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Soil mineral nitrogen benefits derived from legumes and comparisons of the apparent recovery of legume or fertiliser nitrogen by wheat

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Abridged title: Nitrogen benefits of legumes

Abstract

The nitrogen (N) contributed by legumes to the nutrition of subsequent cereal crops is an important component of profitability and sustainability. Additionally, few Australian grain-growers routinely monitor soil N fertility prior to cropping. A simple approach to estimate the contribution of legumes to soil N availability and crop N uptake would assist farmers in N fertiliser decision-making. Soil and crop N data generated from 16 dryland cropping experiments conducted in eastern Australia between 1989 and 2016 involving legume grain crops, brown-manured (BM) treatments (where legumes were terminated prior to maturity with herbicide), and wheat, barley, or canola were examined to explore soil N availability and crop N uptake in these systems.

Soil mineral N measured immediately prior to sowing a following wheat crop were significantly higher ($P<0.05$) after 31 of the 33 legume treatments than detected after the adjacent non-legume controls. Average soil mineral N benefits, defined as the difference in soil mineral N after legumes compared to non-legume controls, did not differ greatly for BM legumes compared with grain crops. The soil mineral N benefits were calculated as 0.15±0.09 kg N/ha per mm fallow rainfall, 9±5 kg N/ha per t/ha legume shoot residue dry matter, 28±11% of the total legume N remaining at the end of the growing season, and 18±9 kg N/ha per t/ha legume grain harvested.

The observed improvements in total N uptake by wheat grown following 20 legume treatments in eight experiments represented a mean apparent recovery of 30±10% of the legume residue N. The increased N accumulation by wheat after legumes reflected higher soil mineral N at sowing, greater in-crop soil N availability and healthier wheat roots recovering more soil N than in non-legume–wheat sequences.

The apparent recovery of fertiliser N was also assessed in the absence of legumes in two experiments. In these studies total N uptake by wheat in response to an additional 51–75 kg fertiliser-N/ha represented a mean apparent recovery of 64±16% of the fertiliser N supplied.

The information gleaned from analysis of 25-years of experimental data provided new insights into the expected improvements in the availability of soil mineral N after legumes and the relative value of legume N for a subsequent wheat crop which can assist grower fertiliser decisions.

Short summary: Soil and crop N data from 33 legume crops in 16 dryland cropping experiments conducted in eastern Australia between 1989 and 2016 identified relationships that can assist farmer decision-making by benchmarking the expected improvements in the availability of soil mineral N after legumes and estimating the relative value of legume N for a subsequent wheat crop.

Key Words: N uptake; pulses; canola; cereals; rotation; sequence

Introduction

The concentrations of soil mineral (i.e. nitrate+ammonium) nitrogen (N) measured prior to sowing a cereal in dryland (rainfed) farming systems depends upon the relative balance between the combined contribution of the carry-over of any mineral N not utilised by the previous crop (*spared N*; Evans *et al.* 1991; Chalk *et al.* 1993; Herridge *et al.* 1995) and the total N mineralised from above- or below-ground plant residues and the soil organic N pool by soil microbes (*total N released*; Murphy *et al.* 1998; Russell and Fillery 1999; Kumar and Goh 2000; Evans *et al.* 2001; Evans *et al.* 2003; Peoples *et al.* 2009). Soil mineral N concentrations are influenced by i) the extent to which crop residues or management influences the use of available soil N by soil microbes for growth (*N immobilised*; Green and Blackmer 1995; Murphy *et al.*1998; Kumar and Goh 2000), ii) assimilation by weeds (*weed N-uptake*; Hunt *et al.* 2013), and iii) leaching, erosion and gaseous losses (*N lost*; Smith *et al.* 1998; Fillery 2001; Schwenke *et al.* 2015) according to the following conceptual equation:

Soil mineral N = [(*spared N*)+(*total N released*)] – [(*N immobilised*)+(*weed N-uptake*)+(*N lost*)]   Equation [1]

Each of these processes can be influenced by:

(i) the duration of the period of fallow between the end of one cropping season and the beginning of the next since this defines the time available for weed growth, and mineralisation or loss processes to occur (Angus *et al.* 2000; Crews and Peoples 2005),

(ii) rainfall amount and distribution during the fallow period as soil moisture regulates soil microbial activity, determines the risk of N losses, and affects weed germination and growth (Fillery 2001; Crews and Peoples 2005; Verburg *et al.* 2012; Hunt *et al*. 2013; Schwenke *et al.* 2015), and

(iii) the quantity of plant residues remaining at the end of the previous growing season and the N content (or C:N ratio "quality" attributes) of those residues. Residue N content determines the amount of N potentially available for mineralisation (Evans *et al.* 2003), and C:N ratio influences whether a net release or immobilisation of mineral N occurs (Russell and Fillery 1999; Kumar and Goh 2000; Fillery 2001).

Many researchers have observed improved grain yields and/or N uptake by cereal crops grown after legumes compared to cereal-after-cereal sequences (e.g. Evans *et al.* 1991; Miller *et al.* 2003; Angus *et al.* 2015). This is usually attributed to elevated availability of soil mineral N and healthier wheat crops recovering more soil N following legumes (Evans *et al.* 2003; Angus *et al.* 2015). But while the principles associated with legume-induced improvements in soil mineral N are well known, previous studies have invariably been undertaken on experimental stations rather than under the prevailing abiotic and biotic constraints and cultural conditions typically experienced by commercial crops in farmers' fields. Furthermore, there have been few attempts to quantify or communicate the impact of legumes on soil N availability in a form that is directly relevant to grain-growers that they can directly apply to inform on-farm decisions about N fertiliser applications.

Australian dryland grain-growers rarely conduct pre-season soil testing, or routinely monitor soil N fertility across their large farms (average farm size is ~ 2,500 ha; Kirkegaard *et al.* 2011). Computer models have been developed that can simulate the complex soil N and water dynamics in rainfed cropping systems (e.g. Probert *et al.* 1995), which have been used to investigate N and water use efficiency in different environments and soil types in response to agronomic practice (e.g. Angus and van Herwaarden 2001; Asseng *et al.* 2001; Kirkegaard and Hunt 2010). Grain-growers can now access the outputs from these sophisticated research tools through subscriptions to internet-based decision support services to predict soil N availability and grain yield prospects in response to different N fertilisation scenarios and seasonal conditions (Hochman *et al.* 2009). However, the costs associated with obtaining useful input data (including soil mineral N) to parametrise the model, and the soil characterisation required to improve the accuracy of predictions, remain major barriers to the widespread adoption of such technologies (Hochman *et al.* 2009). Currently there are no convenient or simple approaches by which farmers and their advisors can benchmark the expected net effect of including a legume in a cropping sequence on the accumulation of soil mineral N prior to sowing the next crop, or to assess how much N from the preceding legume might subsequently be assimilated by the following crop.

This paper presents crop production and soil N data from 16 dryland cropping experiments undertaken at different locations across eastern Australia between 1989 and 2015 which aimed to quantify the N benefits derived from including legumes in cereal dominated cropping sequences. In these investigations soil mineral N concentrations immediately prior to sowing wheat for the next growing season were compared for soils after legumes and after various non-legume control treatments such as wheat (*Triticum aestivum*), barley (*Hordeum vulgare*), or canola (*Brassica napus*). The productivity and N accumulated by the following wheat crop was quantified. These studies differ from many previous investigations as the majority of the experiments where conducted in farmers' fields in partnership with individual grain-growers and local grower associations. The aim of this participatory research was to ensure relevance of the outcomes to farmer stakeholders, and to engage directly with rural communities in the communication and adoption of the results.

The collated crop and soil data from these experiments were used to assess the potential of different approaches to develop 'rules-of-thumb' that could be directly applied by farmers and their advisors as guides to the anticipated soil mineral N benefits derived from legumes harvested for grain (28 comparisons), or where legumes had been brown manured (BM) with herbicide prior to grain-filling (a strategy increasingly being deployed by farmers to control herbicide-resistant weeds and to enhance soil N availability; five comparisons). The apparent recoveries of the legume N by a following wheat crop were calculated for eight experiments (20 estimates), and the recoveries of legume N were directly compared to the apparent uptake of fertiliser N applied to wheat when grown after a preceding wheat or canola crop in two experiments (three estimates).

Materials and Methods

Site locations and treatments

The effects of 28 legume grain crops and five BM legume crops on soil mineral N were quantified in 16 experiments conducted during a 25 year period. These studies were undertaken in partnership with

FarmLink, Riverine Plains, Birchip Cropping Group (BCG), Mackillop Farm Management Group (MFMG), Mallee Sustainable Farming (MSF), or Eyre Peninsula Farming Systems grower groups, and/or in collaboration with the NSW Department of Primary Industries, Victorian Department of Economic Development, Jobs, Transport and Resources, or the South Australian Research and Development Institute.

The experimental sites were located on different soil types across northern and southern New South Wales (NSW), Victoria (Vic), and South Australia (SA; Table 1). Legume treatments included field pea (*Pisum sativum*), chickpea (*Cicer arietinum*), lupin (*Lupinus angustifolius*), faba bean (*Vicia faba*), lentil (*Lens culinaris*), and vetch (*Vicia sativa*). The non-legume control was wheat in nine experiments, barley in one (Breeza 1997), and canola in two studies (Tamworth 2009 and Loxton 2015). Three experiments included both wheat and canola as non-legume controls (Hopetoun 2009, Junee Reefs 2011, and Naracoorte 2011), but only the wheat data were used for subsequent calculations of soil mineral N benefits. During the 25 years, the various cropping treatments experienced both above- and below-average rainfall during the growing season (April-October) and in the post-harvest summer-autumn fallow (Table 1). Such variation in rainfall is typical of the dryland cropping systems of eastern Australia (Kirkegaard and Hunt 2010; Angus and Grace 2017).

Three of the 16 experiments were undertaken on research stations (Breeza 1997, Tamworth 2009, and Wagga Wagga 2011), but the remaining 13 investigations were conducted in farmers' fields. It should be noted that standard farmer practice in rainfed cropping systems of Australia changed considerably during the 25 years of experimentation with respect to the use of fertiliser N for wheat production, which increased from average applications of <5 kg N/ha to ~45 kg N/ha in recent times (Angus and Grace 2017). In 13 experiments the non-legume control received no N fertiliser N, but in three studies wheat and/or canola treatments had N fertiliser applied (49 kg N/ha at Junee Reefs 2011, 80 kg N/ha at Wagga Wagga 2011, 40 kg N/ha at Naracoorte 2011) to provide realistic comparisons of productivity and relative economics performance of the different cropping systems for the grain-growers engaged in the participatory research activities.

The experimental protocols of studies conducted prior to 2011 have previously been described (Herridge *et al.* 1995; Marcellos *et al.* 1998; Felton *et al.* 1998; Khan *et al.* 2003; Smith *et al.* 2004;

Schwenke *et al.* 2015; Denton *et al.* 2017). Comprehensive details for the other investigations will not documented here, but all followed similar basic designs to the aforementioned studies. Most experimental plots consisted of at least six crop rows of plants sown 0.2–0.3 m apart in a randomized complete block design in plots 10 – 35 m long with three or four replicates sown in either late-April to early-May (legume crops and canola) or mid to late-May (wheat). In all cases weeds were controlled with registered herbicides using recommended commercial practices during the summer-autumn fallow between crops and during the growth of the following wheat so neither the accumulation of soil mineral N nor the subsequent uptake of N by wheat were confounded by the presence of weeds.

While wheat was subsequently sown into all legume and non-legume plots in May of the following growing season, to quantify the impact of legumes for all 16 experiments, N analyses of the wheat grain and stubble were only undertaken for 11 studies. In three of these experiments (one at Tamworth 2010 and two at Culcairn 2011), wheat growth was not constrained by N availability and there were no responses to either legume pre-treatments or applications of fertiliser N. The N uptake data presented in the current paper come from the remaining eight experiments and represented 16 legume grain crops and four BM pre-treatments where significant wheat responses were observed (North Star 1990 and 1993, Breeza 1998, Gundibindjal 2001 and 2002, Junee Reefs 2012, Wagga Wagga 2012, and Loxton 2016).

The application of fertiliser N to wheat after wheat was included as an additional treatment to allow direct comparisons of the apparent recoveries of legume and fertiliser N in only two of these experiments (Junee Reefs and Wagga Wagga 2012). At Junee Reefs the wheat sown into the 2011 legume treatment plots received starter fertiliser of 25 kg/ha mono-ammonium phosphate (2.5 kg N/ha), and was top-dressed with 46 kg/ha urea-N at stem elongation. The 2011 wheat and canola plots were each split into 2 × 10 m sub-plots with one half receiving exactly the same N fertiliser treatment as the legume plots, and the other half being top-dressed with an additional 51 kg urea-N/ha (i.e. supplied with a total 97 kg urea-N/ha) just prior to stem elongation. At Wagga Wagga the wheat sown into the 2011 legume plots received no N fertiliser while the 2011 wheat plots received either no fertiliser or 75 kg fertiliser-N/ha (25 kg urea-N/ha at sowing with a further 50 kg N/ha at stem elongation).

Measurements

Above-ground biomass, grain yield and crop N

Shoot dry matter (DM) was determined immediately prior to legume BM termination, or around four weeks later in the case of the grain crops, at the time of peak biomass during late-pod fill and prior to leaf senescence, by removing all plants in the middle four rows from four 1 m sections of row from each plot. Shoot DM was measured after drying subsamples at 70 ºC. Another sampling of above-ground biomass of grain crops was undertaken just prior to maturity, which was separated into grain and vegetative residues. Dried shoot and grain samples were analysed for N and carbon (C) contents and ^{15}N abundance using a 20-20 stable isotope mass spectrometer (Europa Scientific, Crewe, UK). Grain yield was subsequently determined by mechanically harvesting at least 5 m of the middle four rows at the centre of each plot.

In the case of wheat sown following the legume and non-legume pre-treatments, all wheat plants were hand harvested close to ground level from four 1m sections of row for each plot or sub-plot at crop maturity to be separated into vegetative and grain components. Wheat stubble and grain DM and N contents were determined as described above to quantify crop N uptake.

Soil mineral N

Treatment plots were sampled for soil mineral N immediately before sowing wheat in the following growing season. The soil mineral N data presented here, and the values subsequently used for calculations, were normalised for the anticipated rooting depth of wheat. At Mildura, Naracoorte, Minnipa and Loxton this represented 0-0.6 m since inhospitable subsoils (high salinity, sodicity or alkalinity), or rocks at these locations effectively restricted root exploration and/or prevented soil sampling deeper in the profile (Nuttall *et al.* 2003; Adcock *et al.* 2007). At all other sites soil mineral N data were calculated to 1.2 m which approximates the average rooting depth of wheat in most eastern Australian soil types where there aren't major subsoil constraints to root growth (average maximum rooting depth of wheat = 1.29 m ± a standard deviation of 0.3 m, n=36; Kirkegaard and Lilley 2007). Examination of the distribution of soil mineral N in the soil profiles of treatments at eight of the experimental sites indicated that mineral N in the top 0.6 m was on average 68±9% of the total mineral N to 1.2 m (n=27), which in turn represented 88±5% of the soil mineral N detected to 1.5 m depth (data not shown). The distribution of soil mineral N in soil profiles following legumes (0-0.6 m equivalent to 66±8% of soil mineral N 0-1.2 m, n=17) was found to be similar to that observed after non-legumes (0-0.6 m equivalent to 71±9% of soil mineral N 0-1.2 m, n=9; data not shown).

Calculations

Total plant N

Since N associated with, or derived from, nodules and roots can represent a significant source of N for subsequent mineralisation (Evans *et al.* 2003; Khan *et al.* 2003; Yasmin *et al.* 2010) and can play a major role in determining the net inputs of fixed N and the N-balances of cropping systems (Evans *et al.* 2001; Walley *et al.* 2007; Peoples *et al.* 2009), below-ground N was estimated for each experimental treatment. For example, it was assumed that 25% of the whole plant N was below-ground for lupin and therefore a root-factor of 1.33 was used to convert shoot N to total plant N as described by Unkovich *et al.* (2010b). Root factors used for the other crops were 1.47 for both field pea and vetch, 1.52 for faba bean, 1.56 for lentil, and 1.82 for chickpea (Unkovich *et al.* 2010b; Yasmin *et al.* 2010). Below-ground N was also estimated for non-legume treatments using root-factors of 1.43 for canola and 1.52 for wheat, respectively (Wichern *et al*. 2008).

Legume N present in shoot and roots of BM treatments was calculated as:

Total BM-N = [(BM shoot DM) × %N/100] × root-factor Equation [2]

The total N accumulated in shoot and roots of grain crops at the end of the growing season was calculated as:

Total crop-N = [(vegetative DM) × %N/100]+[(grain DM) × %N/100] × root-factor Equation [3]

Legume N_2 fixation

Plants were harvested either from a 1 m length at each end of non-legume control plots which received no fertiliser N (Junee Reefs, Wagga Wagga, Naracoorte), or from unfertilised non-legume treatments included in the experimental design, at the same time as legume samplings at BM termination or peak biomass measurements. These non-N_2-fixing plants provided "reference" sampling of the ^{15}N natural abundance ($\delta^{15}N$) of plant-available soil N which was then compared to the $\delta^{15}N$ determinations for legume shoots to estimate the proportion of the legume N derived from atmospheric N_2 (%Ndfa; Unkovich *et al*. 2008):

$$\%Ndfa = 100 \times (\delta^{15}N\ soil - \delta^{15}N\ legume) / (\delta^{15}N\ soil - B) \qquad \text{Equation [4]}$$

The factor *B* included in Equation [4] represents the $\delta^{15}N$ of the shoots of the different legume species when entirely reliant upon N_2 fixation for growth. The *B* values used for calculations were average determinations from several glasshouse studies where nodulated plants were grown in N-free culture media and were -1.75‰ for chickpea, -0.79‰ for vetch, -0.66‰ for field pea, -0.57‰ for lupin, -0.56‰ for lentil, and -0.50‰ for faba bean (Unkovich *et al.* 2008).

The $\delta^{15}N$ values for the shoots of reference plants were >2‰ at all sites which is considered the lowest reference value where reliable estimates of %Ndfa can still be obtained (Unkovich *et al*. 2008). Estimates of fixed N have been previously reported for a number of the experiments included in these? analyses (Herridge *et al.* 1995; Marcellos *et al.* 1998; Khan *et al.* 2003; Schwenke *et al.* 2015; Denton *et al.* 2017). Little variation in legume or reference plant $\delta^{15}N$ was observed at any of the experimental sites with the standard errors of the means generally falling between ± 0.2–0.6‰ and the standard variation of the resulting mean %Ndfa determination for each legume treatment was typically ± 3–8%. Total amounts of N_2 fixed during the growing season were calculated from %Ndfa determination and measures of legume shoot N as:

$$\text{Amount of } N_2 \text{ fixed} = [(\text{legume shoot DM}) \times \%N/100] \times (\%Ndfa/100) \times \text{root-factor} \qquad \text{Equation [5]}$$

Residue N

For legume BM treatments, Equation [2] was used to estimate total residue N. In the case of crops grown for grain the total amounts of N remaining in crop vegetative residues and roots following grain harvest at the end of the growing season was calculated as:

$$\text{Total residue N} = (\text{total crop N}) - (\text{grain N removed}) \qquad \text{Equation [6]}$$

Soil mineral N benefits of legumes

The net effect of growing legumes on available soil N (i.e. the integrated effect of all the factors described in Equation [1]) were determined from the differences in soil mineral N data after legumes and non-legume controls measured just prior to sowing wheat across all treatments in autumn the following year. Soil mineral N benefits are likely to be strongly influenced by rainfall during fallow, …... The observed soil mineral N benefits of legumes were expressed in four different ways (Equations 7 to 10) as the basis of developing simple predictions:

(a) Mineral N benefit per mm fallow rainfall (kg N/ha per mm)

$$= [(\text{mineral } N_{\text{after legume}}) - (\text{mineral } N_{\text{after non-legume}})]/(\text{fallow rain}) \qquad \text{Equation [7]}$$

where fallow rainfall (mm) represented the cumulative total between legume grain or BM harvest and sowing of the following wheat crop. In most studies where legume grain crops were grown, the fallow period began in late November or early December and finished in May.

(b) Mineral N benefit per t shoot residue DM (kg N/t shoot residue DM)

$$= [(\text{mineral } N_{\text{after legume}}) - (\text{mineral } N_{\text{after non-legume}})]/(\text{legume shoot residue DM}) \qquad \text{Equation [8]}$$

where shoot residue DM = (peak biomass DM) – (grain yield)

(c) Mineral N benefit per t legume grain yield (kg N/t legume grain yield)

$$= [(\text{mineral } N_{\text{after legume}}) - (\text{mineral } N_{\text{after non-legume}})] /(\text{legume grain yield}) \qquad \text{Equation [9]}$$

(d) Soil mineral N benefit expressed as % total residue N

$$= 100\times [(\text{mineral } N_{\text{after legume}}) - (\text{mineral } N_{\text{after non-legume}})] /(\text{total legume residue N}) \qquad \text{Equation [10]}$$

where total legume residue N was determined from either Equation [2] for BM or [6] for grain crops.

Apparent recovery of legume and fertiliser N

The apparent recoveries of legume or fertiliser N by the first wheat crop grown after the legume and non-legume (wheat, barley or canola) treatments were calculated as:

Apparent recovery of legume N (% total residue N)

$$= 100\times [(\text{wheat } N_{\text{after legume}}) - (\text{wheat } N_{\text{after non-legume}})] /(\text{total legume residue N}) \qquad \text{Equation [11]}$$

where wheat N represented an estimate of total N in the shoots + roots calculated as described in Equation [2 or 3?].

Apparent recovery of fertiliser N (% additional N applied)

$$= 100\times [(\text{wheat N uptake } N_{R2}) - (\text{wheat N uptake } N_{R1})] /(N_{R2} - N_{R1}) \qquad \text{Equation [12]}$$

where wheat N represented an estimate of the total N present in shoots + roots, and N_{R1} or N_{R2} represent two different rates of fertiliser N applied to wheat grown after a non-legume. In the case of the Junee

Reefs experiment N_{R1} = 49 kg N/ha, and N_{R2} = 100 kg N/ha, so the recovery of N referred to just the additional top-dressed 51 kg fertiliser-N/ha. At Wagga Wagga N_{R1} = 0 kg N/ha and N_{R2} = 75 kg N/ha.

Statistical analyses

Throughout the paper mean values are presented along with ± standard variations to provide measures of variability. Analysis of variance was undertaken of the soil mineral N, crop DM and N data for each experimental site/year to provide least significant difference (LSD) determinations ($P<0.05$). However, similar statistical analyses were not so easily done for the derived estimates of soil mineral N benefits obtained using Equations [7]–[10]. As soil mineral N? and residue DM or N provided the basis of the estimates, significant differences in these main factors were considered sufficient to confer differences in soil mineral N benefit. Regression analysis was used to evaluate the predictive potential of different rules-of-thumb and to explore the relationship between legume grain yield and residue N.

Results

Legume growth and N accumulation

Data for shoot residue and grain DM, N accumulation, N_2 fixation, and calculations of net inputs of total residue N collated from all 16 experiments conducted in different locations, years, soil types and environments across the eastern Australian dryland cropping zone are presented in Table 2. Field pea grain crops were the most frequently used legume treatment (included in eight experiments), while vetch grown for grain was used the least (only once).

Large differences in growing season rainfall (GSR, 92–447 mm; Table 1) for the different experiments resulted in the accumulation of a wide range of legume shoot residue DM (1.4–9.8 t/ha), and grain yields (0.5–3.9 t/ha; Table 2). The harvest index (i.e. grain yield as a proportion of total above-ground DM calculated from Table 2) for the different legume crops grown for grain ranged from 0.17?–0.20 (field pea and chickpea at Loxton in 2015 which experienced a heat wave during grain-filling) to 0.53–0.57 (faba bean at Breeza in 1997 and lentil at Junee Reefs 2011), and were 0.25–0.40 for the remaining 20 of the 24 crops (mean 0.34±0.09 across all crops), values within the range commonly observed for Australian legume crops (Unkovich *et al.* 2010a).

Measures of N_2 fixation were determined for 24 of the 28 legume grain crops and all five BM legume treatments. Legume reliance upon N_2 fixation for growth (%Ndfa) ranged from 4–85% (mean 55±18%; Table 2) that resulted in 3–235 kg N/ha (mean 86±58 kg N/ha) of above-ground N fixed (detailed data not presented). On average 14±5 kg N was calculated to be fixed for every t of above-ground DM accumulated. This was somewhat lower than previously indicated in a meta-analysis of N_2 fixation by crop legumes in Australian agriculture (21 kg shoot N fixed/t shoot DM across all crops), but was similar to species specific determinations for chickpea and lupin reported in the same publication (11 and 14 kg shoot N fixed/t shoot DM, respectively; Unkovich *et al.* 2010b). These two crops represented 38% (11) of the legume treatments shown in Table 2 where measures of N_2 fixation were recorded. Total inputs of fixed N (i.e. fixed N in above-ground biomass and nodulated roots) were estimated to be 6–338 kg N/ha (mean 126±83 kg N/ha), yet despite the wide variation in N_2 fixation across legume species, locations and years, the amounts of N_2 fixed exceeded the N removed in grain for 19 of the 24 grain crops (Table 2).

The N contents of the legume shoot residues remaining after grain harvest was higher, and C:N ratios were typically lower (0.9–1.4% N, C:N ratios 33–56), than either canola (0.7–0.8% N, C:N ratio 50–60), or wheat and barley stubble (0.3–0.6% N, C:N ratio 75–160). Overall the N concentrations were highest and C:N ratios lowest in the BM treatments where the crops were terminated prior to maturity (2.3–2.6% N, C:N ratios 17–23; detailed data not shown). The estimates of net inputs of legume N associated with the vegetative residues and nodulated roots at the end of the growing season ranged 52–330 kg N/ha for crops grown for grain and 210–323 kg N/ha for BM legumes (Table 2). Comparisons of the contributions by individual grain crops in different experiments suggested that the largest net inputs of total residue N were often achieved by faba bean and lupin (Table 2).

Trends in available soil N

Soil mineral N concentrations measured after the non-legume controls in autumn varied widely from 36–141 kg N/ha, which presumably reflected key differences in inherent background fertility at the various study sites and rainfall during the preceding fallow period, but were on average 68±25 kg N/ha after wheat (n=13), 59 kg N/ha after barley (n=1) and 90±30 kg N/ha canola (n=5; Table 3).

Soil mineral N was significantly greater (*P*<0.05) after legumes than the non-legume controls for 26 of the 28 legume crops harvested for grain (non-significant data occurred following field pea and lentil at Loxton in 2015), and all five BM treatments (Table 3). The difference in autumn soil mineral N after legume BM crops and non-legume treatments ranged 43–86 kg N/ha (mean 60±16 kg N/ha, n=5) and 11–89 kg N/ha (mean 35±20 kg N/ha, n=26) following legume grain crops (Tables 4 and 5).

Soil mineral N benefits

The derived soil mineral N benefits, calculated for the 31 legume treatments where autumn measures of soil mineral N were significantly different from non-legume controls, were equivalent to 0.03–0.36 kg N/ha per mm fallow rainfall (mean 0.15±0.08), 3–20 kg N/t above-ground residue DM (mean 9±4), with 13–48% of the N remaining in vegetative and below-ground legume residue at the end of the previous growing season (mean 27±10%; Table 4). Despite large differences in crop performance, inputs of residue N, GSR and fallow rainfall across the 16 (15?) experiments (Tables 1 and 2), average values for the derived mineral N benefits were remarkedly similar between legume species, irrespective ofuse for grain or BM (Table 5).

In recognition of a previous observation that the size of the effects of lupin rotations on subsequent wheat in Western Australia was related to the grain yield by the lupin crop (Seymour *et al.* 2012), our data were further examined to ascertain whether there might be a useful relationship between legume grain yield and subsequent soil mineral N benefit. Values ranged from 7–46 kg additional soil mineral N/t legume grain harvested, with estimates for 17 of the 26 grain crops of between 11 and 26 kg N/t grain. The overall mean was 18±9 kg mineral N/t legume grain (Table 4).

In the absence of an independent data set to validate these four derived relationships, the accuracy of the different rules-of-thumb were evaluated by using them to predict soil mineral N benefits and comparing those predictions to measured mineral N benefits across the 16 experiments. The fraction of the variance explained by the various rules-of-thumb were then determined from r^2 assessments obtained from regression analyses of predicted soil mineral N benefits *vs* actual data. On the basis of such analyses it was suggested that:

0.15 (after grain crops) or 0.12 (after BM) × mm fallow rainfall explained 0.24 of the variance, 9 × t shoot residue explained 0.35 of the variance,

18 × t grain explained 0.27 of the variance, and

28% (after grain crops) or 24% (after BM) × total legume residue N explained 0.57 of the variance.

Other relationships based on fallow rainfall combined with either grain yield, shoot DM or residue N were also examined, but in all instances comparisons of predicted vs actual indicated that the ability to calculate subsequent soil mineral N was not improved. Indeed regression analysis of all approaches that combined more than one parameter suggested that <0.05 of the variance was explained by the process.

It was concluded that as a predictive tool the individual rules-of-thumb could only provide a rough approximation of the expected soil mineral N benefit. Of the four different expressions, predictions based on legume residue N were likely to be the most reliable because?. Unfortunately, residue N is a particularly difficult parameter for farmers to measure directly. Since grain yield is usually related to above-ground residue biomass (Evans *et al.* 2001; Walley *et al.* 2007; Unkovich *et al.* 2010a), the data were examined to ascertain whether grain yield might also provide a guide to the amount of total residue N remaining at the end of the legume growing season. Analyses of the experimental data were confounded by the clustering of yield below 1.5 t/ha and above 2.5 t/ha (solid circles; Fig. 1), and an apparent outlier (faba bean at Culcairn 2010, solid triangle; Fig. 1), so data for a further 20 legume crops obtained from previously published (Marcellos *et al.* 1998; McCallum *et al.* 2000; Peoples *et al.* 2001; Denton *et al.* 2017) and unpublished sources for another 16 experimental studies conducted in Victoria and NSW between 1995 and 2014 (open circles; Fig. 1) were included to improve the prospects of devising a relationship between grain yield and residue N. These additional data exhibited a degree of variation in residue N across similar yield values, presumably reflecting differences in seasonal conditions and harvest index. This was despite restricting the selection of data to legume crops with a similar range in harvest index to that measured for the majority of treatments in the original 16 experiments (i.e. between 0.25 and 0.40). Regression analysis of the combined data in Figure 1 indicated that total residue N = [54 + (30 × legume grain yield)] (r^2 = 0.56). Therefore, another more slightly complex rule-of-thumb for estimating the additional soil mineral N derived from crop legumes would be: 0.28 × [54 + (30 × t legume grain harvested)].

Apparent recovery of legume N by the following wheat crop

Pre-sowing soil mineral N following all legume and non-legume treatments, and subsequent measures of wheat total N uptake from eight of the 16 experiments are presented in Figure 2 and Table 6. Wheat N at harvest failed to exceed soil mineral N at sowing (i.e. fell below the 1 : 1 line depicted in Fig. 2) at only two locations, North Star 1993 and Breeza 1998. The lower than expected wheat uptake at Breeza could have been associated with potential? measured? denitrification losses of N from the soil as a result of waterlogging due to the record high rainfall experienced during the growing season (866 mm compared to 334 mm long-term average; Tables 1 and 6). Examination of the data across all trials and treatments except Junee Reefs (where applications of fertiliser N would have confounded the calculations), indicated that on average wheat accumulated 1.34±0.67 kg N/ha for every 1 kg/ha of soil mineral N present at sowing. Given that the ratio of wheat N uptake : pre-sowing soil mineral N exceeded 2.5 : 1 at one location (Gundibindjal 2001), and fell between 1.1–1.7 : 1 across all other experiments and treatments, it was concluded that soil N mineralisation during crop growth contributed to wheat N uptake at most locations in most years (Fig. 2).

All legume treatments significantly increased ($P<0.05$) above-ground biomass (0.60.7?–4.5 t/ha mean 2.4±1.2 t/ha) and total N uptake (8–86 kg N/ha, mean 38±23 kg N/ha) of the subsequent wheat crop above that achieved by wheat grown after a non-legume control (Table 6). However, the improved wheat N uptake and crop growth was not always translated into grain, and yield was significantly greater ($P<0.05$) than the neighbouring wheat on wheat, barley, or canola treatments following only 16 of the 20 legume pre-cropping treatments. The increased N uptake represented apparent recoveries of legume N by wheat equivalent to 12–48% of the residue N estimated to be remaining at the end of the previous growing season (mean 30±10%; Table 6).

The potential contribution to wheat N uptake of greater N mineralisation after legumes was explored by calculating the apparent crop recoveries of the additional soil mineral N present at sowing from the data in Table 6 using the following Equation [13]:

Apparent recovery of soil mineral N (%) =

$$100\times [(\text{wheat } N_{\text{after legume}}) - (\text{wheat } N_{\text{after non-legume}})] / [(\text{mineral } N_{\text{after legume}}) - (\text{mineral } N_{\text{after non-legume}})]$$

The resulting determinations of the apparent recoveries of the additional pre-sowing soil mineral N after legumes exceeded 100% (i.e. the increased wheat N uptake exceeded the additional soil mineral N present at sowing) for 10 of the 20 legume treatments which confirmed the presumed importance of the contribution of soil N mineralisation to crop N uptake.

Comparisons of recoveries of legume N with fertiliser N

The effect of legumes on wheat N uptake could only be directly compared to fertiliser N in two experiments. In these, applications of fertiliser N increased total N uptake by wheat grown after canola or wheat by 25 and 31 kg N/ha, respectively at Junee Reefs, or by 61 kg N/ha, at the Wagga Wagga site (Table 7). The apparent recoveries of the fertiliser N were calculated to be equivalent to 49–81% of the fertiliser N supplied (mean 64±16%; Table 7). While these determinations of apparent recoveries of fertiliser N by wheat were somewhat higher than the recovery of legume N in the same experiments (mean recovery of 30±5% of residue N from nine legume treatments), the additional quantity of N accumulated by wheat in response to fertiliser N was lower than observed after all legume treatments (38–84 kg N/ha) at Junee Reefs (Table 5) and the BM crops (69–86 kg N/ha) at Wagga Wagga.

Discussion

Effect of legumes on soil mineral N

In keeping with the findings of other previous studies undertaken in Australia and elsewhere in the world, concentrations of soil mineral N were significantly greater following legumes compared to after non-legumes (e.g. Evans *et al.* 2003; Miller *et al.* 2003; Angus *et al.* 2015). In absolute terms the magnitude of the effect of legumes varied across locations, years and whether the crop was harvested for grain or terminated during spring as a BM crop (Tables 3–5). With legume grain crops, the influence of rainfall within the growing season on biomass production (Evans *et al.* 2001), and rainfall during the subsequent fallow, was such that improvements in soil mineral N tended to be lowest after lentil and vetch grain crops, and highest after faba bean (Table 5), which was consistent with faba bean's reputation as a species with a capacity for the accumulation of high biomass and the symbiotic fixation of large amounts of atmospheric N_2 (Peoples *et al.* 2009; Jensen *et al.* 2010).

As previously noted by other researchers (e.g. Evans *et al.* 2003) the impact of BM legumes on soil mineral N was generally greater than where legumes were grown for grain (Tables 4 and 5). The elevated concentrations of soil mineral N after BM treatments reflected the greater net inputs of residue N (Table 5), the higher concentration of N and lower C:N ratio of above-ground vegetative material (Russell and Fillery 1999; Fillery 2001; Evans *et al.* 2003), and longer period available for mineralisation (Angus *et al.* 2000). However, reasons for the unusually high soil mineral N following the chickpea grain crop at Junee Reefs, compared to the lupin and field pea BM crops (Table 4), remains unresolved. Some of the additional soil mineral N could have arisen from chickpea's tendency to be less efficient at recovering soil mineral N during growth than wheat (Herridge *et al.* 1995). Unfortunately, soil mineral N was not determined following chickpea grain harvest so the presence of unutilized 'spared' mineral N cannot be confirmed in the Junee Reefs experiment. Chickpea is also known to partition a larger proportion of the plant N below-ground in nodules than most other legume species (Unkovich and Pate 2000; Khan *et al.* 2003). Nodules tend to have high N contents (4–7% N) and low C:N ratios which is conducive to rapid decomposition rates (Kumar and Goh 2000; Zhu and Cheng, 2012), so it is possible that the observed effect of chickpea on soil N dynamics could have reflected a higher nodule load combined with high rainfall during the summer-autumn fallow period to stimulate microbial activity and mineralisation processes, even though chickpea demonstrated lower %Ndfa than other legumes (Tables 1 and 2).

Soil mineral N benefits derived from legumes

Pre-season soil testing for soil mineral N is the most accurate data that grain-growers and their advisors can utilise to make informed decision-making about fertiliser N applications for wheat in light of long-range weather forecasts for the coming growing season and grain prices. Unfortunately, few Australian farmers routinely monitor soil N availability in their fields prior to cropping. In the absence of pre-season soil testing, the most valuable information that could be provided to farmers would be some means of predicting the soil mineral N prior to sowing wheat which could be used as a basis for decisions about rates of N fertiliser to apply to meet target yields and grain quality. The large location and year variability in the soil mineral N data observed following the non-legume control treatments in all 16 experiments (Table 3) exemplifies the underlying influence that different soil types, soil organic N contents, and preceding rainfall can have on the end result, and emphasises the challenge in devising such a tool. However, it was hoped that through the interrogation of data collated from 25 years of cropping systems studies that it might be possible to identify some simple relationships that could be utilised to benchmark the likely incremental improvement in soil mineral N as a result of growing a N_2-fixing legume rather than a non-legume.

Two key parameters used in the calculations of soil mineral N benefits of legumes (Tables 4 and 5) that all farmers routinely monitor or measure are rainfall and grain yield. Therefore, of the four potential measures of mineral N benefits examined here, relationships described as 0.15 kg N per mm fallow rainfall, and 18 × t legume grain yield (Table 5), are the ones that could most easily be applied by farmers. Given the relationship between grain yield and shoot DM reported here (i.e. average harvest index = 0.34±0.09), growers might also be able to estimate shoot residue biomass by assuming grain yield generally represents around one-third of above-ground biomass (i.e. shoot residue DM = 2 × t legume grain yield). By combining this knowledge with the estimate of 9 kg additional mineral N/t shoot residue DM (Table 5) the added contribution of crop legumes to soil mineral N could also be calculated to approximate: 18 × t legume grain harvested. However, the most reliable estimate of soil mineral N benefit is likely to be calculated on the basis of net inputs of total residue N after legume cropping (28% residue N; Table 5). By utilising the relationship between grain yield and legume residue N presented in Figure 1, soil mineral N benefit calculated on a % N residue basis could also be re-expressed in a form that farmers can extrapolate from grain yield as: 0.28 × [54 + (30 × t legume grain harvested)].

The soil mineral N benefits reported here were generally lower than equivalent determinations from other studies involving pasture legumes in southern NSW and northern Victoria where soil N availability has been examined following the removal of lucerne (alfalfa, *Medicago sativa*) prior to cropping (0.5 kg N/ha per mm of rainfall during the fallow period following stand termination; Angus *et al.* 2000), or after a range of legume species and pasture mixtures (an additional 15 kg mineral N/additional t of legume forage DM accumulated during a three-year pasture phase; Peoples *et al.* 2004, or 20 kg mineral N/ t of legume forage DM grown in the year immediately prior to cropping; Harris *et* *al.* 2006). This is perhaps not surprising given that pastures in south-eastern Australia tend to be grown for at least two to three years before returning to a cropping phase (Kirkegaard *et al*. 2011), with the legume components of those pastures fixing atmospheric N_2, and foliage N being recycled back onto the pasture via livestock urine and faeces, to contribute to the soil N fertility during the entire period (Fillery 2001; Peoples *et al* 2004; Angus and Peoples 2012). In contrast, legumes in a cropping sequence are present for only part of a single year, and a much larger portion of accumulated plant N is removed at grain harvest than removed or lost from grazed pasture systems (Evans *et al.* 2001; Peoples *et al* 2009; Peoples *et al.* 2012).

Although the relationships between increased soil mineral N and either fallow rainfall, shoot residue DM or total residue N calculated following BM legumes were similar to those developed for legume crops grown for grain (Table 5), the potential value of these different rules-of-thumb is not so straightforward. Brown manuring of legumes by farmers is primarily used as a strategy to reduce the seed set of herbicide-resistant grass weeds, with any improvements in soil N availability being seen as an additional bonus. What impact grass residues within the BM legume biomass has on soil N dynamics is yet to be quantified, but studies examining different mixtures of grass and clover pasture species suggest that potentially there could be large effects on N mineralisation and immobilisation processes (de Neergaard *et al.* 2002; Peoples *et al.* 2004). Furthermore, the timing of crop termination during the growing season will almost certainly influence the subsequent accumulation of soil mineral N, and the relative balance of the available N derived from either soil organic matter or legume residues (Angus *et al.* 2000; Peoples *et al.* 2004). So it is difficult to envisage single relationships that could be applied to a range of situations without further research to examine the influence of such variables. It will also be necessary to devise some way for grain-growers to conveniently estimate shoot biomass and residue N for BM crops. Measurements of crop height could perhaps suffice for legume species with erect growth habits (Evans and Heenan 1998).

Apparent recoveries of legume and fertiliser N by wheat

While the observed range of estimates of the apparent recovery of legume N by wheat was large (12–48%), 15 16? of the 20 determinations fell between 21-39%, and the mean represented 30±10% across all legume treatments (Table 6). This provides new insights into the value of including legumes in a cropping sequence in the rainfed grains belt of eastern Australia. Applying a similar approach to the one described above to estimate total residue N from grain yield, farmers could also calculate the likely recovery of legume N by a following wheat crop as: $0.30 \times [54 + (30 \times \text{t legume grain harvested})]$. The data strongly suggested that for most crops wheat's enhanced N uptake reflected improvements in N availability both prior to sowing wheat and during crop growth. A reduced incidence of cereal root disease following a legume break crop was also likely to have assisted wheat's ability to more fully exploit the soil mineral N pool (Kirkegaard *et al.* 2011; Angus *et al.* 2015).

The mean apparent total recovery of fertiliser N by wheat calculated from two experiments conducted in southern NSW (64%; Table 7) was comparable to the mean value previously reported for wheat in Australia (38% on a shoot basis, which is equivalent to 58% when re-calculated as total shoot + root N, n=42; Krupnik *et al.* 2004). That the apparent recoveries of fertiliser N were higher than calculated for legume residue N in the same studies (30 %, Table 6) was not surprising given that either two-thirds (Wagga Wagga), or >90% of the fertiliser N applied (Junee Reefs) was supplied at the stem elongation phase of crop development immediately prior to a period of high plant demand for N (Crews and Peoples 2005), and that only a fraction of the organic legume N would have become available for crop uptake (Murphy *et al.* 1998; Russell and Fillery 1999; Fillery 2001; Peoples *et al.* 2009). However, it should be noted that the available soil N generated after legume cropping should be just as effective a source of N to support wheat growth as N released from fertiliser, and can potentially represent a larger pool of inorganic N for crop uptake.

Conclusions

In the absence of any direct measures of soil mineral N, the four predictive relationships reported here could be used by grain-growers and their advisors in the dryland cropping areas of eastern Australia to estimate the additional pre-sowing soil mineral N following legume grain crops as they can be calculated directly or indirectly from readily-available information such as rainfall and legume grain yield. Growers could also potentially apply the relationship developed in the current paper to estimate N remaining in legume residues from grain yield to benchmark the subsequent recovery of legume N by a wheat crop. Recognising that none of the relationships will provide perfect predictions, and acknowledging that there are potential consequences in over- or under-estimating available N and wheat N uptake, it is recommended that all five expressions be used as a means of providing some measure of uncertainty. The risks of either under-fertilising in a wet growing season and not realising yield potential, or over-supplying fertiliser N to wheat when there is a prolonged period of drought during spring which can lead to yield reductions due to haying-off (van Herwaarden *et al.* 1998), would also be lowered, and the efficiency of fertiliser N uptake improved, if decisions on applications of N fertiliser can be delayed until later in the growing season when there is more confidence about anticipated rainfall (Crews and Peoples 2005).

More experimentation following the accumulation of soil mineral N and crop recovery of N after legumes still needs to be undertaken across different soil types, farming practices (especially for BM treatments) and years to evaluate and validate the preliminary rules-of-thumb proposed here and to further refine the various relationships. Studies should also be initiated to explore whether similar approaches to those described in the current paper might usefully deployed in dryland grain production systems beyond eastern Australia.

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