Nitrogen use efficiency of ^{15}N urea applied to wheat based on fertiliser timing and use of inhibitors

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Abstract Improving fertiliser nitrogen (N) use efficiency is essential to increase productivity and avoid environmental damage. Using a ^{15}N mass balance approach, we investigated the effects of five N fertiliser management strategies to test the hypothesis that increasing uptake of applied N by wheat improves productivity and reduces loss of N in a semi-arid environment. Three experiments were conducted between 2012 and 2014. Treatments included urea application (50 kg N/ha) at sowing with and without nitrification inhibitor (3,4-dimethylpyrazole phosphate, DMPP) and surface broadcast with and without urease inhibitor (n-butyl thiophosphoric triamide, NBPT) at the end of tillering plus an unfertilised control. It was found that deferring fertiliser application until the end of tillering decreased losses of fertiliser N (35–52%) through increasing uptake by the crop and or recovery in the soil at harvest, while maintaining yield except when rainfall following application was low. In this case, deferring application reduced fertiliser uptake (− 71%) and grain yield (− 18%) and increased recovery of N in the soil (+ 121%). Use of DMPP or NBPT reduced N loss where seasonal conditions were conducive to denitrification during winter (DMPP) and volatilisation or denitrification later in the season (NBPT). Their effect on grain yield was less significant; DMPP increased yield (+ 3–31%) in all years and NBPT increased yield (+ 7–11%) in 2 of 3 years compared to unamended urea. The majority of crop N uptake was supplied from soil reserves and as a result, crop recovery of applied N was not strongly related to grain yield response.

Keywords Nitrification inhibitor · Urease inhibitor · Recovery efficiency · Physiological efficiency · Agronomic efficiency

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Introduction

Nitrogen (N) fertiliser is a key input in dryland grains production systems, however its use can result in

environmental damage via greenhouse gas emissions as well as eutrophication of waterways. Combined with an expanding world population there is a need to identify practices that can improve the efficiency with which N fertilisers are utilised (Zhang et al. 2015). Nitrogen use efficiency can be measured in a variety of ways; at its most basic level the agronomic efficiency (AE) of fertiliser application is measured as the increase in grain yield divided by the rate of application (Dobermann 2007). Increases in AE of fertiliser application can be achieved through a combination of either increased crop recovery of applied N (crop recovery efficiency, RE_c), or increased yield gain per unit of fertiliser N uptake (physiological efficiency, PE). It is therefore possible that by increasing RE_c, loss of applied N can be reduced while simultaneously improving productivity.

Gardner and Drinkwater (2009) indicated that across a range of environments and production systems, RE_c averages 33% in the year of application, while in Australian grains production systems RE_c averages 44% (Angus and Grace 2017). The amount of rainfall following application is a major determinant of these observations through its effect on loss mechanisms as well as the ability of the crop to access applied N. Soil factors such as organic matter, pH and texture can also have a significant effect on these processes (Cameron et al. 2013), interacting with climate and by extension RE_c. Improvements to RE_c will therefore require better temporal matching of N supply to periods of high crop demand and avoiding periods where the risk of loss increases.

Options to manipulate temporal supply of fertiliser N include altered timing of application and the use of inhibitors. Altering the timing of fertiliser application can increase uptake of applied N through a combination of reducing loss (Harris et al. 2016) and better matching supply to periods when demand and ability of the crop to compete for N is higher (Kirda et al. 2001; Limaux et al. 1999). However, delaying application of N to wheat beyond tillering may result in a trade-off between grain yield and protein (Fischer et al. 1993; McDonald 1989).

Inhibitor treated fertilisers also influence the temporal supply of fertiliser N. Urease inhibitors such as NBPT slow the hydrolysis of urea into ammonium (NH_4^+) (Chen et al. 2008) thereby delaying crop access to applied N and reducing potential for volatilisation loss (Turner et al. 2010). Nitrification

inhibitors such as DMPP slow the oxidation of NH_4^+ to nitrite thereby restricting nitrate (NO_3^-) supply and reducing the likelihood of denitrification and leaching (Friedl et al. 2017; Weiske et al. 2001; Yu et al. 2007). While both nitrification and urease inhibitors have been shown to increase RE_c the impact on yield has been less consistent (Abalos et al. 2014), often resulting in limited increases in grain yield (Freney et al. 1992; Harris et al. 2016; Xu et al. 2000; Zhang et al. 2010), although this may in part relate to a lack of studies investigating their use at sub-optimal N rates where the benefit of reducing N loss is likely to be higher (Rose et al. 2018). Furthermore, studies comparing both timing and the use of inhibitors to improve NUE of wheat in semi-arid environments are limited despite the evidence for significant loss of applied N in such environments (Armstrong et al. 1998; Patra et al. 1996; Pilbeam et al. 1997). Further investigation is therefore required to improve NUE in such environments.

This paper outlines findings from a 3-year study investigating options to mitigate loss of fertiliser N and improve NUE of rainfed wheat grown on alkaline clay soils in a temperate, semi-arid environment. The methods tested include altered timing of application (either incorporated at sowing or surface applied at the end of tillering) and the use of inhibitor (nitrification at sowing and urease at the end of tillering) treated urea in order to test the following hypotheses:

1. Crop uptake will be increased, and loss of applied N decreased through controlling temporal supply of mineral N (using timing of application or inhibitors) to avoid periods where the risk of losses are high and coincide with periods of greater crop demand.
2. Increasing crop uptake and reducing loss of applied N through the manipulation of temporal N supply will also increase wheat yield, thereby eliciting the importance of RE_c to AE for wheat grown in a temperate, semi-arid environment.

Materials and methods

Experimental sites

Wheat productivity and NUE was measured at three sites in the Wimmera region of Victoria, Australia

between 2012 and 2014. Experiments were conducted at Taylors Lake (36° 47′ 7″ S, 142° 23′ 41″ E) in 2012 and 2013 and at the Victorian State Government Plant Breeding Centre near Horsham (36° 44′ 49″ S, 142° 6′ 48″ E) in 2014. The Wimmera region is characterised by hot, dry summers and mild winters with the majority of rain falling during winter. Mean annual rainfall is 440 mm at Taylors Lake and 423 mm at Horsham (Bureau of Meteorology 2017). All sites had a strong history of continuous cropping including common vetch (*Vicia sativa*) followed by wheat (*Triticum aestivum*), faba bean (*Vicia faba*) followed by canola (*Brassica napus*) and field pea (*Pisum sativum*) followed by barley (*Hordeum vulgare*) grown in the 2 years prior to experimentation for the 2012, 2013 and 2014 experimental sites respectively.

Experimental design

Experiments were randomised complete block designs including five replicates nested within a larger N management trial of the same design. Treatments included four N fertiliser management strategies plus a zero N control. Fertilised treatments were applied at a rate of 50 kg N/ha which is representative of rates applied to commercial crops in the region. Fertiliser strategies tested were: N applied as urea banded 25 mm below the seed at sowing (50 N), DMPP treated urea banded 25 mm below the seed at sowing (50 N DMPP), urea surface applied at the end of tillering/Zadoks growth stage 30 (Zadoks et al. 1974), (0:50 N) and NBPT treated urea surface applied at the end of tillering (0:50 N NBPT).

Soil characterisation

All sites were located on grey vertosol soils (Isbell 2002) characterised by a clay texture containing low to moderate levels of carbon (total and organic) and N, with neutral topsoils, increasing in pH down the profile. Soil properties were measured by taking a composite sample of three soil cores to a depth of 1.2 m from each replicate. Additional cores were taken from the broader trial (for the treatments listed above) at harvest and analysed for soil water and mineral N. Soil cores were dried at either 105 °C (soil moisture analysis) or 40 °C (chemical and physical analysis) until constant weight prior to analysis. Electrical conductivity was low throughout most of

the upper profile but increased significantly at all sites below 0.4 m as did soil exchangeable sodium in 2013 and 2014. Further details of the 2012 soil profile are contained in Table 1, further details of the 2013 and 2014 soil profiles can be found in Wallace et al. 2018.

Soil water filled pore space (WFPS) was also monitored for the 50 N plots at 9 am daily to a depth of 0.05 m using ML2 theta probes connected to Delta-T GP1 data loggers (Delta-T Devices Limited, Cambridge, UK). Theta probes were calibrated based on the manufacturer's method (Delta T, 2004) and WFPS calculated according to the method of Linn and Doran (1984).

Crop management

Experimental sites were managed similarly to the surrounding paddock; in 2012 and 2013 previous crop residues were burnt, while in 2014 light cultivation was applied prior to sowing. In 2013, 28 kg N/ha was surface applied as urea 1 month prior to sowing in accordance with management of the surrounding paddock by the host farmer. All plots received 128 kg/ha double superphosphate (21 kg P/ha, 5.1 kg S/ha) banded with the seed. Sowing of wheat *cvs.* Gregory, Grenade Cl Plus and Scout was conducted on 6 June, 27 May and 20 May in 2012, 2013 and 2014 respectively. Weeds, pests and disease were controlled using a variety of chemicals depending on seasonal conditions and in line with best management practice. In 2014, rainfall was significantly below the long-term average (Fig. 2) and to avoid the potential for crop failure, equivalent to 31 mm of rainfall was applied to the plots. Irrigation was spread across 3 days; 19, 20 and 22 September to minimise the potential for waterlogging.

^{15}N mass balance

Steel micro-plots (0.5 × 0.3 m) were inserted into an unfertilised area near the end of each plot to a depth of 0.2 m. Each micro-plot contained two rows of wheat, spaced 0.26 m apart and following emergence, plant populations were thinned to 125 plants/m^2. ^{15}N enriched (10% a.e.) urea was dissolved in water and applied using a pipette at a rate of between 5 and 20 mL of solution containing 1.7 g urea per micro-plot; equivalent to 50 kg N/ha. Where required, DMPP or NBPT was added to the solution at a

Table 1 Selected chemical and physical soil characteristics for the 2012 experimental site at Taylors Lake, Victoria

Soil depth (m)	Total carbon[a] (g/kg)	Total organic carbon[b] (g/kg)	Total nitrogen[a] (g/kg)	pH (CaCl$_2$)[c]	Electrical conductivity[d] (dS/m)	Clay[e] (%)	Silt[e] (%)	Sand[e] (%)	Bulk density[f] (g/cm^3)
0–0.1	11.3 (0.96)	9.6 (0.87)	1.2 (0.03)	7.50 (0.06)	0.17 (0.01)	48.13 (1.70)	43.71 (1.26)	8.17 (0.46)	1.10
0.1–0.2	9.0 (0.25)	6.7 (0.38)	1.0 (0.03)	7.86 (0.06)	0.19 (0.02)	56.93 (1.74)	34.41 (1.32)	8.65 (0.99)	1.19
0.2–0.4	11.0 (1.82)	6.5 (0.23)	0.9 (0.03)	8.12 (0.03)	0.30 (0.02)	50.38 (2.77)	32.00 (4.11)	17.62 (6.10)	1.16
0.4–0.8	9.8 (0.71)	4.3 (0.39)	0.7 (0.03)	8.52 (0.02)	0.66 (0.01)	46.93 (10.84)	41.35 (10.25)	11.72 (0.60)	1.09
0.8–1.2	6.7 (1.76)	1.8 (0.24)	0.5 (0.03)	8.48 (0.03)	2.70 (0.36)	58.54 (2.10)	26.95 (1.08)	14.51 (2.72)	1.09

Values presented in parentheses represent the standard error of the mean

[a] Measured by combustion of air dry soils using LECO combustion analyser (n = 3)

[b] Measured by the method of Walkley and Black (1934) (n = 3)

[c] Measured in 0.01 M CaCl$_2$ using a glass electrode and a 1:5 soil to extract ratio (n = 5)

[d] Measured in water using a probe and a 1:5 soil to extract ratio (n = 5)

[e] Measured using a laser particle size analyser (Malvern Mastersizer 2000, Malvern, UK) (n = 3)

[f] Measured using large (> 75 mm diameter) in-tact cores (n = 2)

minimum of the manufacturers' recommended rate and thoroughly mixed prior to application. Fertiliser was applied immediately after sowing (50 N and 50 N DMPP treatments) and at growth stage 30 corresponding to; 3 September, 18 August and 21 August in 2012, 2013 and 2014 respectively (0:50 N and 0:50 N NBPT treatments). Where urea was applied at sowing, a narrow furrow was formed immediately beside and 25 mm below the depth of the seed to replicate as far as possible, urea placement within the broader experimental plots. The furrow was closed following urea application. Where urea was applied at growth stage 30, it was applied as evenly as possible across the soil surface.

The wheat in each micro-plot was harvested, dried at 70 °C and manually threshed to separate grain and straw before fine grinding in preparation for analysis. Soils from within each micro-plot were completely excavated at depths of 0–0.1 and 0.1–0.2 m with additional cores taken from 0.2 to 0.4 m. Soils were weighed and then dried at 40 °C before being thoroughly mixed and subsampled prior to fine grinding in preparation for analysis. Additional subsamples were dried at 105 °C to determine soil water content of both the fresh and 40 °C dried material. Plant and soil samples were analysed for total N and atom % ^{15}N by a continuous flow isotope ratio mass-spectrometer (SERCON 20-22), with samples from the 0 N plots used to quantify natural abundance of ^{15}N.

In addition to biomass samples taken from the ^{15}N microplots, samples were also collected from the surrounding plots to measure above ground biomass and N content during the growing season. Single quadrats (1 m^2 in size) were collected from each plot during tillering and at anthesis. Plant samples were dried at 70 °C and weighed before being fine ground in preparation for analysis of total N by dry combustion using a LECO combustion analyser.

Weather measurements

Weather data was collected at each experimental site using an automated weather station (MEA, Magill) with daily totals (rainfall), maximums and minimums (temperature) taken at 9 am. Where technical problems interfered with the collection of data, daily results were substituted with data from the nearest Bureau of Meteorology monitoring site (Bureau of Meteorology 2017).

Data analysis

Fertiliser recovery in soil and plant material and the proportion of N derived from fertiliser was calculated using the approach of Malhi et al. (2004). Indices of NUE were calculated according to those outlined by Dobermann (2007) where:
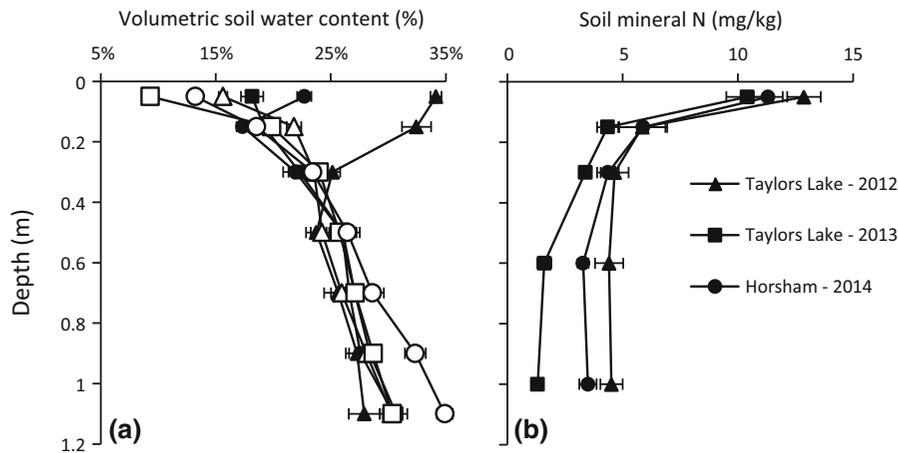
Fig. 1 Volumetric soil water content (%) immediately prior to sowing (closed captions) and averaged across treatments at harvest (open captions) (**a**), soil mineral N content (mg/kg) at sowing (**b**) to a depth of 1.2 m at Taylors Lake in 2012 and 2013 and Horsham in 2014. Error bars represent standard error of the mean (n = 5 at sowing, n = 25 at harvest)

N recovery efficiency by the crop (RE_c)

$\quad$ = fertiliser N uptake ÷ amount of N applied

Physiological efficiency (PE)

$\quad$ = (yield − yield of unfertilised control)

$\qquad$ ÷ fertiliser N uptake

Agronomic efficiency $(AE) = RE_c \times PE$

$\quad$ = (Yield − yield of unfertilised control)

$\qquad$ ÷ amount of N applied

Statistical analysis was undertaken using analysis of variance to determine significant differences between treatments and years at a 5% least significant difference test. Where required, square root or natural logarithmic transformation was undertaken to maintain normality of the dataset prior to analysis. Pearson correlation analysis using a two-sided test was undertaken to identify relationships between explanatory variables and aboveground biomass, grain yield, and fertiliser recovery at harvest. All analysis was undertaken using GenStat 17 Edition (Payne et al. 2014).

Results

Soil conditions prior to sowing

Soil water content prior to sowing was rarely significantly higher than at harvest. The exception was at shallower depths; particularly in 2012 when rainfall in May and June increased topsoil water content above the 'air-dry' values observed at harvest (Fig. 1). Total soil mineral N (NO_3^- and NH_4^+) to a depth of 1.2 m was low across all sites prior to sowing; 71 kg/ha in 2012, 39 kg/ha in 2013 and 57 kg/ha in 2014 to a depth of 1.2 m. Distribution of mineral N throughout the soil profile varied between sites (Fig. 1). In 2012 and 2014, 55% of profile mineral N was present below 0.4 m while in 2013, 65% of profile mineral N was present at depths less than 0.4 m.

Rainfall and temperature

Total annual rainfall varied widely across the 3 years of experimentation; 331 mm (decile 2: representing the lowest 20% of the historical record) and 393 mm (decile 4) at Taylors Lake in 2012 and 2013 respectively and 253 mm (decile 1) at Horsham in 2014. Growing season rainfall (measured from April to October) was 257 mm (decile 3), 329 mm (decile 6) and 166 mm (decile 1) in 2012, 2013 and 2014 respectively. In 2012, the majority of rain fell between May and September, while in 2013 this period extended to October (Fig. 2). In 2014, rainfall during late winter and spring was well below average with most occurring between April and July.

Temperature stress due to either frost or heat near the time of anthesis was minimal across all 3 years. In 2012, one frost (minimum temperature < 2.2 °C) was measured within ± 10 days of anthesis. During 2013
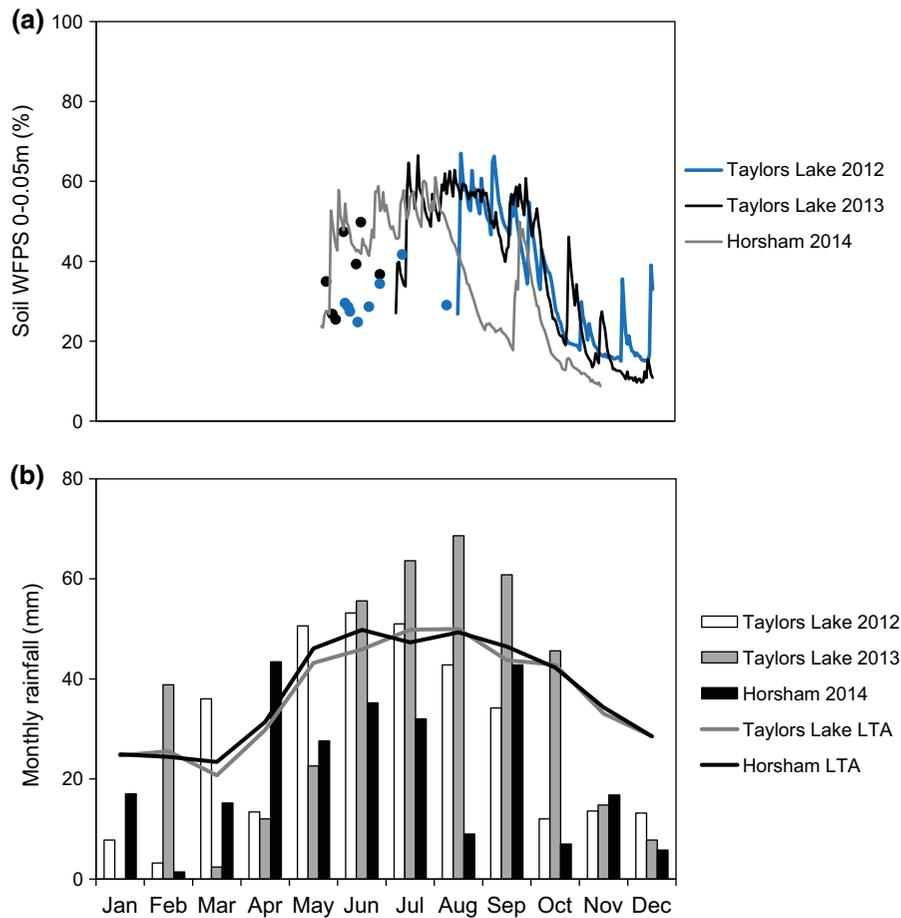
Fig. 2 Daily soil WFPS (0–0.05 m) during the growing season (**a**) and monthly rainfall totals during the experimental period and long-term average (LTA) at Taylors Lake and Horsham (**b**). Rainfall data for September 2014 includes 31 mm of irrigation. Soil WFPS prior to 14-August 2012 and 6-July 2013 based on soil cores, thereafter measured using continuous logging soil moisture probes

and 2014 there were five and four mild frosts respectively, however no major frosts (< -1.5 °C) were observed. Heat stress events (maximum temperature > 32 °C) were measured on two occasions within ± 10 days of anthesis in 2012 (reaching a maximum of 33.2 °C) and not in any other year.

Crop response to applied N

Applying N fertiliser increased crop N uptake throughout the growing season at mid-tillering ($+ 22$–180%) and anthesis ($+ 73$–165%) and continuing through to harvest ($+ 30$–172%). Application of unamended urea significantly ($P < 0.05$) increased crop N uptake at mid-tillering compared with DMPP amended urea and increased N uptake at anthesis

compared with application at GS30 in 2012 and 2014. It also increased above-ground biomass ($+ 24$–92%) and grain yield ($+ 15$–87%) across all years at harvest (Table 2) with significant interactive effects observed between year and treatment ($P = 0.018$ and < 0.001 respectively). Highest biomass and grain yields were observed in 2012 and 2013; in 2012 biomass and grain yield were significantly higher ($P < 0.05$) for the 50 N DMPP treatment than all others, while in 2013 all fertilised treatments resulted in similar yield and biomass with the exception of 0:50 N which yielded significantly ($P < 0.05$) less than 0:50 N NBPT. This is in contrast with 2014 when applying N at sowing resulted in a significant increase ($P < 0.05$) in biomass and a trend towards higher grain yield when compared to application at the end of tillering.

Table 2 Crop N uptake, above ground biomass, grain yield and physiological efficiency in response to treatment at Taylors Lake in 2012 and 2013 and at Horsham in 2014

| Treatment	Total N uptake at mid-tillering (kg/ha)^	Total N uptake at anthesis (kg/ha)^	N uptake from soil at harvest (kg/ha)	N uptake from fertiliser at harvest (kg/ha)	Total N uptake at harvest (kg/ha)	Biomass (kg/ha)	Grain yield (kg/ha)	Fertiliser N harvest index (%)	Physiological efficiency# (kg/kg)
2012									
0 N	15[b]	37[c]	47[b]	–	47[c]	7446[c]	2456[c]	–	–
50 N	30[a]	73[a]	45[b]	24[a]	69[b]	10473[b]	3501[b]	69[b]	42
50 N DMPP	20[b]	67[ab]	63[a]	26[a]	89[a]	12532[a]	4589[a]	70[b]	82
0:50 N	–	61[b]	59[a]	28[a]	87[a]	10251[b]	3940[b]	75[a]	54
0:50 N NBPT	–	58[b]	47[b]	26[a]	73[b]	9466[b]	3672[b]	76[a]	53
2013									
0 N	18	55[b]	65[c]	–	65[b]	8412[b]	3281[c]	–	–
50 N	22	101[a]	68[bc]	16[c]	84[b]	10818[a]	4480[ab]	71	64
50 N DMPP	22	95[a]	73[abc]	18[c]	91[b]	11243[a]	4625[ab]	73	73
0:50 N	–	102[a]	77[ab]	24[b]	101[a]	10398[a]	4177[b]	71	33
0:50 N NBPT	–	91[a]	82[a]	28[a]	109[a]	11054[a]	4651[a]	76	46
2014									
0 N	10[c]	23[b]	25[c]	–	25[c]	3133[b]	1467[c]	–	–
50 N	28[a]	56[a]	41[ab]	21[a]	62[a]	5839[a]	2046[ab]	70[b]	28
50 N DMPP	23[b]	61[a]	45[a]	22[a]	67[a]	6010[a]	2194[a]	70[b]	33
0:50 N	–	26[b]	32[bc]	6[b]	38[b]	3961[b]	1687[bc]	87[a]	29
0:50 N NBPT	–	32[b]	31[bc]	8[b]	38[b]	3985[b]	1825[abc]	91[a]	50
*LSD ($P < 0.05$) year × treatment	5.2	11.9	11.9	3.5	12.2	1223	462	6.2	n.s.

Treatment means within a given year with the same letter are not significantly different ($P < 0.05$)

ns no statistically significant interaction between year and treatment ($P < 0.05$)

^Data collected from main experimental plots. All other data collected at harvest from ^{15}N microplots

#Physiological efficiency = (yield − yield of 0 N control) ÷ N uptake from fertiliser (Dobermann 2007)

*When comparing means within the same year
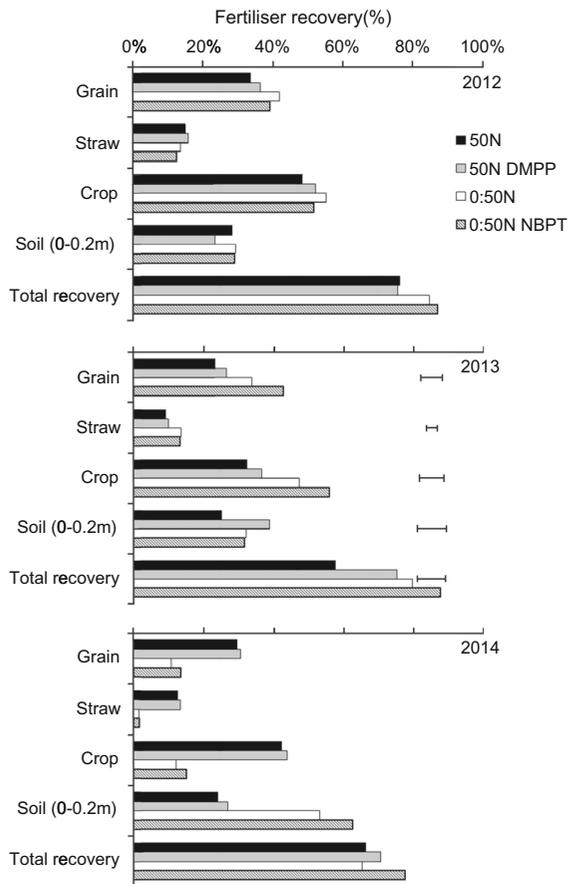
Fig. 3 Recovery (%) of applied N at harvest in grain, straw, aboveground biomass (crop), soil (0–0.2 m) and in total (sum of crop and soil) in relation to N fertiliser treatment at Taylors Lake in 2012 and 2013 and Horsham in 2014. Error bars represent least significant ($P < 0.05$) differences for each variable when comparing treatments within a given year

For fertilised treatments, biomass and grain yield were significantly ($P < 0.001$) correlated with total N uptake ($r = 0.85$ and $r = 0.86$ respectively). Application of DMPP treated urea at sowing or unamended urea at GS30 significantly ($P < 0.05$) increased uptake of soil N ($+ 25–34\%$) compared to the unfertilised control in 2012. Similar observations were made where N was applied at GS30 in 2013 ($+ 19–26\%$) and where urea was applied at sowing in 2014 ($+ 65–84\%$). The proportion of N derived from fertiliser (data not shown) across all fertilised treatments ranged from 16 to 35% and showed no significant ($P > 0.05$) correlation with grain yield across all years. There was a trend ($P = 0.11$) towards higher PE of applied N with the addition of DMPP

compared to unamended urea applied at sowing; 63 kg/kg *cf.* 45 kg/kg, but differences in PE due to treatment were otherwise limited.

Recovery of applied N

Total recovery (crop + soil) of applied N was significantly affected by an interaction of treatment and year (year × treatment: $P = 0.002$). Applying unamended urea at GS30 significantly ($P < 0.05$) increased total recovery (Fig. 3) by 11–38% compared with application at sowing (50 N) in 2012 and 2013 although there was no difference in 2014. Application of DMPP in 2013 and NBPT in 2014 significantly ($P < 0.05$) increased total recovery by 31% and 19% respectively, compared with unamended urea applied at the same time. Across the entire dataset, total recovery was significantly correlated ($P < 0.001$, $r = 0.55$) with RE_c, however this relationship changed between years. In 2012 a significant correlation ($P < 0.05$) was observed between total recovery and both soil recovery efficiency (RE_s) and RE_c with the highest correlation observed for RE_s ($r = 0.70$ *cf.* $r = 0.54$). In 2013, total recovery was more highly correlated with RE_c than RE_s ($r = 0.86$ *cf.* $r = 0.57$) and in 2014 total recovery was only significantly ($P < 0.05$) correlated with RE_s ($r = 0.52$).

Recovery of applied N by the crop, was highest in 2012 and 2013, averaging 52% and 43% respectively compared with 28% in 2014. In-season application of unamended urea significantly ($P < 0.05$) increased RE_c in 2013 and decreased RE_c in 2014 compared to application at sowing ($+ 46\%$ and $- 71\%$ respectively). Application of NBPT amended urea at GS30 significantly increased RE_c in 2013 ($+ 18\%$) compared to unamended urea. Changes in RE_c were significantly ($P < 0.001$) correlated with recovery in both the grain and straw. Applying N at GS30 significantly ($P < 0.05$) increased fertiliser N harvest index (Table 2) compared to application at sowing in 2012 and 2014. In 2012, 69% of the fertiliser N taken up by the crop was recovered in grain when applied at sowing compared with 75% at GS30 and in 2014 this relationship was stronger; increasing from 70 at sowing to 87% at GS30.

Movement of fertiliser derived N through the soil profile (as indicated by recovery at depths of 0–0.1 m and 0.1–0.2 m) varied significantly depending on year and treatment ($P = < 0.001$ and 0.001 respectively,

Table 3 Recovery of applied N in soil at depths of 0–0.1 m and 0.1–0.2 m and atom % ^{15}N of soil at a depth of 0.2–0.4 m at Taylors Lake in 2012 and 2013 and Horsham in 2014

Year	Treatment	Recovery in soil (0–0.1 m)	Recovery in soil (0.1–0.2 m)	Atom % ^{15}N soil (0.2–0.4 m) (%)
2012	0 N	–	–	0.369
	50 N	22.4%[ab]	5.9%[a]	0.386
	50 N DMPP	17.6%[b]	5.9%[a]	0.378
	0:50 N	27.8%[a]	1.6%[b]	0.372
	0:50 N NBPT	27.8%[a]	1.6%[b]	0.374
2013	0 N	–	–	0.367
	50 N	20.1%[b]	5.2%[b]	0.384
	50 N DMPP	31.8%[a]	6.9%[a]	0.380
	0:50 N	25.0%[ab]	5.7%[ab]	0.375
	0:50 N NBPT	26.8%[ab]	4.8%[b]	0.377
2014	0 N	–	–	0.369
	50 N	18.5%[c]	5.5%[a]	0.375
	50 N DMPP	23.1%[c]	3.8%[b]	0.375
	0:50 N	50.8%[b]	2.3%[bc]	0.371
	0:50 N NBPT	61.0%[a]	1.6%[c]	0.372
*LSD (P < 0.05) year × treatment		7.9%	1.7%	n.s.

Treatment means within a given year with the same letter are not significantly different (P < 0.05)

*When comparing means within the same year

Table 3). Fertiliser recovery at a depth of 0–0.1 m increased by 175% when unamended urea was applied at GS30 compared with sowing in 2014. Application of DMPP at sowing in 2013 and NBPT at GS30 in 2014 also significantly (P < 0.05) increased N recovery (+ 58% and + 20% respectively) at a depth of 0–0.1 m compared with unamended urea applied at the same time. Application of unamended urea at GS30 significantly (P < 0.05) reduced fertiliser recovery at 0.1–0.2 m compared to application at sowing in both 2012 (− 72%) and 2014 (− 59%) whereas application of DMPP at sowing only reduced recovery in 2014 (− 32%) compared to unamended urea.

Across all years, application of N resulted in significant (P < 0.05) increases in soil atom % ^{15}N at a depth of 0.2–0.4 m compared to the unfertilised control indicating movement of fertiliser N to this depth. Addition of DMPP at sowing or application of unamended urea at GS30 significantly reduced atom % ^{15}N at a depth of 0.2–0.4 m compared with unamended urea applied at sowing. A trend (P = 0.05) towards interactive effects of year and treatment on atom % ^{15}N at 0.2–0.4 m was also observed (Table 3). In 2012 and 2013 applying DMPP amended urea at sowing or unamended urea at GS30 reduced atom % ^{15}N at 0.2–0.4 m (1–2% and 2–4% respectively) compared with unamended urea at sowing, however this did not occur in 2014.

Effect of fertiliser management on nitrogen use efficiency

Agronomic efficiency of N application varied significantly (P < 0.01) with an interaction between year and treatment (Table 4). In 2012, application of unamended urea at GS30 and the use of DMPP at sowing, significantly (P < 0.05) increased AE

Table 4 Agronomic efficiency (kg/kg) of N fertiliser application at Taylors Lake in 2012 and 2013 and Horsham in 2014

Treatment	2012	2013	2014
50 N	20.5[c]	22.2[ab]	11.6[ab]
50 N DMPP	42.5[a]	25.1[a]	14.5[a]
0:50 N	29.5[b]	16.2[b]	4.4[b]
0:50 N NBPT	24.1[bc]	25.6[a]	7.4[ab]

*Treatment means within a given year with the same letter are not significantly different ($P < 0.05$), LSD (year × treatment) = 8.6 kg/kg

Agronomic efficiency = (yield − yield of 0 N control) ÷ N application rate (Dobermann 2007)

*When comparing means within the same year

(+ 44% and + 107% respectively) compared with unamended urea applied at sowing. In 2013, application of NBPT increased AE (+ 59%) compared with unamended urea applied at GS30 while there was an insignificant trend towards reduced AE from application of N at GS30 in 2014. Changes in AE were strongly, positively related to changes in PE (Fig. 4, $P < 0.001$, Slope = 0.33, $R^2 = 0.68$), but less so with changes in RE_c ($P < 0.001$, Slope = 0.50, $R^2 = 0.25$), while a weak negative relationship was observed between AE and RE_s ($P < 0.001$, Slope = − 0.52, $R^2 = 0.18$). There was no significant relationship between AE and total recovery of applied N ($P = 0.13$).

Discussion

Uptake of fertiliser N and crop response were affected by management and season

Uptake of fertiliser N generally ranged from 40 to 60% of the N applied which is at the higher end of similar studies (Abdel Monem et al. 2010; Armstrong et al. 1998; Corbeels et al. 1999; Kirda et al. 2001; Pilbeam et al. 1997). However, lower uptake of fertiliser N was observed when N was applied at sowing in 2013 or at GS30 in 2014 (Table 2); likely related to soil conditions following application. In 2013, applying N at sowing coincided with wet winter conditions (Fig. 2) conducive to N loss from denitrification (Barton et al. 1999; Parton et al. 1996) at a time when crop demand for N was low (Angus 2001). Conversely in 2014, N application at GS30 was followed by minimal rainfall for an extended period (7.8 mm over 20 days), potentially slowing ammonification (Savant et al.

1987), reducing mobility (Fenn and Miyamoto 1981) and availability (Dunbabin et al. 2009) of applied N and increasing the risk of ammonia (NH_3) volatilisation (Pan et al. 2016a; Turner et al. 2012).

Depending on the season and management, fertiliser application also resulted in a significant added nitrogen interaction (ANI) which can be associated with increased mineralisation of soil organic N and/or crop effects such as stimulation of root growth increasing exploitation of soil reserves (Liu et al. 2017). ANI effects were observed where urea was applied at sowing in 2014 and at GS30 in 2012 and 2013. However, it is uncertain whether the ANI observed for N applied at GS30 was due to a true ANI or the lack of fertiliser N early in the season, resulting in greater exploitation of soil N. The reduced N uptake observed at mid-tillering and anthesis suggest the later cause in 2012 (Table 2). Conversely, in 2014 urea application at GS30 did not increase soil N uptake, presumably due to reduced availability and low uptake of fertiliser N applied at GS30. A significant ANI and a reduction in N uptake at mid-tillering was also observed where DMPP amended urea was applied at sowing in 2012; suggesting that the use of DMPP may have slowed crop uptake of applied N. Previous studies have shown that banded urea can produce localised ammonium concentrations that inhibit root growth (Pan et al. 2016b; Passioura and Wetselaar 1972; Wetselaar et al. 1972). In the current study, the addition of DMPP may have maintained N in the ammonium form and inhibited root access to fertiliser N, forcing greater exploitation of soil N similar to delaying application until GS30 in 2012.

Fertiliser addition and the corresponding increases in crop N uptake consistently lead to increased biomass and grain yield, although the magnitude of
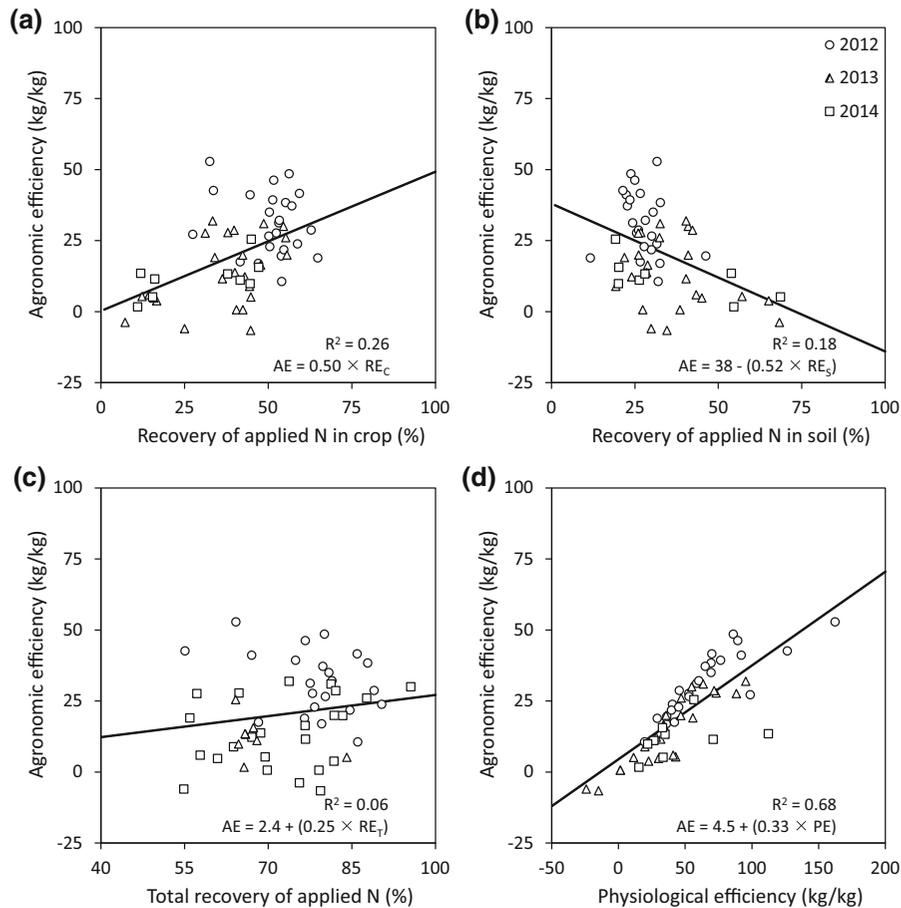
Fig. 4 Relationship between agronomic efficiency of N fertiliser application (kg/kg) and recovery of applied N (%) in crop (**a**), soil (**b**), total (**c**), physiological efficiency (**d**) of N fertiliser application (kg/kg). Solid lines represent the result of linear regression between the two factors

response varied with season and management. In 2012 and 2013, delaying N application until GS30 affected neither biomass nor grain yield. Delaying fertiliser application from sowing until GS30 coincides with increased growth rates, N demand (Limaux et al. 1999) and root mass (Shi et al. 2012; Tran and Tremblay 2000), likely resulting in the observed increases in RE_c. Previous studies have shown that delaying N application from sowing until GS30 can have limited effect on grain yield (Fischer et al. 1993; López-Bellido et al. 2005) while others have showed that early N deficiency (prior to GS30) can stimulate grain yield (Ravier et al. 2017). However, delaying N application until GS30 in 2014 significantly reduced shoot biomass and there was a trend towards lower grain yield, reiterating the importance of considering

soil conditions following application and its effect on availability and loss of applied N.

The use of inhibitors rarely produced significant increases in grain yield; the exceptions were applying DMPP treated urea at sowing in 2012 and NBPT treated urea at GS30 in 2013 (Table 2). However, there was a trend towards increased RE_c (+ 9%), N uptake (+ 9%) and yield (+ 5%) from the use of DMPP treated urea at sowing in 2013 and 2014 compared with unamended urea. These findings are similar to a meta-analysis by Abalos et al. (2014) which showed that use of DMPP or NBPT increased RE_c by 8% and 12% respectively and increased productivity by between 1 and 10% across a range of crops and environments. However, it should be noted that the relative benefit of using inhibitors is likely to

be higher in treatments receiving sub-optimal rates of N fertiliser (Rose et al. 2018). In the current study, peak grain yield across the broader experimental site generally occurred at a rate of 50 kg N/ha applied at sowing; the exception was in 2012 when this increased to 100 kg N/ha coinciding with a significant yield benefit from addition of DMPP to urea at sowing.

Loss of fertiliser N can be significant depending on management and season

The current study indicated that loss of fertiliser N from rain-fed wheat, grown in a semi-arid environment ranged between 13 and 42%, which aligns with similar studies (Angus and Grace 2017; Chen et al. 2008; Hancock et al. 2011; Patra et al. 1996; Pilbeam et al. 1997) and varied with an interaction of management and season. Highest loss of fertiliser N (42%) was recorded where unamended urea was applied at sowing in 2013. This was probably due to denitrification as significant volatilisation was unlikely due to the depth of application (Rochette et al. 2013). There was also limited evidence for movement of applied N to depths below 0.2 m (Table 3), supporting previous studies which also indicate low risk of leaching in similar environments and soils (Di and Cameron 2002; Poss et al. 1995). Furthermore, elevated soil WFPS conducive to denitrification (Granli and Bøckman 1994) was measured during winter and early spring 2013 resulting in elevated nitrous oxide emissions from the same treatment (Wallace et al. 2018). While the quantity of N lost via N_2O flux was minor, previous studies have shown that $N_2:N_2O$ ratios can be high depending on soil type and WFPS (Friedl et al. 2016; Weier et al. 1993).

In 2012 and 2013 total recovery of fertiliser N was higher when applied at GS30 rather than at sowing (Fig. 3), avoiding denitrification earlier in the season and coinciding with high crop N demand. These findings were similar to those of Kirda et al. (2001) and Harris et al. (2016) but in contrast with those of Hancock et al. (2011) where the risk of early season denitrification was negligible, but the risk of volatilisation of N applied in-season increased due to insufficient rainfall. The amount of rainfall required to mitigate volatilisation losses varies with soil texture, pH and initial soil moisture levels (Fillery and Khimashia 2016). Volatilisation loss can also be influenced by urea formulation (liquid *cf.* granular),

however such effects are also dependent on soil and environmental conditions at and following application (Titko et al. 1987; Watson et al. 1992). In this environment and on a similar soil type, Turner et al. (2012) measured volatilisation losses of 13–23% of the N applied over 15–18 days when post application rainfall was limited. In the current study, rainfall in the 10 days following N application was 17 mm and total fertiliser loss was 15% in 2012. While in 2013 40 mm of rainfall was received in the 10 days prior to application and 23 mm in the 10 days following; increasing soil WFPS and the likelihood of denitrification, but reducing volatilisation risk, resulting in fertiliser N loss of 20%.

Adding DMPP to urea applied at sowing in 2013 increased total recovery (+ 31%) compared to unamended urea; similar to Harris et al. (2016) in an environment where fertiliser N loss was high (poorly drained chromosol, high rainfall), but in contrast to De Antoni et al. (2014) where loss was minimal (well-drained oxisol, subtropical environment). Despite exhibiting similar soil WFPS to 2013, adding DMPP did not increase total recovery in 2012. Partitioning between crop and soil sinks indicates that RE_c of N applied at sowing was lower in 2013 (32–37%) than in 2012 (49–52%), increasing the proportion of fertiliser N susceptible to denitrification which may have resulted in the observed benefit from DMPP addition. Adding NBPT to urea applied at GS30 increased total recovery of applied N in 2013 (+ 10%) and 2014 (+ 19%) compared to unamended urea. While NBPT has been shown to slow ammonification (Turner et al. 2010) and reduce volatilisation loss (Sanz-Cobena et al. 2012; Suter et al. 2011), these findings contrast with those of Zhang et al. (2010) and Abdel Monem et al. (2010) which showed limited scope to reduce N loss through use of urease inhibitors. In the current study it is likely that addition of NBPT reduced the potential for denitrification following application (Abalos et al. 2012). Conversely, in 2014 rainfall following fertiliser application at GS30 was low (8 mm in the 20 days post application) and combined with dry soil conditions at application ($\theta_v \approx 24\%$, 0–0.05 m) it is possible that applying urea in solution may have increased the risk of ammonia volatilisation compared with a granular formulation, leading to the observed reduction in N loss following addition of NBPT.

Implications for fertiliser management in dryland, semi-arid cropping systems

The current study indicates that loss of fertiliser N and AE in this environment and soil type varies markedly depending on fertiliser management and seasonal conditions. Given the low predictability of seasonal conditions in this environment (Hayman et al. 2007), making widely applicable recommendations to mitigate N loss and improve productivity is difficult. While increases in AE can be attributed to increased RE_c or increased PE, in the current study AE was more strongly related to PE than RE_c (Fig. 4), in contrast with Bell et al. (2015) and Doyle and Holford (1993) which highlighted the importance of fertiliser RE_c to AE. However, these studies were conducted in northern (sub-tropical) Australia where long-term cereal cultivation has been linked to declining soil N reserves (Dalal and Mayer 1986), increasing reliance on fertiliser N to achieve basic crop productivity levels. Whereas in the Victorian Wimmera, greater use of N_2 fixing grain legumes has partially buffered against long-term declines in soil N supplies (Armstrong et al. 2018) and the proportion of crop N uptake derived from fertiliser in the current study was low (a range of 16–35%) resulting in the observed disconnection between RE_c of fertiliser N and AE.

In addition to considering fertiliser management in a tactical context, growers must also consider longer term N dynamics. The current study showed the potential for significant carry-over (53–62%) of applied N where crop uptake was low due to dry seasonal conditions, such as occurred in 2014 (Fig. 3). While the availability of this unused N to the subsequent crop is dependent on immobilisation, mineralisation and loss processes, it has been shown that where drought hinders crop uptake in the year of application, up to 29% of the applied N can remain available to the following crop (Feigenbaum et al. 1984). An international review by Smith and Chalk (2018) indicated that across a range of environmental conditions this figure tends to be low ($< 11\%$) in the first crop after application and decreases over time. Nonetheless, over the long-term, repeated carryover of unused N could help to maintain higher soil total N levels, resulting in enhanced supply of soil mineral N thereby making a return on fertiliser investments made in poor seasons.

Conclusion

This study has shown that application of fertiliser N is vital to the productivity of rainfed wheat in a semi-arid environment, even in years where rainfall is limited, but losses can be significant (up to 42% of the N applied). Deferring fertiliser application until GS30 significantly reduced fertiliser N loss in situations where rainfall occurred following application. However, when rainfall following application was limited, a large proportion of the fertiliser N remained in the soil and yield response tended to be lower. The use of nitrification or urease inhibitors also showed potential to reduce loss of applied N and in some cases, improve productivity. However, this effect was not consistent, making it difficult to predict best management practices in a highly variable climate. This observation may be related to the N rates tested, which were often at the upper end of the N response curve. The practical use of inhibitors in similar semi-arid environments also requires careful consideration of their cost in comparison to potential productivity increases.

While all of the wheat crops tested were highly responsive to N application, the majority of crop N uptake was sourced from the soil (65–84%). As a result, RE_c of applied N was not strongly related to increases in grain yield. We suggest that while loss of fertiliser N can be reduced through manipulating excess mineral N supply (either by timing of application or use of inhibitors), further improvements in coupling RE_c of fertiliser N with grain yield may be achieved through synchronising N supply to the timing of crop N demand. To achieve this, a better understanding of the dynamics of soil N supply must be coupled with knowledge of crop N demand at a given point in the season. While the current study considered both timing and use of inhibitors, optimising N placement (for example deep, mid-row or side banding) may also offer opportunity to improve crop NUE and reduce losses of fertiliser N to the environment.

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