when the amounts of N released from the organic residues exceed microbial growth requirements.

Nowadays, agriculture uses 100 Mt of fertilizer N via the Haber-Bosch process (Erisman et al., 2008) which consumes a large amount of fossil fuels and produces an enormous amount of CO_2. About half of the fertilizer-N applied to fields is used by crops in year of application, while the rest either carries over in mineral or organic forms for use by subsequent crops or is lost to the environment (Udvardi et al., 2015).

1.2 Australian grain production systems

Australian grain production is characterized by the predominant use of winter cereals (wheat *Triticum aestivum*, barley *Hordeum vulgare* and oats *Avena sativa*) produced across a wide area in a number of distinct agroecological zones with differing climate, soil characteristics and farming systems (Angus and Good, 2004). Australian grain growing regions have semi-arid or subhumid climates and almost all crops are rainfed (French and Schultz, 1984); winters are mostly mild and there is no snow. The mild winter temperatures means that crops can be sown as early as February until July although almost all crops are sown from late April through to June. The lowest mean daily temperatures vary from 8 ˚C in the south to 15 ˚C in the northeast and by the time of wheat flowering, temperatures range from 12˚C in the south to 18 ˚C in the northeast and by maturity from 18 ˚C to 23˚C (Angus and Good, 2004).

Australian rainfall is highly variable due to the effects of various large-scale climate systems (Sturman and Tapper, 1996) which manifest not only in the distribution of rainfall but also in its seasonality (Singh and Luly, 1991). In the north, almost all rainfall is concentrated in the summer but most participation falls during winter in southern parts (Fletcher and Moreno, 2012). The central part of the continent is outstandingly dry with large parts receiving less than 200 mm of rainfall annually (Nicholls et al., 1997).

1.2.1 Nitrogen use in Australian grain production systems

Soil organic carbon (SOC) is essential for biological, chemical, and physical processes which offers crucial information on changes in soil fertility and land degradation (Wang et al., 2018). Soil organic carbon levels are often high in a soil that have not been altered through erosion or grain removal. However, in commercial agriculture, much of the created dry matter is removed or burned, which may cause erosion and speed up losses (Rasmussen and Collins, 1991). Most agricultural soils in Australia have already reached extremely low levels of (SOC). In Australia, intensive farming and horticulture have reduced (SOC) levels from an estimated 3% to less than 1% (Joseph et al., 2007).

The only source of N for the first century of cereal crop production in Australia was from mining of organic soil N through continuous cropping then through fallow-crop sequences (Donald, 1965). In the second half of the 20th century, declining levels of soil N were replenished by biological N_2-fixation by legumes in improved pastures that were grown in sequence with cereal crops (Henzell, 2007). Soil total N was maintained at a steady state when about half of the farm areas contained legume-based pastures (Angus and Peoples, 2012). Most of Australia's wheat crop was grown in rotation with legume pastures in that time, and the perception that legumes could supply all of the N for following wheat crops had been a major factor in reducing the use of N fertilizer in Australian wheat production (McDonald, 1989). Therefore, historically little N fertilizer had been applied to the major dryland crops in southern Australia (Angus and Grace, 2017). Before the 1990s, the number of sheep and the wheat area in Australia was relatively stable, but from the 1990s up to the present sheep numbers declined rapidly and wheat area increased (Hunt et al., 2021). Consequently, the N supplied to crops by pastures through biological N_2-fixation declined and in order for yields to be maintained N supply had to be provided by fertilizers. Whilst the growth of fertilizer N usage in Australia was slow compared to the rest of the world before the mid-1990s due to N inputs from legume pastures, for the rest of that decade there was a rapid increase in N-fertilizer use, mostly as inputs to wheat and other dry-land grain crops (Angus and Grace, 2017).

With an ongoing shift to continuous cropping, grain production and maintenance of soil organic matter are increasingly reliant on applications of N fertilizer (Angus and Grace, 2017) and many crop yields are N-limited (Hochman and Horan, 2018; Lawes et al., 2021). Fertilizer N applications on most farms are currently lower than N offtake in grain, meaning that soil organic N is being mined (Norton, 2016). Due to N-mining by continuous cropping and in order to achieve crops water-limited potential yield, the current application rate of N fertilizer which is ~45 kg N ha^{-1} need to be doubled in the next few decades (Sadras and Angus, 2006). Hochman and Horan (2018) estimate that 40% of the substantial gap between farm yield and water-limited potential yield that exists in Australia is due to N deficiency, indicating that higher

applications are required to achieve potential yields and preserve soil organic matter. Cereals such as rice, maize and wheat use only 50% or less of the applied N for producing above ground biomass in the year of application (Krupnik et al., 2004). Average uptake of fertilizer N in the year of application under Australian conditions is 40% (Angus and Grace, 2017). Following fertilizer N application, N is incorporated into the soil mineral N pool (NH_4^+ and NO_3^-), where some is incorporated into microbial biomass, some taken up by plants and some lost to the environment via loss pathways described above. One of the main reasons for the poor efficiency of fertilizer N use is that much of the N can be lost from the soil-plant system (Ladha et al., 2005). So, better understanding of the inefficiencies associated with nitrogen fertilizer application is essential to increase nitrogen-use-efficiency and yield.

1.3 Defining NUE

The efficiency with which N is used for crop production can be defined and measured in different ways that have been well described by (Good et al., 2004) who consider both yield and grain protein responses to fertilizer N (Fischer, 1993). The numerator in most of definitions is either total aboveground shoot dry weight (SDW) or grain yield, but the denominator varies to suit the purpose and context of specific studies. NUE was defined by (Steenbjerg and Jakobsen, 1963) as SDW divided by N content of shoots (Nshoot).

A more useful definition for this thesis is provided by (Moll et al., 1982) as grain yield (GY) per unit of the total available N to the crop (Ntot).

$$NUE = \frac{GY}{Ntot}$$

Ntot is comprised of mineral N available in the soil at planting (Nsoil), N fertilizer added to the crop (Nfert), and N that mineralizes out of the soil organic pool during crop growth (Nmin).

$$Ntot = Nsoil + Nfert + Nmin$$

NUE by this definition then comprises two key components: N uptake efficiency (NUpE), which is defined as the amount of Ntot taken up by the crop, and N utilization efficiency (NUtE), which is the efficiency of assimilation or remobilization of Nshoot to produce grain (Good et al., 2004; Dobermann, 2005; Foulkes et al., 2009; Gastal et al., 2015; Han et al., 2015). Another calculation for NUE is system efficiency (NUEsys) which can be calculated as total N uptake by crop divided by total N available in the soil profile (Delgado and Shaffer, 2008). A broad measure of the efficiency of grain production in relation to the N fertilizer applications is partial factor productivity (PFP) of N which is calculated as the grain yield divided by fertilizer N applied.

$$PFP\ NUE = \frac{\text{Grain Y}}{\text{N rate}}$$

Although definitions of NUE are well established in different sources, its estimation is more complex (Bouchet et al., 2016). NUE is separated to different component indicators by agronomists (Novoa and Loomis, 1981). Whilst Nsoil and Nfert are easily estimated via relatively robust methods, Nmin is difficult to estimate under field conditions (Dunsford et al., 2015).

1.4 Crop residue (stubble) management

The most abundant form of plant biomass for microbial decomposition in cropping systems are crop residues (stubble, stover), the non-grain biomass which remains on the surface after a grain crop is harvested (Tisdale et al., 1985). Retaining crop residue on the soil surface or incorporating into the soil are associated with different tillage practices which has a significant effect on the soil physical and biological environment. No-tillage (NT) is a part of conservation agriculture (CA) which improves soil ecosystems compared with conventional tillage farming system by extreme reduction of soil disturbance combined with residue retention (Hobbs et al., 2008).

Addition of crop residue can influence the availability of N to the crop. For instance, legume residue with low C:N ratio (~25:1) can result in net N mineralization, whereas cereal residue which has high C:N (~80:1) can immobilize N during the decomposition process (Govaerts et al., 2006). Generally, the benefits of residue retention such as increased topsoil aggregation and protection from erosion and soil loss offset any negative effects due to N immobilization. Conservation agriculture (CA) has been an important recent part of the evolution of Australian grain growing (Llewellyn and Ouzman, 2019). Since the 1970s, management of arable land has been transformed in Australia when crop residues were burnt and fields were cultivated many times before planting (Cornish et al., 2020). Adoption of no-till cropping system in Australia has promoted the intensification of crop production due to reduced level of soil disturbance during tillage operations which reduces the labour and machine hours required for crop production (Mc Tainsh, 2001; Idol, 2015). In Australia, unexpectedly high herbicide resistance risks create a unique adoption environment for reduced tillage cropping systems (Llewellyn et al., 2002). Today, a significant majority of Australian farmers grow their crops using CA principles (Friedrich et al., 2009; Llewellyn et al., 2012). These principles include minimum or zero tillage, partial or full retention of crop residues to maintain soil cover (Friedrich et al., 2012) which has been a major transformation in Australian rainfed agriculture over the past 40-50 years (Thomas et al., 2007).

Since 1990, most of the Australia's wheat has been produced in NT farming systems with all crop residues (stubble) retained (Llewellyn et al., 2012). Crop residues including cereals affect biological fertility of soil whether they are incorporated or retained on the soil surface due to their high C:N ratio (Smith et al., 1992). The addition of crop residues is expected to stimulate microbial activity (Doran, 1980; Kirkegaard et al., 2008) and it has been reported to have a negative effect on N availability (Taylor et al., 1989). Cayuela et al. (2009) found that addition of wheat residue led to a rapid immobilization of N that affected microbial biomass size and activity and subsequent N mineralization. Researchers have reported the effects of crop residues on N mineralization dynamics (Janssen, 1996; Trinsoutrot et al., 2000).

The addition of residue with C:N ratio greater than 30:1 can result in net N immobilization (Sims, 1990). The problem is not just with residue incorporation, it also occurs in surface-retained and standing residue systems (Kirkegaard et al., 2018). Field results suggest immobilization rates of 5-13 kg N ha^{-1} per t ha^{-1} of wheat residue (Mary et al., 1996) although Angus et al. (2020a) reported that where residue was burnt about 90% of N in the residue is lost. Generally, field crops remove 70% to 80% of their N requirements from the soil during the vegetative growth stage, so little crop N is sourced directly from residue breakdown (Gupta, 2016; Kirkegaard et al., 2018). Therefore, yields will be negatively affected if not enough N is available during this growing stage (Cassman et al., 1997). Immobilization during crop growth reduces the synchrony between soil mineral N release and crop uptake (Singh et al., 2007). Kissel et al. (1977) also concluded that N immobilization was an important mechanism in reducing the crop available NO_3^- by the end of the cropping season. This author also mentioned that N immobilization became significant when soil surface temperature increased. Bird et al. (2001) found that grain fertilizer N recovery decreased when residue was retained compared with burning residue due to immobilization.

1.4.1 Nutrient availability

The decomposition of crop residue can provide an important source of N to the plants and other organisms (Grzyb et al., 2020). Amino acids and amino sugars are identified as an organic compound in soil which are readily mineralized under aerobic conditions (Stevenson, 1982). During mineralization, under microbial activity, organic N changes to amino acids first then converts into NH_4^+. This mineralized NH_4^+ can be immobilized or nitrified to NO_3^- and utilised by other microorganisms as N or an energy source (Bolan and Hedley, 2003).

Releasing crop available N can be affected by the method of residue application. Compared to surface retention, the incorporation of residues into the soil accelerates the rate of decomposition which increases the N availability to crops (Yadvinder et al., 2005; Sarker et al., 2019). The retention of crop residues may

lead to reduced fertilizer requirements over the long-term depending on the quality and quantity of residue (Page et al., 2013; Sahu et al., 2015). For example, Kirkegaard et al. (2018) found that wheat residue provides only 1-6% of the N requirements of the subsequent crop due to its high C:N ratio. Furthermore, where high C:N ratio of residue applied or retained, N immobilization can occur in the short term at the time of higher crop N demand. Therefore, in order to meet the crop demand additional fertilizer N will be required to offset this temporary N deficiency. Kirkegaard et al. (2018) recommended that application of more N (5 kg N per t ha^{-1} of cereal residue) in residue retained systems is essential to avoid impacts of N immobilization on crop yield.

While many studies have been conducted on N cycling response to crop residue and tillage, there is a gap between these short-term mechanistic studies and longer-term field-based research. Thus, there is a need for longer-term field-based studies to better quantify long term implications of N cycling, rate of N release from crop residue and the pathways of N loss to help farmers and researchers make more informed decisions about fertilizer N application and maximise NUE.

1.5 Crop model and farming system management

As with crop production systems globally, increasing crop yields in south eastern Australia will require increasing inputs of fertilizer N (Hochman and Horan, 2018). An increase in fertilizer NUE is required to minimise the environmentally harmful effects of this intensification (Angus and Grace, 2017). Therefore, a knowledge of the N inefficiency mechanisms operating in crop production in south eastern Australia is necessary to develop strategies to reduce inefficiency sources and maximise crop yield and NUE (Bacon and Freney, 1989). To achieve this, quantification and understanding of the relative importance of the different sources of N fertilizer inefficiency are required. Most field studies to-date that have tried to achieve this have used ^{15}N-labelled fertilizer to quantify losses. The use of N fertilizer labelled with the ^{15}N isotope allows the fate of applied N to be studied in detail, with the difference between fertilizer applied and that recovered in the plant or remaining in the soil after harvest assumed to be lost to the environment. Angus and Grace (2017) summarised Australian studies that used isotope labelled ^{15}N fertilizer to track the fate of fertilizer N applied to grain crops. Averaged across all studies, 41% of the applied fertilizer was in aboveground crop tissue, 34% in soil and 22% was not recovered and is assumed lost by N inefficiency sources such as denitrification and leaching (Angus and Grace, 2017).

Most of the studies were conducted prior to the widespread adoption of residue retention (Llewellyn et al., 2012) and recent changes in rainfall pattern (Pook et al., 2009; Cai et al., 2012) and at higher rainfall sites not representative of the low-rainfall environment that comprise the majority of the Australian cropping

belt. Cropping systems models which are able to simulate crop growth and soil N processes accurately can be a useful tool to understand and improve management in farming systems (McCrown et al., 1996). Simulation allows simultaneous evaluation of all sources of inefficiency, conducted over many sites and seasons, and imposition of experimental treatments to assess the impact on NUE.

1.6 Factors that influence nitrogen immobilization

1.6.1 Timing of nitrogen application

Fertilizer applied at sowing is more prone to losses and immobilization than when it is applied before the time of high crop N demand (Hyytiäinen et al., 2011). When N fertilizer is applied at sowing, microbes have ready access to mineral N without competition from juvenile plants and NO_3^- is susceptible to immobilization and loss. Applying fertilizer N at early stem-elongation just before the period of most rapid crop uptake allows plants to compete more effectively with microbes, and minimises the amount and duration of NO_3^- in the soil susceptible to loss. Harris et al. (2016) found that N applied at the beginning of stem-elongation was more efficient than N applied at sowing. Recous and Machet (1999) found that wheat was better at competing with microorganisms for the available N during stem-elongation than tillering and after anthesis which was not always due to large amount of fertilizer being immobilized in the soil but losses.

1.6.2 Crop species

Nitrification is a key step in the global N cycle which converts NH_4^+ to NO_3^- and is considered as the first step in several pathways that lead to losses of N from agricultural systems. Some plant species have different abilities to affect microbial dynamics in the soil by releasing chemicals from their roots that influence functional populations of soil microorganisms such as ammonia oxidisers thereby decreasing nitrification rates (O'Sullivan et al., 2016). There is evidence that crop species such as canola differ in their ability to compete with microbes for soil N (Ryan et al., 2006). O'Sullivan et al. (2016) showed that wheat required less N fertilizer when grown after canola compared to after pasture, and that canola reduced nitrification and increased immobilization and remobilisation rates of N in the rhizosphere compared to wheat. These effects from canola on the rhizosphere biology may provide benefits to crop production if immobilized fertilizer N is remobilised and acquired by plants.

1.6.3 Nitrogen placement

Urea-based fertilizers can be broadcast on the soil surface, mid-row banded (MRB), deep banded or surface banded. Broadcast application of N is most subject to immobilization in the presence of high C:N ratio residues because it increases the contact between the fertilizer N and soil microorganism (Nyborg and Malhi, 1989). The effectiveness of nitrogen fertilizer use should be improved by putting nitrogen fertilizers in a

particular area in the soil. Leaching of nitrate will be limited when there is a high concentration of roots in the region where nitrate is generated (Passioura and Wetselaar, 1972). Wetselaar et al. (1972) demonstrated that if a certain amount of fertilizer is applied to a specific area of soil, the rate of nitrate generation per unit area can be regulated by banding the fertilizer and altering the band spacing.

Mid-row banding has the advantage of delaying nitrification of fertilizer by suppressing nitrifying microbes at high concentration of NH_4^+ resulting in slow releasing of N to the crops (Sandral et al., 2017). It also physically separates fertilizer N from residues and large populations of soil microorganisms. Mid-row banding can reduce nutrient loss from the root zone by increasing crop uptake and decrease the risk of N immobilization loss due to leaching or denitrification. Malhi and Nyborg (1991) found that plant ^{15}N recovery was increased by banding compared to broadcasting. Maddux et al. (1991) also reported that 38.9% of the urea N in broadcast application and 24.5% in banded application was immobilized. Norton et al. (2003) found that MRB at sowing provided higher yields than broadcast by sowing. Sandral et al. (2017) also concluded that the apparent fertilizer ^{15}N recovery in grain was higher from MRB urea than from urea broadcast by sowing.

Hofman and Cleemput (2004) showed that N immobilization ranged from 45 kg N ha^{-1} to 112 kg N ha^{-1} in different organic matter incorporated to the soil. Van Den Bossche et al. (2009) suggested that N immobilization increased from 6.85 kg N ha^{-1} at day 0 to 17 kg N ha^{-1} at day 98 by adding maize residues. Blankenau et al. (2000) also concluded that the highest amount of N immobilization was found between tillering and stem elongation of the crop which amounted to 54–97% of the total N immobilized at harvest. King et al. (2001) reported that N immobilization was around (30 kg/ha^{-1}) in the soil when crop growth was most rapid. This author also indicated the fact that applied N did not continue to be immobilized when the crop was not present showed that the crop itself, rather than fundamental soil characteristics, was mostly responsible for immobilization. The amount of available N in soils is influenced by a variety of factors, including soil characteristics (soil texture and organic matter), environmental factors such as rainfall and temperature patterns and farming managements such as tillage, N application rates, timing and method of application as well as residue characteristics (Nahm, 2005). Ahmad et al. (1969) concluded that the process of immobilizing N happened quickly even at low temperatures such as 10 °C, while the process of releasing N progressed significantly at higher temperatures. Mineralization of soil organic N is affected by a wide range of factors, including soil total N (TN) contents, microbial biomass C (MBC) and microbial biomass N (MBN) contents, organic C and the C:N ratio (Barrett and Burke, 2000; Booth et al., 2005). Another factor affects N availability is soil pH (Yansheng et al., 2020). (Bordeleau and Prévost, 1994; Gómez-Rey and González-Prieto, 2015) showed that neutral and slightly alkaline soil (pH < 8.4) increased microbial

NH_4^+ immobilization due to the stimulation of microbial metabolism. Cheng et al. (2013) elucidated that higher C:N ratio of residues increased microbial activity in the soil which likely enhanced microbial immobilization of N with increasing soil pH as a result of high demand for inorganic N for microbial growth. Crop growth rate determines the demand for N, and changes in growth rates during the growing season will be reflected in the pattern of N uptake (McDonald and Hooper, 2013). In rainfed Mediterranean, N fertilizer recovery and efficiency in wheat crops increased when fertilizer was applied as mid-season prior to stem elongation, while application of N in the fall, before sowing, produced poor efficiency (López-Bellido et al., 2005). Blankenau et al. (2002) also showed that recovery of N from the first application at tillering was lower than recovery of N from later N applications.

1.7 Methods for investigating nitrogen transfer in soil-plant systems

Nitrogen has 15 radioactive isotopes and two stable isotopes ^{14}N and ^{15}N. The short half-life time of the radioactive isotopes makes them unstable for most biological investigation (He et al., 2009). Due to the greater atomic mass of the ^{15}N isotope, this isotope is used to trace N transfer over short periods of time (Dawson et al., 2002). Stable isotope (^{15}N) techniques have been developed for determining the fate of N in plant-soil system (Khan, 2000). These techniques give more accurate assessment of below-ground N which is taking by crops or microorganisms in the soil. Another goal of ^{15}N labelled studies is to understand the N dynamics in the soil-plant system and the factors that affect N loss such as N leaching or N immobilization (Jia et al., 2011). The enriched ^{15}N materials are highly expensive to be applied on a large scale, thus their use in field plot experiments is appropriate where only small amounts of ^{15}N are applied (Follett, 2001). The labelled N fertilizer applied to the soil can be taken by crops, remain in the soil or lost from the soil-plant system. Uptake in plants and soil can be measured via analytical techniques, and the balance of unrecovered tracer is assumed lost.

Many reliable reviews have been published on techniques containing ^{15}N enriched and ^{15}N natural abundance studies. For instance, residual fertilizer N value (Smith and Chalk, 2018), N fertilizer use efficiency (Chalk et al., 2015) and N mineralization and immobilization (Murphy et al., 2003). The isotopic techniques are futile in tracing changes within the soil organic N pools in long-term studies because of the high dilution of the ^{15}N by soil organic N (Smith and Chalk, 2018). Follett (2001) showed that treatments with large amounts of crop residues had lower crop ^{15}N recoveries particularly when residue remained on the soil surface.

1.8 The aims of this PhD study

Thus, the question remains: can immobilization of fertilizer N be reduced or overcome to increase NUE in residue retained cropping systems? This thesis uses simulation modelling, field and controlled environment experiments to (i) study the sources of N loss in an area with specific amounts of N fertilizers, in different rainfall and residue retention practices, (ii) investigate whether suppressing immobilized microorganisms is responsible for acquiring more soil mineral nitrogen in canola than wheat in the presence of C-rich wheat residues, and (iii) examine if different N fertilizer placement, N timing application and species can help crops to access more N fertilizer in the presence /absence of wheat residue. The thesis consists of five chapters which include this general introduction, three experimental chapters and a general discussion. The aims of each of these chapters are described below.

Chapter 2 aimed to quantify sources of N inefficiency across a rainfall gradient and a range of fertilizer N inputs in modern farming systems with and without residue retention in Mediterranean southeast Australia by using the APSIM (Holzworth et al., 2014) cropping model. We hypothesised that the denitrification and leaching will become greater sources of inefficiency at the higher rainfall sites and at higher rates of N fertilizer application and that N immobilization will be greater in all treatments where residue is retained.

Chapter 3 was a pot experiment which aimed to determine if canola and wheat differed in their capacity to capture applied fertilizer N in the presence of C-rich crop residues, and whether this was related to a reduction in the immobilization of N in canola rhizospheres resulting from changes in microbial community composition or activity. We hypothesised that reduced microbial biomass N (MBN) derived from fertilizer in the canola rhizosphere would enable canola to obtain more fertilizer N compared to wheat.

Chapter 4 reports two field experiments which examined the effects of different species (wheat vs. canola), N placement (mid-row banding vs. surface application) and N timing (sowing vs. mid-season at early stem-elongation) on crop N uptake when residue was either retained or burnt. In this study, we also used ^{15}N isotope techniques to track N in the plant-soil-microbe system. We hypothesised that canola would better compete with microbes for mineral N and mid-season and deep placement of N in the soil would be more beneficial in residue retained systems compared to surface application.

The thesis concludes with a general discussion and highlights potential areas for future research.

Chapter 2. Modelled quantification of different sources of nitrogen inefficiency in semi-arid cropping systems

2.1 Abstract

Most dryland grain growers in Australia retain all or most of their crop residues to protect the soil from erosion and to improve water conservation, but retaining residues with a high (C:N) ratio can affect N availability to crops. A simulation experiment was conducted to investigate the effects of N fertilizer application rate and residue retention on soil N dynamics. The simulation used seven N fertilizer application rates (0, 25, 50, 75, 100, 150 and 200 kg N ha^{-1}) to wheat (*Triticum aestivum*) over 27 years (1990-2016) at four locations across a gradient in annual rainfall (Mildura 283 mm, Birchip 325 mm, Longerenong 384 mm, Lake Bolac 547 mm) in Victoria, Australia. Nitrogen immobilization, denitrification and N leaching loss were predicted and collectively defined as sources of N inefficiency. When residues were retained, immobilization was predicted to be the biggest source of inefficiency at all simulated sites at N application rates currently used by growers. Leaching became a bigger source of inefficiency at one site with low soil water-holding capacity, but only at N rates much higher than would currently be commercially applied. The higher rates of N fertilizer resulted in high levels of nitrate (NO_3^-) accumulating in the soil which is susceptible to leaching. Denitrification was an appreciable source of inefficiency at higher rainfall sites. Further research is necessary to evaluate strategies to minimise immobilization of N in semi-arid cropping systems.

2.2 Introduction

World population and food demand will both continue to increase in the next three decades at least. Fischer and Connor (2018) conclude that staple grain crop yields need to increase by 1.1% p.a. relative to 2010 levels to meet increasing demand, but future yield gains must be supported with environmentally-appropriate inputs of plant nutrients (Tilman, 1999).

Nitrogen (N) is an essential macro-nutrient for plant growth and a key agricultural input that has been increasingly used in food production since the invention of the Haber-Bosch process to reduce atmospheric N_2 gas to reactive forms of N (Lemaire et al., 2018b). Nitrogen is also the most limiting nutrient for non-leguminous crop production in most agricultural areas in the world (Ågren et al., 2012). Rapid increases in global yields achieved over the last half century have been supported by large increases in application of N fertilizers (Tilman, 1999). However, N can be lost easily from the soil-plant system.

Semi-arid and arid lands comprise one-third of the global land area and are extensively used for agricultural production. Wheat (*Triticum aestivum*) is grown in Australia in climates that range from subtropical to semi-arid Mediterranean with the majority being produced in the semi-arid Mediterranean regions of the states of Western Australia, South Australia and Victoria and the semi-arid temperate regions of southern New South Wales.

Hochman and Horan (2018) estimate that 40% of the substantial gap between farm yield and water-limited potential yield that exists in Australia is due to N deficiency, indicating that higher applications are required to achieve potential yields. As crop yield is now reliant on fertilizer N, which is costly and can be detrimental to the environment, it is vital to use fertilizers as efficiently as possible. A better understanding of the inefficiencies associated with N fertilizer application is therefore essential to increase N-use efficiency (NUE) and yield.

The efficiency with which N is used for crop production can be defined and measured in different ways that have been well described by Good et al. (2004). The numerator in most definitions of NUE is either total above-ground shoot dry weight or grain yield, but the denominator varies to suit the purpose and context of specific studies. In all indices, poor efficiency of N is either a result of cycling or loss mechanisms in the soil environment such as immobilization, leaching, volatilization and denitrification or inefficiency within the plant such as poor N uptake or transfer to grain. In this study, we refer only to the cycling and loss mechanisms which represent long-term unavailability (immobilization) or loss (leaching, volatilization, denitrification) from the soil-plant system and define these collectively as sources of N inefficiency as they reduce NUE by most accepted definitions. These are detailed below.

Ammonia volatilization is a chemical process that occurs at the soil surface when ammonium (NH_4^+) from urea or any sources of ammonium is converted to ammonia gas (NH_3) if the soil pH is high enough. As most of the cropping areas in southern Australia are non-irrigated, farmers are dependent on rainfall to move surface-applied fertilizers into the soil where these are safe from being volatilized (Fenn and Miyamoto, 1981).

Denitrification is an anaerobic respiratory process performed by many bacterial species (van Spanning et al., 2007). It mostly occurs in waterlogged conditions when bacteria must use oxygen from sources such as (NO_3^-), thereby converting N-containing molecules to gaseous forms. Heavy rainfall after mineralization provides conditions that favour denitrification (Pu et al., 2001).

Nitrogen can also be lost from the root zone of annual crops through leaching (Aulakh and Malhi, 2005). This is influenced by the amount and pattern of rainfall relative to evapotranspiration and soil plant-available water capacity. Excessive N fertilization causes accumulation of mineral N in soil, largely in the form of (NO_3^-), which can be readily leached.

Nitrogen immobilization is defined as the transformation of inorganic N forms such as NO_3^-, nitrite (NO_2^-), NH_3 and NH_4^+ into organic forms which are not available to plants (Zagal and Persson, 1994). This process is undertaken by heterotrophic soil microorganisms that use C from plant residues for energy but require N to form their cellular structures. When these organisms die and begin to decompose, they form the humus fraction of soil organic matter (SOM). The balance between N immobilization and mineralization is dependent on the C:N of the organic material added to the soil. Residues with high C:N ratio such as those of cereals (~80:1) immobilize more N than residues with low C:N ratio such as those of legume crops (~25:1) (Moritsuka et al., 2004). Environmental factors including soil temperature, soil water content and drying and rewetting events also greatly influence immobilization and mineralization rates (Stanford and Epstein, 1974). The net balance between N mineralization and immobilization determines how much N becomes available for crops during the growing season. The mismatch between the timing of N mineralization and the time of peak crop demand is likely a key factor for low NUE (Diacono et al., 2019).

The duration of N immobilization and the time of N release may substantially reduce plant available N (Christensen, 1985). Immobilization during crop growth represents intraspecific competition between plants and microorganisms for mineral N. Microorganisms outcompete plants for mineral N, particularly at low N concentrations (Kuzyakov and Xu, 2013). The short lifecycle of microorganisms means that immobilized N is re-mineralized, whereas the longer lifecycle of most plants ensures a unidirectional flow of N which allows plants to compete over the longer term. However, if immobilization induces crop N deficiency during periods of growth critical to yield due to soil mineral N being insufficient to meet plant demand, then yields will be reduced (Kumar and Goh, 1999). The addition of wheat straw to topsoil has been shown to result in 5 months of net immobilization, and N accumulation and yield of wheat decreased as a result (Ichir and Ismaili, 2002). Immobilization of N by retention and incorporation of wheat and rice residues has been shown to depress both wheat and rice yields (3.7 and 4.5 t ha^{-1}, respectively) compared to residue removal (4.1 and 5.6 t /ha) (Beri et al., 1995).

The time taken for immobilized fertilizer N to become available for crop production also has economic implications. The half-life of immobilized N is estimated to be decades (Angus and Grace, 2017), meaning that the return on investment from yield increases due to re-mineralized N from fertilizer would be greatly

diminished by discounting to allow for the time-dependent value of cash. In summary, the advantages and disadvantages of immobilization in farming systems reflect the dilemma that soil organic matter is primarily useful for crop production when it is being mineralized, but immobilization is the 'fee' required to build this resource, which comes at a cost to crop production (Janzen, 2006). Whilst immobilization has been demonstrated to reduce crop yields and NUE, it can also prevent N losses via leaching, denitrification and volatilization by storing N in less mobile forms (Thomsen and Christensen, 1998). Immobilization is also necessary for the accumulation of soil organic matter and storage of soil organic C due to the stoichiometric relationship between C and N in soil organic matter (Kirkby et al., 2014). Whilst accumulation of soil organic matter can offset anthropogenic C emissions, its primary role in crop production is the provision of N from mineralization (Schjønning et al., 2018) and this function can be partially substituted by applications of N fertilizer (Oldfield et al., 2020).

Since 1990, most of the Australia's wheat has been produced in no-till farming systems with all crop residues (stubble) retained (Llewellyn et al., 2012). Crop residues are a source of organic C for soil microorganisms and increase microbial activity, resulting in N immobilization (Doran, 1980). Immobilized N is not lost from the plant-soil system and has no negative offsite environmental consequences. However, the half-life of immobilized N can be up to decades, making it an important source of inefficiency from a crop production perspective (Angus and Grace, 2017).

Knowledge of sources of N inefficiency is necessary to develop strategies to reduce losses of N, maximise NUE and ultimately increase crop yield (Bacon and Freney, 1989). To achieve this, quantification and understanding of the relative importance of the different sources of N-fertilizer inefficiency in different environments are required. Most studies in south-east Australia were conducted prior to the widespread adoption of no-till and residue retention during the 1990s and 2000s (Llewellyn et al., 2012) and recent changes in rainfall pattern (Pook et al., 2009; Cai et al., 2012). Many of these studies were also conducted at higher-rainfall sites not representative of the low-rainfall environment that comprise most of the Australian cropping belt (Angus and Grace, 2017).

Cropping systems models which are able to simulate crop growth and soil N processes accurately can be a useful tool to understand and improve management in farming systems. Simulation allows simultaneous evaluation of all sources of N inefficiency, conducted over many sites and seasons, and imposition of experimental treatments to assess the impact on production and environmental losses. Here we use the Agricultural Production Systems sIMulator (APSIM) (Holzworth et al., 2014) to quantify sources of N inefficiency across a rainfall gradient and a range of fertilizer N inputs in modern farming systems with and

without residue retention in Mediterranean southeast Australia. APSIM is the most extensively validated crop production model available for the environment of interest. We hypothesise that a) denitrification and leaching will become greater sources of inefficiency at the higher rainfall sites and at higher rates of N fertilizer application and b) immobilization will be greater in all treatments where residue is retained.

2.3 Materials and Methods

We applied the Agricultural Production Systems sIMulator (APSIM), which is based on a set of biophysical modules that simulate biological and physical processes in farming systems (Holzworth et al., 2014), to quantify the relative importance of immobilization, denitrification and leaching as sources of N inefficiency in wheat-based farming systems in southeast Australia. We conducted simulations over 27 years at four locations in the state of Victoria across a rainfall gradient, with two residue treatments (with and without retention of crop residues) and seven fertilizer N rates to capture interactions between site and seasons, and how residue retention influences N cycling. Results are considered in terms of sources of N for crop production (mineral N at sowing, net in-crop mineralization) and temporary (immobilization) and permanent (offtake in grain, offtake in removed residue, leaching and denitrification) inefficiency or loss of N from the soil-plant system. APSIM 7.9 (Holzworth et al. 2014) was used to model soil water, N dynamics and crop growth. We considered four different sites (Mildura, Birchip, Longerenong and Lake Bolac *(Meier et al., 2021)*) which are located in the state of Victoria and vary in soil type and the amount of annual rainfall they receive (Figure 2-1, Table 2-1).

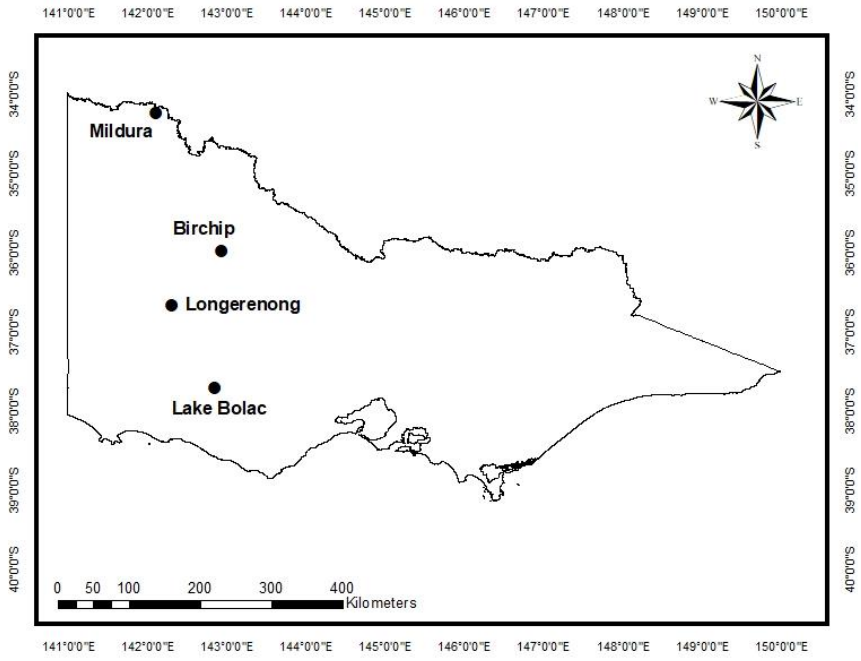

Figure 2-1 The location of simulated sites in Victoria, Australia.

Table 2-1 Details of meteorological stations and soil types used at each site for the period of 1990–2017.

Sites	Station number	Station name	Latitude	Longitude	Average annual rainfall (mm)	Average minimum temperature (°C)	Average Maximum temperature (°C)	[1]APSoil number	Plant available water (PAW) capacity	Organic C (OC) % (0-10 cm)
Mildura	076031	Mildura Airport	34.24 ° S	142.09 ° E	283	11.0	24.6	1097	68	0.41
Birchip	077088	Birchip Cropping Group	35.99 ° S	142.92 ° E	325	8.6	30.3	573	255	1.56
Longerenong	079028	Longerenong	36.67 ° S	142.30 ° E	384	7.8	21.6	1008	348	1.30
Lake Bolac	089016	Lake Bolac (Post office)	37.71 ° S	142.84 ° E	547	7.7	20.2	914	163	0.65

[1] A full list of soil properties is available for corresponding APSoil numbers at https://www.apsim.info/apsim-model/apsoil/ (accessed on 7 June 2021)

The APSIM modules used in the study were NWHEAT, SOILWAT, SOILN, RESIDUE and MANAGER. These modules are described on the APSIM website (www.apsim.info) and are well validated for wheat growth and development (Asseng et al., 1998) and simulation of soil water and N dynamics (Probert et al., 1998; Keating and McCown, 2001). The ability of APSIM to simulate N cycling and losses has been specifically validated against field data for leaching (Asseng et al., 1998) and denitrification (Mielenz et al., 2016; Smith et al., 2019), and numerous studies have demonstrated its relevance to commercial crops in south east Australia (Carberry et al., 2009; Hochman et al., 2009; van Rees et al., 2014).

Crop production was simulated from 1 January 1980 until 31 December 2016 and the first ten years of the simulation were discarded to remove effects of model initialisation. The time period used for analysis (1990-2016) coincides with the break-point in water-limited potential yields identified by Hochman and Horan (2018). Climate data for each location were obtained from the SILO website:

(https://legacy.longpaddock.qld.gov.au/silo/).

Parameters used for simulation included the fertilizer type (urea-N), which was applied at sowing at all sites, the sowing date (1 May), wheat variety (early-mid cultivar which uses base cultivar parameters with vernalization and photoperiod sensitivity adjusted to 1.5 and 3.0 respectively), row spacing (250 mm) and plant population (150 plants m^{-2}). Treatments in the simulation experiment were seven N rates (0, 25, 50, 75, 100, 150 and 200 kg N ha^{-1}) and two residue treatments (retained and removed) which were applied in factorial combination. In the residue-removed systems, surface organic matter was reset to 0 kg ha^{-1} on 1 January in each year of the simulation. During the entire simulation period, soil N and soil water variables were not reset. This approach allows legacy effects of different N fertilizer rates to impact results in following years (Sultan et al., 2014). In the retained treatment, residue accumulated and was only reduced through natural decay, but 70% of all residues were incorporated at sowing each year to represent levels of incorporation of no-till seeding equipment (Lobb et al., 2008). The soil types were selected from APSoil to be representative of each region and the maximum soil depth for each site was 1.3 m deep (Table 2-1).

Outputs from APSIM and the period over which they were obtained are described in (Table 2-1). Mineral N as nitrate was calculated at sowing in APSIM. Net N mineralization was calculated as the annual (1 January to 31 December) N mineralization from soil organic matter pools plus N mineralization from surface residues minus N immobilization from both soil organic matter pools and surface residues. APSIM allows the estimated loss of NO_3^- through denitrification and leaching to be calculated, but it does not predict N loss by NH_3 volatilization (Fillery and Khimashia, 2016). Loss of N by volatilization was therefore not calculated in this study.

Table 2-2 Output variables from APSIM and the seasonal timing at which they were obtained or period of calculation. All soil variables are summed over the maximum rooting depth of the soil (1.3 m)

Output variable	APSIM output terms	Period of calculation
Mineral N (kg ha^{-1})	$nh_4()$ + $no_3()$	Instantaneous (sowing)
Net mineralization (kg ha^{-1})	annual_min + dlt_n_min_res	Annually and from sowing to harvest (in-crop)
N offtake in grain (kg ha^{-1})	grain_n	Instantaneous (harvest)
N remaining in Residues (kg ha^{-1})	N_uptake_stover	Instantaneous (harvest)
N immobilization (kg ha^{-1})	annual_n_min_res	Annually
Denitrification (kg ha^{-1})	annual_no_3_dnit	Annually
N leaching (kg ha^{-1})	annual_leach_no_3	Annually

2.4 Results

2.4.1 Mineral N at sowing and net in-crop N mineralization

Increasing rates of N fertilizer increased mineral N at sowing (Figure 2-2) and net in-crop N mineralization (Figure 2-3), both of which are important sources of N for crop production. In the case of N at sowing, there was a threshold level of fertilizer application at each site above which increases were rapid and large. This coincided with N balance becoming positive (Figure 2-4). Residue retention had a small positive effect on the concentration of mineral N in soil at Birchip, Longerenong and Lake Bolac at higher rates of N application. Residue retention increased net in-crop N mineralization at all sites.

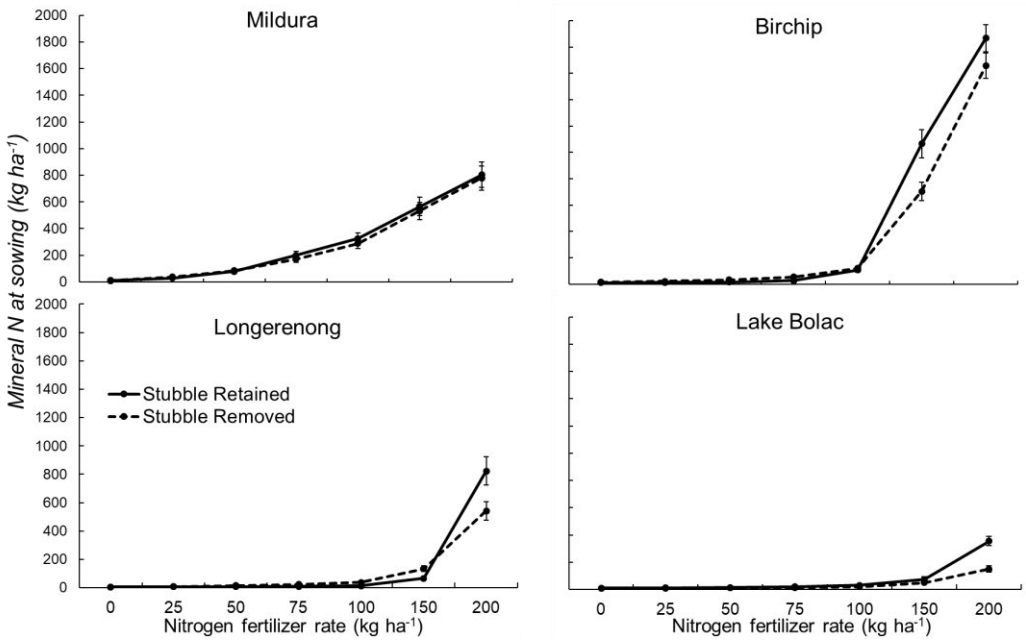

Figure 2-2 Effects of N application rate and residue retention (retained vs. removed) on the mean (1990-2016) mineral N at sowing in the soil profile (0-130 cm) at four sites. Error bars represent ± standard error of the mean over the years of the simulation.

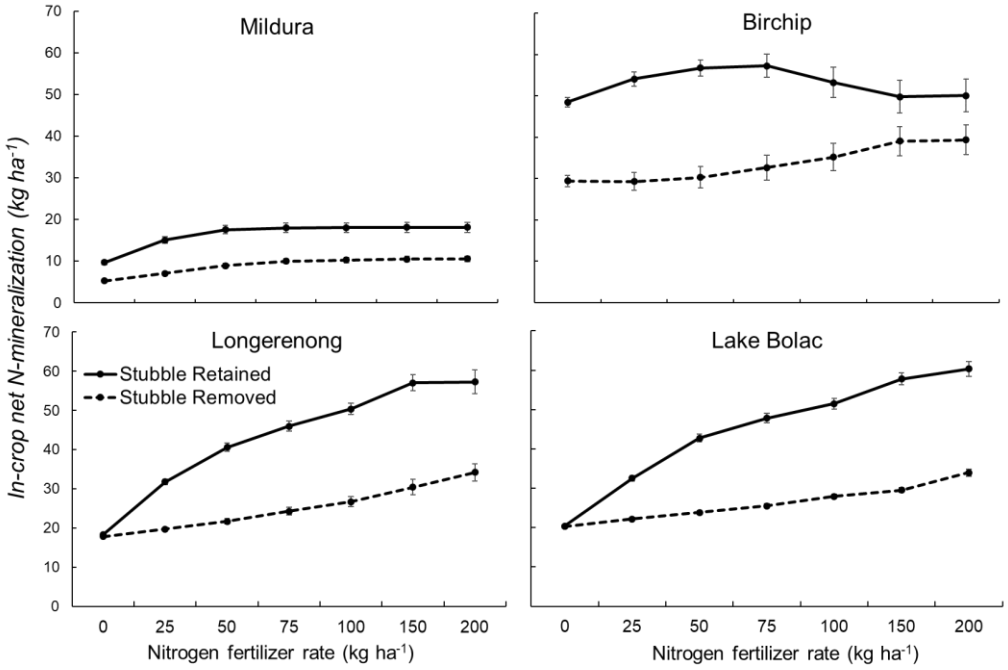

Figure 2-3 Effects of N application rate and residue retention (retained vs. removed) on the mean (1990-2016) net in-crop N mineralization. Error bars represent ± standard error of the mean over the years of the simulations.

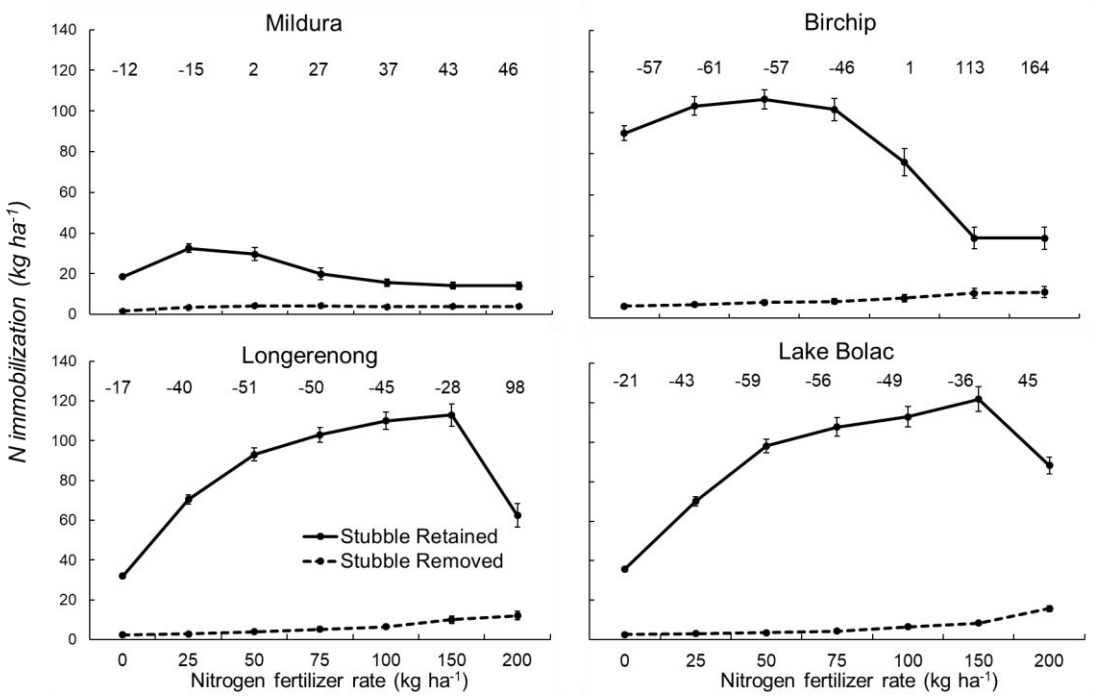

Figure 2-4 Effects of N application rate and residue retention (retained vs. removed) on mean (1990-2016) annual N immobilization at four field sites to 130 cm depth. Error bars represent ± standard error of the mean over the years of the simulation. Numbers at the top of the graphs are the N balance (fertilizer input minus offtake in grain, leaching and denitrification) in kg ha^{-1} of the retained treatments.

2.4.2 Immobilization

Figure 2-4 shows changes in mean annual immobilization at the four sites with increasing N fertilizer rates, as well as changes to the soil N balance (N inputs minus N loss from offtake in grain, leaching and denitrification) for the residue retained system. Immobilization decreased substantially once a positive N balance was achieved, likely reflecting lower C:N ratios of crop residues. Retaining residue increased N immobilization substantially at all sites but this varied with N rate. Where residue was retained, N immobilization initially increased with higher N rates due to increasing C inputs as residue biomass increased. However, it started to decline after a level of N input that coincided with attainment of a neutral N balance (N inputs equal N offtake in grain, denitrification and leaching). Neutral N balance was reached at fertilizer rates of approximately 50 kg N ha^{-1} at Mildura, 75 kg N ha^{-1} at Birchip and 200 kg N ha^{-1} per year at Lake Bolac and Longerenong. This was associated with a decline in C:N ratios of residue once N inputs exceeded outputs (

Figure **2-9** Supplementary). Where residues were removed, N immobilization increased with increasing N rates, but at a much lower level than seen when residue was retained.

2.4.3 N offtake in grain

Grain N offtake increased with increasing N fertilizer rate to 50 kg ha^{-1} at Mildura, 100 kg ha^{-1} at Birchip and 150 kg ha^{-1} at the other two sites (Figure 2-5). These thresholds generally corresponded to the maximum grain yield at each site. Compared to other sites, Mildura had the lowest grain N offtake, reflecting the lower yield. Residue retention did not significantly influence grain N offtake except at Birchip where grain N offtake was generally higher in the residue retained than removed treatment. At all sites, seasonal variation in grain offtake increased (wider standard error bars) with increasing N rates as yields approach water-limited potential and climate-induced variability in yield is expressed.

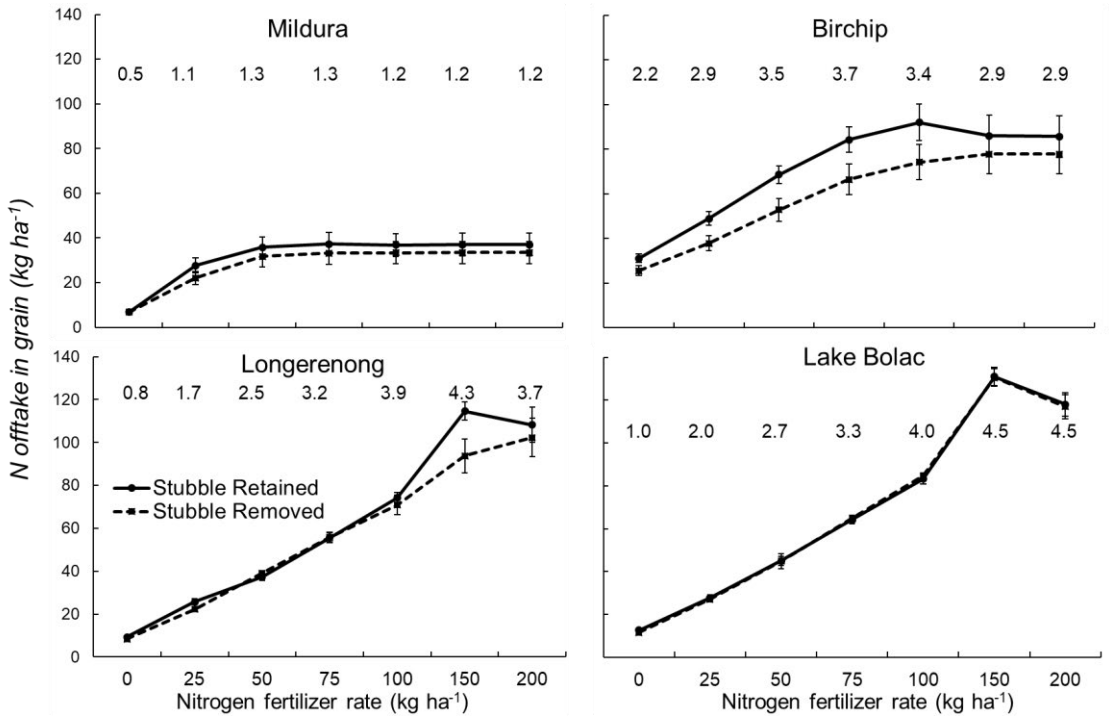

Figure 2-5 Effects of N application rate and residue retention (retained vs. removed) on the mean (1990-2016) N offtake in grain at four field sites. Error bars represent ± standard error of the mean over the years of the simulation. Numbers at the top of the graphs are the average grain yield (t ha^{-1}) of the retained treatment.

2.4.4 Denitrification

Annual denitrification generally increased with increasing N rate (Figure 2-6). At the lower rainfall sites of Mildura and Birchip, annual denitrification was insignificant (below 2 kg ha^{-1}) for both retained and removed residue systems at all N rates. At the higher rainfall sites of Longerenong and Lake Bolac, denitrification became appreciable at higher N rates, and was higher in the residue retained than removed treatments.

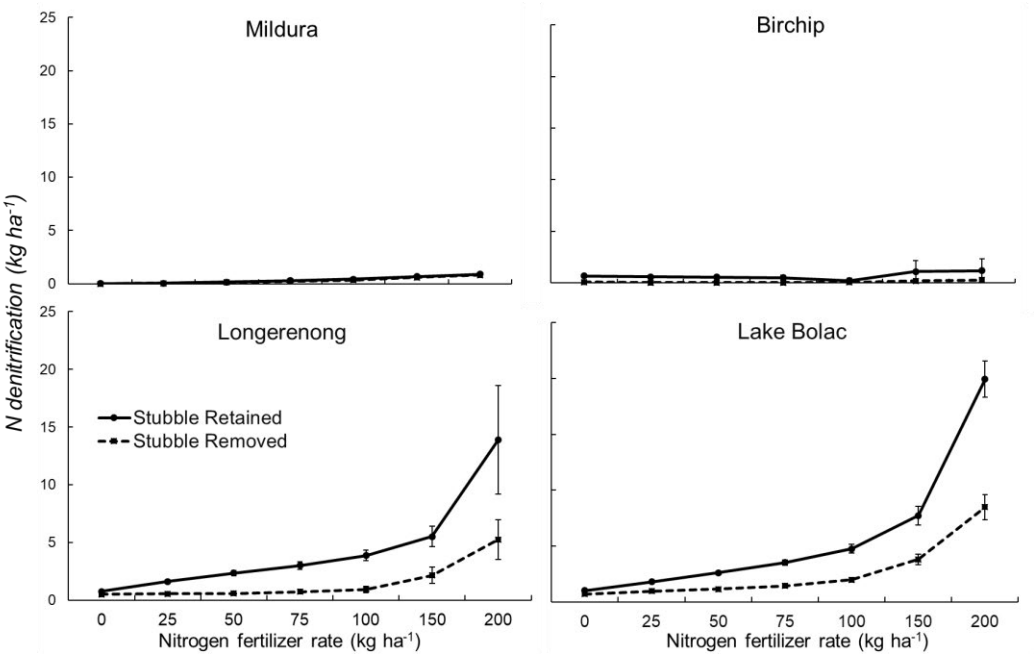

Figure 2-6 Effects of N application rate and residue retention (retained vs. removed) on mean (1990–2016) annual denitrification at four field sites. Error bars represent ± standard error of the mean over the years of the simulation.

2.4.5 Nitrate leaching

There was no difference in nitrate leaching between residue retained and removed treatments at any site (Figure 2-7). Although rainfall at Mildura was very low, leaching loss was the highest at this site compared to other sites. This was due to low water limited yield at this site and thus low offtake of N in grain which resulted in large amounts of mineral N (mainly nitrate, data not shown) accumulating in the soil (Figure 2-2). This nitrate was susceptible to leaching during episodic events of high soil moisture, exacerbated by the sandy and thus low plant-available water capacity soil at this site (Table 2-1).

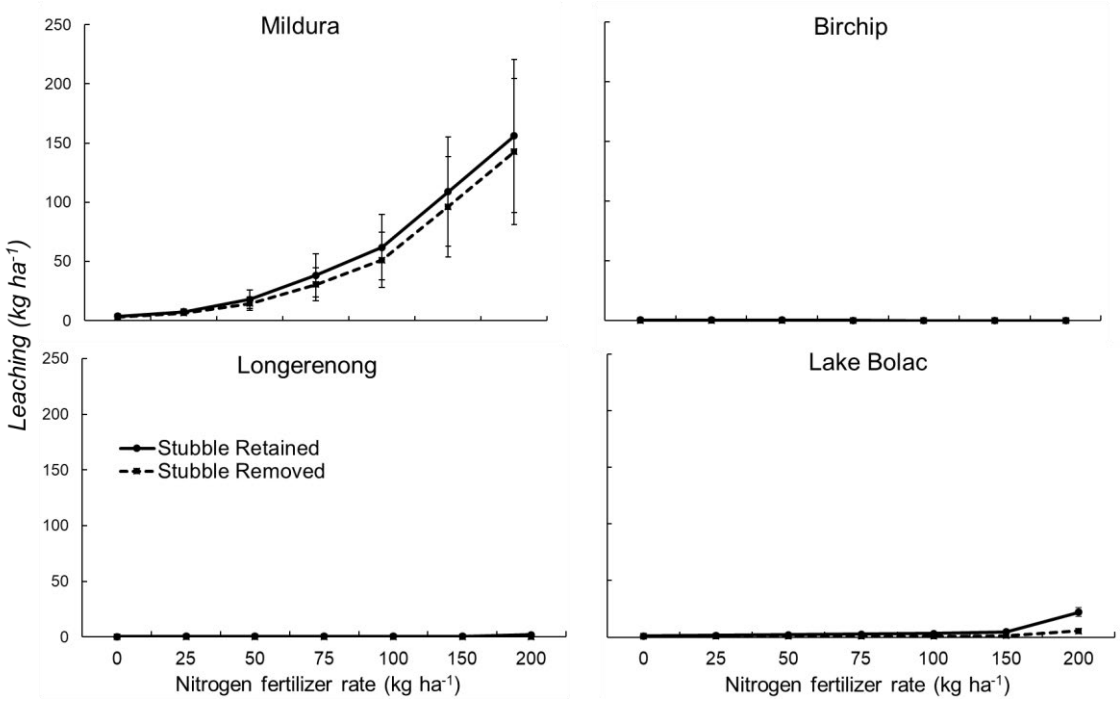

Figure 2-7 Effects of N application rate and residue retention (retained vs. removed) on the mean (1990–2016) N leaching at four field sites. Error bars represent ± standard error of the mean over the years of the simulation.

2.4.6 Nitrogen remaining in crop residues

The four sites displayed different patterns of N remaining in crop residues in response to N fertilizer rates (Figure 2-8), which are only shown for the removed residue treatment. No increase in residue N was observed with N rates larger than 75 kg N ha^{-1} at Mildura and 150 kg N ha^{-1} at Birchip. Residue N continued to respond positively up to the highest rate at Longerenong and Lake Bolac where water supply was not as limiting to plant growth. In the case of the residue removed treatment, N content of crop residues quantifies the permanent loss of N from the soil-plant system due to removal of crop residues, whereas in residue retained treatments, N in residues remains indirectly available to future crops through mineralization (data not shown).

2.4.7 Total N-inefficiency in residue retained and removed systems

Figure 2-8 shows the relative magnitudes of all sources of inefficiency in both residue retained and removed treatments at four simulation sites. Nitrogen immobilization was the largest source of inefficiency at all sites when residue was retained except Mildura, where nitrate leaching was the largest source of inefficiency in

both retained and removed residue systems. Residue removal was the greatest source of N loss in residue-removed systems at Birchip, Longerenong and Lake Bolac at all N rates.

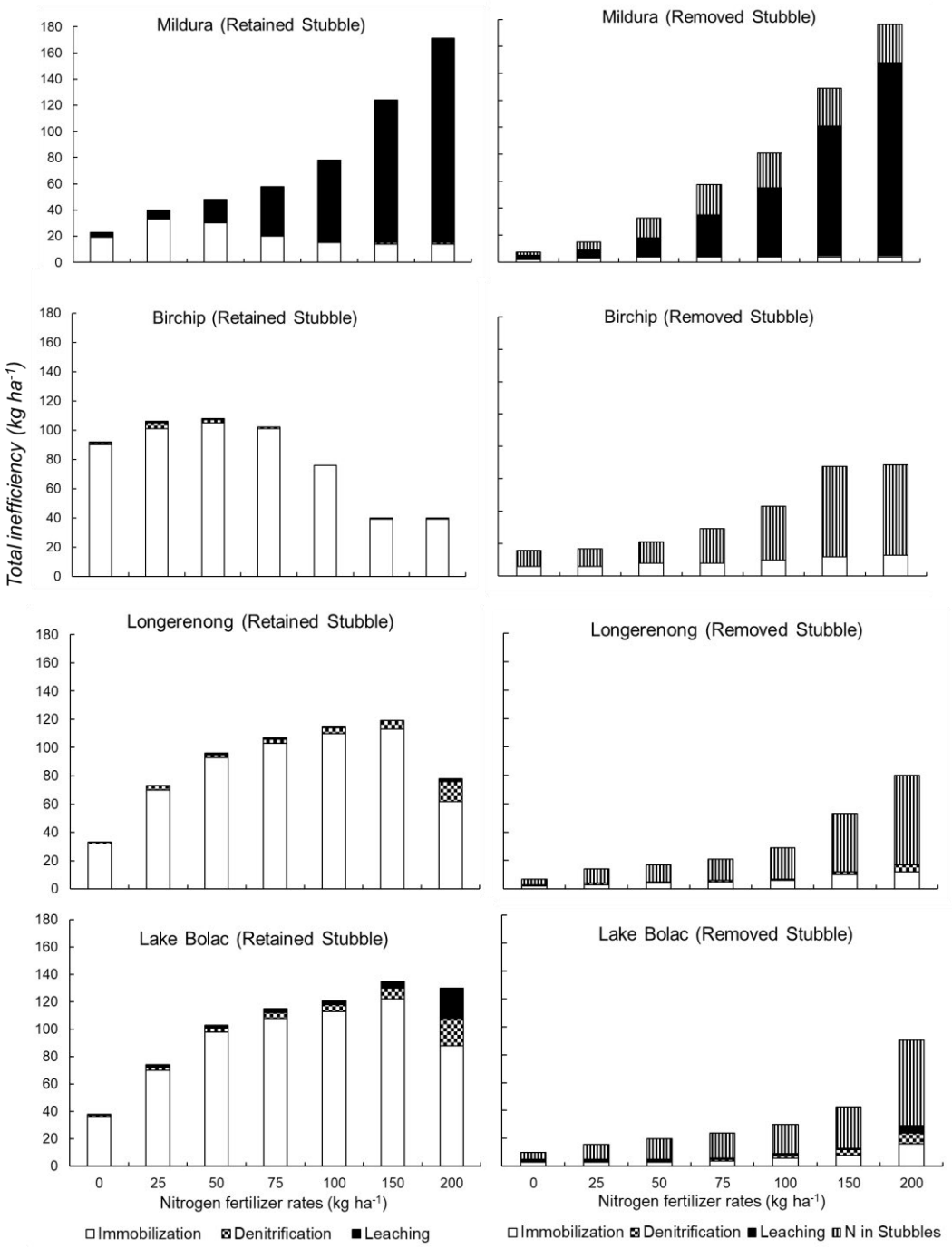

Figure 2-8 Total N inefficiency sources at different N rates with retained and removed residue.

2.5 Discussion

This study provides new insights relating to sources of N inefficiency in Mediterranean cropping systems with retained residues. Crop N balance was changed substantially by both residue and N treatments, but the nature of this change was different in each rainfall × soil type environment. The greatest source of N inefficiency in residue retained treatments was immobilization at most N rates and simulated sites.

Immobilization does not represent a complete loss of N from the soil-plant system as does leaching and denitrification. It is also the counterpoint of mineralization, and our results show that treatments with higher levels of immobilization also have higher levels of mineralization (Figure 2-3 and Figure 2-4). However, it is the temporal dynamics of immobilization combined with seasonal patterns of crop growth that can make it detrimental from a production perspective and a considerable source of inefficiency (Recous et al., 1995).

Given that N immobilization is the biggest source of inefficiency in semi-arid cereal cropping systems with retained residue, fertilizer NUE will be increased by management strategies that reduce immobilization. This could be achieved via improved synchronisation of N availability with crop N demand through a combination of N fertilizer placement and timing of application. The risk of losing N when fertilizer is all applied at sowing is high due to low crop N uptake during early stages of growth. Nitrate from fertilizer applied during this time is vulnerable to loss by leaching, denitrification and particularly immobilization (Angus and Grace, 2017).

During vegetative growth, crop plants are not large enough to compete with microbial immobilizers and most N immobilization occurs at this stage (Barłóg and Grzebisz, 2004). Immobilization and other losses may be reduced by deferring N fertilizer applications to coincide with the period of rapid crop uptake, the beginning of which coincides with the start of stem-elongation. Field experiments have shown that, in environments similar to Lake Bolac (Harris et al., 2016) and Longerenong (Wallace et al., 2020), deferring N application to early stem-elongation could increase plant recovery of ^{15}N-labelled fertilizer in comparison to applications at sowing, assumed to be due to a reduction in losses. Immobilization of N fertilizer is greatest during early growth and crops use N most efficiently when N is applied at stem-elongation (Sowers et al., 1994; Blankenau et al., 2000; Tran and Tremblay, 2000; López-Bellido et al., 2005).

The placement of fertilizer N below the surface layer of soil and away from residues with high C:N ratio can also increase N availability and plant uptake by decreasing immobilization (Sharpe et al., 1988). Banding N fertilizer can also provide greater NUE than broadcasting by reducing N losses from volatilization and holding N as ammonium and nitrate for longer, which reduces losses from leaching and

denitrification (Lemke et al., 2009). Banding N separates the residues from the microbial population which is concentrated in the surface of soil and can significantly suppress immobilization (Paul et al., 2001). Deep banding allows crops to recover more fertilizer N than broadcasting, particularly under early-season drought when deep placed N is more accessible to plants (Malhi and Nyborg, 1985; Hartman and Nyborg, 1989; Rao and Dao, 1996). There is currently little adoption of mid-row banding in western Victoria, and this practice has potential to improve NUE in the region (Wallace et al., 2017).

There is also evidence that grain crop species differ in their ability to compete with microbes for soil N. For instance, levels of soil mineral N are significantly higher after canola in comparison to cereals (Ryan et al., 2006), and one of the reasons for this is reduced microbial immobilization of N (Brown and Morra, 2009). Microbial populations in the canola rhizosphere are relatively low, resulting in less N immobilization in comparison to continuous wheat (McKenzie et al., 1995). Growers in semi-arid environments could reduce immobilization by growing more N-competitive species such as canola in situations where immobilization is likely to be high e.g. following high yielding cereal crops with large residue loads.

The low levels of loss due to leaching and denitrification across all environments at rates of fertilizer likely to be used by growers (<50 kg N ha^{-1} in the case of Mildura) found in this study are consistent with other experimental and simulation findings across similar rainfall gradient (Harris et al., 2016; Smith et al., 2019; Wallace et al., 2020). The higher denitrification in the residue retained treatments reflects that the denitrification rate generally increases when more C is available (Lalisse-Grundmann et al., 1988). Crop residues also increase the supply of energy available to denitrifying organisms which stimulates denitrification in a residue retained system under anerobic conditions (Aulakh et al., 1984). Higher denitrification at Longerenong and Lake Bolac is due to a combination of climate and soil factors. Soils with high clay content and low porosity are prone to waterlogging and therefore anaerobic conditions (Palta et al., 2014). Soil surface textures were sand and sandy clay loam at Mildura and Birchip, respectively, and clay and clay loam at Longerenong and Lake Bolac. Denitrification was not induced by higher soil nitrate concentration at Mildura and Birchip, suggesting that these environments have inherently low denitrification. Leaching losses increased rapidly with increasing N rate at Mildura. The potential of N loss through leaching increases when nitrate accumulates, as happened at this site due to lower offtake in grain. Furthermore, this site has sandy soil with a low water-holding capacity (Table 2-1) which increases susceptibility to leaching (Shepherd and Bennett, 1998; Asseng et al., 2008).

2.6 Conclusion

This study has shown that N fertilization and retention of residue significantly affects N dynamics of crop production systems in south-east of Australia. Our results suggest that immobilization of fertilizer N is by far the largest source of N inefficiency in residue retained cropping systems in Victoria at levels of N input currently used by farmers. Losses of leaching and denitrification tend to be low but vary with site. At the lower-yielding site of Mildura, which had a sandy soil with low plant-available water capacity, losses of N were observed at high fertilizer rates due to leaching, caused by increased accumulation of soil nitrate due to the low offtake of N in grain. Denitrification became appreciable at higher rainfall sites with heavier textured soils when residue was retained, and N applications increased. Management strategies that seek to reduce immobilization of fertilizer N (e.g., deferred N applications and mid-row or deep banding) should be experimentally evaluated for their capacity to increase NUE and their suitability for on-farm adoption.

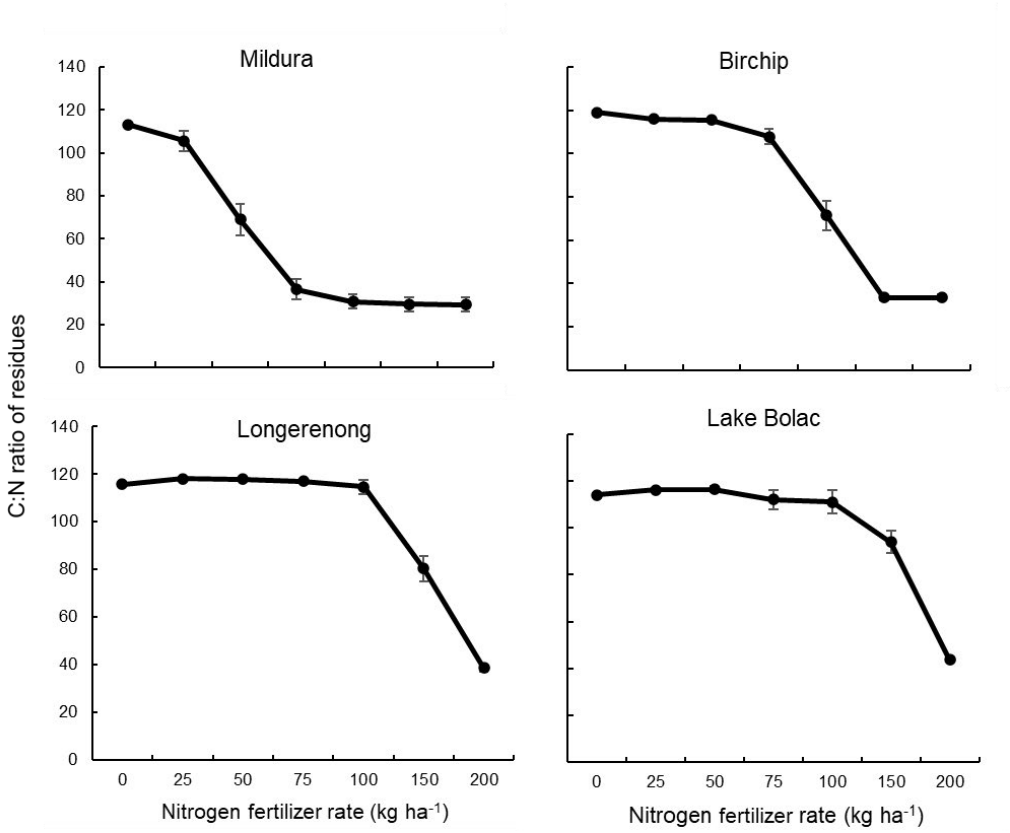

Figure 2-9 Supplementary C:N ratios of residue in retained residue at four simulation sites with different N application. Error bars represent ± standard error of the mean over the years of the simulation.

Chapter 3. Nitrogen fertilizer immobilization and uptake in the rhizospheres of wheat and canola

3.1 Abstract

Immobilization of fertilizer nitrogen (N) by soil microorganisms can reduce N availability to crops, decreasing growth and yield. To date, few studies have focussed on the effect of different plant species on immobilization of fertilizer N. Canola (*Brassica napus*) is known to influence the soil microbiome and increase mineral N in soil for future crops compared with cereals. We tested the hypothesis that canola can reduce immobilization of fertilizer N by influencing the composition of the rhizosphere microbiome. To investigate this, we conducted a glasshouse soil column experiment comparing N fertilizer uptake between canola and wheat (*Triticum aestivium*) and partitioning of fertilizer N between plants and microorganisms. Plants were grown in soil to which high C:N ratio wheat residues and ^{15}N-labelled urea fertilizer were applied. There was no difference between wheat and canola in fertilizer N uptake despite differences in fungal community composition and the C metabolising enzyme alpha-glucosidase in the rhizosphere. Canola obtained more soil-derived N than wheat. There was no significant difference in the rhizosphere bacterial communities present between wheat and canola and unplanted controls. Our results highlight the capacity of canola to increase mineralization of soil N compared with wheat although the study could not describe the microbial community which facilitated this increase.

3.2 Introduction

Nitrogen (N) deficiency is considered the main impediment to achieving water limited potential yield in Australian wheat (*Triticum aestivum*) production systems (Hochman and Horan, 2018). Farmers are often conservative with fertilizer application rates due to climate variability and other risk factors which influence yield (Asseng et al., 2012). In addition to inadequate application rates, Australian wheat crops are estimated to use only 40% of N fertilizer in the season it is applied (Angus and Grace, 2017). Identifying ways to increase crop nitrogen use efficiency (NUE) provides the dual benefit of reducing the rate required for fertilizer application while reducing the risk of potentially damaging N losses to the environment.

A recent simulation study projected that microbial immobilization was the main source of N fertilizer inefficiency in southern Australian cropping systems with retained residue (Nasrollahi et al., 2021). Widespread adoption of no-till cropping in Australian intensive cereal systems (Llewellyn et al., 2012) has resulted in the persistence of significant amounts of C-rich crop residues. Rates of immobilization are particularly high in grain production systems where residues are retained, because the presence of high C:N

ratio cereal crop residues encourages microbes to utilise available N to support residue decomposition (Hodge et al., 2000).

Immobilization and mineralization of N are opposing processes of the soil N cycle and determine the distribution of N between mineral and organic forms. Mineralization converts soil organic matter (SOM) containing N into mineral N, predominantly NH_4^+. The size of the microbial biomass is often reported as the major factor determining N mineralization (Colman and Schimel, 2013). Similarly, it is suggested that the microbial community composition may influence mineralization of SOM (Falchini et al., 2003). Mineralization and immobilization are processes which are carried out by most of the microbial population rather than by specific phyla (Nannipieri et al., 2003). The nutritional demand of the microbial population is likely a key variable influencing both processes, especially when microorganisms are stimulated by the addition of external nutrients (Blagodatskaya and Kuzyakov, 2008). The universal requirement of organisms for N as a macronutrient ensures that in environments with low N availability the competition for available N will be high.

Uptake of soil nutrients by microorganisms is essential for nutrient cycling into plant available forms (Li et al., 2014). However, immobilization reduces the availability of mineral N, an important N source for plants. If all N fertilizer is applied at sowing, this early competition for N may reduce the vigour of young crops potentially affecting yield compared to split N application at different crop stages. Fertilizer applications that coincide with times of high crop demand (i.e. stem-elongation in wheat) can increase plant competitiveness for applied N. However, the abundance of C-rich crop residues ensures that some fertilizer is still immobilized. Nitrogen partitioning in the soil-plant system between microorganisms and plants is thought to change over time, with microbes accumulating more in the short term and plants more in the long term (Kuzyakov and Xu, 2013). The spatiotemporal context of the competition for resources and the influence of environmental factors upon N transformations results in a highly dynamic interaction between plants and microorganisms. To date, few studies have investigated the effect of plant species upon microbial immobilization directly.

Canola (*Brassica napus*) is a common break crop grown in rotation with wheat (Angus et al., 2015). The break crop effect provided by canola to subsequent wheat crops was initially thought to be due to suppression of root pathogens by glucosinolates, a secondary metabolite produced by *Brassica* species (Angus et al., 1991). However, Kirkegaard et al. (1999) demonstrated that mineral N accumulation following a canola crop was higher than following a cereal crop, and often increased when compared to a legume crop. Ryan et al. (2006) attributed this increase in mineral N following canola to changes in the

microbial community composition which emerged from differences in the chemical composition of plant residues. Studies have shown that canola root tissues can influence individual soil microorganisms such as nitrogen fixing *Azospirillum* sp. (Kirkegaard et al., 1999) and the soil borne pathogen *Gaeumannomyces graminis* (Angus et al., 1994). They also appear to influence specific groups of soil microorganisms such as ammonia oxidisers (O'Sullivan et al., 2016). O'Sullivan et al. (2016) showed that wheat required less N fertilizer when grown after canola compared with after pasture, and that canola reduced nitrification and increased immobilization and remobilisation rates of N in the rhizosphere compared with wheat. These effects of canola on the rhizosphere biology may provide benefits to crop production if immobilized fertilizer N is mineralized and acquired by plants in season. Canola suppresses microbial activity, decreases microbial biomass which results in a temporary modification of the microbial community in the soil (Omirou et al., 2011; Potgieter et al., 2013). A biocidal secondary metabolite of canola, ITCs could be a potential mechanism of soil microbial biomass reduction (Hansen et al., 2019). Hansen et al. (2018) showed that less fungi, mycorrhizae, and total microbial biomass observed in wheat-canola rotation than wheat-wheat.

The focus of this study was to determine if canola and wheat differed in their capacity to capture applied fertilizer N in the presence of C-rich crop residues, and whether this was related to a reduction in the immobilization of N in canola rhizospheres as a result of induced changes in microbial community composition and activity. We hypothesised that reduced microbial biomass N (MBN) derived from fertilizer in the canola rhizosphere would enable canola to obtain more fertilizer N compared to wheat.

3.3 Materials and Methods

3.3.1 General

The experiment took place in a glasshouse at AgriBio, Bundoora, Victoria, Australia. The soil used throughout the experiment was collected from Normanville, Victoria, Australia (35°50'39" S, 143°44'56" E). The soil used was a Vertic Calcarosol (Isbell, 2016) with a sandy clay loam texture, bulk density of 1.2 g cm^{-3}, and pH of 6.5 (1:5 0.01 mol L^{-1} $CaCl_2$). Initial soil total N was 1.17 g kg^{-1} (± 0.04) which is similar to other dryland, long-term cropping sites in Australia (Kirkby et al., 2011). Total mineral N ($NH_4^+ + NO_3^-$) of the soil was 22.1 ± 5.6 mg kg^{-1}. Approximately 500 kg of surface soil (0–15 cm) was collected in April 2018 from a paddock which had grown a wheat crop in the previous growing season. Wheat residue with a C:N ratio of 70:1 was also collected from this site at the time of soil collection. Glasshouse temperature was 22 °C during daylight hours (14 h) and 14 °C at night (10 h). Light was supplemented using sodium halide lamps during the 14-h daylight period if external irradiance fell below 170 W m^{-2}.

3.3.2 Experimental design

The experiment was a balanced two-way factorial randomised complete block design with four replicates, two crop species (wheat and canola), and two urea (CH_4N_2O) nitrogen fertilization rates (low, 5 kg N ha^{-1} equivalent and moderate, 61 kg N ha^{-1} equivalent commercial rate for Australian dryland grain cropping systems). This design was replicated for both harvest times (anthesis and maturity). Unplanted control treatments were given the same N rates; however, they were not included in the balanced two-way factorial experimental design. Due to the herbicide history of the collection site, wheat (cv. Razor CL plus, Australian Grain Technologies) and canola (hybrid 44Y90 CL, Pioneer Seeds) cultivars with Clearfield imidazolinone tolerance were selected to ensure that residual herbicide did not adversely influence plant growth.

Plants were grown in PVC columns containing 12.7 kg of air-dried soil sieved to ≤2 mm. Soil was wet and maintained at ~80% field capacity (20.5% w/w) during plant growth. The top 10 cm of soil had 18 g of ground (5 mm sieve) wheat residue incorporated uniformly (equivalent to 10 t ha^{-1}). The incorporation of high-C wheat residue into surface soil with low mineral N (12 kg N ha^{-1} equivalent) was to encourage a competitive environment for fertilizer N uptake between plants and microorganisms. Columns were 60 cm tall to ensure roots were not confined to the upper 10 cm of columns where the residue was added. Additionally, the top 10 cm received basal elemental nutrients (54 K, 49 Ca, 41 P, 33 S, 5 Mg, 5 Mn, 2 Zn, 1.5 Cu, 0.8 Fe, 0.1 Mo mg kg^{-1}) to ensure no limitations of other nutrients for plant growth. Nutrient solutions were mixed thoroughly through the top 10 cm before planting. Seeds were germinated on wet paper towel in a Petri dish and sown into the surface soil. Wheat plants were sown to a depth of 20 mm and canola at 15 mm. Unplanted controls received the same nutrient application and residue incorporation rates.

The two N fertilizer rates, low (10 mg N) and moderate (116 mg N) were applied to each of the crops. The N fertilizer used throughout was urea, with 1% enrichment with ^{15}N to trace the fate of fertilizer N in plants and soil. The N fertilizer was first applied when three leaves had fully emerged on wheat plants, and the moderate-N treatment had two subsequent applications 14 days apart (38.6 mg at each application). The N fertilizer was applied to the soil surface with the water required to maintain moisture content. Plant and soil samples were taken when wheat reached anthesis (Z 65) (Zadoks et al., 1974) 57 days after sowing, and maturity-harvest ripe (Z89-92) 116 days after sowing. Canola plants were harvested at the same time as wheat and were not physiologically mature at the final harvest due to the indeterminate nature of canola. We estimate the canola to be at 30% pods ripe stage (83) (Meier et al., 2009). Soil from the unplanted control was also sampled at the same time.

3.3.3 Plant sampling and analysis

Senesced leaves were collected during the growth period as they detached from the plants to prevent decomposition. At harvest, leaves, spikes (wheat), and pods (canola) were removed at the abscission layer between the respective tissue and the stem, and stems were then cut and removed at the soil surface. Spikes (wheat) and pods (canola) were analysed with immature grain *in situ* for the anthesis harvest, while the mature grain was threshed at maturity and analysed separately. All of the sampled tissues described above were included in the measurement of shoot N.

After sampling, all partitioned plant tissues were placed in an oven at 70 °C for 48 h. Each section of the shoot material was then weighed. All plant material was then ground and milled in preparation for nutrient analysis. The concentration of N in plant tissues from the anthesis sampling time was determined through a 2400 Series II CHNS/O Elemental Analyser (PerkinElmer, Waltham, MA, USA). Plant tissue and soil N and ^{15}N% at the maturity sampling time were determined via mass spectrometry using an IRMS hydra 20-20 isotope ratio mass spectrometer (Sercon Limited, Cheshire, UK) combined with an ANCA-S/L sample preparation unit (Europa Scientific, Crewe, UK).

The percentage of applied ^{15}N fertilizer uptake by plants (uptake, %) was calculated using the following formula (Conyers et al., 2011):

$$\text{Uptake (\%)} = 100 \times [(TN)(c - 0.3663)]/[(FN)(f - 0.3663)]$$

where, TN is the total amount of N in the plants (mg per column), c is the ^{15}N% of the plant samples, 0.3663 is the assumed natural abundance of ^{15}N, FN is the total amount of fertilizer N applied per column (mg), and f is the ^{15}N% in the fertilizer. This value was then used to calculate fertilizer N uptake by plants in mg.

3.3.4 Soil sampling and analysis

After plants were harvested, rhizosphere soil was collected from the top 0–10 cm in each column. Loosely bound soil was shaken from the roots and tightly bound soil within 5 mm of the root surface was collected (rhizosphere soil). Bulk soil (≥5 mm from root surface) from the 0–10 cm and 10–60 cm sections were sampled separately, and all soil samples were sieved to <2 mm prior to analysis.

Soil pH was measured in 0.01 mol L^{-1} $CaCl_2$ (soil:solution of 1:5), after being shaken end-on-end for an hour and centrifuged (at 3000 rpm for 5 min) as per method 4B1 of (Rayment and Lyons, 2011). Moisture content of soil sections was determined at each harvest by weighing soil prior to and after 24-hour oven drying at 105 °C. The values obtained were used to correct nutrient measurements on a mass-basis of oven

dry soil. All of the following soil results were calibrated to be shown as mass-basis of dry soil as per method 2A1 of (Rayment and Lyons, 2011).

Analysis of soil nitrate and ammonium proceeded following extraction with 2 mol L^{-1} KCl (shaking end-on-end for 1 h), centrifugation (3000 rpm for 5 min), and filtering supernatant through Whatman #42 filter paper (Whatman International, Maidstone, England). Extracts were stored at -20°C before determination of extractable nitrate and ammonium using the flow-injection analyser via Quikchem 8500 Series II (Lachat Instruments, Loveland, CO, USA) system as detailed in the Quikchem manuals (12-10706-2-F and 12-107 04-1-B, respectively).

MBN in rhizosphere soil was determined by using a variation of the chloroform fumigation-extraction method as described by (Vance et al., 1987), within 24 hours of sampling. Briefly, fumigated samples were incubated in a desiccator with approximately 50 ml of ethanol-free chloroform for 24 hours at 25 °C in darkness. Fumigated and unfumigated soil samples were extracted using 0.05 mol L^{-1} K_2SO_4 solution in a ratio of 1:5 soil to solution. A 0.05 mol L^{-1} K_2SO_4 solution was used instead of 0.5 M K_2SO_4 for extraction due to the low ^{15}N enrichment rate and to reduce salt content of freeze-dried samples to allow better detection of the isotope ratio. Samples were shaken end-over-end for 1 hour followed by filtration through Whatman #42 filter paper. Following storage at -20 °C, extracts for MBN were freeze-dried and salt extracts were then analysed via IRMS hydra 20-20 isotope ratio mass spectrometer (Sercon Limited, Cheshire, UK) combined with an ANCA-S/L sample preparation unit (Europa Scientific, Crewe, UK). MBN is expressed as the difference between fumigated and unfumigated samples.

Extracellular enzyme activity of cellulose decomposing enzymes in the rhizosphere soil was determined by high-throughput fluorometric measurement of 4-methylumbelliferone labelled substrates as outlined by (Bell et al., 2013). Four enzymes were measured to assess the activity of the decomposer community present in each treatment to detect differences between plant species. These were alpha-glucosidase (AG), beta-glucosidase (BG), cellobiase (CB) and beta-xylosidase (XYL). Soil was incubated at 25 °C for 3 hours and fluorescence of microplates was detected using a CLARIOstar microplate reader (BMG LABTECH, Ortenburg, Germany).

Microbial DNA extraction was performed using the Earth Microbiome Protocol (Marotz et al., 2017). A step-by-step description of this protocol is outlined in https://www.protocols.io/view/earth-microbiome-project-emp-high-throughput-htp-d-pdmdi46. DNA extraction and 16S rRNA and ITS diversity profiling were performed by the Australian Genome Research Facility on an Illumina MiSeq platform (San Diego, CA, USA). A 300-bp target was amplified from the V3–V4 region of the 16S rRNA gene using primers

341F (5'- CCTAY GGGRB GCASC AG) and 806R (5'- GACTA CNNGG GTATC TAAT) (Yu et al., 2005) and an approximately 230-bp target was amplified from the ITS1–ITS2 region of the internal transcribed spacer (ITS) using primers ITS1f (5'- CTT GGT CAT TTA GAG GAA GTA A) and ITS2 (5'- GCT GCG TTC TTC ATC GAT GC) (White et al., 1990; Gardes and Bruns, 1993).

Raw, demultiplexed fastq files were re-barcoded, joined and quality filtered using the UPARSE pipeline (Edgar, 2013). Joined paired-end reads were quality-filtered by discarding reads with total expected errors >1 and removing singletons. Operational taxonomic units (OTUs) were clustered with a minimum cluster size >2 and 97% similarity cut off using the UPARSE-OTU greedy heuristic clustering algorithm. Taxonomic assignments were performed using the USEARCH UTAX algorithm with reference databases created using the RDP 16S (version 16) and UNITE ITS (version 7) training datasets (available at https://www.drive5.com/usearch/ accessed 8 November 2021). The minimum percentage identity required for an OTU to consider a database match a hit was 80%. All OTUs with a taxonomic confidence threshold less than 80% were denoted as 'unassigned'. The OTUs identified as chloroplasts and mitochondrial DNA were removed from the data set. A phylogenetic tree was constructed using the UPGMA algorithm in MUSCLE (Edgar, 2004).

3.3.5 Statistical analysis

GenStat version 19 (VSN International Limited, Hampstead, UK) was used for all statistical analyses of plant and soil data. Shapiro-Wilk test for normality was completed on each variate before analysis of variance (ANOVA) was performed and homogeneity of variance was assessed via plotting residuals and fitted values. Two-way analysis of variance (ANOVA) was used to assess differences in plant species and N treatment data collected at both sampling times. The results for the soil enzyme assay which included the controls as part of an unbalance experimental design were analysed using the unbalanced ANOVA function.

Bar charts of relative abundance of bacterial and fungal phyla were produced in R version 3.5.3 (R Core Team 2018) using package phyloseq (McMurdie and Holmes, 2013) with the assistance of ggplot2 (Wickham, 2009) and RColorBrewer (Neuwirth, 2014). Before plotting, spurious reads were removed using a 0.005% relative abundance cut-off (Bokulich et al., 2013). Weighted and unweighted UniFrac distances (Lozupone and Knight, 2005) between samples were calculated from raw data, normalised to relative abundance, and then the relationships and differences between treatments were visualised with principal coordinates analysis.

R package 'mvabund' (Wang et al., 2012) was used to test multivariate hypotheses about treatment effects and univariate hypotheses about species-by-species effects (i.e. the detection of differentially abundant

OUTs). For this procedure, unrarefied sequence counts were modelled on negative binomial distributions in the generalized linear models and P-values were adjusted to control for the family-wise error rate.

3.4 Results

3.4.1 Differences in N partitioning between soil microorganisms and plant shoots

There was no difference in the total MBN in the wheat and canola rhizosphere soils (Figure 3-1). Fertilizer N made up more of the MBN in the moderate-N treatments (Figure 3-1). The proportion of applied N acquired by microbes in the low-N treatment was higher (Table 3-1). There was no interaction effect of total MBN to plant species or N treatment (Figure 3-1), although the microbial uptake of fertilizer N in mg increased with application rate for both plant treatments ($p \leq 0.001$). Despite no differences in MBN between low moderate N application rates, there is a notable reduction in standard error of the moderate-N application rate compared with the low rate for both plant species (Figure 3-1).

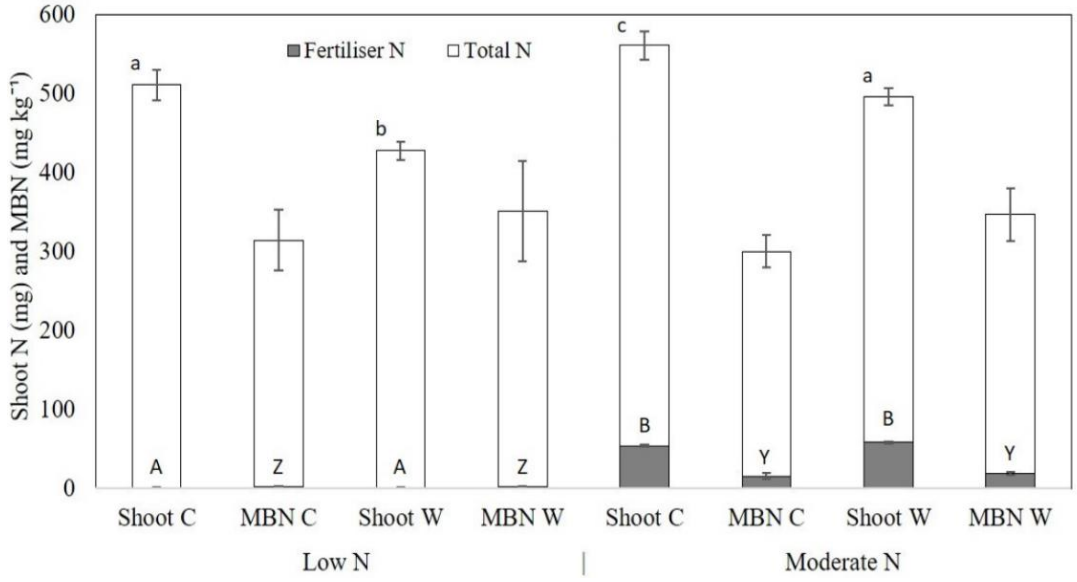

Figure 3-1 Mean N content (mg) of wheat and canola (W and C, respectively) shoots and mean microbial biomass N (MBN, mg kg^{-1}) of rhizosphere soil at maturity, showing N sourced from fertilizer (dark grey) and existing soil N pools (white). Error bars represent mean ± standard error. Significant difference, $p \leq 0.05$, indicated by lower case a-c for total plant N, upper case A, B for plant N derived from fertilizer N, and uppercase Z and Y, for MBN derived from fertilizer N. Letters not added where no significant differences occurred.

The mean total N content of plant shoots (mg) at maturity was higher for canola plants (canola=536, wheat=462, $p = 0.002$) and both plant species showed an increase in N content in response to increased N fertilization (low=469, moderate=529, $p = 0.006$) (Figure 3-1). There was an increase in the fertilizer N acquired by both species with increased N application ($p \leq 0.001$); however, there was no significant difference between plant species (Figure 3-1). Whilst the proportion of fertilizer N in wheat shoots was

higher than canola (Table 3-1), this did not equal an increase in total uptake of N due to the difference in overall N content (Figure 3-1).

Both canola and wheat had similar shoot biomass at anthesis (Table 3-1), but wheat had higher biomass at maturity (Table 3-1). The addition of N increased shoot biomass at both anthesis and maturity (Table 3-1). The concentration of N in plant shoots was higher in wheat at anthesis (Table 3-1) but higher in canola at maturity (Table 3-1). Both plant species fell below the threshold for adequate N nutrition as defined by Reuter and Robinson (1997) of 1.50% and 1.55%, respectively, for whole shoot N. Thus, in both low- and moderate-N treatments, plants were N-deficient.

3.4.2 Changes in wheat and canola rhizospheres

There was little change in the rhizosphere pH and total mineral N (NO_3^- and NH_4^+ at 0–10 cm depth) of treatments at anthesis and maturity (Table 3-1), except for a small increase in total mineral N in the surface soil of canola treatments at maturity (Table 3-1). The concentration of total N in the rhizosphere soil decreased with increased N application (Table 3-1). Rhizosphere total N decreased in the moderate-N canola treatment compared with the low N treatment, whilst in both wheat treatments rhizosphere total N remained close to the crop species mean (Table 3-1).

Table 3-1 Main and treatment effects with statistical significance ($p \leq 0.05$) of plant shoot biomass, shoot N content, applied fertilizer N in plant shoots, applied fertilizer N in MBN, 0–10 cm total mineral N (NO_3^- and NH_4^+), rhizosphere total N mg kg^{-1}, and rhizosphere pH at anthesis and/or maturity. ns denotes no significant main or treatment effects.

Harvest		Anthesis				Maturity					
Harvest	Plant species	Plant shoot biomass (g)	Shoot N content (%)	0-10 cm Total mineral N (mg kg^{-1})	Rhizosphere pH	Plant shoot biomass (g)	Nitrogen in plant shoots from fertilizer (%)	Nitrogen in MBN from fertilizer (%)	0-10 cm total mineral N (mg kg^{-1})	Rhizosphere total N (mg kg^{-1})	Rhizosphere pH
Crop species	Wheat	28.3	1.19	1.0	6.51	47.9	6.0	6.8	1.07	1575	6.24
	Canola	28.5	1.07	1.2	6.67	44.2	4.9	6.1	1.52	1554	6.18
	p value	ns	<0.001	ns	<0.001	<0.001	<0.001	ns	0.022	ns	ns
N treatment	Low	27.5	1.09	1.0	6.58	43.1	0.2	8.3	1.24	1621	6.22
	Moderate	29.3	1.17	1.1	6.66	49.0	10.7	4.6	1.35	1507	6.19
	p value	<0.001	0.006	ns	ns	<0.001	<0.001	0.003	ns	0.009	ns
Crop species × N treatment	Low wheat	-	-	-	-	-	0.2	-	-	1591	-
	Low canola	-	-	-	-	-	0.2	-	-	1651	-
	High wheat	-	-	-	-	-	11.7	-	-	1558	-
	High canola	-	-	-	-	-	9.7	-	-	1457	-
	p value	ns	ns	ns	ns	ns	<0.001	ns	ns	0.035	ns
	LSD (p = 0.05)	-	-	-	-	-	0.35	-	-	104	-

There was no significant difference in the relative abundance of the bacterial phyla present in the rhizosphere of crop species and the unplanted controls (Figure 3-2). This remained so throughout taxonomic ranks to the genus level. The relative abundance of fungal phyla (Figure 3-2 b) between treatments was significantly different ($p \leq 0.05$). This significance remained in all taxonomic ranks down to the genus level. There was a large decrease in the presence of Chytridiomycota in the rhizosphere of crop species compared with the unplanted control (Figure 3-2 b). Basidiomycota increased in the planted treatments compared with the unplanted treatments and made up a larger proportion of the fungal community in the wheat rhizosphere compared with the canola rhizosphere (Figure 3-2 b).

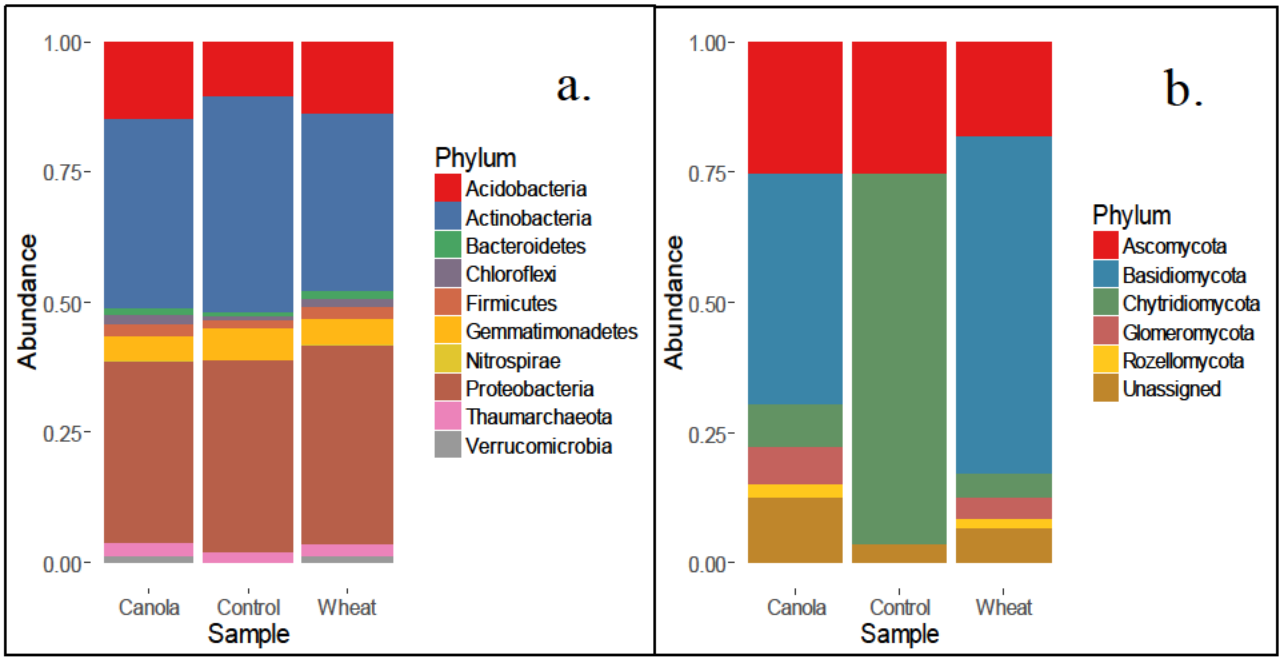

Figure 3-2 Relative abundance of bacterial (a) and fungal (b) phyla in the rhizosphere soil of crop species and unplanted controls at maturity. Phyla with mean relative abundance <0.001% are not shown.

Visualisation of the microbial data showed that the first two principal coordinates explained the majority of variation in rhizosphere samples (Figure 3-3 a–d). The unplanted controls show separation from the planted treatments on at least one of the first two axis for both the weighted and unweighted UniFrac PCoA, demonstrating plants as major determinants for both fungal and bacterial community composition and structure in the rhizosphere. Figure 3-3 a–c shows a noticeable variation between the planted treatments with plant species having no clear effect on structure. However, the unweighted PCoA for fungal communities did indicate plant species as an explanatory variable for differences in community composition for fungi (Figure 3-3 d). More variation in the microbial communities is explained using weighted UniFrac distances, considering the phylogenetic distance and abundance of OTUs, compared with unweighted UniFrac distances that consider only the species present (approximately 76% and 50%, respectively).

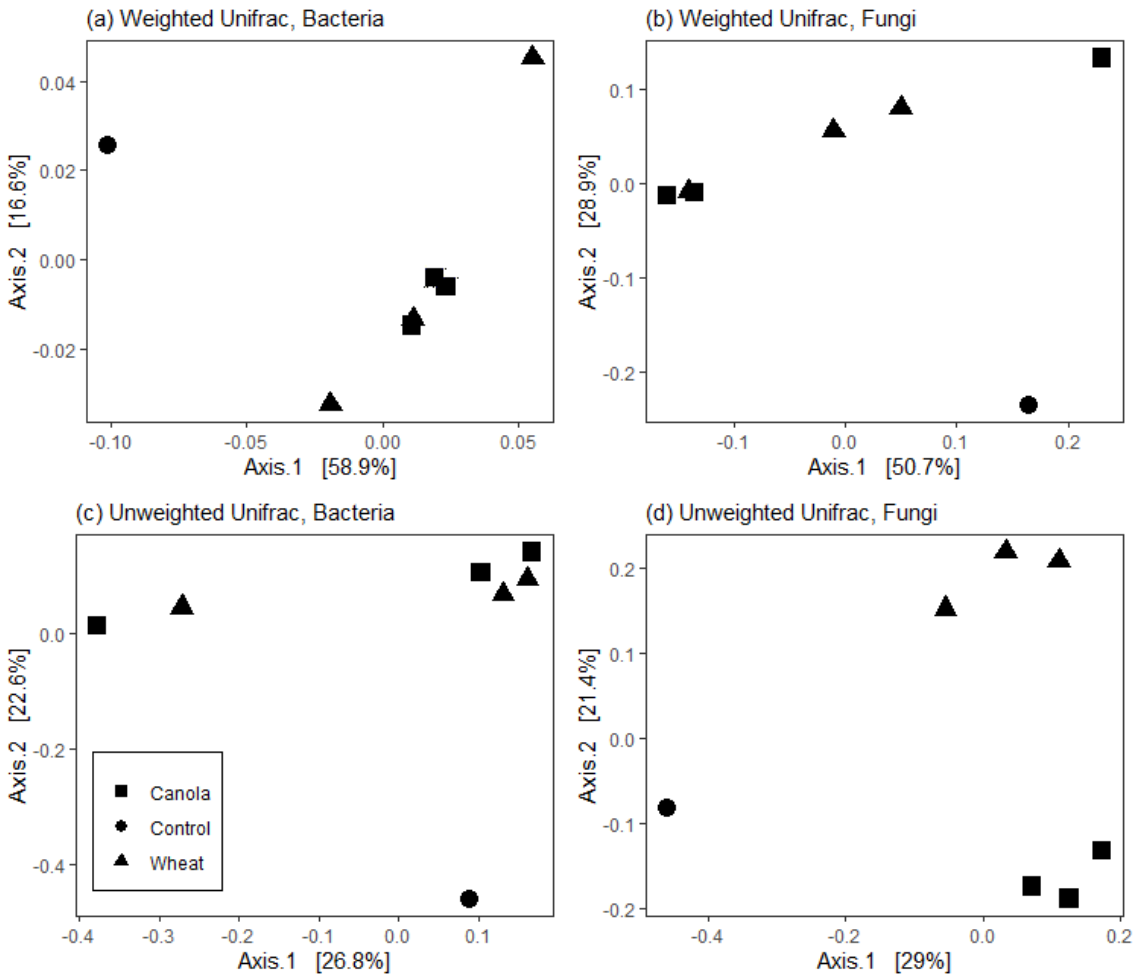

Figure 3-3 Ordination of principal coordinates analysis of weighted (a,b) and unweighted (c,d) UniFrac distances between bacterial (a,c) and fungal (b,d) communities in the rhizosphere of wheat and canola plants at maturity and no plant controls from the moderate N treatments.

There was no difference in the rhizosphere enzyme activities measured except for AG (Figure 3-4). An interaction effect occurred between plant species and N rate (p = 0.036), which lead to a decrease in AG activity in the low-N wheat treatment and no significant difference between species in the moderate N treatment. There was also a noticeable increase in the variation of most enzyme activities (as shown by the increase in the standard error) in the planted moderate N treatments compared with the planted low N treatments (Figure 3-4).

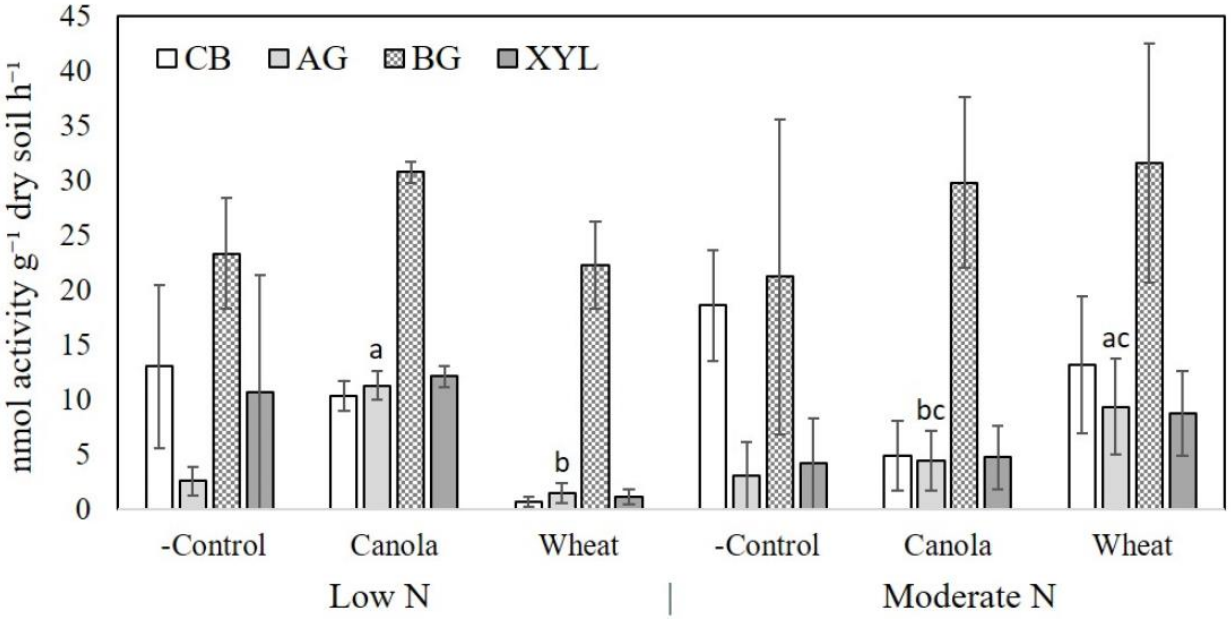

Figure 3-4 Mean extracellular enzyme activity (nmol activity g^{-1} dry soil h^{-1}) of cellulose decomposing enzymes (cellobiase, CB; alpha-glucosidase, AG; beta-glucosidase, BG; and beta-xylosidase, XYL) within the rhizosphere of wheat and canola plants and negative unplanted control for low-N and moderate-N treatments at maturity. Error bars represent mean ± standard error. Letters indicate significant differences ($p \leq 0.05$) between treatments. Letters not added to enzymes where no differences between treatments occurred.

Table 3-2 The relative abundance of fungal OTUs belonging to different trophic modes following wheat, canola and control treatments.

Fungal groups	wheat	canola	control
- (unknown)	38%	49%	12%
Pathotroph	2%	2%	40%
Pathotroph-Saprotroph	4%	4%	1%
Pathotroph-Saprotroph-Symbiotroph	6%	4%	8%
Saprotroph	39%	23%	24%
Saprotroph-Symbiotroph	0%	1%	10%
Symbiotroph	10%	17%	5%

3.5 Discussion

The aim of this study was to ascertain whether canola could acquire more fertilizer N than wheat when grown in soil containing C-rich residues by modifying the rhizosphere communities and reducing immobilization. The results suggest that the competitive ability of wheat and canola to acquire fertilizer N over soil microorganisms is similar despite some differences detected in the rhizosphere microbial populations. The amount of fertilizer N assimilated into plant shoots was similar for both species at both N

application rates. Fertilizer N assimilated into the rhizosphere microbial biomass was likewise similar for both plant species at each N rate. The flow of fertilizer N through the soil–plant system therefore appeared to proceed in a comparable manner for both plant species. It is expected that the proportion of fertilizer acquired by plants increased over the growth period. The temporal advantage of plants over microorganisms in attaining N is due to differences in life/death cycle rates, and the capacity of plants to further accumulate mineralized nutrients (Kuzyakov and Xu, 2013). In our experiment, the amount of N fertilizer in plant shoots and the rhizosphere microbial biomass at maturity was more dependent on the N application rate than on plant species.

The increased amount of existing soil N in canola shoots and rhizosphere microbial biomass suggests that canola may better attain N from soil. The total mass of shoot N not originating from fertilizer was consistently higher in canola shoots compared with wheat (mean difference ~76 mg). Whilst there was no statistically significant difference between the rhizosphere MBN attained from soil, the mean for canola was lower than that of wheat (mean difference ~39 mg kg^{-1}), and the variation decreased with moderate N fertilizer application (reduced standard error with increased N, Figure 3-1). This reduced variation suggests that the competition for N may have been decreasing with increased N availability from fertilizer application. Differences in rhizosphere MBN between wheat and canola may be detectible when adequate N is present or with increased replication of treatments. Given that both plant species were deficient in total shoot N at maturity, it appears that the whole system was N-stressed throughout the experiment. Therefore, the lower mean MBN obtained from soil in the canola rhizosphere may have partially contributed to the increase in canola shoot N. Whilst this mechanism is speculative, the plant shoot N differences between canola and wheat show that canola was consistently better at obtaining soil N.

The results of Ryan et al. (2006) suggest that the composition of *Brassica* tissues and root exudates select soil microbial communities which facilitate increased mineralization of N compared with non-*Brassica* crops. Our results showed that the rhizosphere fungal communities of wheat and canola differed at plant maturity and that the total mineral N in the surface 0–10 cm was higher for canola than wheat (albeit by a small margin). Increased mineralization of soil N throughout the growth period may have facilitated the increase in soil N assimilation seen in canola shoots. Whilst the relative size of the fungal and bacterial populations was not determined, the lack of difference between the rhizosphere bacterial communities suggests that the fungal communities present made an important contribution to the increase in soil N mineralization in the canola treatments.

It is well documented that bacterial rhizosphere communities associated with plant species differ and can even vary between cultivars (Donn et al., 2015). The specificity of fungal communities to plant species is not as evident in the literature (Berg and Smalla, 2009). However, individual species such as mycorrhizae are closely associated to specific plant species (Philippot et al., 2013). We found differences in the relative abundance of fungal phyla present in wheat and canola rhizospheres but no difference in bacterial phyla. Selective pressure for decomposers to proliferate in the surface 10 cm of soil in our treatments would have been high due to the incorporation of C-rich wheat residues. Bacteria may have been outcompeted for soil resources as the most abundant C source was exploited by fungi. Fungi may also suppress bacteria via the production of antibacterial compounds (Boer et al., 2005) which may have adversely affected bacterial growth near hyphae. Basidiomycetes and actinomycetes made up approximately ~69% of the rhizosphere fungal communities of canola (44 and 25%, respectively) and ~82% of wheat plants (64 and 18%, respectively) and microbial degradation of lignin is largely confined to these two phyla (Worrall et al., 1997). Lignin content of wheat straw is variable depending on the cultivar and growth conditions. Summerell and Burgess (1989) examined wheat straw decomposition in the field and the laboratory and reported initial lignin contents of wheat straw ranging from 5.2–11.2% dry matter, which increased as the wheat straw decomposed. As such, elevated lignin content of wheat straw may have enabled basidiomycetes and actinomycetes to flourish, whilst the suppression of bacteria by fungi reduced their proliferation.

The importance of soil microbial communities to N mineralization is contentious as this process is facilitated by most soil microbial species. Blagodatskaya and Kuzyakov (2008) reasoned that differences in the taxonomic composition of the microbial population needs to be linked to differences in the functional activity of the community present to determine the capacity for differential substrate decomposition, including SOM mineralization. We did not see any differences in the enzyme activities measured in soil except for AG, which degrades disaccharides into glucose. Increased activity of extracellular cellulose and lignin degrading enzymes have been linked to increased SOM decomposition (Carreiro et al., 2000; Fontaine and Barot, 2005). We did not observe any difference in BG or CB activity amongst treatments, which are associated with cellulose decomposition, and did not measure ligninolytic activity of rhizosphere soil at maturity.

The fungal relative abundance data suggests that lignin decomposers were dominant in the community at plant maturity. The proportion of the fungal community which was lignin-degrading was larger in the wheat treatments as was the predicted activity of saprotrophs (Table 3-2). These facts do not support the premise that the canola microbial community was responsible for increased mineralization. However, the description of the microbial communities and enzyme activities provided here represent the rhizosphere conditions and

microbes present at maturity and not throughout plant growth, which would have facilitated increased soil N availability to canola. It is expected that microbial communities and enzyme activities changed over time with the successive generations of soil microorganisms in response to the changing rhizosphere conditions during plant growth. As such, whilst the singular sampling date reasonably reports the proportion of fertilizer N in the plant and microbial biomass, it does not allow for the determination of the microbial communities that resulted in the differences in soil N assimilation by plants.

Some of the difference in N content between canola and wheat may be due to the differences in reproductive development and duration of the total growing period. As mentioned previously, plants attain a greater proportion of available N than microorganisms with increased time. Canola has an indeterminate life cycle resulting in a period of prolonged flowering before senescence (Lilley et al., 2019). Conversely, wheat is determinate with highly synchronous flowering and faster senescence. The indeterminate habit of canola means that it still had living shoot and root tissue at the sampling time of maturity for wheat in this experiment. Therefore, it is likely that some of the difference in total N content of plant shoots can be attributed to the prolonged growth period of canola. The extent to which this affected the results, however, is unclear as no difference was observed in fertilizer N uptake, which is also expected to increase if this factor was important. In field conditions, canola growth will be largely terminated when windrowed or desiccated with herbicides, with any increased N-uptake occurring through this mechanism being retained in the system as plant residues.

3.6 Conclusion

The partitioning of fertilizer N into plants and microorganisms appears to occur similarly in wheat and canola in the conditions observed throughout this experiment. Whilst the conditions used are not fully representative of those in the field, the results provide some insight into the source of N acquired by wheat and canola when immobilization pressure is high. No difference in fertilizer uptake was evident between the canola and wheat plants, and immobilization of N fertilizer did not differ in plant rhizospheres. Canola obtained an elevated amount of N from existing soil sources. Reduced mean MBN acquired from soil in the canola rhizosphere may account for some of the difference between the two plant species. Whilst no significant difference in rhizosphere MBN between wheat and canola treatments was observed, insufficient N supply to the system is likely the reason. Differences in the enzyme activities and relative abundance of soil microorganisms were present, although they did not appear to correlate with increased mineralization of soil N by canola. This discrepancy is possibly due to the sampling date not correlating with the actual mineralization events that lead to increased soil N uptake by canola. Future investigations into the effects of plant species on soil N cycling would benefit from considering the influence of microbial succession in

the rhizosphere, and how plant development affects nutrient partitioning. Whilst shorter studies assessing differences before reproductive growth of plants are valuable, they lose a crucial period of increased plant nutrient demand which impacts rhizosphere interactions. As such, including a larger number of sampling intervals would provide insights into these interactions over time. Additionally, including treatments with adequate N for plant growth would allow clearer quantification of the ability of plants to influence soil microorganisms and their requirement for N.

Chapter 4. Nitrogen immobilization and management in the presence of crop residue in Australian cropping systems

4.1 Abstract

Nitrogen (N) is the nutrient most limiting crop production in the world. Low recovery rates and large losses of N fertilizer have increased the environmental cost of food production with environmentally harmful offsite effects. To achieve the global food production goals, more efficient use of fertilizer N which seeks to maximise crop uptake will be critical. Most grain growers in Australia retain crop residues to protect soils from wind and water erosion. Residues with low C:N ratio such as those of wheat (*Triticum aestivum*) cause N-immobilization by soil microorganisms which can negatively affect crop growth, particularly if it occurs at times of high crop N demand. Field trials in 2018 and 2019 were established at Narraport, Birchip and Kalkee in Victoria, Australia to evaluate agronomic interventions intended to reduce N immobilization in residue retained cropping systems. These included crop species choice (wheat vs. canola), timing of N application (sowing vs. mid-season at early stem-elongation) and placement of N (broadcast vs. deep banded). A comparison of crop species (wheat vs. canola, *Brassica napus*) found that canola could accumulate more N, but that this was likely due to longer life cycle rather than suppression of immobilizing soil organisms. Timing of N application (sowing vs. mid-season at early stem-elongation) interacted with species; applications at sowing increased N uptake in canola and mid-season increased N uptake in wheat. In-crop mid-row banding improved crop recovery of ^{15}N-labelled fertilizer in both species compared to broadcast application. Microbial biomass N decreased following canola in the presence of residue compared to wheat which associated with the ability of canola to compete with soil microbes. Further research is required to monitor the levels of *Brassica* species isothiocyanates (ITCs) release in soil and their efficacy on microorganisms in different crop growing stages and study N dynamics with different species in soils of different pH.

4.2 Introduction

Nitrogen (N) is the most limiting nutrient for crop production. Synthetic N fertilizer is required to produce enough food to feed the current global population. Human reliance on N fertilizer will increase with future population growth. Following European colonisation in the late 18th century, grain crop production in Australia relied on N mineralized from native soil organic N. The practice of fallowing assisted in exploiting this resource (Angus and Grace, 2017). Crop yields began to decline at the end of the 19th century as reserves of soil N and other key nutrients began to be exhausted (Kirkegaard and Hunt, 2010). The introduction of pasture legumes in the middle of the 20th century that could be grown in sequence with crops provided a

new source of N from rhizobial N fixation (Donald, 1965). However, in the early 1990s, the wool price dropped and the number of sheep and area of legume pasture in Australia decreased and area of crop increased (Martin et al., 2006; Hunt et al., 2021). This caused a decrease in the proportion of N supplied to crops by legume pastures. Crop yields were able to be maintained by a three-fold increase in use of N fertilizer and grain production currently relies on substantial input of N fertilizer which averages 45 kg ha^{-1} N per year (Angus and Grace, 2017).

Adoption of conservation agriculture in Australia increased rapidly during 1990s due to reduced fuel and labour costs, reduced seeding times and reduced risk of soil erosion (Llewellyn and Ouzman, 2019). In Australia 23.5 million hectares of winter crops are now grown using conservation agriculture principles (Bellotti and Rochecouste, 2014). One of these principles is the part or total retention of crop residues. Crop residues retained on the soil surface can have positive impact on soil structure, nutrient cycling, and C cycling (Magdoff and Weil, 2004).

Despite all the benefits of conservation farming systems, crop residue retention does modify N cycles in a way that can be detrimental to crop production. Cereal crop residues contain more C than N and have high C:N ratio (typically 80:1). Thus, they provide a good source of energy (C) for soil microorganisms, but when microorganisms start decomposing residues, they require mineral N to sustain growth. The N contained in high C:N ratio residue is not sufficient to meet demand, and microbes will immobilize mineral N from the soil. This can include mineral N derived from applied N fertilizer. This immobilization can lead to crop N deficiency, especially early in the growing season when plants are not big enough to compete with microbes, and this has been observed to depress crop yield in some circumstances (Beri et al., 1995; Ichir and Ismaili, 2002). Whilst the N has only been temporarily immobilized by the soil microbial biomass and will be re-mineralized at a later time, it is the acute mismatch between crop demand and N supply caused by immobilization which can reduce crop N uptake and yield in residue retained production systems (Angus, 2001; Liu et al., 2019). A recent simulation study projected that microbial immobilization was the main source of N fertilizer inefficiency in southern Australian cropping systems with retained residues at N application rates currently used by growers (Nasrollahi et al., 2021).

There will be negative economic and environmental impacts associated with the application of N fertilizers and one approach is to improve N-use efficiency (NUE) (Angus and Grace, 2017). In order to achieve water-limited potential yield, more N fertilizer will be required in the future (Sadras and Angus, 2006; Angus and Grace, 2017). Hence, the match between N supply and crop demand will help to improve NUE and less exposure of N fertilizer to immobilization and other losses. In the presence of high C:N ratio of residue,

broadcasting of N fertilizer on the soil surface puts it in close proximity to decaying residues and prone to immobilization. Placing N fertilizers below the soil surface and away from crop residue (deep banding) can reduce immobilization losses by reducing the contact between the fertilizer and soil microorganisms and also decomposing residue (Fenn and Miyamoto, 1981). Wetselaar et al. (1972) suggested that with increased concentration of fertilizer in sub-surface banded N, nitrification will be drastically reduced by high ammonium concentration which slow the conversion of fertilizer N into nitrate (NO_3^-) which may also help prevent immobilization and other losses. Deep banding also puts N into deeper layers of soil which stay moist for longer in dryland systems and can prolong plant access to N relative to broadcasting where N can be trapped away from plant access in dry surface layers of soil (Ma et al., 2009).

The greatest demand for N is when crops are growing rapidly and their leaves are expanding. The greatest N uptake in cereals happens from the beginning of stem-elongation (GS30-31) (Zadoks et al., 1974), the most rapid phase of crop growth (Davidson et al., 1987). Inorganic N levels decline relatively rapidly after applying fertilizer as a result of either crop uptake or immobilization (Recous and Machet, 1999). In the presence of retained residues, applying fertilizer N at sowing gives microbes an opportunity to use N with little competition from the juvenile crop. Therefore, applying fertilizer N at sowing can decrease the availability of N to the crops through immobilization leading to reduced recovery of fertilizer N by a crop (King et al., 2001). Deferring application of fertilizer N to the timing of most rapid crop N uptake allows plants to compete more effectively with microbes and can improve crop recovery (Limaux et al., 1999; Kirda et al., 2001; Harris et al., 2016).

The ability of plants to compete with microbes for mineral N also varies with species. Canola (*Brassica napus*) is the most widely grown broad-leaf grain crop in Australia and its use has increased rapidly in the wetter parts of the southern wheat-belt due to significant yield benefits to subsequent cereal crops through reductions in disease and weed burdens (Angus et al., 2015). In addition to these benefits, availability of soil mineral N has been shown to be higher following canola (Kirkegaard et al., 1999). *Brassica* crops such as canola contain glucosinolates in their tissues which release biocidal compounds (isothiocyanates) during decay when glucosinolates are hydrolysed in soil (Choesin and Boerner, 1991). This can influence the diversity and abundance of microorganisms in the rhizosphere which can have a substantial effect on N cycling (Kirkegaard et al., 1999; Ryan et al., 2006). The accumulation of mineral N following canola can be associated with the release of microbial N in soil (Thompson, 1990) or by a reduction of microorganism activity which reduces the immobilization of mineral N during plant growth (Kirkegaard et al., 1999).

In the present study, we aimed to evaluate agronomic interventions intended to reduce N immobilization and increase crop N uptake in residue retained cropping systems. These included crop species choice (wheat vs. canola), timing of N application (sowing vs. mid-season at early stem-elongation) and placement of N (broadcast vs. deep banded). We hypothesise that canola will suppress microbial immobilization and as a result accumulate more N than wheat in the presence of residue with high C:N ratio. Also, crop N uptake will be higher and immobilization lower in the presence of retained residues when N fertilizer is applied at early stem-elongation compared to at sowing, and when deep-banded compared to broadcast.

4.3 Materials and methods

4.3.1 Field sites

Field experiments were conducted in northwest Victoria, Australia near Narraport (35°56' S ,143°03' E), in 2018, and Birchip (35°57' S, 142°49' E) and Kalkee (36°33' S, 142°12' E) in 2019. The soils at Narraport and Birchip were Calcarosols with a texture of sandy clay loam in the topsoil and a pH of 8.0, and Kalkee was a grey Vertosol with a texture of clay loam in the topsoil (Isbell, 2016) and a pH of 7.6. Previous crops were wheat at Narraport in 2017, barley at Birchip in 2018 and durum wheat at Kalkee in 2018. Soil bulk density was assumed to be 1.2 (t m^{-3}) (MacEwan et al., 2010) and this figure was used for all calculations of soil mineral N.

The amount of residues remaining at each site from the previous crop was estimated at Birchip and Kalkee by collecting all residues within 4 quadrats 0.5 × 0.6 m at Birchip and 4 quadrats 0.42 × 0.5 m at Kalkee and drying at 70°C to constant weight. Remaining residues were estimated to be 1.0 t ha^{-1} at Birchip and 2.0 t ha^{-1} at Kalkee. Residues remaining at Narraport were estimated to be 4.0 t ha^{-1} based on yield of the previous crop and assuming harvesting index of 0.4. Initial soil mineral N to 1 m depth prior to sowing was estimated from bulked soil cores to be 70, 20 and 30 kg N ha^{-1} at Narraport, Birchip and Kalkee, respectively. Total monthly rainfall during the growing season (April-October) in the years of the experiments were 138 mm at Narraport in 2018, and 178 mm at Birchip in 2019 and 254 mm at Kalkee in 2019 (Figure 4-1). The mean April-October maximum temperature at Narraport was 19 °C in 2018 and at Birchip 15 °C and at Kalkee 18 °C in 2019. The mean minimum temperature during the growing season was 5 °C at Narraport and Kalkee and 6 °C at Birchip.

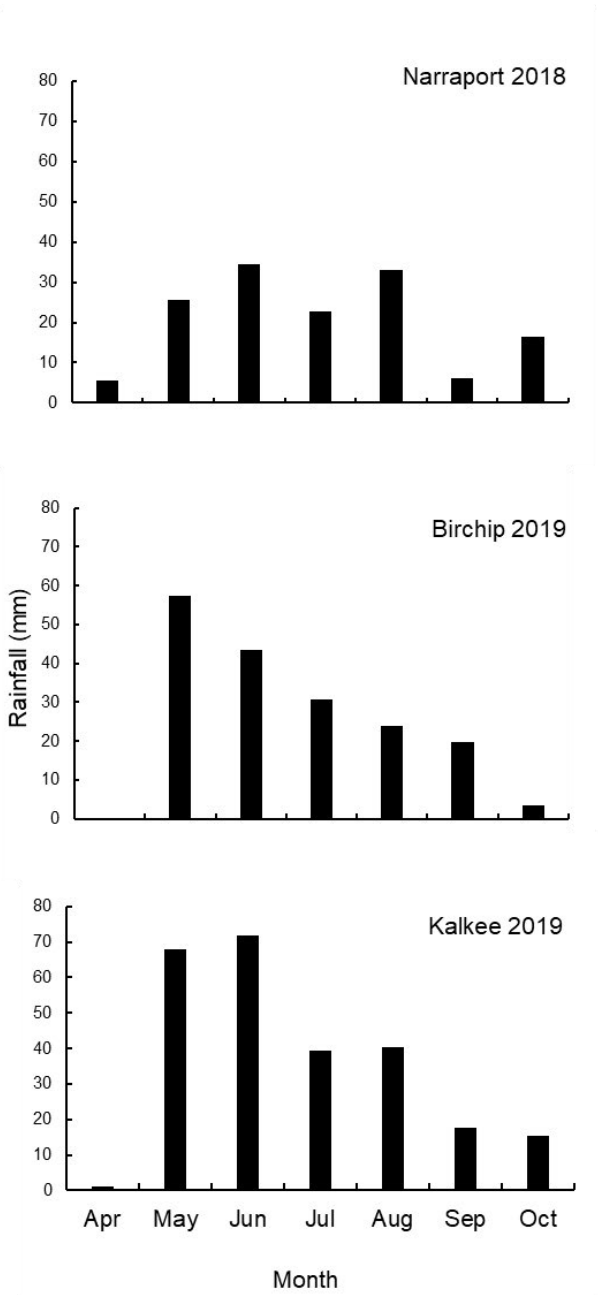

Figure 4-1 Monthly rainfall at Narraport in 2018 and Birchip and Kalkee in 2019.

4.3.2 Experiment 1 (Experimental treatments)

The field experiments were conducted in a rainfed system and consisted of eight treatments (Table 4-1) arranged in a factorial split-split plot design (main-plot residue, sub-plot species, sub-sub-plot placement × timing) with treatments randomly arranged in four blocks (replications) with a plot size of 12 m long and 1.8 m wide.

Table 4-1 Management factors and treatments used in Experiment 1.

Management factor	Treatment 1	Treatment 2
Residues	Residues burnt	Residues retained
Species	Wheat	Canola
N fertilizer timing	Sowing	Mid-season at early stem-elongation
N fertilizer placement	Broadcast	Deep banded

Each experiment also contained 16 control plots (residue × species) to which no N fertilizer was applied. The wheat cultivar used in all experiments was Kord CL and canola hybrid 44Y90 CL. Crops were sown on 25 May 2018 at Narraport, 16 and 21 May 2019 at Birchip and Kalkee. Nitrogen applied at sowing and early stem-elongation (Z31) each time as urea (CH_4N_2O) at the rate of 108 kg ha^{-1} providing 50 kg N ha^{-1}. In this experiment we compared two methods of N supply to wheat and canola. At sowing N was banded below the seed or surface spread and incorporated by sowing and it was mid-row banded (MRB) or surface spread at early stem-elongation (mid-season) at Narraport in 2018. Nitrogen was also MRB or surface spread at sowing and mid-season at Birchip and Kalkee in 2019. Nitrogen was mid-season 85 days after sowing at all experimental sites. It was either spread by hand on the soil surface or MRB with a twin-disc mid-row bander (Wallace, 2019). Banding would have been approximately 5 cm depth which was about the limit of the machine due to the weight/downforce function of the unit.

4.3.3 Biomass collection

Biomass cuts were taken in both species when wheat was at early stem-elongation (GS30-31, July), anthesis (mid-late September) and when both species were physiologically mature (late November to early December). Only early stem-elongation and anthesis samples were taken at Narraport in 2018 as crops failed to produce grain due to low rainfall. Plant samples were collected from 4 rows of each plot (0.6 m^2) at first biomass cut and from 2 rows (0.3 m^2) at the second and third cuts. Samples from the first biomass cut were dried whole at 70 °C for 72 hours. The second and third biomass cuts were partitioned to stems, heads/pods and leaves, and then oven-dried at 70 °C for 72 hours. The canola and wheat biomass at maturity after

harvest were threshed using a stationary thresher and the grains were separated from the chaff ready to be analysed.

4.3.4 Experiment 2 (Experimental treatments)

Experiment 2 was imposed on 1.2 × 1.2 m microplots (four central rows) within the 16 control plots to which no N fertilizer was added in Experiment 1. Each control plot contained two microplots including 4 rows (Figure 4-2). Soil in each of the microplots received ^{15}N-labelled urea (9.15% ^{15}N abundance) and it was applied to the surface of all 4 rows as a surface broadcast (Figure 4-2). In the other microplots, two horizontally cuts between rows 1 and 2 and between rows 3 and 4 were made to 5 cm depth and ^{15}N-labelled urea added to those cuts and soil was then filled and considered as a mid-row banded treatment. This gave a factorial treatment structure with residue, species and placement as factors. All ^{15}N-labelled fertilizer was applied at early stem-elongation (57 days after sowing). Each microplot received 15.60 g labelled urea dissolved in 400 ml water to give an application rate of 50 kg N ha^{-1}.

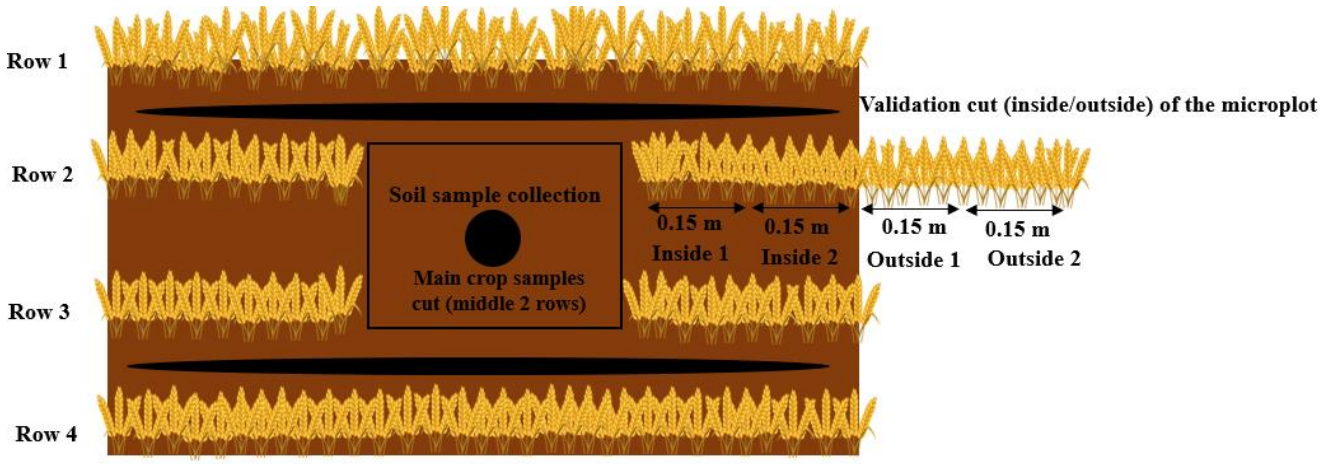

Figure 4-2 Diagram showing the positioning of crop rows, ^{15}N placement in the banded treatments, maturity biomass and soil sample area, validation sample areas in microplots in Experiment 2.

4.3.5 Validation of ^{15}N methodology

The assumption for the validity of our experimental design was that ^{15}N was homogeneously distributed within the centre of the microplots. In our experiment we did not use any physical barriers which can be placed around microplots to reduce transmission risk of ^{15}N. For this reason, crop samples were collected from inside and outside of the ^{15}N microplot areas at Birchip to detect potential lateral movement (Figure 4-2). The samples from inside were taken 0.15 m distance from the centre of the quadrat called “inside 1”

and 0.15 m from inside 1 which called "inside 2" (Figure 4-2). The outside samples were collected 0.15 m distance from the border of quadrat called "outside 1" and 0.15 m distance from outside 1 which called "outside 2" (Figure 4-2).

4.3.6 Crop sampling from the ^{15}N microplot

The main crop samples and validation samples at maturity were taken from the middle 2 rows (0.3 m^2) of each microplots and inside and outside areas respectively. Samples were dried at 70 °C for 48 hours for determination of dry matter. All above-ground plant materials were then ground and ball milled prior to nutrient analysis. Total N and ^{15}N were analysed using a Sercon 20-22 Isotope Ratio Mass Spectrometer with a Europa GSL Sample Preparation system.

4.3.7 Soil sampling

One intact soil core (40 mm) was collected from the centre of each plot following harvest and divided into depths 0-10; 10-40; 40-70- and 70-100 cm depth. Soil samples from ^{15}N microplots were also collected from the centre of each microplots with the same depths (Figure 4-2). All samples were kept at 4°C in sealed plastic bags as soon as they were taken in the field and were then stored in a -20 °C freezer (^{15}N samples were kept at 4 °C) until analysis. Preliminary analysis was done on all depths of soil samples which were collected from microplots, and then full analysis was only done to 40 cm depth.

4.3.8 Analytical procedures

The N% of plant tissues from the anthesis sampling time was determined through a 2400 Series II CHNS/O Elemental Analyser (PerkinElmer, Waltham, USA). Soil samples from both experiments were dried at 40°C. The dried soil was sieved, ground and thoroughly mixed. Analysis of soil NO_3^- and NH_4^+ proceeded following extraction with 0.5 M K_2SO_4 (shaking end-on-end for 1 hour), centrifugation (3000 rpm for 5 min) and filtering supernatant through Whatman #42 filter paper (Whatman International, Maidstone, England). Extracts were stored at -20 ^oC before determination of extractable NO_3^- and NH_4^+ using the flow-injection analyser via Quikchem 8500 Series II (Lachat Instruments, Loveland, USA) system as detailed in the Quikchem manuals (12-10706-2-F and 12-107-04-1- B, respectively).

Topsoil (0-10 cm) microbial biomass was determined by the chloroform fumigation-extraction method as described by Vance et al. (1987). Briefly, 16 g of soil was used for the fumigated treatments which were incubated in a desiccator with approximately 50 ml of ethanol-free chloroform for 24 hours at 25 ^oC in darkness. Fumigated and non-fumigated soil samples were extracted using 0.5 M K_2SO_4 solution in a soil-to-solution ratio of 1:5 (8 g soil: 40 ml extraction). Samples were extracted by filtration through Whatman #42 and extracts were kept in -20 ^oC. Organic C in the extracts was measured using a TOC analyser (GE

Sievers Innovox Laboratory, Boulder, USA). Microbial biomass C is expressed as the difference between fumigated and non-fumigated samples. A conversion factor (k factor) of 0.45 was used for calculating the biomass from the extraction (Jenkinson, 1988).

4.3.9 Calculation of ^{15}N samples

The percentage recovery of applied fertilizer ^{15}N in plants, plant fertilizer uptake and the residual soil fertilizer was calculated according to the following formula (Conyers et al., 2011),

$$X\% = 100 \times [(TN)(c - 0.3663)]/[(FN)(f - 0.3663)] \quad (1)$$

where TN is the total quantity of N in the plants (mg N per quadrat), c is the $^{15}N\%$ in the plant samples, FN is the total amount of fertilizer N applied (mg N per quadrat), f is the $^{15}N\%$ in the fertilizer (9.15 %) and 0.3663 is the assumed natural abundance of ^{15}N.

4.3.10 Statistical analyses

The experiments were a split-split plot design with residue treatment assigned to whole plot, species assigned to sub-plot and N timing and placement assigned to sub-sub-plot level. Plant and soil data (total ^{15}N recovery, crop biomass, crop yield, N uptake, grain protein and microbial biomass C) were analysed using linear mixed models with residual maximum likelihood estimation using the software GenStat version 19 (VSN International Limited, Hampstead, UK). Treatment variances were homogeneous and residuals fitted normal distributions. Differences between untreated controls and experimental treatments were analysed to confirm that adding nitrogen significantly affected the response variable, then untreated controls were discarded, and the data analysed as a balanced split-split plot design to investigate difference between treatments. Significant differences between means of main factor effects and their interactions were compared at 0.05 level of significance using Fisher's least significant difference.

4.4 Results

4.4.1 Narraport (2018)

Canola had higher N uptake, shoot N concentration and shoot biomass at early stem-elongation than wheat at Narraport in 2018 (Table 4-2). Broadcast N application increased N uptake and shoot biomass compared to banding. There was a significant interaction between crop species and residue on shoot biomass and N uptake. Burning increased shoot biomass and N uptake relative to retaining residue in canola but had no effect in wheat (Table 4-2).

Table 4-2 Main effects, selected interactions and statistical significance ($p \leq 0.05$) of species, N placement and residue on shoot biomass, shoot N concentration and shoot N uptake at early stem-elongation at Narraport in 2018.

		Shoot biomass (kg ha^{-1})	Shoot N concentration (mg g^{-1})	Above-ground crop N uptake (kg ha^{-1})
Species	Wheat	422	42	18
	Canola	584	50	28
	p value	0.024	<0.001	<0.001
N placement	Banded	396	43	19
	Broadcast	610	42	27
	p value	<0.001	0.704	<0.001
Residue	Retained	466	48	22
	Burnt	540	44	24
	p value	0.31	0.044	0.367
Residue × species	Retained Wheat	445	43	19
	Burnt Wheat	399	41	16
	Retained Canola	487	53	25
	Burnt Canola	682	47	32
	p value	0.016	0.142	0.022
	LSD (p = 0.05)	147	4	5

Canola had higher N uptake and shoot N concentration than wheat at anthesis at Narraport in 2018 (Table 4-3). Mid-season N at early stem-elongation decreased N uptake and shoot N concentration compared to application N at sowing. Reduced crop N uptake of mid-season N was reflected in higher (p = 0.006) soil mineral N measured at the end of the season in mid-season treatments (86 kg ha^{-1}) compared to sowing treatments (57 kg ha^{-1}). There was a significant interaction between crop species and N timing on shoot N concentration and N uptake (Table 4-3). Mid-season decreased N uptake and shoot N concentration in canola compared to sowing application. A significant interaction was observed between N timing and N placement on shoot biomass and shoot N concentration. Broadcasting N at sowing increased biomass compared to broadcasting at mid-season. Banding N at mid-season decreased shoot N concentration compared with banding N at sowing.

Table 4-3 Main effects, selected interactions and statistical significance ($p \leq 0.05$) of species and N timing on shoot biomass, shoot N concentration and N uptake at anthesis at Narraport in 2018.

		Shoot biomass (kg ha^{-1})	Shoot N concentration (mg g^{-1})	Above-ground crop N uptake (kg ha^{-1})
Species	Wheat	1346	20	27
	Canola	1117	44	49
	p value	0.084	<0.001	<0.001
N timing	Sowing	1294	35	44
	Mid-season	1169	29	32
	p value	0.196	<0.001	<0.001
N Placement	Broadcast	1266	32	39
	Banded	1197	32	37
	p value	0.480	0.696	0.465
Residue	Burnt	1245	31	38
	Retained	1218	33	38
	p value	0.783	0.136	0.989
Timing × species	Sowing canola	1219	49	60
	Mid-season canola	1016	38	38
	Sowing wheat	1370	22	29
	Mid-season wheat	1322	19	25
	p value	0.422	<0.001	0.003
	LSD (p = 0.05)	306	3	8
Timing × placement	Sowing banded	1142	36	42
	Mid-season banded	1253	27	32
	Sowing broadcast	1447	34	46
	Mid-season broadcast	1084	30	32
	p value	0.017	0.009	0.518
	LSD (p = 0.05)	270	3	8

4.4.2 Birchip (2019)

Wheat had higher (p = 0.039) shoot biomass at early stem-elongation (712 kg ha^{-1}) compared to canola (613 kg ha^{-1}) at Birchip in 2019, but there was no significant effect of species on shoot N concentration or N uptake at this time. In contrast, by anthesis canola had higher shoot N concentration (19 vs. 13 mg g^{-1}, p < 0.001) and N uptake (86 vs. 58 kg ha^{-1}, p < 0.001) compared to wheat but there was no difference in biomass. The difference between species in shoot N concentration and N uptake persisted until maturity, but by this point wheat had significantly more biomass than canola (Table 4-4). Nitrogen application at sowing increased shoot biomass and N uptake at maturity compared to mid-season (Table 4-5). There was a significant interaction between crop species and N timing on N uptake and shoot biomass. Applying N at sowing compared to mid-season increased biomass and N uptake in canola, but there was no significant effect of N timing in wheat.

Table 4-4 Main effects, selected interactions and statistical significance ($p \leq 0.05$) of species and N timing on shoot biomass, shoot N concentration and shoot N uptake at maturity at Birchip in 2019.

		Shoot biomass (kg ha^{-1})	Shoot N concentration (mg g^{-1})	Above-ground crop N uptake (kg ha^{-1})
Species	Canola	8939	33	305
	Wheat	10327	21	200
	p value	0.002	<0.001	<0.001
N timing	Sowing	10153	27	270
	Mid-season	9114	26	234
	p value	0.018	0.756	0.018
Species × timing	Canola sowing	10061	33	344
	Canola mid-season	7817	32	265
	Wheat sowing	10244	21	196
	Wheat mid-season	10410	20	204
	p value	0.007	0.940	0.005
	LSD (p = 0.05)	1206	4	41

Canola had higher ($p < 0.001$) grain N concentration and grain N content than wheat at maturity despite lower grain yield (Table 4-5). Banding increased grain yield and N content relative to broadcasting. There was an interaction between crop species and N timing on grain yield, N concentration and N content. Mid-season increased grain yield, grain N concentration and content in wheat relative to sowing application, but decreased yield and N concentration and had no effect on N uptake in canola. There was no significant difference in soil mineral N at this site following harvest.

Table 4-5 Main effects, selected interactions and statistical significance ($p \leq 0.05$) of species and N placement on grain yield, grain N concentration, grain N content and hand cut grain yield at maturity at Birchip in 2019.

		Hand cut grain yield (kg ha^{-1})	Grain N concentration (mg g^{-1})	Grain N content (kg ha^{-1})
Species	Canola	2586	30	74
	Wheat	3687	16	61
	p value	<0.001	<0.001	0.003
N Placement	Banded	3264	23	72
	Broadcast	3008	23	63
	p value	0.010	0.237	0.039
Species × timing	Canola sowing	2844	31	78
	Canola mid-season	2327	30	71
	Wheat sowing	3393	16	53
	Wheat mid-season	3981	17	68
	p value	<0.001	0.021	0.017
	LSD (p = 0.05)	435	1	12

4.4.3 Kalkee (2019)

There was no significant difference in biomass, N concentration or N uptake between crop species at early stem-elongation at Kalkee in 2019 (Table 4-6). There was a main effect of residue treatment on biomass and N content, but most importantly, there was an interaction between residue and crop species at early stem-elongation (Table 4-6). Burning residue significantly increased shoot biomass and N uptake of canola but had no effect on those of wheat.

Table 4-6 Main effects, selected interactions and statistical significance ($p \leq 0.05$) of species and residue on shoot biomass, shoot N concentration and shoot N uptake at early stem-elongation at Kalkee in 2019.

		Shoot biomass (kg ha^{-1})	Shoot N concentration (mg g^{-1})	Above-ground crop N uptake (kg ha^{-1})
Species	Canola	249	50	13
	Wheat	274	53	15
	p value	0.377	0.090	0.341
Residue	Burnt	322	50	17
	Retained	201	53	11
	p value	<0.001	0.242	0.005
Residue × species	Burnt canola	362	47	19
	Retained canola	137	52	8
	Burnt wheat	283	54	16
	Retained wheat	265	53	15
	p value	0.001	0.202	0.014
	LSD (p = 0.05)	80	6	6

The main effect of species and residue on some parameters remained at anthesis (Table 4-7), but the interaction between the two was no longer significant (p = 0.961).

Table 4-7 Main effects, selected interactions and statistical significance ($p \leq 0.05$) of species and residue on shoot biomass, shoot N concentration and shoot N uptake at anthesis at Kalkee in 2019.

		Shoot Biomass (kg ha^{-1})	Shoot N concentration (mg g^{-1})	Above-ground crop N uptake (kg ha^{-1})
Species	Canola	3509	21	75
	Wheat	4241	28	121
	p value	<0.001	0.003	<0.001
Residue	Burnt	3877	22	95
	Retained	3874	26	101
	p value	0.986	0.017	0.256

At maturity shoot biomass was significantly higher in wheat than canola but canola had higher shoot N concentration and N uptake (Table 4-8). Shoot N concentration was significantly greater with banding compared to broadcasting (28 vs. 23 mg g^{-1}, p = 0.004) but there was no difference in biomass or N uptake (Table 4-8). There was a significant interaction between crop species, N timing and placement. In canola,

banding at early stem-elongation was most effective at promoting N concentration, but banding at sowing was more effective in wheat. The differences between means were small, and there was no effect on biomass or N uptake.

Table 4-8 Main effects, selected interactions and statistical significance ($p \leq 0.05$) of species and N placement on shoot biomass, shoot N concentration and N uptake at maturity at Kalkee in 2019.

		Shoot biomass (kg ha^{-1})	Shoot N concentration (mg g^{-1})	Above-ground crop N uptake (kg ha^{-1})
Species	Canola	6788	34	227
	Wheat	8641	22	187
	p value	<0.001	<0.001	0.001
N Placement	Banded	7650	28	212
	Broadcast	7779	23	203
	p value	0.717	0.004	0.449
Species × timing × placement	Canola sowing banded	7349	33	244
	Canola sowing broadcast	6435	34	218
	Canola mid-season banded	6197	35	213
	Canola mid-season broadcast	7172	33	234
	Wheat sowing banded	8528	24	205
	Wheat sowing broadcast	8288	20	167
	Wheat mid-season banded	8252	22	184
	Wheat mid-season broadcast	9222	21	191
	p value	0.507	0.008	0.963
	LSD ($p = 0.05$)	1433	2	47

Canola yielded less grain than wheat but had higher grain N concentration and equivalent grain N content (Table 4-9). Grain yield was significantly ($p = 0.025$) higher in mid-season treatments relative to sowing application (2817 vs. 2546 kg ha^{-1}, $p = 0.025$), but there was no effect on grain N concentration or N content. Banding increased grain N concentration (32 vs. 21 mg kg^{-1}, $p = 0.008$), but not yield or grain N content. There were no significant differences in soil mineral N after harvest.

Table 4-9 Main effects ($p \leq 0.05$) of species on grain yield, grain N concentration and grain N content at maturity at Kalkee in 2019.

		Hand cut grain yield (kg ha^{-1})	Grain N concentration (mg g^{-1})	Grain N content (kg ha^{-1})
Species	Canola	2002	31	61
	Wheat	3362	18	60
	p value	<0.001	<0.001	0.809

4.4.4 Experiment 2

4.4.4.1 Plant biomass, grain yield and nitrogen uptake

Wheat yield obtained from the microplots was significantly higher than canola at Birchip and Kalkee (Table 4-10 and Table 4-11). Canola yield was 54% lower than wheat yield at Kalkee. It was 70% higher at the Kalkee site than the Birchip site. At Birchip, canola had higher shoot N concentration, grain N concentration and content, and N uptake (Table 4-10). At Kalkee, canola had higher shoot N concentration and grain N concentration, but not grain N content or N uptake. At Kalkee banding N significantly increased grain yield, shoot biomass, grain N content and N uptake compared to broadcast application, but there was no effect at Birchip. There was a significant interaction between residue and N placement on biomass, and a near-significant effect on yield and N uptake at Kalkee. Banding increased biomass to a greater extent when residue was retained compared to when it was burnt, and yield and N uptake followed the same pattern.

Table 4-10 Main effects and statistical significance ($p \leq 0.05$) of species on grain yield, shoot biomass, shoot N concentration, grain N concertation, grain N content and total N at Birchip in 2019.

		Shoot biomass (kg ha^{-1})	Grain yield (kg ha^{-1})	Shoot N concentration (mg g^{-1})	Grain N concentration (%)	Grain N content (kg ha^{-1})	Above-ground N uptake (kg ha^{-1})
Species	Wheat	10773	3728	19	1.63	61	77
	Canola	9404	2607	33	3.02	82	94
	p value	0.008	<0.001	<0.001	<0.001	0.002	0.025

Table 4-11 Main effects, selected interactions and statistical significance ($p \leq 0.05$) of species on the fate of urea-^{15}N grain yield, dry biomass, shoot N concentration, grain N concertation, grain N content and total N at Kalkee in 2019.

		Shoot biomass (kg ha^{-1})	Grain yield (kg ha^{-1})	Shoot N concentration (mg g^{-1})	Grain N concentration (%)	Grain N content (kg ha^{-1})	Above-ground N uptake (kg ha^{-1})
Species	Wheat	8679	3456	20	1.79	57	73
	Canola	6591	1887	33	3.08	60	69
	p value	<0.001	<0.001	<0.001	<0.001	0.485	0.302
N placement	Banded	8543	3021	27	2.50	67	81
	Broadcast	6727	2321	26	2.37	50	61
	p value	<0.001	<0.001	0.060	0.147	<0.001	<0.001
Residue × placement	Burnt banded	8048	2825	27	2.55	61	74
	Burnt broadcast	7129	2414	26	2.33	50	62
	Retained banded	9037	3218	27	2.46	73	89
	Retained broadcast	6325	2229	27	2.42	50	60
	p value	0.045	0.066	0.566	0.591	0.076	0.058
	LSD (p=0.05)	1236	437	2	0.1	10	12

There was some movement of ^{15}N to areas outside the application area, but this declined rapidly after 15 cm outside the quadrat and was approaching natural abundance by 30 cm outside the quadrat. This suggests that wheat plants access little ^{15}N from further than 30 cm, and that plant samples taken from the centre of the microplot should be an accurate estimate of plant recovery. The ^{15}N% in the plant samples collected from outside 1 was 23% and the one from outside 2 contained 7% of ^{15}N. Samples from inside 1 and inside 2 contained 32% and 38% of ^{15}N respectively (Figure 4-3).

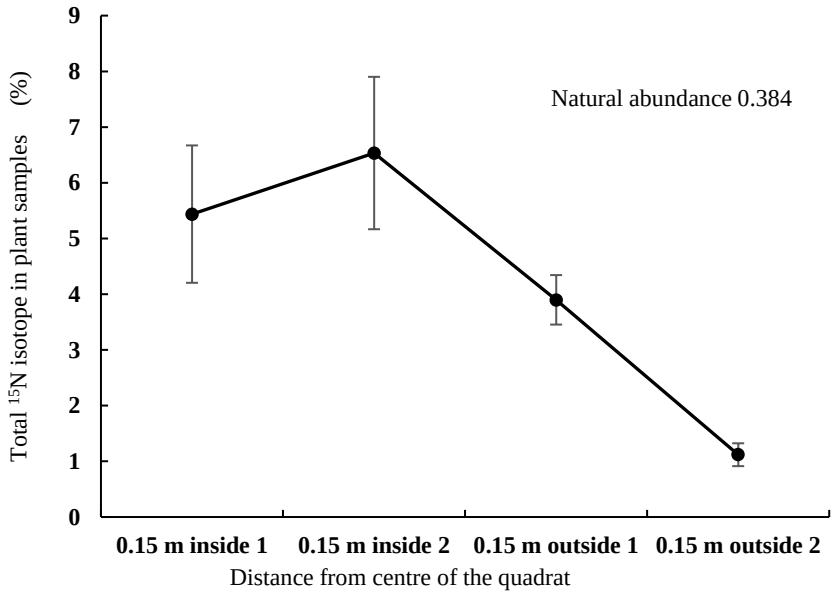

Figure 4-3 Urea-^{15}N isotope in plant samples inside/outside of quadrat with the natural abundance of 0.384 at Birchip in 2019. Error bars represent mean ± standard error.

There was some movement of ^{15}N to the soil below 40 cm depth of soil. The ^{15}N recovered below 40 cm depth was 7% at Birchip and 20% at Kalkee (Table 4-12 and Table 4-13). Most residual ^{15}N-urea remained above 40 cm depth which was 93% at Birchip and 80% at Kalkee. In general, results showed that the concentration of residual fertilizer ^{15}N-urea decreased along with depth (Table 4-12 and Table 4-13). In our experiment, movement of ^{15}N tracer below 40 cm was one of the main factors leading to an underestimation of actual rates of ^{15}N fertilizer recovery in plants.

Table 4-12 Urea-^{15}N content of sub-samples to the depth of 0-100 cm soil at Birchip in 2019.

Residue	Species	Placement	Depth (cm)	Residual fertilizer urea-^{15}N (kg ha^{-1})	SE
Burnt	Wheat	Broadcast	0-10	6.4	0.105
Burnt	Wheat	Broadcast	10-40	0.6	0.022
Burnt	Wheat	Broadcast	40-70	0.2	0.001
Burnt	Wheat	Broadcast	70-100	0.2	0.005

Table 4-13 Urea-^{15}N content of sub-samples to the depth of 0-100 cm soil at Kalkee in 2019.

Residue	Species	Placement	Depth (cm)	Residual fertilizer urea-^{15}N (kg ha^{-1})	SE
Burnt	Wheat	Broadcast	0-10	1.2	0.011
Burnt	Wheat	Broadcast	10-40	1.1	0.035
Burnt	Wheat	Broadcast	40-70	0.5	0.009
Burnt	Wheat	Broadcast	70-100	0.1	0.002

4.4.4.2 Plant ^{15}N recovery

Compared to surface application, mid-row banding significantly increased total N recovery in the plant-soil system at both Birchip and at Kalkee compared to surface application (Table 4-14 and Table 4-15). There was also an interaction between crop species and N placement on plant ^{15}N recovery at Birchip. Mid-row banding, relative to the surface application, increased ^{15}N recovery in canola to a greater extent than in wheat at Birchip (Table 4-14). There was no interaction between species and placement at Kalkee, but the percentage of total N recovery in the plant-soil system was higher in wheat compared to canola despite no difference in plant ^{15}N recovery (Table 4-15).

4.4.4.3 Distribution of residual urea-^{15}N in soil

The residual urea-^{15}N in the 0-40 cm of soil was significantly greater following wheat compared to canola at Birchip (Table 4-14). The same result was observed at Kalkee where the residual urea-^{15}N was significantly higher in wheat plots than canola plots (Table 4-15). Mid-row banding N significantly ($p <$ 0.001) increased residual urea-^{15}N in the soil at Birchip and Kalkee. At Birchip there was a significant interaction between crop species and N placement. Banding N increased soil N recovery in wheat but not canola.

Table 4-14 Main effects, selected interactions and statistical significance ($p \leq 0.05$) of N placement on total urea-^{15}N recovery and plant N recovery at Birchip in 2019.

		Total N recovery (%) (Plant+Soil)	Plant ^{15}N recovery (%)	Residual fertilizer urea-^{15}N (kg ha^{-1}) (0-40 cm)
Species	Wheat	57	39	20
	Canola	53	42	13
	p value	0.296	0.185	0.003
N placement	Banded	67	49	21
	Broadcast	43	32	12
	p value	<0.001	<0.001	<0.001
Species × placement	Canola banded	65	53	14
	Canola broadcast	42	31	12
	Wheat banded	70	44	29
	Wheat broadcast	44	33	12
	p value	0.740	0.047	0.005
	LSD (p = 0.05)	14	11	7

Table 4-15 Statistical significance ($p \leq 0.05$) of species and N placement on total urea-^{15}N recovery and plant N recovery at Kalkee in 2019.

		Total N recovery (%) (Plant+Soil)	Plant ^{15}N recovery (%)	Residual fertilizer urea-^{15}N (kg ha^{-1}) (0-40 cm)
Species	Wheat	64	46	21
	Canola	51	40	12
	p value	0.015	0.089	0.018
N placement	Banded	75	52	25
	Broadcast	40	35	7
	p value	<0.001	<0.001	<0.001

4.4.4.4 Microbial biomass

There was an interaction between residue and crop species on MBN at Birchip (Table 4-16) but there was no significant difference observed in microbial biomass C (MBC) (Table 4-17). The MBN was significantly higher under retained residue in wheat compared to canola. A significant interaction was found between residue and N placement at Kalkee (Table 4-18). When the residue was retained, mid-row banding significantly (p = 0.027) increased MBN (10 mg kg^{-1}) compared to surface broadcast (4 mg kg^{-1}).

Table 4-16 Statistical significance ($p \leq 0.05$) of species and residue on microbial biomass N (MBN) at Birchip in 2019.

		MBN (mg kg^{-1})
Residue × species	Burnt canola	7
	Burnt wheat	4
	Retained canola	5
	Retained wheat	12
	p value	0.041
	LSD ($p = 0.05$)	7

Table 4-17 Selected interaction of residue and species on microbial biomass C (MBC) at Birchip in 2019.

		MBC (mg kg^{-1})
Residue × species	Burnt canola	269
	Burnt wheat	285
	Retained canola	267
	Retained wheat	326
	p value	0.294
	LSD ($p = 0.05$)	85

Table 4-18 Selected interaction of residue and placement on microbial biomass N (MBN) at Kalkee in 2019.

		MBN (mg kg^{-1})
Residue × placement	Burnt banded	5
	Burnt broadcast	6
	Retained banded	10
	Retained broadcast	4
	p value	0.027
	LSD ($p = 0.05$)	5

4.5 Discussion

4.5.1 Experiment 1

4.5.1.1 Species × residue interaction

There was a negative impact of retained wheat residue on canola biomass and above-ground N uptake at stem-elongation at Narraport in 2018 and Kalkee in 2019. Retained wheat residue has been reported to reduce crop establishment and slow seedling growth in canola (Bruce et al., 2003). Residue did not reduce plant establishment in our experiment (Table 4-19 Supplementary), but it is likely that the cooler microclimate produced by residue and the different positions of apical meristems in wheat and canola described by Bruce et al. (2003) slowed early growth in canola, which led to lower initial biomass and N uptake. At Birchip in 2019, canola had less biomass than wheat when measured at early stem-elongation, but there was no interaction between species and residue, possibly due to the very low residue load at this site.

At Narraport and Birchip, canola had higher tissue N concentration and thus higher total N uptake by anthesis. At Kalkee, canola still had lower N uptake by anthesis, but higher at maturity. Whilst higher N uptake in canola over the length of the season is consistent with our hypothesis that canola would better compete with microbes for mineral N, there was no interaction with residue retention, indicating that higher N demand or longer life cycle could be responsible for the difference between species rather than competitive ability (Rigby et al., 2021).

4.5.1.2 Species × Timing interaction

At Narraport, application of N at sowing was more effective at increasing N uptake than mid-season at early stem-elongation in both species. This was contrary to our hypothesis that N uptake would be higher if fertilizer was applied at early stem-elongation as rapidly growing plants could better compete for N. This result was likely due to the very dry season in 2018 and little (2 mm) rainfall in the month following mid-season application. Significant rainfall is required following mid-season urea for effective N recovery in both wheat and canola to move the fertilizer into the soil (Motley et al., 2001). This result is consistent with the variable yield response to mid-season vs. sowing application reported by Porker et al. (2019) in a similar environment, who found that sowing application was more effective at promoting yield in dry environments and mid-season more effective in wet environments. Banding was able to offset the biomass reduction due to mid-season application by placing urea below the soil surface where it is more accessible to roots without follow-up rainfall (Ma et al., 2009). Canola was more sensitive to N timing than wheat.

At Birchip in a season with more rainfall in the month following mid-season (31 mm), wheat biomass and N uptake at maturity were unaffected by N timing, whereas mid-season reduced both in canola. Similarly, Kaefer et al. (2015) observed that mid-season increased grain yield and grain N content in wheat but decreased both in canola. They found an advantage with a single application of N at sowing, compared with the application as mid-season.

There are several plausible reasons why canola may be more sensitive to N timing than wheat. This could include differences in surface root proliferation and the ability of the two different species to effectively extract N from the surface layers of soil. The root length density of wheat and canola has been studied by Cutforth et al. (2013) who found that the percentage of roots above the 40 cm depth was about 64% for spring wheat and 60% for canola and higher root length density below 100 cm was found in canola compared to wheat. Differences in above-ground crop architecture may also affect how urea granules, rainfall or dew may distribute across the row and interrow when broadcast. The more upright architecture of wheat may cause both to concentrate in the crop row, facilitating plant uptake (Richards et al., 2019). Perhaps the most likely explanation is the different species' pattern of N uptake. By early stem-elongation, canola has reached maximum leaf area, and N uptake begins to slow whilst wheat is still producing leaves on the main stem as well as tillers and is just at the start of the period of most rapid N uptake (Mason and Brennan, 1998). Mid-season at the start of stem-elongation therefore coincides with the start of the period of high N demand in wheat, but declining N demand in canola. Which of these mechanisms is responsible for this observation needs to be tested in further research, but a firm recommendation for growers would be to apply most N fertilizer to canola at sowing and wheat during stem-elongation, particularly in higher rainfall environments.

4.5.1.3 Effect of banding

Grain N content and yield were greater when N was banded compared to broadcast application at Birchip. At Kalkee banding increased shoot N concentration at anthesis, but this was the only significant main effect. The interaction with species and timing suggested that banding increased N uptake in wheat at early stem elongation and canola at sowing, but there was no effect at inverse timing. There was no interaction between banding and residue, which does not support our hypothesis that banding would be more beneficial in residue retained systems.

4.5.2 Experiment 2

4.5.2.1 Effects of N placement and crop species

Mid-row banding significantly increased shoot biomass, grain yield, grain N content and total N compared to surface broadcast at Kalkee (Table 4-15). Delay in nitrification by concentrating ammonium in bands

reduces the risk of losses by denitrification and leaching (Wetselaar et al., 1973; Angus et al., 2014). Banding N increased total N recovery (67%) at Birchip and (75%) at Kalkee. Similarly, Malhi and Nyborg (1992) found that when urea was banded compared to broadcasting, N recovery and yield were both increased. Sandral et al. (2017) reported that grain recovery of N from mid-row banded urea applied at sowing was greater (52%) than broadcasting at stem-elongation (45%) and at sowing (36%). Wallace (2019) found that banding N significantly increased total recovery of applied N from 60% to 80%. Chen et al. (2016) reported that at the N rates of 60 and 240 kg ha^{-1} the residual ^{15}N was higher for banding (34 and 109 kg ha^{-1}, respectively) than for surface broadcast application (30 and 88 k/ha, respectively). The positive interaction between crop species and N placement at Birchip showed that mid-row banding in canola significantly increased plant ^{15}N recovery (53%) compared to broadcasting (31%) (Table 4-14). This was probably due to the high fertilizer N uptake by canola which led to much lower residual fertilizer urea ^{15}N remaining in the soil under canola grown than wheat at Birchip and Kalkee (Table 4-14 and Table 4-15). During stem elongation, N uptake is most rapid and unlike wheat, canola is able to continue N uptake past this time (Norton, 2016). No interaction observed between species and N placement on plant ^{15}N recovery and residual fertilizer ^{15}N at Kalkee. This indicates that the effects of species on plant N recovery and residual fertilizer do not depend on the N placement. It is hard to say why this interaction between species and placement did not happen at Kalkee but this could be affected by a different amount of rainfall both experimental sites received after N application, soil moisture and N content at the time of N application, longer growing season at Kalkee compared to Birchip and also the higher residue load at this site.

No significant difference was found between treatments in microbial biomass C (MBC) in the top 10 cm soil. However, MBN was significantly affected by the interaction of residue and crop species at Birchip and residue and N placement at Kalkee. The MBN was significantly higher in residue retained treatments following wheat (12 mg kg^{-1}) than when residue was burned (4 mg kg^{-1}) at Birchip. Sustained increases in microbial biomass have been observed following many seasons of residue incorporation compared with burning (Powlson et al., 1987; Thompson, 1992; Bird et al., 2001). Residue retention with banding N significantly increased MBN content (10 mg kg^{-1}) compared to surface broadcast (4 mg kg^{-1}) at Kalkee (Table 4-18), which is in contrary to our hypothesis that banding N reduces immobilization when residue is retained. At Birchip when the residue was retained, MBN was higher under wheat than under canola (Table 4-16), which was consistent with our hypothesis that canola would better compete for N with microbes and provide a condition for less immobilization. Greater residual fertilizer urea ^{15}N remained in the soil when N was banded at Kalkee after harvest, implying that any improved N uptake due to banding was likely due to reduced losses rather than reduced immobilization.

4.6 Conclusion

It is clear from this study that the retention of crop residue increases the microbial biomass which in turn affects the N dynamics. Banding N showed improved crop N uptake and better N recovery, which is not necessarily related to a decrease in N immobilization but through reduced losses or improved plant uptake in drying soil. Lower MBN observed under canola than under wheat when residue was retained, suggests that canola is able to compete with microbes to obtain more N, but this could be due to its longer life cycle rather than suppression of N-immobilizing microorganisms. The study also suggests that the effect of application method on N-use efficiency depends on crop species; the best results of N uptake with N banding at sowing in canola and surface N application at stem-elongation in wheat. The soils we used in this study were Calcarosols with sandy clay loam topsoil and grey Vertosol with clay loam topsoil which were all very alkaline whilst previous research has been focused on acid soils (loam and sandy loam) with lower pH. Further research in this area could be focussed on the possible effects of soils with different pH and different crop species on N dynamics.

Table 4-19 Supplementary Statistical significance ($p \leq 0.05$) of species and residue on plant establishment at Birchip in 2019.

		Plant establishment (plant m^{-2})
Species	Wheat	135
	Canola	33
	p value	<0.001
Residue	Burnt	85
	Retained	83
	p value	0.471

Chapter 5. General discussion

5.1 Key findings

This thesis investigated the efficiency of N fertilizer applied to dryland crops in the semi-arid Mediterranean cropping systems of south-eastern Australia in presence of retained cereal residues. It aimed to determine whether immobilization of fertilizer N could be reduced or overcome to increase NUE in residue retained cropping systems.

Chapter 2 investigated the effects of N fertilizer application rates and residue retention on soil N dynamics using APSIM crop simulation. The greatest source of N inefficiency in residue retained treatments was immobilization at most N rates and simulated sites in Victoria, at levels of N input currently used by farmers. This chapter provided new insights relating to sources of N inefficiency in semi-arid cropping systems where crop N balance was changed considerably by residue and N treatments.

Chapter 3 investigated the effects of canola and wheat crops, and their respective rhizosphere microbiomes, on immobilization of fertilizer N in the presence of wheat residue. It was found that the ability of wheat and canola to obtain fertilizer N over soil microorganisms is similar, despite some differences observed in the rhizosphere microbial populations. Results showed that canola was better at obtaining soil N than wheat. The temporal advantage of canola over microorganisms in attaining N was probably due to differences in crop life cycles and the capacity of plants to further accumulate mineralized nutrients, rather than the manipulation of the rhizosphere microbiome.

Chapter 4 studied management factors which aimed to reduce immobilization and increase crop access to N in the presence of retained crop residues. Nitrogen application at sowing was found to increase N uptake in canola, whereas mid-season at early stem-elongation increased N uptake in wheat. Higher total accumulation of N was also detected in canola compared to wheat; however this was most likely not due to an ability of canola to suppress immobilization of soil organisms, but rather due to canola having a longer life cycle than wheat. Mid-row banding improved crop N uptake, but this was not necessarily related to a reduction in immobilization. This probably was due to reduced losses or better access to N being in moist soil rather than trapped in surface dry layers of soil.

Collectively, this body of work provides evidence that N uptake, N recovery and N availability to crops can be substantially affected by residue retention, timing of fertilizer application, fertilizer placement and the ability of crops to acquire fertilizer N. However, differences in NUE were not due to a reduction in N

immobilization and so growers are instead advised to use strategies that overcome acute N deficiency such as additional N fertilizer applications.

5.2 Nitrogen immobilization

When residues were retained, N immobilization was the greatest source of N inefficiency. Immobilization initially increased with greater N rates due to accumulated carbon inputs as crop biomass increased (Chapter 2). Different patterns of rainfall throughout the season lead to varying soil moisture contents, which may have an impact on C and N dynamics (Sänger et al., 2010). This pattern can also affect the number of days when the soil moisture content is high enough for denitrification to occur. Greater denitrification was expected at Longerenong and Lake Bolac where the rainfall amount was higher than other locations and the soils were clay in texture. Megonigal et al. (1993) showed that soils with a high clay content reduced oxygen rates even where soil was not saturated and prepared an environment for a high level of denitrification.

Understanding of crop N uptake from soil during their growth will help to improve N management. This data is critical for creating optimal management practises and N decision that aim to improve crop production, profitability, and sustainability (Hocking et al., 2002). It was previously found that after canola, the soil mineral N status is higher than after cereals (Kirkegaard et al., 1999) which is consistent with the results from Chapter 3 that the total mineral N in the surface 0-10 cm at maturity was higher for canola than wheat. This was despite no significant difference present in the rhizosphere bacterial communities of wheat and canola. Plant uptake of nitrogen fertilizer was high, despite having a theoretically high potential for immobilization in all treatments due to the addition of wheat residue. Between canola and wheat plants, there was no noticeable difference in the uptake of fertilizer, and plant rhizospheres did not display any variation in the immobilization of N fertilizer. Therefore, this does not support the notion that canola microbial communities were responsible for decreased immobilization or increased mineralization. Similarly, the field results (Chapter 4) showed the highest N uptake in canola, resulting in lower soil residual N following canola when compared to wheat. In this experiment, mid-row banding N significantly increased MBN content in retained residue compared to surface broadcast which doesn't support our hypothesis that banding N reduces immobilization when residue is retained. Mid-row banding N resulted in enhanced crop N uptake and greater N recovery, which is not necessarily related to a reduction in N immobilization but is likely due to reduced losses or better plant N uptake. The pot experiment was conducted in conditions where soil water content, temperature and light were controlled. Thus, it was expected that the observation of microbial biomass C and N was not quite same as that observed at the different field sites. Soil microbial activities can be modified through drought, soil moisture, soil N content, temperature and any small changes in the soil structure (Pang et al., 2009). The results of Helliwell et al. (2014) showed that changes in the soil

porous architecture and soil volume can change microbial activities which can in turn affect soil structure and influence water and nutrient delivery to plant roots.

Soil microorganisms compete for mineral N with crop roots, resulting in N immobilization (Myrold and Bottomley, 2008). Therefore, increasing plant root uptake while decreasing microbial uptake may increase NUE (Lester et al., 2016). The ability of crop to take up N can be improved by banding rather than broadcasting fertilizer (Sandral et al., 2017; Wallace, 2019). Better recovery of N applied by mid-row banding was observed in canola more than wheat. This positive interaction between crop species and N placement, and lower residual fertilizer N remaining after canola, could be related to the high fertilizer N uptake by canola (Nuttall et al., 1989). This has implications for developing appropriate recommendations that match crop nutrient requirements to fertilizer and minimise nutrient loss (Johnston and Bruulsema, 2014).

Plants and microbes compete for mineral N over a period of days to weeks as "short-term" cycling and over months to years as "long-term" cycling (Perakis and Hedin, 2001). If N remineralizes within a few months it might profitably contribute to the subsequent crop, but if it takes longer than this the delay will be costly (Angus and Grace, 2017). Reichel et al. (2018) showed that after application of 4.5 t ha^{-1} of wheat residue, 42 kg N ha^{-1} was immobilized during a short period of time and then two-thirds of this amount was released to the soil within 4 months. Nitrogen availability from soil can change quickly and testing soil mineral N status will reflect the ability of the soil to supply available N to the crops (Heckman, 2002). As previously mentioned, a larger number of sampling times would provide a better knowledge of all interactions between plant and soil over the growing season. Soil samples from field experiments were collected only at maturity, whereas samples should be taken at different crop growing stages such as before and after anthesis when crop N demand is high. Crops at each growth stages may also influence soil microbial dynamics by changing the distribution of organic inputs from roots and residues (Franzluebbers et al., 1995). However, accurately measuring mineral N variability and its high spatial unevenness would require regular and intensive soil sampling, which is not realistic and is very costly for large scale experiments. According to the results from (Chapter 3), although there was a difference between enzyme activities and relative abundance of soil microorganisms, they did not seem to be related to canola's higher mineralization of soil N. The sampling date may not have coincided with the actual mineralization events that led to enhanced soil N uptake by canola, which would explain the discrepancy.

5.3 Manipulation by management

Residue retention (Chapter 4) decreased shoot biomass and crop N uptake at early stem-elongation but not at anthesis which indicated that N was possibly released and present for crop uptake at this growing stage compared with N at early stem-elongation. Other researchers have reported more efficient use of N between stem elongation and anthesis (Palta and Fillery, 1993; Cui et al., 2011). In addition, it was previously thought that applying N at stem-elongation as rapidly growing plants could better uptake N; however, this thesis instead demonstrated that canola was more sensitive to applying N at sowing rather than stem-elongation likely due to the different period of N demand in canola and wheat. Mid-season at the start of stem-elongation coincides with the start of the period of high N demand in wheat, but declining N demand in canola (Palta and Fillery, 1993; López-Bellido et al., 2006). These mechanisms need to be tested in further research. The difference between our results and those of Kirkegaard et al. (1999) could be due to the soil characteristics such as different topsoil texture or pH. Lower nutrient availability and accessibility of plant residues to microbes and binding of nutrients observed in clay soil by (Pal and Marschner, 2016). The soils we used in (Chapter 4) study were Calcarosols with sandy clay loam topsoil and grey Vertosol with clay loam topsoil which were all very alkaline whilst the study of Kirkegaard et al. (1999) was focused on acid soils (loam and sandy loam) with lower pH. Soil pH affects all physical, chemical and biological soil properties (Brady et al., 2008). Aciego Pietri and Brookes (2008) confirmed that the soil organic matter and pH stimulated microbial biomass growth. Aciego Pietri and Brookes (2009) also observed the higher soil microbial biomass and microbial community structure in higher soil pH. Further research in this area could be focussed on the possible effects of soils with different pH and different crop species on N dynamics.

5.4 General recommendations

N fertilizer investment is frequently the largest input expense for Australian grain growers, and low commodity prices and growing input costs place further focus on better defining soil N availability and N fertilizer management (Ryan, 2010). Nitrogen use efficiency, potential N loss mechanisms, and the performance of various fertilizer application methods must all be considered when making profitable and productive N fertilizer decisions. Grain growers are advised to complete a soil test (e.g. soil pH, organic matter, mineral N content and moisture content) to rooting depth before sowing and during the growing season (Robson and Abbott, 1989; Majumdar et al., 2013). Growers are recommended to split applications of fertilizer N based on the soil testing data and weather conditions, which will affect crop N uptake and soil N supply (Schröder, 1999). According to our data, canola will uptake N more effectively if fertilizer is applied at sowing, and wheat N uptake will be maximised when N fertilizer is applied at early stem elongation. Higher N content in plant and soil with mid-row banding of N fertilizer in residue-retained

systems provided evidence of a growth restriction caused by retained residues. Thus, growers are recommended to band their N fertilizers instead of surface application (Angus et al., 2020b).

5.5 Further research

There are still many unknowns regarding N cycling, N fertilizer use efficiency and crop N uptake. As mentioned above, future studies need to investigate in more detail the effects of fertilizer timing and placement on NUE in crops with different life cycles. The interacting effects of rainfall patterns and soil characteristics on N dynamics also warrant further investigation. We recommend field experiments and simulation modelling to assist in answering these questions around how best to overcome N deficiency caused by immobilization.

In terms of plant-soil interactions, further studies should consider root characteristics (including root exudates and root system architecture) to determine the belowground dynamics of crop N uptake efficiency in the different species. More research is required to understand seasonal variations in the response of the soil microbial community to crop residue and N fertilizer, and the influence of different crop species on the belowground soil microbial profiles over time via factors such as nutrient composition (Van Der Heijden et al., 2008). These detailed mechanistic questions might be investigated using both field and controlled environment experiments.

5.6 Conclusion

In summary, simulation modelling indicated that immobilization is the largest source of N inefficiency when residues are retained in the semi-arid cropping systems of south-eastern Australia. Whilst management factors including crop species, N timing and N placement were all demonstrated to affect crop N uptake, this was not convincingly achieved through the targeted mechanism of a reduction in N immobilization. Given the poor efficacy of management to reduce N immobilization, growers should instead accept immobilization as a necessary cost for the maintenance of soil organic N and subsequent mineralization. Any acute N deficiency caused by immobilization is perhaps best offset by targeted additional inputs of N fertilizer, and how to best achieve this would be a fruitful subject for further research.

Appendix

A1 Journal publications from thesis chapters

Nasrollahi, N., Hunt, J., Tang, C., Cann, D., 2021. Modelled Quantification of Different Sources of Nitrogen Inefficiency in Semi-Arid Cropping Systems. Agronomy 11, 1222. (Chapter 2)

Rigby, B.A., **Nasrollahi, N**., Celestina, C., Hunt, J.R., Kirkegaard, J.A., Tang, C., 2021. Nitrogen Fertilizer Immobilization and Uptake in the Rhizospheres of Wheat and Canola. Agronomy 11, 2507. (Chapter 3)

A2 Conference presentation

Nasrollahi, N., Hunt, J., Tang, C., Cann, D., 2019. Quantifying sources of N inefficiency in Mediterranean semi-arid cropping systems. Agronomy Australia Conference, 25 – 29 August 2019, Wagga Wagga, Australia 2019.

A3 Declaration of publication contributions

A3. 1 Chapter 2

Nasrollahi, N., Hunt, J., Tang, C., Cann, D. 2021. Modelled quantification of different sources of nitrogen inefficiency in semi-arid cropping systems. Agronomy 11, 1222.

Statement of contribution: Conceptualization, N.N. and J.H; methodology, N.N, J.H. and D.C; formal analysis, N.N; investigation, N.N; data curation, N.N; writing-original draft preparation, N.N; writing-review and editing, D.C, J.H and C.T; visualization, N.N, J.H, C.T; supervision, J.H. and C.T; funding acquisition, J.H. All authors have read and agreed to the published version of the manuscript.

Niloufar Nasrollahi

James Hunt

Caixian Tang

David Cann

A3. 2 Chapter 3

Rigby, B.A., Nasrollahi, N., Celestina, C., Hunt, J.R., Kirkegaard, J.A., Tang, C., 2021. Nitrogen fertilizer immobilization and uptake in the rhizospheres of wheat and canola. Agronomy 11, 2507.

Statement of contribution: Conceptualization, J.R.H., J.A.K., N.N. and B.A.R; methodology, B.A.R., N.N., J.R.H., C.T. and C.C.; validation, J.R.H., C.T. and C.C.; formal analysis, B.A.R., N.N. and C.C.; investigation, B.A.R., N.N. and C.C.; resources, J.R.H., C.T. and C.C.; data curation, B.A.R., N.N. and C.C.; writing-original draft preparation, B.A.R.; writing-review and editing, B.A.R., N.N., C.C., J.R.H., J.A.K. and C.T.; visualization, B.A.R and C.C; supervision, J.R.H and C.T.; project administration, B.A.R., N.N. and J.R.H.; funding acquisition, J.R.H. and C.T. All authors have read and agreed to the published version of the manuscript.

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