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Nitrogen fixation in Australian dairy systems: review and prospect

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Abstract. Quantitative measurement of N_2 fixation has rarely been conducted in Australian dairy pastures. The available data indicate that annual N_2 fixation rates in Australian dairy pastures are generally low, due to low pasture legume content. With typical legume contents of grazed pastures less than 30% of total pasture biomass production, annual N_2 fixation in herbage is usually much less than $50\,kg\,ha^{-1}\,year^{-1}$. Other factors which are likely to be able to contribute to increased N_2 fixation input (rhizobia, mineral N management, soil acidity, soil water contents) will have little impact until such time as legume contents are increased. In contrast, for some hay systems, such as those using lucerne, N_2 fixation input is shown to be high ($200–300\,kg\,ha^{-1}\,year^{-1}$).

While pasture clover contents remain low there is little value in study or measurement of N_2 fixation, nor in complex modelling, as N_2 fixation will be of little quantitative importance. However, where legume contents, and thus potential N_2 fixation are increased, there is scope for investigation into potential increases in N input from this source, which is invariably linked to fertiliser application, the management of grazing and the N returns in urine and dung. These are the major influences on sward N dynamics and legume N_2 fixation. The inoculant rhizobia used for white clover in Australia (TA1) is likely to be suboptimal. Isolated in Tasmania in 1953 it has been shown to be inferior in N_2 fixation compared with other strains on several occasions. Root pests and diseases are likely to be prevalent and impact directly on clover root growth and perhaps nodulation.

Modelling is often used to describe the probable influence of management and/or climate on the operation of agricultural systems. Reliable modelling of N_2 fixation requires capacity to integrate the effects of grazing and pasture composition on soil mineral N dynamics, the influence of this mineral N on nodulation and on suppression of N_2 fixation, and environmental and management influences on soil rhizobial populations. Currently no models have demonstrated this capacity. At present, a suitably calibrated regression model is probably a good option for modelling N_2 fixation in Australian dairy pastures.

Environmental benefits ensuing from increasing N_2 fixation and substituting this for fertiliser N are likely to be greater off-farm (reduced GHG emissions at site of fertiliser manufacture) than on, if current fertiliser management is optimal. Nevertheless substituting fixed N for fertiliser N would have modest environmental and feed efficiency benefits.

Introduction

Nitrogen (N) in plants is the primary N source for animal and milk protein production in dairy systems. Biological dinitrogen (N_2) fixation is the process whereby specialised microorganisms are able to convert N_2 from the atmosphere into ammonia (NH_3) via the enzyme nitrogenase. This 'fixed' N can then be incorporated into microbial and plant protein. This is a very important process because, along with fertiliser N (industrial N fixation), it provides the main entry point for N into agricultural systems. There are four principal forms of N_2 fixation which relate to the type of N_2-fixing bacteria and to the strength of their relationship with plants. Some bacteria fix N_2 in a *free-living* state, while others fix N_2 in association with plants. The associations with plants range from rather loose associations around plant roots (*associative*), *endophytic* N_2-fixing bacteria residing in the vascular tissues of some grasses, and finally, highly-evolved, complex *symbioses*, involving morphological changes of both microbe and plant in specialised root structures (nodules). In legume symbioses the

N_2-fixing bacteria pass all of the fixed NH_3 directly on to their plant hosts which incorporate it into plant protein. The N_2-fixing symbioses with legume plants (e.g. clovers, medics, peas, beans) are the most important because they are more highly evolved and able to fix much greater amounts of N than the other associations. For example, symbiotic N_2 fixation can provide for all of the N requirements of pasture legumes, while for pasture grasses the N_2-fixing associations are unlikely to be able to provide more than 10% of grass N demand, even under optimal conditions.

The objective of the present review is to document the state of knowledge of N_2 fixation in dairy pastures in Australia and to indicate potential areas of research which might increase the value of N_2 fixation in Australian dairy systems. While the review is clearly directed at Australian field studies, the limited Australian research requires recourse to salient reviews or critical information from studies elsewhere. This review focuses primarily on perennial high-rainfall or irrigated legume pastures where much of the Australian dairy industry is located.

Thorough reviews on N_2 fixation in annual legume pastures can be found elsewhere (e.g. Unkovich *et al.* 1997; Peoples *et al.* 1998, 2001; Peoples and Baldock 2001) and Unkovich *et al.* (1998) provide a detailed study of N dynamics in grazed annual clover pastures.

Some of the more pertinent reviews on N_2 fixation in grazed perennial pastures include Haynes and Williams (1993), Jarvis *et al.* (1995), Ledgard and Steele (1992), Ledgard (2001), Menneer *et al.* (2004), while the reviews of Carlsson and Huss-Danell (2003) and Cuttle *et al.* (2003) are also quite useful. Eckard (1998) provides salient background to the N dynamics of dairy pastures in Australia and likely responses to N fertiliser application but does not explicitly deal with N_2 fixation.

Operation of the N_2-fixing legume symbiosis under field conditions

Symbiotic N_2 fixation is a complex process involving two organisms in a dynamic partnership subject to a range of environmental and management influences. While the physiological operation of the symbioses are generally understood (Schulze 2004; Garg and Geetanjali 2007), an ability to predict N_2 fixation under field conditions requires site-specific knowledge of partner and symbiotic responses to relevant local environmental and management parameters (Russelle 2008). Interactions between grazing and competition for light with grasses exert considerable influence on the balance between grasses and legumes in pasture systems but this will not be considered in detail here. Readers are referred to Schwinning and Parsons (1996).

Mineral N depresses N_2 fixation

While legumes have the capacity to fix atmospheric N_2 via their symbioses with rhizobia, they are also able to take up soil mineral N like non-legume plants. Indeed they have a preference for use of soil mineral N such that the nodulation and N_2 fixation processes are downregulated or turned off in the presence of significant concentrations of mineral N (see Streeter 1988). The dynamic relationship between these factors is illustrated in Fig. 1 for two annual pasture legumes grown under controlled (glasshouse) conditions. The figure highlights that (*i*) both nodulation and N_2 fixation are downregulated by available mineral N (*ii*) small amounts of mineral N can stimulate growth, nodulation and N_2 fixation, and (*iii*) there are significant differences between species in the extent of these relationships. Although it is not illustrated here, the same legume species with different rhizobia may also vary in their sensitivity to soil mineral N (Unkovich and Pate 1998).

In the context of grazed dairy pastures, this means that returns of N in urine and dung will suppress N_2 fixation if most of the resultant mineral N is not taken up by companion grasses. Similarly, application of fertiliser N to legume pastures will suppress clover N_2 fixation (see e.g. Ledgard *et al.* 1996, 2001). Regardless of fertiliser N application, this phenomenon is most likely to occur under urine patches which may result in concentrations of readily mineralisable N equivalent to $\geq 1000 \, kg \, ha^{-1}$ (Haynes and Williams 1993). Such mineral N concentrations would be expected to suppress N_2 fixation and nodulation for some months. Soil nitrate concentrations high
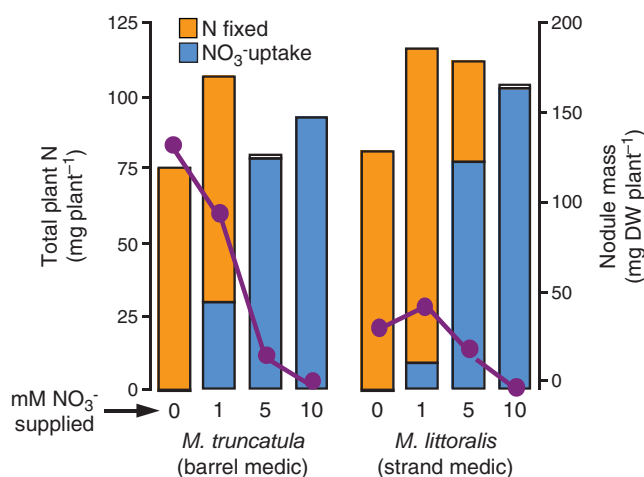

Fig. 1. Relationship between mineral N (nitrate) supply and nodulation (right *y*-axis) and N_2 fixation for two annual pasture legumes. From Pate and Unkovich (1999).

enough to suppress nodulation and N_2 fixation may also arise in rain-fed pastures at the end of summer and into autumn, particularly in pastures containing annual species (see Unkovich *et al.* 1998).

In a study in northern Victoria, Mundy (1987) used a ^{15}N tracer to follow fertiliser uptake and N_2 fixation in an irrigated white clover/ryegrass pasture following the application of 5 or $100 \, kg \, N \, ha^{-1}$ (Fig. 2). Pasture clover content was reduced from 40% in the $5 \, kg \, N \, ha^{-1}$ treatment to 20% with $100 \, kg \, N$ fertiliser applied. However, total mineral N uptake by clover was not reduced but fertiliser N uptake was substituted for N_2 fixation, which was reduced from 74 to 45% of clover herbage N. The authors indicated that this suppression of N_2 fixation continued for at least 10 weeks. These data demonstrate the dynamic interaction between soil mineral N availability, clover and grass growth, and symbiotic N_2 fixation, even in the absence of grazing animals. Increased availability of soil mineral N reduces the competitive advantage of N_2-fixing legumes under low soil mineral N supply, switches off legume N_2 fixation and reduces pasture clover content.
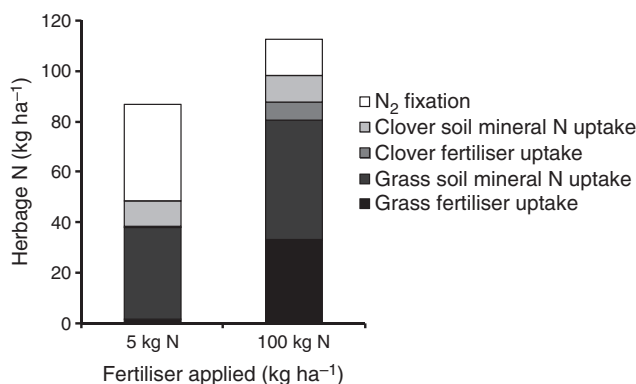

Fig. 2. Sources of herbage N in an irrigated white clover/ryegrass pasture in northern Victoria 35 days after the application of 5 or $100 \, kg \, ha^{-1}$ N fertiliser. Plotted from the data of Mundy (1987).

A second example of the effect of N fertiliser application on N$_2$ fixation in an irrigated white clover/ryegrass dairy pasture from northern Victoria is shown in Fig. 3. Following application of 100 kg N ha^{-1}, N$_2$ fixation remained at ~50% of that for unfertilised pasture over the following 2 weeks.

In a study of a rain-fed white clover pasture in western Victoria (McKenzie *et al*. 1998), application of 45 kg N ha^{-1} had no measurable impact on N$_2$ fixation, regardless of fertiliser type (Table 1). However, in this case, differences between treatments might not be expected since prior grazing may have provided much more mineral N than the modest fertiliser application, and this effect may last many months (Menneer *et al*. 2004) and, furthermore, very low legume content (9%) and thus low N$_2$ fixation (2–4 kg ha^{-1}) would mask potential treatment effects on measured N$_2$ fixation.

In a second experiment McKenzie *et al*. (1998) applied 0–60 kg N ha^{-1} to the pasture and N$_2$ fixed ranged from 0.8–3.7 kg N ha^{-1}. While these authors indicated a positive linear response to increasing N fertiliser N application, this seems an unlikely conclusion given the difficulties of measuring such small differences in N$_2$ fixation at the field level (Unkovich *et al*. 2008). The results of these two experiments highlight the limited value in measuring N$_2$ fixation in low clover content pastures.

Soil water limitations to N$_2$ fixation

N$_2$ fixation activity of legume nodules declines under high soil water contents associated with flood irrigation (Mundy *et al*. 1988) or water logging, possibly a consequence of the production of ethanol in nodules under anoxic conditions (Sprent and Gallacher 1976). Decreases in soil oxygen availability and subsequent declines in N$_2$ fixation may also result from pugging or increased bulk densities under grazing (Menneer *et al*. 2001). Nitrogenase activity also declines at low soil water contents, and probably more so than plant growth (Davey and Simpson 1990), although it is difficult to separate these as it is often unclear whether N$_2$ fixation activity reduction is due to reduced plant demand for N or a reduced supply of photosynthate to the nodules. Nodule activity declines with water stress, but can only recover if the water stress is moderate (Sprent 1971). Recommencement of N$_2$ fixation after more severe stress requires regrowth of existing nodules (3–4 days, Engin and Sprent 1973), but after drought, initiation and growth of completely new nodules is required, which would take longer (5–10 days, Davey and Simpson 1990).

An example of the sensitivity of the N$_2$-fixing nodule to soil water content is given in Fig. 4, which shows nitrogenase activity for two irrigated white clover pastures in northern Victoria. The two sites had different soil bulk densities, thus different pore space, and presumably oxygen availability, but the relative effects of soil water content were maintained. For irrigated systems there is thus a challenge to maintain soil water content within the non-limiting range to maximise N$_2$ fixation activity.
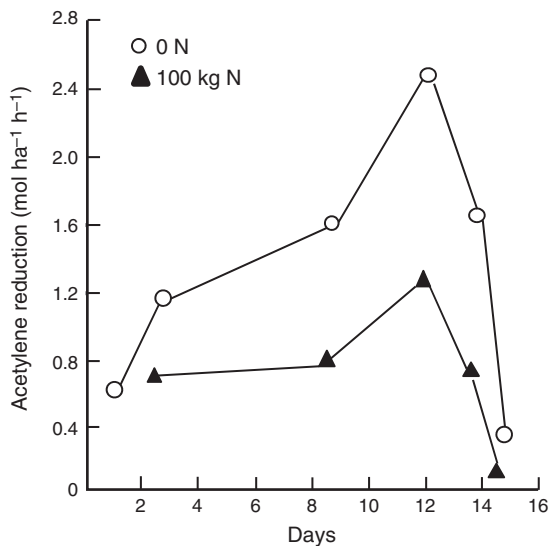

Fig. 3. The sensitivity of N$_2$ fixation (relative nitrogenase activity) to applied N in irrigated white clover pasture in northern Victoria. Redrawn from Mundy *et al*. (1988).

Table 1. Response of a ryegrass/white clover pasture to fertilisers measured 37 days after application

All fertiliser treatments were applied at a rate of 45 kg N ha^{-1}. From McKenzie *et al*. (1998)

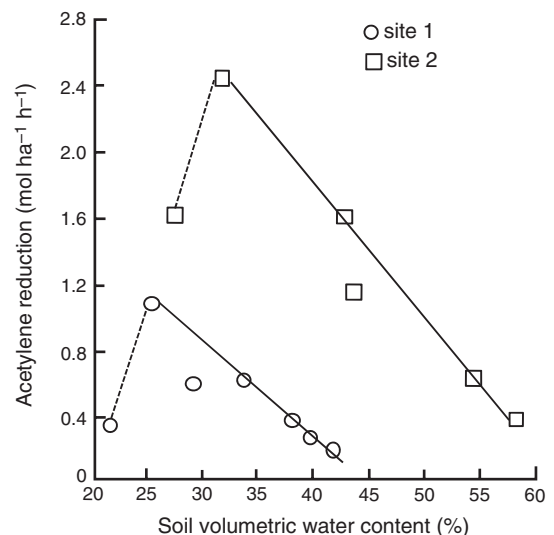| Fertiliser	%Ndfa	N fixed (kg ha^{-1})
None	69	3.6
PKS	60	2.5
Urea	58	1.9
Pastureboosta blend	59	2.0
Ammonium nitrate	65	3.0
Di-ammonium phosphate	65	2.9
Ammonium sulfate	64	2.7
Ammonium nitrate and sulfur	70	4.1
Urea + PKS	69	3.5
Pastureboosta + PKS	66	3.5
Ammonium nitrate + PKS	64	2.6
DAP + PKS	64	2.7
Ammonium sulfate + PKS	61	2.6
Ammonium nitrate and sulfur + PKS	66	3.4

Fig. 4. The sensitivity of symbiotic N fixation (relative nitrogenase activity) to soil water content. Redrawn from Mundy *et al*. (1988).

White clover may be more susceptible to water stress than other perennial pasture legume species and lucerne more tolerant (Kelly *et al*. 1989; Neal *et al*. 2009), Ostrowski (1972) considered white clover to be more susceptible to water than heat stress. This is a probable explanation for observed increases in pasture growth in summer in high-rainfall or irrigated (Kelly and O'Brien 1992) environments when clover contents are increased, and potential increases in N_2 fixation during the warmer months of the year (see Eckard 1998, 2001). The low drought tolerance of white clover may be a significant cause of its poor persistence in many systems, even under irrigated conditions where white clover may only maintain maximal growth for 4–5 days after irrigation (Mason *et al*. 1987). Pasture clover content thus appears to be higher with more frequent irrigations (Dunbabin *et al*. 1997). Compared with other perennial legumes, lucerne may be more tolerant of water stress, producing greater biomass than five other perennial legumes when grown under deficit irrigation (Neal *et al*. 2009).

Temperature and N_2 fixation

There is considerable inconsistency in the literature relating temperature to N_2 fixation in white clover. Whitehead (1995) suggests that N_2 fixation ceases below a soil temperature of 9°C, but other evidence indicates that it occurs over a wider range of temperatures (~2−40°C), and is relatively insensitive to temperature over quite a wide range (15−30°C) (Liu *et al*. 2010). Provided that there is adequate water available, white clover can maintain a constant N_2 fixation rate over the 20–33° temperature range (Ryle *et al*. 1989) and thus the summer temperatures experienced in the Australian dairy regions should not be prohibitive to N_2 fixation. Low temperatures may affect N_2 fixation less than NO_3^- uptake (Hatch and Macduff 1991). While Bouchart *et al*. (1998) reported that N_2 fixation in white clover declined more than NH_4^+ uptake at low temperatures (6°C), they also showed that this was due to reduced clover N demand, not to a direct effect of low temperature on N_2 fixation *per se*. Temperatures as low as 7°C were not limiting to N_2 fixation in white clover (Svenning and MacDuff 1996). In the study of three white clover pastures in western Victoria (Riffkin *et al*. 1997), dependence of white clover on N_2 fixation did not decline during the winter months. Dart and Day (1971) found that most of the legume species studied (including red clover and lucerne) continued to fix N_2 down to 2°C, and N_2 fixation in lucerne was maintained up to 37°C (white clover was not included in the study). Nodulation and N_2 fixation in lucerne was suggested to cease below 8°C (Bordeleau and Prévost 1994) but this is not consistent with other reports. Temperature *per se* is thus unlikely to have any significant direct influence on N_2 fixation at the field level under Australian dairy climates, although clearly it will exert indirect influence via effects on plant development, plant water relations, and on the mineralisation of soil N.

Rhizobia and N_2 fixation

The microsymbiont bacteria contained in commercial inoculants that partner the primary pasture legumes in Australian dairy systems are given in Table 2. Here it can be seen that while development of legume inoculants has continued for lucerne and annual *Trifolium* species, there has been no development of rhizobial inoculants for perennial *Trifolium* species since the initial release of TA1 in ca. 1954.

Inoculant rhizobia for perennial Trifolium *species*

The current rhizobia used in the commercial inoculant for white (*Trifolium repens*), red (*Trifolium pratense*) and strawberry (*Trifolium fragiferum*) clovers in Australia was isolated in Tasmania, and first tested on clovers in 1953 (Paton 1957). Initially named BA-Tas, it was renamed TA1 (Waters 1957). In combination with strain NA30 it was recommended for use as the commercial inoculant for clovers at that time (Waters 1957), primarily because it was effective on a wide range of annual and perennial *Trifolium* species (Paton 1957). Although TA1 was later shown to be poorly competitive with native rhizobia (Brockwell *et al*. 1972) on alpine soils, it had appeared to fare better in agricultural soils (Dudman and Brockwell 1968). Strain NA30 was later annexed from the culture (Brockwell and Gibson 1968) and TA1 remains the single strain in the Group B commercial inoculant for white clover available today (Pulsford and Bullard 1997). *Rhizobium leguminosarum* bv. *trifolii* strain TA1 became a benchmark organism, and studies deploying this strain of rhizobia developed into a voluminous literature internationally, but little of this relates to its field performance in N_2 fixation, particularly with the varieties of white clover grown in Australia. As far as I am able to ascertain this has in fact not been examined, although it has been shown to be less effective in N_2 fixation on clovers than a range of other field isolates on several occasions (see Brockwell and Gibson 1968; Riffkin *et al*. 1999a). Under laboratory conditions Gibson *et al*. (1975) found that very few field isolates could match its N_2-fixing effectiveness. Meanwhile, there is a strong tendency for self selection of suitable rhizobia in the field (Baird 1955; Brockwell *et al*. 1972), and this may be reflected in the superior performance of some field isolates in western Victoria when compared with TA1 (Riffkin *et al*. 1999b). There is little doubt that significant improvements could be made with respect to the N_2 fixation effectiveness of the microsymbiont used for white clover in Australia, however, while legume contents of dairy pastures remain low, there may be little benefit realised from such improvement.

Table 2.   **Rhizobia used in Australian commercial inoculants for legumes used in dairy systems**

Inoculant group	Rhizobia	Strain	Recommended legume hosts
B	*Rhizobium leguminosarum* bv. *trifolii*	TA1, (since ca. 1954)	Perennial *Trifolium* spp. (white, red, strawberry clover)
AL	*Sinorhizobium meliloti*	RRI128 (since 2001)	Lucerne
C	*Rhizobium leguminosarum* bv. *trifolii*	WSM1325 (since 2005)	Annual *Trifolium* spp.

Inoculant rhizobia for lucerne

Nodulation and rhizobiology of lucerne in Australia has seen more attention than white clover, probably because lucerne also has important roles outside of the dairy industry. These studies have generally shown lucerne to nodulate well and fix N with the range of rhizobia that persist in agricultural soils in Australia (Bowman *et al*. 1998; Ballard *et al*. 2003), and in this respect lucerne may be more gregarious than some other *Medicago* species (Ballard *et al*. 2003).

N₂ fixation, soil acidity and salinity

The average soil pH on 44 dairy farms across the country in the survey of Gourley *et al*. (2010) was 5.3 (CaCl₂), and 4.8 across 71 dairy farms in western Victoria (Riffkin *et al*. 1999*a*). At such low soil pH rhizobia (Richardson and Simpson 1989) and nodulation (Munns 1965*b*) are likely to be severely compromised. Effects may be primarily manifest through poor survival of rhizobia at low pH (Richardson and Simpson 1989; Ballard *et al*. 2003) and/or inhibition of legume nodulation by toxic aluminium (Unkovich *et al*. 1996). The white clover inoculant strain (TA1) was shown to be less persistent in acid soil than five of six other strains in a field comparison on annual clovers (Watkin *et al*. 2000), so this rhizobia may be relatively sensitive to low soil pH. Consistent with this, in the survey of Riffkin *et al*. (1999*a*) clover dependence on N₂ fixation was negatively correlated with rhizobial numbers on light textured soils (mean pH 4.6) but not on medium textured soils (mean pH 4.9), and the amount of N₂ fixed positively correlated with soil pH on light but not medium textured soils. Although lucerne has been shown to 'select' compatible, effective rhizobia under acid soil conditions (Ballard *et al*. 2003), N₂ fixation will most likely be suboptimal under the typical soil pH of Australian dairy farms. While rhizobial partners more able to withstand acid soil conditions can be identified (Howieson *et al*. 1991), these are not a long-term solution to the problem of acid soil development which requires the addition of lime to provide improved soil chemical conditions for plant growth, legume N₂ fixation (Howieson and Ballard 2004) and general soil health. Lucerne is generally considered more susceptible to problems of low soil pH than some other legume species and nodule establishment can be an issue (Munns 1965*a*).

Irrigation of a white clover/ryegrass pasture with saline water reduced clover growth but not grass growth (Smith *et al*. 1993), yet N₂ fixation did not appear to be impaired at the salinities encountered (5 dS m⁻¹). Similarly long-term applications of sewage sludge to soils under dairy pasture in NSW did not impair the operation of white clover symbioses or the effectiveness of the naturalised soil rhizobia (Munn *et al*. 1997).

Pests and diseases

A range of parasitic nematodes are known to infect white clover across the dairy zone, and to reduce root growth and nodulation (McLeish *et al*. 1997), with bacterial feeding nematodes being particularly important as pasture legume content increases (Yeates and Stirling 2008). Some pests feed directly on clover nodules (Gerard 2001) and in this case would severely compromise N₂ fixation capacity. Although specific, direct effects of pests and diseases on N₂ fixation have not been studied (quantified) in the field, the density of some nematode species was correlated with the amount of N₂ fixed and the dependence of white clover on N₂ fixation on light textured soils in a field survey in western Victoria (Riffkin *et al*. 1999*a*). This would imply that nematodes might be reducing white clover N₂ fixation in this region. Where the white clover content of pastures are higher this may constitute a significant restraint on N₂ fixation potential.

Quantitative estimates of N₂ fixation in Australian dairy pastures

Interpreting N₂ fixation data in grazed pasture systems

Before examining the available quantitative data on N₂ fixation in dairy pastures it is worth considering a framework for interpretation of symbiotic N₂ fixation field data. From a systems point of view the key elements are the interactive effects of soil mineral N, clover : grass ratio, and grazing pressure, on N₂ fixation as shown in Fig. 5. This highlights that (1) N₂ fixation generally tops up clover N demand where it cannot first be satisfied by soil mineral N supply, (2) grasses and other non-legumes are stronger competitors for mineral N than legumes and thus the mineral N demand of non-legumes tends to be met first, (3) the N returns in urine and dung from grazing animals, and fertiliser N, result in increased mineral N in the soil which tends to favour growth of grasses over legumes and to reduce N₂ fixation directly, but contrary to this (4) at the lower end of the grazing spectrum, increased grazing intensity may favour the growth of clover over grass due to reduced shading of the clover, and (5) when clover content is lower it is forced to depend more on N₂ fixation for its N requirement because more of the mineral N will be taken by the larger grass component.

One must be careful when interpreting N₂ fixation data, for example a clover pasture fixing 100% of its N might be considered excellent, but if the total clover production is only say 500 kg ha⁻¹ then only the tiny amount of 12 kg N ha⁻¹ might be fixed. Conversely, if only 20 kg N were fixed this might be quite acceptable for a pasture with a clover yield of 10 t ha⁻¹, in which case %Ndfa would be low but total clover N might be a respectable 300 kg N ha⁻¹. Unambiguous data on N₂ fixation for pastures thus must include information on clover total N or dry matter, as well as the amount of N₂ fixed, and the proportional dependence on N₂ fixation (%Ndfa).
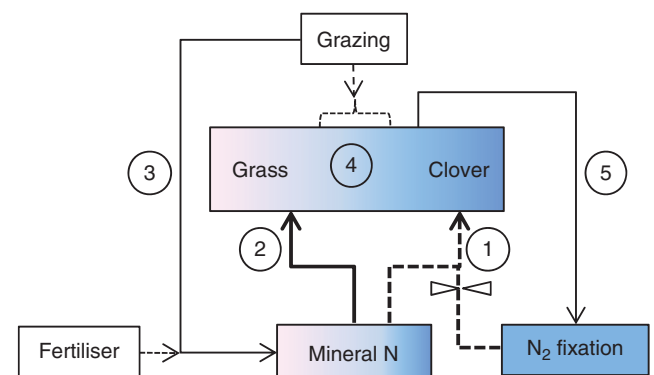

Fig. 5. Key influences on N₂ fixation in a grazed clover/grass pasture.

Potential (maximum) N$_2$ fixation is established by legume total dry matter production (Fig. 6), with the realisation of this potential primarily determined by mineral N availability, soil fertility [primarily phosphorus (P)], and the abundance and competence of the microsymbiont rhizobia. The figure shows that a clover production of 14 t ha^{-1} could potentially sponsor up to 700 kg of N$_2$ fixation annually.
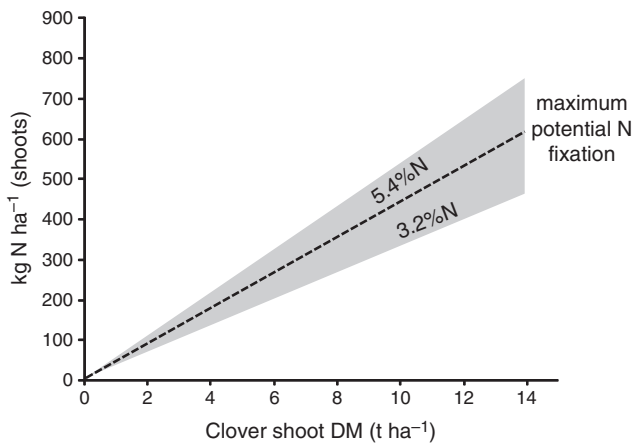

Fig. 6. Potential N$_2$ fixation by clover herbage is set by clover total N in herbage, a function of herbage dry matter and N concentration. The indicated upper and lower limits around the central line result from the range in N content (%) observed for white clover across 71 dairy pastures in south-west Victoria (Riffkin *et al.* 1999*a*). The slope of the dotted line is 44.8 kg t^{-1} (based on a mean N concentration of 4.48%).

Problems of measurement

Methods for field measurement of N$_2$ fixation have been detailed in Unkovich *et al.* (2008) and summarised by Peoples *et al.* (2009). These reports highlight that there are substantial obstacles to the reliable quantification of N$_2$ fixation in the field and no available methodology is optimal. Those methods which use the stable isotope ^{15}N are considered the more reliable and also give time integrated values. The natural ^{15}N abundance (δ^{15}N) methodology is currently the most widely deployed approach for field measurement of N$_2$ fixation in temperate legumes. Under controlled conditions the relative activity of the N-fixing enzyme, nitrogenase, can be compared in different treatments using the acetylene reduction assay (e.g. Mundy *et al.* 1988), and while the assay can be applied to field samples it cannot provide reliable quantitative estimates of symbiotic N$_2$ fixation at the field (kg ha^{-1} year^{-1}) scale (Unkovich *et al.* 2008). Other non-isotopic techniques (N difference, N balance, regression equations) do not measure N$_2$ fixation directly but rely on a suite of assumptions that are very often invalid, and this reduces their usefulness in many situations. Regression equations relating clover growth to the amount of N$_2$ fixed are becoming popular (e.g. Ledgard *et al.* 1999; Eckard *et al.* 2001*a*; Carlsson and Huss-Danell 2003; Gourley *et al.* 2010) but these may not be as widely applicable as one might hope. This approach is considered in more detail in *Modelling N$_2$ fixation in dairy systems*, but results of their application in Australia are not considered to constitute measurements of N$_2$ fixation in the present review. Studies reporting quantitative field estimates of N$_2$ fixation in Australian dairy systems are outlined in Table 3.

Table 3.   Studies quantifying legume N$_2$ fixation in Australian dairy, high-rainfall or irrigated perennial pastures

Reference	Location	Notes
White clover		
Riffkin *et al.* (1999*a*) (see also Riffkin *et al.* 1997)	South-west Victoria	Survey of 71 pastures, qualitative (%Ndfa) rather than quantitative (kg N ha^{-1}), methodology: δ^{15}N
Riffkin *et al.* (1999*b*) (see also Riffkin *et al.* 1997)	South-west Victoria	Three sites, quantitative seasonal and annual estimates, methodology: δ^{15}N
Pakrou and Dillon (2000)	South-east South Australia	Compared perennial and annual grazed pastures, quantitative annual estimates, methodology: δ^{15}N
I. R. P. Fillery, pers. comm. 2012	South-west Western Australia	Six farms, quantitative annual estimates, methodology: δ^{15}N
McKenzie *et al.* (1998)	South-west Victoria	One site, N fertiliser rates, quantitative for 3 months after N applications, only 9% clover, methodology: δ^{15}N
Mundy *et al.* (1988)	North Victoria	One site, varied soil water content and N fertiliser rate, semiquantitative, measurement period of hours extrapolated to days, methodology: acetylene reduction
Mundy (1987)	North Victoria	Fertiliser N rates, 70 days, methodology: ^{15}N isotope dilution
Smith *et al.* (1993)	North Victoria	Irrigation rates with saline water, quantitative seasonal (6 months), methodology: ^{15}N isotope dilution
Peoples *et al.* (1995)	New South Wales	Irrigation frequency, legume content comparisons, 109 days, methodology: δ^{15}N
Lucerne		
Yang *et al.* (2011)	South-east South Australia	Surveyed 20 irrigated lucerne hay fields, quantitative (seasonal) estimates, methodology: δ^{15}N
Gault *et al.* (1995)	Australian Capital Territory	Irrigated lucerne, fertiliser and inoculation treatments, quantitative annual estimates for 3 years, methodology: δ^{15}N
Brockwell *et al.* (1995)	Australian Capital Territory	Irrigated lucerne, fertiliser and inoculation treatments, quantitative seasonal estimate, methodology: δ^{15}N

Accounting for whole-plant N

From the point of view of dairy production the N contained in legume roots that might have been input from N_2 fixation may not be as important as in cropping systems (see e.g. Khan *et al*. 2003). However, it represents a N input to the system and as such can provide for fertility build up and N supply to companion grasses when roots senesce and the N becomes more readily available for microorganisms. This may be particularly important when studying N balances or when modelling mineral N availability in dairy pasture soils. None of the reports in Table 3 include measurement of the total N in legume roots, a task which remains an ongoing challenge (McNeill *et al*. 1997). In the absence of such measurement the pragmatic approach has been to apply fixed ratios of shoot : root N and multiply these by the amount of shoot N fixed to get total N_2 fixation (Unkovich *et al*. 2010). However, in the absence of the aforementioned root N measurements (see also Wichern *et al*. 2008) it is difficult to have confidence in the ratios proposed. For white clover a multiplication factor of 1.7 times herbage N was proposed for estimating total clover N (herbage + stolons + roots, Jørgensen and Ledgard 1997) and this has been applied in several studies (e.g. Peoples *et al*. 2001; Eckard *et al*. 2007), however, most of the Jorgensen and Ledgard data came from pot studies where the plants were ungrazed/uncut and only grown for a few weeks. How such leaf/stolon + root N ratios might relate to field ratios for grazed perennial clover is unclear. Although they had one contrasting data point for a grazed field experiment this was not compared with the glasshouse experiments, although they were plotted on the same graph. In a field study of subterranean clover using mowing, McNeill *et al*. (1997) estimated below-ground plant N and came up with a similar 1.75 ratio for estimating total plant N. Unkovich *et al*. (2010) give a value of 2.0 for lucerne, based on a pot study. It is not clear how such multiplication factors might apply across grazing/cutting regimes, soils, water availabilities, soil fertilities or species, and thus some caution must be exercised in their use. Nevertheless application of these approximate ratios might result in a more accurate estimate of total N_2 fixed than if they were not applied at all and root N was ignored.

Grazed white clover pastures

I have only been able to find 12 reports of field measurement of N_2 fixation in Australian high-rainfall/irrigated perennial pastures (Table 3), although there are several other reports on rain-fed, annual or lower rainfall, perennial pastures (see Peoples and Baldock 2001).

Annual inputs

For white clover, only four of the datasets in Table 3 (Peoples *et al*. 1995; Riffkin *et al*. 1999b; Pakrou and Dillon 2000; I. R. P. Fillery, pers. comm. 2012) include annual N_2 fixation estimates, the remainder of the datasets are for shorter periods of time. The work of Riffkin *et al*. (1999b) demonstrated at three rain-fed sites in south-west Victoria, that N_2 fixation was primarily limited by the low legume (white clover) content, averaging only 8% across the three sites. Thus annual N_2 fixation input in herbage was only 19–22 kg N ha⁻¹, with the total amount (including roots) being perhaps ~1.7 times this

(Jørgensen and Ledgard 1997) at 32–37 kg N ha⁻¹ year⁻¹. These values may be slightly under the average for the region, with an average clover content double these (19%) across 71 dairy pastures examined (Riffkin *et al*. 1999a), and with values of up to 50% of pasture herbage as clover recorded.

In a recent study in Western Australian dairy pastures (Table 4), similar low legume contents constrained N_2 fixation to 2–87 kg ha⁻¹ year⁻¹ across 2 years and six farmlets (I. R. P. Fillery, pers. comm. 2012). The higher value in Farmlet 6 was for a perennial pasture whereas the other pastures contained annual legumes.

The most comprehensive study of the N stocks and flows in an Australian dairy pasture comes from the work of Pakrou and Dillon (2000). This study is invaluable because it used isotopic measurement of N_2 fixation rather than estimation as has been used in several other N balance studies (e.g. Eckard *et al*. 2001a, 2007; Gourley *et al*. 2007). The South Australian study by Pakrou and Dillon (2000) compared a perennial, irrigated white clover/ryegrass pasture (Fig. 7) with a rain-fed, annual subterranean clover-based pasture. In contrast to the abovementioned studies, this involved the sowing of a white clover/ryegrass pasture and comparing this irrigated pasture with an adjacent, rain-fed, unrenovated annual *Trifolium* pasture. In the irrigated white clover pasture, legume content was just above 50%, and in the rain-fed annual pasture ~25%. Both pastures were grazed by cows, with utilisation rates around 70%.

Over the 12-month study period the irrigated white clover pasture fixed 231 kg N ha⁻¹ in the harvested herbage whereas the annual subterranean clover based pasture only fixed 75 kg N ha⁻¹ (Fig. 7). The difference between the two pastures was clearly due to the increased productivity of the white clover pasture with irrigation, to the longer growing season afforded by this irrigation, and to the high clover content when compared with the annual pasture. In the annual pasture, grass N uptake dominated the accumulation of herbage N whereas in the perennial pasture clover accounted for 66% of total herbage N. In the annual pasture, soil mineral N uptake by herbage totalled 208 kg ha⁻¹ for the growing season while N_2 fixation contributed only 75 kg ha⁻¹, clearly soil mineral N supply provided for the bulk of plant N requirements, thus limiting N_2 fixation. The key element of these results is the substantial fixation of N_2 when pasture productivity (17.2 t ha⁻¹) and clover content (>50%) are high. Interestingly although productivity of the annual pasture (12.2 t ha⁻¹) was 70% of the irrigated perennial pasture, herbage N accumulation totalled only 47% of that of the perennial pasture. Why the N concentration in

Table 4. N_2 fixation by clover in farmlets in feed allocated to each herd. Figures in parentheses are shoot N_2 fixation × 1.75 to account for N_2 fixation above- and belowground, based on the work of McNeill *et al*. (1997)

| Year | N_2 fixation in clover (kg N ha⁻¹) allocated to each farmlet | | | | | |
	1	2	3	4	5	6[A]
2006	8 (14)	4 (7)	7 (12)	3 (5)	2 (4)	87 (152)
2007	18 (32)	10 (18)	9 (16)	5 (9)	6 (11)	50 (88)

[A]Farmlet 6 is a perennial legume (white clover) pasture, the others contain annual legumes. Data from Dairy Australia Greener Pastures project, per Ian Fillery, CSIRO.
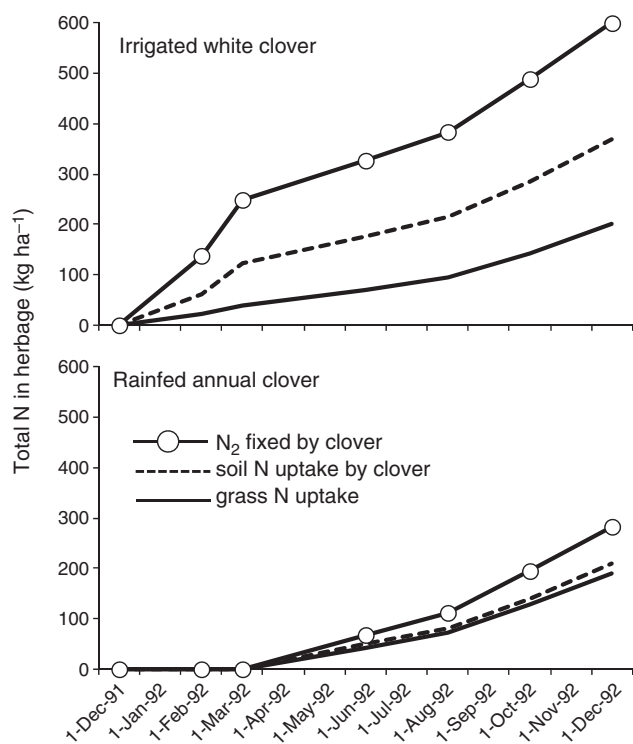
Fig. 7. Cumulative plant N acquisition over time in an irrigated white clover and rain-fed annual clover pasture in the south-east of South Australia. Plotted from the data of Pakrou and Dillon (2000).

herbage was lower in the annual pasture is not clear, but might relate to differential grazing management (see Unkovich *et al.* 1998).

The final quantitative estimate of N_2 fixation in a white clover pasture is that of Peoples *et al.* (1995), comparing a clover-dominant (85%) with a grass-dominant (60%) pasture over 109 days, with low or high irrigation frequency. Few details of the experiment are given in the Peoples *et al.* (1995) review paper. Results are as one might anticipate, with greater total N accumulation in both pastures under lower soil water deficits, and greater N_2 fixation with higher pasture clover content and clover N yield (Table 5).

Based on the survey of Riffkin *et al.* (1999*a*) white clover dependence on N_2 fixation in Australian dairy pastures is typically ~65%, indicating reasonable N_2-fixing capacity, however, the actual amounts of N_2 fixed are very much limited by low clover dry matter production as a consequence of low clover content in most pastures. Much higher rates of N_2 fixation are achievable,

with up to 294 kg N ha^{-1} year^{-1} being recorded for a recently sown, irrigated white clover pasture (Pakrou and Dillon 2000). In a review of perennial forage legumes in temperate/boreal environments, Carlsson and Huss-Danell (2003) report N_2 fixation by white clover to be up to 545 kg N ha^{-1} year^{-1}, but this did not include data on white clover from Australia. Mason *et al.* (1987) measured irrigated pure white clover pasture annual dry matter production of almost 23 t ha^{-1} in northern Victoria, which, according to Fig. 6 would provide for potential annual N_2 fixation of >1000 kg N ha^{-1}. This is higher than any value in the literature for any N_2-fixing system, but nevertheless shows that the potential with this species is very high. In current dairy systems this potential is not being realised due to low pasture legume contents.

The Achilles heel: low white clover content of pastures

Similar low white clover contents of pastures were reported earlier in a survey of Australian temperate pastures (Pearson *et al.* 1997; Hill and Donald 1998), and also earlier in Victoria (Ward and Quigley 1992). It would thus appear that pasture clover contents, and potential N_2 fixation in Australian perennial pastures has probably not improved in almost 20 years, regardless of the increased application of fertiliser N. Farmers appear reluctant to resow legumes (Ward and Quigley 1992). It may well be that for well managed, N fertilised, intensively grazed perennial ryegrass/white clover pastures, that equilibrium clover contents are around 20% resulting in the fixation of no more than ~100 kg N ha^{-1} year^{-1}, similar to that observed in the UK (Parsons *et al.* 1991; Andrews *et al.* 2007) and NZ (Woodfield and Clark 2009), although Jarvis (1993) suggested that in the UK, dairy pastures were typically much lower in both clover content (<10%) and the amount of N_2 fixed (10 kg ha^{-1} year^{-1}). These low clover contents are likely to be suboptimal in terms of dairy production (Woodfield and Clark 2009) as well as N_2 fixation and thus efforts to increase N_2 fixation should be rewarded with increased milk production efficiency.

In the absence of cattle grazing and the associated deposition of high rates of urine and dung, which increase soil mineral N and most likely depress N_2 fixation (Haynes and Williams 1993; Ledgard *et al.* 1999), dependence on N_2 fixation may be higher. For example, in the irrigated pure lucerne systems of south-eastern Australia (Yang *et al.* 2011) lucerne dependence on N_2 fixation averaged 65% and annual N_2 fixation in herbage estimated to be >200 kg N ha^{-1}.

Lucerne hay systems

While grazed lucerne pastures are used in Australian dairy systems they are of relatively minor importance compared with white clover/ryegrass pastures, but nevertheless important in the production of hay that feeds directly into the dairy system. Table 3 indicates just three studies quantifying N_2 fixation of irrigated lucerne in Australia, with the only two of those (Brockwell *et al.* 1995; Gault *et al.* 1995) providing annual N_2 fixation estimates being experimental sites in the ACT.

Gault *et al.* (1995) measured N_2 fixation using $\delta^{15}N$ natural abundance, in newly established, irrigated lucerne stands cut for hay, over a 3-year period. Experimental treatments were (1) no rhizobial inoculation and superphosphate only in the year of

Table 5. N_2 fixation by white clover over 109 days in clover-dominant (85%) or grass-dominant (60%) pastures irrigated after 60 mm (high) or 120 mm (low) evaporation (from Peoples *et al.* 1995)

Pasture type	Irrigation frequency	Clover N yield (kg ha^{-1})	N fixed (%)	N fixed (kg ha^{-1})
Clover dominant	Low	108	61	66
	High	145	62	90
Grass dominant	Low	66	67	44
	High	93	71	66

sowing $(9 \, kg \, P \, ha^{-1})$, (2) rhizobial inoculation plus annual applications of superphosphate, and (3) no rhizobial inoculation, but with annual application of superphosphate and N fertiliser $(33 \, kg \, N \, ha^{-1})$. Dry matter production and N_2 fixation increased dramatically after the first year (Fig. 8), reaching $284 \, kg \, N \, ha \, year^{-1}$ for the inoculated and P fertilised treatment in the third year, although this was only marginally more than for the second year for all treatments $(269–275 \, kg \, N \, ha^{-1})$. In the third year, the uninoculated treatment, which had not received annual applications of P fertiliser fixed much less than the other treatments. The authors estimated that total N_2 fixation (including root N) over the 3-year period to exceed $1400 \, kg \, N \, ha^{-1}$ in the annual P fertilised treatments.

This study shows the potential for N_2 fixation in irrigated lucerne is very high, provided that attention is paid to crop nutrition. The removal of $10–12 \, t \, ha^{-1} \, year^{-1}$ of hay exports significant quantities of nutrients, aside from N, and these would need to be replaced if growth and N_2 fixation is to continue uninhibited.

The above treatments were also applied to a 4-year-old lucerne stand at the same site (Brockwell *et al*. 1995) and N_2 fixation

ranged from 83 to 97 $kg \, N \, ha^{-1}$ over the 6-month period of study, giving a nominal annual rate similar to that of Gault *et al*. (1995). From the data of Fig. 9 it would appear that N_2 fixation continues unabated at a constant rate over the warmer months where irrigation water is applied.

The final example of field measures of N_2 fixation in lucerne systems comes from Yang *et al*. (2011) who surveyed N_2 fixation in 18 irrigated lucerne hay fields in the south-east of South Australia. The estimates of N_2 fixation were for standing dry matter at the time of sampling, in a system which typically has three hay cuts per year. Mean N_2 fixation in standing biomass (Table 6) was 73 $kg \, N \, ha^{-1}$, or 65% of lucerne herbage N. What time period these values might represent was not able to be established, but the authors considered that, on average, annual values were likely to be 3 times those observed, giving a value very similar to the annual N_2 fixation indications from the studies of Brockwell *et al*. (1995) and Gault *et al*. (1995). The South Australian study also indicated that these lucerne stands continued to fix N_2 many years (>25) after they were established.

Together these data indicate that irrigated lucerne hay crop systems continue to fix considerable amounts of N over time. In contrast to grazed white clover systems, these hay systems export substantial quantities of N in herbage. Furthermore, they are often only grazed lightly such that the build up of soil mineral N does not occur to the extent that is seen in intensively grazed white clover pastures. In this case it is not the legume species which are driving the massive differences in N_2 fixation input between lucerne and white clover, but rather the presence of the animals, and the differential management of the systems in which the legumes are utilised.

Grazing and N_2 fixation

A detailed review of the impacts of grazing animals on legume N_2 fixation are given in Menneer *et al*. (2004). The key element of grazed dairy systems is the excretion by cattle of at least 75%
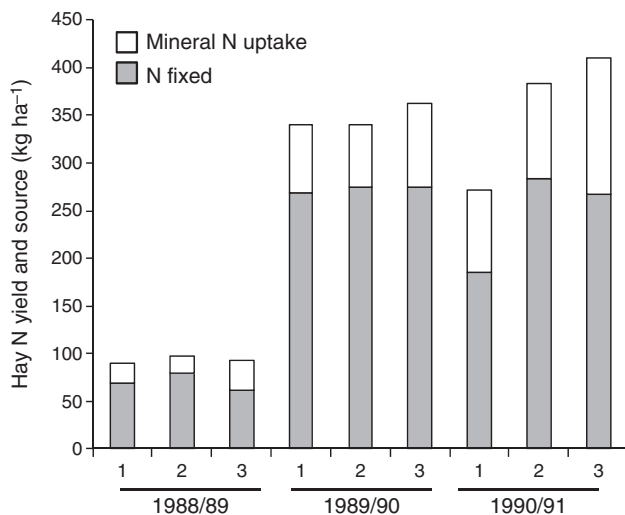
Fig. 8. Sources of N for irrigated lucerne hay in the first 3 years after establishment, (1) uninoculated and superphosphate only in the year of sowing $(9 \, kg \, P \, ha^{-1})$, (2) inoculated plus annual applications of superphosphate, and (3) uninoculated, annual application of superphosphate and N fertiliser $(33 \, kg \, N \, ha^{-1})$. Plotted from the data of Gault *et al*. (1995).

Table 6. Summary of N_2 fixation data from a survey of 18 irrigated lucerne stands cut for hay in the south-east of South Australia (from Yang *et al*. 2011)

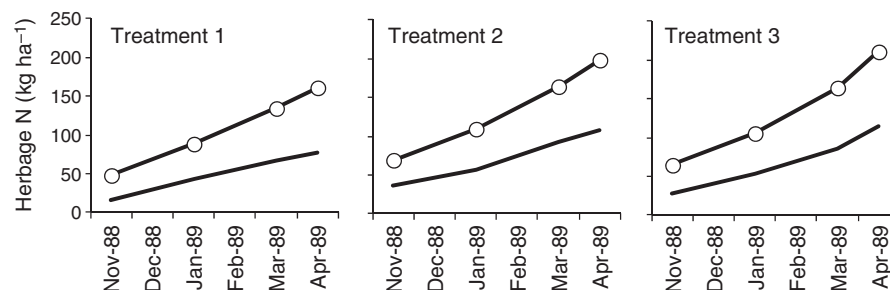|	%Ndfa	N fixed	Mineral N uptake
Mean	65	73	44
Min.	33	33	9
Max.	90	122	90

Fig. 9. Cumulative seasonal N_2 fixation and mineral N uptake in a 4-year-old irrigated lucerne stand grown for hay. Treatments same as for Fig. 8. Plotted from the data of Brockwell *et al*. (1995).

of the N they ingest from herbage as urine and dung (Whitehead 1995). Maximal N_2 fixation is likely to come from well managed hay systems rather than grazed systems, this is because optimal clover content can be more easily managed and the urinary and dung N returns do not suppress N_2 fixation. However, this does not mean that ungrazed systems will have greater N_2 fixation than grazed systems. Ungrazed mixtures of clover and grass are likely to become grass dominant with shading reducing clover growth and N_2 fixation (Sanford *et al.* 1995). In a study of an annual subterranean clover pasture grazed by sheep in Western Australia (Unkovich *et al.* 1998) a more heavily grazed pasture had lower grass growth and greater N_2 fixation than a lightly grazed pasture. While increased grazing pressure can favour clover growth over grasses, in practise the magnitude of this generally appears quite small as the effect occurs at the lighter end of grazing intensities (Doyle *et al.* 2000). Increased grazing pressure usually increases the N (protein) content of clover (Unkovich *et al.* 1998), and indeed other pasture species (Kelly *et al.* 2005). The work of Pakrou and Dillon (2000) highlights the significance of the mineral N flux under grazing. Under irrigated, grazed white clover pasture, the flux of N through the soil mineral N pool was estimated to be 687 kg N ha^{-1}, more than half of which was derived from animal returns. This study also highlights the significant role that N_2 fixation can play when there is a high clover content, even in the presence of intensive grazing. Under the annual pasture, mineralisation of soil organic N was driving the available N pool, being no higher when the animals were on the pasture than when they there were absent (see Pakrou and Dillon 2000). Nevertheless excretory N returns from grazing animals showed up as the key influence on sward N dynamics and N_2 fixation in these two dairy systems.

   In terms of N_2 fixation the key elements to note in the perennial pasture of Pakrou and Dillon (2000) are:

- The legume (white clover) content was high (57%) because the pasture had been sown only 2 years before, this is atypical for Australian dairy pastures where legumes contents are commonly <20%,
- Because the legume content and legume dry matter production (9.8 t ha^{-1}) was high, N_2 fixation was also high (236 kg N ha^{-1}), excluding an additional 59 kg ha^{-1} (25%) estimated for clover roots,
- After mineralisation (687 kg N ha^{-1}), cattle intake (419 kg N ha^{-1}) and grass mineral N uptake (389 kg N ha^{-1}), N_2 fixation was the fourth highest N flux in the system, and

- N_2 fixation was greater than the combined N losses estimated from leaching, NH_3 volatilisation and denitrification (209 kg N ha^{-1}) and thus the system appeared to be in an approximate N balance, despite there being no N fertiliser inputs.

The key elements to note for the annual, rain-fed pasture were:

- N_2 fixation was much lower than for the white clover-based pasture because (a) subterranean clover is an annual and only grows for part of the year (b) the clover content (25%) was less than half that of the perennial pasture, and (c) the annual pasture was rain-fed, not irrigated,
- Most of the clover N was fixed (80%),
- The system had a marginally negative N balance overall, and
- The fixation rate of 100 kg N ha^{-1} year^{-1} in this pasture is higher than for Australian dairy pastures generally because there were no fertiliser N inputs.

Differences in N_2 fixation capacity between species and cultivars

Differences between legume cultivars are unlikely to be of quantitative importance for N_2 fixation input in Australian dairy systems. However, where differences in clover productivity are expressed then those cultivars with greater shoot biomass would fix more N. While this has not been examined specifically for Australian cultivars (N_2 fixation has not been considered in the Australian white clover breeding program (Carol Harris, NSW DPI, pers. comm. 2012)), data on nine white clover cultivars from New Zealand (Ledgard *et al.* 1996) indicated that differences between cultivars in the amount of N_2 fixed are related to dry matter production driven differences in clover total N accumulation, rather than to inherent differences in the N_2 fixation efficiency or shoot N concentration (Fig. 10). While all three of these variables are used to calculate the amount of N fixed, it is clearly legume dry matter production which is the driving force in this dataset, and indeed in most others (Unkovich *et al.* 2010).

   In an earlier study of differences in N_2 fixation between white clover cultivars in New Zealand (Ledgard *et al.* 1990), it was concluded that there were no inherent differences in the capacity of different cultivars to fix N, and thus that N_2 fixation was not a basis for substituting one for another. Generally speaking, in breeding for maximum dry matter or total N accumulation, clover breeding programs might indirectly select for maximal N_2 fixation. However, this does not mean that N_2 fixation is optimal nor has been selected for, because it may well be that
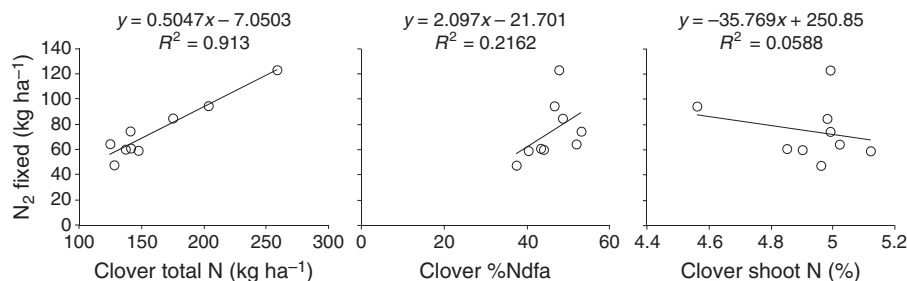
Fig. 10.   Correlation between clover shoot total N, clover dependence on N_2 fixation (%Ndfa) or clover shoot N concentration (%,) and the amount of N_2 fixed (kg ha^{-1}) for nine white clover cultivars in New Zealand. Plotted from the data of Ledgard *et al.* (1996).

even with the best available plant material, N$_2$ fixation could still be limiting growth, due, for example to poorly effective rhizobia. With respect to cultivar performance in N$_2$ fixation, in several pasture legume species it has been shown that there is a strong interaction between legume cultivar and rhizobium strain, such that optimal N$_2$ fixation potential is achieved with specific combinations of pasture legume cultivar and rhizobial strain (see e.g. Ballard *et al.* 2003).

Differences in N$_2$ fixation between species of legume will be driven as much by differential management of species/systems and environment, as by inherent differences between legume species.

Modelling N$_2$ fixation in dairy systems

Because field measurement of biological N$_2$ fixation is complex and expensive (Unkovich *et al.* 2008) modelling approaches to estimate N$_2$ fixation hold significant attraction. The basis for model design can be either empirical (e.g. Høgh-Jensen *et al.* 2004; Unkovich *et al.* 2010) or dynamic mechanistic (e.g. Boote *et al.* 2008). Empirical approaches tend to correlate measured N$_2$ fixation rates with other, more easily measured pasture properties, fit regression equations to the resulting dataset, and then apply those regressions elsewhere in time or space. Dynamic simulation models attempt to mimic the primary biological and physical processes driving plant growth (Sinclair and Seligman 1996), including N$_2$ fixation, and usually attempt to be universally applicable upon local parameterisation. Such so called mechanistic or dynamic simulation models are usually only semi-mechanistic as they typically include some empirical approaches. Liu *et al.* (2010) reviewed a large number of approaches to modelling N$_2$ fixation and the reader is referred to this thorough exposé of N$_2$ fixation modelling, the detail of which is outside the scope of the present review.

Empirical relationships

An example of a typical empirical model for estimating N$_2$ fixation is given in Fig. 11, which relates legume shoot dry matter production to the amount of N$_2$ fixed. This figure is for herbage N fixed, an additional fraction can be added for fixed N possibly contained in roots.
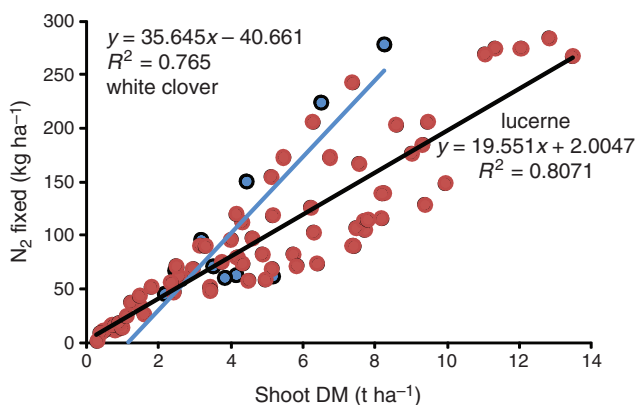
The pros and cons of such approaches are detailed in and Liu *et al.* (2010) and Unkovich *et al.* (2010). The primary limitation of such approaches are that, aside from the influence of dry matter production, they are naive to other possible drivers of N$_2$ fixation, such as soil fertility, temperature, water availability, grazing intensity, non-legume pasture content, and microsymbiont performance. The net effect of such factors are of course inherent in the observed data and so have been captured for the data points presented. The problem is that once the regression is applied in another situation (time or place), these inherent effects may not apply at the application place/time.

Carlsson and Huss-Danell (2003) found significantly different regressions for grazed and mown white clover pastures, and thus the regressions are not transferable between such management regimes. Relationships which have been developed elsewhere (e.g. Ledgard *et al.* 1999) and applied in Australia (e.g. Eckard *et al.* 2001a, 2007) are thus fraught with danger, especially if applied too specifically. Such regressions have no experience beyond their derivation dataset and thus other regressions might have equal validity. For example Carlsson and Huss-Danell (2003) gave linear regressions between white clover dry matter and N fixed accounting for 91% (clover/grass) to 55% (legume monocultures) of the measured amount of N$_2$ fixed, without accounting for N fertiliser application.

Examples such as that in Fig. 11 may approximate behaviour across regions but are unlikely to be correct at any given point and should only be applied at the scale at which the regression is derived. That is, if the data are derived for a range of treatments within a single field or farm, they could not be reliably extrapolated outside of that field or farm. Conversely, regression across a range of fields or regions might usefully be applied across such a scale, but is not likely to apply at sub field or region scale. The regressions cannot be reliably used in situations where they have no previous experience. In this way they are different to dynamic simulation models which often respond to local environmental and management influences.

In the study of Ledgard *et al.* (2001) the white clover N concentration did not drop below 4.5%, whereas this was close to the average for 71 pastures investigated in Victoria (Riffkin *et al.* 1999a) and in the analysis of broader Australian data by Unkovich *et al.* (2010) the mean shoot [N] for white clover was given as 3.2%, which could account for a significant difference in the slope of the regression lines. Indeed Fig. 12 looks much like

Fig. 11. Correlation between clover shoot dry matter and the amount of N$_2$ fixed, and fitted regression equations for white clover ⊙ and lucerne ⬤ grown in Australia. Data from Unkovich *et al.* (2010).
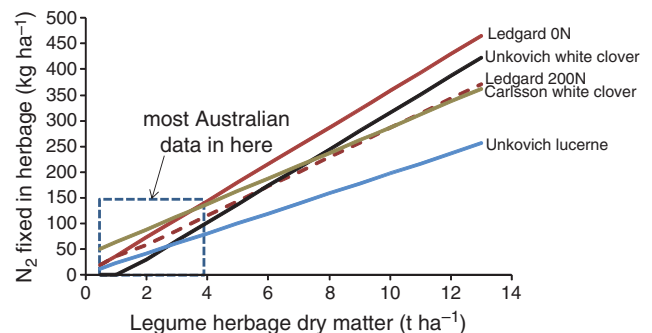

Fig. 12. Comparison of different regression equations used to estimate N$_2$ fixation in white clover or lucerne from clover shoot dry matter. Details of the regressions are given in Table 7.

Table 7. Regression equations collated from the literature relating clover herbage dry matter (kg ha^{-1}) to N$_2$ fixation in shoots for a range of perennial legumes

Such equations have been used to estimate N$_2$ fixation from legume shoot dry matter

Reference	Legume	Regression
Ledgard (2001)	White clover	= DM * (0.0358 − 3.59 * 10^{-5} * N fertiliser rate)
Carlsson and Huss-Danell (2003)	White clover (generic)	= DM * 0.025 + 37.2
	White clover (monoculture)	= DM * 0.016 + 57.9
	White clover (mixtures)	= DM * 0.031 + 23.9
	Red clover (generic)	= DM * 0.023 + 8.4
	Red clover (monoculture)	= DM * 0.016 + 16.5
	Red clover (mixtures)	= DM * 0.026 + 7.4
	Lucerne (generic)	= DM * 0.012 + 38.8
	Lucerne (monoculture)	= DM * 0.0013 + 12.3
	Lucerne (mixtures)	= DM * 0.0021 + 16.9
Unkovich *et al*. (2010)	White clover	= DM * 0.036 − 40.661
	Lucerne	= DM * 0.0196 + 2.007

Fig. 6. Furthermore, the clover N$_2$ fixation in the Ledgard study did not exceed 94 kg N ha^{-1} whereas in the Unkovich dataset the maximum was 278 kg N ha^{-1} and in the Carlsson dataset it exceeded 400 kg N ha^{-1} year^{-1}. As much of the evidence indicates that clover content and clover dry matter production are low in Australia ($\leq$4 t ha^{-1}) the relevant part of Fig. 12 is near the origin. At 2 t ha^{-1} clover dry matter N$_2$ fixation would range from 30 to 87 kg N ha^{-1} year^{-1} depending on which regression equation was used. Further complications arise because in some instances significant N$_2$ fixation would be indicated with no clover dry matter (Fig. 12, Carlsson regression). This can occur with regressions when they are extended beyond their experience, or where the responses may indeed not be linear, as is likely to be the case at the lower end of the range when soil mineral N will become increasingly important.

Given that a value of ~4.5% N for herbage seems typical for grazed white clover (Fig. 6 and Ledgard *et al*. 2001), a shoot : root

N ratio of 1.7 (Jørgensen and Ledgard 1997) and the average dependence of white clover on N$_2$ fixation in western Victoria of 65% (Riffkin *et al*. 1999b), this implies a total N$_2$ fixation for current systems averaging 50 kg t^{-1} clover shoot dry matter, or in shoots only 29 kg t^{-1} herbage. While this might provide a useful rule of thumb for pastures of low (<25%) legume content from which the data have been derived, for higher legume content dairy pastures other factors may play a part in changing %Ndfa or herbage N concentration and thus alter the relationship between dry matter and N$_2$ fixed.

Dynamic simulation models

In the review of Liu *et al*. (2010) of nine mechanistic/process based models of N$_2$ fixation, commonalities were the scaling of a maximum daily N$_2$ fixation rate as a function of some combination of temperature, soil water, soil mineral N, plant carbon availability, and plant development stage. The implementation of these various factors in a range of models are shown in Table 8. Eight of the models have been used for perennial legume pasture species (white clover or lucerne).

When reviewing models the first consideration is the purpose/ objective of the modelling required. There are many models, either specifically for N$_2$ fixation, or which have N$_2$ fixation as a component, but each has been built with a different specific purpose in mind. For the present purposes it is assumed that the modelling objective is to quantify changes in legume N$_2$ fixation in response to management and climate, rather than legume physiological responses to climate and management. Relevant pasture simulation models which have been used in Australia are given in Table 9, along with their N$_2$ fixation simulation capacity.

In GrassGro the potential N$_2$ fixation rate is calculated as the total plant N demand less N translocated from belowground reserves and N recycled from shaded leaves, multiplied by a factor for the development of nodules in early growth. This potential rate is then scaled back by low water content and high mineral N, weighted according to a nodule depth distribution (Andrew Moore, CSIRO, pers. comm.).

In the DairyMod tool, N$_2$ fixation is linked directly to photosynthesis and a value of 6 mg C respired/mg N fixed used as a carbon cost, thus reducing growth of N$_2$-fixing clover compared with non-fixing clover. An earlier version of the model constrained N$_2$-dependent clover to 0.6 of the growth

Table 8. Factors used to scale maximum daily N$_2$ fixation rate in various 'mechanistic' N$_2$ fixation models

Adapted from Liu *et al*. (2010) with SGS/DairyMod added and an indication of whether the model has been used for white clover ✓ (* or lucerne)

Model	Temp.	Water	Mineral N	Plant C	Growth stage	White clover	Reference
Sinclair	–	✓	–	✓	–	–	Sinclair (1986)
EPIC	–	✓	–	–	–	–	Cabelguenne *et al*. (1999)
Hurley	✓	✓	✓	✓	–	✓	Thornley (2001)
Schwinning	–	–	✓	✓	–	✓	Schwinning and Parsons (1996)
CropGro	✓	✓	–	✓	✓	–	Boote *et al*. (2008)
SOILN	✓	✓	✓	–	–	✓	Wu and McGechan (1999)
APSIM	–	✓	✓	–	✓	*	Robertson *et al*. (2002)
Soussana	–	–	✓	–	–	✓	Soussana *et al*. (2002)
STICS	✓	✓	✓	–	✓	✓	Brisson *et al*. (2009)
GrassGro	–	✓	✓	–	✓	*	Moore *et al*. (1997)
SGS/DairyMod	–	–	✓	–	–	✓	Johnson *et al*. (2008)

Table 9. Legume N$_2$ fixation simulation capacity of dynamic pasture models used in Australia

Model	N$_2$ fixation functions	Reference
GrassGro	A fraction of the net remaining demand for N, affected by nodule mass, developmental stage, soil moisture availability and NO$_3^-$-N	Moore *et al.* (1997); A. Moore, pers. comm.
DairyMod	A minimum of 20% of legume N is from fixation, where mineral N cannot meet legume N demand then N$_2$ fixation tops up herbage N to the optimal shoot [N], but constrained by plant C availability	Johnson (2005); Johnson *et al.* (2008); I. Johnson, pers. comm.
SGS Pasture Model	As above	Johnson *et al.* (2003)**;** Johnson (2005)
APSIM	A function of daily growth rate, up to a maximum daily N$_2$ fixation rate, with a legume-specific factor for relative suppression of N$_2$ fixation by soil mineral N	Robertson *et al.* (2002)
GRASP	Does not incorporate N$_2$ fixation	McKeon *et al.* (1982)

of mineral N-dependent clover, although I think this has now been removed. A minimum of 20% of legume N comes from N$_2$ fixation under all conditions. Legumes are not limited for N, with N$_2$ fixation topping herbage N up to the optimal value (Johnson 2005). Graham (2008) provides a thorough review of the DairyMod tool although does not discuss legume N$_2$ fixation.

In APSIM (Robertson *et al.* 2002) N$_2$ fixation occurs when there is insufficient mineral N to meet plant N demand, but the sensitivity with which N$_2$ fixation is switched on in the presence of mineral N, being a cultivar specific parameter. While the model does not currently have an interaction between soil mineral N and nodulation, the N$_2$ fixation routines are currently being revised and nodule mass will be developed into an integral part of the N$_2$ fixation simulation routines.

While DairyMod, APSIM and GrassGro have the capacity to model N$_2$ fixation I can find no published model output showing N$_2$ fixation by pasture or crop legumes, or a comparison of model output with measured N$_2$ fixation data. While the models often show good correlation of model simulated and measured dry matter production or total N, the validity of these models nevertheless remains essentially untested in terms of N$_2$ fixation. The N$_2$ fixation routines in both APSIM and DairyMod are currently being revised [M. Robertson (CSIRO) and I. Johnson (IMJ), pers. comms].

None of these models include any consideration of the population dynamics, effectiveness or environmental responses of the microsymbiont and thus will be unable to simulate responses of the symbiosis to management and environment in the field. Those models which ignore the microsymbiont dynamics will inevitably have limited capacity over time. There is no physiological process-based model tested for specific study of N$_2$ fixation in Australian dairy systems. Until the N$_2$ fixation routines in the available models have been tested against measured data they offer no more in terms of predictive N$_2$ fixation capacity than a suitably calibrated empirical model.

Environmental costs and benefits of N$_2$ fixation

A fair assessment of the environmental costs and benefits of legume N$_2$ fixation in dairy systems can only be achieved with consideration of a gamut of factors impinging on the environmental balance sheet for a dairy farm. While this is beyond the scope of the present review we can briefly consider some of the issues feeding into and out of legume N in dairy farming systems. More thorough environmental analyses of

dairy farming systems can be found in a recent volume (de Klein *et al.* 2008; Kleinman and Soder 2008; Nash and Barlow 2008) and a range of other relevant articles (Ridley *et al.* 2004; Andrews *et al.* 2007; Ledgard *et al.* 2009; Woodfield and Clark 2009).

Urinary N returns from dairy cattle concentrate soluble N at very high rates and provide the primary point of soluble N excess and thus the greatest opportunity for environmental impact. Generally to minimise losses of N via denitrification, leaching or NH$_3$ volatilisation a 'tight' N cycle is required, necessitating the maintenance of some N limited grass to 'mop up' available N (Parsons *et al.* 1991). However, it is generally thought that a system with slightly N-deficient grass may limit feed quantity and quality and is not considered optimised in terms of animal production (Eckard 2001). This is thus not usually recommended from a milk production perspective but nevertheless could provide significant environmental benefits.

In a study in the UK, Andrews *et al.* (2007) considered the relative merits of (*i*) an unfertilised perennial ryegrass/white clover pasture (*ii*) a perennial ryegrass pasture receiving 200 kg N ha^{-1} year^{-1}, and (*iii*) a perennial ryegrass only pasture supplied with 350–400 kg N ha^{-1} year^{-1}. From a N cycling and NO$_3^-$ leaching perspective, pastures (*i*) and (*ii*) were considered equal as the unfertilised pasture had similar N input from N$_2$ fixation, and with a similar grazing regime the amount of N cycling through the animals was about the same. The pasture with the higher fertiliser N addition rate (*iii*) was considered to have a greater N footprint due to increased leaching and nitrous oxide emissions. Generally it was considered that with similar N inputs, pasture productivity and grazing intensity the environmental N footprint would be about the same; that is there may be no inherent advantage in N$_2$ fixation *per se* in terms of N cycling impacts. The analysis of Andrews *et al.* (2007) did not include the magnitude of N$_2$ fixed in clover roots and thus may have underestimated the difference in N inputs between treatments.

Generally then, if contrasting systems (grass versus grass/clover) are equally as productive and have the same stocking rates or animal products output, they are likely to have very similar environmental costs/benefits. This is because most of the environmental footprint from dairy systems comes from the livestock N returns not the N input *per se*. While substitution of fertiliser N with clover fixed N might improve the environmental balance sheet on farm, the benefit is likely to be marginal where best practise fertiliser management is already being used.

Excretal N is the primary source of nitrous oxide (N_2O) emissions from dairy systems (de Klein *et al.* 2008; Ledgard *et al.* 2009). Although legume N_2 fixation was previously thought to contribute directly to N_2O emissions, this has been shown not to be the case (Rochette and Janzen 2005) and so the direct N_2O footprint of legume fixed N_2 is minimal. If one were also to include energy costs of urea fertiliser manufacture (0.73–2.14 kg CO_2-e kg^{-1} Ledgard *et al.* 2011), then substituting fixed N_2 for fertiliser N should have some GHG mitigation potential (Ledgard *et al.* 2009), but not if pasture clover contents are low. Andrews *et al.* (2007) considered that savings in CO_2-e by substituting 200 kg N for fixed N would be negligible on a global scale but very significant on a ha^{-1} scale. Nitrogen fertiliser manufacture accounts for ~1% of total global CO_2-e emissions. In the future if legumes with condensed tannins become available (Woodfield and Clark 2009) additional GHG benefits should accrue in terms of reduced CH_4 emissions.

High land-use intensity in the dairy industry is the primary cause of environmental problems resulting from excess N (de Klein *et al.* 2008). While similar, well managed clover/grass and grass only pastures are likely to have the same local environmental impact, whole system or life cycle analysis suggests that overall, pastures which contain N_2-fixing legumes would have a lower net environmental impact than N-fertilised pastures (Ledgard *et al.* 2009). While ungrazed legume-dominant hay systems would appear to have a much lower environmental impact than intensively grazed pastures as the primary animal driven mineral N fluxes would be avoided, this ignores the fact that the hay will still be fed to animals and the excretal N returned elsewhere. Although in this case it might be more effectively managed.

Managing N_2 fixation in Australian dairy pastures – where to from here?

Australian dairy systems have made the inevitable drift from the exploitation of legume N in extended grazing systems to short rotational grazing of N fertilised pastures that has characterised the development of intensive, modern dairy systems elsewhere in the world. This is due to a perceived increase in system efficiency by increasing the stocking rate to utilise more of the pasture, and then supplementing the otherwise underfed cows (Lemerle *et al.* 1992). Such a system increases the return of urinary and dung N to pastures, further reducing legume content. It is this high intensity grazing rate that is exerting significant influences on N_2 fixation by clover, through defoliation, treading and returns of urinary N which cause direct reductions in N_2-fixing (nitrogenase) activity and in clover persistence. However, as legumes have several special benefits to dairy cows and to farming systems, they are likely to have a continuing, perhaps increasing role in dairy systems in the future, provided that investment is made in the appropriate areas.

The clover contents of typical dairy pastures are clearly below the optimum required for effective N_2 fixation input, and perhaps below what might be optimal in terms of animal nutrition and milk production (Harris *et al.* 1997). Thus efforts to increase N_2 fixation should be rewarded with both improved animal production efficiency, and environmental benefits. Generally lower rates of N application and moderate intensity grazing

favour white clover persistence and abundance in mixed pastures (Kelly *et al.* 2005). Legume herbage has distinct advantages over grasses in terms of animal production and warrants inclusion in dairy pasture systems. The fact that clover is able to obtain its own N requirements from the atmosphere provides an opportunity to reduce input costs and the environmental impact of dairy agriculture.

In high-rainfall and irrigated pastures, clover contents should be able to be increased, with multiple benefits, including N_2 fixation. However, under rain-fed conditions where summer droughts occur, perennial legume persistence and N_2 fixation are likely to be more difficult to maintain, and occasional resowing could be required. Housed animal systems with cut and carry forage could be more reliant on legumes and N_2 fixation, whereas intensively grazed pastures will inevitably have lower clover contents, higher returns of urinary and dung N (intensified through the addition of supplementary feeding when pasture supply is limited), increasing the downward pressure on legumes and N_2 fixation.

Eckard *et al.* (2001*b*) points out that reduced N fertiliser use and increased dependence on legumes has now occurred in Europe, a trend which might follow here, whether this alone will be sufficient to boost pasture legume contents and N_2 fixation to the required level is not clear. It is likely to also require lower stocking rates which is somewhat anachronistic to the current management paradigm in Australian dairy systems, which focuses on pasture utilisation efficiency rather than N-use efficiency. In any event if pasture legume contents are increased there will be a requirement for monitoring of legume growth and N_2 fixation to ascertain whether the other factors highlighted begin to constrain N_2 fixation (rhizobia effectiveness, nematodes, grazing intensity and excretal N returns). One alternative option worth exploring might be the spatial separation of clover and grass (Chapman *et al.* 2007; Woodfield and Clark 2009), with potential increases in N_2 fixation input and scope for spatial management of fertiliser, and improved milk production. Differences in the N_2-fixing potential (growth) of white clover cultivars are likely, as are differences in responses to available N (Doyle *et al.* 2000) but these have not been explicitly explored for Australian clover varieties.
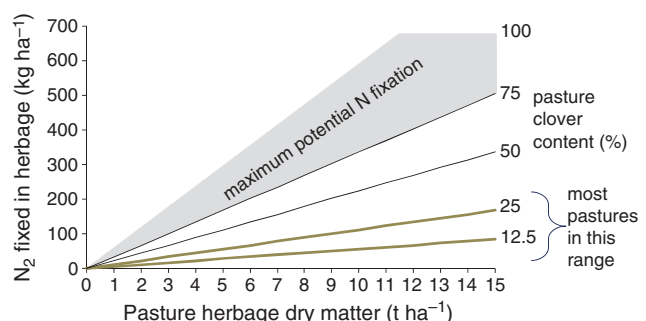

Fig. 13. Potential N fixation by clover assuming herbage N content of 4.48 and 100% dependence on N_2 fixation for a range of pasture clover contents. Maximum potential N_2 fixation is ~700 kg N ha^{-1} $year^{-1}$ depending on clover N content. Most Australian dairy pastures have a clover content below 25% and a %Ndfa of ~65%, so actual N_2 fixation in clover herbage must typically be much less than 80 kg N ha^{-1} $year^{-1}$.

Complex dynamic simulation models are probably not required to predict the likely outcome of changes in pasture legume content in terms of N₂ fixation. This should be able to be modelled relatively simply, or with simple regression models such as that shown in Fig. 13. The DairyMod, APSIM and GrassGro models all have some capacity for N₂ fixation simulation, but this is yet to be exploited. A comparison of model outputs in terms of N₂ fixation against measured data are required to ascertain if the current models have anything to offer.

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References

Andrews M, Scholefield D, Abberton MT, McKenzie BA, Hodge S, Raven JA (2007) Use of white clover as an alternative to nitrogen fertiliser for dairy pastures in nitrate vulnerable zones in the UK: productivity, environmental impact and economic considerations. *Annals of Applied Biology* **151**, 11–23. doi:10.1111/j.1744-7348.2007.00137.x

Baird KJ (1955) Clover root-nodule bacteria in the New England region on NSW. *Australian Journal of Agricultural Research* **6**, 15–26. doi:10.1071/AR9550015

Ballard RA, Shepherd BR, Charman N (2003) Nodulation and growth of pasture legumes with naturalised soil rhizobia. 3. Lucerne (*Medicago sativa* L.). *Australian Journal of Experimental Agriculture* **43**, 135–140. doi:10.1071/EA02047

Boote K, Hoogenboom G, Jones J, Ingram K (2008) Modeling nitrogen fixation and its relationship to nitrogen uptake in the CROPGRO model. In 'Quantifying and understanding plant nitrogen uptake for systems modeling'. (Eds L Ma, L Ahuja, V Bruulsema) pp. 13–46. (CRC Press: Florence, SC)

Bordeleau L, Prévost D (1994) Nodulation and nitrogen fixation in extreme environments. *Plant and Soil* **161**, 115–125. doi:10.1007/BF02183092

Bouchart V, Macduff JH, Ourry A, Svenning MM, Gay AP, Simon JC, Boucaud J (1998) Seasonal pattern of accumulation and effects of low temperatures on storage compounds in *Trifolium repens*. *Physiologia Plantarum* **104**, 65–74. doi:10.1034/j.1399-3054.1998.1040109.x

Bowman A, Hebb D, Munnich D, Brockwell J (1998) Rhizobium as a factor in the re-establishment of legume-based pastures on clay soils of the wheat belt of north-western New South Wales. *Australian Journal of Experimental Agriculture* **38**, 555–566. doi:10.1071/EA98072

Brisson N, Launay M, Mary B, Beaudoin N (2009) Nitrogen transformations. In 'Conceptual basis, formalisations and parameterization of the STICS crop model'. (Eds N Brisson, M Launay, B Mary, N Beaudoin) pp. 141–165. (Quæ: Versailles Cedex)

Brockwell J, Gibson A (1968) Root-nodule bacteria for some cultivated spceis of *Trifolium*. *Joural of The Australian Institute of Agricultural Science* **34**, 224–227.

Brockwell J, Bryant WG, Gault RR (1972) Ecological studies of root-nodule bacteria introduced into field environments. 3. Persistence of *Rhizobium trifolii* in association with white clover at high elevations. *Australian Journal of Experimental Agriculture* **12**, 407–413. doi:10.1071/EA9720407

Brockwell J, Gault R, Peoples M, Turner G, Lilley D, Bergersen F (1995) N₂ fixation in irrigated lucerne grown for hay. *Soil Biology & Biochemistry* **27**, 589–594. doi:10.1016/0038-0717(95)98636-3

Cabelguenne M, Debaeke P, Bouniols A (1999) EPICphase, a version of the EPIC model simulating the effects of water and nitrogen stress on biomass and yield, taking account of developmental stages: validation on maize, sunflower, sorghum, soybean and winter wheat. *Agricultural Systems* **60**, 175–196. doi:10.1016/S0308-521X(99)00027-X

Carlsson G, Huss-Danell K (2003) Nitrogen fixation in perennial forage legumes in the field. *Plant and Soil* **253**, 353–372. doi:10.1023/A:1024847017371

Chapman D, Parsons A, Cosgrove G, Barker D, Marotti D, Venning K, Rutter S, Hill J, Thompson A (2007) Impacts of spatial patterns in pasture on animal grazing behaviour, intake and performance. *Crop Science* **47**, 399–415. doi:10.2135/cropsci2006.01.0036

Cuttle S, Shepherd M, Goodlass G (2003) 'A review of leguminous fertility-building crops, with particular refence to nitrogen fixation and utilisation.' (Institute of Grassland & Environmental Research: Aberystwyth)

Dart PJ, Day JM (1971) Effects of incubation temperature and oxygen tension on nitrogenase activity of legume root nodules. *Plant & Soil* **35**, 167–184. doi:10.1007/BF02661849

Davey AG, Simpson RJ (1990) Nitrogen fixation by subterranean clover at varying stages of nodule dehydration I. Carbohydrate status and short-term recovery of nodulated root respiration. *Journal of Experimental Botany* **41**, 1175–1187. doi:10.1093/jxb/41.9.1175

de Klein C, Pinares-Patino C, Waghorn G (2008) Greenhouse gas emissions. In 'Environmental impacts of pasture-based farming'. (Ed. R McDowell) pp. 1–33. (CAB International: Wallingford, UK)

Doyle P, Stockdale C, Lawson A, Choenm D (2000) 'Pastures for dairy production in Victoria.' (Department of Natural Resources and Environment: Tatura, Vic.)

Dudman WF, Brockwell J (1968) Ecological studies of root-nodule bacteria introduced into field environments. I. A survey of field performance of clover inoculants by gel immune diffusion serology. *Australian Journal of Agricultural Research* **19**, 739–747. doi:10.1071/AR9680739

Dunbabin JS, Hume IH, Ireson ME (1997) Effects of irrigation frequency and transient waterlogging on the production of a perennial ryegrass/white clover pasture. *Australian Journal of Experimental Agriculture* **37**, 165–171. doi:10.1071/EA96057

Eckard R (1998) 'A critical review of research on the nitrogen nutrition of dairy pastures in Victoria.' (The University of Melbourne and Agriculture Victoria: Ellinbank)

Eckard R (2001) 'Best management practices for nitrogen in intensive pasture production systems.' (University of Melbourne: Melbourne)

Eckard R, Chapman D, White R, Smith A, Chen D, Durling P (2001*a*) Nitrogen balances in high rainfall, temperate dairy pastures of south eastern Australia. In 'Proceedings of the XIX International Grasslands Congress'. São Pedro, Brazil. (Eds JA Gomide, WRS Mattos, SC Da Silva) pp. 169–170. (Fundacao Estudos Agrarios Luiz Queiroz (FEALQ), Piracicaba, Brazil)

Eckard R, McKenzie F, Lane P (2001*b*) The 'Pendulum Paradigm' – trends in nitrogen fertiliser use on temperate grass/clover pastures. In 'Proceedings of the XVIII International Grasslands Congress'. Manitoba, Canada. (Eds JG Buchanan-Smith, LD Bailey, P McCaughey) pp. 30–33. (Extension Service, Saskatchewan Agriculture and Food: Saskatchewan, Canada)

Eckard RJ, Chapman DF, White RE (2007) Nitrogen balances in temperate perennial grass and clover dairy pastures in south-eastern Australia. *Australian Journal of Agricultural Research* **58**, 1167–1173. doi:10.1071/AR07022

Engin M, Sprent J (1973) Effects of water stress on growth and nitrogen-fixing activity of *Trifolium repens*. *New Phytologist* **72**, 117–126. doi:10.1111/j.1469-8137.1973.tb02016.x

Garg N, Geetanjali (2007) Symbiotic nitrogen fixation in legume nodules: process and signaling. A review. *Agronomy for Sustainable Development* **27**, 59–68. doi:10.1051/agro:2006030

Gault RR, Peoples MB, Turner GL, Lilley D, Brockwell J, Bergersen FJ (1995) Nitrogen fixation by irrigated lucerne during the first three years after establishment. *Australian Journal of Agricultural Research* **46**, 1401–1425. doi:10.1071/AR9951401

Gerard PJ (2001) Dependence of *Sitona lepidus* (Coleoptera: Curculionidae) larvae on abundance of white clover Rhizobium nodules. *Bulletin of Entomological Research* **91**, 149–152.

Gibson AH, Curnow B, Bergersen FJ, Brockwell J, Robinson C (1975) Studies of field populations of *Rhizobium*: effectiveness of strains of *Rhizobium trifolii* associated with *Trifolium subterraneu*m L. pastures in southeastern Australia. *Soil Biology & Biochemistry* **7**, 95–102. doi:10.1016/0038-0717(75)90005-X

Gourley C, Powell J, Dougherty W, Weaver D (2007) Nutrient budgeting as an approach to improve nutrient management on Australian dairy farms. *Australian Journal of Experimental Agriculture* **47**, 1064–1074. doi:10.1071/EA07017

Gourley C, Dougherty W, Aarons S, Hannah S (2010) 'Accounting for nutrients on Australian dairy farms.' (DPI Victoria: Melbourne)

Graham M (2008) Biophysical modelling and performance measurement. In 'AARES 52nd Annual Conference'. Canberra. p. 25. (Australian Agricultural and Resource Economics Society: Canberra)

Harris S, Clark D, Auldist M, Waugh C, Laboyre P (1997) Optimum white clover content for dairy pastures. *Proceedings of the New Zealand Grassland Association* **59**, 29–33.

Hatch DJ, Macduff JH (1991) Concurrent rates of N₂ fixation, nitrate and ammonium uptake by white clover in response to growth at different root temperatures. *Annals of Botany* **67**, 265–274.

Haynes RJ, Williams PH (1993) Nutrient cycling and soil fertility in the grazed pasture ecosystem. *Advances in Agronomy* **49**, 119–199. doi:10.1016/S0065-2113(08)60794-4

Hill MJ, Donald GE (1998) 'Australian Temperate Pastures Database.' (CD-ROM) (CSIRO: Canberra)

Høgh-Jensen H, Loges R, Jørgensen FV, Vinther FP, Jensen ES (2004) An empirical model for quantification of symbiotic nitrogen fixation in grass-clover mixtures. *Agricultural Systems* **82**, 181–194. doi:10.1016/j.agsy.2003.12.003

Howieson J, Ballard R (2004) Optimising the legume symbiosis in stressful and competitive environments within southern Australia – some contemporary thoughts. *Soil Biology & Biochemistry* **36**, 1261–1273. doi:10.1016/j.soilbio.2004.04.008

Howieson JG, Ewing MA, Thorn CW, Revell CK (1991) Increased yield in annual species of Medicago grown in acidic soil in response to inoculation with acid tolerant *Rhizobium meliloti*. In 'Plant–soil interactions at low pH'. (Ed. RJ Wright) pp. 589–595. (Kluwer Academic Publishers: Dordrecht, The Netherlands)

Jarvis S (1993) Nitrogen cycling and losses from dairy farms. *Soil Use and Management* **9**, 99–104. doi:10.1111/j.1475-2743.1993.tb00937.x

Jarvis SC, Scholefield D, Pain B (1995) Nitrogen cycling in grazing systems. In 'Nitrogen fertilization and the environment'. (Ed. PE Bacon) pp. 381–419. (Marcel Dekker Inc.: New York)

Johnson I (2005) 'Simulation models in agriculture and plant science.' (IMJ Consultants Pty Ltd: Melbourne) Available at: www.imj.com.au/consultancy/wfsat/wfsat.html

Johnson IR, Lodge GM, White RE (2003) The Sustainable Grazing Systems Pasture Model: description, philosophy and application to the SGS National Experiment. *Australian Journal of Experimental Agriculture* **43**, 711–728. doi:10.1071/EA02213

Johnson I, Chapman D, Snow V, Eckard R, Parsons A, Lambert M, Cullen B (2008) DairyMod and EcoMod: biophysical pastoral simulation models for Australia and New Zealand. *Australian Journal of Experimental Agriculture* **48**, 621–631. doi:10.1071/EA07133

Jørgensen FV, Ledgard SF (1997) Contribution from stolons and roots to estimates of the total amount of N₂ fixed by white clover (*Trifolium repens* L.). *Annals of Botany* **80**, 641–648. doi:10.1006/anbo.1997.0501

Kelly B, O'Brien G (1992) Management of legumes for high herbage production. *Proceedings of the Australian Society of Animal Production* **19**, 343–345.

Kelly K, Mason W, Simpson R (1989) Effects of irrigation on the productivity of white clover, red clover and lucerne in northern Victoria. In '5th Australian Agronomy Conference'. Perth, W. Aust. (Ed. MW Perry) p. 601. (Australian Society of Agronomy: Perth)

Kelly KB, Stockdale CR, Mason WK (2005) Effects of initial sowing rate and subsequent grazing management on the growth and clover content of irrigated white clover–perennial ryegrass swards in northern Victoria. *Australian Journal of Experimental Agriculture* **45**, 1595–1602. doi:10.1071/EA04121

Khan DF, Peoples M, Schwenke G, Felton WL, Chen D, Herridge DF (2003) Effects of below-ground nitrogen on N balances of field-grown fababean, chickpea and barley. *Australian Journal of Agricultural Research* **54**, 333–340. doi:10.1071/AR02105

Kleinman P, Soder K (2008) Impact of hybrid dairy systems on air, soil and water quality: focus on nitrogen and phosphorus cycling. In 'Environmental impacts of pasture-based farming'. (Ed. R McDowell) pp. 248–276. (CAB International: Wallingford, UK)

Ledgard SF (2001) Nitrogen cycling in low input legume-based agriculture, with emphasis on legume/grass pastures. *Plant and Soil* **228**, 43–59. doi:10.1023/A:1004810620983

Ledgard SF, Steele KW (1992) Biological nitrogen fixation in legume/grass pastures. *Plant and Soil* **141**, 137–153. doi:10.1007/BF00011314

Ledgard SF, Brier GJ, Upsdell MP (1990) Effect of clover cultivar on production and nitrogen fixation in clover ryegrass swards under dairy cow grazing. *New Zealand Journal of Agricultural Research* **33**, 243–249.

Ledgard SF, Sprosen MS, Steele KW (1996) Nitrogen fixation by nine white clover cultivars in grazed pasture, as affected by nitrogen fertilization. *Plant and Soil* **178**, 193–203. doi:10.1007/BF00011583

Ledgard SF, Penno JW, Sprosen MS (1999) Nitrogen inputs and losses from clover/grass pastures grazed by dairy cows, as affected by nitrogen fertilizer application. *The Journal of Agricultural Science* **132**, 215–225. doi:10.1017/S002185969800625X

Ledgard SF, Sprosen MS, Penno JW, Rajendram GS (2001) Nitrogen fixation by white clover in pastures grazed by dairy cows: temporal variation and effects of nitrogen fertilization. *Plant and Soil* **229**, 177–187. doi:10.1023/A:1004833804002

Ledgard S, Schils R, Eriksen J, Luo J (2009) Environmental impacts of grazed clover/grass pastures. *Irish Journal of Agricultural and Food Research* **48**, 209–226.

Ledgard S, Boyes M, Brentrup F (2011) 'Life cycle assessment of local and imported fertilisers used on New Zealand farms.' (Fertilizer and Lime Research Centre: Palmerston North)

Lemerle C, Stockdale C, Moate P, Rogers G (1992) Use of supplements with legumes. *Proceedings of the Australian Society of Animal Production* **19**, 347–349.

Liu Y, Wu L, Baddeley JA, Watson CA (2010) Models of biological nitrogen fixation of legumes. A review. *Agronomy for Sustainable Development* **2**, 883–905.

Mason W, Kelly K, Blaikie S, Stockdale R (1987) New directions for irrigated pastures. In 'Proceedings of the 4th Australian Agronomy Conference: Responding to Change'. (Ed. TG Reeves) pp. 100–117. (Australian Society of Agronomy: Melbourne)

McKenzie FR, Jacobs JL, Riffkin P, Kearney G (1998) Influence of single applications of nitrogen on white clover nitrogen fixation in autumn and winter dairy pastures in south-west Victoria, Australia. *African Journal of Range & Forage Science* **15**, 1–6. doi:10.1080/10220119.1998.9647933

McKeon G, Rickert K, Ash A, Cooksley D, Scattini W (1982) Pasture production model. *Proceedings of the Australian Society for Animal Production* **14**, 201–204.

McLeish LJ, Berg GN, Hinch JM, Nambiar LV, Norton MR (1997) Plant parasitic nematodes in white clover and soil from white clover pastures in Australia. *Australian Journal of Experimental Agriculture* **37**, 75–82. doi:10.1071/EA96046

McNeill AM, Zhu C, Fillery I (1997) Use of *in situ* ^{15}N-labelling to estimate the total below-ground nitrogen of pasture legumes in intact plant-soil systems. *Australian Journal of Agricultural Research* **48**, 295–304. doi:10.1071/A96097

Menneer J, Ledgard S, McLay C, Silvester W (2001) What impact does dairy cow pugging have on clover N$_2$ fixation and long-term farm production? *Proceedings of the New Zealand Grassland Association* **63**, 63–67.

Menneer JC, Ledgard S, McLay C, Silvester W (2004) The impact of grazing animals on N$_2$ fixation in legume-based pastures and management options for improvement. In 'Advances in agronomy'. pp. 181–241. (Academic Press: New York)

Moore AD, Donnelly JR, Freer M (1997) GRAZPLAN: decision support systems for Australian grazing enterprises. III. Pasture growth and soil moisture submodels, and the GrassGro DSS. *Agricultural Systems* **55**, 535–582. doi:10.1016/S0308-521X(97)00023-1

Mundy G (1987) The nitrogen nutrition of irrigated perennial pasture during spring. In 'Nitrogen cycling in temperate agricultural systems'. (Eds P Bacon, J Evans, R Storrier, A Taylor) pp. 185–190. (Australian Society of Soil Science: Griffiths, NSW)

Mundy GN, Jones HR, Mason WK (1988) Nitrogen fixation activity by white clover pastures during flood irrigation cycles. *Australian Journal of Agricultural Research* **39**, 409–414.

Munn KJ, Evans J, Chalk PM, Morris SG, Whatmuff M (1997) Symbiotic effectiveness of *Rhizobium trifolii* and mineralisation of legume nitrogen in response to past amendment of a soil with sewage sludge. *Journal of Sustainable Agriculture* **11**, 23–37. doi:10.1300/J064v11n01_04

Munns DN (1965a) Interactions of lime with nitrogen and phosphate on growth of *Medicago sativa* and *Trifolium subterraneum*. *Australian Journal of Agricultural Research* **16**, 733–742. doi:10.1071/AR9650733

Munns DN (1965b) Soil acidity and growth of a legume I. Interactions of lime with nitrogen and phosphate on growth of *Medicago sativa* L. and *Trifolium subterraneum* L. *Australian Journal of Agricultural Research* **16**, 733–741. doi:10.1071/AR9650733

Nash D, Barlow K (2008) Impacts of irrigated dairying on the environment. In 'Environmental impacts of pasture-based farming'. (Ed. R McDowell) pp. 232–248. (CAB International: Wallingford, UK)

Neal JS, Fulkerson WJ, Lawrie R, Barchia I (2009) Difference in yield and persistence among perennial forages used by the dairy industry under optimum and deficit irrigation. *Crop & Pasture Science* **60**, 1071–1087. doi:10.1071/CP09059

Ostrowski H (1972) White clover (*Trifolium repens*) in sub-tropical Australia – a review. *Tropical Grasslands* **6**, 97–106.

Pakrou N, Dillon P (2000) Key processes of the nitrogen cycle in an irrigated and a non-irrigated grazed pasture. *Plant and Soil* **224**, 231–250. doi:10.1023/A:1004838323594

Parsons AJ, Orr RJ, Penning PD, Lockyer DR, Ryden JC (1991) Uptake, cycling and fate of nitrogen in grass-clover swards continuously grazed by sheep. *Journal of Agricultural Science, Cambridge* **116**, 47–61. doi:10.1017/S0021859600076139

Pate JS, Unkovich MJ (1999) Measuring symbiotic nitrogen fixation – case studies of natural and agricultural ecosystems in a Western Australian setting. In 'Advances in physiological plant ecology'. (Eds MC Press, JD Scholes, MG Barker). (Blackwell Scientific: Oxford, UK)

Paton D (1957) Rhizobium strain trials on clovers in Tasmania 1955/56. *Rhizobium Newsletter* **2**, 19–20.

Pearson CJ, Brown R, Collins WJ, Archer KA, Wood MS, Petersen C, Bootle B (1997) An Australian temperate pastures database. *Australian Journal of Agricultural Research* **48**, 453–465. doi:10.1071/A96095

Peoples M, Baldock J (2001) Nitrogen dynamics of pastures: nitrogen fixation inputs, the impact of legumes on soil nitrogen to Australian farming systems. *Australian Journal of Experimental Agriculture* **41**, 327–346. doi:10.1071/EA99139

Peoples MB, Ladha JK, Herridge DF (1995) Enhancing legume N$_2$ fixation through plant and soil management. *Plant and Soil* **174**, 83–101. doi:10.1007/BF00032242

Peoples MB, Gault RR, Scammell GJ, Dear BS, Virgona J, Sandral G, Paul J, Wolfe EC, Angus JF (1998) Effect of pasture management on the contributions of fixed N to the N economy of ley-farming systems. *Australian Journal of Agricultural Research* **49**, 459–474. doi:10.1071/A97014

Peoples MB, Bowman A, Gault R, Herridge D, McCallum M, McCormick K, Norton R, Rochester I, Scammell G, Schwenke G (2001) Factors regulating the contributions of fixed nitrogen by pasture and crop legumes to different farming systems of eastern Australia. *Plant and Soil* **228**, 29–41. doi:10.1023/A:1004799703040

Peoples M, Unkovich M, Herridge D (2009) Measuring symbiotic nitrogen fixation by legumes. In 'Nitrogen fixation in crop production'. (Eds D Emerich, H Krishnan) pp. 125–170. (American Society of Agronomy, Crop Science Society of America, Soil Science Society of America: Madison, WI)

Pulsford D, Bullard G (1997) Genetic resources research – a perspective from the root-nodule bacteria industry. *Tropical Grasslands* **31**, 300–303.

Richardson AE, Simpson RJ (1989) Acid-tolerance and symbiotic effectiveness of *Rhizobium trifolii* associated with a *Trifolium subterraneum* L.-based pasture growing in an acid soil. *Soil Biology & Biochemistry* **21**, 87–96. doi:10.1016/0038-0717(89)90016-3

Ridley AM, Mele PM, Beverly CR (2004) Legume-based farming in Southern Australia: developing sustainable systems to meet environmental challenges. *Soil Biology & Biochemistry* **36**, 1213–1221. doi:10.1016/j.soilbio.2004.04.007

Riffkin P, Quigley P, Cameron F (1997) 'Improving the white clover feedbase by optimising nitrogen fixation.' (Dairy Research & Development Corporation: Melbourne)

Riffkin P, Cameron F, Kearney G, Quigley P, Gault R, Peoples M, Thies J (1999a) Factors associated with biological nitrogen fixation in dairy pastures in south-western Victoria. *Australian Journal of Agricultural Research* **50**, 261–272. doi:10.1071/A98035

Riffkin P, Quigley P, Cameron F, Peoples M, Thies J (1999b) Annual nitrogen fixation in grazed dairy pastures in south-western Victoria. *Australian Journal of Agricultural Research* **50**, 273–281. doi:10.1071/A98034

Robertson M, Carberry P, Huth N, Turpin J, Probert M, Poulton P, Bell M, Wright G, Yeates S, Brinsmead R (2002) Simulation of growth and development of diverse legume species in APSIM. *Australian Journal of Agricultural Research* **53**, 429–446. doi:10.1071/AR01106

Rochette P, Janzen H (2005) Towards a revised coefficient for estimating N$_2$O emissions from legumes. *Nutrient Cycling in Agroecosystems* **73**, 171–179. doi:10.1007/s10705-005-0357-9

Russelle M (2008) Biological dinitrogen fixation in agriculture. In 'Nitrogen in agricultural systems'. (Eds JS Schepers, W Raun) pp. 281–360. (ASA, CSSA, SSSA: Madison, WI)

Ryle GJA, Powell CE, Timbrell MK, Gordon AJ (1989) Effect of temperature on nitrogenase activity in white clover. *Journal of Experimental Botany* **40**, 733–739. doi:10.1093/jxb/40.7.733

Sanford P, Pate JS, Unkovich MJ, Thompson AN (1995) Nitrogen fixation in grazed and ungrazed subterranean clover pasture in south-west Australia assessed by the ^{15}N natural abundance technique. *Australian Journal of Agricultural Research* **46**, 1427–1443. doi:10.1071/AR9951427

Schulze J (2004) How are nitrogen fixation rates regulated in legumes? *Journal of Plant Nutrition and Soil Science* **167**, 125–137. doi:10.1002/jpln.200320358

Schwinning S, Parsons AJ (1996) Analysis of the coexistence mechanisms for grasses and legumes in grazing systems. *Journal of Ecology* **84**, 799–813. doi:10.2307/2960553

Sinclair T (1986) Water and nitrogen limitations in soybean grain production I. Model development. *Field Crops Research* **15**, 125–141. doi:10.1016/0378-4290(86)90082-1

Sinclair TR, Seligman NG (1996) Crop modeling: from infancy to maturity. *Agronomy Journal* **88**, 698–704. doi:10.2134/agronj1996.00021962008800050004x

Smith CJ, Chalk PM, Noble CL, Prendergast JB, Robertson F (1993) Nitrogen fixation in a white clover-grass pasture irrigated with saline groundwater. *Irrigation Science* **13**, 189–194. doi:10.1007/BF00190035

Soussana JF, Minchin FR, Macduff JH, Raistrick N, Abberton MT, Michaelson-Yeates TPT (2002) A simple model of feedback regulation for nitrate uptake and N_2 fixation in contrasting phenotypes of white clover. *Annals of Botany* **90**, 139–147. doi:10.1093/aob/mcf161

Sprent J (1971) Effects of water stress on nitrogen fixation in root nodules. *Plant and Soil* **35**, 225–228. doi:10.1007/BF02661853

Sprent J, Gallacher A (1976) Anaerobisis in soybean root nodules under water stress. *Soil Biology & Biochemistry* **8**, 317–320. doi:10.1016/0038-0717(76)90063-8

Streeter J (1988) Inhibition of legume nodule formation and N_2 fixation by nitrate. *Critical Reviews in Plant Sciences* **7**, 1–23. doi:10.1080/07352688809382257

Svenning MM, MacDuff JH (1996) Low root temperature retardation of the mineral nitrogen induced decline in N_2 fixation by a northern ecotype of white clover. *Annals of Botany* **77**, 615–621. doi:10.1093/aob/77.6.615

Thornley JHM (2001) Simulating grass-legume dynamics: a phenomenological submodel. *Annals of Botany* **88**, 905–913. doi:10.1006/anbo.2001.1529

Unkovich MJ, Pate JS (1998) Symbiotic effectiveness and tolerance to early season nitrate availability in indigenous populations of subterranean clover rhizobia from SW Australian pastures. *Soil Biology & Biochemistry* **30**, 1435–1443. doi:10.1016/S0038-0717(97)00258-7

Unkovich MJ, Sanford P, Pate JS (1996) Nodulation and nitrogen fixation by subterranean clover in acid soils as influenced by lime application, toxic aluminium, soil mineral N, and competition from annual ryegrass. *Soil Biology & Biochemistry* **28**, 639–648. doi:10.1016/0038-0717(95)00174-3

Unkovich MJ, Pate JS, Sanford P (1997) Nitrogen fixation by annual legumes in Australian Mediterranean agriculture. *Australian Journal of Agricultural Research* **48**, 267–293. doi:10.1071/A96099

Unkovich MJ, Sanford P, Pate JS, Hyder M (1998) Effects of grazing on plant and soil nitrogen relations of pasture-crop rotations. *Australian Journal of Agricultural Research* **49**, 475–485. doi:10.1071/A97071

Unkovich M, Herridge DF, Peoples MB, Boddey RM, Cadisch G, Giller K, Alves B, Chalk PM (2008) 'Measuring plant-associated nitrogen fixation in agricultural systems.' ACIAR Monograph No.136. (Australian Centre for International Agricultural Research: Canberra)

Unkovich M, Baldock J, Peoples M (2010) Prospects and problems of simple linear models for estimating symbiotic N_2 fixation by crop and pasture legumes. *Plant and Soil* **329**, 75–89. doi:10.1007/s11104-009-0136-5

Ward G, Quigley P (1992) The botanical composition of high-rainfall pastures in south-west Victoria. In 'Looking back: planning ahead. Proceedings of the 6th Australian Agronomy Conference'. (Eds K Hutchinson, P Vickery) p. 530. (Australian Society of Agronomy: Armidale)

Waters L (1957) Rhizobium strain trials at Lismore 1956. *Rhizobium Newsletter* **2**, 19–20.

Watkin ELJ, O'Hara GW, Howieson JG, Glenn AR (2000) Identification of tolerance to soil acidity in inoculant strains of *Rhizobium leguminosarum* bv. trifolii. *Soil Biology & Biochemistry* **32**, 1393–1403. doi:10.1016/S0038-0717(00)00057-2

Whitehead DC (1995) 'Grassland nitrogen.' (CAB International: Wallingford, UK)

Wichern F, Eberhardt E, Mayer J, Joergensen RG, Müller T (2008) Nitrogen rhizodeposition in agricultural crops: methods, estimates and future prospects. *Soil Biology & Biochemistry* **40**, 30–48. doi:10.1016/j.soilbio.2007.08.010

Woodfield D, Clark D (2009) Do forage legumes have a role in modern dairy farming systems? *Irish Journal of Agricultural and Food Research* **48**, 137–147.

Wu L, McGechan MB (1999) Simulation of nitrogen uptake, fixation and leaching in a grass/white clover mixture. *Grass and Forage Science* **54**, 30–41. doi:10.1046/j.1365-2494.1999.00145.x

Yang H, Unkovich MJ, McNeill AM, Wang X (2011) Symbiotic N_2 fixation and nitrate utilisation in irrigated lucerne (*Medicago sativa*) systems. *Biology and Fertility of Soils* **47**, 377–385. doi:10.1007/s00374-011-0543-7

Yeates GW, Stirling GR (2008) Regional patterns among soil nematode assemblages in Australasian pastures and effects of management practices. *Australasian Plant Pathology* **37**, 298–307. doi:10.1071/AP08028