THE UNIVERSITY OF ADELAIDE
AUSTRALIA

Managing crop residues and nitrogen fertiliser to improve wheat yield potential in water-limited environments

Thesis submitted to The University of Adelaide in fulfilment of the requirements for the degree of Doctor of Philosophy

Maria del Pilar Muschietti Piana

School of Agriculture, Food and Wine

The University of Adelaide

October 2020

TABLE OF CONTENTS

CHAPTER 1. General introduction and literature review

APPENDIX. Supplementary material chapter by chapter

ABSTRACT

Nitrogen (N) supply to rain-fed crops is becoming increasingly challenging due to the decline in organic N reserves. In low-rainfall wheat cropping systems, low crop N uptake has been linked to asynchrony in soil N supply through mineralisation. This is especially true on sandy soils of south eastern Australia which have a low N supply capacity and are considered highly 'risky' in a management context. When the N released from soil and residues is insufficient, and/or the timing of biological supply is not well matched with crop demand, management of N inputs to the soil (i.e. legume residue addition and/or fertiliser N) is essential to achieve yield potential and to return a neutral soil N balance for environmental sustainability. The general aim of this thesis was to improve our understanding of the seasonal pattern of the soil N supply capacity *via* mineralisation for increased wheat N uptake and grain yield, by combining N inputs from different crop residues (removed, wheat or lupin) and fertiliser N inputs (nil, or low, or high N) in a low-rainfall sandy soil environment.

Field experiments were conducted over 2 years (2015-2016) at low-rainfall Kandosols based on-farm in the Mallee environment of South Australia. The temporal patterns of the soil profile mineral N and plant available water to 100 cm depth, wheat aerial biomass and N uptake were measured in both years (Chapter 2). In 2016 we also measured the disease incidence as a key environmental variable. There was 35 kg ha^{-1} more soil mineral N to 100 cm depth following lupin compared with wheat residues at the end of the fallow in both years. In a below average rainfall season (Decile 4), wheat biomass produced on lupin residues was responsive to fertiliser N input with soil profile mineral N depleted by increased crop N uptake early in the season. In an above average rainfall season (Decile 9), a higher soil mineral N supply increased actual and potential grain yield, total biomass, N uptake, harvest index and water use efficiency of wheat, regardless of the source of N (legume N/fertiliser N). These experiments showed that the combination of lupin residues with N fertiliser application increased soil profile mineral N at early growth stages, providing a greater soil N supply at

the time of high wheat N demand, and the inclusion of a legume in the rotation is critical for improving the N supply to wheat, with added disease break benefits (Chapter 2).

The 2016 field experiment involved the quantification of decomposition rates and N release from wheat and lupin residues over the fallow and the subsequent wheat crop growing season with and without fertiliser N application. It also involved measurements of the temporal patterns of the surface soil mineral N, potentially mineralisable N, microbial biomass N, dissolved organic N and with temperature and rainfall as key environmental variables in all treatments (Chapter 3). Residue decomposition and N release over the fallow and the wheat growing season was measured in the field using litterbags with wheat or lupin residues. Fertiliser N input treatments at wheat crop sowing time and surface soil N pools were measured at key growth stages. A higher potential N supply to wheat following lupin residues at early stages was evidenced through greater decomposition rates and N release *via* mineralisation than wheat residues, which resulted in increased surface soil N pools. This experiment showed that when lupin residues are combined with fertiliser N application, the N supply capacity to wheat is improved during the growing season measured as mineralised N, dissolved organic N and potentially mineralisable N, relative to wheat residues combined with fertiliser N

The last experiment (Chapter 4) was conducted under controlled conditions to directly assess (using ^{15}N labelled fertiliser) the role of N fertiliser on the supply of N to wheat N through soil mineral and biological pools. This experiment measured the role of the N fertiliser combined with wheat, lupin, or no stubble incorporation. Wheat plants were grown in a glasshouse and sampled at 3 critical wheat growth stages (tillering, first node, booting) to determine wheat and ^{15}N uptake. Soil samples were collected at sowing, tillering, first node and booting to determine mineral N, microbial biomass N, dissolved organic N, and potentially mineralisable N on subsets of samples. This study indicated that the presence of early N immobilisation (between sowing and tillering) in all the treatments without ^{15}N

fertiliser limited N availability for wheat uptake in the subsequent period (between tillering and first node), when fertiliser N appeared critical to maximise N supply for plant requirements. It was found that up to 38% of the ^{15}N fertiliser applied at sowing was incorporated into the soil microbial biomass pool. Therefore, the fertiliser N was critical to relieve short-term inherent N limitations for both plant and microbial growth, and to supply the longer-term biological pools (microbial biomass) to support subsequent mineralisation potential. This study also showed that reducing the energy limitation to the microbial pool through inputs of carbon from stubble was critical to ensure fertiliser N supplied sufficient N to satisfy plant demand later in the growing period.

This research contributes to a greater knowledge of the main factors affecting soil N dynamics relative to wheat N nutrition and yield, quantifying the N supply from soil and fertiliser and the N accumulation in wheat biomass (roots, shoots and grain) at critical phenological stages in a low rainfall sand. Further research will require measurements of the contribution of different legumes combined with varying fertiliser N rates for a complete assessment of the impacts that could be achieved, and examination of the effect on the main soil N pools driving N supply to wheat N uptake across several seasons and/or in different soil types.

DECLARATION

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in my name in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

In addition, I certify that no part of this work will, in the future, be used in a submission in my name for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint award of this degree.

The author acknowledges that copyright of published works contained within this thesis resides with the copyright holder(s) of those works.

I give permission for the digital version of my thesis to be made available on the web, via the University's digital research repository, the Library Search and also through web search engines, unless permission has been granted by the University to restrict access for a period of time.

Maria del Pilar Muschietti Piana (PhD Candidate)

Signed:

Date: 17 January 2021

PUBLICATIONS ARISING FROM THIS THESIS

Peer-reviewed Journal articles

Muschietti-Piana, M.P., T.M. McBeath, A.M. McNeill, P.A. Cipriotti and V.V.S.R. Gupta. 2021. Nitrogen fertiliser input increases potentially mineralisable and dissolved organic nitrogen more after lupin than wheat residues in a sandy surface soil. Submitted to *Journal of Soil Science and Plant Nutrition* (revisions requested on 10 January 2021).

Muschietti-Piana, M.P., T.M. McBeath, A.M. McNeill, P.A. Cipriotti and V.V.S.R. Gupta. 2020. Combined nitrogen input from legume residues and fertiliser improves early nitrogen supply and uptake by wheat. *Journal of Plant Nutrition and Soil Science* 183: 355–366.

Muschietti-Piana, M.P., T.M. McBeath, A.M. McNeill, P.A. Cipriotti and V.V.S.R. Gupta. 2020. Early growing season immobilisation affects post-tillering wheat nitrogen uptake from crop stubble and ^{15}N fertiliser in a sandy soil. *Soil Research,* online early publication 17 November 2020. DOI: https://doi.org/10.1071/SR20158.

Conference papers

McBeath, T.M., **M.P. Muschietti Piana**, M. Moodie, A. Ware, R. Llewellyn and V. Gupta. 2019. Benefits to wheat and canola from upfront nitrogen fertiliser even when following a legume. In: Proceedings of the 2019 Agronomy Australia Conference, Wagga Wagga, Australia.

Muschietti-Piana, M.P., T.M. McBeath, A.M. McNeill, P.A. Cipriotti and V.V.S.R. Gupta. 2016. Closing the nitrogen supply and demand gap using legume residue combined with fertiliser nitrogen input. In: Proceedings of the 2016 International Nitrogen

Initiative Conference, Solutions to improve nitrogen use efficiency for the world, Melbourne, Australia.

Technical articles

Gupta, V.R.S, **M.P. Muschietti-Piana**, L. Bell and T. McBeath. 2020. Biology of nitrogen release from pulses. In: Proceedings of the GRDC Grains Research Update for Advisors, Adelaide, South Australia.

Muschietti-Piana, M.P., T.M. McBeath, V.V.S.R. Gupta, A.M. McNeill, B. Davoren and W. Shoobridge. 2017. Combined nitrogen inputs from lupin stubble and fertiliser improves wheat productivity on sands. Mallee Sustainable Farming Compendium 2017. 4 pp. http://www.msfp.org.au/combined-nitrogen-inputs-lupin-stubble-fertiliser-improves-wheat-productivity-sands.

Muschietti-Piana, M.P., T.M. McBeath, V.V.S.R. Gupta, A.M. McNeill, B. Davoren and W. Shoobridge. 2016. Legume residue with fertiliser nitrogen input to better match nitrogen supply with demand on sands. In: Proceedings Mallee Sustainable Farming, Karoonda Field Day 2016 Booklet, pp. 30–33. http://www.msfp.org.au/wp-content/uploads/2016.08.04-Karoonda-FD-booklet.pdf.

ACKNOWLEDGEMENTS

To all my colleagues and friends of the Soil Group; Ron Smernik for the writing group, Cameron Grant during the CCSP, Murray Unkovich for the magical Pivot Tables.

I could not have completed my PhD without the support of my family and friends. My sisters and soul mates, Mili and Vicky, whose backpacks are always ready for that 3-hugged encounter, here, there and everywhere. Mili, you always find the right words in key times. Thanks for helping me to find my balance and think far and beyond. Vicky, you are the one who pushed me harder to make this experience abroad true. Thanks for making myself question about love, life, profession. My Primiga, a gifted soul plenty of love. My dearest Chincho´s, Molly, Dondo, Olli and Martu for feeding me with laugh and joy, my best in-laws, Tom and Mauri.

To my Mom in the Earth for her roots, to my Dad in the Sky for his wings. You both provided me a range of tools and possibilities to explore the world and beyond.

Thanks to my partner, Robertinho, for filling with colors this last stage, for showing me other ways of thinking, with you I grow up as a woman and as a person.

Thanks to those who walked with me during this journey. You were indispensable in my Aussie life, for calming me down when things were hard to deal. Thanks to Francisco, Andrew, Martin, Sabela, Gennady, Paola, Fien, Olivia, Rachna, Martina, Lila, Monica, Vico, Peter, Mariana, Rodrigo. Thanks for your love, friendship, and endless support.

To Waite Arboretum for making my day, every day on my way to campus, its flora and fauna. Thanks to my mates, to my soul mates, to my aussie and non-aussie mates, and to every single creature I had the chance to cross path with.

STRUCTURE OF THE THESIS

My Thesis comprises five chapters in total and is structured as a combination of chapters that have been either published or submitted to a journal.

Chapter 1 introduces this Thesis and provides an overview of the literature that frames the research focus of this study, highlighting the main advances along with the gaps in knowledge to date. Both the research questions and the respective aims of study are given in this chapter.

Chapter 2 comprises a paper that has been published in the *Journal of Plant Nutrition and Soil Science*. This paper is based on field experimentation conducted at 2 different locations in the same fields in 2015 and 2016 on a low-rainfall sandy soil. This chapter provides an estimation of the soil N supply capacity relative to wheat N uptake at key growth stages, under combinations of crop residue (removed, wheat or lupin) and fertiliser N inputs. The disease incidence is also considered as a key environmental variable affecting wheat crop attributes and yield.

Chapter 3 presents a paper submitted to *Journal of Soil Science and Plant Nutrition,* based on a field-based experiment conducted in 2016. This chapter examines the decomposition rates and N release from wheat and lupin residues over the fallow and the subsequent wheat crop growing season with and without fertiliser N application in a litterbag experiment. In addition, the temporal patterns of the surface soil mineral and biological N pools along with environmental variables (rainfall and temperature) at plot level are also examined.

Chapter 4 comprises a paper that has been published in *Soil Research* based on an experiment conducted under controlled conditions in a glasshouse in 2017. This paper examines soil mineral and biological N pools that influence N supply relative to wheat N uptake at early stages, and the recovery by wheat of ^{15}N-labelled fertiliser applied at sowing in a sandy soil with wheat, or lupin or no stubble incorporation combined with and without ^{15}N fertiliser addition.

Chapter 5 consists of a summary of the main findings in my Thesis, a general discussion and recommendations for future research.

CHAPTER 1

General Introduction and

Literature Review

1. Introduction

A rise in food demand to feed a global population of about 9 billion as expected by 2050 (Godfray et al., 2010) requires 100 to 110% increases of crop production of major cereals (Tilman et al., 2011) such as wheat (*Triticum aestivum* L.). Worldwide wheat consumption has doubled in the past four decades, and 15.4% of annual world consumption is traded on the international market. Wheat is Australia's main cereal crop, which represents 56% of the cultivated area and contributes 10-15% of the total world market (ABS, 2014). About 70% of wheat is cultivated in dryland systems worldwide, and in Australia is almost entirely rain fed (~91%, ABS, 2014; Portmann et al., 2010). Crop production and yield in the Australian wheat belt is restricted by water availability and use efficiency (Sadras and Angus, 2006). Water supply in dryland systems is predominantly a function of seasonal conditions (Hochman and Horan, 2018). The principal climatic factors affecting wheat yields are the timing and amount of rainfall, the frequency and severity of periods of high temperatures (hot spells) during grain filling, and the incidence of frost events especially around ear emergence (Richards et al., 2014).

Research on wheat production in water-limited environments of Australia has triggered a new emphasis on improving resource use efficiency (French and Schultz, 1984; Kirkegaard et al., 2014; Passioura, 2006). In a seminal study, French and Schultz (1984) provided a valuable benchmark to which farmers can aspire considering the inter-seasonal variability in rainfall (Passioura and Angus, 2010). In Fig. 1 the line reflects the expected yield potential for a given water use of current wheat cultivars; the slope of this line is considered to be the upper limit of crop transpiration efficiency for grain yield, and it is about 20 kg grain ha^{-1} per mm^{-1} of water transpired (French and Schultz, 1984). This benchmark was reappraised at 22 kg grain ha^{-1} per mm^{-1} with a minimum of 60 mm of evaporation (Sadras and Angus, 2006) but later Sadras and Lawson (2013) reported a value of 25 kg $ha^{-1}mm^{-1}$ owing to improved new cultivars and rising atmospheric carbon (C) dioxide concentration. However, this benchmark

will depend on vapour pressure deficit (Rodriguez and Sadras, 2007). A major finding of French and Schultz's study (1984) is that farmers' yields are below the water-limited yields (circles below the boundary line in Fig. 1), and the factors controlling these lower yields relative to the potential include weeds, root diseases, frost and heat shock, poor nutrition, soil acidity, salinity, waterlogging, and other issues (McDonald et al., 2012; Richards et al., 2014). A recent evaluation suggests that leading growers are achieving yields at 80% of this benchmark which is considered the 'attainable' yield but a gap remains for many (Van Rees et al. 2014).
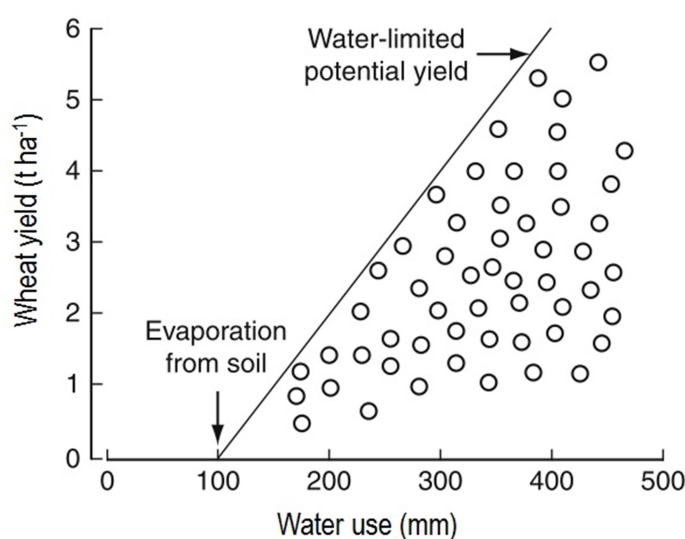
Figure 1. A schematic representation of the relationship between wheat yields and water use (or seasonal rainfall plus stored soil moisture) on farms. The solid line reflects wheat yield with water as the only limitation. The slope of the line is about 20 kg grain ha^{-1} mm^{-1}. The intercept of this line on the x-axis represents the loss of water by direct evaporation from soil. The circles depict examples of individual paddock yields. Extracted from Passioura and Angus (2010).

Nitrogen (N) is the most limiting factor after water for cereal-based systems and N management is vital for improving resource use efficiency (Chen et al., 2008). Inadequate N

supply has been identified as a key driver of wheat yield gaps (Hochman and Horan, 2018), especially where soils have low organic C content and N supply capacity and are considered highly 'risky' in a management context (Gupta et al., 2011). The Mallee region in south-eastern Australia is dominated by a Mediterranean type-climate with low and erratic rainfall (annual average rainfall 250–350 mm), a short winter growing season, and wheat N uptake is driven by a unique combination of soil and climatic attributes (Sadras, 2002).

The Mallee region of south-eastern Australia is a key grain growing zone in Australia dominated by cereal cropping and pastures. Native vegetation of the region consisted largely of Eucalypt mallee woodlands and shrublands, which are multi-branched from ground level, usually with a flattened canopy (Specht, 1972). Soils in the Mallee are highly variable with a typical dune–swale landscape with light sandy soils on the dunes and heavier loam or clay soils in the swale (Conner, 2004). The undulating plains of the region are characterised by low parallel sandridges underlain by calcrete, which frequently outcrops in the swales (Specht, 1972). Variation in crop production in the Mallee is affected by the inherent variation in soil fertility, subsoil constraints, plant available water capacity, which might be exacerbated under climate change (Connor, 2004). Sandy soils in the Mallee are characterised by organic matter contents of less than 1%, low N supply capacity, and the presence of soil-borne diseases and sub-soil constraints that restrict root growth and access to water and (deep) mineral N; which in combination lead to limited wheat crop N uptake (Gupta et al., 2011).

The variability in N supply and uptake for wheat is mainly driven by environmental (i.e. climate, soil attributes) and management factors (i.e. fertiliser N, crop rotation). Cropping systems which are more complex or diverse (i.e. mixed, break crops) are likely to be more resilient and less dependent on external inputs. Legume crops (grain and/or pasture) play a key role in providing significant benefits for sustainable cereal-based agriculture (McBeath et al., 2015; Seymour et al., 2012; St Luce et al., 2020). Reducing the uncertainty in both soil N

supply and crop uptake is vital to optimise fertiliser N inputs and reduce the associated environmental and economic costs.

Little is known about how residues and N fertiliser might interact to improve N supply and crop growth and grain yield (St Luce et al., 2016). Manipulating in-crop mineral N availability through legume residues combined with fertiliser N inputs is vital to close the N-derived yield gap of rainfed wheat (Bryan et al., 2014; McBeath et al., 2015) by improving N use efficiency (Angus and Grace, 2017; St Luce et al., 2020). This becomes critical for low-rainfall cropping systems, where limitations to N uptake by wheat have been linked to an asynchrony between plant N demand and soil mineral N supply through mineralisation (Hoyle and Murphy, 2011), and where the interaction between water and N constitutes a primary driver in the timing of N supply (Sadras 2005).

1.1 Drivers of wheat nitrogen demand

Overall, N demand capacity in a cropping system refers to the combination of the microbial requirement for N, and N for crop uptake. Wheat crop N demand can be defined by the amount of N used to achieve a target yield and grain protein content, the amount of N in straw and roots, and the unavoidable N losses *via* leaching (rooting zone) and/or atmospheric (Angus, 2001). Early supplementation of N is critical to achieve wheat yield potential in soils with low N reserves (soil mineral N to 30 cm depth below 80 kg ha^{-1}) since the peak of wheat N demand occurs between tillering and anthesis. Yield potential is set in this period and N treatments tend to confer their greatest advantage to crop yield at this stage, i.e. ensuring a better crop establishment, tiller production and formation. In southern Australia, canopy management to ensure that pre- and post-anthesis growth are balanced to achieve efficient conversion of water into grain, thus optimising wheat growth for grain yield, is usually based on practices such as including legumes in the rotation with wheat, and applying fertiliser N at sowing in low N fertility scenarios (Poole and Hunt, 2014). After ensuring adequate N for

crop establishment and early development, the period between the growth stages of stem extension and anthesis (Zadoks et al., 1974) is recognised to be critical in terms of water and N supply for reducing wheat yield variation in the Mallee (Sadras et al., 2012).

Both N supply and demand fluxes are strongly affected by environmental (i.e. climate, soil attributes) and management factors (Crews and Peoples, 2005; Peoples et al., 2009). Managing for synchrony, here defined as the close balance between the quantity, timing and position of N supply relative to crop N demand (Myers et al., 1994) is vital for sustainable farming systems. Increased N supply can result from increased soil microbial biomass, higher non-symbiotic N fixation, and the associated higher mineralisation potential (Gupta and Roget, 2004). Greater amounts of stubble added after wheat crop harvest can delay the timing of net N mineralisation (Gupta et al., 2011). However, periods of asynchrony can be found if N release from soil organic matter or crop residues occurs at a time that crop demand is restricted, so that N availability would exceed crop requirements, or similarly, if N supply is insufficient to meet crop requirements (Myers et al., 1994).

The magnitude of the asynchrony between N supply and demand is particularly significant where the N supply is less than optimal during critical early growth stages defining yield potential, namely, prior to terminal spikelet and/or during tiller initiation (Hoyle and Murphy, 2011). In south-eastern Australia the peak of N demand for rain-fed wheat generally occurs in late winter and spring when the crop is growing rapidly (Angus, 2001). However, wheat N demand is difficult to predict as it can vary from season to season (Hoyle and Murphy, 2011), and thus, synchronising N supply with crop demand is still an issue of major concern in these environments (Gupta et al., 2011).

Nitrogen use efficiency expresses the grain yield per unit of N supplied and is defined by two main components: the N uptake efficiency, which explains the extraction of N from soil; and the N utilisation efficiency, related to the conversion of the assimilated N into grain yield (Moll et al., 1982). The main factors driving N uptake by roots are the nitrate (NO_3^-) content

in the soil solution, the volume of soil exploited by roots, and root features such as the size, the density, the efficiency in absorbing NO_3^-, and the crop vigour (Lawlor, 2002; Liao et al., 2004; Palta and Watt, 2009). Accordingly, to increase N uptake by a crop under low NO_3^- levels, transporters of high affinity with NO_3^-, a greater root length and surface area per volume of soil are needed. Added to this, considerable NO_3^- accumulation in cells is required, and even more when metabolism is limited as a result of a delayed photosynthesis (Lawlor, 2002). As root size is expanded by growth, N supply becomes a limiting factor for root development, and then for crop production. Thus, N uptake efficiency is determined by the (genetic) yield potential, and is also affected by environmental conditions such as NO_3^- supply. Wheat responses to non-limiting and limited N supply are shown in Fig. 2 (Gastal et al., 2014). Biomass production demands sufficient levels of N to reach the asymptote of the response curve, but limitations to N uptake can create a gap in the relationship between N supplied and biomass production (Lawlor, 2002).
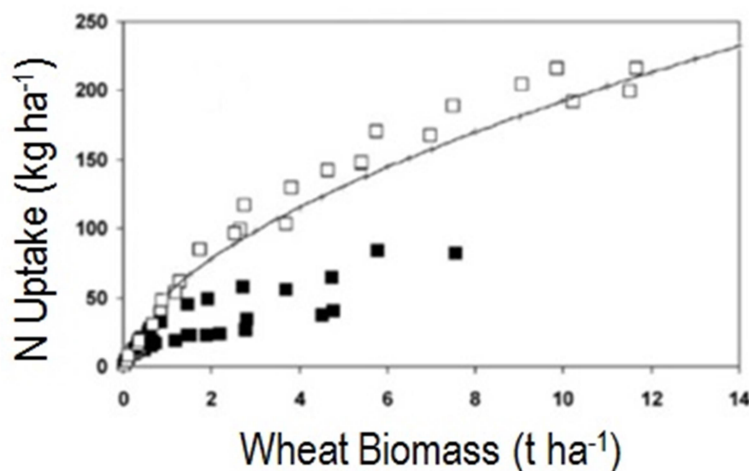
Figure 2. The figure illustrates the relationship between N uptake and wheat crop biomass for non-limited (□) and limited N (■) supply in wheat crops measured under field conditions. Extracted from Gastal et al. (2014).

Plant-based diagnostic methods for determining direct and water-induced N deficiency are one of the most important diagnostic tools for improving N management efficiency (Gastal et al., 2014; Gonzalez-Dugo et al., 2010; Lemaire and Gastal, 2009; Naud et al., 2008). Some methods to quantify the N status of the crop based on N dilution curves defining biomass as a component of the N uptake, e.g. the N nutrition index (NNI), have been proposed (Lemaire and Gastal, 2009; Sadras and Lemaire, 2014). The NNI is calculated as the ratio between the actual plant N concentration of the crop and the critical plant N concentration (%Nc) corresponding to the actual crop mass (Lemaire and Gastal, 2009). The actual Nc in a crop decreases as the crop mass increases, still under favourable N supply (Greenwood et al., 1986), and it is characterised by a negative power function (Lemaire and Gastal, 1997) of plant % Nc against crop mass throughout the vegetative growing stage (before anthesis). This dilution curve is used to segregate the actual N status under supra-optimal N (luxury consumption), and sub-optimal N (deficient supply).

Knowing the plant N status at target stages of the crop growth period is a prerequisite for studying crop response to N deficiency, as the same rate of N application could lead to variable plant N status, depending on plant growth potential, soil N mineralisation rate and soil N availability (Lemaire and Gastal, 2009). In low rainfall environments, vigorous early growth of cereals has been shown to improve grain yield and water use efficiency. This is particularly true for dryland wheat grown in Mediterranean-type climate of south-western Australia, where light-textured surface soils predominate (Turner and Nicolas, 1987). In soils of Western Australia, studies that examined the effect of N fertiliser on early vegetative growth in wheat have shown that 60–95% of wheat grain N at harvest came from remobilisation of N stored in roots and shoots before anthesis (Palta and Fillery, 1995). In such environments, determining crop N status (e.g. through NNI) at anthesis is too late for management of N fertiliser aimed at yield improvement (Naud et al., 2008). To predict the evolution of crop N status at different growth stages, the use of wheat crop models together

with *in situ* crop measurements is a valuable approach to assess N use efficiency for wheat (Naud et al., 2008).

Zhao et al. (2014) assessed the effect of potential differences in threshold Nc on wheat growth with The Agricultural Production Systems sIMulator (APSIM, Keating et al., 2003) and found that the Nc as a function of growth stage development was a more robust approach than the Nc against crop biomass. These findings are significant for simulation models addressing wheat N demand-supply relationships. These authors highlighted the necessity of additional experimentation for validating the derived Nc (Zhao et al., 2014). More recently, Hoogmoed and Sadras (2018) assessed the combined effects of water stress, phenology, partitioning of biomass, and water-soluble carbohydrates, as driven by environment and variety, on the Nc of wheat crops. The biomass dilution model developed for well-watered crops overestimated N deficiency of water-stressed crops, and a biomass-based model was conceptually more justified than developmental models. They have concluded that N-biomass dilution curves need to account for genotypic and environmental sources of variation in biomass allocation, including phenology and water soluble C (Hoogmoed and Sadras 2018).

Crop measurements of wheat N status at different growth stages in the low rainfall (<350 mm per year, Cawood and McDonald, 1996) zone of South Australia are still very limited (Hoogmoed et al., 2018). Further studies in these fragile environments are crucial to contribute input data for crop modelling to better understand and predict wheat N demand for a given growing season.

1.2 Soil nitrogen supply for wheat crop

Management of N is a valuable tool in order to attain the yield potential for rain-fed wheat (Hochman and Horan, 2018; van Rees et al., 2014). Soil N supply capacity, in terms of quantity and timing for release of mineral N, is largely regulated by the amount and quality of

crop residues, the size of the particulate organic matter pool, the degree of soil-residue contact, and microbial turnover (Kumar and Goh, 2000).

In cereal dominated cropping systems, the challenge for soil management is to maintain adequate N available for plant growth while minimising losses to the environment (Angus, 2001; Follett et al., 1991). It is vital to better understand the nature of the processes involved in soil N dynamics affecting crop N availability for sustainable cropping systems. Since N cycling and turnover is strongly affected by management practices within agro-ecosystems, soil N content is highly variable in cultivated soils and fluctuates over time, particularly when fertiliser is applied (e.g. soil solution concentration can range from 620 to 11,300 µM) (Marschner and Rengel, 2012). To meet the N demand of soil-grown plants, this nutrient must reach the root surface, and movement or transport in the soil solution mainly mediates this. For that reason, the concentration of N in the soil solution is critical for its supply to roots.

The following diagram represents the relationships between the pools and forms of N in natural and agro-ecosystems with emphasis on the plant-soil N cycle and pathways for N transformation mediated by physiological processes (Fig. 3). The largest N pool in the plant root zone is in the soil organic matter but is mostly not readily available for plant uptake, as this organic N must be released to form mineral N prior to plant uptake. Decomposition of soil organic matter is a complex process, mainly regulated by soil biota resulting in nutrient release in mineral forms and carbon (C) loss from soil *via* CO_2 respiration. Decomposition depends on plant inputs and soil organic matter turnover providing the available C required for maintaining soil biological activity. Soil biological processes are inextricably linked to the location, quantity and quality of available organic C resources and nutrients in soil (Murphy et al., 2011). The microbial biomass, although being a small N pool, is defined as a critical pool in the soil internal N cycle (Jarvis et al., 1996), with the living microbial biomass providing the enzymes for decomposition and the dead microbial biomass providing the labile fraction of soil N (McNeill and Unkovich, 2007). For example, in a wide range of temperate

soils with different cropping sequences, soil microbial biomass N accounted for 0.5–15.3% (3–108 kg N ha^{-1}) of the total N in the surface of the soil (Anderson and Domsch, 1990; Jenkinson and Ladd, 1981). In soils of the low-rainfall zone of the Mallee region in South Australia soil microbial biomass N in the surface layer (0–10 cm) ranged between 21–34 kg N ha^{-1} in Lowaldie (McBeath et al., 2019) and between 29–49 kg N ha^{-1} in Waikerie (Gupta and Roget, 2004).
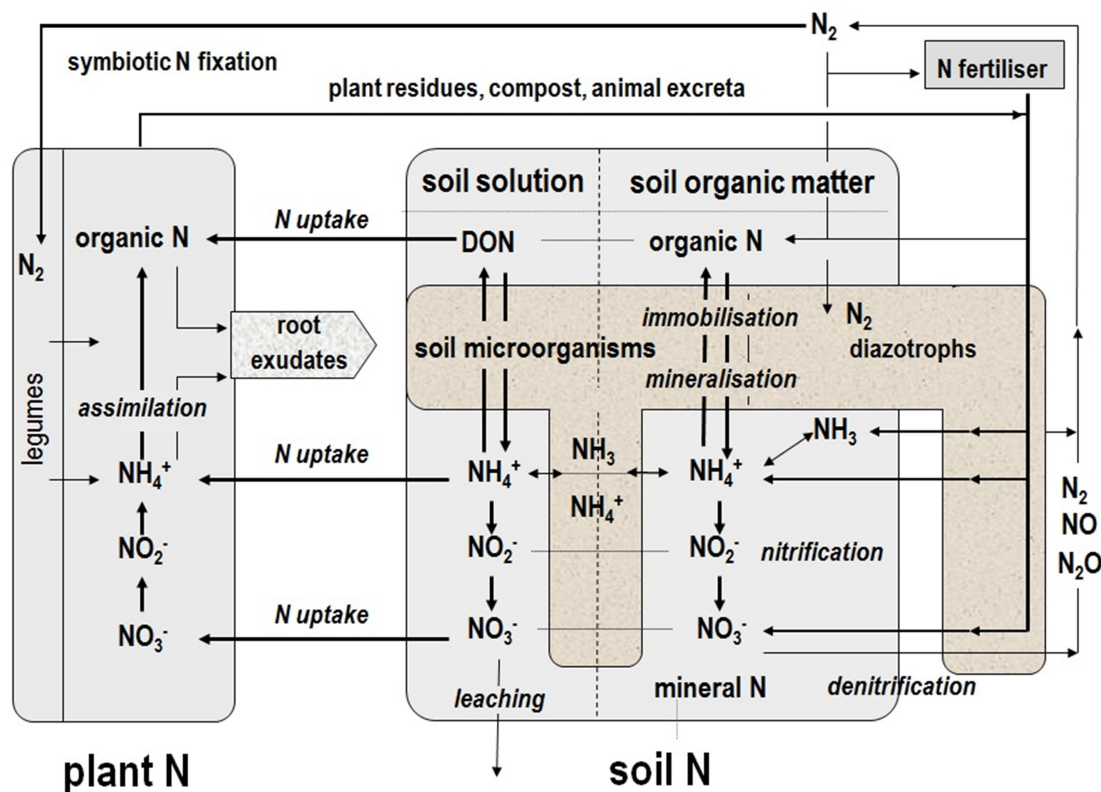
Figure 3. The plant-soil N cycle in natural and managed ecosystems (DON = dissolved organic nitrogen). Extracted from Richardson et al. (2009).

As shown in Fig. 3, mineralisation-immobilisation turnover is a vital process for the continuous transfer of mineral N into organic materials through incorporation of N into soil microbial biomass and subsequent release (mineralisation) of immobilised N into the soluble mineral N pool (McNeill and Unkovich, 2007). Consequently, mineralisation-immobilisation

is considered a dominant process for plant N availability. Quantification of the dynamics of mineralisation and immobilisation, and the key drivers of these processes, is crucial to the successful management of low- and medium-input cropping systems.

Mineralised N is the major source of plant-available N in most natural and managed ecosystems. Mineralisation of N from soil organic matter and previous residues of crop and pastures supplies around 70% of the N uptake by wheat crops in Australia (Angus, 2001). Mineralisation and immobilisation in soil occurs simultaneously, with the relative extent of each process defining whether net mineralisation or net immobilisation prevails (Jarvis et al., 1996). Net N mineralisation is the balance of gross N mineralisation and microbial N immobilisation (Yokobe et al., 2018). Net mineralisation is the difference in the size of the ammonium and nitrate pool at two points in time, while gross N mineralisation is the total flux of N into the ammonium pool over the same time period. As a result, gross N mineralisation is much larger as it also includes ammonium that has been consumed (immobilised) by microbes (Murphy et al., 1998). Net immobilisation occurs when the size of the soil microbial biomass increases due to substrate addition or residue incorporation and N demand exceeds that available from soil inorganic N or fertiliser (Cabrera et al., 2005; Gupta et al., 2011; Kumar and Goh, 1999). Previous field experimentation have indicated that the microbes' physiological performance and the diffusion of nutrients were influenced by rainfall conditions, which in turn altered soil N cycling and availability (Chen et al., 2017). However, other studies have suggested that net N mineralisation was not responsive to precipitation under field conditions due to the drought-resistant ability of microorganisms (Yuste et al. 2014).

Microbial activities not only drive the mineralisation of soil organic N formed from crop stubbles but also utilise N applied as fertiliser (immobilisation) as well as converting some forms of fertiliser N (e.g. urea) into plant available forms. A better understanding of the processes controlling immobilisation and mineralisation of the various N inputs is vital for

improving N use efficiency by crops. Indeed, more specific information on the contribution of soil biology to N processes including the microbial dynamics that affect mineralisation rates and soil organic matter turnover has been identified as key for the development of successful agronomic strategies (or decisions) aimed at synchronising N inputs and crop N demand or use (Roberts *et al.* 2015). The rate of N mineralisation is generally thought to reach a maximum between 15–35 °C (Stanford et al., 1975); this process is usually enhanced with optimal soil moisture content for microbial activity (matric potentials between -10 to -50kPa), reaching its maximum at field capacity (Angus et al., 1998; Sommers et al., 1981).

Soils in the semi-arid wheat-belt of southern Australia are subjected to repeated cycles of drying-rewetting (often referred to as 'Birch effect') (Amato et al., 1984; Gupta et al., 2011), so a clear understanding of the effect of soil water stress on N mineralisation is needed. Cycles of drying and rewetting of a soil in the absence of plants can cause N mineralisation flushes (Cabrera, 1993; Haynes, 1986) with each successive cycle resulting in a smaller flush as the biologically available C is consumed. The subsequent effect of rewetting on mineralisation generally increases with the humus content, the dryness of the soil, the duration of the soil dryness, and increasing soil temperatures (Fig. 4) (Haynes, 1986). Drying-rewetting cycles and rainfall events have shown stimulatory effects on microbial activity and N mineralisation (Adcock, 2005; Fierer and Schimel, 2002; Sadras and Baldock, 2003).

Microbial activity in dry soils is usually low, owing to a reduced substrate diffusion and water uptake, and adapted microorganisms can accumulate osmolytes to reduce water loss from cells (Boot, 2011). Rewetting a dry soil can result in respiration flushes due to higher substrate availability from osmolytes released, cell burst and exposure of previously occluded organic matter (Borken and Matzner, 2009; Fierer and Schimel, 2002). Nevertheless, drying and rewetting does not have a consistent stimulatory effect on cumulative respiration and nutrient release (Mikha et al., 2005; Shi and Marschner, 2014). For example, it was found that drying stimulated the subsequent mineralisation of C and N from soil humus, but it retarded

mineralisation of C and N from fresh plant materials (van Schreven, 1968). While in other studies, no effect of drying and rewetting was observed on the decomposition of ^{14}C-labelled lignin (Haider and Martin, 1981), and net N mineralisation was significantly less for the dry-rewetting cycles, compared with continuous wetting treatments. Additionally, Mikha et al. (2005) found a reduction in mineralised N during the first 24 h after each rewetting period, which could be caused by increased microbial activity after rewetting the dry soil, resulting in N immobilisation.
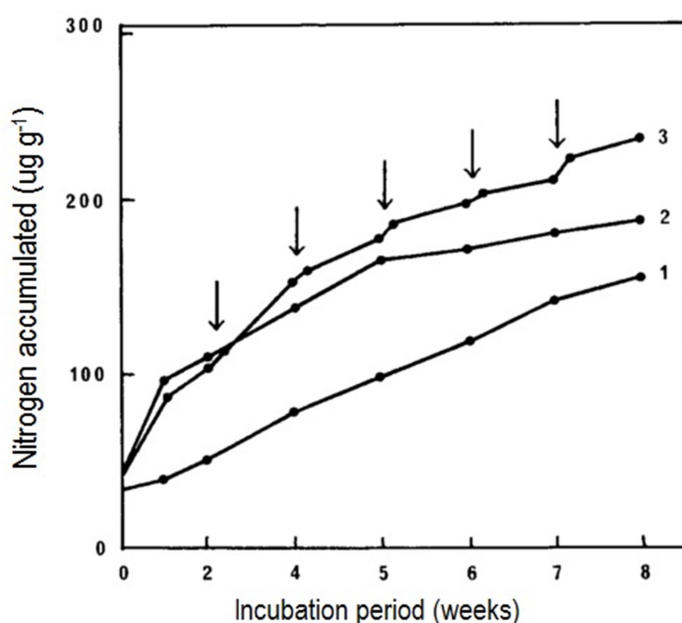
Figure 4. The effect of drying-rewetting cycles on mineral N accumulation in soil samples during incubation: (1) fresh moist soil incubated continuously; (2) soil initially dried at 35 °C, then rewetted and incubated continuously; (3) soil periodically dried at 35 °C and rewetted during incubation (van Schereven, 1986). Arrows indicate drying and rewetting points in time. Extracted from Haynes (1986).

Crop residues and fertiliser addition to soils can stimulate soil organic N mineralisation (Azam et al., 1991). This stimulation has been called the 'priming effect' (Bingeman et al., 1953) or 'added nitrogen interaction' (ANI) (Jenkinson et al., 1985; Pare and Gregorich,

1999). While the 'priming' effect is generally associated with some sort of substrate addition, the 'term ANI or priming effect' has not been used to describe the effect of changes in environmental factors such as drying and rewetting (Jenkinson et al., 1985).

Nitrogen cycling and availability can also be affected by N fertilisation strategies with a cumulative effect over time. Between 50–54% of the N fertiliser applied in a growing season usually remains in the soil (Ladd and Amato, 1986), and as a result, N derived from fertiliser continues to affect soil N dynamics after a crop is harvested. The extent of this effect is debated as some have found that the amount of N mineralised increased with higher quantities of N applied, and that more N was mineralised from fertilised than unfertilised soils (Gill et al., 1995; Kolberg et al., 1997; Yan et al., 2006) due to a greater N availability with N applications (Ladd et al., 1994). In contrast, others noted no effect of N fertilisation on N mineralisation (Franzluebbers et al., 1994). Even more controversial, a negative interaction between fertiliser and mineralisation has been documented (Carpenter-Boggs et al., 2000; Wienhold and Halvorson, 1999). A possible explanation is that soils receiving N application, have rapid short-term mineralisation which decreases the long-term availability of C and N pools. That is to say, N fertilisation can alter the soil N supplied by reducing the time or residence of crop residues in soil. Alternatively, more residue produced with higher N rates can result in more N immobilised during decomposition (Carpenter-Boggs et al., 2000; Wienhold and Halvorson, 1999).

Organisms involved in N mineralisation are active during both the crop growing season and the summer fallow. In water-limited Mediterranean-type environments, N mineralisation during summer is related to intermittent rainfall periods only (Sadras and Baldock, 2003) and processes slowing the accumulation of mineral N are preferred to reduce losses to the environment (Follet et al., 1991). In cropping systems of South Australia, large rainfall events (> 25–40 mm), especially over summer, are needed for most soil biota to contribute significantly to biological functions. In these systems the temperature during the summer is

generally high (>25°C) hence, smaller rainfall events (e.g. 5–10 mm) when received intermittently do not maintain soil moisture in the surface soil for sufficient periods to stimulate all groups of microorganisms (e.g. 'nitrifying bacteria') and soil fauna (e.g. protozoa) to be able to contribute to biological functions. Such small rainfall events at high soil temperatures can only maintain soil moisture optimal for biological activities for less than 24 hours (Gupta et al., 2011). However, the relationship between N mineralisation and rainfall in-field conditions is difficult to determine due to the seasonal variation in temperature and evaporative demand (Sadras and Baldock, 2003).

Fluctuations in the amount of potentially mineralisable N and microbial biomass N can be found during the wheat-growing season, with the lowest levels generally found in late spring and early summer. The seasonal pattern of N mineralisation during wheat growth in southern Australia can vary from 0.2 kg N ha^{-1} day^{-1} during winter to 1 kg ha^{-1} day^{-1} during optimal soil moisture in spring, decreasing to 0 kg ha^{-1} day^{-1} as the soil dries. At the same time, the N demand from a cereal can be 5 kg ha^{-1} day^{-1} in spring (Angus, 2001). However, the amount of net N mineralised from soil and crop residues is variable, especially during fallow (Angus et al., 1998), and different ranges have been reported (Table 1).

Table 1. Mean ranges of soil net N mineralisation (kg N ha^{-1} day^{-1}) plus crop residues in field experiments conducted in Australia during fallow or break crop phase, and during wheat growing season indicating variability of reported values.

Crop Sequence	Fallow or Break Crop Net N mineralisation (kg N ha^{-1} day^{-1})	Wheat Growing Season	Soil Type and Location	Reference
pasture-wheat, lupin-wheat	0.15–0.24	0.3–0.4	Yellow sands, Moora, Western Australia	Anderson et al. (1998a)
wheat-canola, wheat-fallow, wheat-peas	-0.2–2	0.53–1.1	Sandy soils, Euston and Waikere, Mallee region, south-eastern Australia	Sadras and Baldock (2003)
pasture-wheat wheat-wheat	0.3–0.9 (wet) 0.08–0.33 (average) -0.14– -0.25 (dry)	0.12–0.28 (average)	Sandy loam, upper Eyre Peninsula, South Australia	Adcock (2005)
wheat-fallow wheat-oat	0.16–0.22 (dry) 0.12–0.17 (dry)		Calcareous soils, Yorke Peninsula, South Australia	Clough (2001)
wheat-pasture (DP) wheat-pasture (HI) wheat-canola (HI)	0.09 0.18 0.11–0.20		Alkaline Calcareous loamy sand soils, Waikerie, South Australia	Roget and Gupta (2004)

DP: district practice with fertiliser rate of 5 kg N ha^{-1}; HI: high fertiliser rate of 27 kg N ha^{-1}. *Negative values indicate N immobilisation.

Recently, Dunsford (2019) conducted field experiments in the low (< 400 mm per year), medium (400–550 mm per year) and high (>550 mm per year) rainfall zones of south-eastern Australia to estimate the contribution of net in-crop mineralisation (measured as the balance of N mineralised from soil organic matter and crop residues, minus N immobilised by soil microbes or lost to the environment) to crop N supply during the wheat or barley (*Hordeum vulgare*) growth phase. The median positive net in-crop N mineralisation value was 33 kg N ha^{-1} and the maximum was 116 kg N ha^{-1}, with growing-season rainfall and soil organic C tending to have the greatest impact on N mineralisation. While the net in-crop N mineralisation supplied an average of 63% of a crop's N demand, results for individual paddocks ranged from 0 to >100%, showing the high variability of mineralisation (Dunsford 2019). A previous study by Dunsford et al. (2015) estimated net in-crop mineralisation values between 24 and 35 kg N ha^{-1} in the low-rainfall zone and between 40 and 113 kg N ha^{-1} in the medium rainfall zone of Victoria over a season. Such variation highlights the challenge faced by advisors and growers when accounting for N mineralisation (both in-crop and between seasons) to formulate N budgets.

Measurements of N mineralisation and immobilisation are scarce for soils in the semi-arid Mallee region of south-eastern Australia, and hence, it is difficult to develop suitable N management strategies based on predictions of soil N supply. There is variable information on the pattern of N mineralisation and effects on N release during residue decomposition and the magnitude of site-specific soil and environmental factors controlling N mineralisation in these environments are still not clearly defined.

1.3 Management of in-season nitrogen supply for wheat crop

1.3.1 Residues as a source of nitrogen

Residues (here defined as mature crop material left in the field after grain harvest) play an important role in the cycling of N to subsequent crops because they can enhance N supply

through the release of N from decomposing materials before and during that crop growth. Decomposition of crop residues is a complex process which is driven by microbial processes and influenced by many factors, especially soil temperature and moisture and residue biochemical quality (Trinsoutrot et al., 2000). Variation in decomposition rates of different crop residues are largely related to differences in chemical composition related to N concentration, soluble organic-C and -N, and C:N ratio, and cellulose, lignin and polyphenol concentration (Adl, 2003). Established relationships between the kinetics of C and N decomposition and the biochemical characteristics of crop residues indicate that the residue C:N and N concentration are the most important factors for estimating the net effects of residue decomposition on soil mineral-N dynamics (Trinsoutrot et al., 2000).

Since the quality and the rate of decomposition of crop residues is determined by its C:N ratio, the C input rate is linked to the decomposition of crop residues, being a process involving the conversion of C and N in microbial tissue (Marschner and Rengel, 2012; McNeill and Unkovich, 2007; Singh and Rengel, 2007). As a general rule, those residues with C:N ratio greater than 30 result in an initial reduction of mineral N content due to net N immobilisation by microorganisms, while those having C:N ratio below 20 lead to increased mineral N and net N mineralisation (Lal et al., 1995). However, residues with similar C:N ratio can release different amounts of N (Cabrera et al., 2005) due to differences in compounds or groups of compounds such as proteins, polyphenols, cellulose-like, hemicellulose-like, lignin-like which were found to be related to N mineralisation (Quemada et al. 1997; Vigil and Kissel, 1995). Additionally, maintaining or improving soil fertility by fertiliser inputs can also maintain or enhance the soil C content (Lal et al., 1995), since the above- and below-ground biomass productivity can be augmented by N additions, increasing C returns (Singh and Rengel, 2007).

Legume residues readily release mineral N due to a low C:N ratio, increasing the soil N pool available for subsequent crops (Ladd and Amato, 1986; Crews and Peoples, 2005). The

potential benefits of legumes to N available for subsequent wheat crops depends on the total amount of N fixed. In soils with low N content the amount of N fixed is proportional to the legume dry matter produced (Ladd and Amato, 1986). In dryland systems of southern Australia significant amounts of mineral N (> 100 kg N ha^{-1}) can be accumulated in soil during the summer-autumn fallows between phases of legumes and wheat (Fillery, 2001). In Australian pasture systems the amounts of foliage N fixed by different annual and perennial legumes cultivated ranged from less than 10 to more than 250 kg N ha^{-1} per year (Peoples et al., 2012). In eastern Australia the differences in autumn soil mineral N after legume brown manured crops and non-legume treatments were in the ranges of 43–86 kg N ha^{-1} and 11–89 kg N ha^{-1} following legume grain crops (Peoples et al., 2017).

Studies using ^{15}N-labelling in these environments have shown rapid decomposition of legume residues and that the legume residue retained in soils decreased with increasing amounts added (Ladd et al., 1983b). Nevertheless, estimating the availability of N to the following crops over a given period of time under field conditions is challenging due to the complexity of the processes involved in residue decomposition, soil C and N dynamics (Singh and Rengel, 2007).

Legume-based pastures cultivated in rotation phases with wheat crops can maintain net N mineralisation. In general terms, during the pasture phase about 25% of the mineral N present in annual legumes is available prior to the subsequent cropping phase. On the assumption that the mineral N is located in the root-zone, and provided the mineralisation period is not followed by a heavy rain capable of leaching N below the root zone, the mineralised N will contribute to the growth of subsequent crops (Angus and Peoples, 2012). Although soil N losses from mineralised residues by leaching or denitrification in Mediterranean-type climates are usually scarce (Fillery, 2001), a lack of synchrony between crop N demand and N availability added to the pattern of rainfall can result in nitrate leaching losses up to 50 kg N ha^{-1} year^{-1} following spells of summer rainfall in the Mallee region of southern Australia

(Roget and Gupta, 2004), or as high as 72 kg N ha^{-1} from lupin (*Lupinus angustifolius*) below-ground residues on deep sandy soils of Western Australia (McNeill and Fillery, 2008).

Nitrogen supply from either pasture or grain legumes is partitioned into soil mineral N and soil organic matter by residue decomposition (Fillery, 2001) affecting the timing for N supply and the N taken up by the following crop (Ladd and Amato, 1986; Ladd et al., 1981, 1983a). Only a small proportion of pasture legume N is recovered by a second wheat crop as the direct benefit of the legume residue that is decomposed does not last beyond the second wheat crop in a cropping sequence (Peoples et al., 2017). Field data indicated that 16% of the pasture legume N from medics (*Medicago littoralis*) is recovered by the first succeeding wheat, with 4% of the legume N being recovered by a second wheat crop (Angus and Peoples, 2012).

In a review of the data from legume-cereals rotations of south-eastern Australia, Evans et al. (2001) found that net N inputs from grain legumes (lupin, pea and chickpea) averaged 47 kg N ha^{-1}, with lupin having the highest average net input of 88 kg N ha^{-1}. It was indicated that the mineralisation of organic N from the legume phase can contribute an average of 20–40 kg N ha^{-1} from stubble and root N to succeeding wheat. Similarly, in temperate soils of Australia, N availability for wheat following legumes was increased on average by 14–16 kg ha^{-1} when compared to cereals as predecessors (Kirkegaard et al., 2008). Nonetheless, it was suggested that in the short-term, in the absence of N fertiliser inputs, N used by wheat is mostly from soil native N rather than from the legume predecessor. However, in the long-term soil N balance, a significant contribution from grain legumes can be expected (Evans et al., 2001).

The main difference between pasture legumes and grain legumes is the greater proportion of biologically fixed N that is removed in the grains (Peoples et al., 2012). However, grain legumes such as narrow-leaf lupin grown in rotation with wheat can contribute to higher yields and protein content in the subsequent wheat phase (Evans et al., 2001) by replenishing the soil organic N reserves through a modest N release from lupin stubble decomposition

(Russell and Fillery, 1999) and/or *via* improved control of soil borne disease and pests (Gupta et al., 2011; McBeath et al., 2015).

The N supplied by legume below-ground residues to subsequent crops comprises an important proportion of total residue N, and the range of approaches that have been used have had varying results with respect to the amount of N that below ground residue N contributes to subsequent crops (Arcand et al., 2014a, b; Foyjunnessa et al., 2018; McNeill and Fillery, 2008; Russell and Fillery, 1996). Glasshouse studies to differentiate between N supplied from above- and below-ground crop residues of pea (*Pisum sativum* L.) and canola (*Brassica napus* L.) to wheat using ^{15}N labelling have reported that the amount of N in wheat derived from below-ground residues was almost twice the amount from above-ground residues (Arcand et al., 2014a). However, while 20–25% of below-ground lupin residue N was available as mineral N for wheat at planting only 10% was recovered by wheat shoots in a study by McNeill and Fillery (2008). In similar studies, the subsequent wheat crop recovered 20% of the below-ground residue N, representing 40% of the N uptake in the subsequent unfertilised wheat crop (Russell and Fillery, 1996); while wheat after pasture legumes recovered 25% and 18% of the below-ground residue N of subterranean clover (*Trifolium subterraneum* L.) and serradella (*Ornithopus compressus* L.), respectively (McNeill et al., 1997).

Similar to above-ground residues, there is a difference in the N contribution of different crop species. Comparison of lentil (*Lens culinaris*) and wheat below-ground residues found that a higher proportion of added ^{15}N from lentil below-ground residue (14.4 *vs*. 8.5%) was recovered in the succeeding wheat crop, indicating that lentil below-ground residues was more readily mineralised than wheat below-ground residues (Arcand et al., 2014b). Recent studies on a sandy soil using ^{15}N labelling have reported that the proportion of lupin below-ground N (37%) released was similar to that for canola below-ground N (33%) but a larger amount of N was released from lupin given the larger below-ground N pool. The proportion of below-ground ^{15}N recovered by wheat after lupin was slightly higher than after canola,

comprising 29.5% of the total wheat N uptake after lupin and 19% after canola (Foyjunnessa et al., 2018). Despite the considerable N mineralisation rates of below-ground residues of lupin (20-27%) reported in some annual-based cropping systems (McNeill and Fillery, 2008, Russell and Fillery, 1999) the asynchrony of mineral N supply and wheat N demand to achieve yield potential is still evident in semi-arid conditions.

1.3.2 The effect of break crops on wheat yield

Several studies have addressed the benefits of break crops to wheat yields, although the magnitude of the yield benefit in rain-fed farming systems of Australia is highly variable. In a review of 167 crop sequence experiments conducted in Western Australia during 1974–2007, Seymour et al. (2012) concluded that wheat after legume break crops generally yielded more than wheat after wheat in 90% of the analysed cases. The yield differences between wheat after lupin and wheat after wheat were variable, and ranged between -1.75 to 3 t ha^{-1} (Seymour et al., 2012). Similarly, recent studies in Mallee environments (Karoonda and Hopetoun) over a 3-year period to identify low risk, profitable break crops and end-uses, demonstrated that the inclusion of legume in rotation break crops increased the yield of the subsequent crop (Fig. 5), resulting in gains of 16–83% for water use efficiency (Browne et al., 2012; McBeath et al., 2015). The magnitude of the cumulative effect of break crops on three subsequent wheat crops at Karoonda was in the order of 1 t ha^{-1} owing to greater N cycling and supply. It also demonstrated the role of N to close the exploitable yield gap in these environments (McBeath et al., 2015; 2019).
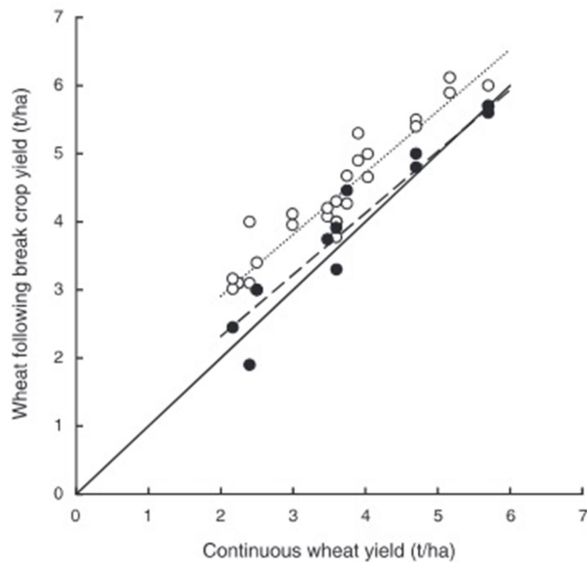
Figure 5. Relationship between wheat yields following break crops (WB) vs. wheat yields following continuous wheat (WW) at Karoonda and Hopetoun (Browne et al., 2012; McBeath et al., 2015). The relationship are parallel lines (P<0.001, r^2=0.90) where wheat yield following a *Brassica* break crop (*) was BW=0.51+0.91WW yield (dashed line), and following a legume break crop was LW=1.1+0.91WW yield (dotted line). Extracted from Kirkegaard et al. (2014).

1.3.3 Fertiliser as a source of nitrogen

Australian rain-fed wheat cropping systems are increasingly mining the soil organic N pool, which increases the amount of fertiliser N required to sustain productivity (Angus and Grace, 2017; Angus et al., 2019). Where in the past legume-based systems were less reliant on fertiliser N inputs (Murphy et al., 1998; Fillery, 2001), it is increasingly the case that these systems require supplemental N from fertiliser and in increasing amounts. This requirement is a response to reduced organic N, improved N harvest index of grain legumes and barriers to effective N fixation (Peoples et al., 2012) which mean that the right amount of N is not available at the right time for subsequent crops (Gupta et al., 2011; Hoyle and Murphy, 2011).

In several Australian rain-fed wheat cropping systems that include legumes in a sequence the N supplied from soil during a given season appears adequate for crop growth, so these systems are less dependent on supplemental N additions through fertiliser (Murphy et al., 1998; Fillery, 2001). Nonetheless, if the N released from soil and residues are insufficient, and/or if the timing of biological supply is not well matched with crop demand, then manipulating N supply with N fertilisers becomes crucial to achieve yield potential (Gupta et al., 2011; Hoyle and Murphy, 2011).

Fertiliser N recommendations are generally based upon the crop yield expectations, amount of mineral N available in the soil at planting, and an estimate of N inputs from previous legumes. Net N mineralisation from organic sources in soil is estimated using knowledge of local soils and climate in order to predict realistic yield goals, N fertilisation requirements, and inherent soil fertility. The time at which immobilisation occurs is also a factor that may influence the mean difference in fertiliser N requirement for cereals (Green and Blackmer, 1995). For instance, it was found that when N limitations occur at early stages of a wheat crop, N deficiencies can be alleviated by supply of fertiliser N (Ravier et al., 2017). Thus, accurate fertiliser N recommendations are vital for cost efficient and environmentally feasible cereal crop production.

In dryland cereal systems of Western Australia, about 30–50% of the N supplied from N fertiliser is taken up by a wheat crop in the year of application (Fillery and McInnes, 1992). In these farming systems on low organic matter soils N is generally known to be the most limiting nutrient, having the greatest fertiliser cost (Chen et al., 2008). Furthermore, the tendency for intensive and extended cropping phases in a sequence and the implicit reduction in biological fixation as a source of N in southern Australia have implications for better N fertiliser management (Angus, 2001).

Managing N fertilisation in environments prone to terminal drought, such as those of the Mallee dryland farming region in south-eastern Australia, relies on trade-offs between

fertiliser cost and the risk of a lack of yield response to N input in dry seasons. In this region, low and erratic rainfall events increase riskiness of grain production, leading to farmers tending to adopt conservative strategies, especially during dry seasons (Sadras, 2002). Nitrogen is often considered a risk-increasing input (Sadras, 2002), and thus, low N rates are adopted in the Mallee region (Asseng et al., 2001; Cossani et al., 2010; Sadras, 2002, Sadras, 2005). Growers' decisions are also affected by apparent low N use efficiency, N and wheat price fluctuations (Monjardino et al., 2013).

Monjardino et al. (2013) have indicated that using higher N rates than the region's average (district practice was between 15–20 kg N ha^{-1}) can improve economic returns on most of the dominant soil types of the Mallee area (dune and slope soils), including those returns to risk-averse farmers. These authors also demonstrated the potential benefits of using variable rate N fertiliser strategies based on site-specific management zones to improve profitability and reduce risk in a variable environment (Monjardino et al., 2013). The soil-specific N fertiliser strategy to better match soil type with inputs levels in this type of environments is being adopted as well as in several other low-rainfall regions (Robertson et al., 2012). Therefore, a better understanding of the soil-specific N supply to support a variable rate N fertiliser strategy is needed.

In Mediterranean environments potential losses from fertiliser N application such as urea are generally influenced by soil moisture and ambient temperature conditions, and so rainfall after N applications would affect movement of the dissolved urea into the soil (Ryan et al., 2009). In field experiments on wheat cropped on Mediterranean soils of Tunisia, the ^{15}N recovery with ammonium sulfate and ammonium nitrate ranged from 18% to 47%, volatilisation losses of 15%, and N derived from fertiliser of 9% and 21% with no leaching was observed in the dry season (El Mhiri and El Sanaa, 1993).

In no-till wheat crops of New South Wales, Australia, N losses *via* volatilisation measured using ^{15}N labelled urea plots ranged from 1–24% of N applied, with the higher losses

occurring when N was applied at crop establishment, and volatilisation was negligible when applied at ear initiation. Denitrification losses accounted for 1–14% of N applied, with the least loss occurring when wheat was fertilised at tillering (Bacon and Freney, 1989). It is important to consider that legume sources of N may also suffer significant losses of N, both during fallow periods and prior to uptake by the subsequent crop (Crews and Peoples, 2005). Studies conducted by Turner et al. (2012) have found that ammonia loss following late-winter and early-spring application of N fertiliser to cereal crops in southern Australia ranged from 1.8 to 23% of N applied and varied with location and fertiliser type, with substantially less volatilisation from ammonium nitrate and ammonium sulfate fertiliser relative to urea.

1.3.4 Identifying the source of nitrogen available for wheat uptake using ^{15}N-labelled techniques

Techniques using ^{15}N labelled tracers are robust tools that allow identification of sources and sinks of N within the soil–plant system such as crop fertiliser N recovery and N immobilisation in soil, as well as N losses (Follett et al., 1991). Application of high rates of N fertiliser can result in a short period of very high N availability in the root zone, often well above root physiological uptake capacity (Brackin et al., 2019). Blankenau et al. (2000) have conducted Mitscherlich's pot experiments under four different ^{15}N fertiliser rates (0, 300, 600, and 900 mg N pot^{-1}) that were equally split at tillering, stem elongation and ear emergence using ^{15}N labelled $Ca(NO_3)_2$ (10.8 at% ^{15}N abundance) for winter wheat on a loamy sand. They have found that ^{15}N fertiliser recovery in wheat roots at milk ripe growth stage was 6% on average, whereas in wheat shoots was 75% on average and increased with increasing ^{15}N fertiliser rates. This high fertiliser N use efficiency with a higher rate might indicate a better plant growth and thus a higher N demand. Also, fertiliser ^{15}N immobilised in the soil increased with increasing fertiliser N rates and between 5–9% of ^{15}N fertiliser was found in the soil microbial biomass N. On the contrary, in studies in winter wheat with different ^{15}N

fertiliser rates application and N splits, ^{15}N fertiliser recovery efficiency in wheat shoots at harvest was 24–41%, and it increased with decreasing ^{15}N fertiliser rates reflecting a lower level of fertiliser N demand (Wang et al., 2006).

Importantly fertiliser N has a residual value and ^{15}N based techniques allow tracing of the fate of fertiliser N over several growing seasons. Three field experiments in Eastern England using ^{15}N-labelled fertiliser on winter wheat were conducted to measure the persistence of the labelled N in soil and stubble at harvest and the availability of this N to up to four subsequent wheat crops. Wheat crop uptake was 6.6%, 3.5%, 2.2% and 2.2% over the 4 years (total of 14.5%); 55% remained in the soil (0–70 cm depth), and 29% was lost from the soil/crop system. Losses of the residual labelled N were more rapid from a sandy soil than from two heavier-textured soils, particularly in the first residual year (Hart et al., 1993). Field experiments in Australian dryland systems to quantify the fate of ^{15}N labelled wheat stubble to the successive wheat crop for up to two growing seasons at three different sites has found that 10–20% was taken up by the following two wheat crops; 35–45% was immobilised by soil microbes, 10–20% remained in residual stubble and 30% was unaccounted for (lost). At a site on a low rainfall Mallee sand, the following wheat crop took up 5% (0.75 kg N ha^{-1}) and 3.5% (0.4 kg N ha^{-1}) of the N derived from the original wheat stubble (representing only 1.2% and 1% of the crop's N requirements) (Gupta et al., 2018). Therefore, the use of ^{15}N labelling techniques can provide valuable information to identify the source of N that is available for wheat uptake in a particular environment under study by tracing N in the soil-plant system.

1.4 Towards improved nitrogen management for wheat crop *via* quantification of in-season nitrogen mineralisation

The mineralisation of N from soil organic matter during the growing season remains a primary source of N for crops (Angus, 2001), especially in the lower input systems in the rain-fed cropping regions of Australia. The measurement of *in situ* N mineralisation is useful

for determining the inherent yield potential and for more accurately determining the yield potential with fertiliser N application (Yan et al., 2006). However, this may require many replicated measurements owing to the large variability of N mineralisation rates due to small-scale spatial heterogeneity (CV~ 43–100%) in key soil characteristics (Clough, 2001; Sadras and Baldock, 2003; Xu et al., 1996). Nevertheless, given that soil N transformations are strongly affected by site-specific conditions of climate, soil micro-environment, and management, *in situ* methods are expected to give more field-relevant estimates than laboratory methods. To study soil mineral N dynamics there is no single methodology for the application of *in situ* methods; site-specific factors and field-testing is essential to adjust the method and to improve the reliability of estimates (Khanna and Raison, 2013).

Field studies of *in situ* N mineralisation in wheat cropping systems are commonly found for temperate environments, where rainfall is spread more evenly across the season than in the Mediterranean semi-arid type rainfed environments, which are characterised by winter dominant rainfall (Sadras et al., 2016). In south-eastern Australia in temperate rainfed environments, Angus et al. (1998) used the *in situ* method (Raison et al., 1987) to measure the in-crop N mineralisation for wheat crops growing on soils with different management history and total N contents. In experiments conducted in the Riverina, the south-west slopes of New South Wales and the tableland environments of the Australian Capital Territory, the N supply for wheat crops yielding less than 3 t ha^{-1} was mainly from mineralisation during crop growth, whereas the input from mineral N accumulated prior to sowing was comparably small. For crops yielding more than 4 t ha^{-1} the N supply was largely from mineral N present in the soil at sowing time. The knowledge gained by using this method is that, during the wheat growing season, crops need to be supplied with a greater amount of N than that from mineralisation (either from fertiliser or from mineral N accumulated during the fallow period), in order to achieve their water-limited yield potential (Angus et al., 1998).

Currently, there is no clarity for dryland farming areas in Mallee environments regarding seasonal in-field patterns of N mineralisation in soil and N release from crop residue decomposition, leading to uncertainty for decision making on fertiliser N applications and yield prediction. Measuring in-field N mineralisation could contribute to a better understanding for manipulating N supply for wheat N uptake in these environments. In this sense, plant and soil sampling at key times (e.g. pre-sowing, tillering) for a given wheat growing season (Fig. 6) can provide reliable estimations of the crop N demand and soil N supply, especially when performed under field conditions.
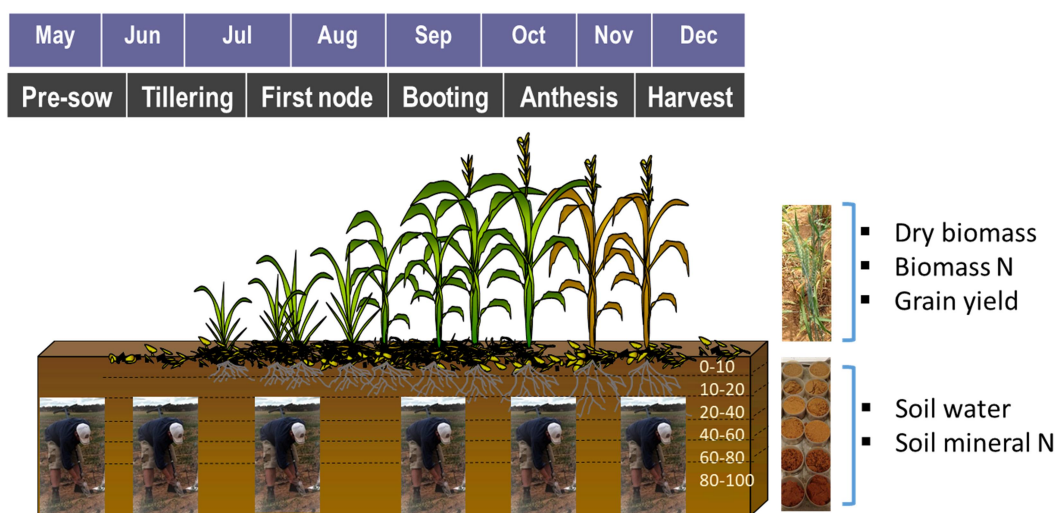
Figure 6. This graph illustrates plant and soil sampling at key times with an approximation of the calendar months for the wheat growing stages (Zadoks et al., 1974) in the low rainfall zone (<350 mm per year) of the Mallee in south-eastern Australia.

2. **Research aims**

The ability to predict the effect of N mineralisation (from soil organic matter and recent residue inputs) on crop performance remains difficult due to the interactions of soil, residue and fertiliser derived N within the complex physico-chemical soil matrix. This project aims to improve our understanding of the seasonal pattern of N mineralisation to supply wheat N

nutrition and to identify strategies that will improve wheat N uptake through N fertilisation inputs and crop rotation systems in low-rainfall environments of south-eastern Australia. Fulfilling this aim will allow the implementation of efficient management strategies to enhance N availability to improve wheat yield potential.

The objectives of this thesis are:

1. To characterise the seasonal pattern of N mineralisation resultant from different fertiliser N input and crop rotation schemes in a low-rainfall environment.
2. To assess the role of N release from different residue types on wheat crop productivity through the effect of timing on N availability.
3. To quantify the impact of combining different crop residue types and fertilisation N inputs on N use efficiency and N uptake by wheat in field conditions.
4. To identify critical wheat growth stages where ensuring N supply will improve wheat N uptake in a low rainfall sandy soil.

3. Theoretical framework and methods

In order to address the aims above mentioned, this PhD project consists of a set of experiments conducted under field and controlled environment conditions. A conceptual model describing the main processes and components addressed in this study is illustrated in Fig. 7.
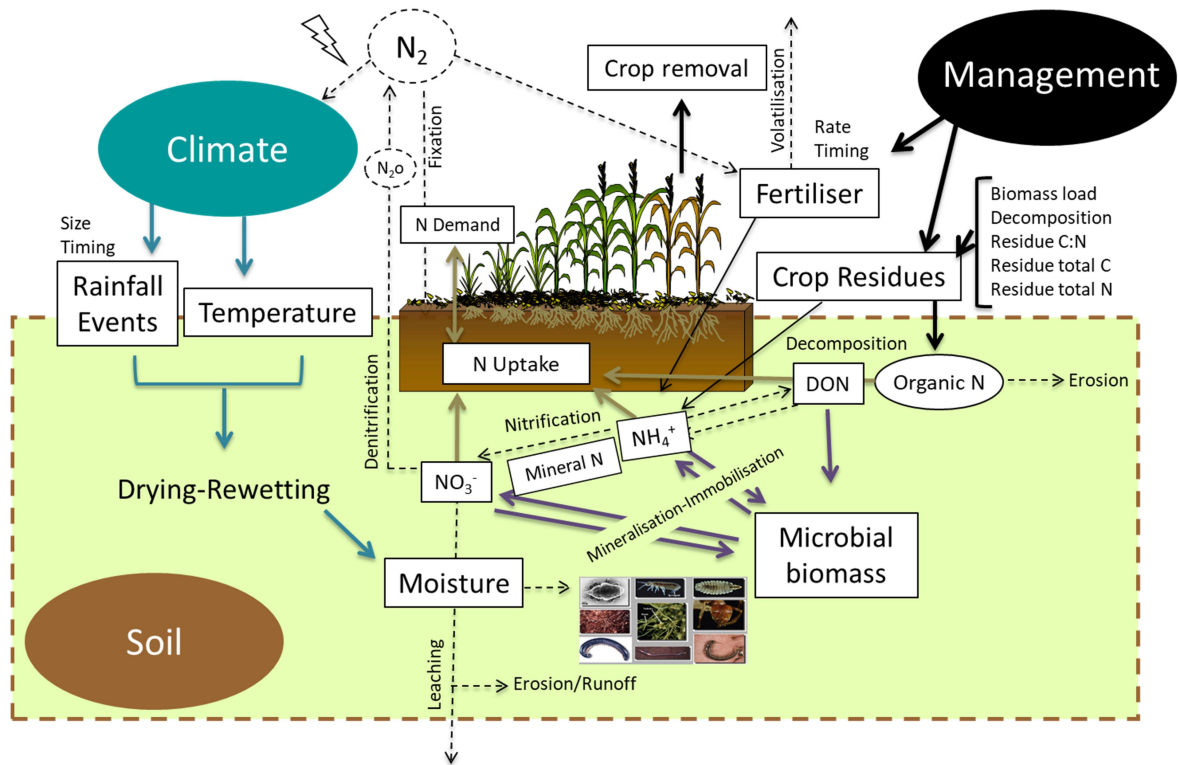
Figure 7. A conceptual model of the key factors affecting N availability for wheat. The main processes and components addressed in this PhD study are outlined by rectangles and indicated with bold arrows (solid lines). The brown dashed line indicates the processes and components within the soil matrix.

4. Significance and contribution to the discipline

This study adds to our current knowledge on manipulating N inputs to meet crop demand for wheat yield potential by choice of plant residues in conjunction with using mineral N fertilisers in low rainfall Mediterranean-type environments. With a better understanding of the seasonal pattern of soil N mineralisation occurring in these fragile environments, farmers may be able to improve the current management of crop sequences and fertiliser for economic and environmental sustainability. The information obtained from this project may enable farmers and advisors to select a better N fertilisation strategy that will improve the N bioavailability from soil at the appropriate time to meet plant demand, and consequently contribute to profitability of wheat production with less risk.

References

ABS, 2014. Australian Bureau of Statistics. Agricultural commodities. Available at
http://www.abs.gov.au/ausstats.

Adcock, D.P. 2005. Soil water and nitrogen dynamics of farming systems on the Upper Eyre
Peninsula, South Australia. PhD Thesis, The University of Adelaide, Adelaide, SA,
Australia.

Adl, S.M. 2003. The ecology of soil decomposition. CABI, Wallingford, Oxon, United
Kingdom.

Amato, M., R. Jackson, J. Butler and J. Ladd. 1984. Decomposition of plant material in
Australian soils. II. Residual organic 14C and 15N from legume plant parts
decomposing under field and laboratory conditions. Soil Research 22: 331-341.
doi:http://dx.doi.org/10.1071/SR9840331.

Anderson, G.C., I.R.P. Fillery, P.J. Dolling and S. Asseng. 1998a. Nitrogen and water flows
under pasture-wheat and lupin-wheat rotations in deep sands in Western Australia. 1.
Nitrogen fixation in legumes, net N mineralisation,and utilisation of soil-derived
nitrogen. Australian Journal of Agricultural Research 49: 329-344.
doi:http://dx.doi.org/10.1071/A97141.

Anderson, T.H. and K.H. Domsch. 1990. Application of eco-physiological quotients (qCO2
and qD) on microbial biomasses from soils of different cropping histories. Soil
Biology and Biochemistry 22: 251-255. doi:http://dx.doi.org/10.1016/0038-
0717(90)90094-G.

Angus J.F. and P.R. Grace. 2017. Nitrogen balance in Australia and nitrogen use efficiency on
Australian farms. Soil Research 55: 435-450.

Angus, J.F. 2001. Nitrogen supply and demand in Australian agriculture. Australian Journal of Experimental Agriculture 41: 277-288. http://dx.doi.org/10.1071/EA00141

Angus, J.F. and M.B. Peoples. 2012. Nitrogen from Australian dryland pastures. Crop and Pasture Science 63: 746-758. doi:http://dx.doi.org/10.1071/CP12161.

Angus, J.F., A.F. van Herwaarden, D.P. Heenan, R.A. Fischer and G.N. Howe. 1998. The source of mineral nitrogen for cereals in south-eastern Australia. Australian Journal of Agricultural Research 49: 511-522. doi:http://dx.doi.org/10.1071/A97125.

Arcand M., R. Lemke, R. Farrell and J.D. Knight. 2014b. Nitrogen supply from belowground residues of lentil and wheat to a subsequent wheat crop. Biology and Fertility Soils 50:507–515.

Arcand, M., J.D. Knight and R. Farrell. 2014a. Differentiating between the supply of N to wheat from above and belowground residues of preceding crops of pea and canola. Biol and Fertility Soils 50:563–570.

Asseng, S., N.C. Turner and B.A. Keating. 2001. Analysis of water- and nitrogen-use efficiency of wheat in a Mediterranean climate. Plant and Soil 233: 127-143. doi:10.1023/A:1010381602223.

Azam F., K. Haider and K.A. Malik. 1991. Transformation of 14C-labeled plant components in soil in relation to immobilization-remineralization o f 5N fertilizer. Plant and Soil 86: 15- 25.

Bacon, P.E. and J.R. Freney. 1989. Nitrogen loss from different tillage systems and the effect on cereal grain yield. Fertiliser Research 20. doi:10.1007/bf01055429.

Bingeman, C.W., J.E. Varner and W.P. Martin. 1953. The effect of the addition of organic materials on the decomposition of an organic soil. Soil Science Society America Proceedings 17: 34-38.

Blankenau, K., H. Kuhlmann and H.W. Olfs. 2000. Effect of increasing rates of 15N labelled fertiliser on recovery of fertiliser N in plant and soil N pools in a pot experiment with winter wheat. Journal of Plant Nutrition and Soil Science 163: 475–480.

Boot, C. 2011. Microbial response to drying and rewetting: osmotic and matric effects. Plant and Soil 348: 99-102. doi:10.1007/s11104-011-0958-9.

Borken, W. and E. Matzner. 2009. Reappraisal of drying and wetting effects on C and N mineralisation and fluxes in soils. Global Change Biology 15: 808-824. doi:10.1111/j.1365-2486.2008.01681.x.

Brackin, R., S. Buckley, R. Pirie and F. Visser. 2019. Predicting nitrogen mineralisation in Australian irrigated cotton cropping systems. Soil Research 57: 247–256.

Browne, C., J.R. Hunt and T.M. McBeath. 2012. Break crops pay in the Victorian Mallee. 16th Australian Society of Agronomy Conference. Capturing opportunities and overcoming obstacles in Australian agronomy. Australian Society of Agronomy/The Regional Institute, Armidale, NSW. p. 6.

Bryan, B.A., D. King and G. Zhao. 2014. Influence of management and environment on Australian wheat: information for sustainable intensification and closing yield gaps. Environmental Research Letters 9: 044005. doi:10.1088/1748-9326/9/4/044005.

Cabrera, M.L. 1993. Modeling the flush of nitrogen mineralisation caused by drying and rewetting soils. Soil Science Society of America Journal 57: 63-66. doi:10.2136/sssaj1993.03615995005700010012x.

Cabrera, M.L., D.E. Kissel and M.F. Vigil. 2005. Nitrogen mineralisation from organic residues: research opportunities. Journal of Environmental Quality 34: 75-79. doi:10.2136/sssaj 1988.03615995005200040024x.

Carpenter-Boggs, L., J.L. Pikul, M.F. Vigil and W.E. Riedell. 2000. Soil nitrogen
 mineralisation influenced by crop rotation and nitrogen fertilization. Soil Science
 Society of America Journal 64: 2038-2045. doi:10.2136/sssaj2000.6462038x.

Cawood, R. and G. McDonald. 1996. Climate of south-eastern Australia. In: R. Cawood,
 editor Climate, Temperature and Crop Production in South Eastern Australia.
 Agriculture Victoria: Melbourne. pp. 21–33.

Chen, D., H. Suter, A. Islam, R. Edis, J.R. Freney and C.N. Walker. 2008. Prospects of
 improving efficiency of fertiliser nitrogen in Australian agriculture: a review of
 enhanced efficiency fertilisers. Soil Research 46: 289-301.
 doi:http://dx.doi.org/10.1071/SR07197.

Chen, J., G. Xiao, Y. Kuzyakov, G.D. Jenerette, Y. Ma, W. Liu, Z. Wang and W. Shen. 2017.
 Soil nitrogen transformation responses to seasonal precipitation changes are regulated
 by changes in functional microbial abundance in a subtropical forest. Biogeosciences
 14:2513–2525.

Clough, A. 2001. Nitrogen and carbon mineralisation in agricultural soils of South Australia.
 PhD Thesis, The University of Adelaide, Adelaide, South Australia, Australia.

Connor, D.J. 2004. Designing cropping systems for efficient use of limited water in southern
 Australia. European Journal of Agronomy 21: 419–431.

Cossani, C.M., G.A. Slafer and R. Savin. 2010. Co-limitation of nitrogen and water, and yield
 and resource-use efficiencies of wheat and barley. Crop and Pasture Science 61: 844-
 851. doi:http://dx.doi.org/10.1071/CP10018.

Crews, T.E. and M.B. Peoples. 2005. Can the synchrony of nitrogen supply and crop demand
 be improved in legume and fertiliser-based agroecosystems? A review. Nutrient
 Cycling in Agroecosystems 72: 101-120. doi:10.1007/s10705-004-6480-1.

Dunsford, K. 2019. Understanding causes of poor grain yield response of wheat to phosphorus and nitrogen fertilisers. PhD Thesis, La Trobe University Victoria, Australia. 179 pp.

Dunsford, K., R.D. Armstrong, C. Tang and P. Sale. 2015. Estimating in-crop nitrogen mineralisation in dryland cropping systems of southern Australia. Proceedings of the 16th Australian Agronomy Conference' Hobart, Tasmania, Australia.

El Mhiri, M., and M. El Sanaa. 1993. Suitability of N fertilisers for rainfed wheat cultivation in the north of Tunisia. Proceedings, Environmentally sound location and crop-specific application of fertilisers in areas of North Africa and the Near East, Cairo and Ismailia, Egypt.

Evans, J., A.M. McNeill, M.J. Unkovich, N.A. Fettell and D.P. Heenan. 2001. Net nitrogen balances for cool-season grain legume crops and contributions to wheat nitrogen uptake: a review. Australian Journal of Experimental Agriculture 41: 347-359. doi:10.1071/ea00036.

Fierer, N. and J.P. Schimel. 2002. Effects of drying–rewetting frequency on soil carbon and nitrogen transformations. Soil Biology and Biochemistry 34: 777-787. doi:http://dx.doi.org/10.1016/S0038-0717(02)00007-X.

Fillery, I.R.P. 2001. The fate of biologically fixed nitrogen in legume-based dryland farming systems: A review. Australian Journal of Experimental Agriculture 41: 361-381. doi:10.1071/EA00126.

Fillery, I.R.P. and K.J. McInnes. 1992. Components of the fertiliser nitrogen balance for wheat production on duplex soils. Australian Journal of Experimental Agriculture 32: 887-899. doi:http://dx.doi.org/10.1071/EA9920887.

Follet, R.F., D.R. Keeney and R.M. Cruse. 1991. Managing nitrogen for groundwater quality and farm profitability. Soil Science Society of America, Wisconsin, United States. 357 pp.

Foyjunnessa, A. McNeill, A. Doolette and S. Mason. 2018. Dual-labelling (15 N and 33 P) provides insights into stoichiometry and release of nitrogen and phosphorus from in situ mature lupin and canola below-ground residues. Plant and Soil 426: 77-93.

Franzluebbers, A.J., F.M. Hons and D.A. Zuberer. 1994. Long-term changes in soil carbon and nitrogen pools in wheat management systems. Soil Science Society of America Journal 58: 1639-1645. doi:10.2136/sssaj1994.03615995005800060009x.

French, R.J. and J.E. Schultz. 1984. Water use efficiency of wheat in a Mediterranean-type environment. I. The relation between yield, water use and climate. Australian Journal of Agricultural Research 35: 743-764. doi:10.1071/AR9840743.

Gastal, F., G. Lemaire, J.L. Durand and G. Louarn. 2014. Quantifying crop responses to nitrogen and avenues to improve nitrogen-use efficiency. In: V.O. Sadras, editor Crop physiology. Elsevier. p. 161-198.

Gill, K., S.C. Jarvis and D.J. Hatch. 1995. Mineralisation of nitrogen in long-term pasture soils: effects of management. Plant and Soil 172: 153-162. doi:10.1007/BF00020869.

Godfray, H.C.J., J.R. Beddington, I.R. Crute, L. Haddad, D. Lawrence, J.F. Muir, et al. 2010. Food security: the challenge of feeding 9 billion people. Science 327: 812-818. doi:10.1126/science.1185383.

Gonzalez-Dugo, V., J.L. Durand and F. Gastal. 2010. Water deficit and nitrogen nutrition of crops. A review. Agronomy for Sustainable Development 30: 529-544. doi:10.1051/agro/2009059.

Green, C.J. and A.M. Blackmer.1995. Residue decomposition effects on nitrogen availability to corn following corn or soybean. Soil Science Society of America Journal 59: 1065–1070.

Greenwood, D.J., J.J. Neeteson and A. Draycott. 1986. Quantitative relationships for the dependence of growth rate of arable crops on their nitrogen content, dry weight and aerial environment. Plant and Soil 91: 281–301. 10.1007/BF02198111.

Gupta, V.V.R.S., T.M. McBeath, J. Kirkegaard, T. Swan and A. Richardson. 2018. 15N stubble experiments at Temora, Horsham and Karoonda. In: J. Kirkegaard, K. Verburg, V.V.R.S. Gupta, T.M McBeath, Z. Luo et al., editors Research outcomes on N-dynamics in stubble retained systems and their implications for N management guidelines, CSIRO, Australia, p.32-37.

Gupta, V.V.S.R. and D. K. Roget. 2004. Understanding soil biota and biological functions: management of soil biota for improved benefits to crop production and environmental health. In: R. Lines-Kelly, editor Proceedings of the Soil Biology in Agriculture Conference,Tamworth Agricultural Institute, Calala, NSW, pp 1–7.

Gupta, V.V.S.R., A.D. Rovira and D.K. Roget. 2011. Principles and management of soil biological factors for sustainable rainfed farming systems. In: P. Tow, I. Cooper, I. Partridge and C. Birch, editors, Rainfed Farming Systems. Springer, Netherlands. p. 149-184.

Haider, K., and Martin, J. P. 1981. Decomposition in soil of specifically C-labelled model and cornstalk lignins and coniferyl alcohol over two years as influenced by drying, re-wetting, and additions of an available substrate. Soil Biology and Biochemistry 13: 447-450. doi:10.1016/0038-0717(81)90032-8.

Hart, P.B.S. D.S. Powlson, P.R. Poulton, A.E. Johnston and D.S. Jenkinson. 1993. The availability of the nitrogen in the crop residues of winter wheat to subsequent crops. The Journal of Agricultural Science 121: 355–362.

Haynes, R.J. 1986. Chapter 2 - The decomposition process: mineralisation, immobilisation, humus formation, and degradation. In: R. J. Haynes, editor Mineral nitrogen in the plant–soil system. Academic Press. p. 52-126.

Hochman, Z. and H. Horan. 2018. Causes of wheat yield gaps and opportunities to advance the water-limited yield frontier in Australia. Field Crops Research 228: 20–30.

Hochman, Z., D.L. Gobbett and H. Horan. 2017. Climate trends account for stalled wheat yields in Australia since 1990. Global Change Biology 23: 2071–2081.

Hoogmoed, M., A. Neuhausb, S. Noack and V.O. Sadras. 2018. Benchmarking wheat yield against crop nitrogen status. Field Crops Research 222: 153-163.

Hoogmoed, M. and V.O. Sadras. 2018. Water stress scatters nitrogen dilution curves in wheat. Frontiers in Plant Science 9: 406.

Hoyle, F.C. and D.V. Murphy. 2006. Seasonal changes in microbial function and diversity associated with stubble retention versus burning. Soil Research 44: 407-423. doi:http://dx.doi.org/10.1071/SR05183.

Jarvis, S.C., E.A. Stockdale, M.A. Shepherd and D.S. Powlson. 1996. Nitrogen mineralisation in temperate agricultural soils: processes and measurement. In: L. S. Donald, editor Advances in agronomy. Academic Press. p. 187-235.

Jenkinson, D.S. and J.N. Ladd. 1981. Microbial biomass in soil: measurement and turnover. In: E.A. Paul and J.N. Ladd, editors Soil biochemistry. Marcel Dekker, New York, United States of America.

Jenkinson, D.S., R.H. Fox and J. H. Rayner. 1985. Interactions between fertilizer nitrogen and soil nitrogen-the so-called "priming" effect'. Journal of Soil Science 36: 425–444.

Keating, B.A., P.S. Carberry, G.L. Hammer, M.E. Probert, M.J. Robertson, D. Holzworth, et al. 2003. An overview of APSIM, a model designed for farming systems simulation. European Journal of Agronomy 18: 267-288. doi:http://dx.doi.org/10.1016/S1161-0301(02)00108-9.

Khanna, P.K. and R.J. Raison. 2013. In situ core methods for estimating soil mineral-N fluxes: Re-evaluation based on 25 years of application and experience. Soil Biology and Biochemistry 64: 203-210. doi:http://dx.doi.org/10.1016/j.soilbio.2012.09.004.

Kirkegaard, J.A., J.R. Hunt, T.M. McBeath, J.M. Lilley, A. Moore, K. Verburg, M.J Robertson., Y.M. Oliver, P.R.Ward, S. Milroy and A.M.Whitbread. 2014. Improving water productivity in the Australian grains industry - A nationally coordinated approach. Crop and Pasture Science 65: 583-601. 10.1071/cp14019.

Kirkegaard, J.A., O. Christen, J. Krupinsky and D. Layzell. 2008. Break crop benefits in temperate wheat production. Field Crops Research 107: 185-195. doi:http://dx.doi.org/10.1016/j.fcr.2008.02.010.

Kolberg, R.L., B. Rouppet, D.G. Westfall and G.A. Peterson. 1997. Evaluation of an in situ net soil nitrogen mineralisation method in dryland agroecosystems Soil Science Society of America Journal 61: 504-508. doi:10.2136/sssaj1997.03615995006100020019x.

Kumar, K. and K.M. Goh. 1999. Crop Residues and Management Practices: Effects on soil quality, soil nitrogen dynamics, crop yield, and nitrogen recovery. In: L.S. Donald, editor Advances in Agronomy. Academic Press. p. 197-319.

Ladd, J.N. and M. Amato. 1986. The fate of nitrogen from legume and fertiliser sources in

    soils successively cropped with wheat under field conditions. Soil Biology and

    Biochemistry 18: 417-425. doi:http://dx.doi.org/10.1016/0038-0717(86)90048-9.

Ladd, J.N., J.M. Oades and M. Amato. 1981. Distribution and recovery of nitrogen from

    legume residues decomposing in soils sown to wheat in the field. Soil Biology and

    Biochemistry 13: 251-256. doi:http://dx.doi.org/10.1016/0038-0717(81)90058-4.

Ladd, J.N., M. Amato, L.K. Zhou and J.E. Schultz. 1994. Differential effects of rotation, plant

    residue and nitrogen fertiliser on microbial biomass and organic matter in an

    Australian alfisol. Soil Biology and Biochemistry 26: 821-831.

    doi:http://dx.doi.org/10.1016/0038-0717(94)90298-4.

Ladd, J.N., M. Amato, R.B. Jackson and J.H.A. Butler. 1983a. Utilization by wheat crops of

    nitrogen from legume residues decomposing in soils in the field. Soil Biology and

    Biochemistry 15: 231-238. doi:http://dx.doi.org/10.1016/0038-0717(83)90064-0.

Ladd, J.N., R.B. Jackson, M. Amato and J.H.A. Butler. 1983b. Decomposition of plant

    material in Australian soils. I. The effect of quantity added on decomposition and on

    residual microbial biomass. Soil Research 21: 563-570.

    doi:http://dx.doi.org/10.1071/SR9830563.

Lal, R. 1995. Soils and global change. Lewis Publishers, Boca Raton, Florida, United States.

Lawlor, D.W. 2002. Carbon and nitrogen assimilation in relation to yield: mechanisms are the

    key to understanding production systems. Journal of Experimental Botany 53: 773-

    787. doi:10.1093/jexbot/53.370.773.

Lemaire, G. and F. Gastal. 1997. N uptake and distribution in plant canopies. In: G. Lemaire,

    editor Diagnosis of the nitrogen status in crops. Springer Berlin Heidelberg. p. 3-43.

Lemaire, G. and F. Gastal. 2009. Chapter 8 - Quantifying Crop Responses to Nitrogen Deficiency and Avenues to Improve Nitrogen Use Efficiency. In: V. Sadras and D. Calderini, editors Crop Physiology. Academic Press, San Diego. p. 171-211.

Liao, M., I.R.P. Fillery and J.A. Palta. 2004. Early vigorous growth is a major factor influencing nitrogen uptake in wheat. Functional Plant Biology 31: 121-129. doi:http://dx.doi.org/10.1071/FP03060.

Marschner, P. and Z. Rengel. 2012. Chapter 12 - Nutrient availability in soils. In: P. Marschner, editor Marschner's mineral nutrition of higher plants (3rd ed.). Academic Press, San Diego. p. 315-330.

McBeath, T.M., V.V.S.R. Gupta, R.S. Llewellyn, C.W. Davoren and A.M. Whitbread. 2015. Break crop effects on wheat production accross soils and seasons in a semi-arid environment. Crop and Pasture Science 66, 566–579. doi:10.1071/CP14166.

Mcbeath, T.M., V.V.S.R.Gupta, R.S. Llewellyn, S.D. Mason, C.W. Davoren, R.L. Correll, B. Jones and A.M. Whitbread. 2019. Combined application of nitrogen and phosphorus to enhance nitrogen use efficiency and close the wheat yield gap on varying soils in semi-arid conditions. Journal of Agronomy and Crop Science 205: 635–646.

McDonald, G.K., J.D. Taylor, A. Verbyla and H. Kuchel. 2012. Assessing the importance of subsoil constraints to yield of wheat and its implications for yield improvement. Crop and Pasture Science 63: 1043-1065. doi:http://dx.doi.org/10.1071/CP12244.

McNeill, A.M. and I.R.P. Fillery. 2008. Field measurement of lupin belowground nitrogen accumulation and recovery in the subsequent cereal-soil system in a semi-arid Mediterranean-type climate. Plant and Soil 302: 297-316. doi:10.1007/s11104-007-9487-y.

McNeill, A.M. and M. Unkovich. 2007. The nitrogen cycle in terrestrial ecosystems. In: P. Marschner and Z. Rengel, editors, nutrient cycling in terrestrial ecosystems. Springer Berlin Heidelberg. p. 37-64.

McNeill, A.M., C. Zhu and I.R.P. Fillery. 1997. Use of in situ 15N-labelling to estimate the total below-ground nitrogen of pasture legumes in intact soil-plant systems. Australian Journal of Agricultural Research 48: 295-304. doi:http://dx.doi.org/10.1071/A96097.

Mikha, M.M., C.W. Rice and G.A. Milliken. 2005. Carbon and nitrogen mineralisation as affected by drying and wetting cycles. Soil Biology and Biochemistry 37: 339-347. doi:http://dx.doi.org/10.1016/j.soilbio.2004.08.003.

Moll, R.H., E.J. Kamprath and W.A. Jackson. 1982. Analysis and interpretation of factors which contribute to efficiency of nitrogen utilization. Agronomy Journal 74: 562-564. doi:10.2134/agronj1982.00021962007400030037x.

Monjardino, M., T.M. McBeath, L. Brennan and R.S. Llewellyn. 2013. Are farmers in low-rainfall cropping regions under-fertilising with nitrogen? A risk analysis. Agricultural Systems 116: 37-51. doi:10.1016/j.agsy.2012.12.007.

Murphy, D.V., I.R.P. Fillery and G.P. Sparling. 1998. Seasonal fluctuations in gross N mineralisation, ammonium consumption, and microbial biomass in a Western Australian soil under different land uses. Australian Journal of Agricultural Research 49: 523-535. doi:10.1071/A97096.

Murphy, D.V., W.R. Cookson, M. Braimbridge, P. Marschner, D.L. Jones, E.A. Stockdale, et al. 2011. Relationships between soil organic matter and the soil microbial biomass (size, functional diversity, and community structure) in crop and pasture systems in a semi-arid environment. Soil Research 49: 582-594. doi:http://dx.doi.org/10.1071/SR11203.

Myers, R.J.K., C.A. Palm, E. Cuevas, I.U.N. Gunatilleke and M. Brossard. 1994. The synchronisation of nutrient mineralisation and plant nutrient demand. In: P. L. Woomer and M. J. Swift, editors The biological management of tropical soil fertility. Wiley-Sayce, Chicester, New York, USA. p. 81-116.

Naud, C., D. Makowski and M.H. Jeuffroy. 2008. Is it useful to combine measurements taken during the growing season with a dynamic model to predict the nitrogen status of winter wheat? European Journal of Agronomy 28: 291-300. doi:http://dx.doi.org/10.1016/j.eja.2007.08.005.

Palta, J.A. and I.R.P. Fillery. 1995. N application increases pre-anthesis contribution of dry matter to grain yield in wheat grown on a duplex soil. Australian Journal of Agricultural Research 46: 507–518.

Palta, J.A. and M. Watt. 2009. Vigorous crop root systems: form and function for improving the capture of water and nutrients. In: V. C. Sadras, D., editor Crop Physiology. Applications for Genetic Improvement and Agronomy. Elsevier. p. 309-325.

Pare, T. and E.G. Gregorich. 1999. Soil textural effects on mineralization of nitrogen from crop residues and the added nitrogen interaction, Communications in Soil Science and Plant Analysis, 30:1-2, 145-157.

Passioura, J.B. 2006. Increasing crop productivity when water is scarce—from breeding to field management. Agricultural Water Management 80: 176-196. doi:http://dx.doi.org/10.1016/j.agwat.2005.07.012.

Passioura, J.B. and J.F. Angus. 2010. Chapter 2 - Improving productivity of crops in water-limited environments. In: L. S. Donald, editor Advances in agronomy. Elsevier, Canberra, Australia. p. 37-75.

Peoples, M.B., H. Hauggaard-Nielsen and E.S. Jensen. 2009. The potential environmental benefits and risks derived rom legumes in rotations. In: H. B. Krishnan, editor

Nitrogen Fixation in Crop Production. American Society of Agronomy, Madison, WI, US. p. 349-385.

Peoples, M.B., J. Brockwell, J.R. Hunt, A.D. Swan, L. Watson, R.C. Hayes, et al. 2012. Factors affecting the potential contributions of N_2 fixation by legumes in Australian pasture systems. Crop and Pasture Science 63: 759-786.

Peoples, M.B., T. Swan, L. Goward, J.R. Hunt, G. Li, G. Schwenke et al. 2017. Soil mineral nitrogen benefits derived from legumes and comparisons of the apparent recovery of legume or fertiliser nitrogen by wheat. Soil Research 55:600-615.

Poole, N. and J. Hunt. 2014. Advancing the management of crop canopies, GRDC Project SFS00017. https://grdc.com.au/__data/assets/pdf_file/0019/126406/advancing-the-management-of-crop-canopies-pdf.pdf.pdf

Portmann, F.T., S. Siebert and P. Döll. 2010. MIRCA2000—Global monthly irrigated and rainfed crop areas around the year 2000: A new high-resolution data set for agricultural and hydrological modeling. Global Biogeochemical Cycles 24: GB1011. doi:10.1029/2008GB003435.

Quemada, M., M.L. Cabrera, and D.V McCracken. 1997. Nitrogen released from surface-applied cover crop residues: evaluating CERES-N. Agron J 89:723–729.

Raison, R.J., M.J. Connell and P.K. Khanna. 1987. Methodology for studying fluxes of soil mineral-N in situ. Soil Biology and Biochemistry 19: 521-530. doi:http://dx.doi.org/10.1016/0038-0717(87)90094-0.

Ravier, C., J.M. Meynard, J.P Cohan, P. Gate and M.H. Jeuffroy. 2017. Early nitrogen deficiencies favor high yield, grain protein content and N use efficiency in wheat. European Journal of Agronomy 89: 16–24.

Richards, R.A., J.R. Hunt, J.A. Kirkegaard and J.B. Passioura. 2014. Yield improvement and adaptation of wheat to water-limited environments in Australia—a case study. Crop and Pasture Science 65: 676-689. doi:http://dx.doi.org/10.1071/CP13426.

Richardson, A., J.M. Barea, A. McNeill and C. Prigent-Combaret. 2009. Acquisition of phosphorus and nitrogen in the rhizosphere and plant growth promotion by microorganisms. Plant and Soil 321: 305-339. doi:10.1007/s11104-009-9895-2.

Roberts, B.A., F.B. Fritschi, W.R. Horwath and S. Bardhan. 2015. Nitrogen mineralisation potential as influenced by microbial biomass, cotton residues and temperature. Journal of Plant Nutrition 38: 311–324.

Robertson, M.J., R.S. Llewellyn, R. Mandel, R. Lawes, R.G.V. Bramley, L. Swift, et al. 2012. Adoption of variable rate fertiliser application in the Australian grains industry: status, issues and prospects. Precision Agriculture 13: 181-199. doi:10.1007/s11119-011-9236-3.

Rodriguez, D. and V.O. Sadras. 2007. The limit to wheat water-use efficiency in eastern Australia. I.* Gradients in the radiation environment and atmospheric demand. Australian Journal of Agricultural Research 58: 287-302. doi:http://dx.doi.org/10.1071/AR06135.

Roget, D.K. and V.V.S.R. Gupta. 2004. Impact of management practices on soil microbial functions in alkaline Mallee soils. In: R. Lines-Kelly, editor Proceedings of the Conference Soil Biology in Agriculture, Tamworth Agricultural Institute, Tamworth, New South Wales, Australia. pp. 33–38.

Russell, C.A. and I.R.P. Fillery. 1996. Estimates of lupin below-ground biomass nitrogen, dry matter, and nitrogen turnover to wheat. Australian Journal of Agricultural Research 47: 1047-1059. doi:http://dx.doi.org/10.1071/AR9961047.

Russell, C.A. and I.R.P. Fillery. 1999. Turnover of nitrogen from components of lupin stubble to wheat in sandy soil. Soil Research 37: 575-592. doi:http://dx.doi.org/10.1071/S98071.

Ryan, J., H. Ibrikci, R. Sommer and A. McNeill. 2009. Nitrogen in rainfed and irrigated cropping systems in the Mediterranean Region. In: D.L. Sparks, D.L. editors Advances in Agronomy, Academic Press, Burlington, Canada.

Sadras, V.O. 2002. Interaction between rainfall and nitrogen fertilisation of wheat in environments prone to terminal drought: Economic and environmental risk analysis. Field Crops Research 77: 201-215. doi:10.1016/S0378-4290(02)00083-7.

Sadras, V.O. 2005. A quantitative top-down view of interactions between stresses: Theory and analysis of nitrogen-water co-limitation in Mediterranean agro-ecosystems. Australian Journal of Agricultural Research 56: 1151-1157. http://dx.doi.org/10.1071/AR05073.

Sadras, V.O. and C. Lawson. 2013. Nitrogen and water-use efficiency of Australian wheat varieties released between 1958 and 2007. European Journal of Agronomy 46: 34-41. doi:10.1016/j.eja.2012.11.008

Sadras, V.O. and G. Lemaire. 2014. Quantifying crop nitrogen status for comparisons of agronomic practices and genotypes. Field Crops Research 164: 54-64. doi:http://dx.doi.org/10.1016/j.fcr.2014.05.006.

Sadras, V.O. and J.A. Baldock. 2003. Influence of size of rainfall events on water-driven processes. II. Soil nitrogen mineralisation in a semi-arid environment. Australian Journal of Agricultural Research 54: 353-361. doi:http://dx.doi.org/10.1071/AR02113.

Sadras, V.O. and J.F. Angus. 2006. Benchmarking water-use efficiency of rainfed wheat in dry environments. Australian Journal of Agricultural Research 57: 847-856. http://dx.doi.org/10.1071/AR05359.

Sadras, V.O., C. Lawson, P. Hooper and G.K. Mcdonald. 2012. Contribution of summer rainfall and nitrogen to the yield and water use efficiency of wheat in Mediterranean-type environments of South Australia. European Journal of Agronomy 36: 41–54.

Sadras, V.O., P.T.Hayman, D. Rodriguez, M. Monjardino, M. Bielich, M. Unkovich, B. Mudge and E. Wang. 2016. Interactions between water and nitrogen in Australian cropping systems: physiological agronomic, economic, breeding and modelling perspectives. Crop and Pasture Science 67: 1019–1053.

Seymour, M., J.A. Kirkegaard, M.B. Peoples, P.F. White and R.J. French. 2012. Break-crop benefits to wheat in Western Australia – insights from over three decades of research. Crop and Pasture Science 63: 1-16. doi:http://dx.doi.org/10.1071/CP11320.

Shi, A. and P. Marschner. 2014. The number of moist days determines respiration in drying and rewetting cycles. Biology and Fertiliy of Soils: 1–9. doi:10.1007/s00374-014-0947-2.

Singh, B. and Z. Rengel. 2007. The role of crop residues in improving soil fertility. In: P. Marschner and Z. Rengel, editors Nutrient cycling in terrestrial ecosystems. Springer, Berlin, Heidelberg, Germany. p. 183–214.

Sommers, L.E., C.M. Gilmour, R.E. Wildung and S.M. Beck. 1981. The effect of water potential on decomposition processes in Soils1. In: J.F. Parr, W.R. Gardner and L.F. Elliott, editors Water potential relations in soil m icrobiology. Soil Science Society of America. pp. 97–117.

Specht, R.L. 1972. The vegetation of South Australia (2nd Edition). Government Printer, Adelaide.

St. Luce M, C.A. Grant, N. Ziadi, B.J. Zebarth, J.T. O'Donovan, R.E. Blackshaw, K.N. Harker, E.N. Johnson,Y. Gan, G.P. Lafond, W.E. May, et al. 2016. Preceding crops

and nitrogen fertilization influence soil nitrogen cycling in no-till canola and wheat cropping systems. Field Crops Research 191: 20–32.

St. Luce, M., R.Lemke, Y. Gan, B. McConkey, W. May, C. Campbell, R.Z. Zentner, H. Wang, R. Kroebel, M. Fernandez and K. Bradt. 2020. Diversifying cropping systems enhances productivity, stability, and nitrogen use efficiency. Agronomy Journal 112: 1517–1536.

Stanford, G., M.H. Frere and R.A. Vander Pol. 1975. Effect of fluctuating temperatures on soil nitrogen mineralisation. Soil Science 119: 222–226.

Tilman, D., C.Balzer, J. Hill, and B.L. Befort. 2011. Global food demand and the sustainable intensification of agriculture. Proceedings of the National Academy of Sciences of the United States of America 108: 20260–20264.

Trinsoutrot, I., S. Recous, B. Bentz, M. Line`res, D. Che`neby and B. Nicolardo. 2000. Biochemical quality of crop residues and carbon and nitrogen mineralization kinetics under non limiting nitrogen conditions. Soil Science Society of America Journal 64: 918–926.

Turner, E., R.E. Edis, D. Chen, J.R. Freney and O. T. Denmead. 2012. Ammonia volatilization from nitrogen fertilizers applied to cereals in two cropping areas of southern Australia. Nutrient Cycling in Agroecosystems 93: 113–126.

Turner, N.C and M.E. Nicolas. 1987. Drought resistance of wheat for light textured soils in a mediterranean climate. In: J.P. Srivastava, I. Porceddu, E. Acevedo and S. Varma, editors Drought Tolerance in Winter Cereals. John Wiley, Chichester, pp. 203–16.

Van Rees, H., T. McClelland, Z. Hochman, P.S. Carberry, J.R. Hunt, N.I. Huth, et al. 2014. Leading farmers in South East Australia have closed the exploitable wheat yield gap: Prospects for further improvement. Field Crops Research 164: 1–11. doi:http://dx.doi.org/10.1016/j.fcr.2014.04.018.

Van Schreven, D.A. 1968. Mineralisation of the carbon and nitrogen of plant material added to soil and of the soil humus during incubation following periodic drying and rewetting of the soil. Plant and Soil 28: 226–245. doi:10.1007/BF01880241.

Vigil, M.F. and D.E. Kissel. 1995. Rate of nitrogen mineralised from incorporated crop residues as influenced by temperature. Soil Sci. Soc. Am. J. 59: 1636–1644. doi:10.2136/sssaj1995.03615995005900060019x.

Wang, E., M.J. Robertson, G.L. Hammer, P.S. Carberry, D. Holzworth, H. Meinke, et al. 2002. Development of a generic crop model template in the cropping system model APSIM. European Journal of Agronomy 18: 121–140. doi:http://dx.doi.org/10.1016/S1161-0301(02)00100-4.

Wienhold, B.J. and A.D. Halvorson. 1999. Nitrogen mineralisation responses to cropping, tillage, and nitrogen rate in the Northern Great Plains. Soil Science Society of America Journal 63: 192–196. doi:10.2136/sssaj1999.03615995006300010027x.

Xu, Z., J. Ladd and D. Elliott. 1996. Soil nitrogen availability in the cereal zone of South Australia .1. Soil organic carbon, total nitrogen, and nitrogen mineralisation rates. Soil Research 34: 937–948. doi:http://dx.doi.org/10.1071/SR9960937.

Yan, D.Z., D.J. Wang, R.J. Sun and J.H. Lin. 2006. N mineralisation as affected by long-term N fertilization and its relationship with crop N uptake. Pedosphere 16: 125–130. doi:http://dx.doi.org/10.1016/S1002-0160(06)60034-9.

Yokobe, T., F. Hyodo and N. Tokuchi N. 2018. Seasonal effects on microbial community structure and nitrogen dynamics in temperate forest soil. Forests 9: 153.

Yuste, J.C., A.J. Fernandez-Gonzalez, M. Fernandez-Lopez, R. Ogaya, J. Penuelas, J. Sardans and F. Lloret. 2014. Strong functional stability of soil microbial communities under semiarid Mediterranean conditions and subjected to long-term shifts in baseline precipitation. Soil Biology and Biochemistry 69: 223–233.

Zadoks, J.C., Chang, T.T. and C.F. Konzak. 1974. A decimal code for the growth stages of cereals. Weed Research 14: 415-421.

Zhao, Z., E. Wang, Z. Wang, H. Zang, Y. Liu and J.F. Angus. 2014. A reappraisal of the critical nitrogen concentration of wheat and its implications on crop modeling. Field Crops Research 164: 65–73. doi:http://dx.doi.org/10.1016/j.fcr.2014.05.004

CHAPTER 2

Combined nitrogen input from legume residues and fertiliser improves early nitrogen supply and uptake by wheat

Supplementary material for this Chapter is provided in the Appendix section of this Thesis

Statement of Authorship

Title of Paper	Combined nitrogen input from legume residues and fertilizer improves early nitrogen supply and uptake by wheat
Publication Status	☑ Published ☐ Accepted for Publication ☐ Submitted for Publication ☐ Unpublished and Unsubmitted w ork w ritten in manuscript style
Publication Details	Muschietti Piana MP, T McBeath, A McNeill, PA Cipriotti & V Gupta. 2020. Combined nitrogen input from legume residues and fertilizer improves early nitrogen supply and uptake by wheat. *Journal of Plant Nutrition & Soil Science* 183, 355–366.

Principal Author

Name of Principal Author (Candidate)	Maria del Pilar Muschietti Piana	
Contribution to the Paper	Performed the experiment and the analysis of all samples, interpreted data, wrote the manuscript, illustrated all figures and tables, and act as the corresponding author.	
Overall percentage (%)	80%	
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.	
Signature		Date 01/09/2020

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

  i.   the candidate's stated contribution to the publication is accurate (as detailed above);

  ii. permission is granted for the candidate in include the publication in the thesis; and

  iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of Co-Author	Therese McBeath	
Contribution to the Paper	Provided advice on experimental design and analysis. Provided edits on several versions of the manuscript.	
Signature		Date 01/09/2020

Name of Co-Author	Ann M. McNeill	
Contribution to the Paper	Provided advice on experimental design, analysis and during manuscript preparation and review.	
Signature		Date 04/09/2020

Name of Co-Author	Pablo Ariel Cipriotti		
Contribution to the Paper	Provided advice on experimental design, statistical analysis and manuscript editing during preparation and review.		
Signature		Date	02/09/2020

Name of Co-Author	Vadakattu V. S. R. Gupta		
Contribution to the Paper	Provided advice on experimental design, analysis and during manuscript preparation and review.		
Signature		Date	02/09/2020

Please cut and paste additional co-author panels here as required.

Combined nitrogen input from legume residues and fertilizer improves early nitrogen supply and uptake by wheat

Pilar Muschietti-Piana[1,2]*, Therese M. McBeath[1], Ann M. McNeill[2], Pablo A. Cipriotti[3], and Vadakattu V. S. R. Gupta[1]

[1] Commonwealth Scientific and Industrial Research Organization (CSIRO) Agriculture and Food, Urrbrae, SA5064, Australia
[2] School of Agriculture, Food and Wine, The University of Adelaide, SA5005, Australia
[3] School of Agriculture-Instituto de Investigaciones Fisiológicas y Ecológicas Vinculadas a la Agricultura (IFEVA), University of Buenos Aires/ Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Av. San Martin 4453, Ciudad Autónoma de Buenos Aires, C1417DSE, Argentina

Abstract

Soil nitrogen (N) supply for wheat N uptake can be manipulated through legume and fertilizer N inputs to achieve yield potential in low-rainfall sandy soil environments. Field experiments over 2 years (2015–2016) were conducted at 2 different sites in a low-rainfall sandy soil to determine the soil N supply capacity relative to wheat N uptake at key growth stages, after a combination of crop residue (removed, wheat or lupin) and fertilizer N (nil, low or high N) treatments were manipulated to improve wheat yield. We measured the temporal patterns of the soil profile mineral N and PAW to 100 cm depth, wheat aerial biomass and N uptake in both years. In 2016 we also measured the disease incidence as a key environmental variable. There was 35 kg ha^{-1} more soil mineral N to 100 cm depth following lupin than wheat residues at the end of the fallow on average in both years. In a below average rainfall season, wheat biomass produced on lupin residues was responsive to N input with soil profile mineral N depleted by increased crop N uptake early in the season. In an above average rainfall season, a higher soil mineral N supply increased actual and potential grain yield, total biomass, N uptake, harvest index and water use efficiency of wheat, regardless of the source of N. Our study showed that the combination of lupin residues with high N rate increased soil profile mineral N at early growth stages, providing a greater soil N supply at the time of high wheat N demand, and the inclusion of a legume in the rotation is critical for improving the N supply to wheat, with added disease break benefits in a low-rainfall sandy soil environment.

Key words: growth stages / lupin / sandy soil / semi-arid environments / soil mineral N

Accepted March 14, 2020

Supporting Information available online

1 Introduction

About 70% of wheat (*Triticum aestivum* L.) is cultivated in dry land systems worldwide and in Australia is almost entirely rain fed (≈ 91%; *ABS*, 2014). Crop production and yield in the Australian wheat belt is therefore restricted by water availability and use efficiency (*Sadras* and *Angus*, 2006). Nitrogen (N) is the most limiting factor after water for cereal-based systems and N management for an adequate N supply, from either the soil or the fertilizer, is crucial for improving resource use efficiency (*Hochman* and *Horan*, 2018). This is especially true in soils having low organic content and N supply capacity, which are considered highly 'risky' in a management context (*Gupta* et al., 2011). The estimated N balance in Australian dryland cropping is positive mainly due to N-fixation by legumes in permanent pastures, and to a lesser extent from crop legumes, representing ≈ 5% of the crop area. However, fertilizer N use efficiency (NUE) remains at less than 50% recovery of fertilizer N in the year of application (*Angus* and *Grace*, 2017). Cropping systems which are more diverse (*i.e.*, break crops) are likely to be more resilient and less dependent on external inputs, and legumes play a key role in providing significant benefits for a sustainable agriculture. It is known that legumes can provide net additions of N into cropping soils, contributing to the N uptake by wheat and reducing N fertilizer requirements (*St. Luce* et al., 2016), improving grain yield, root disease incidence and weed control to the following cereals (*Seymour* et al., 2012).

About 70% of wheat N uptake in Australia is supplied by soil N mineralization (*Angus* and *Grace*, 2017). In the sandy soils of south-eastern Australia both the low N supply capacity and issues of asynchrony in the timing of N supply *via* mineralization relative to peak crop N uptake means that wheat is persistently lacking in N. Soil N supply capacity is mostly regulated by the amount and type of crop residues, the degree of soil-residue contact and microbial turnover (*Gupta* et al., 2011, 2012). Wheat N uptake is determined by the amount of N input used to achieve a target yield and the content, N in grain, straw and roots, and the N losses from the rooting

www.plant-soil.com

zone. Both N supply and uptake fluxes are highly variable as they are strongly affected by environmental (*i.e.*, climate, diseases) and management factors (*i.e.*, fertilizer N, crop rotation). So managing for synchrony between soil N supply and crop N uptake is vital for sustainable farming systems (*Hangs* et al., 2013).

Field studies conducted in Canadian soils have demonstrated that preceding legumes increase the apparent in crop N mineralization and soil mineral N prior to sowing wheat, reducing the reliance on fertilizer N inputs when compared to preceding wheat (*St. Luce* et al., 2016). Experiments in rain fed wheat of southern Australia have reported significant boosts to soil mineral N (> 100 kg N ha^{-1}) following legume phases (*Peoples* et al., 2017). However, estimating soil mineral N that will be available to the following wheat to meet crop's N demand at critical growth stages under field conditions is challenging due to the complexity of soil N dynamics and residue decomposition along with environmental variability. A knowledge gap still remains about the interactive effects of both crop residues and fertilizer N inputs on soil N supply for wheat N uptake (*St. Luce* et al., 2016), particularly in low N fertility and poorly buffered sandy soils of dry environments (*Gupta* et al., 2011).

Our study is focused on the low rainfall cropping zone of the Mallee environment in South Australia, characterized by unreliable rainfall patterns with a mean annual rainfall of ≈ 250–350 mm, of which ≈ 70% falls during May to October crop growing season. Fertilizer N rates are typically low and opportunities for late season application can be limited by a lack of rainfall events, leading to uncertainty for farmers when deciding on fertilizer N applications in this risky environment (*Monjardino* et al., 2013). Research in Mallee environments have demonstrated that the inclusion of a legume break crop increased the subsequent wheat yield (*McBeath* et al., 2015), due to greater N cycling and supply, and reduced soil-borne diseases (*Gupta* et al., 2012). Manipulating in crop mineral N availability through legume residues combined with fertilizer N inputs is vital to improve wheat yield by improving NUE in dry environments (*Angus* and *Grace*, 2017). Field experiments over 2 years (2015–2016) were conducted in 2 different sites in a low-rainfall sandy soil to determine the soil N supply capacity relative to wheat N uptake at key growth stages after a combination of crop residue (removed, wheat or lupin) and fertilizer N inputs (nil, low or high N) were manipulated to improve wheat yield. To identify the key drivers controlling wheat NUE we measured the temporal patterns of the soil mineral N and plant available water to 100 cm depth, and wheat N uptake in both years. In 2016, we also measured the biotic stress (diseases) as a key environmental variable.

2 Material and methods

2.1 Experimental site characterization, design and treatments

Experiments were conducted over 2 years in 2 different sites (Site 1 in 2015, Site 2 in 2016) in a representative field of the Mallee environment of the low rainfall cropping zone, at Low-

aldie (33°59.616S, 13°619.51E), South Australia. Both sites (situated ≈ 50 m apart) were positioned on a deep sandy soil on the dune (Kandosol; *Isbell*, 2002), with residues from the respective previous cropping season (Supplementary S.1.1): wheat residue retained or removed (removal of wheat aerial biomass with a low cut and rake, hereafter referred as 'removed residue') and lupin residue retained. We were unable to fully randomize the residue treatments in Site 1 due to operational and experimental conditions which were then facilitated in Site 2, to allow residue treatments to be randomized in a split-plot design (Supplementary Fig. S1.3). In Site 1, we utilized 3 residue management areas that were established in 2014 (wheat retained, wheat removed, and lupin retained) with physical separation of ≈ 15 m between each other, requiring that the areas were statistically analyzed as independent experiments (Supplementary Tab. S1.2.1). In the 2015 growing season, each residue management experiment comprised 8 plots with two different fertilizer N treatments (low or high N) with 4 replicates in a completely randomized design. In 2016 growing season, the residue treatments (removed, wheat, or lupin) and fertilizer N input treatments (nil, low, high N) were fully randomized in a split-plot design with four replicates.

For soil characterization (Supplementary Tabs. S.1.2.2 and S1.2.3), deep soil cores were collected from four of each residue experiment on March 17, 2015 in Site 1 and from all replicates for each residue experiment on June 01, 2015 in Site 2. In each plot, two deep soil cores to 100 cm depth were collected (with a hand auger in Site 1, with machine-corer in Site 2), segmented and combined for each layer. All cores were divided into depths of 0–10, 10–20, 20–40, 40–60, 60–80, 80–100 cm. Soil samples were sieved (< 2 mm), mixed and stored at –16°C prior to determining physical and chemical properties in the laboratory (Supplementary S.1.2).

Soils were characterized in both sites for particle size analysis with the pipette method in Site 1 (*McKenzie* et al., 2002) and using mid-infrared (MIR) spectroscopy in Site 2 (*Viscarra Rossel* et al., 2006), bulk density (*Burk* and *Dalgliesh*, 2008), pH and electrical conductivity (1:5, soil:water suspension), and cation exchange capacity (*Rayment* and *Lyons*, 2011), total organic C in surface soil (0–10 cm depth) by dry combustion using Leco TruMAC (2000-CNS, Leco Australia Pty. Ltd.), and to 10–100 cm depth (in 5 intervals) using MIR. Plant available water (PAW) capacity was estimated as the difference between the water content at field capacity and the crop lower limit (*Burk* and *Dalgliesh*, 2008). The field capacity was measured in the laboratory with a suction plate as the soil water content at a suction pressure of –10 kPa, while the crop lower limit was assessed in the experimental plots, using the driest soil water content observed during the growing season. Soils had a total PAW capacity (0–100 cm depth) of 96 mm in Site 1 and of 110 mm in Site 2. In both sites, there was an apparent texture change with increasing clay content from 40 cm depth increasing the water holding capacity. Soil samples (0–10 cm) were also analyzed for extractable phosphorus, potassium (*Colwell*, 1963) and sulfur (KCl-40, *Rayment* and *Lyons*, 2011). In both sites, soils had a neutral to alkaline pH increasing at depth with a low cation exchange capacity, organic C but moderate levels of extractable phosphorus,

potassium and sulfur. The electrical conductivity was low (< 0.9 dS m^{-1}) but exchangeable sodium (> 6%) was likely to restrict crop productivity below 40 cm depth, and boron (> 15 mg kg^{-1}) below 80 cm (*Peverill* et al., 1999) (Supplementary Tabs. S1.2.2–3).

2.2 Daily rainfall and air temperature in the experimental sites

The climate at the sites is Mediterranean-type with a mean annual rainfall of 337 mm and of 227 mm during May–October wheat growing season (historical records 1957–2014). Daily rainfall and air temperature were monitored by two weather stations: HOBO® Event Data Logger-H07-002-04 (USA) and MEA103 Mk 3 automatic station (Australia). In 2015 growing season rainfall was below the historical average (195 mm out of an annual of 334 mm). Total rainfall during November 2015 was 67 mm (with five rainfall events) but only two rainfall events were higher than 10 mm that might have caused leaching below 150 cm of soil depth. So there was a potential rainfall leaching event on November 04 of 31 mm and on November 13, 2015, of 25 mm. Average daily temperature was < 15°C from late March to early September, outside these periods it ranged between 15–35°C and was above average records. In 2016 growing season rainfall was above average (314 mm out of an annual of 474 mm), but it was below average during February and April. During September 2016 total rainfall was 114 mm (with fourteen rainfall events) but only one event of 33 mm might have caused leaching below 150 cm of soil depth and occurred on September 07, 2016. Mean daily temperature was < 17°C from late April to mid-October, outside these periods it ranged between 16–28°C (Fig. 1).
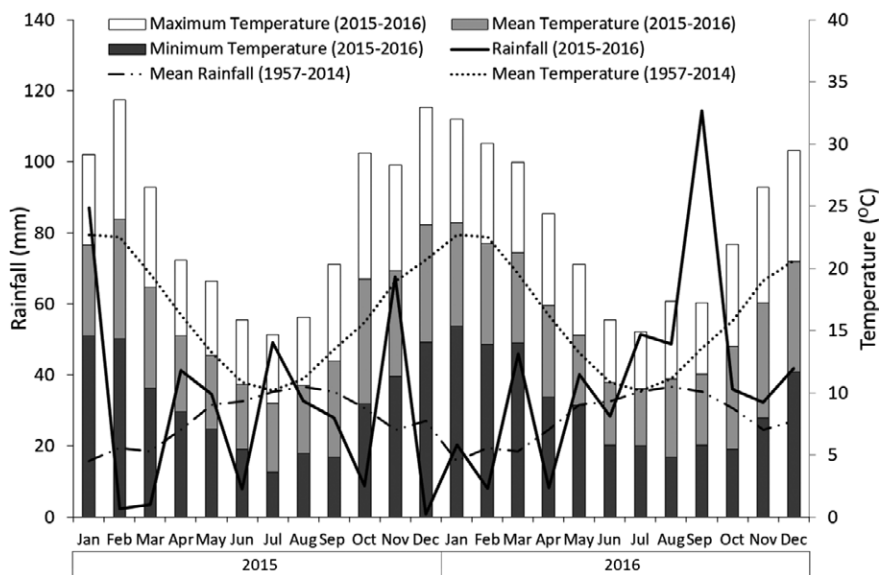
2.3 Soil mineral nitrogen and water profiles at the fallow season

The (autumn) fallow at Site 1 comprised the start of the experiment and the wheat sowing time (March 17 to May 21, 2015), whereas at Site 2, the whole of the fallow period was measured, between the harvest of the preceding crop and the sowing of the following wheat (December 02, 2015, to June 02, 2016). Fallow periods were characterized by high temperatures and low rainfall which prevents growth of annual crop and pasture species (Fig. 1), so the soil was left uncultivated in both sites. Soil samples collected from each plot at the start and end of the fallow in each site were processed (as in section 2.1) prior to soil water and mineral N (ammonium + nitrate) analysis. Soil gravimetric water content was measured from the difference in weight between the samples as collected and when oven-dried (at 105°C to constant weight) and converted to volumetric water content using the measured bulk density for each soil layer. Soils were extracted for mineral N by shaking with 2 mol L^{-1} KCl (1:3, soil:solution) on an orbital shaker at 25°C for 1 h and filtered through Whatman No. 1. Nitrate and ammonium N concentration of extracts were analyzed colorimetrically (*Miranda* et al., 2001) using SynergyMX microplate reader. Soil water was expressed as PAW (%, v/v), as the difference between the actual water content and the crop lower limit (*Burk* and *Dalgliesh*, 2008).

2.4 Estimation of fallow net nitrogen mineralization

Net N mineralization (kg ha^{-1}) during the fallow was estimated according to Eq. (1):

$$M = \sum_{i=0}^{n} S_i - \sum_{i=0}^{n} F_i, \tag{1}$$

where *M* is the net mineralization (kg N ha^{-1}), *S* is the sum of soil mineral N (kg N ha^{-1}) at wheat sowing time (May 21, 2015 for Site 1, and June 02, 2016 for Site 2), *F* sum of soil mineral N (kg N ha^{-1}) at the start of fallow (March 17, 2015 for Site 1, and December 02, 2015 for Site 2), and *i* is the sampling depth to 100 cm.

2.5 Residue load and quality prior to sowing

One quadrat sample of wheat or lupin residue was collected (May 21, 2015, in Site 1, and May 30, 2016, in Site 2) randomly in each respective plot using a 0.32 m^2 quadrat (0.56 m × 0.56 m). Dry biomass weights (forced-air oven at 60°C for 48 h) from each quadrat were recorded and averaged to quantify the aerial residue load in each residue experiment/ treatment. Samples were ground to pass a 0.5-mm sieve and subsamples (0.5 g) were ashed in a muffle furnace at 550°C for 16 h. The ash content was used to adjust the sample dry weights to an ash-free dry

Figure 1: Monthly rainfall (mm) and air temperature (mean, maximum, minimum, °C) recorded with two weather stations located near the experimental sites during 2015–2016. Historical monthly mean rainfall and air temperature (1957–2014) was obtained from the SILO database (https://www.longpaddock.qld.gov.au/).

weight basis to correct for any contaminating soil in the sample. Total C and N in wheat and lupin residues were determined by high temperature combustion in an atmosphere of oxygen using Leco TruMAC (Tab. 1).

2.6 Agronomic management of the sites

In both years, wheat was sown at a 70 kg seed ha^{-1} rate with a 7-row conventional plot seeder with narrow points (50-mm wings) and press-wheels spaced 0.25 m apart. Wheat was sown on May 21, 2015 (cv. Mace) in Site 1 and on June 02, 2016 (cv. Scepter) in Site 2, within the optimal window when surface soil water was adequate to allow even establishment. In both years wheat seeds were treated with Vibrance and Reldan (1000 gwt = 46.5 g) for the prevention of fungal diseases and pre-emergent herbicide was applied prior to sowing. In 2015, fertilizers were applied at sowing, banded below the seed as 50 kg ha^{-1} of di-ammonium phosphate and 24 kg of urea (to supply ≈ 10 kg P ha and 20 kg N ha^{-1}). To reduce the risk of N toxicity at sowing, the remainder of the N dose for the high N treatment was applied on June 16, 2015, as 76 kg ha^{-1} of urea (to supply ≈ 35 kg N ha^{-1}). In 2016, fertilizers were applied at sowing by banding below the seed a blend of 49 kg ha^{-1} of triple superphosphate to supply rates of ≈ 10 kg P ha^{-1}, and 43 or 87 kg ha^{-1} of urea to supply rates of 20 or 40 kg N ha^{-1}, respectively. In both years all plots received trace element fertilizers to ensure that nutrients other than N were not limiting, and wheat was harvested following final sampling using a plot-harvester (Supplementary Tab. S1.2.1). In 2016, 20 plant samples were randomly collected at 7 weeks after sowing from wheat residue with no fertilizer N plots, which were the most susceptible to root disease (*e.g.*, *Rhizoctonia Solani*). Roots were washed and rated for disease on a 0–5 scale: 0, no disease to 5, all primary roots infected and severely truncated (*McDonald* and *Rovira*, 1985). At the wheat grain filling stage several plots

evidenced patches of white heads, a symptom of crown rot (*Fusarium pseudograminearum* and *F. culmorum*). So at 23 weeks after sowing, the severity of diseases were monitored in each plot by counting the number of wheat plants with white heads and the total number of heads 8 times in 0.5 m of crop row (≈ 1 m^2 per plot). The severity of the disease was expressed as the mean proportion of crop heads present as white heads for each treatment.

2.7 Wheat aerial biomass and nitrogen uptake, soil mineral nitrogen and water profiles at key wheat growth stages

Wheat plant samples were collected in all plots (4 × 0.5 m rows, ≈ 0.5 m^2 area in 2015, and 8 × 0.5 m rows, ≈ 1 m^2 area in 2016) for determining aerial biomass, and N content at 5 growth stages (*Zadoks* et al., 1974), and for yield and grain N at maturity. Aerial biomass and grain yield were expressed on a dry matter basis. Plants were oven dried at 60°C to a constant weight, ground, sieved (1 mm mesh) and analyzed for total C and N (Leco TruMAC), and N uptake (stubble, grain) was determined by the percentage of total N in each fraction multiplied by the biomass weight and converted to kg N ha^{-1}. In both years, deep soil cores were collected in all plots prior to sowing and at 5 growth stages, and were segmented and combined for each layer, stored, processed and analyzed for soil mineral N and PAW (as in section 2.3). Sampling details provided in the Supplementary S1.4.

2.8 Estimated in crop net nitrogen mineralization

In both years, the net N mineralization (kg ha^{-1}) during each growing season was estimated as Eq. (2), assuming that there was no movement of soil mineral N below 100 cm depth and there was no wheat N uptake of soil mineral N from below 100 cm depth.

Table 1: Soil and residues main characteristics at the start of the fallow and end of the fallow at the experimental sites. # = mean values of 4 replicates ± standard deviation, * = mean values of 8 replicates ± standard deviation, and ∧ = mean values of 12 replicates ± standard deviation. In 2016, columns with different letters indicate significant differences between residue treatment means (*p* < 0.05). NA = not applicable, [S] = March 17, 2015, in Site 1, December 02, 2015, in Site 2, [E] = May 20, 2015, in Site 1, June 01, 2016, in Site 2.

	2015 (Site 1)			2016 (Site 2)		
	Removed	**Wheat**	**Lupin**	**Removed**	**Wheat**	**Lupin**
Sum of soil mineral N (0–100 cm, kg ha^{-1}) at start of the fallow[S]	67 ± 6*	60 ± 4*	73 ± 4*	NA	26 ± 4 b ∧	69 ± 6 a ∧
Sum of soil mineral N (0–100 cm, kg ha^{-1}) end of the fallow[E]	47 ± 2*	36 ± 5*	63 ± 4*	100 ± 2 b ∧	98 ± 11 b ∧	129 ± 12 a ∧
Sum of plant available water (0–100 cm, mm) end of the fallow[E]	62 ± 2*	62 ± 3*	59 ± 2*	59 ± 4 a ∧	59 ± 3 a ∧	65 ± 5 a ∧
Estimated fallow net N mineralization (0–100 cm, kg ha^{-1})	−18 ± 6#	−31 ± 11#	−11 ± 9#	NA	73 ± 21 a#	53 ± 26 a#
Residue load (t ha^{-1})	NA	4 ± 1*	6 ± 2*	NA	5 ± 1 a ∧	1 ± 1 b ∧
Residue total C (g kg^{-1})	NA	417 ± 11*	439 ± 15*	NA	439 a ∧	415 a ∧
Residue total N (g kg^{-1})	NA	5 ± 0*	11 ± 1*	NA	5 ± 1 a ∧	8 ± 3 a ∧
Residue C:N ratio	NA	79 ± 5*	38 ± 2*	NA	45 ± 9 a ∧	37 ± 6 b ∧

$$M = \sum_{i=0}^{n} H_i - \sum_{i=0}^{n} S_i + \sum_{i=0}^{n} U - \sum_{r=0}^{n} F_r, \qquad (2)$$

where M is the estimated net N mineralization (kg ha^{-1}), H is the sum of soil mineral N (kg ha^{-1}) at harvest (November 30, 2015 in Site 1, December 07, 2016 in Site 2), S is the sum of soil mineral N (kg ha^{-1}) at sowing time (May 21, 2015 in Site 1, June 02, 2016 in Site 2), i is the sampling depth to 100 cm, U is total wheat N uptake (kg ha^{-1}), F is fertilizer N and r is the N input (kg ha^{-1}).

2.9 Wheat crop attributes at harvest

The harvest index was calculated as the ratio of harvested grain to total aerial biomass (dry matter basis). Water use efficiency (WUE, kg grain ha^{-1} mm^{-1}) was calculated as the product of grain yield (kg ha^{-1}) and water use (mm); water use was calculated as the difference of PAW (mm) at sowing and at harvest (to rooting depth of 60 cm in 2015, and to 100 cm soil depth in 2016) *plus* the growing season rainfall (mm). The N use efficiency (NUE) for grain yield (kg grain kg N^{-1}) was calculated as the product of the N uptake and N utilization efficiency; with the N uptake efficiency as the ratio of N uptake (stubble + grain) to soil mineral N at sowing *plus* the fertilizer N input, and the N utilization efficiency as the ratio of grain yield to N uptake (stubble + grain) (*Moll* et al., 1982). Wheat yield potential (kg ha^{-1}) was estimated as Eq. (3):

$$Yield\ potential = [water\ use(mm) - evaporation(mm)] \\ \times WUE\ benchmark(kg\ grain\ ha^{-1}mm^{-1}), \qquad (3)$$

where *evaporation* is 110 mm (*French* and *Schultz*, 1984) and *WUE benchmark* is 22 kg grain ha^{-1} mm^{-1} (*Sadras* and *Angus*, 2006).

2.10 Statistical analysis

In 2015, within each residue experiment, the effect of 'time'(as 'growth stage') and 'fertilizer N' on each measured variable (*e.g*., wheat biomass) was analyzed using ANOVA for repeated measures designs with linear mixed effects models (lme) in R (*R Core Team*, 2015). The 'growth stage' and 'N rate' were used as fixed effects, and the 'replicate' as a random effect. Where ANOVA results showed a significant effect of growth stage × N rate, the comparisons between treatment means were performed with DGC (Di Rienzo, Guzman, Casanoves) test ($p < 0.05$; *Rienzo* et al., 2002). In 2016, for all treatments ('residue' and/or 'fertilizer N'), the effect growth stage on each measured variable was analyzed using ANOVA for repeated measures design with linear mixed effects models (lme) in R (*R Core Team,* 2015). Residue and fertilizer N treatments were used as fixed effects, and block, main plot and sub-plot as a random effect. Where ANOVA results (Supplementary Tab. S2.2) showed a significant effect of the residue or fertilizer N treatment, growth stage and/or their interactions, the comparison between treatment means were performed with DGC test ($p < 0.05$). In both years, paired t tests ($p < 0.05$) were performed when there was only

1 factor to compare treatment differences; *e.g*., for '20 *vs*. 55N' within each residue experiment. Relationships between soil, crop and environmental variables (*i.e*., rainfall) were assessed by correlation analysis with Infostat v.7.

3 Results

3.1 Soil and residue conditions in each residue experiment/treatment during the fallow season

Overall, results from our field experiments over two years have shown that there was more soil profile mineral N after lupin than after wheat residues, both at the start and end of the fallow periods considered for each site, but the estimated fallow net N mineralization was similar between wheat and lupin residues. The PAW in the soil profile showed a similar pattern in all treatments, at the start and end of the fallow, tending to increase up to ≈ 80 cm soil depth (Supplementary Figs. S3.1 and S3.2). At the time of wheat crop sowing in 2015, lupin residues appeared to have similar values of biomass load, greater N concentration, and narrower C:N ratio than in the wheat residues experiment, whereas in 2016 lupin residues showed a lower load and C:N than wheat residues (Tab. 1).

3.2 Wheat aerial biomass and nitrogen uptake, and soil mineral nitrogen at key growth stages

In 2015, wheat showed no response in plant growth or N uptake to a higher fertilizer N rate over the growing season in either the removed (Fig. 2a, d) or the wheat (Fig. 2b, e) residue experiments. But in the lupin residue experiment, wheat had a clear response in plant growth to the 55N rate at advanced growth stages. Wheat aerial biomass was 2.5 fold higher at booting and anthesis compared to that with 20N rate (Fig. 2c), resulting in higher N uptake ($p = 0.04$; Fig. 2f).

In the wheat residue experiment with the 55N rate, higher soil mineral N ($p < 0.001$) remained at the end of booting (Fig. 4b). Greater soil mineral N was observed under lupin residues at first node and booting with 20N rate ($p = 0.03$; Fig. 4c), corresponding with lower aerial biomass. The greater N uptake by wheat with the 55N rate can be attributed in part to a greater extraction of mineral N and water from the soil profile, as the mineral N and PAW were higher for the 20N rate at harvest within the 60–80 cm soil depth (Fig. S3.1c, f). Total soil mineral N (0–60 cm) at harvest was higher with 20N than with 55N rate (32 ± 3 *vs*. 24 ± 2 kg ha^{-1}, respectively; Fig. 4c).

In 2016, wheat aerial biomass was higher after lupin than after removed and wheat residues between first node and anthesis, regardless of the fertilizer N input (Fig. 3a), and the magnitude of this difference was higher as the season progressed to anthesis. The fertilizer N inputs resulted in a higher wheat aerial biomass between tillering and anthesis regardless of the residue treatment (Fig. 3b). At first node, the wheat N uptake was higher after lupin (+19 kg N ha^{-1}) and removed residues (+6 kg N ha^{-1}) than after wheat residues, regardless of the fertilizer N input ($p < 0.0001$; Fig. 3c). The difference in wheat N uptake was highest at flag leaf with 34, 14, and
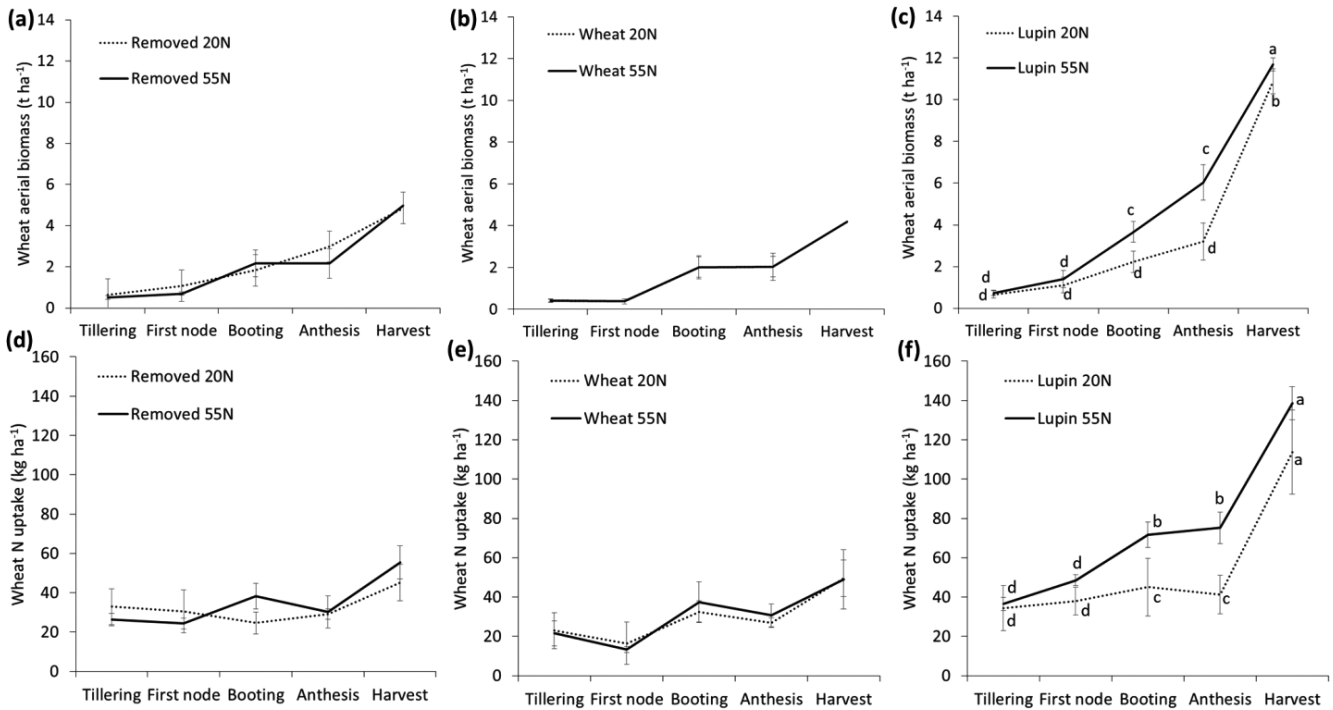
Figure 2: Wheat aerial biomass and N uptake in 2015 (Site 1) for the removed (a, d), wheat (b, e), and lupin (c, f) residue management experiments with 20 or 55 kg N ha^{-1} rates at different growth stages. Different letters indicate significant differences of the mean for interaction 'growth stage *x* 'N rate' (DGC test, *p* < 0.05).
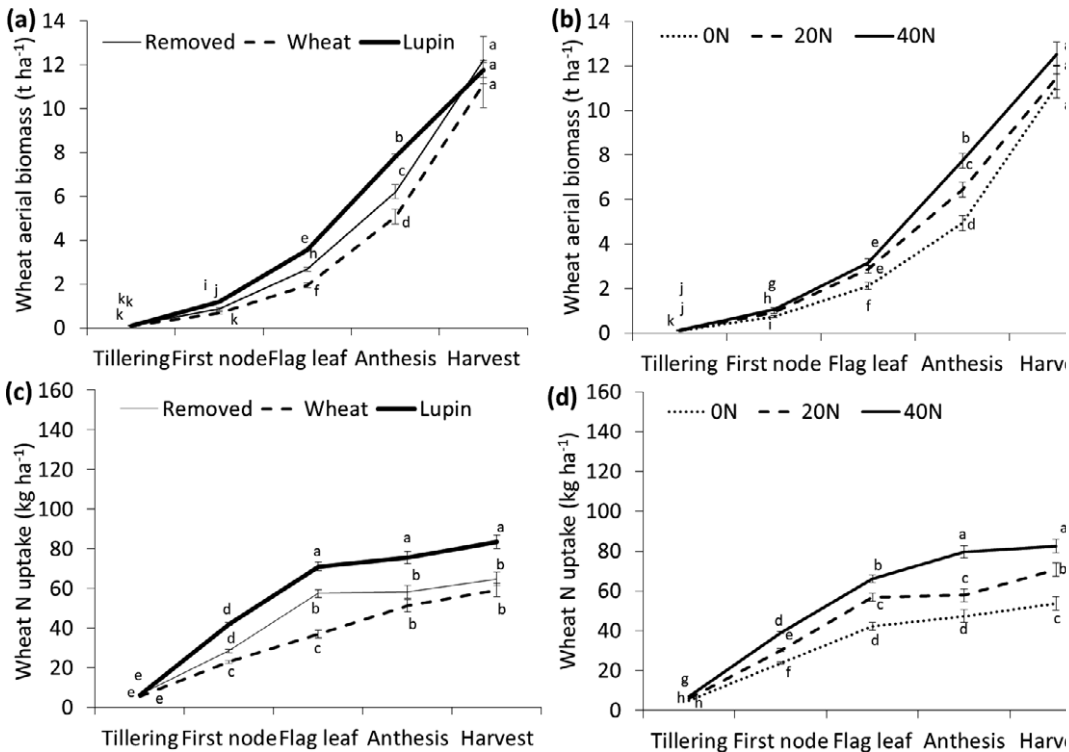
Figure 3: Wheat aerial biomass (a) and N uptake (c) in 2016 (Site 2) in the removed, wheat and lupin residues at different growth stages, wheat aerial biomass (b), and N uptake (d) for 0, 20, or 40 kg N ha^{-1} inputs at different growth stages. Different letters indicate significant differences among means for the interaction 'residue treatment' *x* 'growth stage' (a, c) and for 'fertilizer N rate' *x* 'growth stage' (DGC test, *p* < 0.0001) (b, d).
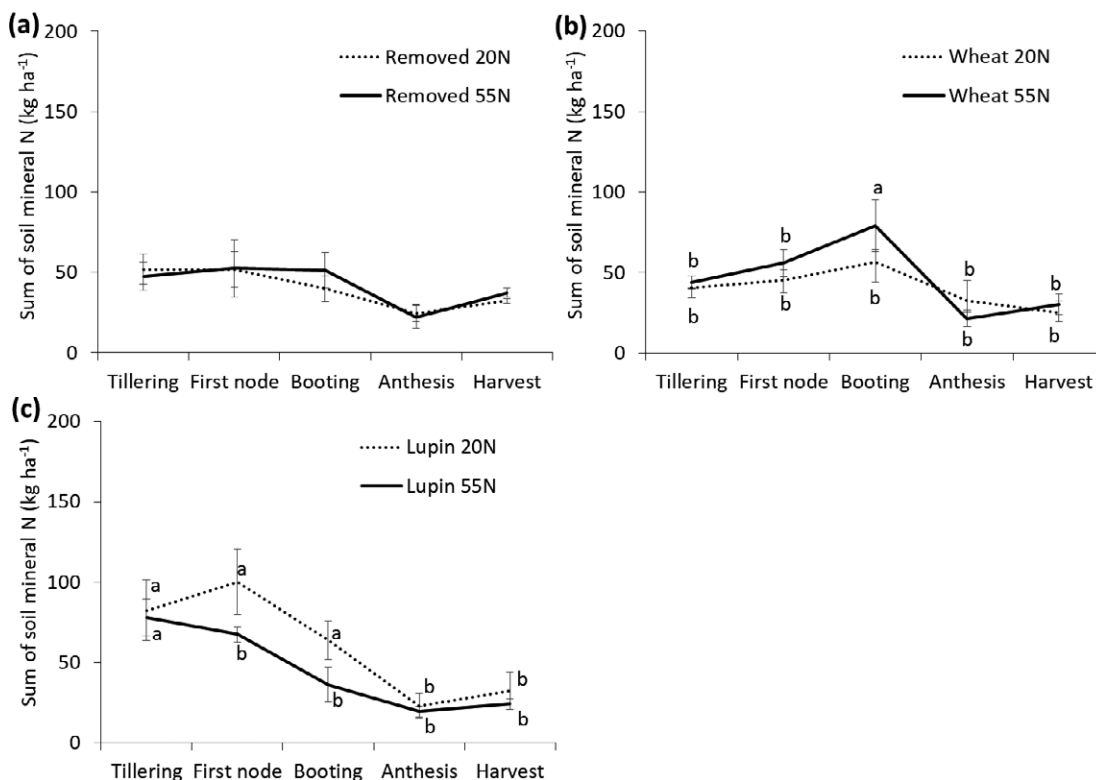
Figure 4: Sum of soil mineral N to rooting depth in 2015 (Site 1) for the removed (a), wheat (b), and lupin (c) residue management experiments with 20 or 55 kg N ha⁻¹ rates at different growth stages. Rooting depth to 40 cm at tillering, first node and booting, and to 60 cm at anthesis and harvest. For (a, b, c) different letters indicate significant differences of the mean for the interaction 'growth stage' *x* 'N rate' (DGC test, *p* < 0.05).

20 kg N ha⁻¹ for lupin *vs*. wheat, lupin *vs*. removed and removed *vs*. wheat residues respectively. From flag leaf onwards, wheat N uptake remained highest after lupin residues (+20 kg N ha⁻¹ higher on average than after wheat and removed residues; Fig. 3c). Regardless of the residue treatment, wheat N uptake was always higher with the 40N input (Fig. 3d), and the difference between 40N *vs*. 0N treatments increased over time. For all treatments and growth stages, wheat N uptake increased 0.5 kg ha⁻¹ on average with every additional unit of fertilizer N input. From first node onwards, wheat N uptake increased with increasing fertilizer N inputs (40N > 20N > 0N) regardless of the residue treatment.

In 2016, soil profile mineral N to 100 cm tended to decrease more rapidly in the lupin residues *plus* fertilizer N inputs as the crop progressed over the season, being highest at tillering (185 ± 6 kg ha⁻¹; Fig. 5c) regardless of the fertilizer N input (Fig. 5a). Between tillering and first node, 61 ± 5 kg ha⁻¹ of soil profile mineral N were depleted in lupin residue treatment compared with 3 ± 5 kg ha⁻¹ and 22 ± 5 kg ha⁻¹ for the removed and wheat residues, respectively, regardless of the fertilizer N input. At flag leaf, wheat+40N had the highest soil profile mineral N (+25 ± 13 kg ha⁻¹ than the other treatments; Fig. 5a–c). At anthesis, the removed+20N and lupin+40N had the lowest soil profile mineral N (–16 ± 2 kg ha⁻¹ than the other treatments; Fig. 5a–c). For all treatments and growth stages, wheat aerial biomass and N uptake were positively correlated with the cumulative rainfall (*r* = 0.96, *p* < 0.001; *r* = 0.79, *p* < 0.001) and temperature (*r* = 0.96, *p* < 0.001;

r = 0.79, *p* < 0.001). Wheat aerial biomass and N uptake were more when the sum of soil profile mineral N was less (*r* = –0.7, *p* < 0.001, *r* = –0.6, *p* < 0.001).

3.3 Wheat crop attributes at harvest

In 2015, grain yields were characterized by relatively high standard errors, particularly in the lupin residue experiment. Wheat yield after lupin residues exceeded 3 t ha⁻¹, whereas wheat yield after wheat or removed residues ranged between 0.9–1.8 t ha⁻¹ (min–max). When either wheat residues were removed or retained, the NUE in grain was higher with the 20N than with the 55N rate (Tab. 2) due to improved N utilization efficiency, which was 9 ± 0 and 4 ± 0 kg grain kg N⁻¹ higher with the 20N than with 55N rate in the removed and wheat residues experiments, respectively.

In 2016, the majority of the wheat crop attributes at harvest had a significant effect of either the 'residue treatment' or 'fertilizer N inputs' terms separately but not of their interaction. The mean grain yield, yield as percentage of potential yield, grain N uptake and harvest index of wheat were highest after lupin residues regardless of the fertilizer N input, and with the 40N rate, regardless of the residue treatment. The NUE and WUE in grain were ≈ 1.6 fold higher after lupin than after wheat or removed residues, and WUE in grain also increased with increasing fertilizer N rates. The WUE in total aerial biomass was highest with the 40N rate (Tab. 2). The combination of residue and fertilizer N treatments was significant for the N
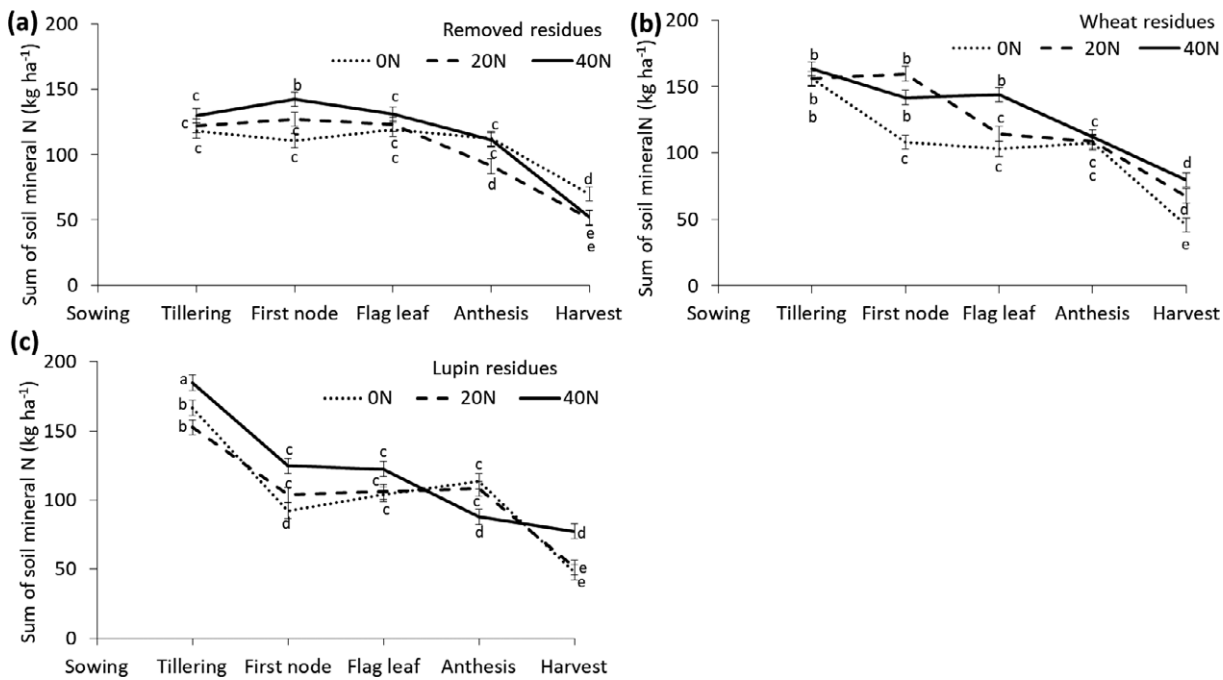
Figure 5: Sum of soil mineral N to 100 cm depth in 2016 (Site 2) in the removed (a), wheat (b), and lupin (c) residues with 0, 20, or 40 kg N ha^{-1} rates at different growth stages. Different letters indicate significant differences among means across all panels (a–c) resulting from the interaction 'residue treatment' *x* 'fertilizer N rate' *x* 'growth stage' (DGC test, *p* < 0.05).

utilization efficiency (45 ± 8 kg grain kg N^{-1} in average for all treatments), being lower (*p* = 0.03) in the removed+0N, removed+20N, wheat+0N, wheat+20N treatments. There was a positive relationship between the majority of crop attributes at harvest in 2016 such as grain yield, harvest index, grain and total N uptake, and N and WUE against the fertilizer N inputs. The WUE in grain was positively correlated with wheat N uptake (*r* = 0.9, *p* < 0.001), harvest index (*r* = 0.7, *p* < 0.001), N use (*r* = 0.9, *p* < 0.001), and N uptake efficiency (*r* = 0.9, *p* < 0.001).

3.4 Disease incidence in wheat crop in 2016

At 7 weeks after sowing, the severity of soil-borne root diseases affecting the wheat crop was assessed to be low in all plots (mean score = 1 meaning < 20% damage). At 23 weeks after sowing, the proportion of white heads showed a significant effect of residue treatment, regardless of the fertilizer N input. Soil-borne root disease incidence at advanced stages of wheat was significantly lower (*p* = 0.005) in the lupin than in the removed or wheat residue treatments. White head incidence was 45 ± 16, 53 ± 20, and 14 ± 10% for the removed, wheat and lupin residue treatments. Disease incidence had a negative correlation with the majority of the crop attributes at harvest, as grain yield (*r* = –0.6, *p* < 0.001), grain N (*r* = –0.6, *p* < 0.001), N uptake (*r* = –0.5, *p* < 0.01), harvest index (*r* = –0.4, *p* < 0.05), NUE (*r* = –0.3, *p* < 0.05), and WUE in grain and total biomass (*r* = –0.6, *p* < 0.001).

4 Discussion

4.1 Drivers of higher soil mineral nitrogen following legumes during the fallow

There was 35 kg ha^{-1} more soil mineral N to 100 cm depth following lupin than wheat residues at the end of the fallow on average in both years. *Peoples* et al. (2017) have indicated that the soil mineral N from legume crops was on average 35 kg N ha^{-1} but varied from 11 to 89 kg N ha^{-1} based on experiments in eastern and southern Australia. The higher soil mineral N after legumes is likely to be the result of one or more factors; the conservation of soil mineral N during lupin growth (*Evans* et al., 2001), higher microbial cycling and more rapid mineralization and/or lower N immobilization of legume residues with a lower C:N ratio than wheat residues (*Peoples* et al., 2017), and/or a net increase in soil mineral N when the N fixed by a legume exceeds the N removed in its grain at harvest (*Evans* et al., 2001). Lupin residues load at the end of the fallow was similar (Site 1) or lower (Site 2) to that for wheat residues, as reported in recent studies (*St. Luce* et al., 2016). This fact, coupled with the lower C:N of lupin residues in our study, support the inference that legume residues decomposed more rapidly (*Gupta* et al., 2011) as the crop senesced and input of shoot material to the soil towards the end of the previous cropping season in both sites, the net result being more mineral N after lupins than after wheat residues at the start of the fallow (*Evans* et al., 2001). But during the fallow there was no apparent difference in the net N mineralized from wheat or lupin residues in both sites, which may be partially attributed to sustained decomposition from wheat residues with a wider C:N than lupin residues, and/or to a similar decomposition between wheat residues and non-leaf material

Table 2: Grain yield, actual yield as percentage of yield potential, grain N uptake, harvest index, estimated net nitrogen mineralization, nitrogen and water use efficiency for wheat at harvest. Mean value of 4 replicates ± standard deviation. In 2015, for each residue experiment, different letters within the same column indicate significant differences of the mean (paired t test, at $^*p < 0.05$, $^{**}p < 0.001$). In 2016, for the residue treatment or for the fertilizer N input, different letters within the same column indicate significant differences of the mean (DGC, at $^*p < 0.05$, $^{**}p < 0.001$). WUE = water use efficiency, NUE = N use efficiency, total biomass = aerial biomass (stubble + grain).

	Treatment	Grain yield	Yield as percentage of potential yield	Grain N uptake	Harvest index	Estimated net N mineralization	NUE in grain	WUE in grain	WUE in total biomass
		(t ha^{-1})	(%)	(kg ha^{-1})		(0–100 cm, kg ha^{-1})	(0–100 cm, kg grain kg N^{-1})	(0–100 cm, kg ha^{-1} mm^{-1})	(0–100 cm, kg ha^{-1} mm^{-1})
2015 (Site 1)					Residue removed experiment				
	20N	1.4 ± 0.2 a	41.1 ± 7.0 a	27.7 ± 4.2 a	0.3 ± 0.0 a	31.1 ± 10.0 a	29.7 ± 5.0 a[*]	9.4 ± 1.6 a	32.1 ± 6.0 a
	55N	1.2 ± 0.3 a	38.0 ± 7.8 a	28.0 ± 5.8 a	0.3 ± 0.0 a	8.8 ± 14.1 a	14.8 ± 4.9 b	7.6 ± 1.8 a	31.3 ± 8.3 a
					Wheat residue experiment				
	20N	1.5 ± 0.2 a	39.9 ± 9.5 a	30.4 ± 7.4 a	0.3 ± 0.0 a	36.6 ± 11.2 a[*]	37.4 ± 11.7 a[*]	9.0 ± 2.1 a	30.1 ± 6.1 a
	55N	1.2 ± 0.2 a	34.0 ± 6.3 a	29.3 ± 5.2 a	0.3 ± 0.0 a	6.5 ± 6.6 b	15.5 ± 3.2 b	8.1 ± 1.4 a	27.2 ± 4.0 a
					Lupin residue experiment				
	20N	3.4 ± 0.8 a	90.0 ± 20.2 a	70.8 ± 14.9 a	0.3 ± 0.0	78.2 ± 12.0 a	55.8 ± 17.1 a	21.6 ± 5.0 b	67.7 ± 13.6 b
	55N	4.2 ± 0.5 a	116.0 ± 14.2 a	85.5 ± 9.3 a	0.4 ± 0.0	57.5 ± 26.0 a	50.5 ± 9.2 a	32.2 ± 5.4 a[**]	90.4 ± 10.4 a[**]
2016 (Site 2)					Residue treatment				
	Removed	2.8 ± 0.2 b	41.9 ± 2.9 b	39.5 ± 5.8 b	0.3 ± 0.0 b	1.6 ± 19.1 a	25.2 ± 2.8 b	9.2 ± 1.0 b	40.5 ± 4.8 a
	Wheat	2.5 ± 0.2 b	36.1 ± 2.9 b	35.9 ± 5.8 b	0.3 ± 0.0 b	5.3 ± 17.8 a	23.6 ± 2.8 b	8.4 ± 1.0 b	37.5 ± 4.8 a
	Lupin	4.4 ± 0.2 a[**]	62.3 ± 2.9 a[**]	65.3 ± 5.8 a[**]	0.4 ± 0.0 a[*]	−1.3 ± 32.2 a	33.1 ± 2.8 a[**]	15.3 ± 1.0 a[**]	41.4 ± 4.8 a
					Fertilizer N input				
	0N	2.5 ± 0.1 c	35.1 ± 2.9 c	36.7 ± 4.1 c	0.2 ± 0.0 b	5.1 ± 31.8 a	22.8 ± 2.0 a	8.2 ± 0.7 c	37.1 ± 2.3 b
	20N	3.3 ± 0.1 b	48.6 ± 2.9 b	47.4 ± 4.1 b	0.3 ± 0.0 a	−2.1 ± 19.4 a	27.8 ± 2.0 a	11.2 ± 0.7 b	38.8 ± 2.3 b
	40N	3.9 ± 0.1 a[**]	56.8 ± 2.9 a[**]	56.6 ± 4.1 a[**]	0.4 ± 0.0 a[*]	2.5 ± 18.1 a	31.7 ± 2.0 a	13.5 ± 0.7 a[**]	43.5 ± 2.3 a[**]

from lupin residues, and/or to the dry periods evidenced during the fallow (*Gupta* et al., 2011). There will be also an unknown contribution to soil N from crop below-ground components, which in the case of the legumes may also be more readily decomposable than for wheat. Indeed, in field studies of a Mediterranean-type environment of Western Australia, *McNeill* and *Fillery* (2008) found that ≈ 20–27% of below-ground lupin residue N was available as mineral N for wheat at sowing on deep sands. They have suggested a factor of 1.5 for conversion of measured aerial lupin N at peak biomass to an estimate of total N accumulation. Based on this factor, the estimated contribution from below-ground lupin biomass N is likely to have been a further 38 ± 14 and 6 ± 5 kg N ha^{-1} in Site 1 and 2, respectively. It is important to note that our study only refers to immediate effects of legume and fertilizer N on N dynamics. Long-term effects on N pools would demand a complete N balance analysis over several seasons.

4.2 Influence of early nitrogen supply on wheat aerial biomass and nitrogen uptake

In both years, the majority of the soil mineral N at sowing time (≈ 70%) was present in the top 30 cm (Supplementary Figs. S3.1–3). It is normally expected that most mineralization reactions in sandy soils occur in the surface soil, since the majority of their total labile C (required to drive mineralization) is contained in the top 10 cm layer (*Gupta* et al., 2019). However, to understand the relationship between soil mineral N flux as a potential source of N to crops and crop uptake of N, it is necessary to sample profile mineral N to the rooting depth during the growing season. In our study, soil profile mineral N decreased over the growing season in both years following crop growth patterns, illustrating the soil N supply–plant demand relationship (*Crews* and *Peoples*, 2005).

In situations of low N fertility, early supplementation with fertilizer N is needed to ensure adequate crop establishment and early development (*Poole* and *Hunt*, 2014). At wheat sowing in 2015, the lupin residue N had an average 66 ± 19 kg ha^{-1}

compared with just 21 ± 3 kg ha^{-1} of the wheat residue N. Also, the surface and profile soil mineral N appeared to be higher for wheat following lupin residues, and this wheat was more responsive to fertilizer N input, with increased utilization of soil mineral N to meet plant demand at the high N rate, notably at the critical stage of tillering. The majority of the profile N in 2015 appeared to be depleted between tillering and booting, and it was faster under a high N rate in line with the higher amount of N uptake. Wheat aerial biomass and N uptake during the 2015 growing season in the removed and wheat residues experiments were not responsive to the different fertilizer N rates. However, the higher N rate in the lupin residue experiment increased surface soil mineral N at tillering, a time of high N demand, improving wheat aerial biomass and N uptake. The lack of response in plant growth to a higher N rate for the wheat following wheat and removed residues could be the result of other environmental factors as root disease incidence which reduced the effective root length curtailing plant ability to access nutrients from soil, thereby inhibiting a response to the increased fertilizer N input (*Gupta* et al., 2012). In Mediterranean-type environments of South Australia, *Sadras* et al. (2012) found that shortage of N reduced wheat biomass and growth rate, affecting wheat biomass at anthesis, and grain number per unit growth rate. Generally, in 2016 the soil profile mineral N during the wheat phase decreased over the season and remained lower under the 20N rate. For wheat after lupin residues there was a clear relationship of N supply and demand with the greatest depletion of soil mineral N at early stages and the highest mineral N was left after the highest N fertility scenario of lupin+40N. The extra 32 kg ha^{-1} of soil profile mineral N after lupin residues at wheat sowing in 2016 corresponded with 34 kg ha^{-1} more wheat N uptake after lupin than after wheat residues by flag leaf. Wheat aerial biomass and N uptake between first node and anthesis were higher in the removed than in the wheat residue treatment, possibly as a result of some net N immobilization that might have occurred due to the wide C:N of wheat residues (*Evans* et al., 2001).

4.3 Yield, water and nitrogen use efficiency by wheat in response to the legume residue and/or fertilizer nitrogen treatments

Understanding the interactive effects of the main limiting factors of wheat production is a challenge in semi-arid environments (*Sadras* et al., 2012), and caution was taken in our study to explain the effect of both biotic and abiotic stressors on the measured variables. Wheat plants were affected by root diseases (*i.e.*, *Rhizoctonia solani, Fusarium*) at grain filling. Such diseases mostly affected wheat residue treatments regardless of the N rate (wheat > removed > lupin) with less severity observed under legume residue treatments (*Gupta* et al., 2012). The healthier wheat root system after a legume break (more vigor and exploration depth) can utilize more water and mineral N from deeper subsoil, minimizing the potential N leaching risk (*St. Luce* et al., 2016). The apparent greater wheat yields after lupin (4.2 t ha^{-1} on average) than after wheat and removed residues (1.2 t ha^{-1} on average) in 2015 along with the significant higher yields with lupin residues in 2016 were indicative of the legume-break effect in the

low fertility Mallee soils (*McBeath* et al., 2015). *Evans* et al. (2001) found that wheat yield benefit from pre-cropping lupin rather than wheat was on average 44% (3 *vs*. 2 t ha^{-1}). Also, the lack of responsiveness of the continuous wheat plots to fertilizer N input under the dry season conditions in 2015, with a low NUE (13–14 kg grain kg N^{-1} *vs*. 44 kg grain kg N^{-1} on lupin residues) in this low fertility environment suggests that this wheat was suffering a range of biotic and/or abiotic stressors (*Gupta* et al., 2012). In the absence of other stressors (*e.g.*, wheat following lupin giving a disease break) wheat with better N nutrition was able to extract more water and from deeper in the profile, particularly at about 80 cm depth (Fig. S3.1), indicative of water by N co-limitation for the continuous wheat plots (*Sadras* et al., 2012).

In both years, the amount of total water used by the crop (from sowing to harvest) was not affected by the residue treatment and/or the fertilizer N inputs, and ranged between 155 and 339 mm (min–max), corresponding with the reported values by *McBeath* et al. (2015) at a nearby location. In 2016, the WUE in grain were also within the range of 7–15 kg grain ha^{-1} mm^{-1} reported by *McBeath* et al. (2015) but were lower than the 22 kg ha^{-1} mm^{-1} benchmark proposed for similar environments (*Sadras* and *Angus*, 2006). This inability to attain the proposed WUE benchmark reflects the vulnerability and typical limitations of these environments (*Sadras* and *Angus*, 2006).

Increasing WUE can increase N use efficiency by the crop, since the latter is driven by the crop response to water and N supply during the growing season (*Hatfield* and *Prueger*, 2004). Both WUE and NUE were positively correlated with the fertilizer N inputs. Higher WUE with higher fertilizer N inputs could be attributed to a greater leaf expansion or a delay in senescence by increased radiation interception of wheat when the N supply was higher (*Brueck*, 2008). *Kim* et al. (2008) found a synergistic relationship between water and N where higher fertilizer N inputs increased the WUE, and higher water supply resulted in a mean increase of 65 and 45% in the NUE from the soil N and fertilizer N respectively, whereby crop N uptake is a function of the water transpired by the crop. As a result, water and N uptake by the roots are driven by the nitrate N in the soil matrix (relative to the pore size) so that the amount of N remaining in the soil (*i.e.*, not taken up by roots) will be less as the water uptake by the crop root system increases. Some studies have indicated a positive effect of fertilizer N on WUE by cereals (*Hatfield* and *Prueger*, 2004) although crop response to fertilizer N inputs differs (*Brueck*, 2008).

Increased N availability can increase crop water extraction but this effect is generally modest (typically $\approx$ 10 mm) in southern Australia because of lack of water at depth, soil chemical constraints or a combination of both. Shortage of N often reduces crop transpiration leaving more residual water at maturity (*Sadras* and *Angus*, 2006). In our study the 0N treatment had the highest sum of PAW to 100 cm depth at harvest in 2016, regardless of the residue treatment (96 ± 4, 87 ± 3, and 83 ± 4 mm for 0, 20, and 40N). A shortage of N can have neutral, positive or negative effects on harvest index and it can respond to the interaction between water and N.

A high N supply can increase harvest index under favorable water conditions but decrease it under water deficit. Our reported values of harvest index were similar to other studies in a Mediterranean environment (*Albrizio* et al., 2010). A high harvest index (> 0.4 in Australia) and WUE indicate that the distribution of pre- and post-anthesis water use and growth was optimal and that the N supply was well matched to water-limited demand (*Poole* and *Hunt*, 2014).

Although the identification of which source of N had the biggest effect on the processes and the measured soil and crop variables was out of the scope in our study, some inferences could be attributed to either the fertilizer N or the residue N. *Peoples* et al. (2017) found that the apparent recovery of legume residue N by wheat averaged $30 \pm 10\%$ and the apparent recovery of fertilizer N in the absence of legumes was $64 \pm 16\%$. In our study, the magnitude of the difference in WUE in grain in 2016 differed between the sources of N; the fertilizer N (kg N^{-1}) increased WUE by 0.14 kg ha^{-1} mm^{-1} per unit N input while the additional soil mineral N supply from the legume residue in the soil profile at sowing increased it by 0.22 kg ha^{-1} mm^{-1} per unit N.

5 Conclusions

The combination of legume (lupin) residues with higher fertilizer N increased soil profile mineral N at early wheat growth stages, providing the crop with a greater soil N supply at the time of high demand. This relationship influenced the ability of wheat to use N (either from the soil or the fertilizer) improving its accumulation in the crop, which was reflected in a higher N utilization efficiency and N partitioning in the grain. Although yield response of wheat to a high N rate was not significant under a below average rainfall season, responses in wheat N uptake throughout the growing season indicated that there was a demand for fertilizer N following legume residue. Wheat crop in an above average rainfall season showed an increased actual and potential grain yield, aerial biomass, N uptake, harvest index and WUE with a higher N supply, regardless of the source of N.

It was demonstrated that the inclusion of a legume in a low-rainfall sandy soil is critical for improving not only the N supply to wheat, but also for reducing the wheat disease severity experienced in continuous cereals. Further study is required to determine the key soil N pools and environmental drivers of soil N supply capacity to improve the synchrony with wheat N uptake in dry environments.

Acknowledgments

References

ABS (2014): Agricultural Production Australia Bureau of Statistics, Canberra, Australia. Available at: www.abs.gov.au/ausstats.

Albrizio, R., *Todorovic, M.*, *Matic, T.*, *Stellacci, A. M.* (2010): Comparing the interactive effects of water and nitrogen on durum wheat and barley grown in a Mediterranean environment. *Field Crops Res.* 115, 179–190.

Angus, J. F., *Grace, P. R.* (2017): Nitrogen balance in Australia and nitrogen use efficiency on Australian farms. *Soil Res.* 55, 435–450.

Brueck, H. (2008): Effects of nitrogen supply on water-use efficiency of higher plants. *J. Plant Nutr. Soil Sci.* 171, 210–219.

Burk, L., *Dalgliesh, N.* (2008): Estimating plant available water capacity-a methodology. CSIRO Sustainable Ecosystems, Canberra, Australia.

Colwell, J. D. (1963): The estimation of phosphorus fertilizer requirements of wheat in Southern New South Wales by soil analysis. *Aust. J. Exp. Agr.* 3, 190–197.

Crews, T. E., *Peoples, M. B.* (2005): Can the synchrony of nitrogen supply and crop demand be improved in legume and fertilizer-based agroecosystems? A review. *Nutr. Cycl. Agroecosys.* 72, 101–120.

Evans, J., *McNeill, A. M.*, *Unkovich, M. J.*, *Fettell, N. A.*, *Heenan, D. P.* (2001): Net nitrogen balances for cool-season grain legume crops and contributions to wheat nitrogen uptake: a review. *Aust. J. Exp. Agr.* 41, 347–359.

French, R. J., *Schultz, J. E.* (1984): Water use efficiency of wheat in a Mediterranean-type environment. I. The relation between yield, water use and climate. *Aust. J. Agric. Res.* 35, 743–764.

Gupta, V. V. S. R., *Llewellyn, R.*, *McBeath, T.*, *Kroker, S.*, *Davoren, W.*, *McKay, A.*, *Ophel-Keller, K.*, *Whitbread, A.* (2012): Break crops for disease and nutrient management in intensive cereal cropping. Proceedings 16th Australian Agronomy Conference, October 14–18, 2012, Armidale, Australia. Available at: http://www.regional.org.au/au/asa/2012/nutrition/7961_vadakattugupta.htm#TopOfPa.

Gupta, V. V. S. R., *Rope, M.*, *Thompson, J.* (2019): Harnessing the Benefits of Soil Biology in Conservation Agriculture, in Pratley, J., Kirkegaard, J. (eds.): Australian Agriculture in 2020: From Conservation to Automation. Agronomy Australia and Charles Sturt University, Wagga Wagga, Australia, pp. 237–253.

Gupta, V. V. S. R., *Rovira, A. D.*, *Roget, D. K.* (2011): Principles and Management of Soil Biological Factors for Sustainable Rainfed Farming Systems, in Tow, P., Cooper, I., Partridge, I., Birch, C. (eds.): Rainfed Farming Systems. Springer, Berlin, Germany, pp. 149–184.

Hangs, R. D., *Schoenau, J. J.*, *Lafond, G. P.* (2013): The effect of nitrogen fertilization and no-till duration on soil nitrogen supply power and post–spring thaw greenhouse-gas emissions. *J. Plant Nutr. Soil Sci.* 176, 227–237.

Hatfield, J. L., *Prueger, J. H.* (2004): Nitrogen over-use, under-use, and efficiency. Proceedings of 4th International Crop Science Congress, September 26–October 01, 2004, Brisbane, Australia. Available at: https://www.ars.usda.gov/research/publications/publication/?seqNo115=165702.

Hochman, Z., *Horan, H.* (2018): Causes of wheat yield gaps and opportunities to advance the water-limited yield frontier in Australia. *Field Crops Res.* 228, 20–30.

Isbell, R. F. (2002): The Australian Soil Classification. CSIRO Publishing, Melbourne, Australia.

Kim, K., *Clay, D. E.*, *Carlson, C. G.*, *Clay, S. A.*, *Trooien, T.* (2008): Do synergistic relationships between nitrogen and water influence the ability of corn to use nitrogen derived from fertilizer and soil? *Agron. J.* 100, 551–556.

McBeath, T. M., *Gupta, V. V. S. R.*, *Llewellyn, R. S.*, *Davoren, C. W.*, *Whitbread, A. M.* (2015): Break crop effects on wheat production across soils and seasons in a semi-arid environment. *Crop Pasture Sci.* 65, 566–579.

McDonald, H. J., *Rovira, A. D.* (1985): Development of Inoculation Technique for *Rhizoctonia solani* and its Application to Screening Cereal Cultivars for Resistance, in Parker, C. A., Rovira, A. D., Moore, K. J., Wong, P. T. W., Kollmorgen, J. F. (eds.): Ecology and Management of Soilborne Plant Pathogens. American Phytopathological Society, St. Paul, MN, USA.

McKenzie, N., *Coughlan, K.*, *Cresswell, H.* (2002): Soil Physical Measurement and Interpretation for Land Evaluation. CSIRO Publishing, Melbourne, Australia.

McNeill, A. M., *Fillery, I. R. P.* (2008): Field measurement of lupin belowground nitrogen accumulation and recovery in the subsequent cereal-soil system in a semi-arid Mediterranean-type climate. *Plant Soil* 302, 297–316.

Miranda, K. M., *Espey, M. G.*, *Wink, D. A.* (2001): A rapid, simple spectrophotometric method for simultaneous detection of nitrate and nitrite. *Nitric Oxide* 5, 62–71.

Moll, R. H., *Kamprath, E. J.*, *Jackson, W. A.* (1982): Analysis and interpretation of factors which contribute to efficiency of nitrogen utilization. *Agr. J.* 74, 562–564.

Monjardino, M., *McBeath, T. M.*, *Brennan, L.*, *Llewellyn, R. S.* (2013): Are farmers in low-rainfall cropping regions under-fertilising with nitrogen? A risk analysis. *Agric. Syst.* 116, 37–51.

Peoples, M. B., *Swan, A. D.*, *Goward, L.*, *Kirkegaard, J. A.*, *Hunt, J. R.*, *Li, G. D.*, *Schwenke, G. D.*, *Herridge, D. F.*, *Moodie, M.*, *Wilhelm, N.*, *Potter, T.*, *Denton, M. D.*, *Browne, C.*, *Phillips, L. A.*, *Khan, D. F.* (2017): Soil mineral nitrogen benefits derived from legumes and comparisons of the apparent recovery of legume or fertilizer nitrogen by wheat. *Soil Res.* 55, 600–615.

Peverill, K., *Reuter, D. J.*, *Sparrow, L. A.* (1999): Soil Analysis: An Interpretation Manual. CSIRO Publishing, Collingwood, Australia.

Poole, N., *Hunt, J.* (2014): Advancing the management of crop canopies. Grains Research and Development Corporation (Project SFS00017), Canberra, Australia.

R Core Team (2015): R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Rayment, G. E., *Lyons, D. J.* (2011): Soil Chemical Methods: Australasia. CSIRO Publishing, Melbourne, Australia.

Rienzo, J. A., *Guzmán, A. W.*, *Casanoves, F.* (2002): A multiple-comparisons method based on the distribution of the root node distance of a binary tree. *J. Agr. Biol. Environ. Stat.* 7, 129–142.

Sadras, V. O., *Lawson, C.*, *Hooper, P.*, *McDonald, G. K.* (2012): Contribution of summer rainfall and nitrogen to the yield and water use efficiency of wheat in Mediterranean-type environments of South Australia. *Euro. J. Agron.* 36, 41–54.

Sadras, V. O., *Angus, J. F.* (2006): Benchmarking water-use efficiency of rainfed wheat in dry environments. *Aust. J. Agric. Res.* 57, 847–856.

Seymour, M., *Kirkegaard, J. A.*, *Peoples, M. B.*, *White, P. F.*, *French, R. J.* (2012): Break-crop benefits to wheat in Western Australia—insights from over three decades of research. *Crop Pasture Sci.* 63, 1–16.

St. Luce, M., *Grant, C. A.*, *Ziadi, N.*, *Zebarth, B. J.*, *O'Donovan, J. T.*, *Blackshaw, R. E.*, *Harker, K. N.*, *Johnson, E. N.*, *Gan, Y.*, *Lafond, G. P.*, *May, W. E.*, *Malhi, S. S.*, *Turkington, T. K.*, *Lupwayi, N. Z.*, *McLaren, D. L.* (2016): Preceding crops and nitrogen fertilization influence soil nitrogen cycling in no-till canola and wheat cropping systems. *Field Crops Res.* 191, 20–32.

Viscarra Rossel, R. A., *Walvoort, D. J. J.*, *Mcbratney, A. B.*, *Janik, L. J.*, *Skjemsta, J. O.* (2006): Visible, near infrared, mid infrared or combined diffuse reflectance spectroscopy for simultaneous assessment of various soil properties. *Geoderma* 131, 59–75.

Zadoks, J. C., *Chang, T. T.*, *Konzak, C. F.* (1974): A decimal code for the growth stages of cereals. *Weed Res.* 14, 415–421.

CHAPTER 3

Nitrogen fertiliser input increases potentially mineralisable and dissolved organic nitrogen more after lupin than wheat residues in a sandy surface soil

Statement of Authorship

Title of Paper	Nitrogen fertiliser input increases potentially mineralisable and dissolved organic nitrogen more after lupin than wheat residues in a sandy surface soil.
Publication Status	☐ Published ☐ Accepted for Publication √ Submitted for Publication ☐ Unpublished and Unsubmitted work written in manuscript style
Publication Details	Muschietti Piana MP, T McBeath, A McNeill, PA Cipriotti & V Gupta. 2021. Nitrogen fertiliser input increases potentially mineralisable and dissolved organic nitrogen more after lupin than wheat residues in a sandy surface soil. Submitted to *Journal of Soil Science and Plant Nutrition* (revisions requested on 10 January 2021)

Principal Author

Name of Principal Author (Candidate)	Maria del Pilar Muschietti Piana		
Contribution to the Paper	Performed the experiment and the analysis of all samples, interpreted data, wrote the manuscript, illustrated all figures and tables, and act as the corresponding author.		
Overall percentage (%)	80%		
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
Signature		Date	01-09-2020

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

  i. the candidate's stated contribution to the publication is accurate (as detailed above);

  ii. permission is granted for the candidate in include the publication in the thesis; and

  iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of Co-Author	Therese McBeath		
Contribution to the Paper	Provided advice on experimental design, analysis, and during manuscript preparation and review.		
Signature		Date	01/09/2020

Name of Co-Author	Ann M. McNeill		
Contribution to the Paper	Provided advice on experimental design, analysis and during manuscript preparation and review.		
Signature		Date	04/09/2020

Name of Co-Author	Pablo Ariel Cipriotti		
Contribution to the Paper	Provided advice on experimental design, statistical analysis and manuscript editing during preparation and review.		
Signature		Date	02/09/2020

Name of Co-Author	Vadakattu V. S. R. Gupta		
Contribution to the Paper	Provided advice on experimental design, analysis and during manuscript preparation and review.		
Signature		Date	02/09/2020

Please cut and paste additional co-author panels here as required.

Nitrogen fertiliser input increases potentially mineralisable and dissolved organic nitrogen more after lupin than wheat residues in a sandy surface soil

Pilar Muschietti Piana [A]*, Therese Marie McBeath* [B]*, Ann Marie McNeill* [A]*, Pablo Ariel Cipriotti* [C]*, and Vadakattu Gupta* [B]

[A] School of Agriculture, Food and Wine, The University of Adelaide, SA5005, Australia

[B] Commonwealth Scientific and Industrial Research Organisation (CSIRO) Agriculture and Food, Urrbrae, SA5064, Australia

[C] School of Agriculture-Instituto de Investigaciones Fisiológicas y Ecológicas Vinculadas a la Agricultura (IFEVA), University of Buenos Aires/Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Av. San Martin 4453, Ciudad Autónoma de Buenos Aires, C1417DSE, Argentina

Corresponding author

Correspondence to Pilar Muschietti Piana, E-mail: muschiet@agro.uba.ar

Abstract

Understanding the combined influence of crop residue and fertiliser-nitrogen (N) inputs on soil N supply factors in relation to critical stages of plant N demand may assist in the formulation of N management strategies for optimising N use-efficiency. In this study, combinations of crop residue treatments and fertiliser-N inputs were manipulated to potentially increase soil-N supply during the wheat growing season on a low-rainfall sandy soil. The aims were to i) quantify decomposition rates and N release from wheat and lupin residues with fertiliser-N (0 or 40 kg N ha^{-1}) inputs; and ii) measure the temporal patterns of the surface soil-N capacity under a combination of different residue (removed, wheat or lupin residues) and fertiliser-N (0, 20, or 40 kg N ha^{-1}) treatments during the growing season of a wheat (*Triticum Aestivum* L.) crop. Residue decomposition and N release during the fallow and the wheat growing season was measured in the field using litterbags with wheat or lupin residues. Fertiliser-N treatments were applied at the time of wheat sowing and surface soil-N supply was measured at key wheat growth stages (seedling, tillering, first node, flag leaf and anthesis). Lupin residues provided a greater N release *via* mineralisation at sowing, seedling and tillering stages, and dissolved organic N (DON) at the seedling stage. Lupin residues had a higher surface soil potentially mineralisable N (PMN) at seedling, tillering and anthesis, and microbial biomass N at first node and flag leaf, than wheat or removed residues. When lupin residues were combined with fertiliser-N there was more surface soil DON and PMN between the seedling and tillering stages, relative to wheat residues combined with fertiliser N. This study showed that N supply capacity to wheat, measured as mineralised N, DON and PMN, is increased during the growing season from legume residues and fertiliser-N inputs, independently and together, in a low-rainfall sandy soil environment.

Keywords

Introduction

Achieving synchrony between nitrogen (N) supply and demand in legume-based or fertiliser-based agro-ecosystems is a major challenge for agriculture based on annual crops (Crews and Peoples 2005). Soil-N supply is driven by the dynamics of several N pools in the soil including microbial biomass N (MBN), mineral N and labile organic-N such as dissolved organic-N (DON). The soil-N supply capacity is highly influenced by agricultural management practices such as the crop residue type (i.e. legume, cereal, oilseed) and fertiliser-N application (Dalias 2015; Gupta *et al.* 2011) and can be measured as potentially mineralisable N (PMN). Estimates of surface soil PMN based on short-term anaerobic incubations are known to reflect the mineralisation capacity of the soil organic matter pools and are often used as a reliable indicator of soil-N supply potential (Murphy *et al.* 2011).

Complex microbial processes control plant residue decomposition and residue-N release constitutes an important source of N for subsequent crops. Crop residue decomposition is influenced by many factors, especially soil temperature and moisture and residue biochemical quality (Trinsoutrot *et al.* 2000).Variation in decomposition rates of different crop residues are largely related to differences in chemical composition in terms of N concentration, soluble organic-C and -N, and C:N ratio, although other properties (e.g. cellulose, lignin, polyphenol concentration) have also been shown to influence stubble decomposition (Adl 2003). Established relationships between the kinetics of C and N decomposition and the biochemical characteristics (residue N, polyphenols concentration) of crop residues indicate that the N concentration and C:N ratio of the residues are the most important factors for estimating the net effects of residue decomposition on soil mineral-N dynamics (Trinsoutrot *et al.* 2000).

Legume residues can readily release N due to their lower C:N ratio and higher N content, when compared to cereal residues (Crews and Peoples 2005). Grain legumes such as narrow leaf lupin (*Lupinus angustifolius*) grown in rotation with wheat (*Triticum aestivum* L.) play an important role in enriching the pools of soil N by N release from lupin residue decomposition (McNeill and Fillery 2008). Legumes can provide significant benefits to a following wheat crop, such as higher yield through higher N supply in soils with low N supply capacity and lower disease incidence (Gupta *et al.* 2011; McBeath *et al.* 2015). The amount of N from legume residues that will be available to the

following cereal is difficult to predict in the field due to the complexity of the residue decomposition process and N cycling in agricultural soils (Singh and Rengel 2007).

In an extensive evaluation of crop and soil N in dryland systems of eastern Australia, Peoples *et al.* (2017) developed some simple predictive relationships for estimating the amount of N that will become available from legume residues for following crops based on rainfall data, legume grain yield or shoot dry matter, and soil mineral N measures at sowing. However, it was acknowledged that further research is required to refine these relationships. Quantifying under field conditions residue decomposition during the fallow period and growing season, and the mineral and biological soil-N pools that contribute to the N supply capacity at the start of the wheat growing season is of vital importance. This information will facilitate a better understanding of N supply from residues and enable improved management of N inputs in semi-arid cropping systems.

The MBN is defined as a small but active and critical pool in the internal N cycle involved in mineralisation-immobilisation turnover (MIT) in soil. The MIT is a vital process for the continuous transfer of mineral N into organic materials through incorporation of N into soil MB and subsequent release (mineralisation) of immobilised N into the soluble mineral-N pool (McNeill and Unkovich 2007). Soil MBN pools play important roles in ecological processing and provide a reservoir of nutrients for crop uptake (Ros *et al.* 2009; Gupta 2016). Soil MBN acts as both a source and sink of N, and its turnover time determines the availability of N for crop uptake and growth (Ros *et al.* 2009).

The DON is a significant soluble-N pool in some soils, containing compounds of various molecular sizes and types. Many DON compounds (i.e. low molecular weight oligomers and monomers) can be taken up directly by both soil microorganisms and plants at rapid rates during a period of minutes to a few hours (Farrell *et al.* 2014). The size and the seasonal changes in both MBN and DON in the surface soil layer (0–10 cm) are likely to be more pronounced than in the deeper layers (Ros *et al.* 2009). In semi-arid sandy soils of Western Australia Murphy *et al.* (1998*a*) investigated the distribution of MBN and the decline in gross N mineralisation and ammonium consumption with soil depth to 50 cm (in 0–10 cm intervals). They have found that 67% of the soil MBN was present in the 0–10 cm layer, and the rates of gross N mineralisation and ammonium consumption were not significant below the 0–10 cm soil layer (Murphy *et al.* 1998*a*). Consequently, the quantification of

surface soil DON and MBN is necessary for an understanding of the N cycling processes linked to soil-derived potential N supply for wheat N uptake.

When the N released from soil and residues is insufficient and/or the timing of N supply is not well matched with crop N demand, fertiliser-N inputs become necessary to meet the N demands for wheat production (Hoyle and Murphy 2011). Measuring the temporal changes of soil-N supply capacity, in both the mineral and organic phases and its associated key drivers, relative to critical wheat growth stages for N demand can provide important clues as to how to best manage N inputs for optimal efficiency (Crews and Peoples 2005). This is especially true for wheat cropped on sandy soil in low-rainfall environments, where N supply capacity is low and microbial turnover plays a significant role in maintaining the mineral-N pool in surface soils (Gupta *et al.* 2011). Low-rainfall sandy environments present unique challenges for the maintenance of soil-N supply capacity for intensive crop production (Sadras 2002; Sadras and Roget 2004). Manipulation of N inputs through fertiliser management (Fillery and McIness 1992, Gupta *et al*. 2018) or from legume residue decomposition (Evans 2001, McBeath 2015, van Vliet *et al.* 2000) has been studied independently for semi-arid environments, but not together.

The study presented here indirectly assesses the relative contribution of different N sources (residue, soil organic matter and fertiliser) to surface soil mineral-N pools and other pools (MBN and DON) that drive soil N supply capacity for wheat N uptake. This assessment aimed to provide a mechanistic understanding of the enhanced wheat N uptake derived from a combination of residues and N fertiliser that has previously been reported for this environment (Muschietti-Piana *et al.* 2020). A combination of crop residue treatments (wheat residue-removed, wheat residue-retained or lupin residue-retained) and fertiliser-N inputs (0, 20 or 40 kg N ha^{-1}) were manipulated to potentially increase soil-N supply at critical wheat growth stages on a low-rainfall sandy soil. Decomposition rates and N release from wheat and lupin residues during the fallow and the subsequent wheat crop growing season with 0 and 40 kg N ha^{-1} fertiliser-N were quantified. Temporal patterns of surface soil mineral N, PMN, MBN, DON were measured in all treatments at key wheat growth stages, along with temperature and rainfall.

Materials and methods

Experimental site characterisation, design and treatments

The experiment duration was from the start of fallow on 2 December 2015 to the end of the wheat growing season on 8 December 2016 in a representative field of the Mallee environment situated at Lowaldie (35.0535° S, 139.9874° E), South Australia. The soil was a deep sand dune (Kandosol; Isbell and NCST 2016). Residue treatments: wheat (*Triticum Aestivum* L.) residue-retained or removed and lupin (*Lupinus angustifolius*) residue-retained were set up using plots from a field experiment in the 2015 cropping season. For the wheat residue-retained treatment, the wheat aboveground residues were retained from the previous wheat crop (cv. Corack) which was harvested on 2 December 2015 with an average grain yield of 1.4 ± 0.3 t ha^{-1} and an estimated residue load of 2.5 ± 0.5 t ha^{-1}. For the lupin residue-retained treatment, lupin aboveground residues were retained from the previous narrow-leafed lupin (cv. Mandelup) crop which was harvested on 2 December 2015 with an average grain yield of 0.5 ± 0.2 t ha^{-1} and an estimated residue load of 1.2 ± 0.5 t ha^{-1}. For the wheat removed treatment (hereafter referred to as 'removed residue' treatment), wheat aboveground residues were removed manually using a low cut to ~ 2 cm and rake on 2 December 2015.

The experiment comprised two components undertaken in the same plots: i) the residue decomposition component during both the fallow (15 December 2015 – 30 May 2016) and the wheat growing season (1 June – 8 December 2016); and ii) the surface soil-N supply capacity component during the wheat growing season only.

The experiment had a split plot design with the crop residue treatment (wheat residue-removed, wheat residue-retained or lupin residue-retained) as the 'main plot' effect (for both 'fallow' and 'growing season') and the fertiliser-N input (0, 20 and 40 kg N ha^{-1}) as the 'sub-plot' effect (for 'growing season' only) with four replicates as blocks. The fertiliser-N treatments were termed as '0N, 20N and 40N' (0, 20 and 40 kg N ha^{-1}). Each fertiliser-N treatment was randomly assigned in plots (1.7 m wide *x* 14 m long).

Wheat (cv. Scepter) was sown on 2 June 2016 at row spacing of 28 cm and managed according to the methods outlined in Muschietti Piana *et al.* (2020). Fertilisers were applied at sowing by banding 4 cm below the seed a blend of 49 kg ha^{-1} of triple superphosphate, and 0, 43 or 87 kg ha^{-1} of urea to supply rates of 0, 20 or 40 kg N ha^{-1} respectively. The experimental site had grown continuous wheat from 2009–2014. The combinations of residue treatments and fertiliser-N inputs are referred to as the 'Residue+fertiliser-N input' (e.g. Wheat+0N). The residue decomposition component of the study

included sampling of all combinations of wheat and lupin residue treatments and the 0N and 40N fertiliser-N treatments, while the surface soil-N supply capacity component included sampling of all available treatments.

Climate and sampling conditions during the experiment

The site has a Mediterranean-type climate with a mean annual rainfall of 337 mm, of which 227 mm typically falls between May and October, the wheat growing season (historical records 1957–2014). Daily rainfall was monitored by two weather stations; HOBO® Event Data Logger-H07-002-04 (Onset Computer Corporation, Bourne, Massachusetts, United States of America) and MEA103 Mk 3® automatic station (Measurement Engineering Australia Pty. Ltd., Magill, South Australia). Daily air temperature data was recorded and was monitored by a Tinytag ultra 2® sensor (Hastings Data Loggers Pty. Ltd, United Kingdom). Annual rainfall for 2016 was 474 mm (Fig. 1), with 123 mm (sum of recorded events > 0.8 mm) during the fallow (15 December 2015–1 June 2016, Table 1) and 316 mm during the growing season resulting in a decile 9 season (2 June–8 December 2016). Mean daily temperature was below 17°C from late April to late October. Outside these periods, mean daily temperature ranged between 16–28°C, and was below the historical records (Fig. 1). Cumulative temperature during the experiment was expressed as thermal time or degree-days (°C d) and was calculated as:

$$\text{Degree days (°C d)} = [(T\mathit{max} + T\mathit{min}) / 2] - T\mathit{base} \tag{1}$$

where $T\mathit{max}$, maximum temperature (°C); $T\mathit{min}$, minimum temperature (°C); $T\mathit{base}$, base temperature at 0°C.
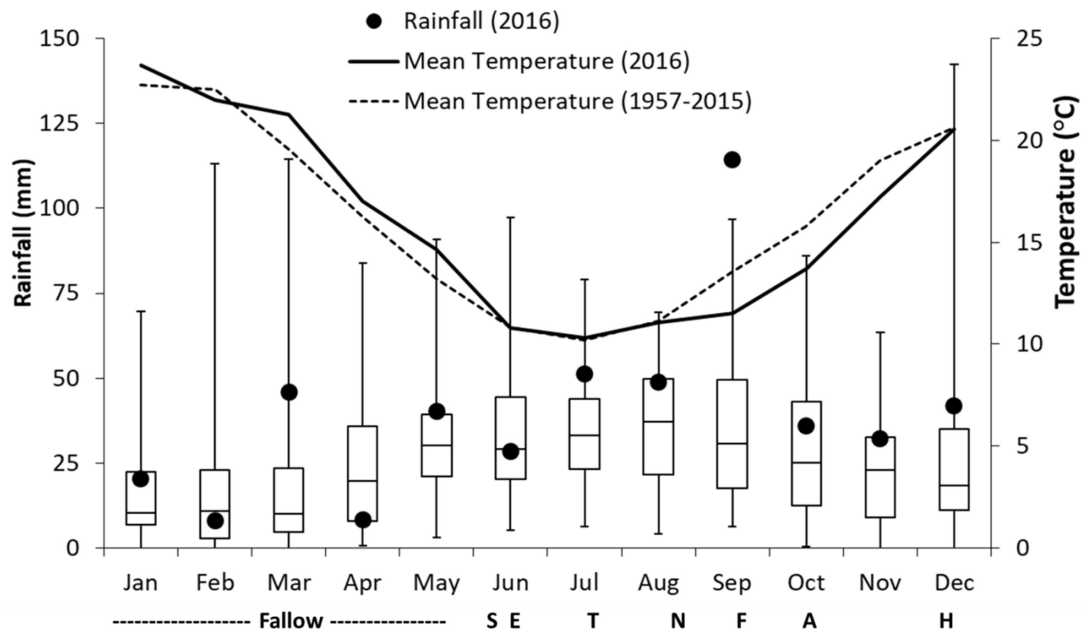
Figure 1. Monthly rainfall (mm) and air temperature (°C) recorded with two weather stations located near the experimental site (2016). Historical daily rainfall and air temperature (1957–2015) were obtained from the SILO database (https://www.longpaddock.qld.gov.au/). Historical rainfall data (mm) is displayed in the box-and-whisker plot, indicating minimum, maximum, median (Q_2), first quartile (Q1), third quartile (Q3) of the dataset (1957–2015). Sampling times on x-axis are indicated as; S, sowing, E, seedling, T, tillering, N, first node, F, flag leaf, A, anthesis, H, harvest.

Table 1. Measured rainfall and number of rainfall events > 10 mm between sampling dates for the duration of the experiment

Sampling dates	Period	Incubation period (days)	Measured rainfall (mm)	Rainfall events >10 mm
15-Dec (2015) – 28 Jan (2016)	Fallow	0–44	12	1
29 Jan – 17 Mar (2016)	Fallow	44–93	59	2
18 Mar – 30 May (2016)	Fallow	93–167	52	1
1 Jun – 16 Jun (2016)	Sowing–Seedling	167–182	7	0
17 Jun – 20 Jul (2016)	Seedling–Tillering	182–218	60	2
1 Jun – 20 Jul (2016)	Sowing–Tillering	167–218	67	2
21 Jul – 22 Aug (2016)	Tillering–First node	218–251	53	2
23 Aug – 15 Sep (2016)	First node–Flag leaf	251–275	64	3
16 Sep – 11 Oct (2016)	Flag leaf–Anthesis	275–301	67	3
12 Oct – 8 Dec (2016)	Anthesis–Harvest	301–359	65	2

Wheat and lupin residue collection, characterisation and decomposition

Residue decomposition during the fallow and the wheat growing season was measured using a litterbag technique (Verhoef 1995). Litterbags (0.13 m *x* 0.12 m) containing wheat and lupin residues were made with a fiberglass-nylon material with a 0.8 mm mesh. Wheat and lupin residues were collected immediately after harvest from the selected plots in which the same litterbag residue treatments were later imposed.

All residues were cut into 2–3 cm pieces, fractionated into plant components (straw, leaves, spikes, etc.) and placed in the field at a rate of 8.5 g per bag in the lupin litterbags (equivalent to a residue load in the litterbag of 5.4 t dry matter ha^{-1}) and 7.0 g per bag in the wheat litterbag (equivalent to a residue load of 4.5 t dry matter ha^{-1}). In each bag, the proportion of each residue component was reproduced as in field conditions: 66% straw and 34% chaff for wheat residues; 49% pods, 43% branches and stems, and 8% leaves and inflorescences for lupin residues. Wheat and lupin residue load in the litterbags

represented the maximum residue load that had been measured under these field conditions. Recorded residue load in field plots during the 2015 season on sandy soils near the experimental site were 4 t ha^{-1} for wheat residues and 6 t ha^{-1} for lupin residues (Muschietti-Piana *et al.* 2020).

The initial residue fractions (straw, pod, stem, leaves) were analysed for C and N content using a TruMAC analyser (2000-CNS, manufactured by Leco Corporation, Michigan, United States of America). Total soluble C and N of wheat and lupin residues were measured in water extracts from 10 g oven-dried equivalent of residue shaken in 30 mL of distilled water (water temperature ~21 °C), centrifuged at 1,252 x g. for 15 min and filtered (Whatman No. 42). Total C and N concentrations of the water extracts were determined using a Thermalox dry combustion analyser (manufactured by Analytical Sciences Ltd., Cambridge, United Kingdom).

At the start of the fallow, ten litterbags were assigned to each plot containing the same residue treatment (lupin or wheat) and in two of the proposed fertiliser-N treatments (0N and 40N0 x four replicates. The *in-situ* aboveground residue was gently removed. The litterbags were then placed adjacent to two intended 2016 crop row positions at 1–2 cm below the surface, and then covered with surface soil in order to ensure contact between the residue and the soil, with a 2 cm spacing between litterbags. Litterbags were collected during the fallow and the growing season at the sampling times outlined in Table 1. The area of the bags was covered with a wire mesh and stapled to the ground, to prevent losses from predators and/or erosion. Prior to sowing the wheat crop on 2 June 2016, the remaining lupin and wheat litterbags were retrieved and stored in paper bags in cool conditions and returned to their original positions in the field after sowing. The individual wet weight of each bag at the start of the experiment was recorded and four wheat litterbags and four lupin litterbags were collected immediately after the bags were placed in the field at the start of the experiment to determine any mass loss due to bag handling and transportation. The wet weight of the litterbags was recorded on collection and ash and moisture content were measured in order to calculate the ash-free weight. At the end of their incubation period all sample bags were collected from the field, transported to the laboratory and stored at 5°C until their analysis. Wheat and lupin residues were removed from the bags, gently agitated on a sieve (0.7 mm) to separate any soil remaining in the bags. Residues were dried at 60 °C for 48 h to determine dry weight, and then ground (< 0.5 mm). One sample of each bag (0.1 g) was calcined in a muffle at 550°C to determine ash content. This value was used to adjust the

biomass in each sample as the remaining ash-free biomass, to account for any soil contamination of each residue sample. The carbon (C) and N content in each sample was measured as described above.

Temporal patterns of surface soil-nitrogen supply capacity at key growth stages

In each plot, eight composite surface soil (0–10 cm) samples were collected from between the crop rows with a hand corer at five key growth stages in 2016 (Table 1): seedling (Z13, 15 June), tillering (Z22, 20 July), first node (Z31, 22 August), flag leaf (Z39, 15 September), anthesis (Z65, 11 October) (Zadoks *et al.* 1974). Soil samples were stored at 4°C prior to extraction and analysis in the laboratory (within two to seven days). All surface samples were sieved (<2 mm), homogenised, and analysed for soil gravimetric water, mineral N, PMN, MBN, and DON.

Soils were extracted for mineral N by shaking with 2 M KCl (1:3, soil: solution) on an orbital shaker for 1 h at 25°C and filtered through Whatman No. 1. Nitrate and ammonium N concentration of extracts were analysed colorimetrically (Miranda *et al.* 2001) using a SynergyMX microplate reader.

Soil PMN was determined using a modification of Keeney and Bremner (1966) with a short-term (seven days) anaerobic incubation at 40 ºC in KCl to match the matrix of mineral N analyses (Gianello and Bremner 1986). For all samples, 5 g dry weight equivalent of soil was mixed with 15 mL of 2 M KCl^{-1} (1:3 soil:solution), centrifuged at 1,252 g. for 15 min, decanted and filtered (Whatman No. 1) at time zero and after seven days of incubation, and ammonium and nitrate N of the extracts was analysed colorimetrically (Miranda *et al.* 2001) using a SynergyMX® microplate reader.

Soil MBN was assessed using chloroform fumigation and extraction (Jenkinson 1977). Prior to the assay any soil samples that were below field capacity moisture were adjusted to field capacity (6% w w^{-1}, as derived from Muschietti-Piana *et al.* 2020) by spraying deionised water to each soil sample in a sealed bag and storing at 4ºC for 48 h prior to fumigation (Sparling and West 1989). Soil samples equivalent to 20 g of dry weight were fumigated with ethanol-free chloroform and stored at 25°C in a dark room for seven days (Sparling and Zhu 1993). Both fumigated and non-fumigated soil samples were extracted by shaking with 2 M KCl (soil/solution ratio 1:3) on an orbital shaker for 1 h at 25°C and filtered through Whatman No. 1. Soil MBN was estimated from the flush of measured ninhydrin-positive compounds between fumigated and non-fumigated soil (Amato and Ladd 1988). A conversion factor was applied to the calculated ninhydrin-positive compound flush in order to derive the MBN

(c=3.715, Sparling and Zhu1993). The KCl extracts of the unfumigated samples were analysed for nitrate N and ammonium N (Miranda *et al.* 2001).

Soil DON was measured in water extracts (10 g oven-dried equivalent shaken in 30 mL of distilled water with a temperature of 21°C) after centrifuging at 1,252 x g for 15 min and filtering (Whatman No. 42). These water extracts were analysed for nitrate N and ammonium N concentration colorimetrically (Miranda *et al.* 2001) using a SynergyMX microplate reader. Total dissolved N was determined in the water extracts using a Thermalox® dry combustion analyser (Analytical Sciences Ltd., Cambridge, United Kingdom). The concentration of DON was determined by subtraction of nitrate N plus ammonium N from the total dissolved N value (Jones and Willett 2006).

Estimated net nitrogen mineralisation

The estimated fallow net N mineralisation (start of fallow to pre-sowing) was calculated as:

$$M(t_2\text{-}t_1) = Ht_2 - St_1 \tag{2}$$

The in-season (sowing to harvest) net N mineralisation (kg ha^{-1}) between any two sampling times (t_1 and t_2), was estimated according to the following equation:

$$M(t_2\text{-}t_1) = Ht_2 - St_1 + U(t_2\text{-}t_1) \tag{3}$$

where M is the estimated net N mineralisation (kg N ha^{-1}) for the period t_1 to t_2, H is the sum of soil mineral N to 10 cm depth (nitrate+ammonium) at t_2 (kg N ha^{-1}), S is the sum of soil mineral N to 10 cm depth (nitrate+ammonium) at t_1 (kg N ha^{-1}), U is wheat plant N uptake (shoot+root, kg N ha^{-1}) for the period t_1 to t_2. Wheat plant N uptake data from Muschietti Piana *et al.* (2020) was used and the wheat N uptake between any two periods is presented in Table 3.

Statistical analysis

Throughout the paper, mean values are presented along with ± standard deviation to provide measures of variability. Data for remaining residue biomass in a litterbag as a proportion of initial biomass

weight (Fig. 2*a*, *b*) was examined in relation to days of incubation by fitting the data to a single exponential decay model for each treatment:

$$\text{Remaining biomass} = b \exp^{-kx} \tag{4}$$

where b is the initial residue biomass in the litterbag, k is the relative decomposition rate (decomposition day^{-1}) and x is the number of days of decomposition.

For the analysis of the changes in residue biomass (Fig. 2*c*, *d*) and C:N (Fig. 2*e*, *f*) over time the residue treatment, fertiliser-N input, time (incubation days) and their second and third order interactions were considered as fixed effects. The plot was used as a random effect including a correlation regressive structure (CAR1) to assess the same plot across different dates (i.e. repeated measures design). Analysis was completed with Infostat v.7 in interface with R with the lme4 package (Bates *et al.* 2015) to linear mixed effects models (R Development Core Team 2018).

For all treatments (residue and/or fertiliser N), the effect of time (as growth stages, or incubation days) on each measured variable (e.g. soil mineral N) was analysed using ANOVAs for repeated measures design with linear mixed effects models (lme4) in R (Bates *et al.* 2015; R Development Core Team 2018). Residue and fertiliser-N treatments were used as fixed effects, and block, main plot and sub-plot as a random effect. Where ANOVA results showed a significant effect of the residue treatment, fertiliser-N input, time and/or their interactions, the *post-hoc* comparisons between treatment means was analysed with Di Rienzo, Guzman, Casanoves (DGC) test (alpha=0.05, Rienzo *et al.* 2002). Relationships between remaining biomass, residue N, residue C:N, soil, crop and environmental variables were assessed by correlation analysis using Pearson's r or Spearman's rho for normal or non-normal data with Infostat v.7 .

Results

Changes in residue decomposition, nitrogen and C:N ratio in litterbags

The decomposition rate (k=decomposition days^{-1}) was faster (p<0.0001) with lupin residues (k=-0.0033 days^{-1}) than with wheat residues (k=-0.0015 days^{-1}) during the entire 359 days of incubation (DOI) for

the experiment (Fig. 2*a*). By the end of the experiment, 70% of the Lupin+0N residue had decomposed and 40% of the Wheat+0N residue had decomposed (Fig. 2*a*). At the start of the experiment the lupin leaves had a greater total N, soluble organic C and N, and lower C:N ratio than the other lupin fractions, and leaves represented 8% of the initial residue mass. Wheat straw had the greatest total C:N and lowest total N and soluble N and straw represented 66% of the initial wheat residue mass (Table 2).

Table 2. Initial quality of each residue fraction used for the litterbags at the start of the experiment (15 December 2015). Residue as a percentage by weight of total residue is shown in brackets.

C, carbon; SOC, total soluble organic C in water extracts; SN, total soluble N in water extracts; SOC, soluble organic C, SON, soluble organic N.

Residue fraction	Total C (mg g^{-1})	Total N (mg g^{-1})	Total C:N	SOC (mg kg^{-1})	SN (mg kg^{-1})	SOC:SON
Wheat chaff (34%)	439	5.6	78	17	0.9	19
Wheat straw (66%)	439	3.4	129	19	0.8	24
Lupin leaves (8%)	402	11.6	35	68	2.8	24
Lupin pods (49%)	423	5.0	85	41	1.2	35
Lupin straw (43%)	421	6.0	70	19	1.1	17
Total wheat residue	439	4.1	107	18	0.8	23
Total lupin residue	421	6.0	70	34	1.3	26

The residue N as % of initial followed a similar pattern over time for both treatments but it remained higher in Wheat+0N from first node to harvest (218–359 DOI, Fig. 2*c*) than in lupin+0N. Residue N % increased from late fallow to wheat sowing (93–167 DOI) in both treatments, and from flag leaf to anthesis (275–301 DOI) in Wheat+0N. By the end of the experiment, 60 and 40% of residue N remained in Wheat+0N and Lupin+0N respectively (Fig. 2*c*). The residue N % and biomass (Fig. 2*c, d*) was used to derive the potential residue N contribution in kg N ha^{-1} (Fig. 2*e, f*) in order to facilitate

comparison with crop N uptake in the main plots. During the whole incubation, there were significant differences in the potential N provided between the residues types (paired t test, bilateral, p=0.01); with lupin residues releasing twice as much N (20±2 kg N ha^{-1}) as wheat residues (10±3 kg N ha^{-1}). Residue N release during the fallow accounted for 40% of the total N release by lupin residues (i.e.8±3 kg N ha^{-1}) and wheat residues released 50% of their total during the fallow (5±3 kg N ha^{-1}) (Fig. 2*e*). Across the whole 360 day period of litterbag incubation the residue C:N ratio tended to decrease in all treatments but was always higher for wheat residue treatments (Figs. 2*g* and 2*h*). There was an apparent increase in C:N ratio of wheat residues during the early fallow (0–44 DOI) but by sowing (167 DOI) the C:N ratio of both wheat and lupin residues had narrowed. The decrease in C:N was more pronounced during late fallow (93–167 DOI; Fig. 2*g*) in conjunction with an increase in residue N concentrations during that period (Fig. 2*c*). Residue C:N ratio decreased over time in all treatments and was always higher for wheat residue treatments (Fig. 2*h*).

For the wheat growing season period (167–359 DOI), the interaction between residue type and fertiliser-N treatment had a strong effect on residue decomposition rates (Fig. 2*b*). Lupin+0N had the highest decomposition rate (*k*= -0.0045 day^{-1}) followed by Lupin+40N (*k*=-0.0029 day^{-1}) and Wheat+40N (*k*=-0.0018 day^{-1}), with Wheat+0N having the slowest decomposition rate (*k*=-0.0014 day^{-1}). It was only at the end of the incubation at the point decomposition was starting to plateau that there was a small difference where Lupin+40N decomposition was slower than Lupin+0N (F=5.879, p=0.0195). For the whole of incubation period residue decomposition had a strong and positive association with cumulative rainfall and degree days (Fig. 3). This relationship was better explained when separated between residue treatments. Lupin residue decomposition against cumulative rainfall had a steeper slope than wheat residues (-0.138 *vs* -0.0855; $F_{1, 14}$=6.93, p-value=0.019). Similarly, lupin residue decomposition against cumulative degree days had a steeper slope than wheat residues (-0.0118 *vs* -0.0074; $F_{1, 14}$=18.25, p=0.0007).
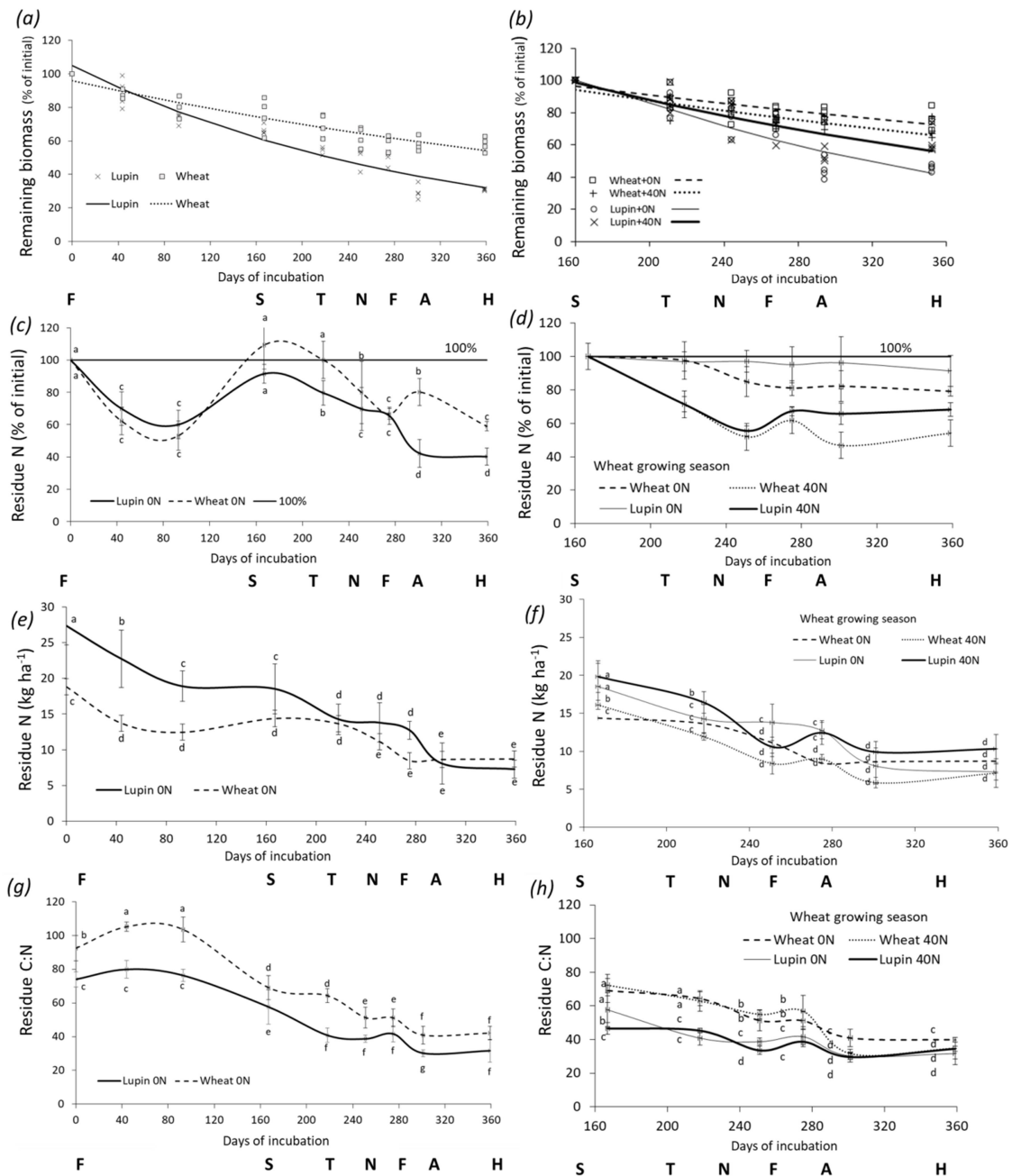
Figure 2. Whole of Incubation (359 days of incubation, fallow to harvest) remaining biomass as % of initial *(a)*, residue N as % of initial *(c),* and residue C:N (e) of wheat and lupin residues. **In-season** (post-fertiliser-N application) remaining biomass as % of initial *(b),* residue N as % of initial *(d)*, and residue C:N *(f)* of wheat and lupin residues fertiliser (0N vs. 40N). For *(a, b)* lines show decay exponential model fitted for treatments that had significant differences and markers show observed values for each treatment. For *(c, e, f)* different letters indicate significant differences for interaction between 'residue treatment', and 'days after first incubation' (DGC test, p<0.05). Sampling times on x-axis are indicated as; F, fallow, S, sowing, T, tillering, N, first node, F, flag leaf, A, anthesis, H, harvest.
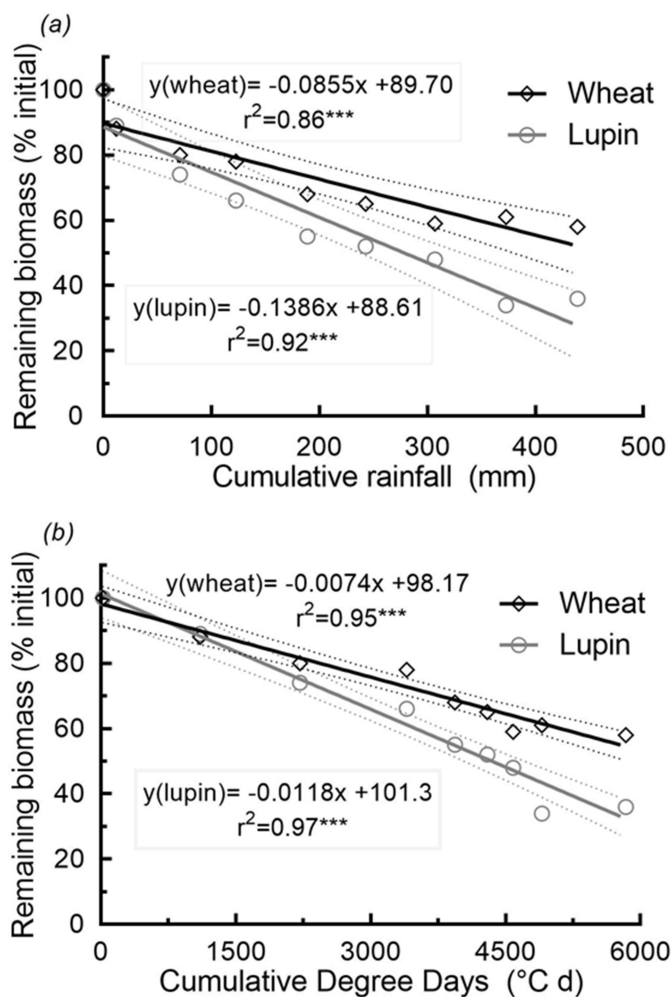
Figure 3. Whole of Incubation (359 days of incubation, fallow to harvest) remaining biomass (as % of initial) against cumulative rainfall (mm) *(a)* and cumulative degree days (°C d) *(b)* for wheat (solid black line) and lupin (solid grey line) residues. Solid lines indicate the fitted model for each residue treatment; dotted grey lines indicate the 95% confidence band for each residue treatment.

Seasonal patterns of surface soil-nitrogen supply capacity from whole plots

During the wheat growing season the surface soil nitrate and ammonium N were responsive to the combination of residue types and fertiliser-N inputs (Fig. 4). Surface soil mineral N averaged across all growth stages was highest for the lupin residue treatments with 5 kg ha^{-1} more surface soil mineral N than the other treatments (p=0.005). This difference represented a gain of 38% and 21% of the surface soil mineral N content at sowing when compared with the removed and wheat residues. At wheat crop sowing, the surface soil nitrate and ammonium N were highest with lupin residues and resulted in an average mineral N (nitrate + ammonium) of 32±3, 23±2 and 13±4 kg N ha^{-1} for lupin >

wheat > removed residues respectively. The surface soil nitrate N followed a similar pattern across all treatments with the greatest depletion of nitrate N between tillering and anthesis. Surface soil ammonium N in the Lupin+40N treatment was higher than all treatments at seedling and tillering and tended to decline from seedling through to anthesis.
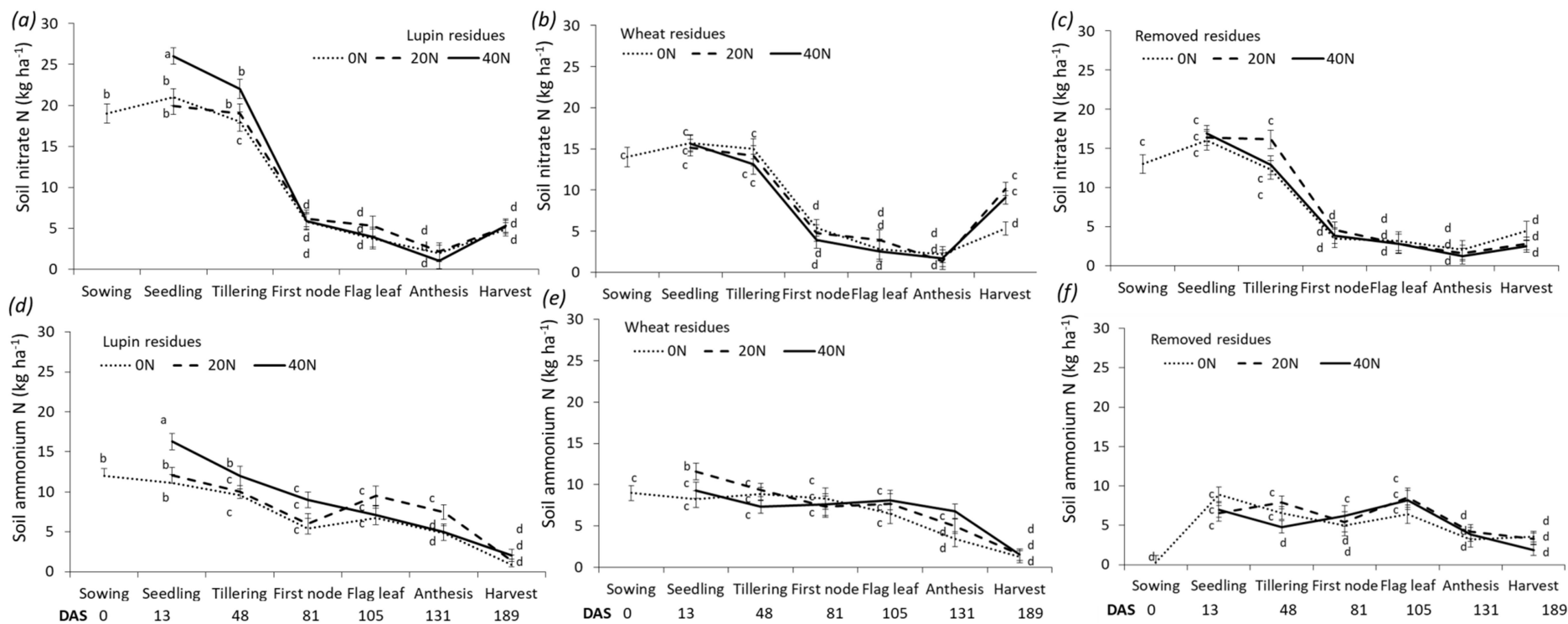
Figure 4. Soil nitrate N to 10 cm depth (kg ha^{-1}) for lupin *(a),* wheat *(b)* and removed *(c)* residues and soil ammonium N to 10 cm depth (kg ha^{-1}) for lupin *(d),* wheat *(e)* and removed *(f)* residues; combined with the fertiliser-N inputs (0, 20, 40 kg ha^{-1}) at different growth stages. DAS (in x-axis) days after sowing. Different letters indicate significant differences across panels of the same row for the interaction between 'residue type', 'fertiliser-N input', and 'growth stage' (DGC test, p<0.05).

Changes in the seasonal patterns of the surface soil PMN, MBN and DON were observed as a result of the interaction between the residue treatment and the fertiliser-N inputs (Fig. 5a–i). Where wheat residues had been removed, the surface soil PMN remained relatively constant with time (Fig. 5c). Under both residue-retained treatments, the surface soil PMN showed a similar pattern, especially when fertiliser-N was applied (Fig. 5a, b). At the seedling stage, surface soil PMN was highest in the Lupin+40N and at tillering in both residue-retained treatments when fertiliser-N was applied. Between first node and flag leaf, surface soil PMN remained constant but had sharp increase between flag leaf and anthesis in the Lupin+0N, Lupin+40N and Wheat+40N treatments.

Surface soil MBN increased during the season from seedling onwards, being highest at flag leaf; then decreased from flag leaf to anthesis. Regardless of the fertiliser-N input, lupin residues had a higher surface soil MBN on average at first node (+10 kg N ha^{-1}) and flag leaf (+11 kg N ha^{-1}) than wheat and removed residues (Fig. 5d–f). In all treatments, surface soil PMN ranged between 10–100 kg N ha^{-1} and MBN between 10–70 N kg ha^{-1}, and both fluctuated around the mean seasonal value by 40%.

Surface soil DON was highest at the seedling stage and then decreased during the growing season in all treatments (Fig. 5g-i). Overall, where wheat residues had been removed, surface soil DON became negligible from tillering onwards but where residue had been retained DON was still measurable at more advanced stages up until flag leaf in the lupin residue treatment and anthesis in the wheat residue treatment (Fig. 5g–i).

The Lupin+40N treatment increased surface soil nitrate N and PMN at seedling and surface soil ammonium N at seedling and tillering, while Lupin+20N and Lupin+40N increased surface soil nitrate N at tillering when compared to all other treatments. Regardless of the fertiliser-N input, lupin residues increased surface soil mineral N (nitrate + ammonium) at sowing, seedling and tillering, PMN at seedling and anthesis, MBN at first node and flag leaf, and DON at seedling when compared to wheat or removed residues. In summary, lupin residues increase mineral N, PMN, MBN and DON but not wheat residues which have similar mineral, DON, PMN and DON to soil where wheat residues had been removed.
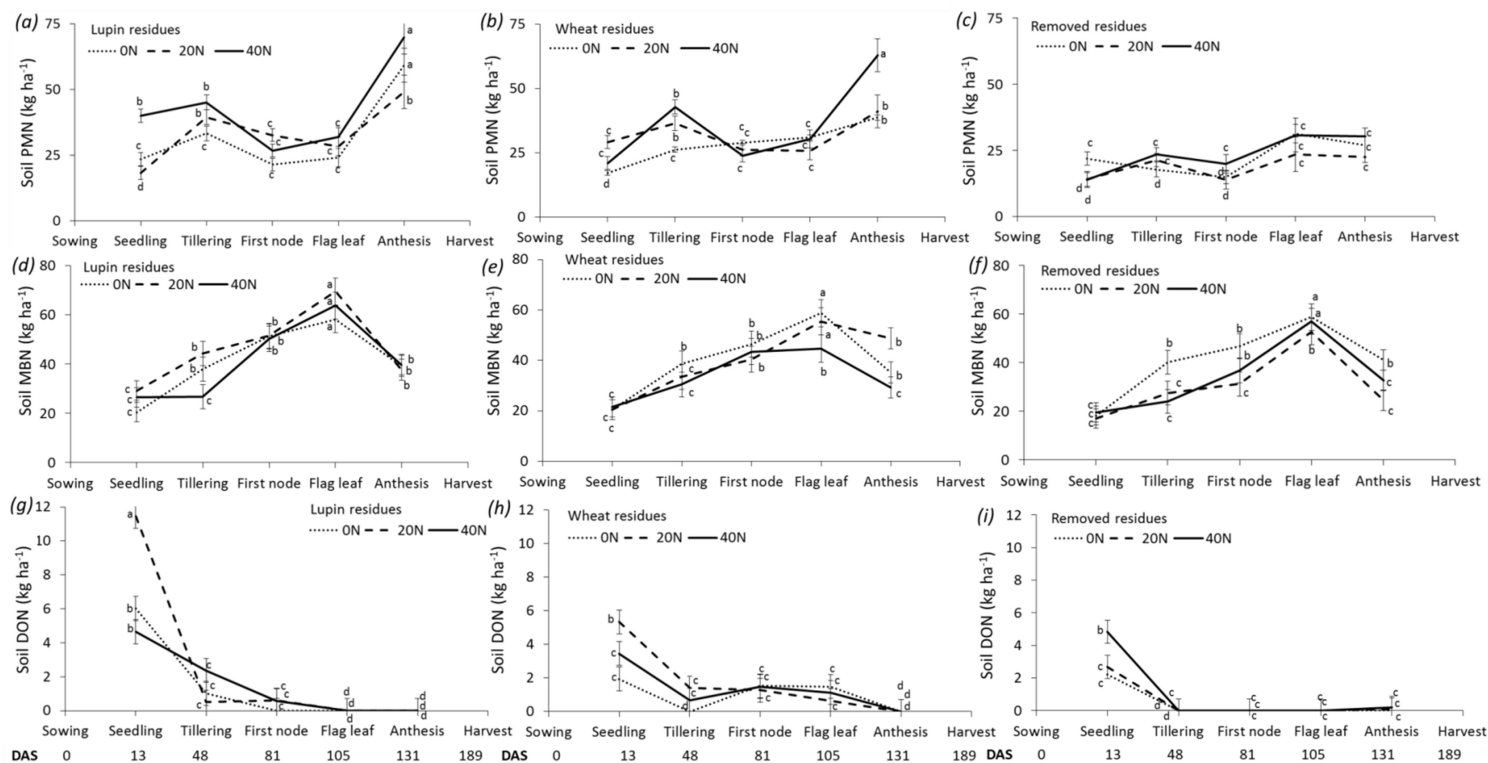
Figure 5. Soil potentially mineralisable-N (PMN) to 10 cm depth (kg ha^{-1}) for lupin *(a)*, wheat *(b)* and removed *(c)* residues; soil MB N (MBN) to 10 cm depth (kg ha^{-1}) for lupin *(d)*, wheat *(e)* and removed *(f)* residues; soil dissolved organic nitrogen (DON) to 10 cm depth (kg ha^{-1}) for lupin *(g)*, wheat *(h)* and removed *(i)* residues; combined with the fertiliser-N inputs (0, 20, 40 kg ha^{-1}) at different growth stages. DAS (in x-axis) days after sowing. Different letters indicate significant differences across panels of the same row for the interaction between 'residue type', 'fertiliser-N input', and 'growth stage' (DGC test, p<0.05).

Estimated net nitrogen mineralisation, surface soil-nitrogen supply capacity, and wheat plant nitrogen uptake in whole-plot residue treatments

The in-season net N mineralisation in the soil surface (0–10 cm) and profile (0–100cm) was estimated only for the unfertilised treatments. Accounting for the fertiliser N input in the estimation of the in-season net N mineralisation as described in Eq. 3 requires reliable data of the fertiliser N uptake efficiency. Otherwise, it might result in an overestimation of the net N mineralisation in the fertilised treatments, and so the net N mineralisation was not estimated in the fertilised N treatments (Table 3). The Lupin+0N treatment had the highest estimated net N mineralisation from fallow to harvest (whole of incubation), from sowing to harvest, from tillering to first node, first node to flag leaf and from anthesis to harvest. During the period from tillering to flag leaf, the estimated net N mineralisation in the soil profile (0–100 cm) was 72 kg N ha^{-1} under wheat after Lupin+0N, but there was net N immobilisation of 15 kg N ha^{-1} after Wheat+0N and increased surface soil MBN. During this period, the estimated net N mineralisation in the surface soil in the Lupin+0N treatment represented 73% of the total in the soil profile for that treatment (Table 3).

Table 3. Changes (Δ) in surface soil (0-10 cm) mineral N, microbial biomass N (MBN) and estimated net N mineralisation/immobilisation (kg N ha^{-1}) between sampling times for the residue treatments without fertiliser-N application. This is compared with the wheat plant N uptake and the estimated net N mineralisation in the soil profile to 100 cm depth (kg N ha^{-1}) data published in Mushcietti Piana et al. (2020)

Mean value of four replicates with standard deviation in parenthesis. Different letters within the same column of each sampling time indicate significant differences between treatment means (DGC test, p<0.05). Values were highlighted in grey to improve visualisation of the significant differences among treatment means.

Treatment	ΔSoil mineral N (0-10 cm)	ΔMBN (0-10 cm)	Estimated net N mineralisation (0-10 cm)	(0-100 cm)*	ΔPlant N uptake*
			Whole Fallow		
Wheat+0N	23 (4) a	NA	23 (4) a	73 (21) a	NA
Lupin+0N	20 (8) a	NA	20 (8) a	53 (26) a	NA
			Fallow to Harvest		
Wheat+0N	4 (2) a	NA	52 (12) b	68 (11) a	48 (12) b
Lupin+0N	-5 (1) b	NA	84 (23) a	75 (26) a	89 (11) a
			Sowing to Harvest		
Removed+0N	-8 (4) a	NA	43 (9) b	22 (16) a	51 (12) b
Wheat+0N	-18 (5) a	NA	30 (9) b	8 (18) a	48 (11) b
Lupin+0N	-30 (4) b	NA	68 (8) a	8 (16) a	89 (11) a
			Sowing to Tillering		
Removed+0N	2 (5) a	40 (5) a	7 (5) a	44 (18) a	5 (1) a
Wheat+0N	0 (4) a	39 (4) a	5 (4) a	65 (12) a	5 (1) a
Lupin+0N	-6 (4) a	38 (5) a	6 (3) a	56 (17) a	5 (1) a
			Tillering to First node		
Removed+0N	-8 (3) a	7 (9) a	7 (7) b	0 (8) b	16 (2) b
Wheat+0N	-5 (4) a	7 (9) a	11 (3) b	-3 (9) b	16 (1) b
Lupin+0N	-2 (4) a	13 (8) a	23 (5) a	32 (9) a	25 (2) a
			First node to Flag leaf		
Removed+0N	0 (3) a	12 (6) a	16 (6) b	25 (4) b	16 (4) b
Wheat+0N	-5 (2) a	12 (4) a	3 (7) b	-12 (6) c	8 (4) c
Lupin+0N	-2 (3) a	7 (6) b	30 (6) a	40 (9) a	32 (5) a
			Flag leaf to Anthesis		
Removed+0N	-3 (3) a	-18 (6) a	0 (3) b	-5 (4) b	4 (2) a
Wheat+0N	-2 (1) a	-24 (5) a	12 (2) a	19 (6) a	15 (3) a
Lupin+0N	-3 (1) a	-19 (5) a	-1 (2) b	4 (4) b	0 (2) b
			Anthesis to Harvest		
Removed+0N	2 (2) a	NA	13 (8) b	-41 (14) a	11 (11) a
Wheat+0N	-1 (1) a	NA	5 (8) b	-66 (12) a	6 (11) a
Lupin+0N	-2 (2) a	NA	34 (10) a	-29 (13) a	36 (12) a

*Data extracted from Muschietti-Piana et al. (2020).

Discussion

Legume residues have a faster decomposition rate and more nitrogen released through mineralisation

The rate of decomposition of lupin residues was faster than that for wheat residues and the magnitude of the difference was more pronounced between first node and harvest of the following wheat crop (251–359 DOI), relative to the earlier period (0–250 DOI). Legume leaves are easily decomposed by soil microorganisms due to a low C:N ratio and lignin content. They immobilise soil mineral N to a lesser extent and enter the net mineralisation phase faster than cereal residues, as indicated in litterbag field studies on a loamy sand (Fosu *et al.* 2007). Litterbag field experiments on decomposition of lupin and wheat residues during summer and autumn in Mediterranean soils of south-western Australia have reported a decomposition rate of 0.0011 day^{-1} for wheat residues (van Vliet *et al.* 2000), like the rate reported here (0.0015 day^{-1}). But, the decomposition rate for lupin residues in this study (0.0033 day^{-1}) was 2.5 times higher than the one reported by van Vliet *et al.* (2000) of 0.0013 day^{-1}. These differences may be due to the lack of rainfall and residue decomposition during the first 55 DOI (January–March) in the van Vliet (2000) study, compared with 59 mm January–March rainfall here. In our present study, cumulative residue decomposition was linked to rainfall and temperature, but the magnitude of its effect was determined by residue quality. Lupin residue decomposition was more responsive to both cumulative rainfall and degree days than wheat residues.

The effect of belowground residues from the previous crop has not been directly determined in this study. The estimated N input of belowground lupin biomass was likely to have been a further 6±5 kg N ha^{-1} based on an estimate of total N accumulation using a 1.5 conversion factor as suggested by McNeill and Fillery (2008). A proportion (10–30%) of this lupin below-ground N input is likely to also have become available as mineral N for the following wheat at sowing (McNeill and Fillery 2008, Ladd *et al.* 1983). Overall the higher N supply to wheat following lupin residues came from both the higher amounts of mineral N at sowing and the N released *via* mineralisation from residue decomposition, which was evidenced by the higher estimated net N mineralisation from tillering to flag leaf (Table 3). The N released from lupin residue decomposition from sowing to harvest could have supplied up to 6±2 kg N ha^{-1} more than N from wheat residues (Fig. 2*e*).

Legume residues increased nitrogen supply and reduced nitrogen immobilisation

The N benefits in legume–cereal rotations are likely to relate to less immobilisation of N during the decomposition of legume compared with cereal residues. Immobilisation of N can be considered a

temporary storage of N that will be then re-mineralised under favourable conditions of increased soil water and temperature and due to microbial predation by protozoa and free-living nematodes (Gupta and Yeates 1997). This may affect the mineral-N supply during critical growing phases of a crop. Research in soils amended with different crop residues under laboratory conditions have shown that the degree of immobilisation of N was more with residues of higher C:N (41 *cv.* 20) (Abera *et al.* 2012). Litterbag data in this study provided evidence of N immobilisation (Fig. 2*c, e*) which was more pronounced with wheat residues that had a higher C:N ratio. This observation also accords with Trinsoutrot *et al.* (2000) who reported that crop residues with higher C:N ratio induced net N immobilisation after 168 DOI.

There were two noticeable increases in residue N as a % of the initial value from late fallow to sowing for both lupin and wheat residues, and from flag leaf to anthesis for wheat residue (Fig. 2*c*). They were probably caused by reciprocal transfer of C and N by decomposer fungi at the soil-litter interface. Previous studies have indicated that fungi can translocate residue-derived C into the underlying soil while simultaneously translocating soil-derived inorganic N into the litter layer (Frey *et al.* 2003). Similar results of fungal mediated-N transport were observed between the surface litter and the N in the soil below in low organic matter Australian agricultural soils under no-tillage cropping systems (Gupta *et al.* 1996). The low N concentration in the residue (4 and 6 mg N g^{-1} for wheat and lupin respectively) is likely to have contributed to the fungal-based translocation of N from soil to the residue resulting in the increase in the amount of N in the residues. As the increasing residue N was measured in all the treatment replicates, it is unlikely that it was due to sampling error, temporal and/or spatial variability in the soil.

The magnitude of the immobilisation of mineral N depends on the MB activity during residue decomposition in the soil (Gupta *et al.* 2011). Generally, N is mineralised and released into the soil during the decomposition of residues with lower C:N ratio, while any mineral N from the residues with higher C:N ratio is consumed by the microorganisms causing immobilisation. Then, part of the MB is lost, causing the MIT and an increase in soil mineral-N content (Akbari *et al.* 2020). In our study, the difference found in the C:N ratio of lupin and wheat residues was due to their difference in N concentration but not in C concentration, similar to the results of van Vliet *et al.* (2000). In line with

our experiment, the observed loss of C in van Vliet *et al.* (2000) followed a similar pattern to that of biomass loss for both lupin and wheat residues.

Surface soil-nitrogen supply capacity is increased by the legume and fertiliser-nitrogen inputs

Several studies (Crews and Peoples 2005, Dalias 2015, St Luce et al. 2016) have demonstrated that growing cereals after legumes reduced the need of N fertiliser and enhance soil productivity compared with continuous cereal production. However, the inherent soil fertility will determine whether legume derived N supply is adequate, or if supplemental fertiliser N is needed.

Surface soil PMN and MBN seasonal values and oscillations were in line with similar studies conducted in soils of Western Australia which reported 50–55% variation across the season (Murphy *et al.* 1998*c*). In our study, both surface soil PMN and MBN appeared to be responsive to cumulative rainfall and degree days in all residue and fertiliser-N treatments. Between flag leaf and anthesis when there were 67 mm of measured rainfall, the temperature increased to >18°C, and when the gravimetric water in the surface soil was maximum in all treatments, surface soil PMN increased in all residue-retained treatments, and surface soil MBN decreased in all residue and fertiliser-N treatments. A sharp increase in the surface soil MBN content was correlated with decreasing surface soil mineral N from seedling to first node with net immobilisation from residue N (as % of initial). Also, soil gravimetric moisture to 10 cm depth from seedling to first node was close to field capacity ($6\pm2\%$ w w^{-1}, on average in all treatments), and maximum at flag leaf with $10\pm2\%$ (w w^{-1}) on average in all treatments (Muschietti Piana *et al.* 2020).

A parallel study at this site showed that the treatments with lupin residue and higher fertiliser-N inputs had consistently higher wheat N uptake (Muschietti Piana *et al.* 2020) associated with soil profile mineral-N flux, and with high fertiliser-N rates alleviating the immobilisation of N effect. This study offers insights into the key drivers of the higher soil profile mineral N and N supply potential enhancing crop N uptake observed in Muschietti-Piana *et al.* (2020). The higher mineralisation of residue N and more surface soil-N supply capacity for the treatments with the highest levels of crop N uptake are likely to have contributed to a better synchrony to match crop N demand at critical growth stages as previously indicated by Crews and Peoples (2005). In Muschietti-Piana *et al.* (2020) there was a gain in plant N uptake following lupin residues by applying 40 kg N ha^{-1} of fertiliser-N relative

to no fertiliser-N addition was 24±6 kg N ha^{-1} at first node and 42±4 kg N ha^{-1} at anthesis. This indicates that legumes in rotation can improve soil-N supply to the following cereal crops with potential benefits of combining them with fertiliser-N in low-rainfall sandy soil environments. There may be avenues to increase N supply from cereal residues as recent research has observed that fertiliser N addition affected wheat residue C:N by stimulating microbial activity, which accelerated degradation of plant residues and increased residue decomposition improving N supply from mineralisation of wheat residues (Akbari *et al*. 2020).

Conclusions

Lupin residues provided a greater N supply capacity through the wheat growing season due to higher decomposition rates and greater net N release. Lupin residues increased surface soil mineral N (nitrate + ammonium) at sowing, seedling and tillering, PMN at seedling and anthesis, MBN at first node and flag leaf, and DON at seedling more than when wheat residues were either removed or retained. The combination of lupin residues with 40N fertiliser N offered some significant gains for N supply capacity early in the season, with the highest surface soil nitrate N at seedling and tillering, PMN at seedling and ammonium N at seedling and tillering.

References

Abera G, Wolde-Meskel E, Bakken L (2012) Carbon and nitrogen mineralisation dynamics in different soils of the tropics amended with legume residues and contrasting soil moisture contents. *Biology and Fertility Soils* **48**, 51–66.

Adl SM (2003) The ecology of soil decomposition. (CABI: Wallingford, Oxon, United Kingdom).

Akbari F, Fallah S, Dahmardeh M, Pessarakli M (2020) Interaction effects of nitrogen and phosphorus fertiliser on nitrogen mineralisation of wheat residues in a calcareous soil. *Journal of Plant Nutrition* **43**, 1–12.

Amato M, Ladd JN (1988) Assay for microbial biomass based on ninhydrin-reactive nitrogen in extracts of fumigated soils. *Soil Biology & Biochemistry* **20**, 107–114.

Bates D, Maechler M, Bolker B, Walker S (2015) Fitting linear mixed-effects models using lme4. *Journal of Statistical Software* **67**, 1-48. <doi:10.18637/jss.v067.i01

Crews TE, Peoples MB (2005) Can the synchrony of nitrogen supply and crop demand be improved in legume and fertiliser-based agroecosystems? A review. *Nutrient Cycling in Agroecosystems* **72**, 101–120.

Dalias P (2015) Grain legume effects on soil-Nitrogen mineralisation potential and wheat productivity in a Mediterranean environment. *Archives of Agronomy and Soil Science* **61**, 461–473.

Evans J, McNeill AM, Unkovich MJ, Fettell NA, Heenan DP (2001) Net nitrogen balances for cool-season grain legume crops and contributions to wheat nitrogen uptake: a review. *Australian Journal of Experimental Agriculture* **41**, 347–359.

Farrell M, Prendergast-Miller M, Jones DL, Hill PW, Condron LM (2014) Soil microbial organic nitrogen uptake is regulated by carbon availability. *Soil Biology & Biochemistry* **37**, 261–267.

Fillery IRP, McInnes KJ (1992) Components of the fertiliser nitrogen balance for wheat production on duplex soils. *Australian Journal of Experimental Agricultu*re **32**, 887–899.

Fosu M, Kunne RF, Vlek PLG (2007) Mineralisation and microbial biomass dynamics during decomposition of four leguminous residues. *Journal of Biological Sciences* **7**, 632–637.

Frey SD, Six J, Elliott ET (2003) Reciprocal transfer of carbon and nitrogen by decomposer fungi at the soil–litter interface. *Soil Biology & Biochemistry* **35**, 1001–1004.

Gianello C, Bremner JM (1986) Comparison of chemical methods of assessing potentially available organic nitrogen in soil. *Communications in Soil Science and Plant Analysis*, **17**, 2, 215–236.

Gupta VVSR, Amato M, Pankhurst CE (1996) Transport of carbon and nitrogen by soil fungi under laboratory conditions: effects of protozoan predation and mineral nitrogen. In 'Proceedings of the XII International Colloquium on Soil Zoology'. (Ed JP Curry). University College Dublin, Ireland.

Gupta VVRS, McBeath, TM, Kirkegaard J, Swan T, Richardson A (2018) 15N stubble experiments at Temora, Horsham and Karoonda. In 'Research outcomes on N-dynamics in stubble retained systems and their implications for N management guidelines.' (Eds J Kirkegaard, K Verburg, VVRS Gupta, T McBeath, Z Luo, E Wang, A Richardson, J Lilley). pp.32–37. (CSIRO Publishing: Australia).

Gupta VVSR, Yeates GW (1997) Soil microfauna as indicators of soil health. (Eds CE Pankhurst, B Doube, VVSR Gupta), pp. 201–233. (CAB International, Oxon, United Kingdom).

Gupta VVSR (2016) Biological factors influence N mineralisation from soil organic matter and crop residues in Australian cropping systems. In 'Proceedings of the 2016 International Nitrogen Initiative Conference', Australia. http://www.ini2016.com/pdf-papers/INI2016_Vadakattu_Gupta.pdf.

Gupta VVSR, Rovira AD, Roget DK (2011) Principles and management of soil biological factors for sustainable rainfed farming systems. In 'Rainfed Farming Systems'. (Eds P Tow, I Cooper, I Partridge, C Birch), pp. 149–184. (Springer, Berlin, Germany).

Hoyle FC, Murphy DV (2011) Influence of organic residues and soil incorporation on temporal measures of microbial biomass and plant available nitrogen. *Plant and Soil* **347**, 53–64.

Isbell RF and National Committee on Soil and Terrain (2016) ´The Australian soil classification.´ 2nd edn (CSIRO Publishing: Melbourne).

Jenkinson DS (1977) The soil biomass. *New Zealand Soil News* **25**, 213–218.

Jones DL, Willett VB (2006). Experimental evaluation of methods to quantify dissolved organic nitrogen (DON) and dissolved organic carbon (DOC) in soil. *Soil Biology and Biochemistry* **38**, 991–999.

Keeney DR, Bremner JM (1966) Comparison and evaluation of laboratory methods of obtaining an index of soil nitrogen availability. *Agronomy Journal* **58**, 498–503.

Ladd JN, Amato M, Jackson RB, Butler JHA (1983) Utilization by wheat crops of nitrogen from legume residues decomposing in soils in the field. *Soil Biology and Biochemistry* **15**, 231–238.

McBeath TM, Gupta VVSR, Llewellyn RS, Davoren CW, Whitbread AM (2015) Break crop effects on wheat production across soils and seasons in a semi-arid environment. *Crop and Pasture Science* **66,** 566–579.

McNeill AM, Fillery IRP (2008) Field measurement of lupin belowground nitrogen accumulation and recovery in the subsequent cereal-soil system in a semi-arid Mediterranean-type climate. *Plant and Soil* **302**, 297–316.

McNeill AM, Unkovich M (2007) The nitrogen cycle in terrestrial ecosystems. In 'Nutrient cycling in terrestrial ecosystems'. (Eds P Marschner, Z Rengel), pp. 37–64. (Springer Verlag: Berlin Heidelberg, Germany).

Miranda KM, Espey MG, Wink DA (2001) A rapid, simple spectrophotometric method for simultaneous detection of nitrate and nitrite. *Nitric Oxide* **5**, 62–71.

Murphy DV, Cookson WR, Braimbridge MP, Marschner P, Jones DL, Stockdale EA, Abbott LK (2011) Relationships between soil organic matter and the soil MB (size, functional diversity, and community structure) in crop and pasture systems in a semi-arid environment. *Soil Research* **49**, 582–594.

Murphy DV, Fillery IRP, Sparling GP (1998*c*) Seasonal fluctuations in gross N mineralisation, ammonium consumption, and MB in a Western Australian soil under different land uses. *Australian Journal of Agricultural Research* **49**, 523–535.

Murphy DV, Sparling GP, Fillery IRP (1998*a*). Stratification of microbial biomass C and N and gross mineralisation with soil depth in two contrasting Western Australian agricultural soils. *Australian Journal of Soil Research* **36**, 45–55.

Muschietti Piana MP, McBeath T, McNeill AM, Cipriotti PAC, Gupta VVSR (2020) Combined nitrogen input from legume residues and fertiliser improves early nitrogen supply and uptake by wheat. *Journal of Plant Nutrition and Soil Science* **183**, 355–366.

Peoples MB, Swan AD, Goward L, Kirkegaard JA, Hunt JR, Li GD, Schwenke GD, Herridge D F., Moodie M, Wilhelm N, Potter T, Denton MD, Browne C, Phillips LA, Khan DF (2017) Soil mineral nitrogen benefits derived from legumes and comparisons of the apparent recovery of legume or fertilizer nitrogen by wheat. *Soil Research.* **55,** 600–615.

R Core Team (2018) R: A language and environment for statistical computing. R Foundation for statistical computing, Vienna, Austria. Available at https://www.r-project.org/ [verified 30 March 2020].

Rienzo JA, Guzman AW, Casanoves F (2002) A multiple-comparisons method based on the distribution of the root node distance of a binary tree. *Journal of Agricultural, Biological, and Environmental Statistics* **7**, 129–142.

Ros GH, Hoffland E, van Kessel C, Temminghoff EJM (2009) Extractable and dissolved soil orgnic nitrogen—a quantitative assessment. *Soil Biology & Biochemistry* **41**, 1029–1039.

Sadras VO (2002) Interaction between rainfall and nitrogen fertilisation of wheat in environments prone to terminal drought: economic and environmental risk analysis. *Field Crops Research* **77**, 201–215.

Sadras VO, Roget DK (2004) Production and environmental aspects of cropping intensification in a semiarid environment of Southeastern Australia. *Agronomy Journal* **96**, 236–246.

Singh B, Rengel Z (2007) The role of crop residues in improving soil fertility. In 'Soil Biology: nutrient Cycling in Terrestrial Ecosystems'. (Eds P Marschner, Z Rengel), pp. 183–214. (Springer-Verlag: Berlin, Germany).

Sparling GP, Zhu C (1993) Evaluation and calibration of biochemical methods to measure microbial biomass C and N in soils from Western Australia. *Soil Biology & Biochemistry* **25**, 1793–1901.

Sparling GP, West AW (1989) Importance of soil water content when estimating soil microbial C, N and P by the fumigation-extraction methods. *Soil Biology & Biochemistry* **21**, 245–253.

St. Luce, M., Grant, C. A., Ziadi, N., Zebarth, B. J., O'Donovan, J. T., Blackshaw, R. E., Harker, K. N., Johnson, E. N., Gan, Y., Lafond, G. P., May, W. E., Malhi, S. S., Turkington, T. K., Lupwayi, N. Z., McLaren, D. L. (2016) Preceding crops and nitrogen fertilization influence

soil nitrogen cycling in no-till canola and wheat cropping systems. *Field Crops Research* **191**, 20–32.

Trinsoutrot I, Recous S, Bentz B, Line`res M, Che`neby D, Nicolardo B (2000) Biochemical quality of crop residues and carbon and nitrogen mineralisation kinetics under non limiting nitrogen conditions. *Soil Science Society of America Journal* **64**, 918–926.

Van Vliet PCJ, Gupta VVSR, Abbott LK (2000) Soil biota and crop residue decomposition during summer andautumn in south-western Australia. *Applied Soil Ecology* **14**, 111–124.

Verhoef HA (1995) Litter bag method. In 'Methods in applied soil microbiology and biochemistry. (Eds K Alef, P Nannipieri), pp. 485-487. (Academic Press: London, United Kingdom).

Zadoks JC, Chang TT, Konzak CF (1974) A decimal code for the growth stages of cereals. *Weed Res*earch **14**, 415–421.

CHAPTER 4

Early growing season immobilisation affects post-tillering wheat nitrogen uptake from crop stubble and ^{15}N fertiliser in a sandy soil

Statement of Authorship

Title of Paper	Early growing season immobilisation affects post-tillering wheat nitrogen uptake from crop stubble and 15N fertiliser in a sandy soil
Publication Status	√ Published Accepted for Publication Submitted for Publication ☐ Unpublished and Unsubmitted work written in manuscript style
Publication Details	Muschietti Piana MP, T McBeath, A McNeill, PA Cipriotti & V Gupta. 2020. Early growing season immobilisation affects post-tillering wheat nitrogen uptake from crop stubble and 15N fertiliser in a sandy soil. *Soil Research,* online early publication 17 November 2020 DOI: https://doi.org/10.1071/SR20158.

Principal Author

Name of Principal Author (Candidate)	Maria del Pilar Muschietti Piana
Contribution to the Paper	Performed the experiment and the analysis of samples, interpreted data, wrote the manuscript, illustrated all figures and tables, and act as the corresponding author.
Overall percentage (%)	80%
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.
Signature	Date 01/09/20

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

    i.   the candidate's stated contribution to the publication is accurate (as detailed above);

    ii.   permission is granted for the candidate in include the publication in the thesis; and

    iii.   the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of Co-Author	Therese McBeath
Contribution to the Paper	Provided advice on experimental design and analysis. Provided edits on several versions of the manuscript including contributing to revisions arising from the journal submission process.
Signature	Date 01/09/20

Name of Co-Author	Ann M. McNeill
Contribution to the Paper	Provided advice on experimental design, analysis and during manuscript preparation and review
Signature	Date 04/09/20

Name of Co-Author	Pablo Ariel Cipriotti
Contribution to the Paper	Provided advice on experimental design, statistical analysis and manuscript editing during preparation and review.
Signature		Date	02/09/2020

Name of Co-Author	Vadakattu V. S. R. Gupta
Contribution to the Paper	Provided advice on experimental design, analysis and during manuscript preparation and review.
Signature		Date	02/09/2020

Please cut and paste additional co-author panels here as required.

Corrigendum

Early growing season immobilisation affects post-tillering wheat nitrogen uptake from crop stubble and ^{15}N fertiliser in a sandy soil

Pilar Muschietti Piana, Therese Marie McBeath, Ann Marie McNeill,
Pablo Ariel Cipriotti, and Vadakattu Gupta

The authors regret that Table 1 contained an incorrect value in the Stubble C column, for Lupin pods + leaves; the value 9107 should have been 911. The corrected table is shown below.

Table 1.   Stubble C and N content in each component used to amend the lupin and wheat stubble treatments

Stubble treatment	Dry weight (g)	Stubble (%)	Stubble load (g pot^{-1})	Stubble C (mg pot^{-1})	Stubble N (mg pot^{-1})	Stubble C:N
Wheat straw	267	72	6	2698	19	145
Wheat chaff + leaves	104	28	2	1063	23	46
Lupin branches	385	80	6	1087	14	79
Lupin pods + leaves	99	20	1	911	28	32
Total wheat stubble	371	100	8	3761	42	90
Total lupin stubble	484	100	7	1997	42	47

Early growing season immobilisation affects post-tillering wheat nitrogen uptake from crop stubble and ^{15}N fertiliser in a sandy soil

Pilar Muschietti Piana [iD][A,B,D]*, Therese Marie McBeath* [iD][B]*, Ann Marie McNeill* [iD][A]*,*
Pablo Ariel Cipriotti [iD][C]*, and Vadakattu Gupta* [iD][B]

[A]School of Agriculture, Food and Wine, The University of Adelaide, SA 5005, Australia.
[B]Commonwealth Scientific and Industrial Research Organisation (CSIRO) Agriculture and Food,
  Urrbrae, SA 5064, Australia.
[C]School of Agriculture-Instituto de Investigaciones Fisiológicas y Ecológicas Vinculadas a la Agricultura (IFEVA),
  University of Buenos Aires/Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET),
  Av. San Martin 4453, Ciudad Autónoma de Buenos Aires, C1417DSE, Argentina.
[D]Corresponding author. Email: muschiet@agro.uba.ar

Abstract.   In semiarid sandy soil environments there is a dual challenge of carbon and nitrogen (N) limitation that needs to be managed to ensure timely supply of N to crops. Management of N inputs to soil using combinations of legume stubble addition and fertiliser N in cereal systems is essential to meet crop demand and maintain N in soil organic matter. The aim of this study was to assess soil mineral and biological N pools that influence N supply and N uptake of wheat at early growth stages. The recovery of ^{15}N-labelled fertiliser by wheat was evaluated using a factorial combination of either wheat, lupin or no stubble incorporated with or without ^{15}N fertiliser in a sandy soil system. Soil and plant samples were collected at sowing, tillering, first node and booting to monitor changes in N pools and ^{15}N uptake by the wheat. Crop stubble incorporation one week before sowing increased biological N pools in the surface soil (0–10 cm). Early N immobilisation (sowing–tillering) in all the treatments without ^{15}N fertiliser may have limited N availability for wheat uptake in the subsequent period (tillering–first node), when fertiliser N appeared critical to maximise N supply for plant requirements. Up to 38% of the ^{15}N fertiliser applied at sowing was incorporated into the soil microbial biomass pool, so that fertiliser N was critical to relieve short-term inherent N limitations for both plant and microbial growth, and to supply the longer-term biological pools (microbial biomass) to support subsequent mineralisation potential. Reducing the energy limitation to the microbial pool through inputs of carbon from stubble was also critical to ensure fertiliser N supplied sufficient N to satisfy plant demand later in the growing period. These results have implications for management decisions on semiarid sandy soil systems that aim to synchronise N from inputs of legume stubbles and fertiliser with crop N demand during early growth stages of wheat.

Keywords: growth stages, Kandosol, microbial biomass, mineralisation–immobilisation, nitrogen recovery efficiency, semiarid environments.

Received 4 June 2020, accepted 25 September 2020, published online 17 November 2020

Introduction

Globally, maintaining supply of nitrogen (N) to low-input rain-fed cereal crops is becoming increasingly challenging due to a decline in soil organic N reserves, particularly in semiarid agroecosystems where yield and response to N is often limited by water (Luo *et al*. 2010; Ghimire *et al*. 2018). Cereal systems in the Australian low-rainfall zone are characterised by low-fertility soils with low N-supply capacity, especially on sandy soils (Gupta *et al*. 2011). The N-supply potential of a soil is a product of soil biological capacity (characterised by the presence and ability of microorganisms to perform N cycling processes), the size of organic N pools and the rate

of microbial turnover. The rate and timing of N mineralisation, including the processes associated with microbial turnover, regulate plant-available mineral N content in soils (Kirkegaard *et al*. 2018; Gupta *et al*. 2019). Nitrogen mineralised from existing or 'native' soil organic matter makes a substantial contribution (~50%) to crop N uptake (Gupta 2016; Angus and Grace 2017) and, particularly in low-input cereal systems, soil mineral N produced by microbial mineralisation of N in recent crop stubble inputs plus native soil organic N are the main source (~70%) of mineral N for crop N uptake (Angus 2001).
   Microbial activity drives the mineralisation of soil organic N formed from crop stubble, utilises N (immobilisation) and

converts some forms of fertiliser N (e.g. urea) into plant-available forms. Therefore, a better understanding of the processes controlling immobilisation and mineralisation of the various N inputs is vital to improve N use efficiency by crops. Indeed, more specific information on the contribution of soil biology to N processes including the microbial dynamics that affect net mineralisation rates and soil organic matter turnover has been identified as critical to develop successful agronomic strategies (or decisions) to better synchronise N inputs with crop N demand (Roberts *et al*. 2015).

Legume stubbles and root residues constitute a significant source of N that can help to prevent nutrient depletion in soils under continuous cereal production systems by sustaining soil organic matter, enhancing biological activity and increasing nutrient availability (Abera *et al*. 2012). In addition to legume stubbles, N supplied by manure and fertiliser can maintain or improve soil N balances, which are essential to maintaining soil organic matter in conservation cropping systems (Abera *et al*. 2012; Smith *et al*. 2019). Research that has focused on the response of soil microbial biomass (MB) to fertiliser N application has shown variable results, generally associated with crop management and environmental factors (Geisseler and Scow 2014). The rates of stubble decomposition can vary between different crops due to differences in their chemical composition such as the stubble N concentration, soluble organic carbon (C) and N, and C : N ratio, but also due to differences in other properties such as cellulose, lignin, and polyphenol concentrations (Adl 2003). The extent that nutrient addition to soil or supply from soil can decrease, increase, or have no effect on microbial activity and consequently on soil organic matter dynamics is also affected by stoichiometry of those nutrients (Coonan *et al*. 2020). The stoichiometry of nutrient supply, such as C : N of the added residues or fertilisers, also regulates the production of microbial enzymes. This is then reflected in the magnitude of the mineralisation of pre-existing soil organic matter by microorganisms to access the amount of C and N necessary for growth, i.e. 'mine' soil organic matter for additional N when C supply is in excess of N supply (Kallenbach *et al*. 2019). A recent study reported that stoichiometrically balanced nutrient supply in agroecosystems can significantly reduce the mineralisation of pre-existing soil organic matter by 24–50% (Coonan *et al*. 2020).

Field data for Australian soils are very limited and either suggest a lack of response by soil MB to fertiliser N application or a decrease in the size of the MB associated with faster turnover of decomposer organisms in the presence of added N (Ladd *et al*. 1994; Bünemann *et al*. 2006). In contrast, an extensive review by Geisseler and Scow (2014) on responses of soil microorganisms to N-fertiliser application in long-term annual cropping experiments reported that in soils with pH > 5, MB increased on average by 15% with fertiliser N compared with unfertilised treatments. Fertiliser N, possibly via a positive influence on crop dry matter accumulation, also increased soil organic C, contributing to the overall increase in MBC. Research in soils of the low-rainfall Mallee environment of South Australia reported that the lower yield potential in sandy soils was conditioned by lower N fertility than in the adjacent clayey soil within the same field. Wheat yield response to fertiliser N application (up

to 80 kg N ha^{-1}) in the sandy soil was greater than in the clayey soil both in wet and dry season conditions (McBeath *et al*. 2019). Further benefits to wheat yield in the low-rainfall sandy soil environments of south-eastern Australia have been in response to increased N inputs from legume crop stubbles or fertiliser N (McBeath *et al*. 2015). Field studies in wheat crops grown on a calcareous sandy dune of South Australia using ^{15}N-labelled medic (*Medicago littoralis*) residues found that residues incorporated in the fallow contributed 15% of N uptake in a following wheat crop by maturity (Ladd *et al*. 1981). However, in all of these studies the contribution to wheat yield from N facilitated by biological pools or the N dynamics during early growth stages was not investigated. Recent research (Muschietti Piana *et al*. 2020) in similar field environments has measured greater surface soil dissolved organic N (DON), potentially mineralisable N (PMN) and microbial biomass N (MBN) during wheat growth where lupin (*Lupinus angustifolius*) stubble was retained compared with where wheat stubble was either removed or retained. Wheat N uptake was also shown to increase 0.5 kg ha^{-1} with every additional 1 kg ha^{-1} of fertiliser N applied, but the contribution of each source (fertiliser vs stubble) to supply N for wheat uptake was not assessed. A knowledge gap remains for these low-rainfall Australian environments concerning the relative importance of organic and inorganic N sources in the short-term N dynamics during early growth stages of wheat, and how these relate to plant N uptake in the same period. Nitrogen supply at early stages is vital for defining yield potential, by ensuring better crop establishment, tiller production and formation, therefore optimising wheat growth for grain yield and protein content (Poole and Hunt 2014). The aim of this study was to assess soil mineral N and biological N pools that influence N supply relative to wheat N uptake at early stages of wheat in a sandy soil system where organic and inorganic N supply was manipulated and N dynamics monitored using ^{15}N fertiliser.

Materials and methods

Experimental design and treatments

Wheat plants (*Triticum aestivum* L. cv. Scepter) were grown in cylindrical pots (15 cm diameter × 20 cm height, 3.5 L volume) with 4594 g of dry soil and sealed at the base with a PVC cap to prevent losses of N through leaching. The pots were well insulated and placed in a glasshouse under controlled conditions as outlined in the glasshouse conditions section. The experiment had a factorial design consisting of three stubble types, two N-fertiliser treatments, three sampling times (i.e. 'growth stages') and four replicates, i.e. 3 × 2 × 3 × 4 = 72 pots. A sandy soil classified as a Kandosol using the Australian Soil Classification (Isbell and NCST 2016) and a Durisol according to the World Reference Base for Soil Resources (IUSS Working Group WRB 2006) typical of a large area of the semiarid Mallee regions in south-eastern Australia, were treated with three different crop stubble treatments – wheat, lupin, and no-stubble – and two different fertiliser N treatments: 'unfertilised' (–N) treatment with no fertiliser N applied throughout the experiment vs 'fertilised' (+N)

treatment with ^{15}N fertiliser applied at wheat sowing time (making a total of six treatments). Plant and soil samples were collected at three different wheat growth stages according to Zadoks *et al.* (1974): tillering (Z2.2), first node (Z3.1) and booting (Z4.9) by destructive sampling (pot removal). An error occurred during the experimental set up for the wheat stubble combined with fertilised N treatment at booting ('wheat+N' treatment) where the ^{15}N labelled fertiliser was not applied. This resulted in the experiment having an incomplete design with six treatments between tillering and first node stages, but five treatments at the booting stage. As a result, analysis of plant and soil variables over time was performed separately according to the growth stages for the six treatments between tillering and first node, and for the five treatments at booting as described in the statistical analysis section.

Soil and crop stubble collection and characterisation

Soils for the experiment were collected from a study site previously characterised (McBeath *et al.* 2015) as a Kandosol soil on the dune (Isbell and NCST 2016) in a field at Lowaldie (35.0535°S, 139.9874°E), 20 km north-east of Karoonda, South Australia. All soils were collected from experimental plots on a sandy soil with either lupin or wheat stubble in the previous cropping season. Soils from the lupin–wheat plots were assigned to the lupin stubble treatments, whereas soils from the wheat–wheat plots were assigned to the wheat and no-stubble treatments. In the field, all soils were collected in two separate layers (0–10 and 10–20 cm) and transported to the laboratory. In the laboratory, each soil was air-dried for 72 h, sieved (<2 mm), homogenised and stored at 4°C before chemical analysis (Table S1, available as Supplementary material) in the laboratory including pH and electrical conductivity in a 1:5 soil water suspension (Rayment and Lyons 2011), cation exchange capacity (Rayment and Lyons 2011), extractable phosphorus (Colwell 1963), potassium and sulfur (KCl-40, Rayment and Lyons 2011) and total C and N by dry combustion using Leco TruMAC analyser (2000-CNS, Leco Australia Pty Ltd, Castle Hills, NSW, Australia). Soil water content at field capacity was measured in the laboratory with a suction plate as the soil water content at –10 kPa (Klute 1986).

Wheat (cv. Scepter) and lupin (*Lupinus angustifolius* cv. Mandelup) aboveground residues (hereinafter referred to as stubble) were collected on 6 September 2017 from a field

experiment in a paddock in a semidecomposed state. Stubbles were separated into components (e.g. straw, leaves and spikes) to determine the proportion of each component as in field conditions (Table 1). In order to determine the initial quality of wheat and lupin stubble, a representative subsample of each stubble was dried at 60°C during 48 h, mixed and then ground (<0.5 mm) before analysis in the laboratory. Each stubble was characterised for chemical composition and analysed for C and N content by high-temperature combustion in an atmosphere of oxygen using a Leco TruMAC analyser (2000-CNS, Leco Australia Pty Ltd, Castle Hills, NSW, Australia). Overall wheat stubble, particularly the straw fraction, had higher C content than lupin stubble. Lupin stubble had a higher N content, especially in the pod and leaf fractions, even though these represented only 20% of the total lupin biomass (Table 1).

Soil pre-incubation and stubble treatment set up

In each pot, air-dried sandy soil (either from the lupin or the wheat sequence) was added one layer at a time (in order 10–20 and 0–10 cm) and packed (2297 g of dry soil in each layer) to achieve a bulk density of 1.3 g cm^{-3}. Due to the water repellent characteristic of the sandy soils under study, all pots were wet to field capacity 12% (w w^{-1} gravimetric water content) during pre-incubation. Two nutrient solutions were applied to all pots to ensure no nutrients other than N were limiting wheat growth. Solution I contained 65 mg K kg^{-1} dry soil, 16 mg S kg^{-1} dry soil and 20 mg P kg^{-1} dry soil as K_2SO_4 and KH_2PO_4; and solution II had 18 mg Cu kg^{-1} dry soil as $CuSO_4 \cdot 5H_2O$, 18 mg Mn kg^{-1} dry soil as $MnCl_2 \cdot 4H_2O$ and 22.5 mg Zn kg^{-1} dry soil as $ZnCl_2$.

All pots were covered with thin plastic film to prevent evaporation and were left to stabilise for 36 h before addition of the stubble treatments (lupin, wheat and no-stubble). Each stubble was cut into 0.5-cm pieces and mixed manually with the soil in the top 5 cm of each pot to simulate disturbance experienced in the field at sowing. Similarly, the control treatment with no stubble was mixed in the top 5 cm layer. Wheat stubble was added at a rate of 8 g (ash-free weight) per pot (equivalent to a stubble load of 5 t ha^{-1}) and legume residue was added at 7 g (ash-free weight) per pot (equivalent to a stubble load of 4 t ha^{-1}) based on in-field stubble load of previous studies in the same field and soil type (Gupta 2016; Muschietti Piana *et al.* 2016).

Table 1.   Stubble C and N content in each component used to amend the lupin and wheat stubble treatments

Stubble treatment	Dry weight (g)	Stubble (%)	Stubble load (g pot^{-1})	Stubble C (mg pot^{-1})	Stubble N (mg pot^{-1})	Stubble C:N
Wheat straw	267	72	6	2698	19	145
Wheat chaff + leaves	104	28	2	1063	23	46
Lupin branches	385	80	6	1087	14	79
Lupin pods + leaves	99	20	1	9107	28	32
Total wheat stubble	371	100	8	3761	42	90
Total lupin stubble	484	100	7	1997	42	47

Sowing, watering regime and N-fertiliser treatments

Six pregerminated seeds of wheat (cv. Scepter) were sown in two rows in each pot and thinned to two plants per pot at Z1.2 (Zadoks *et al.* 1974). Nitrogen fertiliser was added to half of the pots the day after sowing by applying 5 mL of ^{15}N-labelled urea (20 atom % [15]N excess) to provide a rate of 70 mg of N pot[-1] (~equivalent to 40 kg N ha^{-1} on a surface area basis), hereafter labelled '+N' treatment. The other pots did not receive any fertiliser N throughout the experiment, hereafter labelled '−N' treatment.

Pots were maintained at 67% field capacity (8% w w[-1]) during plant growth throughout the experiment. The weight of each pot was recorded every two days and deionised water added to replace that lost by evapotranspiration. This watering frequency did not allow for soil to rapidly or substantially dry out or reach wilting point at any time. Soil gravimetric water content in each layer was measured from the difference in weight between the samples as collected and when oven-dried at 105°C to constant weight. Soil gravimetric water remained similar for all treatments at different growth stages with a mean value of 9 ± 2% w w[-1] in the 0–10 cm layer and 8 ± 2% w w[-1] in the 10–20 cm layer (Fig. S1), well above the crop lower limit of 1% w w[-1].

Glasshouse conditions

Plants were grown in a naturally lit glasshouse in the southern hemisphere during spring (22 September to 30 October) under ambient temperature and relative humidity conditions. Pots were randomised weekly to reduce any sustained location of specific effects on individual pots from inherent lighting and temperature variations. Air temperature ranged within 18–28°C for the duration of the experiment. Cumulative thermal time or degree-days (°C d) was used to identify the duration of each growth stage and was calculated as the sum of the average daily temperature as the following Eqn 1:

$$Degree - days(°Cd) = [(Tmax + Tmin)/2] - Tbase \quad (1)$$

where $Tmax$ is maximum temperature (°C), $Tmin$ is minimum temperature (°C) and $Tbase$ is base temperature assumed as 0°C.

Average daily temperature was 23°C d at sowing, 21°C d at tillering, 22°C d at first node and 20°C d at booting, with a cumulative thermal time of 834°C d from sowing to booting. Relative humidity ranged within 35–81%.

Pre-sowing soil sampling and analyses for N in soil mineral and biological pools

Prior to sowing wheat and one week after initiation of the stubble treatments, 12 pots (three stubble treatments × four replicates) were retrieved from the glasshouse to determine the soil chemical properties (Table S2). Soil samples were removed from each pot, sectioned into 0–10 and 10–20 cm depth and stored in sealed plastic bags at 4°C before extraction and analysis in the laboratory (within 2–7 days). All samples were homogenised, mixed, subsampled and analysed for soil gravimetric water (%, w w[-1]) and mineral N in both layers; surface soil (only in 0–10 cm layer) for PMN, MBN and DON (Table 2). Soil gravimetric water content in each layer was measured as indicated above.

Table 2.   Soil C and N characterisation in each layer (0–10 and 10–20 cm) before wheat sowing and one week after the addition of three stubble treatments (wheat, lupin, and no-stubble)

Mean value of four replicates with standard deviation in parentheses. Values within the same row followed by different letters indicate significant differences between stubble treatments (DGC test, $P < 0.05$). Mineral N, nitrate N + ammonium N; MBN, microbial biomass N; DON, dissolved organic N; PMN, potentially mineralisable N. Values are highlighted in bold to improve visualisation of the significant differences among treatment means

Stubble treatment	No-stubble	Wheat	Lupin
Soil layer 0–10 cm			
Organic C (mg pot[-1])	17 255 (980) a	16 900 (880) a	16 040 (881) a
Organic N (mg pot[-1])	1337 (110) a	1365 (103) a	1297 (100) a
C : N ratio	13 (1) a	13 (1) a	13 (1) a
Nitrate N (mg pot[-1])	**14 (1) a**	7 (1) b	**12 (1) a**
Ammonium N (mg pot[-1])	8 (2) a	8 (5) a	6 (2) a
Mineral N (mg pot[-1])	22 (2) a	15 (6) a	18 (2) a
MBN (mg pot[-1])	14 (1) b	**25 (1) a**	**26 (6) a**
DON (mg pot[-1])	2 (1) b	**5 (1) a**	**4 (1) a**
PMN (mg pot[-1])	37 (8) b	55 (7) b	**75 (4) a**
Gravimetric water % (w w[-1])	10 (1) a	12 (1) a	13 (1) a
Soil layer 10–20 cm			
Organic C (mg pot[-1])	10 851 (885) a	9732 (790) a	9434 (780) a
Organic N (mg pot[-1])	805 (145) a	722 (100) a	785 (100) a
C : N ratio	14 (1) a	14 (1) a	12 (1) a
Nitrate N (mg pot[-1])	22 (4) a	20 (5) a	18 (1) a
Ammonium N (mg pot[-1])	1 (2) a	1 (1) a	1 (1) a
Mineral N (mg pot[-1])	24 (4) a	21 (4) a	20 (1) a
Gravimetric water % (w w[-1])	10 (1) a	10 (1) a	11 (1) a
Soil layer 0–20 cm			
Ammonium + nitrate (mg pot[-1])	45 (2) a	36 (4) a	38 (6) a

Soils were extracted by shaking with 2 M KCl (1 : 3 soil : solution ratio) on an orbital shaker for 1 h at 25°C and filtered to <11 μm (Whatman No. 1). Soil nitrate N and ammonium N were analysed colourimetrically on the extracts (Miranda *et al.* 2001) using a SpectraMax® M4 microplate reader.

Surface soil PMN was determined by a short-term (7 days) anaerobic incubation at 40°C (Keeney and Bremner 1966). For all samples, 5 g of dry weight equivalent of soil was mixed with 15 mL of water (1 : 3 soil : water ratio), centrifuged at 1252 *g* for 15 min, decanted and filtered (Whatman No. 1) at time zero and after 7 days of incubation. Ammonium N was analysed colourimetrically on the water extracts (Miranda *et al.* 2001) using a SpectraMax® M4 microplate reader.

Surface soil MBN was assessed using chloroform fumigation and extraction techniques (Jenkinson 1977; Brookes *et al.* 1985). Soil samples equivalent to 20 g of dry weight were fumigated with ethanol-free chloroform and stored at 25°C in a dark room for 7 days (Sparling and Zhu 1993). Both fumigated and paired nonfumigated soil samples were extracted by shaking with 2 M KCl (1 : 3 soil : solution ratio) on an orbital shaker for 1 h at 25°C and filtered (Whatman No. 1). Soil MBN was estimated from the flush of ninhydrin-positive compounds between the extracts of the fumigated and nonfumigated soil (Amato and Ladd 1988). A conversion factor (*c*) of the ninhydrin-positive compounds flux was used to derive the MBN (*c* = 3.715, Sparling and Zhu 1993). The KCl extracts of the unfumigated samples were also analysed for nitrate N and ammonium N (Miranda *et al.* 2001).

Surface soil DON was measured in the water extracts (10 g oven-dried equivalent) shaken in 30 mL of distilled water after centrifuging at 1252 *g* for 15 min and filtering (Whatman No. 42). These water extracts were analysed for nitrate N and ammonium N concentration colourimetrically (Miranda *et al.* 2001) using a SynergyMX® microplate reader. Total dissolved N was determined in the water extracts using a Thermalox® dry combustion analyser (Analytical Sciences Ltd, Cambridge, UK). The concentration of DON was determined by subtraction of nitrate N and ammonium N from the total dissolved N value (Jones and Willett 2006). Soil mineral N, MBN, DON and PMN were expressed in mg kg^{-1} of dry soil and then converted to mg N pot^{-1}.

Plant and soil sampling at different wheat growth stages

Plants and soils were sampled at three different wheat growth stages (Zadoks *et al.* 1974): tillering (Z2.2), first node (Z3.1), and booting (Z4.9). At each sampling time, 24 pots (six treatments × four replicates) were retrieved from the glasshouse for soil and plant analysis in the laboratory (Table 2, Table S2). In each pot, wheat aerial biomass was cut, and placed in paper bags, and soils were sectioned into 0–10 and 10–20 cm depth and sampled with a scoop in each layer. Wheat root material in each soil depth was placed in a separate tray to recover all the roots (free from soil) using tweezers, gently rinsed using a wash bottle with reverse-osmosis water and placed in paper bags. Any loss of ^{15}N during root washing was highly likely to be an extremely small amount relative to the amount of ^{15}N incorporated within the roots. A majority of the N in the roots would be insoluble N

forms incorporated into cellular structures (Hertenberger and Wanek 2004). All soil samples were homogenised, mixed, subsampled and analysed for soil gravimetric water and mineral N in both layers, and for surface soil MBN (0–10 cm) as indicated above. For each treatment, plant material was dried via lyophilisation for at least 48 h to determine aerial and root biomass on a dry matter basis. These were ground to a uniform fine-grained (talc-like) texture using a Spex ball-mill to less than 250-μm particle size before determining ^{15}N and ^{14}N content in each plant fraction using an isotope ratio mass spectrometer (20–22 IRMS, Sercon Ltd, Crewe, UK) coupled to a Sercon GSL prep unit. Samples were analysed against known plant-based secondary standards and a gradient of secondary standards containing different amounts of N. These standards were calibrated against IAEA N1 ammonium sulfate primary standards.

Nitrogen uptake, N derived from fertiliser and ^{15}N fertiliser recovery by wheat

For all treatments, N uptake (mg N pot^{-1}) in wheat shoots and roots at each sampling time (Table 2) was calculated from the percentage of total N in each plant fraction multiplied by the dry biomass weight (mg). The proportion of the N derived from the fertiliser (%Ndff) for shoots and roots was determined by dividing the atom% ^{15}N excess value of each plant fraction by the atom% ^{15}N excess of the fertiliser and multiplying by 100. The quantity of ^{15}N derived from fertiliser (QNdff, mg ^{15}N pot^{-1}) in each plant fraction was determined as the product of %Ndff and total N of the dry biomass for that plant fraction. The proportion of the ^{15}N-labelled fertiliser recovered in the two plants per pot (shoots plus roots) was defined as '^{15}N recovery efficiency' and calculated using the equation by Hauck and Bremner (1976):

$$^{15}\text{N recovery efficiency}(\%) = [\text{QNdff}(\text{shoots} + \text{roots})/ \tag{2}$$
$$^{15}\text{N fertiliser rate}] \times 100$$

where ^{15}N fertiliser rate equals 70 mg N pot^{-1} and QNdff is the quantity of plant N derived from the ^{15}N − labelled fertiliser.

Estimated net N mineralisation

The net N mineralisation (mg pot^{-1}) between any two sampling times (t_1 and t_2) was estimated according to the following equation:

$$M(t_2 - t_1) = Ht_2 - St_1 + U(t_2 - t_1) - \text{QNdff} r(t_2 - t_1) \tag{3}$$

where *M* is the estimated net N mineralisation (mg pot^{-1}) for the period t_1 to t_2, *H* is the sum of soil mineral N to 20 cm depth (nitrate + ammonium) at t_2 (mg pot^{-1}), *S* is the sum of soil mineral N to 20 cm depth (nitrate + ammonium) at t_1 (mg pot^{-1}), *U* is whole plant N uptake (shoot + root, mg pot^{-1}) for the period t_1 to t_2 and *r* is the fertiliser N input (70 mg pot^{-1}).

Statistical analysis

One-way ANOVAs were used for the analysis of variables at sowing (e.g. DON) for all stubble treatments. Where results

showed a significant effect from global *F*-tests for the stubble treatment, the comparisons between treatment means were performed with the DGC (Di Rienzo, Guzman, Casanoves) *post-hoc* test (DGC test, α = 0.05, Rienzo *et al.* 2002).

For the analysis of soil variables (e.g. soil mineral N) in each layer (0–10 and 10–20 cm), and wheat biomass and ^{15}N uptake (e.g. in shoots), the effect of the 'stubble treatment' combined with the 'fertiliser N' and 'growth stages' (tillering and first node stages), with 'growth stage' and their second- and third-order interactions as fixed effects. However, for the last two response variables (i.e. wheat biomass and N uptake) the heterogeneity of variance in relation to 'growth stage' was included in analysis since the data did not meet the assumption of homoscedasticity of variance. This analysis was conducted with the Infostat v.7 in interface with R with the nlme function to produce linear mixed effects models (R Core Team 2018).

One-way ANOVAs were used for the analysis of plant and soil variables at the booting stage (where there was a missing treatment) and, where results showed a significant effect of the treatment, the comparison between treatment means was performed with DGC test (α = 0.05, Rienzo *et al.* 2002). When there was only one factor to compare treatment differences, paired *t*-tests were (α = 0.05) performed with Infostat ver.7 software, e.g. at the booting stage. Paired *t*-tests were performed between lupin and no-stubble treatments on the following variables: shoot and root Ndff (%), shoot and root QNdff (mg ^{15}N pot^{-1}) and fertiliser ^{15}N recovery efficiency (%).

Results

Soil N pools at wheat sowing time in stubble treatments

At the time of sowing, all stubble treatments recorded similar soil organic C and total N, mineral N and gravimetric water content in both layers, except for nitrate N (0–10 cm) in the wheat stubble treatment which was 6 ± 1 mg pot^{-1} lower than in the other treatments (Table 2). Surface soil MBN and DON were higher by 11 ± 1 (+40%) and 2 ± 1 (+50%) mg pot^{-1} with wheat and lupin stubble respectively than with no stubble incorporation. Surface soil PMN in the lupin stubble treatment was 29 ± 9 mg pot^{-1} (+62%) higher on average than in the other treatments.

Soil mineral and MBN between stubble and fertiliser N treatments at different growth stages

There were limited treatment effects on soil mineral N throughout the experiment (Table S3). Soil mineral N at tillering was an average of 16 ± 5 mg N pot^{-1} in all treatments (24 mg N pot^{-1} less than at sowing time), and by booting it averaged 11 ± 1 mg N pot^{-1} (29 mg N pot^{-1} less than at sowing time). Regardless of the stubble treatment, adding fertiliser N resulted in higher soil mineral N at tillering. Although soil nitrate N in the 0–10 cm layer was 6 ± 1 mg pot^{-1} higher in the no-stubble+N treatment than in the other two treatments at tillering, it decreased by sampling at the first node stage to the same concentration as the other treatments and thereafter remained unchanged to booting with a mean value of 5 ± 1 mg N pot^{-1} in each layer (Table S3).

At tillering, surface soil MBN was affected by the combination of stubble treatment and fertiliser N (Fig. 1), and also individually by either the fertiliser N or the stubble treatment. Surface soil MBN was highest in all +N treatments (41 ± 4 mg pot^{-1} on average), regardless of stubble treatment. In the absence of fertiliser N, surface soil MBN in both lupin and wheat stubble treatments was higher than when no stubble had been incorporated. At booting, surface soil MBN was higher in the lupin stubble+N and no-stubble+N with 22 ± 2 mg pot^{-1} more on average than in the other treatments (Fig. 1).

Wheat plant biomass and N uptake at different growth stages

Whole wheat plant (shoot + root) biomass increased in all treatments between tillering and first node (Fig. 2) as did shoot and root biomass individually (data not shown). At first node, all stubble treatments with +N had a higher whole plant biomass than stubble treatments with –N, and the no-stubble+N had the highest whole plant biomass of all
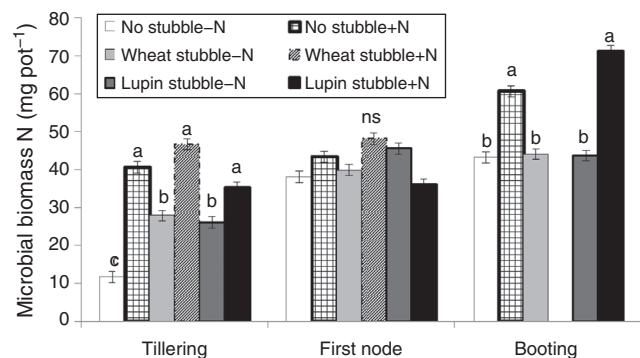
Fig. 1. Microbial biomass N (mg pot^{-1}) for the 0–10 cm soil depth for the stubble and N-fertiliser treatments at different growth stages. Within tillering and first node, different letters indicate significant differences for the interaction of 'stubble treatment' and 'fertiliser N' (DGC test, $P < 0.05$). At booting, different letters indicate significant differences between treatments (DGC test, $P < 0.05$).
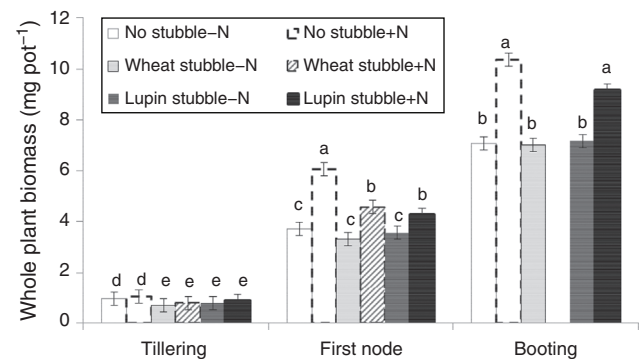
Fig. 2. Whole wheat plant (shoot + root) biomass for the treatments at different growth stages. Between tillering and first node, different letters indicate significant differences for the interaction of 'stubble treatment', 'fertiliser N' and 'growth stage' (DGC test, $P < 0.05$). At booting, different letters indicate significant differences between treatments (DGC test, $P < 0.05$).

treatments (Fig. 2). At both the tillering and first node stages, shoot biomass was higher for +N than –N treatment, regardless of stubble treatment (data not shown). Root biomass was highest in the +N treatment at first node (Table S3). At booting, in the lupin stubble+N and no-stubble+N treatments, shoot biomass (data not shown) was on average $49 \pm 1\%$ higher and whole plant (shoot + root) biomass was $38 \pm 1\%$ higher than in the other treatments (Fig. 2).

Regardless of stubble treatment, plant N uptake was always higher in the +N than –N treatments, this difference was $22 \pm 1\%$ higher at tillering and $44 \pm 1\%$ at first node (Fig. 3b). Shoot N uptake was $26 \pm 3\%$ higher on average in +N than –N treatments at tillering (data not shown) and the magnitude of this difference was $41 \pm 2\%$ at first node (data not shown). Root N uptake was 4 ± 3 mg N pot^{-1} higher ($42 \pm 3\%$) in +N than –N treatments at first node (data not shown). At booting, plant N uptake was highest in the lupin stubble+N and no-stubble+N treatments, and the magnitude of the difference was $35 \pm 1\%$ higher on average than the other treatments (Fig. 3a).

Nitrogen derived from fertiliser and ^{15}N recovery efficiency by wheat

Fertiliser contributed $24 \pm 1\%$ of the N in the shoots of wheat across all treatments and growth stages (Fig. 4a). Wheat root

Fig. 3. (*a*) Whole wheat plant (shoot + root) N uptake for the treatments at different growth stages. Between tillering and first node, differences between the interaction of 'stubble treatment', 'fertiliser N' and 'growth stage' were not significant (ns, DGC test). At booting, different letters indicate significant differences between trea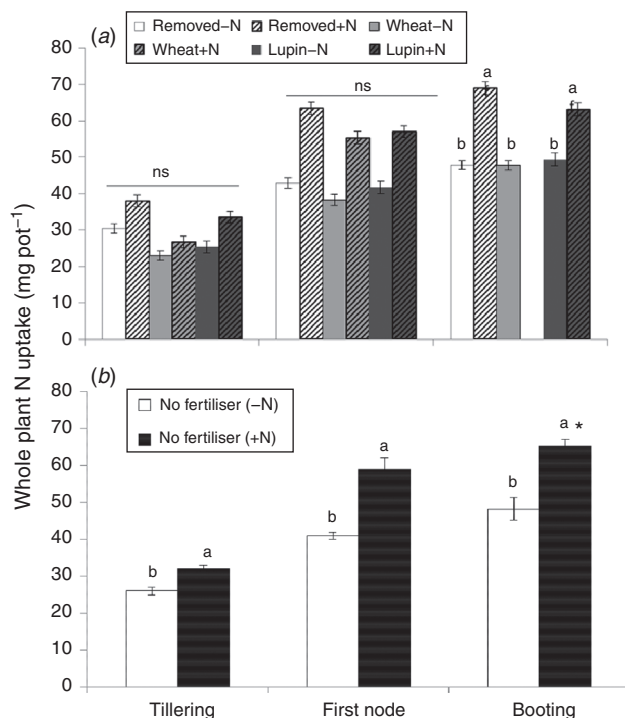tments (DGC test, $P < 0.05$). (*b*) Whole wheat plant (shoot + root) N uptake for the fertilised (+N) vs unfertilised (–N) treatments at different growth stages, where different letters indicate significant differences between 'fertiliser N' treatments (DGC test, $P < 0.05$). *Mean value of eight replicates (missing treatment).

Ndff was also higher in the no-stubble than in the stubble incorporated treatments at both growth stages (Fig. 4b). The quantity of N derived from the ^{15}N fertiliser (QNdff, mg ^{15}N pot^{-1}) in plant shoots was highest in the no-stubble treatment at first node, $6 \pm 2\%$ higher on average than when stubbles were incorporated (Fig. 4c). Across tillering and first node, the ^{15}N fertiliser recovery efficiency ranged within 4–25% with mean of $12 \pm 6\%$, and was lowest at tillering in all treatments ($6 \pm 2\%$) and highest ($21 \pm 2\%$) at first node in the no-stubble treatment (Fig. 4e).

Shoot Ndff was $18 \pm 11\%$ on average at booting in the no-stubble treatment and was similar to the lupin stubble treatment, which was $14 \pm 1\%$ on average (Fig. 5a). Root Ndff was higher in the no-stubble than the lupin stubble treatment ($19 \pm 1\%$ vs $10 \pm 1\%$). Root QNdff (Fig. 5b) was higher in the no-stubble than in the lupin stubble treatment (4 ± 1 vs 2 ± 1 mg ^{15}N pot^{-1}). There were however no significant differences in the ^{15}N fertiliser recovery efficiency by wheat plants at booting between lupin and no-stubble treatments, with a mean of $14 \pm 2\%$ (data not shown).

Dynamics between growth stages for estimated net N mineralisation, soil mineral N and MBN pools, wheat plant N uptake and quantity of plant N derived from fertiliser

The depletion in soil mineral N over the whole experiment between sowing to booting was less for the wheat stubble–N, lupin stubble–N and lupin stubble+N treatments than for the no-stubble treatment with or without fertiliser N. Whole plant N uptake and the increase in surface soil MBN for this same period were greater for the no-stubble+N and lupin stubble+N treatments than the other treatments (Table 3).

For the period between sowing and tillering, the increase in surface soil MBN was greater in the no-stubble+N and wheat stubble+N treatments, and changed little for all the other treatments. Where fertiliser N was applied (+N), there was an estimated net mineralisation of N between sowing and tillering across all treatments, whereas there was net N immobilisation in all –N treatments (Table 3). Between tillering to first node, the change in soil mineral N across the treatments was considerably less in magnitude (range –12 to +4 mg pot^{-1}) compared with the previous period (–17 to –32 mg pot^{-1}). There was net depletion in the +N treatments but a slight increase in the –N treatments, regardless of stubble treatment. Regardless of residue treatment, wheat N uptake for this period was 26 ± 2 mg pot^{-1} higher on average where fertiliser N had been applied than where it had not.

The QNdff for wheat in the no-stubble+N treatment for the period between tillering and first node was 2.3 times higher on average than in the other treatments, which had similar QNdff values to the previous sowing–tillering growth period. The increase in surface soil MBN was 19 ± 2 mg pot^{-1} greater on average for the no-stubble–N and lupin stubble–N than all other treatments. The +N treatments generally had much smaller increases in surface soil MBN during this period than the –N treatments. All treatments resulted in net N mineralisation, ranging within 13–22 mg pot^{-1} apart from the no-stubble+N treatment which was significantly lower (Table 3).
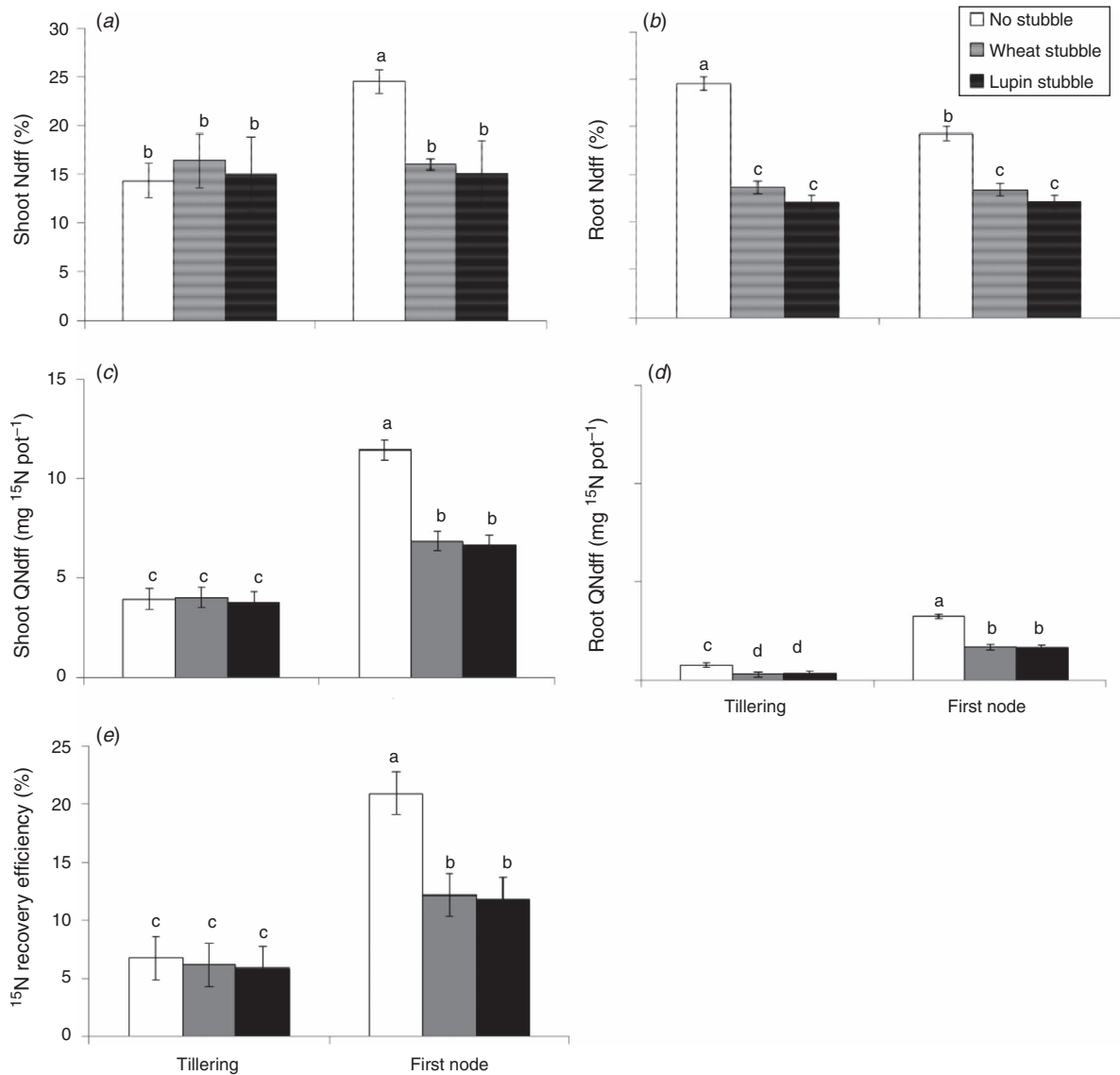
Fig. 4. (*a*) Shoot Ndff, (*b*) shoot QNdff, (*c*) root Ndff, (*d*) root QNdff and (*e*) ^{15}N fertiliser recovery efficiency by wheat plants for all stubble treatments across tillering and first node, where different letters indicate significant differences for the interaction of 'stubble treatment' and 'growth stage' (DGC test, $P < 0.05$). Ndff, ^{15}N derived from fertiliser; QNdff, quantity of N derived from fertiliser.
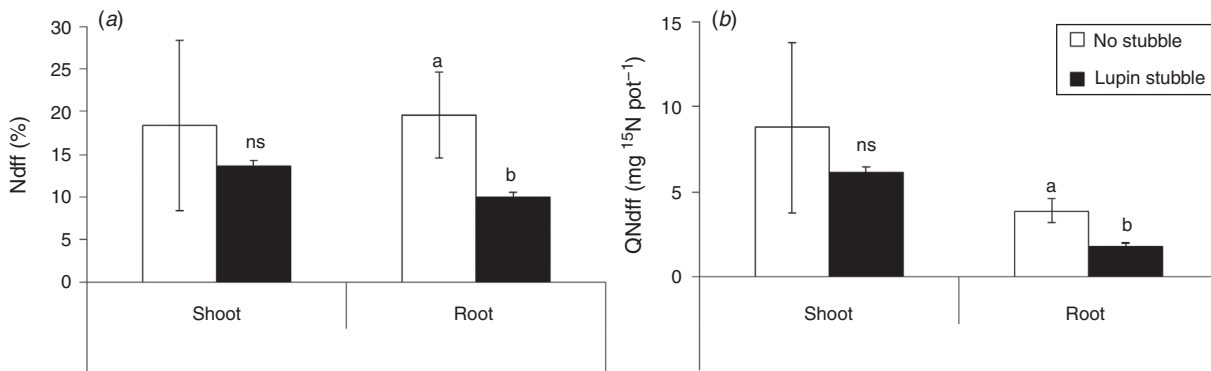
Fig. 5. Wheat plant shoot/root (*a*) Ndff and (*b*) QNdff for lupin and no-stubble treatments at booting, where different letters indicate significant differences between treatments (DGC test, $P < 0.05$, paired *t*-test). Ndff, ^{15}N derived from fertiliser; QNdff, quantity of N derived from fertiliser.

Table 3. Whole wheat plant N uptake (mg pot^{-1}), quantity of the plant N derived from fertiliser (QNdff), changes (Δ) in soil mineral N (0–20 cm) and microbial biomass N (0–10 cm, MBN), and estimated net N mineralisation between sampling times for all treatments

Mean value of four replicates with standard deviation in parenthesis. Different letters within the same column of each sampling time indicate significant differences between treatment means (DGC test, $P < 0.05$). Values are highlighted in bold to improve visualisation of the significant differences among treatment means

Treatments	Whole plant N uptake	QNdff (plant)	Δ Soil mineral N (0–20 cm)	Δ MBN (0–10 cm)	Estimated net N mineralisation/ immobilisation
			mg pot^{-1}		
		Sowing to booting			
No-stubble–N	48 (4) b		–34 (4) b	29 (4) b	14 (6) a
No-stubble+N	**67 (3) a**	13 (6) a	–34 (5) b	**46 (2) a**	20 (10) a
Wheat stubble–N	48 (4) b		**–25 (3) a**	19 (4) b	23 (2) a
Lupin stubble–N	49 (3) b		**–27 (2) a**	18 (6) b	23 (4) a
Lupin stubble+N	**64 (2) a**	8 (1) a	**–27 (2) a**	45 (3) a	28 (2) a
		Sowing to tillering			
No-stubble–N	30 (1) a		–32 (4) a	–3 (10) b	–1 (7) b
No-stubble+N	38 (3) a	5 (2) a	–19 (8) a	**26 (6) a**	**15 (3) a**
Wheat stubble–N	23 (2) a		–24 (2) a	2 (4) b	–1 (3) b
Wheat stubble+N	27 (4) a	4 (1) a	–17 (8) a	**21 (7) a**	**5 (5) a**
Lupin stubble–N	25 (1) a		–30 (1) a	0 (8) b	–4 (2) b
Lupin stubble+N	33 (2) a	4 (2) a	–22 (3) a	9 (2) b	**7 (24) a**
		Tillering to first node			
No-stubble–N	12 (3) b		1 (4) a	**26 (10) a**	13 (4) a
No-stubble+N	**27 (4) a**	10 (2) a	**–12 (3) b**	3 (5) b	**4 (4) b**
Wheat stubble–N	15 (3) b		2 (2) a	12 (2) b	17 (2) b
Wheat stubble+N	**27 (5) a**	4 (1) b	–5 (7) a	1 (8) b	18 (4) b
Lupin stubble–N	19 (3) b		3 (2) a	**19 (3) a**	22 (2) b
Lupin stubble+N	**24 (4) a**	4 (4) b	–2 (3) a	1 (7) b	17 (3) b
		First node to booting			
No-stubble–N	5 (4) a		–3 (0) a	5 (9) b	2 (2) a
No-stubble+N	2 (3) a	0 (1) a	–2 (0) a	17 (4) b	–0.8 (2) a
Wheat stubble–N	10 (4) a		–3 (0) a	4 (14) b	6 (7) a
Lupin stubble–N	5 (5) a		–2 (2) a	–2 (9) b	2 (10) a
Lupin stubble+N	7 (4) a	0 (2) a	–3 (2) a	**35 (3) a**	3 (2) a

During the period from first node to booting, the depletion in soil mineral N was relatively small (not dissimilar to the previous period). Increases in whole wheat plant N uptake was smaller than for the earlier growth stages and did not significantly vary between treatments. The QNdff was negligible for both measured treatments (lupin stubble+N and no-stubble+N).

Overall, the highest proportion of N in the whole wheat plant biomass across all treatments and growth stages was derived from nonfertiliser N sources, i.e. taken up from 'native' soil N pools (Fig. 6). By booting, fertiliser N represented $19 \pm 3\%$ of wheat plant N in the no-stubble+N treatment (Fig. 6*a*) and $13 \pm 2\%$ in the lupin stubble+N treatment (Fig. 6*c*). Across all stubble treatments and growth stages, surface soil MBN represented a large pool of N with 42 ± 13 mg pot^{-1} on average with a range of 5–73 mg pot^{-1}, whereas soil mineral N (0–20 cm) content ranged within 8–31 with a mean of 14 ± 4 mg pot^{-1} (Fig. 6).

Discussion

Increased soil biological N pools and net N mineralisation after ^{15}N fertiliser application improved early N uptake by wheat plant

Adding organic matter as stubble rapidly affected the soil biological N pools, with apparent effects 7 days after application to soil. At sowing, surface soil DON and MBN in the lupin and wheat stubble treatments were almost double those where no stubble had been added. This reflected the rapid effect on soil biological activity from the input of C and N as residues to this sandy low organic matter soil. Other studies in coarse-textured soils also reported rapid increases in MBN due to the incorporation of easily degradable organic stubbles (Sall *et al.* 2006). By wheat tillering stage, stubble addition increased surface soil MBN regardless of the fertiliser N treatment, and the no-stubble–N treatment had the lowest surface soil MBN. The application of fertiliser N similarly
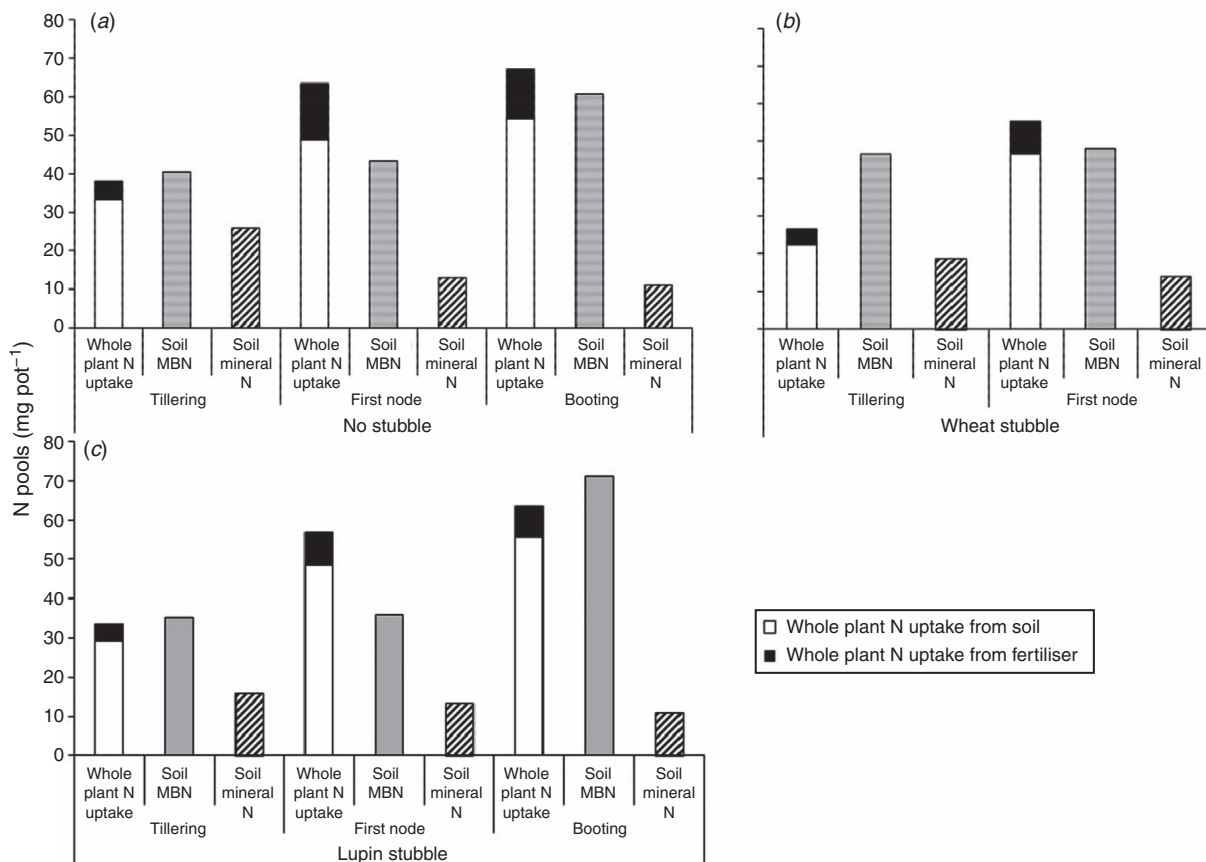
Fig. 6. (*a*) Plant and soil N pools (mg pot^{-1}) at different wheat growth stages for the no-stubble, (*b*) wheat stubble, and (*c*) lupin stubble with fertiliser N (+N). Wheat stubble treatment data at booting not available (missing data). MBN, soil microbial biomass N at 0–10 cm depth; mineral N, soil mineral N (nitrate + ammonium) at 0–20 cm depth; and whole plant N uptake, root + shoot N uptake.

increased MBN regardless of stubble treatment. It could be inferred that microbial growth was promoted by N derived from the residues or the fertiliser N. Previous studies from these (and other) semiarid rain-fed areas have indicated that microbes were both C and N limited in similar sandy soils (Gupta *et al*. 2011).

In dryland agroecosystems, the application of wheat straw can significantly increase MBN within a few weeks of stubble addition, perhaps by removing both C and N limitations (Singh and Singh 1993). An overall increase in the MBN during different crop and fallow periods over 2 years occurred when wheat straw was combined with fertiliser N application. It was inferred that straw alone, with a high C:N, might decompose and release nutrients slowly, but when supplemented with fertiliser it decomposed more rapidly (Singh and Singh 1993). The C input from the wheat stubble (3761 mg C pot^{-1}, equivalent to 4 t C ha^{-1}) represented 22% of the total C measured in the top 0–10 cm depth at sowing compared with 12% for the lupin stubble, but both stubbles contained similar amounts of N (42 mg pot^{-1}). In both wheat and lupin stubble treatments, the N input from fertiliser represented 5% of the total soil N content at sowing, and the N input from stubble represented 3% relative to existing soil N. Hence, higher surface soil PMN in the lupin stubble treatment at sowing was likely to result from the inputs of lupin stubble, which had

a lower C:N, including the branches and stems with C:N of 79:1 and the pods and leaves with C:N of 32:1. It is widely reported that the lower C:N of legumes can increase mineralisation compared with cereal residues (Abera *et al*. 2012).

Regardless of stubble treatment, applying fertiliser N at sowing resulted in net N mineralisation in early growth stages (sowing to tillering), increases in the MBN pool and depletion of the soil mineral N pool. Although the uptake of fertiliser N was a relatively small component of total wheat N uptake, wheat N content was greater than without fertiliser N, as was wheat plant biomass. The effects of stubble addition on increased biological activity (MBN) of the soil at sowing were still evident at tillering in the absence of fertiliser N, although the size of the MBN pools had not changed since sowing despite net N immobilisation for this period. Thus, uptake of N by wheat grown without fertiliser N for this initial growth period (sowing to tillering), regardless of stubble treatment, depended mostly on depletion of soil mineral N present at sowing, and was generally less than wheat grown with fertiliser N.

The time at which immobilisation occurs is a factor that could influence the mean difference in fertiliser N requirement for cereals (Green and Blackmer 1995). Under the conditions

of this study, fertiliser N appeared critical to maximise N supply to meet crop requirements by the first node stage when differences in plant biomass and N uptake by wheat were greatest between treatments. The lower wheat N uptake by tillering (Fig. 3*b*) for the –N fertiliser treatments demonstrated an N limitation that appeared to be alleviated by supply of fertiliser N (Ravier *et al*. 2017), regardless of the stubble treatment. Fertiliser N appeared to improve the synchrony of supply with demand by the wheat plant for this period albeit indirectly via stimulation of microbial N cycling or N mineralisation, potentially some contribution from a positive priming effect (Kuzyakov 2010; Fang *et al*. 2020). In subsequent growth stages (first node and booting), wheat N uptake in the +N treatment exceeded that by the –N, regardless of whether stubbles were incorporated, or whether the stubbles were lupin or wheat. Previous research on biological processes and N mineralisation in crop rotations, in a semiarid environment of Western Australia, found that neither crop stubble type nor stubble incorporation method significantly altered the timing or pattern of soil N supply for wheat N uptake, rather climatic factors were the major drivers (Hoyle and Murphy 2011).

Clearly the extrapolation of N uptake findings from this glasshouse study to crops under field conditions needs to be undertaken with caution given the environmental differences. Although the growth rate of wheat in this glasshouse study appears rapid, the rate of development follows the normal requirements for thermal time quoted for field conditions. The cumulative degree-days from sowing to booting in our study (834°C d) were similar to those recently reported from emergence to heading in field conditions (~965°C d) in a review paper on phenology of wheat adaptation to different environments (Hyles *et al*. 2020). Daily temperature at each growth stage were within the ranges for minimum (base), optimum and maximum temperatures for different phenological phases and stages in wheat, as indicated by Porter and Gawith (1999) in their review study on wheat crop responses to extreme temperatures. Furthermore, the importance of N fertiliser for early wheat growth in this soil and the contribution of lupin crop residues to increased biological pools are findings supported by our previous field observations (Muschietti Piana *et al*. unpubl. data).

Apparent lack of a legume residue effect on soil N supply and wheat N uptake

The results from this study indicated no effect of the stubble treatment on wheat N uptake or soil N-supply capacity, apart from evidence of a higher PMN where lupin stubble had been incorporated at sowing. The unexpected lack of legume residue effect could partly be due to the use of semidecomposed legume stubble in this glasshouse experiment that may have already lost labile C before collection from the field since it had a C : N of 47. The greater C : N of the added stubble could have potentially induced a positive priming effect through accelerated turnover of soil MB when C supply is greater than N supply (Kuzyakov *et al*. 2000). Indeed, previous field-based research (Muschietti Piana *et al*. unpubl. data) found that lupin

stubbles collected at crop maturity with C : N of 72 provided a greater N supply to wheat at early stages through higher decomposition rates and N release via mineralisation, which resulted in increased surface soil N pools. As a result of legume residues with a stronger C limitation, the period of immobilisation in the –N treatments was shorter in this controlled environment study (18 days from sowing to tillering) than that observed *in situ* under field conditions (120 days from late fallow to flag leaf) (Muschietti Piana *et al*. unpubl. data).

An increase in surface soil MBN with stubble addition and fertiliser N application indicates that there were both C and N limitations present in this system, and this was observed with both cereal and legume stubble despite their differing C : N. Priming of native soil organic matter when either stubble or fertiliser N were added to the soil (Kuzyakov *et al*. 2000) may have been additional factors contributing to the observed increased soil MBN and greater plant N uptake. This priming could have resulted in no apparent influence of legume stubble relative to wheat stubble on plant N uptake or N mineralisation, and also contributed to lower ^{15}N fertiliser recovery.

The greater C : N of the partially decomposed residues tested here could have induced a priming effect through accelerated turnover of MB when C supply was greater than N supply. However, the priming effect on N release from soil organic matter may first contribute to the N requirement of microorganisms as reflected in the measurement higher MBN. Russell and Fillery (1999) reported that 'only the leaf contained sufficient quantities of mineralisable N to be an important source of N for wheat', meaning in that any increase in soil MBN would likely have come from soil organic matter N; whereas, in the fertiliser N treatment, fertiliser N would have satisfied some of the MBN demand. The increase in MBN with legume residues, some of which would have come from fertiliser suggests the overall effects of greater C : N on N release for plant uptake are a product of multiple processes. In the absence of measurements of microbial turnover (i.e. measurements of microbial activity) there is no direct evidence for the priming effect. However, the work reported by Kuzyakov (2010) suggests that this would occur under the conditions of greater C : N. From first node to booting, the low amount of N mineralisation and the changes in the soil MBN observed in all treatments are indicative of inherent C and N limitations, inferring that the fertiliser N may remain tied up in the soil MBN pool until given more C inputs.

In a laboratory study using a saline sandy loam soil amended with lupin residues, the greater C availability in the soil amended with lupin residue supported a more active MB with greater N demand, therefore promoting immobilisation of nitrate, compared with the unamended control soil. The addition of inorganic N as nitrate in combination with the lupin residue stimulated microbial activity, thus increasing N availability in the soil (Elgharably and Marschner 2011). The longer-term fate of the immobilised N and whether it could become plant-available later in the crop growth cycle was not determined from this short-term glasshouse pot study. A recent study under a controlled environment found that the release of

plant-available N was maintained or increased in both the wheat and canola (*Brassica napus*) residue-amended treatments, due to the stimulation of soil organic matter mineralisation via positive priming by the plant residues. Crop residue inputs released plant-available nutrients from the soil reserves, more than the inherent crop residue nutrient content, after significant decomposition (Sarker *et al.* 2019). If this process can deliver significant proportions of plant N uptake, it may explain why fertiliser N uptake represented a small proportion of plant N uptake despite it generating a significant system response.

Differences in the ^{15}N fertiliser recovery by wheat at different growth stages in response to stubble treatment

Soil N was the major source of N for plant uptake. An average of 52 ± 3 mg pot^{-1} was acquired from the soil N pool by wheat plants in the two treatments measured (lupin and no-stubble) at booting (Fig. 6), and this compared with a total of 42 ± 2 mg pot^{-1} soil mineral N present at sowing and the remaining N derived from soil microbial N turnover. Only 10 ± 2 mg of the 70 mg pot^{-1} applied as ^{15}N-labelled fertiliser at sowing was found in the wheat at booting. These results suggest that there is proportionally more crop acquisition of native N in this sandy soil, from mineral N present at sowing plus in-crop N mineralisation than fertiliser-derived N. Recent research using a ^{15}N mass balance approach to test the effects of different N-fertiliser management strategies on wheat N uptake and productivity in a clay soils in a semiarid environment of south-eastern Australia also found that the majority (65–84%) of crop N uptake was supplied from soil reserves (Wallace *et al.* 2020).

The proportion of applied fertiliser N taken up by wheat by booting in this study is relatively small compared with the values generally reported for wheat at grain maturity (Blankenau *et al.* 2000; Wang *et al.* 2016; Smith *et al.* 2019). However, the fertiliser recoveries measured in this study are within ranges reported from field studies in the semiarid Mediterranean region; 6–22% in central Turkey (Halitligil *et al.* 2000) and 8–22% in Syria, with 31% of the ^{15}N fertiliser remaining in the 0–20 cm soil layer (Pilbeam *et al.* 1997*a*, 1997*b*). Studies in winter wheat with different applications of ^{15}N fertiliser rates and different timing of N application had ^{15}N fertiliser recovery efficiency of 24–41% in wheat shoots at harvest – this increased with decreasing ^{15}N fertiliser rates, reflecting a low level of fertiliser N demand (Wang *et al.* 2016). Meta-analysis showed that increases in crop yield from the use of organic amendments can be 60% greater than in the nonamended control, while combined use of organic amendments and fertiliser N can result in a yield increase of 114% (Chivenge *et al.* 2011).

The addition of stubble inhibited the recovery of fertiliser N. Wheat plants in the no-stubble treatment had the highest % N derived from fertiliser over the course of the experiment and the highest ^{15}N recovery efficiency at first node, indicative of less demand from the soil MB for fertiliser N where no recent residue was added. This suggestion is supported by the significantly higher plant uptake of fertiliser N in the no-

stubble treatment between tillering and first node coupled with very low N mineralisation and negligible increase in MBN in the treatment for that same period. Further to this, although the surface soil MBN at sowing was 22 mg pot^{-1} in all stubble treatments it had increased by an average of 44 mg pot^{-1} where fertiliser N was applied and by 22 mg pot^{-1} without fertiliser N at booting. Even though the ^{15}N in the soil MB was not measured, it could be inferred that some of this increase in N might have come from fertiliser N stored in the soil MB for later release, given the N limitation of the sandy soil.

The synchronisation of the demand for N by the crop with the ability of the soil to supply N is a major determinant of fertiliser N recovery. In a previous field study, wheat crop N uptake at tillering, first node and flag leaf represented 10%, 45% and 79% of total wheat N uptake at harvest respectively (Muschietti Piana *et al.* 2020). In contrast, most of wheat N uptake in the current glasshouse study occurred by first node (Table 3). This suggests that, despite being added, the availability of the fertiliser N applied before sowing and available for crop uptake was significantly impaired because measurements of shoot N concentration indicated that plants experienced some N deficiency between first node and booting. Shoot N concentration was 4.4%, 1.9% and 1.0% at tillering, first node and booting respectively, compared to corresponding reported critical N values for wheat in Australia of 4.0%, 2.7% and 1.8% (Reuter and Robinson 1997). The ability of a crop to tolerate N deficiency depends primarily on the time at which the deficiency occurs (Ravier *et al.* 2017). Particularly at the beginning of the wheat growing season (~tillering–first node), the sensitivity of the crop to N deficiency, in terms of biomass production and grain yield, may be greater than at other stages, since crop biomass and leaf area index might have a more determinant effect at this early growth stage (Ravier *et al.* 2017).

Conclusions

Incorporating aboveground residues one week before sowing had a rapid but relatively short-term effect on soil biological activity (MBN, DON and PMN) in this low organic matter sandy soil. Early immobilisation of N between sowing and tillering, in the absence of N-fertiliser addition, limited the N availability for wheat uptake in the subsequent growth period (tillering to first node), when the direct and indirect effects of fertiliser N appeared critical to maximise N supply to the plant. This rapid effect of fertiliser N on soil biological activity appeared to improve wheat plant biomass and N uptake in the subsequent vegetative stages of wheat (tillering to first node). However, wheat N uptake during this period was mainly derived from soil native N pools with only a small proportion (15–16%) sourced from the fertiliser N, although this proportion increased when no stubble was added. A large proportion (up to 38%) of the ^{15}N fertiliser applied at sowing was incorporated into MBN, with this proportion increasing as the experiment continued. The increase in surface soil MBN with both stubble and ^{15}N fertiliser treatments indicated an inherent C and N limitation in this sandy low organic matter soil, and both cereal and legume stubble stimulated the

biological pools of soil N. Whether the N immobilised in these biological pools becomes available to crops later in the season or if it may be sequestered as relatively stable organic matter remains to be determined, suggesting the need for further field-based experiments.

Conflicts of interest

The authors declare no conflicts of interest.

Acknowledgements

References

Abera G, Wolde-Meskel E, Bakken L (2012) Carbon and nitrogen mineralisation dynamics in different soils of the tropics amended with legume stubbles and contrasting soil moisture contents. *Biology and Fertility of Soils* **48**, 51–66. doi:10.1007/s00374-011-0607-8

Adl SM (2003) 'The ecology of soil decomposition.' (CABI: Wallingford, Oxon, UK)

Amato M, Ladd JN (1988) Assay for microbial biomass based on ninhydrin-reactive nitrogen in extracts of fumigated soils. *Soil Biology & Biochemistry* **20**, 107–114. doi:10.1016/0038-0717(88)90134-4

Angus JF (2001) Nitrogen supply and demand in Australian agriculture. *Australian Journal of Experimental Agriculture* **41**, 277–288. doi:10.1071/EA00141

Angus JF, Grace PR (2017) Nitrogen balance in Australia and nitrogen use efficiency on Australian farms. *Soil Research* **55**, 435–450. doi:10.1071/SR16325

Blankenau K, Kuhlmann H, Olfs HW (2000) Effect of increasing rates of ^{15}N labelled fertiliser on recovery of fertiliser N in plant and soil N pools in a pot experiment with winter wheat. *Journal of Plant Nutrition and Soil Science* **163**, 475–480. doi:10.1002/1522-2624(200010)163:5<475::AID-JPLN475>3.0.CO;2-W

Brookes PC, Landman A, Pruden G, Jenkinson DS (1985) Chloroform fumigation and the release of soil nitrogen: a rapid direct extraction method to measure microbial biomass nitrogen in soil. *Soil Biology & Biochemistry* **17**, 837–842. doi:10.1016/0038-0717(85)90144-0

Bünemann EK, Schwenke G, Van Zwieten L (2006) Impact of agricultural inputs on soil organisms- a review. *Australian Journal of Soil Research* **44**, 379–406. doi:10.1071/SR05125

Chivenge P, Vanlauwe B, Six J (2011) Does the combined application of organic and mineral nutrient sources influence maize productivity? A meta-analysis. *Plant and Soil* **342**, 1–30. doi:10.1007/s11104-010-0626-5

Colwell JD (1963) The estimation of phosphorus fertiliser requirements of wheat in Southern New South Wales by soil analysis. *Australian Journal of Experimental Agriculture* **3**, 190–197. doi:10.1071/EA9630190

Coonan EC, Kirkby CA, Kirkegaard JA, Amidy MR, Strong CL, Richardson AE (2020) Microorganisms and nutrient stoichiometry as mediators of soil organic matter dynamics. *Nutrient Cycling in Agroecosystems* **117**, 273–298. doi:10.1007/s10705-020-10076-8

Elgharably A, Marschner P (2011) Microbial activity and biomass and N and P availability in a saline sandy loam amended with inorganic N and lupin residues. *European Journal of Soil Biology* **47**, 310–315. doi:10.1016/j.ejsobi.2011.07.005

Fang Y, Singh BP, Farrell M, Van Zwieten L, Armstrong R, Chen C, Mohammad B, Avakkoli E (2020) Balanced nutrient stoichiometry of

organic amendments enhances carbon priming in a poorly structured sodic subsoil. *Soil Biology & Biochemistry* **145**, 107800. doi:10.1016/j.soilbio.2020.107800

Geisseler D, Scow DM (2014) Long-term effects of mineral fertilisers on soil microorganisms - a review. *Soil Biology & Biochemistry* **75**, 54–63. doi:10.1016/j.soilbio.2014.03.023

Ghimire R, Machado S, Bista P (2018) Decline in soil organic carbon and nitrogen limits yield in wheat-fallow systems. *Plant and Soil* **422**, 423–435. doi:10.1007/s11104-017-3470-z

Green C, Blackmer A (1995) Residue decomposition effects on nitrogen availability to corn following corn or soybean. *Soil Science Society of America Journal* **59**, 1065–1070. doi:10.2136/sssaj1995.03615995005900040016x

Gupta VVSR (2016) Biological factors influence N mineralisation from soil organic matter and crop residues in Australian cropping systems. In 'Proceedings of the 2016 International Nitrogen Initiative Conference, Melbourne, Australia'. Available at http://www.ini2016.com/pdf-papers/INI2016_Vadakattu_Gupta.pdf [verified 9 May 2018]

Gupta VVSR, Rovira AD, Roget DK (2011) Principles and management of soil biological factors for sustainable rainfed farming systems. In 'Rainfed farming systems'. (Eds P Tow, I Cooper, I Partridge, C Birch) pp. 149–184. (Springer: Berlin, Germany)

Gupta VVSR, Roper M, Thompson J (2019) Harnessing the benefits of soil biology in conservation agriculture. In 'Australian agriculture in 2020: From conservation to automation'. (Eds J Pratley and J Kirkegaard) pp. 237–253. (Agronomy Australia and Charles Sturt University: Wagga Wagga).

Halitligil B, Akin A, Bilgin N, Deni Y, Ögret K, Altinel B, Isik Y (2000) Effect of nitrogen fertilisation on yield and nitrogen and water use efficiencies of winter wheat (durum and bread) varieties grown under conditions found in Central Anatolia. *Biology and Fertility of Soils* **31**, 175–182. doi:10.1007/s003740050642

Hauck RD, Bremner JM (1976) Use of tracer for soil and fertiliser nitrogen research. *Advances in Agronomy* **28**, 219–266. doi:10.1016/S0065-2113(08)60556-8

Hertenberger G, Wanek W (2004) Evaluation of methods to measure differential ^{15}N labeling of soil and root N pools for studies of root exudation. *Rapid Communications in Mass Spectrometry* **18**, 2415–2425. doi:10.1002/rcm.1615

Hoyle FC, Murphy DV (2011) Influence of organic residues and soil incorporation on temporal measures of microbial biomass and plant available nitrogen. *Plant and Soil* **347**, 53–64. doi:10.1007/s11104-011-0922-8

Hyles J, Bloomfield MT, Hunt JR, Trethowan RM, Trevaskis M (2020) Phenology and related traits for wheat adaptation. *Heredity* doi:10.1038/s41437-020-0320-1

Isbell RF, National Committee on Soil and Terrain (2016) 'The Australian soil classification.' 2nd edn. (CSIRO Publishing: Melbourne)

IUSS Working Group WRB (2006) 'World reference base for soil resources.' World Soil Resources Reports 2006 No 103. (FAO: Rome, Italy)

Jenkinson DS (1977) The soil biomass. *New Zealand Soil News* **25**, 213–218.

Jones DL, Willett VB (2006) Experimental evaluation of methods to quantify dissolved organic nitrogen (DON) and dissolved organic carbon (DOC) in soil. *Soil Biology & Biochemistry* **38**, 991–999. doi:10.1016/j.soilbio.2005.08.012

Kallenbach CM, Wallenstein MD, Schipanksi ME, Grandy AS (2019) Managing agroecosystems for soil microbial carbon use efficiency: ecological unknowns potential outcomes, and a path forward. *Frontiers in Microbiology* doi:10.3389/fmicb.2019.01146

Keeney DR, Bremner JM (1966) Comparison and evaluation of laboratory methods of obtaining an index of soil nitrogen availability. *Agronomy Journal* **58**, 498–503. doi:10.2134/agronj1966.00021962005800050013x

Kirkegaard J, Verburg K, Gupta V, McBeath T, Luo Z, Wang E, Richardson A, Lilley J (2018) 'Research outcomes on N-dynamics in stubble retained systems and their implications for N management guidelines.' (CSIRO: Australia)

Klute A (1986) Water retention: laboratory methods. In 'Methods of soil analysis. I. Physical and mineralogical methods'. (Ed CA Black). pp. 901–926 (ASA: Madison, WI, USA)

Kuzyakov Y (2010) Priming effects: Interactions between living and dead organic matter. *Soil Biology & Biochemistry* **42**, 1363–1371. doi:10.1016/j.soilbio.2010.04.003

Kuzyakov Y, Friedel JK, Stahr K (2000) Review of mechanisms and quantification of priming effects. *Soil Biology & Biochemistry* **32**, 1485–1498. doi:10.1016/S0038-0717(00)00084-5

Ladd JN, Oades JM, Amato M (1981) Distribution and recovery of nitrogen from legume residues decomposing in soils sown to wheat in the field. *Soil Biology & Biochemistry* **13**, 251–256. doi:10.1016/0038-0717(81)90058-4

Ladd JN, Amato M, Li-Kai Z, Schultz JE (1994) Differential effects of rotation, plant residue and nitrogen fertiliser on microbial biomass and organic matter in an Australian Alfisol. *Soil Biology & Biochemistry* **26**, 821–831. doi:10.1016/0038-0717(94)90298-4

Luo Z, Wang E, Sun OJ (2010) Soil carbon change and its responses to agricultural practices in Australian agro-ecosystems: a review and synthesis. *Geoderma* **155**, 211–223. doi:10.1016/j.geoderma.2009.12.012

McBeath TM, Gupta VVSR, Llewellyn RS, Davoren CW, Whitbread AM (2015) Break crop effects on wheat production across soils and seasons in a semi-arid environment. *Crop and Pasture Science* **66**, 566–579. doi:10.1071/CP14166

McBeath TM, Gupta VVSR, Llewellyn RS, Mason SD, Davoren CW, Correll RL, Jones B, Whitbread AM (2019) Combined application of nitrogen and phosphorus to enhance nitrogen use efficiency and close the wheat yield gap on varying soils in semi-arid conditions. *Journal Agronomy & Crop Science* **205**, 635–646. doi:10.1111/jac.12357

Miranda KM, Espey MG, Wink DA (2001) A rapid, simple spectrophotometric method for simultaneous detection of nitrate and nitrite. *Nitric Oxide* **5**, 62–71. doi:10.1006/niox.2000.0319

Muschietti Piana MB, McBeath TM, McNeill AM, Cipriotti PA, Gupta VVSR (2016). Closing the nitrogen supply and demand gap using legume residue combined with fertiliser nitrogen input. In 'Proceedings of the 2016 International Nitrogen Initiative Conference: Solutions to improve nitrogen use efficiency for the world' Melbourne, Australia. Available at http://www.ini2016.com/pdf-papers/INI2016_MuschiettiPiana_Pilar.pdf [verified 9 May 2018]

Muschietti Piana MP, McBeath TM, McNeill AM, Cipriotti PA, Gupta VVSR (2020) Combined nitrogen input from legume residues and fertiliser improves early nitrogen supply and uptake by wheat. *Journal of Plant Nutrition and Soil Science* **183**, 355–366. doi:10.1002/jpln.202000002

Pilbeam CJ, Wood M, Jones MJ (1997*a*) Proportion of total nitrogen and fixed nitrogen in shoots of lentil and chickpea grown in a Mediterranean-type environment. *Experimental Agriculture* **33**, 139–148. doi:10.1017/S0014479797000045

Pilbeam CJ, McNeill AM, Harris HC, Swift RS (1997*b*) Effect of fertiliser rate and form on the recovery of ^{15}N-labelled fertiliser applied to wheat in Syria. *The Journal of Agricultural Science* **128**, 415–424. doi:10.1017/S0021859697004371

Poole N, Hunt J (2014) Advancing the management of crop canopies, GRDC Project SFS00017.

Porter JR, Gawith M (1999) Temperatures and the growth and development of wheat: a review. *European Journal of Agronomy* **10**, 23–36. doi:10.1016/S1161-0301(98)00047-1

R Core Team (2018) R: A language and environment for statistical computing. R Foundation for statistical computing, Vienna, Austria. Available at https://www.r-project.org/ [verified 30 June 2020].

Ravier C, Meynard JM, Cohan JP, Gate P, Jeuffroy MH (2017) Early nitrogen deficiencies favor high yield, grain protein content and N use efficiency in wheat. *European Journal of Agronomy* **89**, 16–24. doi:10.1016/j.eja.2017.06.002

Rayment GE, Lyons DJ (2011) 'Soil chemical methods: Australasia.' (CSIRO Publishing: Melbourne)

Reuter D, Robinson JB (1997) 'Plant analysis: an interpretation manual.' (CSIRO Publishing: Melbourne)

Rienzo JA, Guzman AW, Casanoves F (2002) A multiple-comparisons method based on the distribution of the root node distance of a binary tree. *Journal of Agricultural Biological & Environmental Statistics* **7**, 129–142. doi:10.1198/10857110260141193

Roberts BA, Fritschi FB, Horwath WR, Bardhan S (2015) Nitrogen mineralisation potential as influenced by microbial biomass, cotton stubbles and temperature *Journal of Plant Nutrition* **38**(3), 311–324. doi:10.1080/01904167.2013.868486

Russell CA, Fillery IRP (1999) Turnover of nitrogen from components of lupin stubble to wheat in sandy soil. *Soil Research* **37**, 575–592. doi:10.1071/S98071

Sall SN, Masse D, Ndou NY, Chott JL (2006) Does cropping modify the decomposition function and the diversity of the soil microbial community of tropical fallow soil? *Applied Soil Ecology* **31**, 211–219. doi:10.1016/j.apsoil.2005.05.007

Sarker JR, Singha BP, Fang Y, Cowiea AL, Dougherty WJ, Collins D, Dalald RC, Singh BK (2019) Tillage history and crop residue input enhanced native carbon mineralisation and nutrient supply in contrasting soils under long-term farming systems. *Soil & Tillage Research* **193**, 71–84. doi:10.1016/j.still.2019.05.027

Singh H, Singh KP (1993) Effect of residue placement and chemical fertiliser on soil microbial biomass under tropical dryland cultivation. *Biology and Fertility of Soils* **16**, 275–281. doi:10.1007/BF00369304

Smith CJ, Hunt JR, Wang E, Macdonald BCT, Xing H, Denmeada OT, Zeglinb S, Zhaoa Z (2019) Using fertiliser to maintain soil inorganic nitrogen can increase dryland wheat yield with little environmental cost. *Agriculture, Ecosystems & Environment* **286**, 106644. doi:10.1016/j.agee.2019.106644

Sparling GP, Zhu C (1993) Evaluation and calibration of biochemical methods to measure microbial biomass C and N in soils from Western Australia. *Soil Biology & Biochemistry* **25**, 1793–1801. doi:10.1016/0038-0717(93)90185-E

Wallace AJ, Armstrong RD, Grace PR, Scheer C, Partington DL (2020) Nitrogen use efficiency of ^{15}N urea applied to wheat based on fertiliser timing and use of inhibitors. *Nutrient Cycling in Agroecosystems* **116**, 41–56. doi:10.1007/s10705-019-10028-x

Wang X, Zhou W, Liang G, Pei X, Li K (2016) The fate of ^{15}N-labelled urea in an alkaline calcareous soil under different N application rates and N splits. *Nutrient Cycling in Agroecosystems* **106**, 311–324. doi:10.1007/s10705-016-9806-x

Zadoks JC, Chang TT, Konzak CF (1974) A decimal code for the growth stages of cereals. *Weed Research* **14**, 415–421. doi:10.1111/j.1365-3180.1974.tb01084.x

Handling Editor: Peter Grace

CHAPTER 5

General Discussion

and

Future Research Direction

1. Summary

Management practices that enable environmental and economic viability of wheat production constitute a big challenge due to the inherent limitations of intensified cropping systems (Sadras and Roget, 2004). Agricultural intensification *via* increasing the number of annual cropping years in a phase/rotation-based farming system may increase the overall grain output, but at the expense of a progressive loss of the soil capacity to sustain higher biological productivity, which in turn may lower wheat yield and increase its inter annual variability and production risks (Ernst et al., 2016). In an extensive long term study in soils of the eastern Pampas of South America, wheat yield loss after the first five years of continuous annual cropping was mainly due to a decline in the soil nutrient supply capacity, expressed as a reduction in potentially mineralisable N, which could be compensated by using additional fertiliser inputs. But thereafter, without those inputs soil physical properties degraded past a threshold that limited wheat yield and reduced nutrient use efficiency and wheat yield loss could no longer be compensated with increased fertiliser nutrient supply (Ernst et al., 2018). Similar trends have been observed in northern grains region of Australia (Angus et al., 2019).

Maintaining productivity while managing the soil resource under conditions of increasing climate risk relies on management options that sustain nutrient and water supply despite low fertility and low buffer capacity (Sadras, 2002). Under low-rainfall intensified cropping systems the implementation of strategic management practices in deep sandy soils on the dunes is of vital importance for sustainable outcomes (Sadras and Roget, 2004). Effective management of sandy soils requires an understanding of the underlying soil constraints and the extent to which these can be improved by available management strategies (Unkovich et al., 2020) that enable sustainable intensification of agriculture (Giller et al., 2015).

Globally, there are more than 400 M ha of sandy soils, with 2.3 M ha of sandy soils under management in the low-medium rainfall cropping zone of south eastern Australia (Unkovich et al., 2020) where a Mediterranean type-climate predominates (Sadras, 2002). Total N

concentrations on these sandy soils are often less than 0.1%, which limits the potential crop supply of N through mineralisation processes (Unkovich et al., 2020). Studies in the Mallee environments of South Australia have found that more intensive and continuous cropping systems reduce the already low fertility of the sandy soils, with the low organic matter content and low rainfall conditions decreasing the soil microbial activity (Roget and Gupta, 1999). Sandy soils with low water holding capacity have lower organic fertility and fewer microsites to protect soil biota, inhibiting suppression of pathogens and other pests and limiting productivity of crops in this environment (Gupta et al., 2011).

Variable application of fertiliser N is one practice that has substantially improved cereal crop productivity on the deep sands. Wheat yield benefits to higher than previously used rates of fertiliser N (up to 80 kg N ha^{-1}) on the sands has been proven across all season types, and the cost of this change can be in part offset by reducing rates on the higher clay content soils which have not shown the same responses (McBeath et al., 2019; Monjardino et al., 2013). Including legumes in the rotation with cereal crops may be required in order to preserve the soil productivity function and reduce production risks (Ernst et al., 2016), enhancing soil fertility through biological N$_2$ fixation (Giller et al., 2015). The benefits of legume break crops for subsequent cereal crops have been proven and largely related to improved N supply (Kirkegaard et al., 2014; McBeath et al., 2015). This thesis explored the potential benefits of combinations of these practices and which part of the growing season would generate the best wheat crop benefit from manipulation of N supply.

A deeper knowledge of the seasonal patterns of soil N supply capacity occurring in these particular environments is a research gap and of vital importance for both farmers and advisors when selecting management strategies. This knowledge can ensure that the crop rotation and N fertilisation schemes chosen will provide the N bioavailability from soil at the appropriate time to meet plant demand. The aim of this thesis was to develop a better understanding of the specific factors controlling soil N supply capacity for wheat N uptake in

a low rainfall sandy soil environment, and how these factors might be manipulated to increase N use efficiency of wheat, and consequently yield potential. This work delivered four key findings:

1. Nitrogen supply from combinations of both legume and fertiliser N sources are critical to maximising the productivity of wheat crops in low rainfall sandy environments.

2. Nitrogen supply from combinations of crop residues and fertiliser is critical in early (pre-first node) growth stages of cereals grown in low fertility and low rainfall conditions.

3. Nitrogen fertiliser input enhances the effect of residues on surface soil N supply capacity in a low fertility sand.

4. Early growing season immobilisation affects post-tillering wheat nitrogen uptake from crop stubble and ^{15}N fertiliser in a sandy soil.

2. Legume residues and fertiliser N are critical to maximising the productivity of wheat crops in low rainfall sandy soils

The combination of lupin residues with fertiliser N input increased soil profile mineral N at early growth stages of subsequent wheat, providing a greater soil N supply at the time of high wheat N demand in the growing seasons of 2015 and 2016. Recent literature suggests that an accumulated legacy of N fertiliser and legume residues is likely responsible for building a soil N reservoir for crop production, explaining yield benefits from diversifying the rotation. This interactive effect is expected to be of greater significance in soils with poor fertility (Taveira et al., 2020).

There was a lack of crop response to fertiliser N inputs following wheat residues, whereas the wheat crop made a better use of soil mineral N in the profile following lupin residues with high fertiliser N inputs. This was in line with the study conducted by Ernst et al. (2018) who

provided evidence for a lower level of responsiveness to fertiliser N application where intensive cropping systems have degraded soil fertility and physical properties. In the Mallee environment, a demand for fertiliser N applied in the season following a grain legume is likely to prevail, but high levels of fertiliser N input risk a lack of a significant yield effect and economic return in below average rainfall seasons as was the case in 2015. However, in above average rainfall seasons, as in the 2016 field experiment, regardless of the fertiliser N input wheat N uptake was higher following lupin residues from first node to harvest, compared with wheat residues. For all treatments and growth stages, wheat N uptake increased by 0.5 kg ha^{-1} with every additional unit of fertiliser N input. From first node onwards, wheat N uptake increased with increasing fertiliser N inputs regardless of the residue treatment. This reflects the benefits of increasing N inputs from either the fertiliser or legume residues for improving plant N uptake under more favorable climate conditions.

The interactive effect of disease pressure on crop response to legume and fertiliser inputs requires consideration. The disease break was a contributing factor when considering the influence of lupin residues on N uptake by following wheat and the interaction with applied N fertiliser. Disease constraints (*Rhizoctonia* root rot and Crown rot) provide a further complex interaction making robust predictions of N mineralisation across seasons and environments difficult. The occurrence of diseases constitutes one of the multiple-constraints for cereal production that prevail in the semi-arid environments of the low-rainfall Mallee, particularly on sandy soil. The proportion of disease incidence in the statistical model for the 2016 field experiment was unable to be included as a covariate because there was no homogeneity of this variable among treatments. However, this effect of disease was indeed considered while interpreting and discussing the results. It is important to highlight that there is strong evidence in the literature for the disease break effect of legume crops in rotation (Evans et al., 2010; Gupta et al., 2012; Seymour et al., 2012).

Measurements that supported our understanding of N supply processes required a combination of deep profile sampling at the beginning and end of the growing season, with shallow sampling throughout the growing season. Deep soil profile mineral N at the time of sowing predicted the potential soil N supply for wheat, while in-crop surface mineral N measurements were able to explain most of the temporal N supply relationships. However, an end of season assessment of profile mineral N and water was key to understanding the effect of residue and fertiliser combinations on crop N uptake. Many previous studies that have assessed the soil N benefits (or supply) from legumes have focused mostly on the soil mineral N content prior to sowing the following cereal (Crews and Peoples, 2005) either deep in the profile (0–120cm) (Peoples et al. 2017), or in the surface (0–20cm) soil layer (Ernst et al., 2018).

The complex interactions between crop, soil, climate and management in field environments present challenges for robust and extrapolatable predictions of N mineralisation (Yin et al., 2020). A consistent model for predicting soil N mineralisation in sandy soil based on the relationship between environmental variables (i.e. rainfall and temperature) and soil N processes was not derived. However, relationships between variables that are useful for understanding the relative importance of different climatic conditions during the fallow and the growing season of wheat for the mineralisation of N in sandy soil were observed. Residue decomposition and N released were accelerated by both the cumulative rainfall and degree days as the season progressed from the start of the fallow to wheat crop harvest.

3. Nitrogen supply from combinations of crop residues and fertiliser is critical in early growth stages of cereals grown in low fertility and low rainfall environments

This Thesis has focused on the identification of temporal changes in the mineralisation–immobilisation processes relevant to critical growth stages of wheat crop. In the published

literature, soil N transformations and potential supply has often been studied in isolation of cereal crop N uptake at critical growth stages. Multiple experiments offered insight into the relationship between soil N supply and plant uptake by matching the timing of soil N pool measurements with critical periods during the fallow and at wheat growth stages for wheat N uptake under field conditions (Evans et al., 2001; Fillery, 2001; Ladd et al., 1983a).

Further to this, where the supply of N to crops has often been independently assessed *via* the manipulation of N fertiliser management or *via* the supply of N from legume residues, this thesis explores the combination of these two factors to facilitate better N supply in low fertility scenarios. Further insight in the controlled environment to trace the effect of N was gained by reproducing the treatments examined in field conditions. The studies undertaken to address this novel information gap were supported by intensive temporal measurements of crop, residue, soil and environmental variables in both the field and controlled environment conditions. Some key relationships between N input and the flow of N through the N cycle during a crop growing season have been identified (Fig.1).

The amount of N from legume residues that will be available to the following cereal is difficult to predict in the field due to the complexity of the residue decomposition process and N cycling in agricultural soils (Singh and Rengel, 2007). Field measurements in this Thesis (Chapter 2) demonstrated that lupin residues can provide 35 kg N ha^{-1} more of soil profile mineral N (0–100 cm) than wheat residues at the time of sowing to the following wheat (Fig. 1). The benefits from lupin compared to wheat residue flowed into the growing season with the estimated net N mineralisation in the soil profile (0–100 cm) of 72 kg N ha^{-1} for lupin residue compared with net N immobilisation of 15 kg ha^{-1} for wheat residue in the critical period for wheat N uptake from tillering to flag leaf (Chapter 2). A further of 6±5 kg N ha^{-1} below-ground lupin residue benefit is estimated (Fig. 1, Chapter 2 and 3).

Work in the low and medium rainfall zone of Victoria near to our study site showed that net in-crop N mineralisation supplied an average of 63% of a crop's N demand, but with a high

level of variation from 0 to >100% (Dunsford 2019). Dunsford et al. (2015) have estimated net in-crop mineralisation values between 24 and 35 kg N ha^{-1} for a growing season in the low-rainfall zone of Victoria. These estimates align with the values measured here in the low rainfall zone of South Australia.

Fertilising the lupin residue system offered further gains, with plant N uptake following lupin residues of 24±6 kg ha^{-1} at first node and 42±4 kg ha^{-1} at anthesis where 40 kg ha^{-1} fertiliser N was added (Chapter 2). As the season progressed the presence of a legume residue reduced the proportion of plant N directly derived from fertiliser (Chapter 4), which was expected because plant N uptake after legume residues was higher than wheat or no residues. At tillering, wheat N uptake (shoot) that derived from fertiliser was 16%, 14% and 14% with lupin, wheat and no stubble treatments respectively. At first node, the proportions were 15% and 14% and 23% for lupin, wheat and no stubble treatments respectively, whereas at booting there were 14% and 18% for lupin and no stubble treatments respectively (Chapter 4). While the proportional contribution of fertiliser to the crop plant may change during the growing season, it is important to recognise that a significant proportion of the added fertiliser N may make an indirect contribution. Up to 38% of the fertiliser N applied was incorporated in the surface soil MBN (Figure 6.1), and while this may initially result in net immobilisation (Chapter 4), subsequent re-mineralisation under beneficial soil moisture and temperature conditions can supply a significant proportion of a crop's N uptake (Elgharably and Marschner, 2011; Gupta et al,. 2011; Green and Blackmer, 1995).
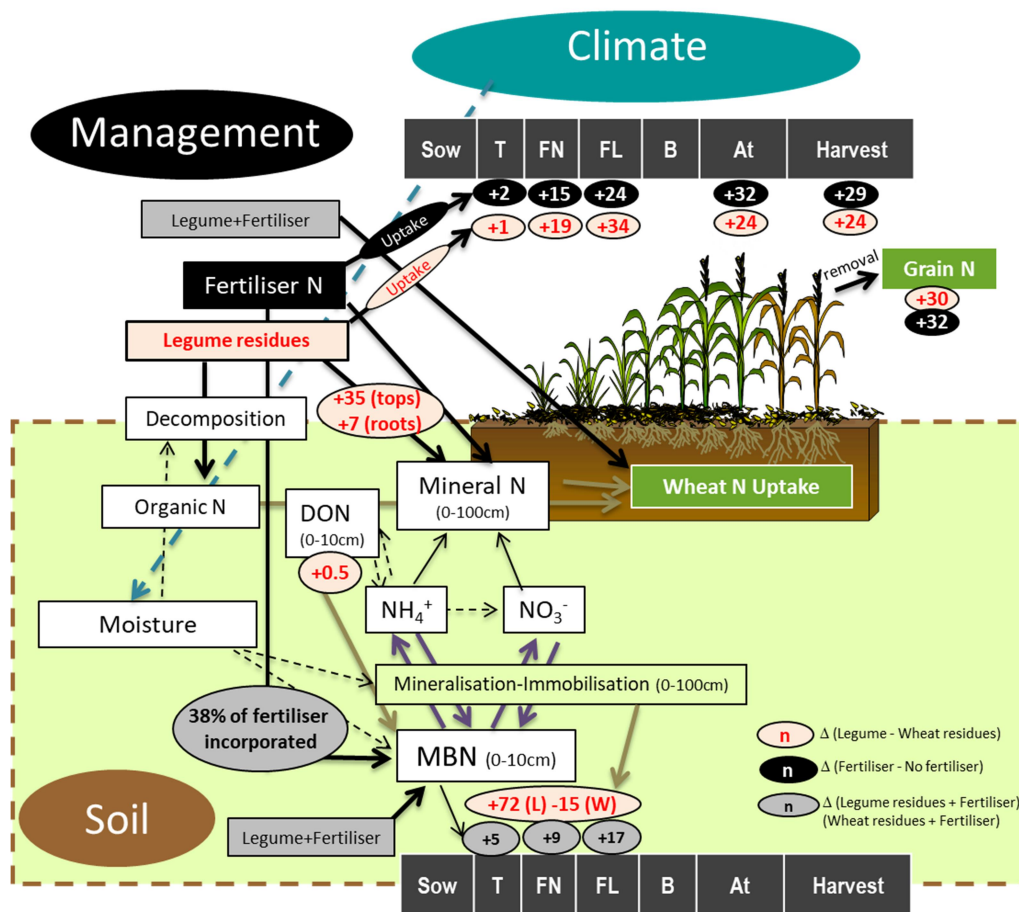
Figure 1. A conceptual model of the key factors affecting N availability for wheat. The main components addressed in this study are outlined by rectangles and the main processes. The ellipses indicate each treatment and their effect on each measured component is indicated with bold arrows (solid lines). Treatments are; legume residue, fertiliser N, and legume residue with fertiliser N application. Dashed arrows indicate processes within the soil matrix. Grey thin arrows (solid lines) indicate the linkage between a component and/or process and/or their respective quantity/proportions. Proportions and numbers indicated in bold are based on measurements in this Thesis. All numbers in circles indicate the mean difference (Δ) in kg N ha^{-1} for; lupin *vs.* wheat residues (in red), for fertiliser N *vs.* no fertiliser application (in black), and for the combination of legume residues with fertiliser N application *vs.* the combination of wheat residues with fertiliser N application (in grey).

NO_3^-=nitrate, NH_4^+=ammonium, DON=dissolved organic N, MBN, microbial biomass N. Wheat growth stages are indicated as; Sow=sowing, T=tillering, FN=first node, FL=flag leaf, B=booting, At=anthesis. Soil depth is indicated as; 0–10cm (surface soil), 0–100 cm=sum of soil profile N

4. Nitrogen fertiliser input enhances the effect of residues on surface soil N supply capacity in a low fertility sand

Lupin residues increased the N supply capacity during the wheat growing season through higher decomposition rates and N release *via* mineralisation. Further to this, lupin residues increased surface soil mineral from sowing to tillering, potentially mineralisable N at seedling and anthesis, microbial biomass N at first node and flag leaf, and dissolved organic N at seedling relative to when wheat residues were either removed or retained, regardless of the fertiliser N input. One of the major contributions from this experiment was that the combination of lupin residues with fertiliser N offered some significant gains for N supply capacity early in the season, with the highest surface soil nitrate N at seedling and tillering, potentially mineralisable N at seedling and surface soil ammonium N at seedling and tillering. This additional insight was gained *via* a focus on surface soil measurements of pertinent chemical and biological N pools in the surface soil, along with litterbag studies of residue decomposition and N release from residues under field conditions. Previous research under laboratory conditions supports these findings with the suggestion that the application of legume residues alone cannot achieve the potential maize (*Zea Mays* L.) crop yield on Tanzanian soils unless managed with frequent application of small doses of mineral fertilisers as top dressing in order to ensure synchrony with crop demand (Baijukya et al., 2006).

Early in the fallow, the patterns of surface soil mineral N fluxes were driven by rewetting of dry soil. A temporary immobilisation occurred in the residue retained experiments which

required two soil wetting periods for net mineralisation to occur. Where residue was removed, a positive N flux occurred after only one wetting period. Seasonal patterns of residue decomposition were linked to rainfall and temperature in 2016, but the magnitude of decomposition was primarily determined by residue quality. Lupin residue decomposition was more responsive to both cumulative rainfall and degree days than wheat residues. Wheat residue decomposition may be limited by lack of nutrients (such as N), more than the lupin residue. Lupin residue has a narrower C:N which facilitates it ability to respond more readily to rainfall and temperature, with the subsequent decomposition providing a readily available source of N (van Vliet et al., 2000). In studies in southern Australian soils the decomposition of legume (aboveground) residues and wheat straw was positively related to rainfall and decomposition of legume residues was more responsive to rainfall than wheat straw. The magnitude of the rainfall effect decreased with decomposition time and it was not significant at 52 weeks for legume residues, whereas for wheat straw the relationship was no longer statistically significant after 10 weeks (Amato et al., 1987). Other studies have indicated that temperature was of greater importance than moisture in controlling the extent of decomposition of added plant materials in the soil surface (Ladd et al,. 1985). Decomposition rates under semi-arid conditions in South Australia were approximately double those reported under temperate conditions in United Kingdom. In a relationship determined by Ladd et al. (1985) the overall rates of decomposition doubled for an 8–9°C increase in mean annual air temperatures but when the mean annual air temperatures differed by less than 2°C it was insufficient to reveal a significant effect of temperature on the extent of decomposition of the plant materials (Amato et al., 1987). These contrasting relationships demonstrate the difficulty with identifying simple predictive models for decomposition of residue in these environments.

5. Early growing season immobilisation affects post-tillering wheat nitrogen uptake from crop stubble and ^{15}N fertiliser in a sandy soil

While the field experiments consistently demonstrated that combinations of fertiliser N and legume residues were required to increase wheat N supply and match demand, there were gaps in our understanding of the relative influence that each source of N had on N supply capacity. From seedling to first node, increasing microbial biomass N in the surface soil measured in the field (2016) was correlated with decreasing surface soil mineral N suggesting net N immobilisation. This pattern coincided with the timing of net immobilisation from residue N. This led us to hypothesise that temporary periods of immobilisation early in the growing season were critical bottlenecks in optimum wheat N supply of low rainfall environments. The use of labelled ^{15}N fertiliser allowed these key questions to be tested under controlled conditions with a focus on the N supply-demand relationship at early stages of wheat plants.

The fertiliser N requirement for wheat can be affected by the timing of N immobilisation (Green and Blackmer, 1995). Regardless of stubble treatment, applying fertiliser N at sowing resulted in net N mineralisation in early growth stages (sowing to tillering), increases in the microbial biomass N pool, and depletion of the soil mineral N pool. Increasingly growers are applying most of their N inputs for average and below average growing seasons at the time of sowing or soon after. This is a response to declining fertility, logistical challenges of topdressing large-scale operations and the lack of reliable rainfall events in the winter months for incorporation of urea. Many have assumed that this shift in practice is a risk increasing behavior but analysis of the evolving system suggests that in many cases this shift is risk-neutral and only risk-increasing in higher fertility scenarios (Monjardino et al., 2013; 2015). In-season application of N in above average seasons remains critical to maximising profit or 'upside' in these environments (McBeath et al., 2020).

A key finding of this experiment was the presence of early N immobilisation from sowing to tillering in all the unfertilised N treatments that may have limited N availability for wheat uptake in the subsequent period from tillering to first node, when the application of fertiliser

N appeared critical to optimise N supply for plant requirements. This result highlights that fertiliser N was critical in the short term for relieving inherent N limitations for both plant growth/N demand and soil N supply/biological capacity, and in the longer term for increasing biological pools (microbial biomass N) that form the key sources of plant available N. Nevertheless, soil N pools were the primary source for wheat N uptake and only a small proportion (15–16%) derived from the ^{15}N fertiliser, but this proportion increased where the wheat stubble had been removed. This finding suggests that there is proportionally more crop acquisition of soil N in this sandy soil, from mineral N present at sowing plus in-crop N mineralisation rather directly derived from the fertiliser applied. Current literature in semi-arid environments of south-eastern Australia indicates that the majority (65–84%) of wheat N uptake is derived from soil N reserves (Angus and Grace, 2001; Wallace et al., 2020).

An interesting finding was that a large proportion (up to 38%) of the ^{15}N fertiliser applied at sowing was incorporated into microbial biomass N as shown in Fig. 6.1, and this proportion increased as the experiment continued. This relates to an inherent carbon and N limitation in the sandy soil based as indicated by the increase in surface soil microbial biomass N with both stubble and ^{15}N fertiliser treatments, and that both cereal and legume stubble stimulated the biological pools of soil N. In the heavier textured soils of semi-arid environments of South Australia, the relatively greater retention of legume biomass N over time was consistent with the net decay of C and N in soil driven by the turnover of biomass C and N. The decay rates were decreased in soils which have the greater capacity to protect decomposer populations when compared to a sandy soil of the dune (Ladd et al., 1985). Whether the N immobilised in these biological pools become available to crops later in the season or if it may be sequestered as relatively stable organic matter remains to be determined, suggesting the need for further field-based experiments.

6. Future research directions

To further develop our understanding of the main drivers improving the synchrony of the N supply-demand relationship for wheat grown in low-rainfall sandy soils, further research in the following areas is recommended:

1. This study highlights the importance of understanding the dynamics of soil mineral N throughout the soil profile prior to wheat sowing and at specific phenological stages to better predict the requirement for N inputs for critical periods in a wheat growing season. Further insight across seasons and environments could be usefully built into predictive models.

2. More elaborate combinations of residue (both legume and non-legume) treatments, fertiliser N rates and key soil types will better identify which management factors can be best manipulated to improve soil N supply capacity across a range of scenarios.

3. In this study the amount of N that is recovered by the wheat crop from the fertiliser was measured under controlled conditions. Further research using dual labelled strategies for labelling both residues and fertiliser in field conditions could allow optimisation of these combined management strategies.

4. Immobilisation reactions early in the growing season appear to be a critical process driving N supply to crops. The process of subsequent mineralisation of the N immobilised in biological pools to supply N to crops later in the season or a competing sequestration of N as relatively stable organic matter requires exploration to determine the net outcome of these immobilisation reactions as influenced by soil type and regional environmental conditions.

5. The use of model-based predictions to simulate residue decomposition and the N mineralisation-immobilisation component in cereal systems is necessary to extrapolate results to other environments under varying climatic and management conditions (Yin et al., 2020). The APSIM model was developed to simulate biophysical process in farming systems for economic and ecological outputs (Keating et al., 2003). Some

studies indicated that APSIM was unable predict the net mineralisation of soil N following lucerne very well and modifications were applied to the parameters of potential decomposition rate of soil organic matter pools, wheat surface residue, and water factors for mineralisation and nitrification (Verburg et al., 2007). Recently, Smith et al. (2019) conducted field studies with ^{15}N labelled fertiliser and APSIM simulations in cereal systems of south-eastern Australia to demonstrate that fertiliser N applied at rates calculated to maintain fixed thresholds of inorganic N (N bank criteria) and top-dressed immediately prior to the period of rapid crop uptake can substantially increase yields and profit in comparison to current practice, even when applications exceed crop demand in some years. This study indicated that the model performance was considered acceptable for simulating the mineral N dynamics, wheat above ground biomass and grain yield, but there were some over-estimations of the wheat N uptake (Smith et al., 2019).

The decomposition rates of legume residues and the amount of net N mineralised from soil and crop residues is variable (Angus et al., 1998), and different ranges have been reported in dryland cereal systems of Australia (Amato et al., 1984; Ladd et al. 1981; Asseng et al., 1998a). It is critical to improve our ability to measure and predict the immobilisation-mineralisation component of the N cycle in cereal systems in order to meaningfully model management changes that will have impact for improved N use efficiency. Recently, a study using a multi-model ensemble reported larger uncertainties for simulating N mineralisation, soil mineral N and N leaching than for simulating plant N uptake, mainly because these variables are influenced by plant-soil interactions and subject to cumulative long-term effects in crop rotations, which makes them more difficult to simulate (Yin et al., 2020).

References

Amato, M., J.N. Ladd, A. Ellington, G. Ford, J.E. Mahoney, A.C. Tayloer, D. Walsgott. 1987. Decomposition of plant material in Australian soils. IV*Decomposition in situ of 14C- and 15N-labeled legume and wheat materials in a range of Southern Australian soils. Australian Journal of Soil Research 25: 95–105.

Angus, J., M. Bell, T. McBeath and C. 2019 Nutrient-management challenges and opportunities in conservation agriculture. In: J. Pratley and J. Kirkegaard, editor Australian Agriculture in 2020: From Conservation to Automation. Agronomy Australia and Charles Sturt University: Wagga Wagga. pp. 221–236.

Angus, J.F. and P.R. Grace. 2017. Nitrogen balance in Australia and nitrogen use efficiency on Australian farms. Soil Research 55: 435–450.

Angus, J.F., A.F. van Herwaarden, D.P. Heenan, R.A. Fischer and G.N. Howe. 1998. The source of mineral nitrogen for cereals in south-eastern Australia. Australian Journal of Agricultural Research 49: 511-522. doi:http://dx.doi.org/10.1071/A97125.

Asseng, S., G.C. Anderson, F.X. Dunin, I.R.P. Fillery, P.J. Dolling and B.A. Keating. 1998a. Use of the APSIM wheat model to predict yield, drainage, and NO3 leaching for a deep sand. Australian Journal of Agricultural Research 49: 363-378. doi:http://dx.doi.org/10.1071/A97095.

Baijukya, F.P., N. Ridder N and K.E. Giller. 2006. Nitrogen release from decomposing residues of leguminous cover crops and their effect on maize yield on depleted soils of Bukoba District, Tanzania. Plant and Soil 279: 77–93.

Crews, T.E. and M.B. Peoples. 2005. Can the synchrony of nitrogen supply and crop demand be improved in legume and fertiliser-based agroecosystems? A review. Nutrient Cycling in Agroecosystems 72: 101–120.

Dunsford, K. 2019. Understanding causes of poor grain yield response of wheat to
phosphorus and nitrogen fertilisers. PhD Thesis, La Trobe University Victoria,
Australia. 179 pp.

Dunsford, K., R.D. Armstrong, C. Tang and P. Sale. 2015. Estimating in-crop nitrogen
mineralisation in dryland cropping systems of southern Australia. Proceedings of the
16th Australian Agronomy Conference' Hobart, Tasmania, Australia.

Elgharably, A., P. Marschner. 2011. Microbial activity and biomass and N and P availability
in a saline sandy loam amended with inorganic N and lupin residues. European
Journal of Soil Biology 47: 310–315.

Ernst, O.R., S. Dogliotti, M. Cadenazzi and A.R. Kemanian. 2018. Shifting crop-pasture
rotations to no-till annual cropping reduces soil quality and wheat yield. Field Crops
Research 217: 180–187.

Ernst, O.R., A.R. Kemanian, S.R. Mazzilli, M. Cadenazzi and S. Dogliotti. 2016. Depressed
attainable wheat yields under continuous annual no-till agriculture suggest declining
soil productivity. Field Crops Research 186: 107–116.

Evans, M.L., G.J. Hollaway, J.I. Dennis, R.L. Correll, and H. Wallwork. 2010. Crop sequence
as a tool for managing populations of Fusarium pseudograminearum and F. culmorum
in south-eastern Australia. Australasian Plant Pathology 39: 376–382.

Evans, J., A.M. McNeill, M.J. Unkovich, N.A. Fettell and D.P. Heenan. 2001. Net nitrogen
balances for cool-season grain legume crops and contributions to wheat nitrogen
uptake: a review. Australian Journal of Experimental Agriculture 41: 347–359.

Fillery, I.R.P. 2001. The fate of biologically fixed nitrogen in legume-based dryland farming
systems: A review. Australian Journal of Experimental Agriculture 41: 361–381.

Giller, K.E., J.A.Andersson, M. Corbeels, J. Kirkegaard, D. Mortensen, O. Erenstein and B. Vanlauwe. 2015. Beyond conservation agriculture. Frontiers in Plant Science 6: 870.

Gupta, V.V.S.R., R. Llewellyn, T.M. McBeath, S. Kroker, W. Davoren, A. McKay, K. Ophel-Keller, and A. Whitbread. 2012. Break crops for disease and nutrient management in intensive cereal cropping. Proceedings 16th Australian Agronomy Conference, Armidale, Australia. Available at:

http://www.regional.g.au/au/asa/2012/nutrition/7961_vadakattugupta.htm#TopOfPa.

Gupta, V.V.S.R., A.D. Rovira and D.K. Roget. 2011. Principles and management of soil biological factors for sustainable rainfed farming systems. In: P. Tow, I. Cooper, I. Partridge and C. Birch, editors, Rainfed Farming Systems. Springer Netherlands. pp. 149–184.

Green, C. and A. Blackmer. 1995. Residue decomposition effects on nitrogen availability to corn following corn or soybean. Soil Science Society American Journal 59: 1065.

Keating, B.A., P.S. Carberry, G.L. Hammer, M.E. Probert, M.J. Robertson, D. Holzworth, et al. 2003. An overview of APSIM, a model designed for farming systems simulation. European Journal of Agronomy 18: 267-288.

Kirkegaard, J.A., J.R. Hunt, T.M. McBeath, J.M. Lilley, A. Moore, K. Verburg, M.J Robertson., Y.M. Oliver, P.R. Ward, S. Milroy and A.M. Whitbread. 2014. Improving water productivity in the Australian grains industry - A nationally coordinated approach. Crop and Pasture Science 65: 583-601. 10.1071/cp14019.

Ladd, J.N., M. Amato, R.B. Jackson and J.H.A. Butler. 1983a. Utilization by wheat crops of nitrogen from legume residues decomposing in soils in the field. Soil Biology and Biochemistry 15: 231–238.

Ladd, J.N., J.M. Oades and M. Amato. 1981. Distribution and recovery of nitrogen from legume residues decomposing in soils sown to wheat in the field. Soil Biology and Biochemistry 13: 251–256.

Ladd, J.N., J.M. Oades, and M. Amato. 1985. Decomposition of plant material in Australian soils. III. Organic residues, biomass, and potential mineralization of C and N from decomposing isotope labelled plant material and soil organic matter. Australian Journal of Soil Research 23: 603–611.

Mcbeath, T.M., V.V.S.R. Gupta, R.S. Llewellyn, S.D. Mason, C.W. Davoren, R.L. Correll, B. Jones and A.M. Whitbread. 2019. Combined application of nitrogen and phosphorus to enhance nitrogen use efficiency and close the wheat yield gap on varying soils in semi-arid conditions. Journal of Agronomy and Crop Science 205: 635–646.

McBeath, T.M., V.V.S.R. Gupta, R.S. Llewellyn, C.W. Davoren and A.M. Whitbread. 2015. Break crop effects on wheat production accrsos soils and seasons in a semi-arid environment. Crop and Pasture Science 66: 566–579. doi:10.1071/CP14166.

McBeath, T.M., Meier, E.A., Ware, A., Kirkegaard, J., Moodie, M., Davoren, B. and E. Hunt. 2020. Agronomic management combining early-sowing on establishment opportunities, cultivar options and adequate nitrogen is critical for canola (*Brassica napus*) productivity and profit in low-rainfall environments. Crop and Pasture Science 71: 807–821.

Monjardino, M., T.M. McBeath, L. Brennan and R.S. Llewellyn. 2013. Are farmers in low-rainfall cropping regions under-fertilising with nitrogen? A risk analysis. Agricultural Systems 116: 37-51. doi:10.1016/j.agsy.2012.12.007.

Monjardino, M., McBeath, T., Ouzman, J. Llewellyn, R. and B. Jones. 2015. Farmer risk-aversion limits closure of yield and profit gaps: A study of nitrogen management in the southern Australian wheatbelt. Agricultural Systems 137: 108–118.

Peoples, M.B., T. Swan, L. Goward, J.R. Hunt, G. Li, G. Schwenke et al. 2017. Soil mineral nitrogen benefits derived from legumes and comparisons of the apparent recovery of legume or fertiliser nitrogen by wheat. Soil Research 55:600-615.

Roget, D. and V.V.R.S. Gupta. 1999. Mallee Sustainable Farming Project - Outcomes from the first year of trials. In: J. Lemon, editor Mallee Soils Seminar. Mallee Agricultural Research and Advisory Committee: Esperance).

Sadras, V.O. 2002. Interaction between rainfall and nitrogen fertilisation of wheat in environments prone to terminal drought: Economic and environmental risk analysis. Field Crops Research 77: 201-215. doi:10.1016/S0378-4290(02)00083-7.

Sadras, V.O. and D.K. Roget. 2004. Production and Environmental aspects of cropping intensification in a semiarid environment of Southeastern Australia. Agronomy Journal 96: 236–246.

Seymour, M., J.A. Kirkegaard, M.B. Peoples, P.F. White and R.J. French. 2012. Break-crop benefits to wheat in Western Australia – insights from over three decades of research. Crop and Pasture Science 63: 1-16.

Singh, B. and Z. Rengel. 2007. The role of crop residues in improving soil fertility. In: P. Marschner and Z. Rengel, editors Nutrient cycling in terrestrial ecosystems. Springer Berlin Heidelberg. Germany. pp. 183-214.

Smith, C.J., J.R. Hunt, E. Wang, B.C.T. Macdonald, H. Xinga, O.T. Denmead, S. Zeglin and Z. Zhao. 2019. Using fertiliser to maintain soil inorganic nitrogen can increase dryland wheat yield with little environmental cost. Agriculture, Ecosystems and Environment 286: 106644.

Taveira, C.J., R.E. Farrell, C. Wagner-Riddle, P.V.F. Machado, B. Deen and K.A. Congreves. 2020. Tracing crop residue N into subsequent crops: Insight from long-term crop rotations that vary in diversity. Field Crops Research 255.

Unkovich, M., T.M. McBeath, R. Llewellyn, J. Hal, V.V.R.S. Gupta and L.M. Macdonald. 2020. Challenges and opportunities for grain farming on sandy soils of semi-arid south and south-eastern Australia. Soil Research 58: 323–334.

Van Vliet, P.C.J., V.V.R.S. Gupta and L.K. Abbott. 2000. Soil biota and crop residue decomposition during summer andautumn in south-western Australia. Applied Soil Ecology 14: 111–124.

Wallace, A.J., R.D. Armstrong, P.R. Grace, C. Scheer and D.L. Partington. 2020. Nitrogen use efficiency of 15N urea applied to wheat based on Fertiliser timing and use of inhibitors. Nutrient Cycling in Agroecosystems 116: 41–56.

Yin, X., K.C. Kersebaum, N. Beaudoin, J. Constantin, F. Chen, G. Louarn, et al. 2020. Uncertainties in simulating N uptake, net N mineralization, soil mineral N and N leaching in European crop rotations using process-based models. Field Crops Research 255.

APPENDIX

142

Supplementary material of Chapter 2

S1. Field experimentation conducted in 2015 (site 1)

Part of this field experiment is comprised in Chapter 2 which was published in the *Journal of Plant Nutrition and Soil Science*

Muschietti Piana, M.P., T.M. McBeath, A.M. McNeill, V.V.R.S. Gupta and P.A. Cipriotti. 2020. Combined nitrogen input from legume residues and fertilizer improves early nitrogen supply and uptake by wheat. Journal of Plant Nutrition and Soil Science 183: 355–366. DOI: 10.1002/jpln.202000002.

Further measurements that were performed in this experiment along with their results and discussion are provided below.

S1.1 Methodological details

S1.1.1 Surface soil mineral nitrogen and plant available water fluxes for the autumn fallow

The fallow period in the Mallee environment is characterised by high temperatures and low rainfall which are not suited to growth of annual crop and pasture species, and the soil is generally left uncultivated. The autumn fallow for this study was defined as the period between 17 March 2015 (start of experiment) and 20 May 2015 (pre-sowing). In addition, during the autumn fallow, soil mineral N (nitrate-N + ammonium-N) content was measured for surface soils (0-10 cm) every 2 or 3 weeks for a total of 5 different sampling dates (Fig. 4) in order to describe patterns of mineral N flux. Soils were sampled using a hand auger (i.d. 3.6 cm) from 4 microplots of each residue experiment and these four samples were a composite consisting of two subsamples randomly taken on-row or off-row of the old crop in each microplot, and then mixed in order to account for the microscale variability (Kolberg et al., 1997). All soil samples were immediately stored at -16°C until their analysis in the laboratory. All samples were homogenised, weighed, and subsamples analysed for soil gravimetric water and mineral N content. Soil gravimetric water content was calculated on

oven-dried samples at 105°C to constant weight and was converted to volumetric water by using the measured bulk density for each soil layer. Soil volumetric water was expressed as plant available water (PAW, %, v v^{-1}), and it was calculated as the difference between the actual volumetric water content in each sampling date and the volumetric water for the 0-10 depth at the crop lower limit as determined in McBeath et al. (2015). Soils were extracted by shaking with 2 mol L KCl^{-1} (soil:solution, ratio 1:3) on an orbital shaker for 1 h at 25°C, and were filtered through Whatman N° 1. Nitrate N and ammonium N in the extracts were analysed colorimetrically (Miranda et al., 2001) using a SynergyMX microplate reader.

S1.1.2 Surface soil water and mineral nitrogen fluxes for the growing season

Growing season surface soil mineral N fluxes (May to November) was measured using an *in situ* method in which open ended PVC tubes were inserted to isolate the soil from plant roots. Undisturbed soil within PVC tubes was sequentially sampled during the growing season (Raison et al., 1987). Two open-ended tubes (7 cm id *x* 10 cm) were inserted between the crop rows in each microplot on 27 May 2015 and were left uncovered to be subjected to the inherent moisture regime during the incubation (2-3-week) period. At the end of each incubation period the two soil tubes were extracted and combined, and a further set of tubes were inserted for the following incubation period. To account for the spatial variation in each microplot, while allowing for multiple sampling dates, two samples were taken and bulked at each sampling date. There were 10 different sampling dates, totaling 20 samples for the whole growing season. Therefore, two samples were just enough to avoid disturbing the soil of the small area comprised by each microplot (1.7 m wide *x* 6 m long). On the sandy soils of the study site at Karoonda leaching of mineral N from the top 10 cm soil layer could occur as calculations indicated rainfall events >7 mm can cause water to drain beyond this depth, very similar to the events (>8 mm) described by Sadras and Baldock (2003) for soils of the Mallee. Potential mineral N leaching past a given soil depth (i.e. >10 cm for surface layer analysis) was calculated for each sampling time as:

$$\text{Soil N depth (mm)} = \frac{[\text{rainfall } (>7 \text{ mm}) \; x \text{ water density } x \; 100]}{[(\text{soil water at FC} - \text{actual soil water}) \; x \text{ bulk density of each layer}]} \qquad \text{Eq (4)}$$

Therefore, the effective rainfall events >7 mm and the number of rainfall events >7 mm within a 24 h period accumulated between sampling times during the autumn fallow and the growing season were recorded based on daily data n order to identify potential N leaching events beyond either the soil surface (>10cm) or beyond the rooting depth. All soils from incubated tubes were processed and analysed for soil gravimetric water and mineral N content as previously described. Daily net mineral N fluxes (kg ha^{-1} day^{-1}) for the surface soil was calculated using the difference in the mineral N content between two consecutive sampling dates divided by the number of days for that sampling interval.

S1.2 Results

S1.2.1 Autumn fallow surface soil mineral nitrogen and plant available water fluxes

S1.2.1.1 Relationship with environmental variables

Soil mineral N and PAW to 10 cm soil depth showed significant variation over the autumn fallow. As a general rule, soil nitrate-N was the dominant form of surface mineral N, and measures of both nitrate-N and ammonium-N were highly variable within each residue experiment. As a result of the variation, a significant shift in N was generally in the order of more than 5 kg N ha^{-1}. Total effective rainfall during the autumn fallow (17 March to 20 May 2015) was 64 mm below average rainfall for the region during that period (decile 4) and individual rainfall events were mainly small with only one event > 7 mm occurring in each of the first, second and third sampling intervals.

There were periods of positive surface soil mineral N flux in response to rainfall events, and the residue removed experiment required only one wetting event to induce a positive surface soil mineral N flux, whereas where wheat residues were retained it required two wetting

events. Overall, the surface soil PAW (0-10 cm) was highest on 20 May 2015 > 6 May 2015 = 22 April 2015 = 9 April 2015 > 17 March 2015 for all residue experiments (Fig. 1), indicative of the pattern of inputs from rainfall and a decreasing potential for soil evaporation losses towards the end of the fallow as temperature decreased (from Fig. 1 in Chapter 2). Surface soil PAW on 9 April was 1.2 times higher than on 17 March 2015 on average in all areas, and with a positive surface soil mineral-N flux on 9 April 2015 under bare soil conditions (wheat removed). From 6 May 2015 to 20 May 2015, surface soil mineral N decreased by 23% in the residue removed experiment, 37% where wheat residue had been retained and 13% in the lupin residue retained experiment, while surface soil PAW increased by 69, 356 and 69% respectively, with 8% v v^{-1} representing 100% of PAW in the surface soil.

Mean temperature decreased from 24°C on 17 March 2015 to 12°C on 22 April 2015 (from Fig. 1 in Chapter 2) and from this point onwards until 20 May 2015 temperature appeared to be the main driver of surface soil mineral N fluxes. However, there were no significant relationships between daily net N mineralisation and both the maximum and mean temperatures across all residue experiments over the autumn fallow season. The correlation analyses that were explored to explain potential relationships between surface soil mineral N and plant available water and environmental variables were generally scattered and the coefficient of determination was low (r^2 <0.6).

S1.2.1.2 Residue removed surface soil mineral nitrogen dynamics

Surface soil mineral N in the residue removed experiment was on average 14±6 kg ha^{-1} with a range between 5-33 kg ha^{-1} (minimum-maximum) and it was lower (p<0.0001) at the start of the autumn fallow than during the remainder of the period. Surface soil mineral N increased by 62% during the period 17 March to 9 April 2015, which resulted in a net release of mineral-N of 4 kg ha^{-1}, and from 9 April to 22 April 2015 it increased by 45% with a mean mineral N flush of 5 kg ha^{-1} (Fig. 1). At the end of the fallow period surface soil mineral N in the residue removed experiment decreased from 20±3 (on 6 May) to 16±1 kg ha^{-1} (on 20

May) (Fig. 1a). Daily net surface soil mineral N fluxes in the residue removed experiment was 0.08 kg ha day^{-1} and ranged between -0.20 to -0.32 kg ha^{-1} day^{-1} (minimum-maximum) over the autumn fallow period.

S1.2.1.3 Wheat residue surface soil mineral nitrogen dynamics

Surface soil mineral N in the wheat residue experiment fluctuated over time (p=0.0008). Mean surface soil mineral N was 17±7 kg ha^{-1} ranged between 5-32 kg ha^{-1} (minimum-maximum). On 22 April surface soil mineral N was 2.4 times higher relative to 9 April (Fig. 1b) representing a flush of 16 kg N ha^{-1}. Towards the end of the autumn fallow period surface soil mineral N decreased from 21±4 (on 6 May) to 13±1 kg ha^{-1} (on 20 May). Daily net surface soil mineral N ranged between -0.34-0.61 kg ha^{-1} day^{-1} (minimum-maximum) throughout the autumn fallow period.

S1.2.1.4 Lupin residue surface soil mineral nitrogen dynamics

Surface soil mineral N where lupin residues had been retained oscillated over the autumn fallow period (p=0.03). A net negative (or neutral) flux of surface soil mineral N of -5±10 kg ha^{-1} was measured for the autumn fallow period. However, mean surface soil mineral N for the lupin residue experiment was 20±6 kg ha^{-1}, ranged between 9-34 kg ha^{-1} (minimum-maximum) and there was 5 kg ha^{-1} more mineral N on average than for the other residue experiments throughout the autumn fallow period (Fig. 1c). Daily net surface soil mineral N fluxes for the lupin residue experiment ranged between -0.36-0.83 kg ha^{-1} day^{-1} (minimum-maximum).
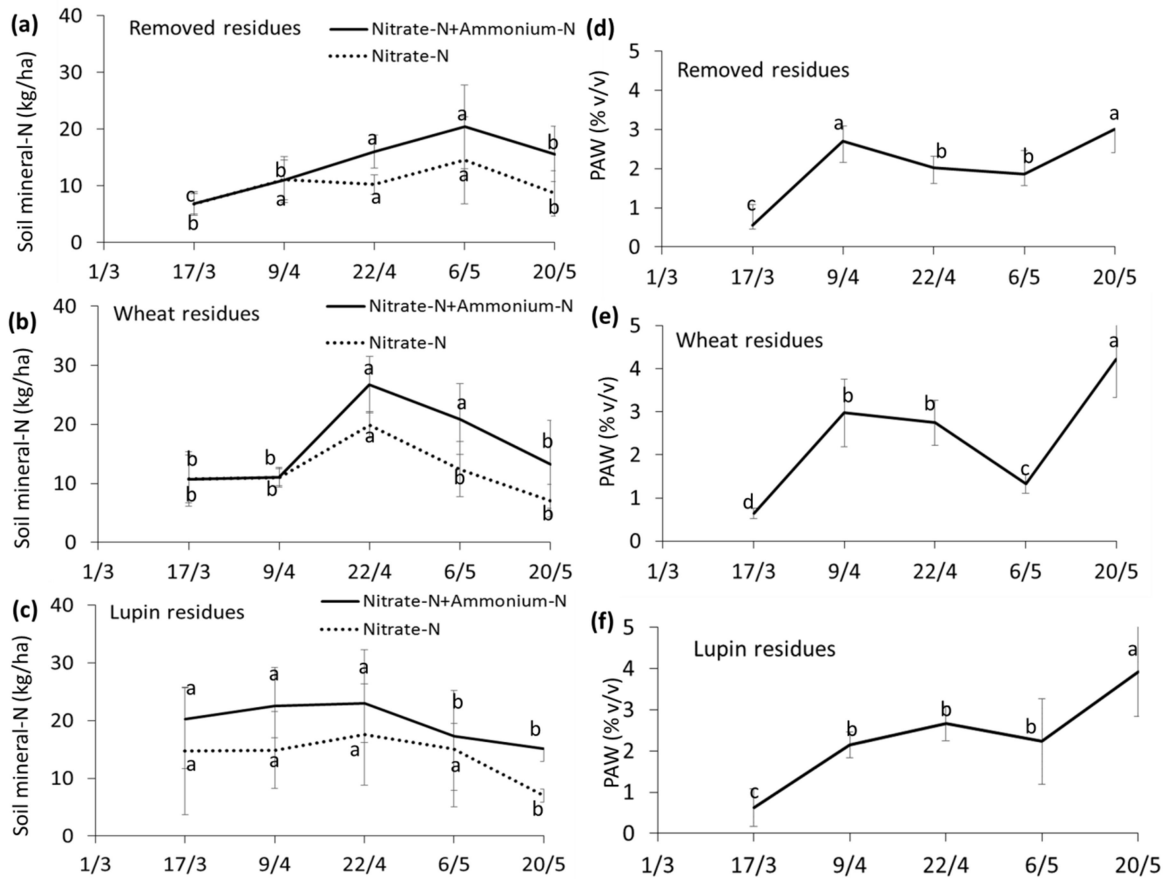
Figure 1. Surface soil (0-10 cm depth) mineral N **(a-c)** and plant available water (PAW %, v v^{-1}) **(d-f)** during the 2015 autumn fallow for the three residue experiments: removed, wheat and lupin. Lines indicate mean value of 4 replicates. Within a residue, time points with different letters indicate significant differences of the mean nitrate-N, Nitrate+Ammonium-N or PAW (DGC test, p<0.05).

S1.2.2 In crop surface soil mineral nitrogen and plant available water fluxes

S1.2.2.1 Relationship with environmental variables

Surface 10 cm soil mineral N was higher with the 55N treatment in all residue experiments (Fig. 2). Also, surface soil mineral N tended to be lower for all three residue management areas at the end of the experiment (30 November 2015) than when the N fertiliser treatments were imposed (16 June 2015), and thus, resulted in negative net flux of surface soil mineral N for the whole season regardless of the fertiliser N treatment. These mean net fluxes of mineral N in the surface soil were -15±3, -12±5, and -17±7 respectively for the residue removed,

wheat and lupin residue experiments (Fig. 2). Surface soil mineral N fluxes during the growing season corresponded with surface soil PAW dynamics, particularly from 16 June to 15 September since higher mineral N was mostly measured when PAW was near or at field capacity (Fig. 2d, e, f). Despite the correlation analysis that were performed between the soil mineral N and plant available water against temperature and rainfall during the wheat growing season, the relationships were not consistent or significant due to the high variability that was observed in the measurements (Fig. 2).

Net positive soil surface mineral N fluxes tended to follow conditions of increased surface soil PAW and temperature during the growing season. Potential surface N leaching events were infrequent and only 5 events >7 mm were recorded over the season. These potential leaching events corresponded with negative surface mineral N fluxes during the season (Fig. 2). Daily in crop surface soil net N mineralisation/immobilisation ranged between -0.37 and 0.95 kg ha^{-1} day^{-1} (minimum-maximum) for all residue experiments. Furthermore, only one rainfall event with potential to cause N leaching beyond the crop rooting depth (>40 cm) was recorded on 2 August and coincided with a negative surface soil mineral-N flux in this sampling interval for all residue experiments.

Surface soil PAW fluxes during the growing season appeared to be driven by rainfall patterns from 27 May to 7 October 2015 with positive fluxes following rainfall events. Whereas from October onwards negative fluxes in surface soil PAW were most likely to have been caused by increased evapotranspiration from the system caused by the high temperatures (Fig. 2) combined with rapid crop growth (Fig. 2 of Chapter 2). In all treatments surface soil PAW was at field capacity (on 4 August 2015) after a 27-day period with 62 mm of rainfall, whereas surface soil PAW was below field capacity between 16 June to 8 July, and from 25 August to 15 September 2015 (Fig. 2). Surface soil PAW was at crop lower limit on 27 May and from 7 October onwards. Surface soil PAW remained at crop lower limit even on 17 November following a period of 67 mm rainfall, probably due to the high evaporation associated with high temperatures at that time of the season (from Fig. 1 of Chapter 2).

Overall, during the season surface soil PAW covered the full range of crop lower limit to field capacity, and in some cases exceeded the predicted field capacity.

S1.2.2.2 Residue removed: surface soil mineral N dynamics in response to fertiliser N

Fertiliser N rate affected mineral N fluxes during the season in the surface soil where residues had been removed (Fig. 2a). There were no differences (p=0.48) in surface soil PAW for the time x N rate interaction but a higher surface soil PAW (p<0.0001) was found between 4 Autust and 15 September 2015 regardless of the N rate (Fig. 2d). Surface soil mineral N content remained mostly unchanged for the low N rate. However, at the high N rate a mineral N flush occurred in the surface soil after 7 October 2015 and surface soil mineral N was thus higher (p=0.003) on 27 October 2015 and on 30 November 2015 (Fig. 2a). An increased temperature on 27 October 2015 resulted in a flush of 16 kg ha^{-1} more (p=0.003) soil mineral N than on 7 October 2015 at the high N rate in the area where wheat residue had been removed (Fig. 2a).

S1.2.2.3 Wheat residue retained: surface soil mineral nitrogen dynamics in response to fertiliser nitrogen

Surface soil mineral N fluxes in the wheat residue retained experiment were unaffected by the fertiliser N rates over the season (p=0.15), and hence both fertiliser N treatments exhibited a similar pattern to wheat residue removed (Fig. 2b). The mineral N in the surface soil varied widely during the season (p=0.0001) from 5-36 kg ha^{-1} (minimum-maximum) and was on average 19±9 kg ha^{-1}. Higher PAW (p=0.0001) in the surface soil layer was observed from 8 July to 15 September, especially with 55N treatment where PAW was at field capacity (Fig. 2e). Surface soil mineral N in the wheat residue retained experiment appeared to be higher on 27 October than on the previous sampling date, especially with 55N treatment where the increment was 17 kg ha^{-1}. Between 7 October and 27 October 2015 the mean temperature changed from 13 to 16°C and the maximum from 20 to 28°C.

S1.2.2.4 Lupin residue retained: surface soil mineral nitrogen dynamics in response to fertiliser nitrogen

Where lupin residue had been retained the fertiliser N rate significantly affected (p<0.0001) surface soil mineral N fluxes throughout the growing season. Flushes in the area with lupin residues occurred on 7 October 2015 with the low N rate, which was 7 kg ha^{-1} higher than on 15 September, and on 27 October with the high N rate that was 21 kg ha^{-1} higher than on 7 October. The in crop N fertiliser application on 16 June for 55N treatment resulted in an increase surface soil mineral N as measured on 4 August, and this was 21 kg ha^{-1} higher (p<0.0001) than the 20N treatment. There were 2 flushes of surface soil mineral N in total in the 20N treatment, with increased amounts measured on 25 August and 7 October. Surface soil mineral N ranged between 9-55 kg ha^{-1} (minimum-maximum) with a mean value of 23±7 vs. 30±5 kg ha^{-1} for the 20N and 55N rates respectively (Fig. 2c). There were no differences (p=0.12) in surface soil PAW for the time x N rate interaction but a higher surface soil PAW (p<0.0001) was found between 4 August and 15 September 2015 regardless of the N rate (Fig. 2f).

152
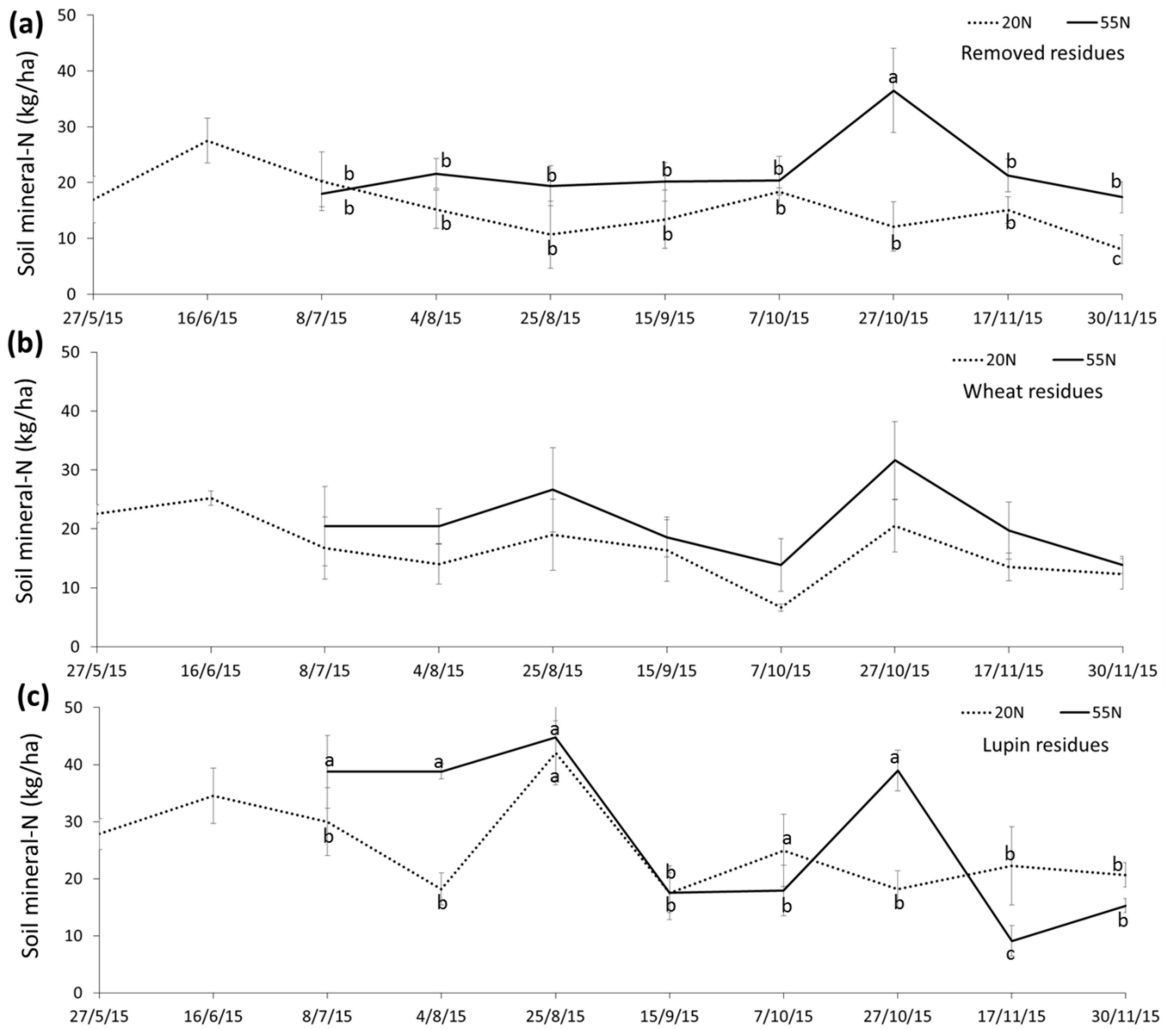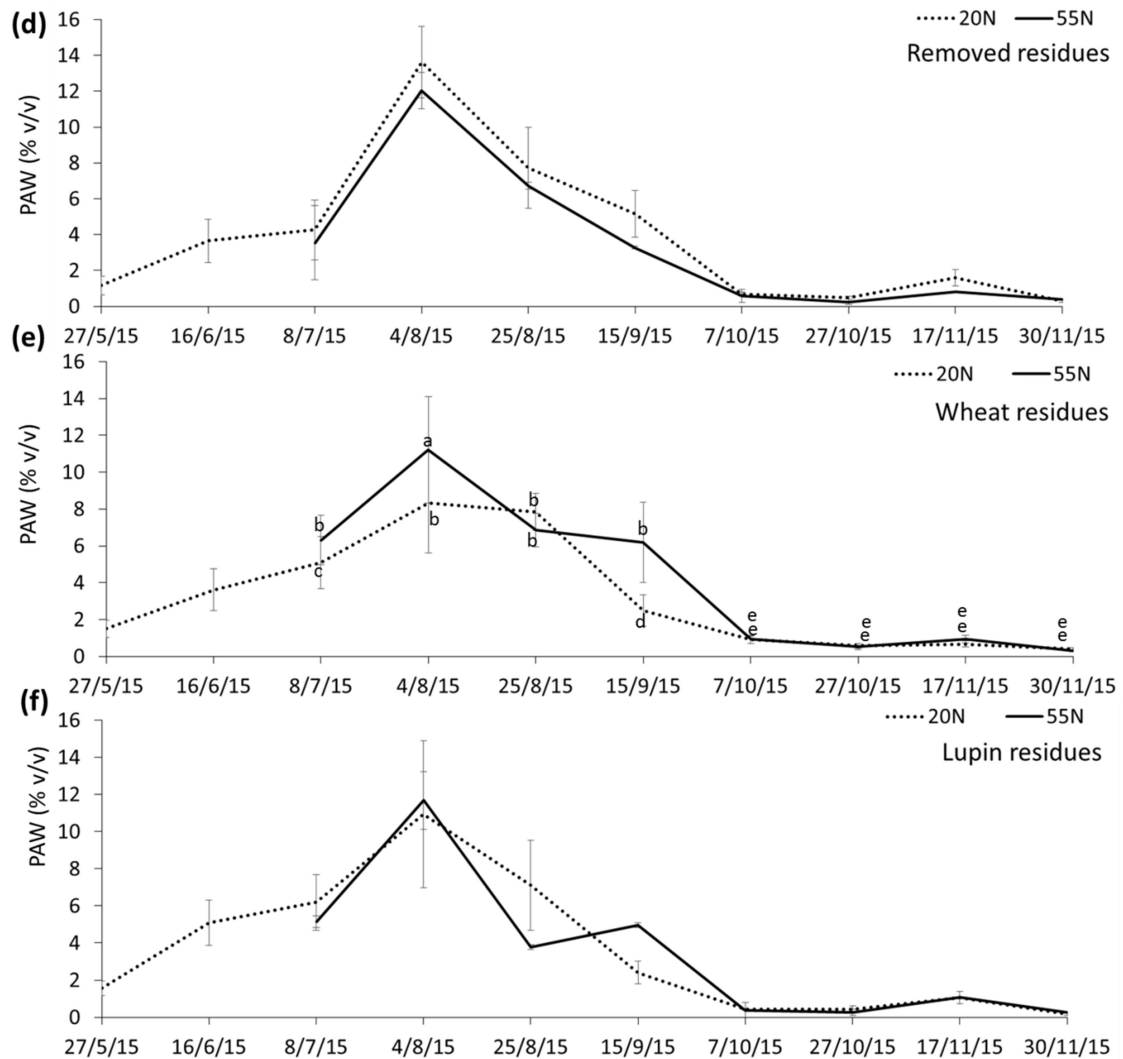
Figure 2. Surface soil mineral N (kg N ha^{-1}) and plant available water (PAW %, v v^{-1}) to 10 cm depth during the 2015 growing season for the residue removed **(a, d respectively)**, wheat residue retained **(b, e respectively)**, and lupin residue retained experiments **(c, f respectively)** given different rates of N fertiliser (20N=20 kg N ha^{-1}, 55N=55 kg N ha^{-1}). From 27 May 15 to 16 June 2015 the lines indicate the mean value of 8 replicates, from 8 July 2015 onwards the lines indicate mean value of 4 replicates, error bars indicate standard deviation of the mean. Figures with different letters indicate significant differences of the mean for the interaction "date x N rate" (DGC test, p<0.05).

S1.3. Discussion

S1.3.1 Soil mineral nitrogen fluxes response to environmental variables during the autumn fallow under the different residue management experiments

Fluxes in surface soil mineral N observed during the autumn fallow were temporally responsive to temperature and soil water conditions which were typical for this Mallee environment. Despite the fact that there was a net negative flux in surface and profile soil mineral N during the autumn fallow for the lupin and wheat retained residue management experiments, some positive surface fluxes occurred immediately following rainfall events. The daily surface net mineral N fluxes range found for lupin residues (-0.4 to -0.8 kg ha^{-1} day^{-1}) were similar to those found during the fallow period with lupin residues on sandy soils of Western Australia (Anderson et al., 1998a). The range of the daily surface net mineral N fluxes (0-10 cm) for removed and wheat residue experiments (-0.34 to -0.61 kg ha^{-1} day^{-1}) were more wide ranging compared with those reported for sandy soils of Australia following wheat residues during a dry fallow period (-0.14 to -0.25 kg ha^{-1} day^{-1}; Clough 2001, Adcock 2005).

It was not possible to broadly describe the relationship between individual environmental parameters and fluxes in surface mineral N, rather there were key parameters at different time points in the fallow season that were likely responsible for fluxes in N. Early in the fallow the patterns of N flux were likely to have been most influenced by rewetting of dry soil. Rewetting a dry soil in the absence of plants can induce N mineralisation flushes (Cabrera, 1993; Haynes, 1986). The magnitude of this flush can be due to the microbial cell lysis that release N-compounds, the conversion of organic N to more soluble N compounds upon soil drying, or the microbial decomposition from the additional substrates derived from breakdown of water-stable soil aggregates (Stevenson and Cole, 1999). Later in the autumn fallow the process of mineralisation slowed as the mean temperatures dropped (Standford et al. 1975), and as the profiles wet up there was potentially leaching (Anderson et al., 1998b, McNeill and Unkovich, 2007) resulting in the net negative flux of N for all residue experiments in the autumn fallow. In all residue experiments, the surface soil mineral N at the end of the autumn fallow (on 20 May 2015) was lower than at the preceding sampling time and corresponded with a potential N leaching event beyond 15 cm soil depth. The higher

surface soil ammonium-N relative to nitrate-N at the end of the fallow further indicates that there may have been nitrate-N losses due to a leaching rainfall event as it was previously indicated (Hoyle and Murphy, 2006), and there were 15 mm of effective rainfall occurring at the end of fallow.

The residue load and quality appeared to have an important effect on the temporal N fluxes. At the time of sowing the subsequent wheat crop mineral N in the soil profile to 100 cm depth in the lupin residue experiment had 22 kg N ha^{-1} more than where wheat residues had been retained or removed, likely due to a greater mineralisation of N from the legumes compared with cereal residues during the fallow. The higher load and wider C:N for wheat residues retained would be expected to result in higher N immobilisation compared with legumes or cereal residue removed. In soils of South Australia, Adcock (2005) reported apparent net N immobilisation during the summer fallow of 21 kg ha^{-1} of nitrate-N in the soil to 80 cm depth following wheat residues when summer fallow rainfall was low (123 mm) and comparable to the one recorded in the present study (131 mm). Below-ground residues make an important contribution to soil organic matter turnover (McNeill and Fillery 2008; Adcock 2005). A positive surface mineral N flux was observed in the removed residue experiment following an increase in soil water on 22 April 2015. However, where wheat residues were retained, a positive flux in surface soil mineral N was only observed after two consecutive periods of soil wetting. This delay was likely due to temporary immobilisation of N in these above ground plant parts (van Schreven, 1968). The increase in the C:N of the crop residues with time over the fallow is indicative of the N released through decomposition of residues over time, with the N in lupin biomass (9-87 kg N ha^{-1}, minimum-maximum) agreeing with previous measurements in low-rainfall sandy environments (Ladd et al., 1983a; Barton et al., 2011; Evans, 2001; Unkovich et al., 2010a). In sandy soils of the Mallee region (Sadras and Baldock, 2003) N mineralisation rate during the fallow phase of canola and wheat (December-March) was mostly explained by the size and number of rainfall events. In a sandy loam soil of South Australia, differences in the amount of summer rainfall explained

the magnitude of the fallow N mineralisation (Adcock, 2005). While these in fallow measurements were able to indicate the key factors that influence N flux, we were not able to develop a broad description of the relationships between environmental factors and mineral N flux, in order to model and predict fallow N mineralisation. Hence, to achieve an objective of understanding soil N supply to meet crop demand, the inability to predict fallow N dynamics necessitates the continued practice of pre- or at- sowing profile N measurements.

S1.3.2 Soil mineral nitrogen and plant available water fluxes during the wheat growing season were responsive to environmental variables and/or fertiliser nitrogen inputs

During the wheat growing season, net positive surface soil mineral N fluxes were associated with significant rainfall events when the temperature was below 15°C, while rainfall events were less important as the temperature increased (Sommers, et al., 1981; Standford et al., 1975). The different trend in soil mineral N fluxes from winter-early spring *vs.* mid-late spring was more evident where legume residues had been retained. In general, the higher mineral N content in the soil from the winter-early spring period (16 June to 15 September 2015) coincided with higher PAW which was close to field capacity, which is commonly found as the optimal soil water condition for mineralisation (Angus, 2001; Vigil et al., 2002). A flush of surface soil mineral N on 27 October 2015 following an increased temperature where the lupin residues had been retained was indicative of an increased rate of decomposition of plant residues and mineralisation processes (Haynes, 1986; Angus, 2001; Vigil et al., 2002).

The lower soil mineral N content at the end of the experiment in all residue experiments could be the result of crop water uptake during the growing season, low microbial activity due to higher soil temperatures (>20°C) in combination with low soil water content (Murphy et al., 1998) that prevailed during October and November. From 7 October to 30 November 2015, the surface PAW was closer to crop lower limit in all residue treatments. Some studies have found that the amount of N mineralised is increased with higher fertiliser N inputs (Gill et al.,

1995) due to a greater N availability for microbial biomass after N applications, and thus, a greater potential for the return through readily degradable plant materials and mineralisation (Gill et al., 1995, Ladd et al., 1994). However, the different rates of applied fertiliser N had no significant effect on surface soil mineral N fluxes where wheat residues had been retained, likely due to the large variance found in the measurements which is a common experience in low rainfall sandy environments (Sadras and Baldock 2003).

S1.4. Conclusion

While the autumn fallow had a negative net soil mineral N flux across residue experiments, there were positive fluxes in surface mineral N at some sampling points in response to variation in both temperature and soil water conditions, but the fallow mineral N flux could not be readily predicted based on the parameters measured. Prediction of the potential soil N supply required profile mineral N measurement at the time of sowing. In crop, surface mineral N measurements were able to explain most of the N supply relationships, but at the end of season assessment of profile mineral N and water was key to understanding the effect of treatment on crop N uptake.

References

Adcock, D.P. 2005. Soil water and nitrogen dynamics of farming systems on the Upper Eyre Peninsula, South Australia. PhD Thesis thesis, The University of Adelaide.

Anderson, G.C., I.R.P. Fillery, P.J. Dolling and S. Asseng. 1998a. Nitrogen and water flows under pasture-wheat and lupin-wheat rotations in deep sands in Western Australia. 1. Nitrogen fixation in legumes, net N mineralisation,and utilisation of soil-derived nitrogen. Australian Journal of Agricultural Research 49, 329-344.

Anderson, G.C., I.R.P. Fillery, F.X. Dunin, P.J. Dolling and S. Asseng. 1998b. Nitrogen and water flows under pasture-wheat and lupin-wheat rotations in deep sands in Western Australia. 2. Drainage and nitrate leaching. Australian Journal of Agricultural Research 49, 345-362.

Angus, J.F. 2001. Nitrogen supply and demand in Australian agriculture. Australian Journal of Experimental Agriculture 41: 277-288. http://dx.doi.org/10.1071/EA00141

Angus, J.F., T.P. Bolger, J.A. Kirkegaard and M.B. Peoples. 2006. Nitrogen mineralisation in relation to previous crops and pastures. Soil Research 44: 355-365. doi:http://dx.doi.org/10.1071/SR05138.

Barton, L., K. Butterbach-Bahl, R. Kiese and D.V. Murphy. 2011. Nitrous oxide fluxes from a grain–legume crop (narrow-leafed lupin) grown in a semiarid climate. Global Change Biology 17, 1153-1166.

Cabrera, M.L. 1993. Modeling the flush of nitrogen mineralization caused by drying and rewetting soils. Soil Science Society of America Journal 57: 63-66. doi:10.2136/sssaj1993.03615995005700010012x.

Clough, A. 2001. Nitrogen and carbon mineralisation in agricultural soils of South Australia. PhD Thesis, The University of Adelaide, Adelaide, SA, Australia.

Evans, J., A.M. McNeill, M.J. Unkovich, N.A. Fettell and D.P. Heenan. 2001. Net nitrogen balances for cool-season grain legume crops and contributions to wheat nitrogen

uptake: a review. Australian Journal of Experimental Agriculture 41: 347-359. doi:10.1071/ea00036.

Gill, K., S.C. Jarvis and D.J. Hatch. 1995. Mineralization of nitrogen in long-term pasture soils: effects of management. Plant and Soil 172: 153-162. doi:10.1007/BF00020869.

Haynes, R.J. 1986. Chapter 2 - The decomposition process: mineralization, immobilization, humus formation, and degradation. In: R. J. Haynes, editor Mineral nitrogen in the plant–soil system. Academic Press. p. 52-126.

Hoyle, F.C. and D.V. Murphy. 2006. Seasonal changes in microbial function and diversity associated with stubble retention versus burning. Soil Research 44: 407-423. doi:http://dx.doi.org/10.1071/SR05183.

Kolberg, R.L., B. Rouppet, D.G. Westfall and G.A. Peterson. 1997. Evaluation of an in situ net soil nitrogen mineralization method in dryland agroecosystems Soil Science Society of America Journal 61: 504-508.doi:10.2136/sssaj1997.03615995006100020019x.

Ladd, J.N., M. Amato, L-K. Zhou and J.E. Schultz. 1994. Differential effects of rotation, plant residue and nitrogen fertiliser on microbial biomass and organic matter in an Australian alfisol. Soil Biology and Biochemistry 26: 821-831.

Ladd, J.N., R.B. Jackson, M. Amato and J.H.A. Butler. 1983a. Decomposition of plant material in Australian soils. I. The effect of quantity added on decomposition and on residual microbial biomass. Soil Research 21: 563-570.

McNeill, A.M. and M. Unkovich. 2007. The nitrogen cycle in terrestrial ecosystems. In: P. Marschner and Z. Rengel, editors, nutrient cycling in terrestrial ecosystems. Springer Berlin Heidelberg. p. 37-64.

McNeill, A.M. and I.R.P. Fillery. 2008. Field measurement of lupin belowground nitrogen accumulation and recovery in the subsequent cereal-soil system in a semi-arid Mediterranean-type climate. Plant and Soil 302: 297-316.

Miranda, K.M., M.G. Espey and D.A. Wink. 2001. A Rapid, Simple Spectrophotometric

    Method for Simultaneous Detection of Nitrate and Nitrite. Nitric Oxide 5: 62-71.

Raison, R.J., M.J. Connell and P.K. Khanna. 1987. Methodology for studying fluxes of soil

    mineral-N in situ. Soil Biology and Biochemistry 19: 521-530.

    doi:http://dx.doi.org/10.1016/0038-0717(87)90094-0.

Sadras, V.O. and J.A. Baldock. 2003. Influence of size of rainfall events on water-driven

    processes. II. Soil nitrogen mineralisation in a semi-arid environment. Australian

    Journal of Agricultural Research 54: 353-361. doi:http://dx.doi.org/10.1071/AR02113.

Sommers, L.E., C.M. Gilmour, R.E. Wildung and S.M. Beck. 1981. The effect of water

    potential on decomposition processes in Soils1. In: J. F. Parr, W. R. Gardner and L. F.

    Elliott, editors Water potential relations in soil m icrobiology. Soil Science Society of

    America. p. 97-117.

Stanford, G. M.H. Frere and R.A. Vander Pol. 1975. Effect of fluctuating temperatures on soil

    nitrogen mineralization. Soil Science 119: 222–226.

Stevenson, F.J. and M.A. Cole. 1999. Cycles of Soil: Carbon, nitrogen, phosphorus, sulfur,

    micronutrients. John Wiley & Sons, Inc., New York.

Unkovich, M.J., J. Baldock and M.B. Peoples. 2010a. Prospects and problems of simple linear

    models for estimating symbiotic N2 fixation by crop and pasture legumes. Plant and

    Soil 329: 75-89.

Van Schreven, D. A. 1968. Mineralisation of the carbon and nitrogen of plant material added

    to soil and of the soil humus during incubation following periodic drying and

    rewetting of the soil. Plant and Soil 28: 226-245. doi:10.1007/BF01880241.

Vigil, M.F., B. Eghball, M.L. Cabrera, B.R. Jakubowski and J.G. Davis. 2002. Accounting for

    seasonal nitrogen mineralisation: An overview. Journal of Soil and Water

    Conservation 57: 464-469.

S.2 Accessory material for paper published

The following data corresponds to the supplementary material of the paper that has been

published in *Journal of Plant Nutrition and Soil Science.*

Muschietti Piana MP, T McBeath, A McNeill, V Gupta, & PA Cipriotti (2020) Combined

nitrogen input from legume residues and fertilizer improves early nitrogen supply and

uptake by wheat. *Journal of Plant Nutrition and Soil Science* 183: 355–366. DOI:

10.1002/jpln.202000002

S2.1. Methodological details

S2.1.1 Crop history of the sites

For the wheat and lupin residue retained experiments in site 1, the wheat aerial residues were

retained from the plots where previous wheat crop (cv. Corack) had been sown on 5 June

2014 at a seed rate of 70 kg ha^{-1} along with 50 kg ha^{-1} of di-ammonium phosphate banded

below the seed and harvested on 2 December 2014. Lupin residues were retained from the

plots where previous narrow-leafed lupin crop (cv. Mandelup) which had been sown on 14

April 2014 at a rate of 100 kg ha^{-1} along with 50 kg ha^{-1} of di-ammonium phosphate banded

below the seed. A biomass of 11 t ha^{-1} was recorded for lupin crop on 22 September 2014

when it was brown manured. For the wheat removed experiment (hereafter referred as

'removed residue experiment') aerial residues were removed manually using a low cut and

rake on 17 February 2015.

For the wheat and lupin residue retained treatments in site 2, the wheat aerial residues were

retained from the previous wheat crop (cv. Corack) which was harvested on 2 December 2015

with an average grain yield from 24 plots of 1.4±0.3 t ha^{-1}. Lupin residues were retained from

the previous narrow-leafed lupin crop (cv. Mandelup) which was harvested on 2 December

2015 with an average grain yield from 12 plots 0.5±0.2 t ha^{-1}. For the wheat removed

treatment (hereafter referred as 'removed residue' treatment), wheat aerial residues were

removed manually using a low cut and rake on 2 December 2015. History of the area of both sites was continuous wheat from 2009-2013.

S2.1.2 Soil sampling and characterisation of the experimental sites

For soil characterisation of physical and chemical properties in site 1 (Table S1.2.2, S1.2.3); deep soil cores were collected from 4 of each residue experiment at the start of the experiment (17 March 2015) using a hand auger (3.6 cm internal diameter). Composite soil samples of the 4 replicates for each depth increment taken from the removed residue experiment were used for particle size analysis (as described below), totaling 6 samples. For the other chemical parameters (Table S1.2.2, S1.2.3), replicates were combined into 1 representative sample at each depth interval for each residue experiment, totaling 18 samples.

In site 2, composite soil samples of 1 replicate of each residue treatment were combined into 1 representative sample at each depth interval, totaling 6 samples, and were used to analyse soil for the chemical parameters described below (Table S1.2.3).

In both sites soils were characterised for; particle size analysis with the pipette method in site 1 (McKenzie et al. 2002) and using (MIR) spectroscopy in site 2 (Viscarra Rossel et al., 2006), bulk density (Burk and Dalgliesh, 2008), pH and electrical conductivity (1:5, soil:water suspension), and cation exchange capacity (Rayment and Lyons, 2011), total organic C in surface soil (0-10 cm depth) by dry combustion using Leco TruMAC (2000-CNS, Leco Australia Pty. Ltd.), and to 10-100 cm depth (in 5 intervals) using MIR (Viscarra Rossel et al., 2006). In site 1, surface soils (0-10 cm) were also analysed for extractable phosphorus, potassium, sulfur, and micronutrients (Table S1.2.2).

In both sites, soils were characterised to 100 cm depth in 6 intervals for bulk density (Table S1.2.3) using a 5 cm internal diameter hand auger, the base of the hole leveled with an auger leveling head and the intact sample taken using a sliding hammer sampler (Burk and Dalgliesh, 2008). In both sites, these samples were used to estimate plant available water

capacity, and in site 2, these samples were also used to charactherise soils for particle size in each layer using MIR (Table S1.2.3).

Table S1.2.1: Site and management details for the experiments in 2015-2016 at Lowaldie, South Australia. DAS=days after sowing.

	2015 (Site 1)	2016 (Site 2)
Plot size	1.7 m wide x 6 m long	1.7 m wide x 14 m long
Fallow period	(Autumn) 17 March to 20 May 2015	(Whole) 2 December 2015 to 1 June 2016
Wheat sowing date (Cultivar)	21 May 2015 (cv. Mace)	2 June 2016 (cv. Scepter)
Seedling rate	70 kg ha^{-1}	70 kg ha^{-1}
Trace elements application: foliar Zn, Cu and Mn sulphate: 400 g ha^{-1} of $CuSO_4$, 3 kg ha of $MnSO_4$ sulphate and 1.5 kg ha^{-1} of $ZnSO_4$ heptahydrate	18 August 2015	25 August 2016
Pre-emergent herbicide formulation	Roundup Ultra® at 1 L ha^{-1}, Trifluralin 480® at 1.5 L ha^{-1}, Avadex Xtra at 1.6 L ha^{-1} and 800 g ha^{-1} sulphate of ammonia, Spreadwet 1000® at 10 mL 100 mL^{-1} water	Roundup Ultra® at 1 L ha^{-1}, Trifluralin 480® at 1.5 L ha^{-1}, Avadex Xtra at 1.6 L ha^{-1} and 800 g ha^{-1} sulphate of ammonia
Growing season rainfall	195 mm Below average (decile 3)	316 mm Above average (decile 9)
Plant and soil sampling at key growth stages (DAS)		
Tillering	8 July (48)	20 July (48)
First node	12 August (83)	22 August (81)
Flag leaf	----	15 September (105)
Booting	10 September (112)	---
Anthesis	1 October (133)	11 October (131)
Physiological maturity (Harvest)	30 November (193)	7 December (188)
Experimental design and treatments	3 different residue management experiments: removed, wheat, lupin, with 2 fertiliser N treatments (randomised) per experiment: 20, 55 kg N ha^{-1} (low or high N rate)	Split plot design with 3 residue treatments (main plot, randomised): removed, wheat, lupin, with 3 fertiliser N treatments (sub-plot, randomised): 0, 20, 40 kg N ha^{-1} (without, low or high N rate)
Fertiliser N inputs	At sowing: 50 kg ha^{-1} as di-ammonium phosphate and 24 kg N ha^{-1} as urea (for the low N	At sowing: 43 or 87 kg ha^{-1} of urea (for the low and high N rates respectively)

rate)

At seedling (26 DAS): 76 kg N
ha^{-1} as urea (for the high N rate)

Table S1.2.2: Soil surface properties of site 1. P=phosphorus, K=potassium, S=Sulfur, Cu=copper, Fe=iron, Mn=manganese, Zn=zinc.

Soil Depth (cm)	C:N ratio	Organic N (%)	Colwell P (mg kg^{-1})	Collwell K (mg kg^{-1})	KCl S (mg kg^{-1})	DTPA Cu (mg kg^{-1})	DTPA Fe (mg kg^{-1})	DTPA Mn (mg kg^{-1})	DTPA Zn (mg kg^{-1})
0-10	12:1	0.05	25	100	3.5	0.4	33.7	3.2	1.5

Table S1.2.3: Soil profile physical and chemical properties of both sites. BD=Bulk density, OC=organic carbon, EC=electrical conductivity, CEC=cation exchange capacity, ESP=exchangeable sodium percentage. For site 1, mean values of 3 different residue experiments ± standard deviation, for site 2, mean values of 3 replicates ± standard deviation.

Depth (cm)	BD (g cm^{-3})	Sand (%)	Clay (%)	OC (%)	pH 1:5 (*)	EC 1:5 (dS m^{-1})	CEC (meq 100g^{-1})	ESP (%)	Boron (mg kg^{-1})
					Site 1 (2015)				
0-10	1.49	95.6	3.3	0.6±0.0	6.1±0.1	0.02±0.0	3.1±0.0	0.7±0.6	0.5±0.1
10-20	1.50	96.7	2.7	0.3±0.0	6.3±0.0	0.03±0.2	3.3±0.0	2.7±1.6	0.5±0.1
20-40	1.64	97.0	2.6	0.2±0.1	6.3±0.1	0.03±0.1	6.7±0.0	3.8±1.4	1.8±1.5
40-60	1.68	92.8	6.9	0.1±0.0	6.5±0.1	0.06±0.1	14.2±0.8	8.2±3.9	8.7±1.5
60-80	1.68	79.2	19.1	0.2±0.1	7.3±0.2	0.16±1.7	19.3±0.1	22.1±5.2	13.0±1.6
80-100	1.50	56.9	34.1	0.1±0.0	8.2±0.0	0.24±0.5	22.0±0.0	23.0±4.5	17.3±1.5
					Site 2 (2016)				
0-10	1.43	99.9	0.1	0.6	7.1	0.11	3.4±0.2	1.9±0.2	0.3±0.2
10-20	1.55	99.8	0.2	0.6	7.3	0.22	3.8±0.0	3.3±0.1	0.4±0.0
20-40	1.65	99.4	1.4	0.4	7.5	0.21	5.0±0.7	4.7±1.3	0.6±0.1
40-60	1.75	85.5	15.0	0.2	8.2	0.15	11.8±0.1	13.2±0.2	3.6±0.3
60-80	1.76	62.9	29.9	0.2	8.7	0.16	33.2±2.0	12.6±0.2	12.8±1.2
80-100	1.48	47.1	38.2	0.4	7.7	0.25	37.6±0.7	14.6±0.1	15.7±0.7

S2.1 Accessory results

Summary of the statistical differences among treatments in both years

Table S2.1.1: Summary showing main factor effects and interactions for residue and/or fertiliser N treatments and/or growth stages in 2015 and 2016, where * is p<0.05 and ** is p<0.001, # is for variables measured at harvest. R=Residue treatment, N=fertiliser N treatment, GS=growth stage, NA=not applicable, NS=not significant, WUE=water use efficiency in grain, NUE=N use efficiency in grain.

| Factors | 2015 (Site 1) | | | | | | | | | 2016 (Site 2) | | | |
| | Removed residue management experiment | | | Wheat residue management experiment | | | Lupin residue management experiment | | | | | | |
	N	GS	Interaction N x GS	N	GS	Interaction N x GS	N	GS	Interaction N x GS	R	N	GS	Interactions
Wheat aerial biomass (t ha^{-1})	NS	NS	NS	NS	NS	NS	**	**	**	**	**	*	R x GS** N x GS**
Wheat N uptake (kg ha^{-1})	NS	NS	NS	NS	NS	NS	*	*	*	**	**	*	R x GS** N x GS**
Sum of soil mineral N (0-100 cm, kg ha^{-1})	NS	NS	NS	NS	NS	NS	*	*	*	*	**	**	R x N x GS**
Sum of plant available water (0-100 cm, mm)	NS	NS	NS	NS	NS	NS	NS	NS	NS	*	NS	*	R x GS** N x GS**
Estimated net N mineralisation (kg ha^{-1})	NS	NA	NA	*	NA	NA	NS	NA	NA	NS	NS	NA	NS
#Grain yield (t ha^{-1})	NS	NA	NA	NS	NA	NA	NS	NA	NA	**	**	NA	NS
#WUE (kg grain ha^{-1} mm^{-1})	NS	NA	NA	NS	NA	NA	NS	NA	NA	**	**	NA	NS
#NUE (kg grain kg N^{-1})	*	NA	NA	*	NA	NA	NS	NA	NA	*	NS	NA	NS
#N utilisation efficiency (kg grain kg N^{-1})	*	NA	NA	*	NA	NA	NS	NA	NA	*	*	NA	R x N*
Diseases (% of incidence)	NA	NA	NA	NA	NA	NA	NA	NA	NA	*	NS	NA	NS

Summary tables of the analysis of variance (ANOVA)

Table S2.2.1: ANOVA outputs for the sum of soil mineral N (kg ha^{-1}) and PAW (mm) to 100 cm depth prior to sowing wheat on 1 June 2016 (site 2). PAW=plant available water, df=degrees of freedom. Treatment effects with significant differences at p<0.05 are highlighted in grey.

Sum soil mineral N prior to sowing	Square sum	df	Mean square	F-value	p-value
Model	12427.46	17	731.03	3.59	0.005
Block (B)	1235.34	3	411.78	2.02	0.1465
Residue treatment(R)	7499.39	2	3749.69	13.7	0.0058
R x B	1642.4	6	273.73	1.35	0.2885
Error	3661.66	18	203.43		
Total	16089.12	35			
Sum PAW prior to sowing	Square sum	df	Mean square	F-value	p-value
Model	18676.64	17	1098.63	6.27	0.0002
Block (B)	4359.99	3	1453.33	0.4	0.1011
Residue treatment (R)	1306.55	2	653.27	0.33	0.7295
R x B	11784.27	6	1964.05	1.21	0.1008
Error	3153	18	175.17		
Total	21829.64	35			

Table S2.2.2: ANOVA outputs for soil mineral N (kg ha^{-1}) and PAW (mm) in the soil profile at depth (cm) for wheat residues vs. lupin residues prior to wheat sowing on 1 June 2016 (site 2). PAW=plant available water, numDF=numerator degrees of freedom, denDF=denominator degrees of freedom. Treatment effects with significant differences at p<0.05 are highlighted in grey.

Soil mineral N at depth prior to sowing	numDF	denDF	F-value	p-value
(Intercept)	1	189	1061.93	<0.0001
Residue treatment (R)	2	6	13.54	0.006
Depth (D)	5	189	12.08	<0.0001
R x D	10	189	6.24	<0.0001
PAW at depth prior to sowing	numDF	denDF	F-value	p-value
(Intercept)	1	189	285.08	<0.0001
Residue treatment (R)	2	6	0.4	0.6854
Depth (D)	5	189	78.91	<0.0001
R x D	10	189	1.01	0.4366

Table S2.2.3: ANOVA outputs for wheat crop attributes (in bold) at wheat harvest in 2016 (site 2). NUE= N use efficiency, WUE=water use efficiency, numDF=numerator degrees of freedom, denDF=denominator degrees of freedom. Treatment effects with significant differences at p<0.05 are highlighted in grey.

Grain yield (t ha^{-1})	numDF	denDF	F-value	p-value
(Intercept)	1	18	209.53	<0.0001
Residue treatment (R)	2	7	45.41	0.0001
Fertiliser N input (N)	2	18	21.71	<0.0001
R x N	4	18	0.65	0.636
Grain N uptake (kg ha^{-1})	numDF	denDF	F-value	p-value
(Intercept)	1	18	60.93	<0.0001
Residue treatment (R)	2	7	21.16	0.0011
Fertiliser N input (N)	2	18	10.72	0.0009
R x N	4	18	0.4	0.8089
Yield as percentage of potential yield (%)	numDF	denDF	F-value	p-value
(Intercept)	1	18	437.59	<0.0001
Residue treatment (R)	2	7	24.2	0.0007
Fertilizer N input (N)	2	18	15.03	0.0001
R x N	4	18	0.63	0.6481
Harvest index	numDF	denDF	F-value	p-value
(Intercept)	1	18	224.07	<0.0001
Residue treatment (R)	2	7	7.41	0.0187
Fertiliser N input (N)	2	18	4.57	0.0248
R x N	4	18	2.24	0.1051
NUE in grain (0-100cm, kg grain kg N^{-1})	numDF	denDF	F-value	p-value
(Intercept)	1	18	88.59	<0.0001
Residue treatment (R)	2	7	9.66	0.0097
Fertiliser N input (N)	2	18	7.88	0.0035
R x N	4	18	0.56	0.693
NUE in total biomass (0-100cm, kg grain kg N^{-1})	numDF	denDF	F-value	p-value
(Intercept)	1	18	134.18	<0.0001
Residue treatment (R)	2	7	1.3	0.332
Fertiliser N input (N)	2	18	0.18	0.834
R x N	4	18	2.21	0.1094
N Uptake efficiency (%)	numDF	denDF	F-value	p-value
(Intercept)	1	18	50.96	<0.0001
Residue treatment (R)	2	7	2.07	0.1965
Fertiliser N input (N)	2	18	1.52	0.2457
R x N	4	18	0.62	0.6513
N utilisation efficiency (%)	numDF	denDF	F-value	p-value
(Intercept)	1	18	854.94	<0.0001
Residue treatment (R)	2	7	2.8	0.1278
Fertiliser N input (N)	2	18	4.69	0.023
R x N	4	18	3.26	0.0356
WUE in grain (0-100cm, kg grain mm^{-1})	numDF	denDF	F-value	p-value
(Intercept)	1	18	107.37	<0.0001
Residue treatment (R)	2	7	31.34	0.0003
Fertiliser N input (N)	2	18	28.36	<0.0001
R x N	4	18	0.92	0.4718
WUE in total biomass (0-100cm, kg grain mm^{-1})	numDF	denDF	F-value	p-value
(Intercept)	1	18	200.15	<0.0001

Residue treatment (R)	2	7	0.18	0.843
Fertiliser N input (N)	2	18	1.0	0.0487
R x N	4	18	2.44	0.0847
Net N mineralisation (0-100cm, kg ha^{-1})	numDF	denDF	F-value	p-value
(Intercept)	1	18	0.04	0.8514
Residue treatment (R)	2	7	0.23	0.8018
Fertiliser N input (N)	2	18	0.48	0.6283
R x N	4	18	2.06	0.1292
Sum of PAW (0-100cm, kg ha^{-1})	numDF	denDF	F-value	p-value
(Intercept)	1	18	790.04	<0.0001
Residue treatment (R)	2	7	0.08	0.9212
Fertiliser N input (N)	2	18	4.42	0.0275
R x N	4	18	2.82	0.0559

Table S2.2.4: ANOVA outputs for wheat biomass (t ha^{-1}) and N uptake (kg ha^{-1}), sum of soil mineral N and PAW (mm) to rooting depth over the 2015 growing season in each residue experiment (site 1). GS=growth stage, N=fertilizer N treatment, PAW=plant available water, numDF=numerator degrees of freedom, denDF=denominator degrees of freedom.
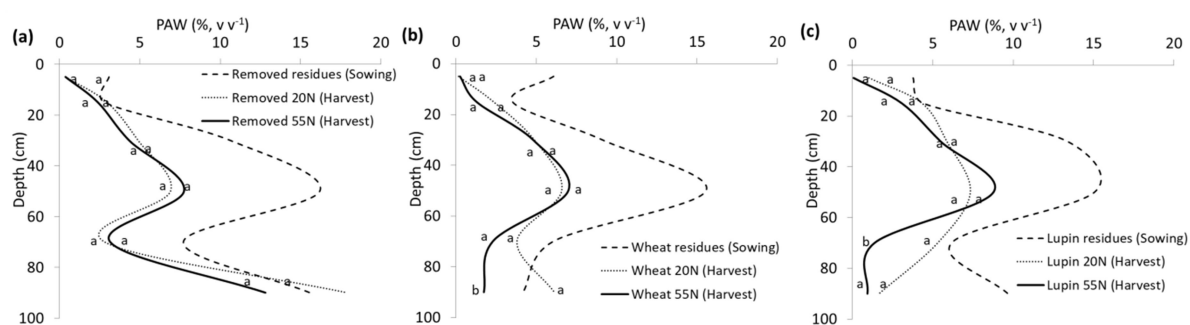
	Removed residue experiment				Wheat residue experiment				Lupin residue experiment			
Wheat biomass	numDF	denDF	F-value	p-value	numDF	denDF	F-value	p-value	numDF	denDF	F-value	p-value
(Intercept)	1	24	188.7	<0.0001	1	24	282.97	<0.0001	1	23	129.92	<0.0001
GS	4	24	75.41	<0.0001	4	24	61.3	<0.0001	4	23	155.27	<0.0001
N	1	6	0.32	0.5937	1	6	0.6	0.4685	1	23	5.18	0.0325
GS x N	4	24	2.37	0.0808	4	24	1.13	0.3675	4	23	9.61	0.0001
Wheat N uptake	numDF	denDF	F-value	p-value	numDF	denDF	F-value	p-value	numDF	denDF	F-value	p-value
(Intercept)	1	24	848.66	<0.0001	1	24	320.43	<0.0001	1	23	762.72	<0.0001
GS	4	24	10.08	0.0001	4	24	23.82	<0.0001	4	23	7.87	0.0017
N	1	6	1.14	0.3272	1	6	0.12	0.7413	1	23	26.87	0.0001
GS x N	4	24	2.9	0.0549	4	24	0.78	0.5481	4	23	3.28	0.0463
Sum soil mineral N	numDF	denDF	F-value	p-value	numDF	denDF	F-value	p-value	numDF	denDF	F-value	p-value
(Intercept)	1	24	239.1	<0.0001	1	24	614.31	<0.0001	1	23	396.58	<0.0001
GS	4	24	56.65	<0.0001	4	24	26.01	<0.0001	4	23	100.47	<0.0001
N	1	6	0.15	0.7102	1	6	3.15	0.1264	1	23	4.83	0.0384
GS x N	4	24	2.9	0.0591	4	24	15.13	<0.0001	4	23	3.6	0.0202
Sum PAW	numDF	denDF	F-value	p-value	numDF	denDF	F-value	p-value	numDF	denDF	F-value	p-value
(Intercept)	1	24	605.22	<0.0001	1	24	4917.04	<0.0001	1	23	2458.93	<0.0001
GS	4	24	39.04	<0.0001	4	24	12.1	<0.0001	4	23	73.08	<0.0001
N	1	6	0.41	0.5442	1	6	2.21	0.1875	1	23	7.35	0.0148
GS x N	4	24	2.56	0.0649	4	24	0.66	0.6268	4	23	2.02	0.1492

Table S2.2.5: ANOVA outputs for wheat biomass (t ha^{-1}) and N uptake (kg ha^{-1}), sum of soil mineral N (kg ha^{-1}) and PAW (mm) to 100 cm depth over the 2016 growing season (site 2). PAW=plant available water, numDF=numerator degrees of freedom, denDF=denominator degrees of freedom. Treatment effects with significant differences at p<0.05 are highlighted in grey.

Wheat biomass	numDF	denDF	F-value	p-value
(Intercept)	1	126	291.33	<0.0001
Residue treatment (R)	2	6	5.81	0.0394
Growth stage (GS)	4	126	727.89	<0.0001
Fertiliser N input (N)	2	126	5.98	0.0033
R x GS	8	126	16.99	<0.0001
R x N	4	126	2.91	0.0243
GS x N	8	126	9.11	<0.0001
R x GS x N	16	126	1.25	0.2421
Wheat N uptake	numDF	denDF	F-value	p-value
(Intercept)	1	124	407.61	<0.0001
Residue treatment (R)	2	6	2.59	0.1547
Growth stage (GS)	4	124	647.23	<0.0001
Fertiliser N input (N)	2	124	23.12	<0.0001
R x GS	8	124	18.19	<0.0001
R x N	4	124	2.39	0.0543
GS x N	8	124	12.77	<0.0001
R x GS x N	16	124	1.45	0.1276
Sum soil mineral N	numDF	denDF	F-value	p-value
(Intercept)	1	108	18837.75	<0.0001
Residue treatment (R)	2	6	15.43	0.0043
Growth stage (GS)	4	108	327	<0.0001
Fertiliser N input (N)	2	18	31.25	<0.0001
R x GS	8	108	17.64	<0.0001
R x N	4	18	5.84	0.0034
GS x N	8	108	7.63	<0.0001
R x GS x N	16	108	3.85	<0.0001
Sum of PAW	numDF	denDF	F-value	p-value
(Intercept)	1	108	3181.53	<0.0001
Residue treatment (R)	2	6	0.07	0.9345
Growth stage (GS)	4	108	386.82	<0.0001
Fertiliser N input (N)	2	18	2.74	0.0915
R x GS	8	108	5.23	<0.0001
R x N	4	18	2.31	0.0978
GS x N	8	108	3.59	0.001
R x GS x N	16	108	2.63	0.1010

Figures of plant available water in the soil profile at depth

Figure S2.1: Plant available water in the soil profile to 100 cm depth for the removed (a), wheat (b), and lupin (c) residue management experiments in site 1 at wheat sowing on 21 May 2015, and with 20, or 55 kg N ha^{-1} rates at wheat harvest on 30 November 2015. Mean values of 8 replicates at sowing, mean value of 4 replicates at harvest. Different letters within each soil layer indicate significant differences of the mean for 20 *vs.* 55 kg N ha^{-1} rates (paired t test, $p < 0.05$).
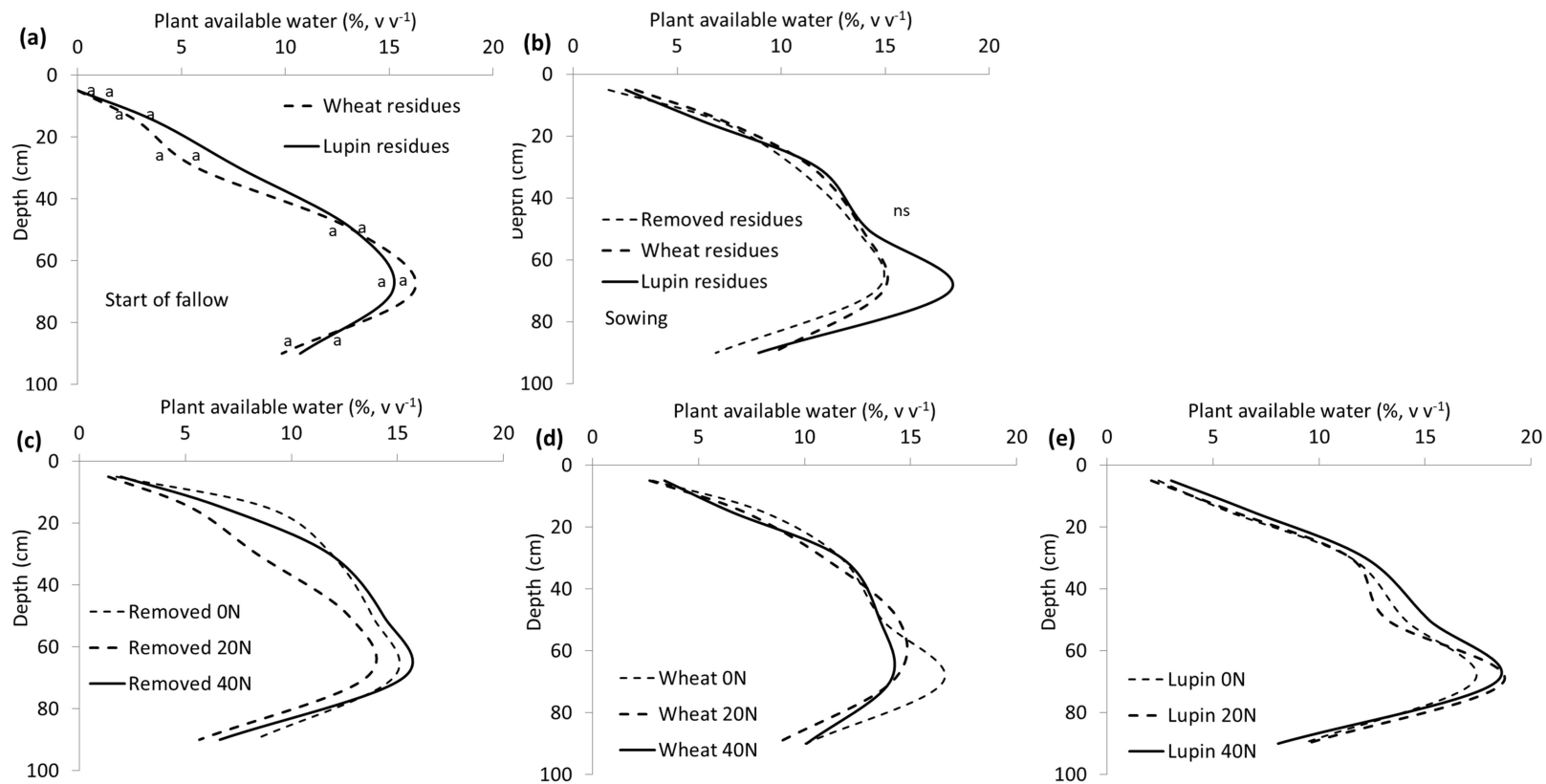
Figure S3.2: Plant available water in the soil profile to 100 cm depth of the residue treatments in site 2 at the start of fallow on 15 December 2015 (a), at wheat sowing on 2 June 2016 (b), and at harvest on 7 December 2016 (c, d, e). For (a, c, d, e) mean values of 4 replicates, for (b) mean value of 12 replicates. For (a) different letters within each soil layer indicate significant differences of the mean between residue treatments (paired t test, p<0.05). For (b) ns=not significant for the interaction between 'depth' and 'residue treatment' (DGC test, p=0.4).

Supplementary material of Chapter 4

The following data corresponds to the supplementary material of the paper that has been accepted for publication in *Soil Research.*

Muschietti Piana, M.P., T.M. McBeath, A.M. McNeill, V.V.R.S. Gupta and P.A. Cipriotti (2020). Early growing season immobilisation affects post-tillering wheat nitrogen uptake from crop stubble and ^{15}N fertiliser in a sandy soil. *Soil Research* (accepted on 25 September 2020).

Table S1. Chemical characteristics of the soils*collected from 0-10cm depth in the field after a lupin or wheat crop and used for the top layer (0-10 cm) in the glasshouse experiment

EC, electrical conductivity; PBI, phosphorus buffering index; P, phosphorus; DTPA, diethylenetriamine pentaacetic acid; DGT-P, diffusive gradient in thin-films-Phosphorus.

Soil history	After wheat	After lupin	Critical values**
pH 1:5 (H_2O)	7.1	7.0	
EC (dS m^{-1})	0.1	0.1	
PBI Index	8.0	12.0	<10
Colwell P (	23.0	23.0	<15-45
Nitrate (mg kg^{-1})	3.6	5.6	10-50 desirable
Ammonium (mg kg^{-1})	1.5	2.3	5 desirable
DTPA Zn (mg kg^{-1})	1.8	1.3	<0.3-0.8
DTPA Cu (mg kg^{-1})	0.4	0.4	<0.2
DTPA Mn (mg kg^{-1})	1.8	1.8	<10
DTPA Fe (ug L^{-1})	17.0	18.0	<5
DGT-P	194	192	<20

*Bulked air-dried soil samples analysed by APAL on 20/9/17. ** According to APAL and documented at: https://grdc.com.au/resources-and-publications/grdc-update-papers/tab-content/grdc-update-papers/2014/08/trace-elements-copper-and-manganese-their-role-requirements-and-options.

Table S2. Soil nitrate N (NO$_3^-$), and ammonium N (NH$_4^+$, mg pot^{-1}) at 0-10 cm depth, and at 10-20 cm depth for all treatments at different wheat growth stages

Mean value of 4 replicates with standard deviation in parenthesis. Different letters indicate significant differences between 'stubble treatment' and 'fertiliser N' within each growth stage (DGC test, $p < 0.05$). NA, not available data. Values were highlighted in grey to improve visualisation of the significant differences among treatment means.

	Tillering		First node		Booting	
	Soil mineral N (mg pot^{-1}) at 0-10 cm depth					
Treatment	NO$_3^-$	NH$_4^+$	NO$_3^-$	NH$_4^+$	NO$_3^-$	NH$_4^+$
No stubble-N	2.8 (1.4) b	6.2 (2.4) a	5.2 (0.3) a	2.4 (0.3) a	4.5 (0.1) a	1.0 (0.1) a
No stubble+N	11.0 (4.0) a	2.9 (0.6) a	4.9 (0.2) a	2.0 (0.1) a	4.3 (0.1) a	0.9 (0.1) a
Wheat-N	4.8 (1.1) b	3.3 (1.1) a	4.9 (0.1) a	2.6 (0.2) a	4.5 (0.2) a	1.2 (0.2) a
Wheat+N	5.1 (1.5) b	3.5 (1.3) a	5.1 (0.1) a	2.3 (0.1) a	NA	NA
Lupin-N	3.5 (1.7) b	4.7 (0.9) a	5.0 (0.1) a	2.1 (0.2) a	4.5 (0.1) a	1.2 (0.2) a
Lupin+N	5.0 (1.5) b	3.6 (1.1) a	4.9 (0.2) a	2.2 (0.0) a	4.3 (0.1) a	1.1 (0.2) a
	Soil mineral N (mg pot^{-1}) at 10-20 cm depth					
Treatment	NO$_3^-$	NH$_4^+$	NO$_3^-$	NH$_4^+$	NO$_3^-$	NH$_4^+$
No stubble-N	1.8 (1.5) a	2.7 (0.9) a	4.4 (0.2) a	2.2 (0.2) a	4.4 (0.2) a	1.1 (0.3) a
No stubble+N	6.1 (3.2) a	6.1 (3.9) a	4.7 (0.1) a	1.5 (0.1) a	4.5 (0.1) a	1.2 (0.0) a
Wheat-N	0.3 (2.2) a	1.6 (1.5) a	4.5 (0.1) a	2.2 (0.1) a	4.3 (0.1) a	1.1 (0.1) a
Wheat+N	2.9 (1.2) a	4.4 (1.5) a	4.9 (0.2) a	2.1 (0.1) a	NA	NA
Lupin-N	1.8 (1.1) a	2.7 (0.4) a	4.3 (0.1) a	2.2 (0.1) a	4.4 (0.3) a	1.1 (0.1) a
Lupin+N	6.1 (1.2) a	6.1 (0.9) a	4.3 (0.1) a	1.5 (0.2) a	4.5 (0.1) a	1.2 (0.2) a
	Sum of soil mineral N (mg pot^{-1}) at 0-20 cm depth					
Treatment	NO$_3^-$+NH$_4^+$		NO$_3^-$+NH$_4^+$		NO$_3^-$+NH$_4^+$	
No stubble-N	13.4 (1.3) b		14.2 (0.2) a		11.0 (0.2) a	
No stubble+N	26.1 (1.4) a		13.1 (0.4) a		11.0 (0.1) a	
Wheat-N	12.2 (1.2) b		14.2 (0.3) a		11.1 (0.2) a	
Wheat+N	18.9 (1.2) b		14.0 (0.2) a		NA	
Lupin-N	10.1 (1.5) b		13.6 (0.2) a		11.1 (0.2) a	
Lupin+N	16.0 (2.1) b		13.6 (0.1) a		10.5 (0.1) a	

Table S3. Wheat plant root biomass (g) for the fertiliser N treatments at tillering and first node wheat growth stages. Mean values with standard error in brackets

+N, plus N with 40 kg N ha^{-1} applied at wheat sowing time, -N, minus N, 0 kg N ha^{-1} no fertiliser N added throughout the experiment. Mean value of 12 replicates with standard deviation in parenthesis. Different letters indicate significant differences between 'growth stage' and 'fertiliser N' (DGC test, $p < 0.05$). Values were highlighted in grey to improve visualisation of the significant differences among treatment means.

	Fertiliser N treatment	+N	-N
Wheat growth stages	Tillering	0.26 (0.02) c	0.25 (0.02) c
	First node	2.64 (0.16) a	1.81 (0.16) b