Changes in N cycling in the rhizosphere of canola lead to decreased N requirements in a following wheat crop.

Cathryn A O'Sullivan[1], Elliott G Duncan[2], Kelley Whisson[1], Karen Treble[1], Margaret M Roper[1], Mark B Peoples[3].
[1] CSIRO Agriculture, Private Bag 5, Wembley, WA 6913, Australia.
[2] Future Industries Institute, University of South Australia, Mawson Lakes Campus, Mawson Lakes, SA 5095, Australia.
[3] CSIRO Agriculture, Clunies Ross St, Black Mountain, ACT 2601, Australia.

Abstract
Growing canola in rotations is known to increase the yield of following cereal crops. In addition to providing a disease break and better weed management, it has been suggested that following crops have lower N fertiliser requirements. The aim of this study was to confirm the lower N requirement of wheat following canola and to postulate possible mechanisms to explain this effect.
In a field study, wheat was grown under several N fertiliser levels in paddocks where either canola or wheat/pasture were grown in the previous year. In parallel, laboratory and glasshouse studies were used to examine the impact of canola growth on nitrification, N mineralisation and N immobilisation rates.
Two seasons of field data in Wongan Hills, WA, and one season in Merredin, WA, showed that wheat had a lower N fertiliser requirement following canola than a wheat or pasture rotation. In the laboratory, nitrification rates were significantly lower in the rhizosphere of canola cv. Hyola 404RR than wheat cv. Janz, while N immobilisation and remobilisation rates were significantly higher.
To explain this we hypothesise that decreased nitrification rates conserve N as NH_4^+ during the canola season leading to increased N immobilisation rates and an elevated organic N pool that is likely to be stored over hot and dry summers in the WA region. In the following season this organic N pool can be remobilised providing an alternative N source for the following crop. Further study is needed to fully explore this concept.

Key Words
Cereal-canola rotations, nitrogen use efficiency, nitrogen cycling

Introduction
Canola has become an increasingly popular rotation crop in the Australian farming system over the last 20 years, with the area cropped increasing from ~100,000 ha in the 1980s to more than 1,000,000 ha in 2008 (GRDC 2009). Growers give several reasons, beyond the value of the oilseed itself, for choosing to plant canola. Most commonly, it provides an important tool in weed management and it is a valuable disease break-crop for cereal production systems (Angus, et al. 2015). In addition, there is evidence that canola may cause changes in the nitrogen cycle that lead to the preservation of N in the soil. Data from a combination of field and laboratory experiments suggests that, during the summer fallow following a canola crop, rates of N immobilisation to the organic N pool and subsequent N mineralisation are increased (Ryan, et al. 2006). Brassicas, including canola, produce glucosinolate compounds that decompose into isothiocyanates when they are released during tissue degradation (Hollister, et al. 2013). Studies have shown that application of some isothiocyanates, and whole Brassica meals, significantly decrease ammonia oxidising microbial populations and associated nitrification rates (Bending and Lincoln 2000, Brown and Morra 2009, Snyder et al. 2010). This could have important implications for N management in cropping systems that include a canola rotation. If canola releases compounds that alter the N cycle by increasing immobilisation of N into the organic pool, then leaching losses of N may be reduced and the stored organic N may provide a reserve of N for the following crop if it is remobilised during the following season.
In this paper we present data to show that a wheat crop grown after a canola crop requires less N fertiliser to achieve its yield potential compared to wheat grown after a pasture or wheat crop. We provide a conceptual model to explain the potential mechanisms.

Methods
Field trials
Three field trials were performed over 2 years at two locations in Western Australia. In 2014, a preliminary trial was carried out at Wongan Hills on paired paddocks in which wheat (*Triticum aestivum*) cv. Mace (80 kg/ha) was grown on paddocks that were previously sown to either canola (cv. Cobbler), or pasture (self-sown medic/rye grass). The paddocks had similar soil types (tenosols) with similar total N but the paddock following canola had higher concentrations of organic C and available P, K, and S at the start of the season.

In 2015, two paired trials were conducted at Wongan Hills, and Merredin in paddocks following canola (cv. Cobbler and GT50 respectively) or wheat (cv. Zippy and Yitpi respectively). At each site the two paddocks were located within 1 km of one another to remove the effect of differences in climate and rainfall on N mineralisation rates; the paired paddocks had a similar soil type (tenosol in Wongan Hills and chromosol in Merredin) with similar total N and organic C but varying background P, K and S concentrations at that start of the wheat season.

In each trial wheat (cv. Mace) was sown at 80 kg ha^{-1} in triplicate for each treatment. Fertiliser N was applied as urea (CH_4N_2O, 46% N; CSBP fertilisers, Kwinana, WA) at rates of 0, 10, 30, 60 and 90 kg N ha^{-1}. Urea was applied as granules in a split application of 10 kg N ha^{-1} at seeding (except for 0 kg N ha^{-1} treatment) and the remainder four weeks later (ZS31) according to common farmer practice in the region (Brennan and Bolland 2009).

Hand cuts were taken from each plot at anthesis (approximately Z64) for biomass measurements. At physiological maturity (GS91) all plots were machine harvested and plot yields determined.

Pot trials and soil incubations to investigate N cycling in canola rhizosphere.

Three varieties of canola (Hyola 404RR, Hyola 559TT and Stingray TT) were grown for 6 weeks in a commercial potting mix with background mineral N of 8.9 ± 2 mg/kg (RichGro, Australia) in root stock pots in a growth cabinet and fertilised with 30 mL of 1 M NH_4NO_3 fertiliser weekly. The potential nitrification rates in soils from under the plants were tested and compared to unplanted pots and pots planted with wheat cv. Janz, (previously shown not to inhibit nitrification) and *Brachiaria humidicola* (a known biological nitrification inhibitor). In a separate soil incubation study, N immobilisation and remobilisation rates were estimated using the ^{15}N dilution technique (Fillery and Recous 2001) in a replicated (n=3) randomised block design using soils that had previously grown canola, wheat or no plant at all.

Results and Discussion

Field trials

In all three field trials, wheat yields following canola reached the rain-limited yield potential at lower applied N levels than wheat following wheat or pasture (Fig 1). In both 2014 and 2015 at Wongan Hills the wheat yield following canola reached the rain-limited yield potential without the need for any N fertiliser, while wheat following pasture needed 10 kg/ha of N to achieve the maximum yield and yield potential in 2014 (Fig 1A); in 2015 yield potential of wheat following wheat was not reached at any fertiliser application rate (Fig 1B). At Merredin in 2015, wheat following canola required between 10 and 30 kg N/ha to achieve yield potential, while wheat following wheat needed 60 kg N/ha to achieve the same yield (Fig 1C).

To achieve this yield increase, wheat following canola appears to have accessed a pool of N other than the mineral N stored in the profile at the start of the season or the fertiliser applied. For example, in 2014 at Wongan Hills, when 0 kg N/ha was applied, N recovered by wheat grown following canola exceeded the mineral N available at the start of the season by approximately 25 kg/ha, whereas wheat following pasture only took up 50% of the available mineral N at the start of the season (Fig 2).

Pot/incubation trials

In pot trials the three canola varieties inhibited nitrification rates by 25.5% to 62% relative to unplanted soils and wheat cv Janz (Fig 3A), with c.v. Hyola 404RR producing the highest level of inhibition followed by c.v. Hyola 555TT and c.v. Stingray. All three canola varieties inhibited nitrification significantly more than wheat c.v. Janz and at similar or higher levels than a known strong biological nitrification inhibitor producer, *Brachiaria humidicola* (Fig 3A).

Application of ^{15}N labelled NH_4NO_3 to soils from pots that had previously grown canola or wheat showed that N immobilisation and N remobilisation rates were significantly elevated in soils from beneath canola ($P<0.05$ for canola) relative to wheat or unplanted soils (Fig 3B).

The improved performance of wheat grown after canola in terms of grain yield (Fig 1); grain N recovery (Fig 2) and grain protein content (data not shown), is consistent with existing literature (See review by Angus et al. (2015)) but the scale of this effect is likely to vary across regions and or seasons. (Seymour et al. 2012, French et al. 2015).

It is possible that some of the yield gains following canola were a result of a disease break as it is known that root pathogens thrive in continuous wheat or pasture-wheat rotations (Angus et al. 2015). However, given that wheat following canola accessed N well in excess of available mineral N at the start of the season, it is likely that the disease-break effect was accompanied by significant changes in N cycling and in particular conservation of N through immobilisation of NH_4^+ into the organic N pool and remobilisation during the following wheat crop.

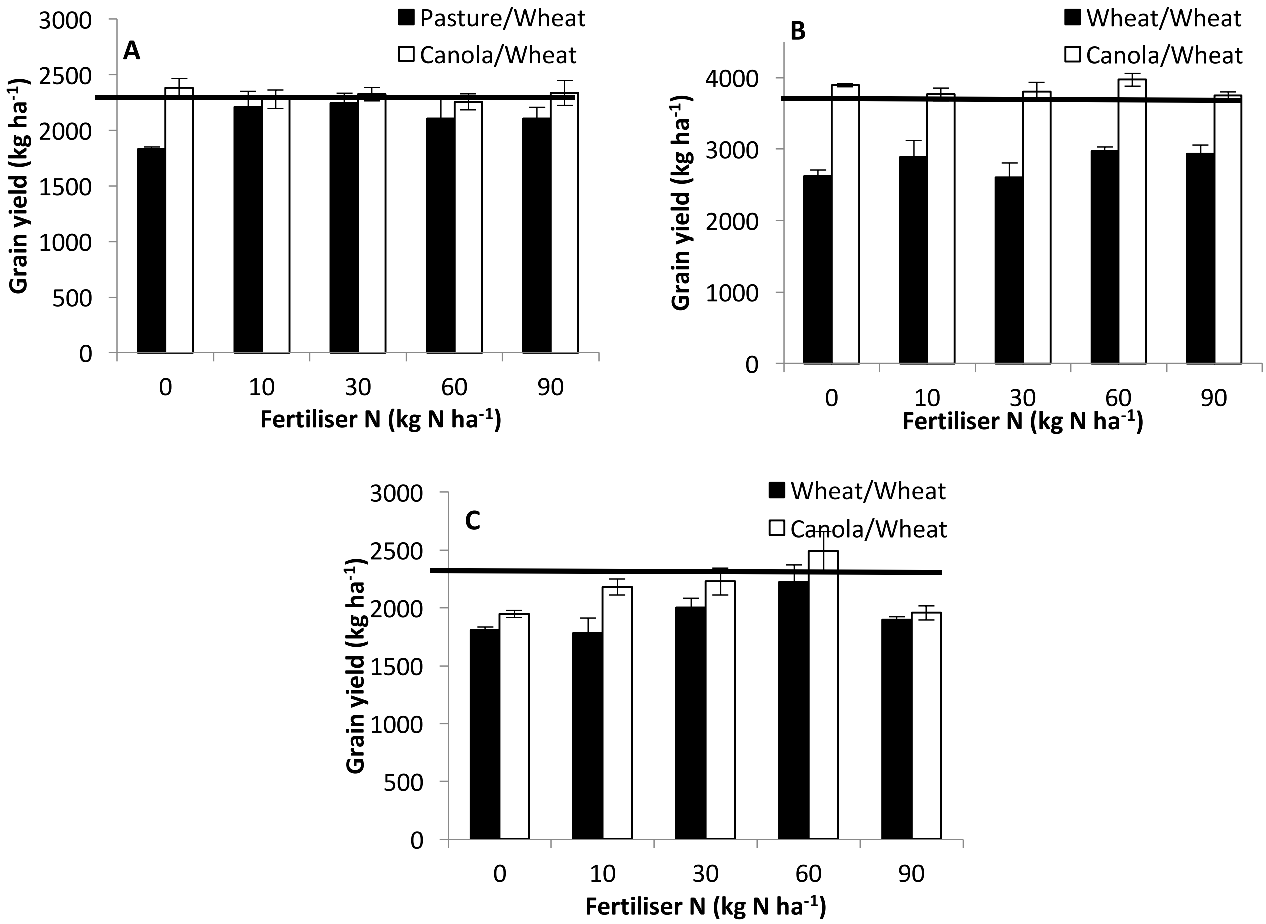

Figure 1. Wheat grain yields (kg ha^{-1}) from the trials performed at Wongan Hills in 2014 (A), 2015 (B) and at Merredin (C) following a canola or wheat crop or pasture the previous year. Values are means ± SE (*n*=3). The solid black line on each chart represents the rainfall limited yield potential for each trial as calculated using the equation by French and Schultz (1984). Yield potentials at each trial were as follows: Wongan Hills 2014 - ~2300 kg ha^{-1}; Wongan Hills 2015 - ~3800 kg ha^{-1}; Merredin - ~2400 kg ha^{-1}.

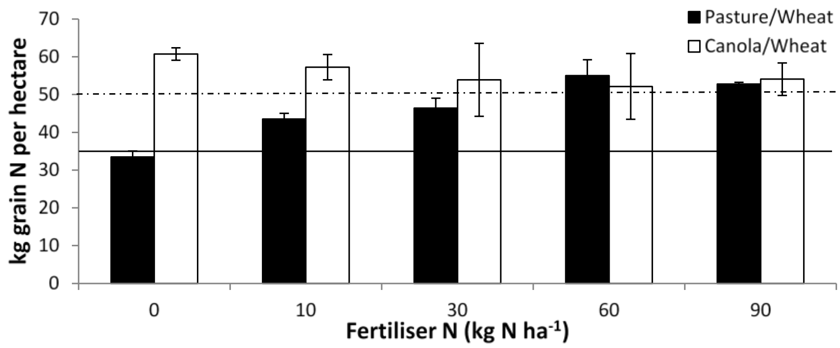

Figure 2: Grain N recovery by wheat cv. Mace at Wongan Hills in 2014 when grown following canola (solid) and pasture (open). There was significant N recovery by wheat following canola even at low levels of N fertiliser applications. In the post-canola plots at 0 kg N/ha and 10 kg N/ha wheat plants recovered more N than was available in the residual mineral N pool at the start of the season, indicating the plants were accessing N from other sources. Solid line = residual mineral N to 30 cm depth at start of season following canola. Dashed line = residual mineral N to 30 cm depth at start of season following pasture.

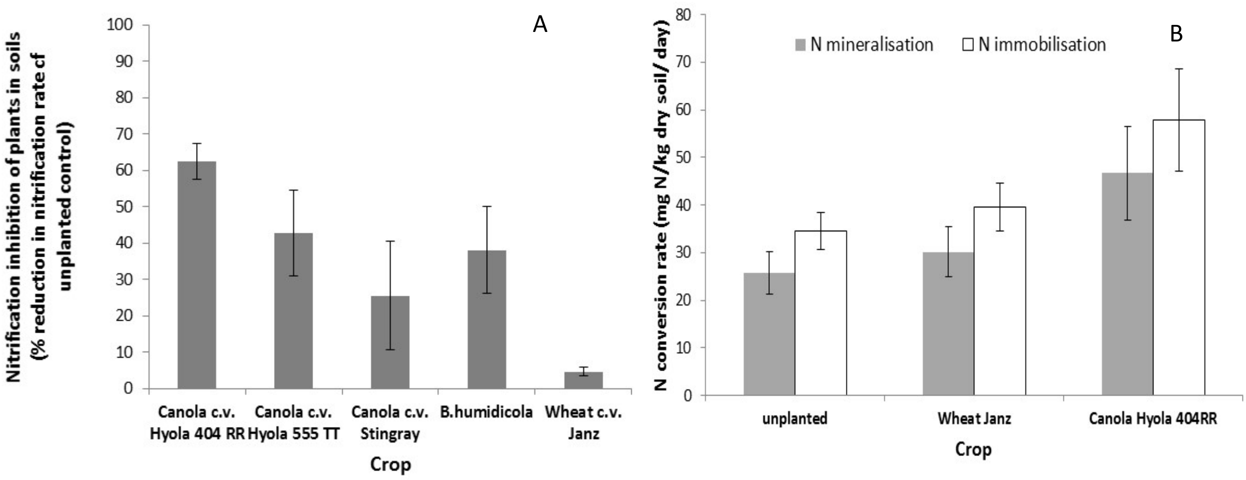

Figure 3: Inhibition of nitrification (A) and stimulation of N immobilisation and N remobilisation (B) in soils planted with three canola varieties compared to wheat cv. Janz, *B. humidicola* or unplanted soils. Error bars indicate 95 % confidence interval from triplicate analyses.

Conclusions

This study has confirmed that wheat grown following a canola rotation requires less N to achieve the same grain yields and N recovery. In addition to the known disease break effect, this could be driven by changes in N cycling in the rhizosphere of the canola. We propose that by preserving N in the NH_4^+ form for longer during the season, canola decreases in-season N losses and allows for higher levels of N immobilisation to the microbial organic N pool. This acts as a store of N which can be remobilised at the onset of rains in the following season and act as an alternative N source for the following crop.

It is possible that the N fertiliser requirements of wheat crops following canola are being significantly over-estimated if the value of the labile organic N pool is not being considered when fertiliser decisions are being made. While this study does not provide definitive proof of the mechanism, it allows us to suggest a conceptual model that is worthy of further study.

References

Angus, J., J. Kirkegaard, J. Hunt, M. Ryan, L. Ohlander and M. Peoples (2015). "Break crops and rotations for wheat." Crop and Pasture Science **66**(6): 523-552.

Bending, G. D. and S. D. Lincoln (2000). "Inhibition of soil nitrifying bacteria communities and their activities by glucosinolate hydrolysis products." Soil Biology & Biochemistry **32**(8-9): 1261-1269.

Brennan, R. and M. Bolland (2009). "Comparing the nitrogen and phosphorus requirements of canola and wheat for grain yield and quality." Crop and Pasture Science **60**(6): 566-577.

Brown, P. D. and M. J. Morra (2009). "Brassicaceae Tissues as Inhibitors of Nitrification in Soil." Journal of Agricultural and Food Chemistry **57**(17): 7706-7711.

Fillery, I. R. P. and S. Recous (2001). "Use of enriched (15)N sources to study soil N transformations." Stable Isotope Techniques in the Study of Biological Processes and Functioning of Ecosystems **40**: 167-194.

French, R. and J. 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**(6): 743-764.

French, R. J., R. S. Malik and M. Seymour (2015). "Crop-sequence effects on productivity in a wheat-based cropping system at Wongan Hills, Western Australia." Crop and Pasture Science **66**(6): 580-593.

GRDC (2009). Canola best practice management guide for south-eastern Australia. D. McCaffery, T. Potter, S. Marcroft and F. Pritchard Eds. Canberra, Australia, Grains Research and Development Corporation.

Hollister, E. B., P. Hu, A. S. Wang, F. M. Hons and T. J. Gentry (2013). "Differential impacts of brassicaceous and nonbrassicaceous oilseed meals on soil bacterial and fungal communities." Fems Micro Ecology **83**(3): 632-641.

Ryan, M. H., J. A. Kirkegaard and J. F. Angus (2006). "Brassica crops stimulate soil mineral N accumulation." Australian Journal of Soil Research **44**(4): 367-377.

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): 1-16.

Snyder, A. J., J. L. Johnson-Maynard and M. J. Morra (2010). "Nitrogen mineralization in soil incubated with N-15-labeled Brassicaceae seed meals." Applied Soil Ecology **46**(1): 73-80.