Proc. Twelfth Ann. Conf. Grassld. Soc. NSW, 1997, pp. 20-29.

Too dry? - Pastures in the wheatbelt:

Nitrogen fixation by pastures in dryland farming systems

Mark Peoples[1], Robert Gault[1], John Angus[1], Alison Bowman[2], John Brockwell[1],
David Kemp[3], Matthew McCallum[4], John Paul[5], Paul Quigley[6], Penny Riffkin[6],
Glen Scammell[7] and Ted Wolfe[5]

[1]*CSIRO Division of Plant Industry, Canberra, ACT 2601*
[2]*NSW Agriculture, Trangie, NSW 2823*
[3]*Orange Agricultural Institute, Orange, NSW 2800*
[4] *Victorian Institute for Dryland Agriculture, Horsham, Vic 3401*
[5] *School of Agriculture, Charles Sturt University, Wagga Wagga, NSW 2650*
[6] *Pastoral and Veterinary Institute, Hamilton, Vic 3300*
[7] *Institute for Integrated Agricultural Development, Rutherglen, Vic 3685*

Summary: Estimates of N_2 fixation by pastures in dryland farming systems range from 5 to 240 kg N/ha/yr for annual legumes and from 35 to 170 kg N/ha/yr for perennial species such as lucerne. The amounts of N_2 fixed are primarily regulated by the legume content and herbage yield of pastures. As a general rule-of-thumb, 25 kg N/ha are fixed for every tonne of legume dry matter produced regardless of the species or the environment in which they are grown. Strategies which favour high legume biomass and N_2 fixation inputs include:

- -rhizobial inoculation the first time a new legume species is sown
- -maintenance of a dense legume stand
- -applications of superphosphate or lime to ameliorate nutritional problems
- -herbicide applications to remove grasses in annual pastures in the year prior to cropping
- -growing lucerne to overcome the year-to-year variability experienced with annual legumes.

However, pasture response to these management treatments may be modified by grazing management through livestock effects on nutrient cycling, pasture productivity and botanical composition.

Role for nitrogen fixation by pastures in the wheat-belt

In the southern cropping zone (South Australia, Victoria and New South Wales), some two-thirds of the arable land is occupied by pasture (largely self-regenerating annual pastures; Wilson and Simpson 1993). Given experimental evidence that symbiotic nitrogen fixation (N_2 fixation) in legume-based pastures can substantially enhance soil nitrogen (N) reserves (Peoples *et al* 1995a), the area under pasture should theoretically be able to supply ample N for following crops. However, there is evidence that the inability of many Australian soils to supply sufficient N is a widespread constraint to cereal production and grain quality (Hamblin and Kyneur 1993).

Although the use of pastures containing subterranean clover (*Trifolium subterraneum*) or annual medics (*Medicago* spp.) has long been considered to be a sustainable and profitable means of maintaining soil fertility in ley-farming systems, there has been a trend over the past two decades towards shorter pasture phases in pasture-crop rotations and continuous cropping. This in part reflected the relative economic returns from livestock enterprises and crop production (McCown *et al.* 1988) and the perceived shortcomings in the ley system associated with problems of pasture decline and soil acidification (Carter *et al.* 1982; Hochman *et al.* 1990). The consequence of a shortened pasture phase and more intensive cropping in the southern cereal-livestock belt has been a general neglect of pastures, increased grazing pressure, a depletion of legume seed reserves, and difficulties for pasture regeneration (Carter *et al.* 1982; Hamblin and Kyneur 1993). Residual effects of crop herbicides on pasture legumes, particularly the effect of sulfonyl ureas on medics have also accelerated pasture decline. Many pastures are now characterised by poorly productive swards dominated by weedy annual species, containing little clover or medic (Wilson and Simpson 1993). The net result has been suboptimal inputs of fixed N_2.

This paper describes the relative impact of improved management of annual pastures or the use of perennial species such as lucerne (*Medicago sativa*) on the potential contributions of fixed N during a pasture phase. Much of the information presented comes from a series of GRDC-funded experiments in ley-farming systems of NSW and Victoria. However, research findings from permanently grazed pastures containing either subterranean clover or white clover (*Trifolium repens*) in the higher rainfall zones will also be included; the management objectives for pastures used primarily for animal production or milk may require different approaches to pasture-crop rotations; however, the principles remain the same.

Measurement of N_2 fixation

The amount of N_2 fixed by a legume in any farming system is determined by the amount of legume N accumulated and the proportion (Pfix) of that legume's N derived from N_2 fixation:

Amount of N fixed = (legume N) x (Pfix)

Therefore, any factor which improves legume N and/or Pfix will increase inputs of fixed N into pastures (Peoples *et al.* 1995b).

Determination of legume N

Measures of legume N are calculated from determinations of legume biomass and tissue N content:

Legume N = (legume dry matter) x (%N)

The information used in the following sections to illustrate the effect of management on legume dry matter production and N_2 fixation refer to changes measured in above-ground biomass during a growing season. This is usually determined by periodically cutting all pasture foliage inside a series quadrat areas of known dimensions (from within stock exclusion cages if grazing animals are present) and then separating the harvested shoot material into legume and non-legume components which are subsequently dried, weighed and analysed for N. It is important to recognise that such measurements will underestimate the total contributions made by N_2 fixation to pasture systems since there is evidence that nodulated roots can play a key role in the N-economy of legume pastures (Gault *et al.* 1995; Peoples *et al.* 1997). Recent research has indicated that the below-ground component of pasture legumes may contain 40-50% of the total plant N (McNeill *et al.* 1997). Therefore, the absolute amounts of N_2 fixed could be twice that measured in shoots.

Determination of Pfix

An estimate of Pfix represents a measure of a legume's reliance upon N_2 fixation for growth. In other words, it provides a means of partitioning legume N into that proportion arising from N_2 fixation, or assimilated from soil N. This can be obtained by analysing plant material for the two stable isotopes of nitrogen, ^{14}N and 15N, using a mass spectrometer. To calculate Pfix, it is necessary for the two sources of N used by the legume for growth (*ie.* soil N and atmospheric N_2) to differ in isotopic concentrations. The heavier isotope ^{15}N occurs in atmospheric N_2 at a constant abundance of 0.3663 atom% ^{15}N (*ie.* for every 100 atoms of N in atmospheric N_2, 99.6337 will be in the form of ^{14}N and 0.3663 will be as ^{15}N). The levels of ^{15}N which occur naturally in many agricultural soils are usually measurably higher than this. The technique most commonly used to estimate Pfix in Australian pastures compares the natural levels of ^{15}N in the legume with other non-N_2-fixing reference plants (*eg.* grass or broad-leaf weeds) also growing the same sward (Unkovich *et al.* 1997). Since the reference plants are totally reliant upon the assimilation of soil N for growth, their ^{15}N composition is taken as a measure of the ^{15}N concentration of plant-available N in the soil. It is assumed that the ^{15}N: ^{14}N composition of a legume will be the same as the reference when the legume is unnodulated, fixes no atmospheric N_2 and draws all of its N from the soil (*ie.* Pfix = 0). As the legume fixes N_2 , its ^{15}N content declines from the level in the reference plant and approaches the ^{15}N composition of atmospheric N_2 as Pfix approaches 100%.

Levels of N_2 fixation observed in commercial pastures

Using the above isotope dilution technique, comprehensive surveys of levels of Pfix in commercial annual and perennial pastures have been undertaken in southern Western Australia, south-western and north-eastern Victoria and southern New South Wales. These on-farm surveys indicated that on average 67 to 84% of legume N was derived from N_2 fixation (Table 1). Yet a sizeable percentage of the pastures examined in each region contained legumes recording Pfix values 65%. Poor N_2 fixation in these pastures has been attributed to nutritional constraints (high levels of tissue aluminium indicative of soil acidity, Sanford *et al.* 1994; high soil N, low available phosphorus and sulphur, Quigley *et al.* 1996), or to environmental stress such as drought (Peoples *et al.* 1997).

A measure of Pfix indicates the legume's capacity to fix N but, provided adequate rhizobia are present in soil, Pfix does not often change much in response to management, and by itself is only a limited guide to symbiotic performance (Peoples *et al.*

Table 1. Comparison of the capacity of pasture legumes from different regions of Australia to fix N based on their relative dependence upon N2 fixation for growth (Pfix).[a]

Region	Legume	Pastures sampled	Mean Pfix (%)	Percentage of pastures in each class:		
				Poor fixation (0-64%)	Adequate fixation (65-80%)	Excellent fixation (81-100%)
WA	Subclover	184	72	29	26	45
Vic	White clover	71	77	18	31	51
Vic/NSW	Subclover	8	18	48	32	60
	Lucerne	39	67	33	41	26

[a] Data from Sanford *et al.* (1994), Quigley *et al.* (1996), and Peoples *et al.* (1997).

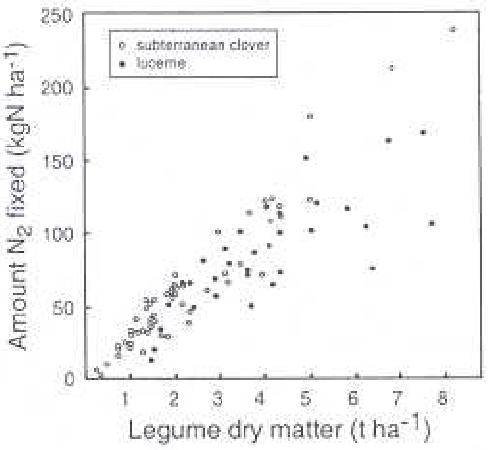

Figure 1. Relationships between the amounts of N2 fixed in annual and perennial pastures and legume dry matter. Subterranean clover (m) and lucerne (l) data were collated from experimental and on-farm pasture sites in north-eastern Victoria and southern NSW as described by Peoples *et al.* (1997).

1997). Legume biomass is closely related to total amounts of N_2 fixed (Figure 1). However, direct comparisons of pastures solely on this basis can be misleading because differences in legume dry matter in pastures are often unrelated to N_2 fixation. A more useful measure of efficiency is available if amounts of N_2 fixed are expressed per unit of legume dry matter produced. Such determinations provide a benchmark by which N_2 fixation can be compared across management treatments, locations, legume species and growing seasons.

Table 2 summarises experimental and on-farm estimates of N_2 fixation calculated in this way for pastures at various localities around New South Wales and Victoria. The data came from pastures in which botanical composition varied from <10 % to >95% legume and covered a range of productivities across different rainfall zones. Yet, despite these inherent differences there was remarkable uniformity in the efficiency of N_2 fixation for both annual (subterranean clover or medic) and perennial-based pastures (lucerne in the cropping zone, white clover under permanent grazing) across most sites examined (Table 2). Relatively few estimates of N_2 fixation fell below 15 kg fixed-N/tonne legume dry matter. Determinations for most pastures were in the range 20 to 30 kg fixed-N/tonne legume. It appeared to make little difference whether pastures were based on annual or perennial legumes, were under permanent grazing or came from ley-farming systems. In all cases the average efficiency with which N_2 was fixed represented around 25 kg fixed-N for each tonne of legume herbage produced (Table 2). Since similar relationships have been observed for pastures in cropping zones in South Australia (Butler 1988) and Western Australia (Bolger *et al.* 1995), it may be possible to use this value as a rule-of-thumb to predict likely inputs of fixed N.

Factors influencing legume biomass and N_2 fixation - strategies to overcome constraints

A pasture legume such as lucerne can be grown over a wide range of environments. However, the amounts of N_2 fixed can vary considerably between different localities (Table 3). Regional limits to N_2 fixation are primarily regulated by climatic constraints to lucerne growth imposed by total rainfall, rainfall distribution, or temperature, but pasture management practices can also play an important role. Potential productivity and N_2 fixation can be raised by removing constraints such as an insufficient or unreliable water supply by growing lucerne with irrigation (Table 3). While this is not an option for most farmers, there are other strategies which can be imposed to manipulate and manage N_2 fixation by dryland pastures.

Presence of effective rhizobia / inoculation

Prior to European settlement, Australian soils contained no root-nodule bacteria (rhizobia) for the majority of agriculturally useful legumes. Since then a great many strains of nodule bacteria have been introduced accidentally or deliberately. Today, the numbers of naturalised rhizobia in most soils are usually adequate to induce nodulation and N_2 fixation by most pasture legumes. Nevertheless, there a number of conditions under which soils may be devoid of effective rhizobia. This can limit the potential for N_2 fixation in pastures (Brockwell *et al.* 1995). The most common hostile soil factor is soil acidity. Low soil pH affects the viability of *Mesorhizobium* (formerly *Rhizobium*) *meliloti*, the

Table 2. Estimates of amounts of N2 fixed for each tonne of legume herbage produced.

Pasture type and localities[a]	Annual rainfall (mm)	Number of pastures	Annual legume[b] Range (kg N-fixed/t)	Annual legume[b] Mean	Perennial legume[c] Range (kg N-fixed/t)	Perennial legume[c] Mean
Permanently grazed						
Timboon, Vic	620-1940	7	10-40	27		
		257			12-53	27
Braidwood, NSW	1059	13	9-21	17		
Orange, NSW	900	9	22-31	27		
Beechworth, Vic	820	13	20-36	27		
Bungendore, NSW	760	13	20-31	25		
Yass, NSW	642-652	4	22-30	26		
Goulburn, NSW	631	2	18-21	20		
Canberra, ACT	631	7	13-32	24		
Overall		325		24		27
Rotated with crop						
Canberra, ACT	631	4			13-24	20
Cootamundra, NSW	625	1	27	27		
		2			19-25	22
Rutherglen, Vic	608	19	18-36	27		
		11			19-31	24
Wagga Wagga, NSW	560	16	9-34	26		
		7			13-31	24
Junee, NSW	504-536	26	8-34	26		
		13			8-33	21
Lockhart, NSW	487	6	14-32	22		
		2			17-36	27
Trangie, NSW	479	14	15-34	27		
Horsham, Vic	423	3	24-31	27		
		5			19-30	25
Overall1		29		26		24

[a] Adapted from data of Peoples *et al.* (1995c, 1997) and includes unpublished findings of Bowman, Gault, Kemp, McCallum, Quigley and Riffkin; [b] All data refer to subterranean clover except for Horsham where the pasture contained annual medic; [c] White clover collected from dairy pastures or lucerne grown in rotation with crop.

root-nodule bacteria for lucerne and annual medics (Brockwell *et al.*1991), and the persistence and distribution of *Rhizobium leguminosarum* bv. *trifolii* (formerly *R. trifolii*), the organism that nodulates subterranean clover (Richardson and Simpson 1988).

Selection of elite strains for rhizobial inoculants or use of legume host-rhizobial strain combinations more tolerant of hostile soil environments may improve the potential establishment of well nodulated pasture legumes (Howieson and Ewing 1986). But typically, responses to inoculation are most likely to occur when appropriate rhizobia are absent from the soil (such as the first time a new legume species is sown in a pasture) or are present only in small numbers, and when soil nitrate is low (Brockwell *et al.* 1995). If populations of infective rhizobia are already resident in soil, they may present a formidable competitive barrier to the establishment of inoculant strains. Inoculation response may be impeded by as few as 10-100 naturalised rhizobia per gram of soil, although 1000 per gram is generally regarded as the threshold above which inoculation is futile. A naturally occurring population of 100 per gram of soil (0-10 cm) is equivalent to $1.5x10^{11}$ rhizobia per hectare. In contrast, white clover seed inoculated at the commercially recommended rate and sown at 5 kg/ha introduces only $5.0x10^{10}$ rhizobia per hectare. In other words, inoculant rhizobia are outnumbered threefold by a soil population of merely 100 per gram and even that assumes no mortality of the inoculant after it has been applied to the seed. Analyses of soils collected from 6 dairying areas of Victoria and New South Wales showed that the majority of sites contained between 2,000 and 900,000 rhizobia per gram of soil able to nodulate white clover (Riffkin, unpublished).

Choice of legume genotype

It is clear that foliar or root diseases will affect plant vigour and depress growth potential, and hence influence N_2 fixation. Productivity losses from 10 to >90% resulting from disease have been reported for pasture legumes. The use of tolerant or resistant legume cultivars is one approach to controlling disease outbreaks (Dear *et al.* 1993) which will contribute to the persistence of a legume component in a pasture and stabilise N_2 fixation inputs. Similarly any varietal or species differences in tolerance to soil acidity or other nutritional constraints to growth and nodulation will influence N_2 fixation (Howieson and Ewing 1986; De Marco *et al.* 1995; Unkovich *et al.* 1997). Regardless of the mechanism for genotypic differences in growth potential,

Table 3. Comparisons of productivity and N_2 fixation by lucerne grown in different environments.

Location	Lucerne dry matter production (t/ha/yr)	Amounts of N fixed (kg N/ha/yr)	Reference
Dryland			
Horsham, Vic	1.00 - 3.30	19 - 90	McCallum, unpubl.
Warra, Qld	2.92 - 3.35	83 - 92	Hossain *et al.* (1995)
Trangie, NSW	2.93 - 5.53	88 - 155	Bowman, unpubl.
Junee, NSW	4.31 - 7.54	113 -167	Peoples *et al.* (1997)
Irrigated			
Canberra, ACT	11.1 - 13.7	306 - 386	Gault *et al.* (1995)

enhanced vigour will generally translate into increased N_2 fixation as illustrated in the example presented in Table 4 for subterranean clover.

Table 4. Effect of cultivar on N_2 fixation and growth by subterranean clover.[a]

Cultivar	Dry matter production (t/ha)	Pfix (%)	Amount of N fixed (kg N/ ha)
Trikkala	2.05	65	40
Leura	3.45	86	88
Enfield	3.12	90	96

[a] Unpublished data of Riffkin and Quigley.

Effect of legume population

The impact of legume numbers on N_2 fixation has been investigated for subterranean clover and lucerne (Table 5). The subterranean clover density study indicated that N_2 fixation was improved as clover density increased. The 20-fold increase in amounts of N_2 fixed between extremes in clover populations arose from significant changes in both legume dry matter production (and hence legume N) and Pfix. Similar results were also observed with lucerne where the amounts of N_2 fixed rose from 64 to 150 kg N/ha as plant populations were increased from 5 to 40 plants/m^2, but in this case inputs of fixed N increased without any major change in Pfix - *ie.* amounts fixed were directly related to changes in lucerne dry matter production. Therefore, periodic resowing of pastures, or undersowing the last crop before a pasture phase is often desirable to ensure dense legume stands.

Table 5. Effect of plant density on legume growth and N_2 fixation.[a]

Species	Plant population (plants/m^2)	Legume DM production (t/ha)	Pfix (%)	Amount of N fixed (kg N/ha)
Subclover	24	0.35	46	3
	137	2.31	63	39
	819	2.75	76	59
Lucerne	5	2.17	52	64
	10	3.09	51	88
	20	4.02	53	117
	40	4.88	55	150

[a] Data from Peoples *et al.* (1997).

Legume nutrition

Nutritional constraints can control a legume's capacity for growth and N_2 fixation. Constraints may be in the form of phytotoxic concentrations of nutrients which inhibit root or shoot growth, such as excessive levels of aluminium and manganese which develop in acid soils, and sodium chloride in the case of saline soil. Alternatively, constraints can result from an inadequate supply of nutrients which can affect growth of the legume host, nodule function, and multiplication and survival of the rhizobial component of the symbiosis. Therefore, soil fertility problems can contribute to the loss of legumes from pastures and poor N_2 fixation (Quigley *et al* 1996). Examples are only presented for soil acidity and phosphorus deficiency although similar results could be expected for an imbalance of any major or minor nutrient.

Soil acidity

Many soils under legume-based improved pastures are acidifying in the winter-dominant, higher rainfall regions (>500mm) of south-eastern Australia. The release of phytotoxic aluminium and manganese into soil solution as pasture soils acidify is believed to be responsible for significant losses of forage production (Hochman *et al.* 1990). Liming has been shown to increase clover growth and amounts of N_2 fixed in permanently grazed pastures with acidic subsoils (Table 6); particularly when phosphorus nutrition was also improved (Peoples *et al.* 1995c). Responses to lime by subterranean clover are not always so dramatic as those depicted in Table 6 (Peoples *et al.* 1997; Unkovich *et al.* 1997). However, applications of lime are essential in many soils for the successful establishment and persistence of other legume species such lucerne and annual medics because of their extreme sensitivity to low pH (De Marco *et al.* 1995).

Phosphorus

Table 6 presents data describing the effect of adding phosphate to responsive pastures where the soils were low in available phosphorus. The observed improvements in total herbage yield in response to superphosphate resulted largely from a

Table 6. Examples of the effect of lime and phosphorus on alleviation of constraints on subterranean clover growth and N_2 fixation in New South Wales pastures.[a]

Nutrient constraint location	Management variable	Total pasture production (t/ha)	Legume DM production (t/ha)	Pasture composition (% clover)	Amount N fixed (kg N/ha)	Increase in N fixed (%)
Excess						
Soil acidity						
Bungendore	- Lime	3.26	1.27	39	34	-
	+ Lime	5.07	1.82	36	57	68
Braidwood	- Lime	4.35	.52	35	32	-
	+ Lime	6.50	3.57	55	72	125
Deficiency						
Phosphorus						
Goulburn	- Superphosphate	3.92	1.69	43	30	-
	+ Superphosphate	5.40	3.90	60	69	130
Bookham	- Superphosphate	2.55	0.23	9	5	-
	+ Superphosphate	3.79	1.93	51	54	980

[a] Data from Peoples *et al.* (1995c, 1997).

stimulation in subterranean clover growth and increased clover content of the pasture. Levels of Pfix (data not shown) were uniformly high (80-98% of clover N derived from N_2 fixation) and were unaffected by either the botanical composition of the pasture or application of superphosphate. Therefore, increased N_2 fixation resulted solely from improvements in clover production. While similar observations have also been reported in other environments (Bolger *et al.* 1995; Peoples *et al.* 1995c) this general pattern of pasture response to phosphorus does not always occur. At one site in southern New South Wales for example, the addition of superphosphate stimulated clover growth and increased the amounts of N_2 fixed in only 1 year of 4 (Figures 2 and 3). In all other years superphosphate boosted growth of annual grasses such as barley grass and silver grass. The relative response of grass and/or clover growth to additional phosphate in different pastures can be influenced by grazing pressure, the initial fertility of the soil and seasonal rainfall conditions (Simpson *et al.* 1974; Ayres *et al.* 1977; Bolger *et al.* 1995).

Effect of removal of annual grasses with herbicide

Pasture plants exposed to a herbicide such as Paraquat as part of a 'winter-cleaning' treatment to remove grasses become desiccated following treatment. Subterranean clover and medics can subsequently regenerate from the crown; grasses do not. As a consequence, grass removal treatments can increase legume content provided the herbicide treatment is applied early enough to allow recovery. The aim is to produce an almost pure legume sward. As well as having a big impact on the carry-over of cereal root disease into a cropping phase, grass control can also significantly improve amounts of N_2 fixed during spring and concentrations of mineral N detected in soil the following autumn (by up to 100 kg N/ha, Peoples *et al.* 1997). Examples of the affect of winter-cleaning on pasture composition and N_2 fixation are presented in Figures 2, 3 and 4.

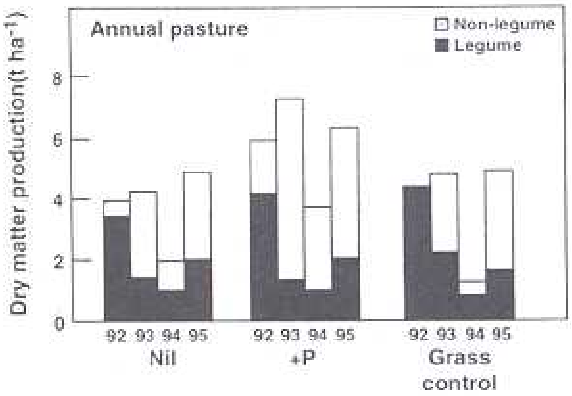

Figure 2. Effect of different management inputs on dry matter production by subterranean clover (black bars) and non-legume components of annual pastures at Junee Reefs, NSW, between 1992 and 1995. Treatments applied to annual pastures included addition of superphosphate (+P) and grass-removal with herbicide (G). Adapted from Peoples *et al.* (1997).

In the skilled hands of researchers at Rutherglen in north-eastern Victoria the timely application of herbicides have routinely increased clover biomass despite a general decrease in total pasture production (Figure 4a). Annual amounts of N_2 fixed were improved by 13 to 57 kg N/ha with winter-cleaning (Figure 4b). The favourable environmental conditions experienced at Rutherglen resulted in high potentials for clover growth by both the nil herbicide and winter-cleaned pastures and in excess of 100 to 200 kg N/ha was fixed during each growing season (Figure 4b). Grass removal treatments under conditions of lower growth potential at Junee Reefs in southern New South Wales initially gave results similar to those observed at Rutherglen (1992 and 1993, Figures 2 and 3), but there was no net benefit from winter-cleaning in the last 2 years of the study. This was attributed to drought in 1994 and to invasion of the herbicide-treated areas by broadleaf weeds in 1995 (Figures 2 and 3).

Grass control during winter can result in areas of

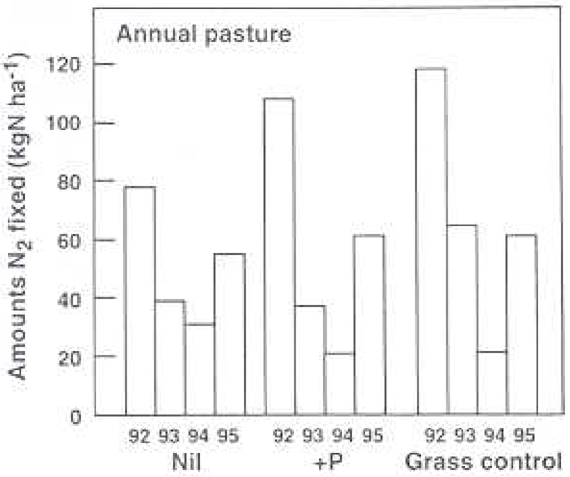

Figure 3. Effect of different management inputs on amounts of N_2 fixed by subterranean clover in annual pastures at Junee Reefs, NSW, between 1992 and 1995. Treatments applied to annual pastures included addition of superphosphate (+P) and grass-removal with herbicide (G). Adapted from Peoples *et al.* (1997).

bare soil at the end of spring which will allow growth of unpalatable weeds after summer rain. But apart from problems with pasture instability and vulnerability of soil to erosion, winter-cleaning creates a potential for the development of herbicide resistance in grasses and difficulties with clover seed set which may affect subsequent pasture regeneration. Therefore, growers' are best advised to winter-clean pastures only in the year immediately prior to cropping.

Comparison of annual pastures with perennial-based systems

The effect of different combinations of common management treatments on the productivity and N_2 fixation by an existing annual pasture was investigated over 4 growing seasons (1992-1995) and compared with the performance of perennial pastures. One of the most striking observations was the dramatic effects of year-to-year shifts in botanical composition, between clover dominance and grassy pastures, on the amounts of N_2 fixed by subterranean clover in annual pastures (Figures 2 and 3, also Rossiter 1966) or by clover growing in association with perennial grasses (Figures 5 and 6). For example around half of the N_2 fixed by subterranean clover over the 4 year period was the result of a single good growing season. However, the lucerne or lucerne/perennial grass mixtures contained greater amounts of legume dry matter and fixed more N than any of the annual pastures in each of the 3 years where direct comparisons were made (Figures 5 and 6). Most of lucerne's advantage was in summer when occasional rainfall events gave immediate growth responses. Figure 7 compares the cumulative performance of annuals over 4 growing seasons with the achievements of lucerne in just 3 years. Although the total amounts of fixed N recovered in annual pastures after 4 years (203-264 kg N/ha) were similar to lucerne/perennial grass mixtures over 3 years (237 kg N/ha), the amounts of N_2 fixed by lucerne as a pure stand was substantially greater (383 kg N/ha, Figure 7). This largely reflected the ability of lucerne-based pastures to maintain a consistently higher legume component and to continue growing

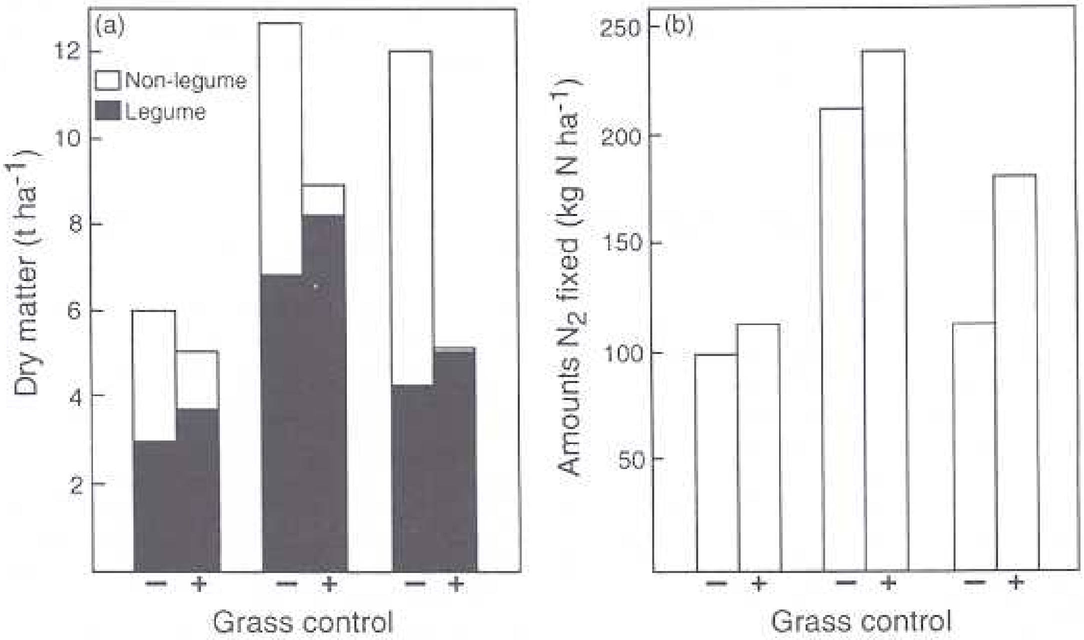

Figure 4. Comparisons of the effect of winter-cleaning to remove grasses on: (a) dry matter production by subterranean clover (black bars) and non-legume components of grazed pasture, and (b) amounts of N_2 fixed at Rutherglen, Victoria, between 1991 and 1993. The + and - symbols indicate treatments with and without herbicide applications. Adapted from Peoples *et al.* (1997).

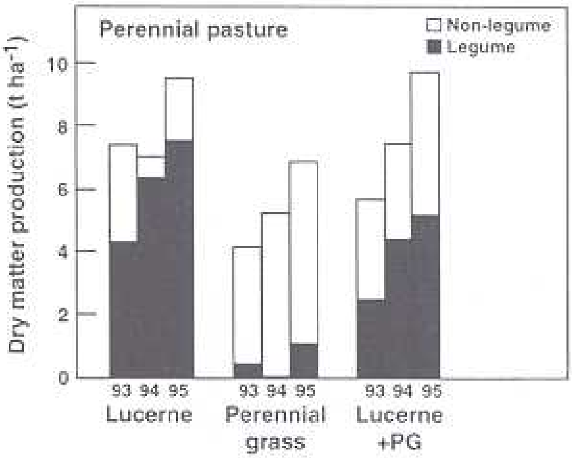

Figure 5. Total dry matter production by legume (black bars) and non-legume components of perennial pastures containing lucerne (Luc) and /or perennial grasses (PG) at Junee Reefs, NSW, between 1993 and 1995. Legume values refer to lucerne (subterranean clover represented <5 % of the total pasture biomass), except in the perennial grass plots where only subterranean clover was present. Adapted from Peoples *et al.* (1997).

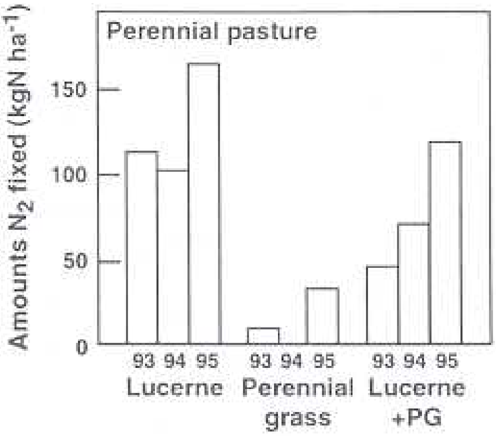

Figure 6. Amounts of N_2 fixed by perennial pastures containing lucerne (Luc) and /or perennial grasses (PG) at Junee Reefs, NSW, between 1993 and 1995. Legume values refer to lucerne (subterranean clover contributed <15 kg N /ha), except in the perennial grass plots where only subterranean clover was present. Adapted from Peoples *et al.* (1997).

and fixing useful amounts of N (71-103 kg N/ha) during the 1994 drought (Figure 6). The average annual input of N_2 fixed by lucerne in this environment (128 kg N/ha/yr) was 90-150% greater than neighbouring subterranean clover-based pastures (50-66 kg N/ha/yr).

Interaction between N2 fixation and grazing animals

The amount of legume required in a pasture to maximise herbage production, optimise animal liveweight gain, and to provide sufficient fixed N to balance the N removed or lost during cycling through the animal-plant-soil system will depend upon Pfix and the extent of forage utilisation by grazing animals (Thomas 1995). However, the most important factor determining net inputs of fixed N in a pasture system will be the maintenance of a high legume content within a pasture, and the persistence of that legume component. New pastures tend to have a high legume content initially, followed in later years by an increase in broad-leaved weeds and grasses. A number of farm surveys of the botanical composition of pastures have concluded that the legume component is often below that required for optimum animal production or to sustain reliable inputs of fixed N_2 (Kemp and Dowling 1991; Wilson and Simpson 1993).

Where pastures become a subsidiary to crop production and are primarily a means of increasing soil fertility and of providing a disease break for following crops, herbicides may be used to induce a high legume content as discussed above. However, stocking rate and grazing system (i.e. continuous, rotational or deferred grazing), livestock type (cattle, sheep or goats) and selective grazing can all influence legume seed set and pasture composition (Rossiter 1966; Ledgard and Steele 1992; Conlan *et al.* 1994; Thomas 1995). This may be influenced by the relative growth responses of the various grass-legume associations, different growth habits and species palatability, climate, and stage during the growing season when grazing occurs. Because of the prostrate growth habit of clovers for example, strategic heavy grazing of a grassy pasture in early spring can shift botanical composition toward legume dominance. As a general rule, undergrazing encourages grass growth and results in pastures with depressed legume content (Kemp *et al.* 1993).

Grazing intensity and animal excreta also affect nutrient cycling and spatial variability (Simpson *et al.* 1974; Ledgard and Steele 1992). Seventy-five to 95% of N ingested by livestock is returned in excreta, typically 30 to 70% as urine, depending upon the N content of the feed. This urinary N represents between 5 and 20 g N/day/head in sheep and 40 to 220 g N/day/head in cattle. Urine is applied to localised areas of pasture at rates equivalent to up to 300 kg N/ha by sheep and 1200 kg N/ha by cattle. The capacity for legumes to fix N in these urine patches can be depressed by up to 90%. The N_2-fixing activity can recover after 30-60 days, although urine stimulation of grass, and resultant competition with the legume, might reduce N_2 fixation per unit area for longer periods. It has been estimated that in an intensive dairy farm, where up to 40% of the land area may be affected by excreta and N_2 fixation may be depressed by 60%, total annual farm inputs from N_2 fixation might be reduced by 20-25% (Ledgard *et al.* 1982, 1992).

General Discussion

When determining how N_2 fixation during a pas-

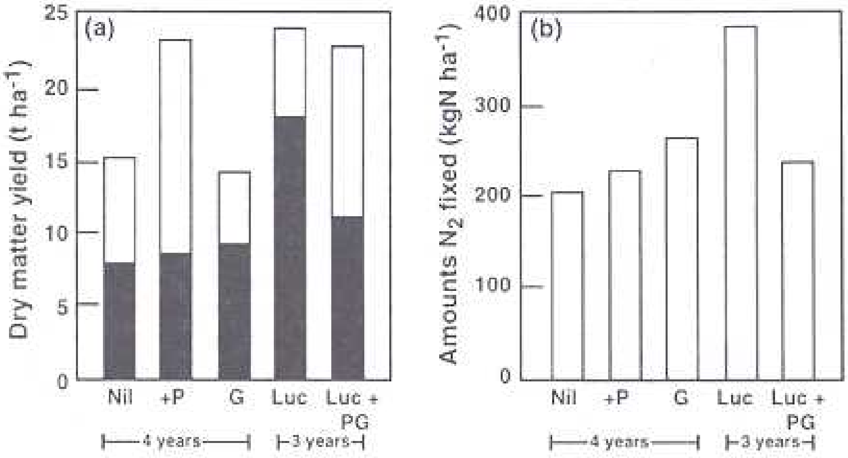

Figure 7. Effect of different management inputs on: (a) cumulative dry matter production by legume (black bars) and non-legume components, and (b) amounts of N_2 fixed over four years of growth of subterranean clover-based annual pastures and three years of pastures containing lucerne (Luc) and /or perennial grasses (PG) at Junee Reefs, NSW. Treatments applied to annual pastures included addition of superphosphate (+P) and grass-removal with herbicide (G). Adapted from Peoples *et al.* (1997).

ture phase might best be enhanced, both pasture productivity and legume content will need to be considered. Provided adequate numbers of effective rhizobia are present in soil, these factors will be the key determinants of the amounts of N_2 fixed. Legume biomass and N_2 fixation inputs can generally be improved with :

- a dense legume stand
- applications of superphosphate or lime to ameliorate nutritional problems
- herbicide applications to remove grasses in annual pastures

Although it is likely that both N_2 fixation inputs and pasture response to these management treatments could be further modified by grazing management through livestock effects on nutrient cycling and soil fertility, pasture productivity and botanical composition (Simpson *et al.* 1974; Steele and Ledgard 1992; Kemp *et al.* 1993; Sanford *et al.* 1995).

High levels of N_2 fixation can be achieved in annual pastures, but there is considerable year-to-year variability due to extreme shifts in the incidence of clover or medic present in pastures. By contrast, pastures containing perennial legume species such as lucerne maintain a more stable legume content and continue to grow and fix N_2 when seasonal rainfall patterns are unsuitable for growth of annuals. Therefore, pastures based on lucerne may have greater potential for consistent growth and N_2 fixation than annual pastures.

Acknowledgments

Data presented in this paper were derived from research supported by the Grains Research & Development Corporation (GRDC projects CSP160, CSP216, CSP132, USC11, DAV67 and DAN157) and Dairy Research and Development Corporation (DRDC project DAV313).

References

Ayres, J.F., McFarlane, J.D., Gilmour, A.R. and McManus, W.R. (1977). Superphosphate requirements of clover-ley farming. I. The effects of topdressing on productivity in the ley phase. *Australian Journal of Agricultural Research*, **28**: 269-285.

Bolger, T.P., Pate, J.S., Unkovich, M.J. and Turner, N.C. (1995). Estimates of seasonal nitrogen fixation of annual subterranean clover-based pastures using the ^{15}N natural abundance technique. *Plant and Soil*, **175**: 57-66.

Brockwell, J., Pilka, A. and Holliday, R.A. (1991) Soil pH is a major determinant of the numbers of naturally occurring *Rhizobium meliloti* in non-cultivated soils in central New South Wales. *Australian Journal of Experimental Agriculture*, **31**: 211-219.

Brockwell, J., Bottomley, P.J. and Thies, J.E. (1995). Manipulation of rhizobia microflora for improving legume productivity and soil fertility: a critical assessment. *Plant and Soil*, **174**: 143-180.

Butler, J.H.A. (1988). Growth and N2 fixation by field grown *Medicago littoralis* in response to added nitrate and competition from *Lolium multiflorum*. *Soil Biology & Biochemistry*, **20**: 863-868.

Carter, E.D., Wolfe, E.C. and Francis, C.M. (1982). Problems with maintaining pastures in the cereal livestock areas of southern Australia. Proceedings of the 2nd Australian Agronomy Conference, Wagga Wagga. pp. 68-100.

Conlan, D.J., Dear, B.S. and Coombes, N.E. (1994) Effect of grazing intensity and number of grazings on herbage production and seed yields of *Trifolium subterraneum*, *Medicago murex*, and *Ornithopus compressus*. *Australian Journal of Experimental Agriculture*, **34**: 181-184.

Dear, B.S., Murray, G.M, Cregan, P.D. and Taylor, P.A. (1993) Comparison of the performance of subterranean clover cultivars in southern New South Wales. 2. Effects of *Phytophora clandestina* and bromoxynil on seedling survival, growth and seed set. *Australian Journal of Experimental Agriculture*, **33**: 591-596.

De Marco, D.G., Li, C.B. and Randall, P.J. (1995) Manganese toxicity in *Trifolium balansae*, *T.respinatum*, *T.subterraneum, Medicago murex, M.polymorpha, M.sativa, Lotus pedunculatus*, and *Ornithopus compressus*: relative tolerance and critical toxicity concentrations. *Australian Journal of Experimental Agriculture*, **35**: 367-374.

Gault, R.R, Peoples, M.B., Turner, G.L., Lilley, D.M., Brockwell, J. and Bergersen, F.J. (1995). Nitrogen fixation by irrigated lucerne during the first three years after establishment. *Australian Journal of Agricultural Research*. **46**: 1401-1425.

Hamblin, A. and Kyneur, G. (1993). 'Trends in Wheat Yields and Soil Fertility in Australia.' (Department of Primary Industries and Energy, Bureau of Resource Sciences. Australian Government Publishing Services, Canberra.).

Hochman, Z., Osborne, G.J., Taylor, P.A. and Cullis, B. (1990). Factors contributing to reduced productivity of subterranean clover (*Trifolium subterranean* L.) pastures on acidic soils. *Australian Journal of Agricultural Research*, **41**: 669-682.

Hossain, S.A., Waring, S.A., Strong, W.M., Dalal, R.C. and Weston, E.J. (1995). Estimates of nitrogen fixation by legumes in alternate cropping systems at Warra, Queensland, using ^{15}N dilution and natural ^{15}N abundance techniques. *Australian Journal of Agricultural Research* **46**: 493-505.

Howieson, J.G. and Ewing, M.A. (1986) Acid tolerance in the *Rhizobium meliloti-Medicago* symbiosis. *Australian Journal of Agricultural Research* **37**: 55-64.

Kemp, D.R. and Doling, P.M. (1991) Species distribution within improved pastures over central NSW in relation to rainfall and altitude. *Australian Journal of Agriculture Research*, **42**: 647-659.

Kemp, D.R., Dowling, P.M. and Michalk, D.L. (1993) Management and legume performance: developing new guidelines for NSW tableland pastures. *Final report to Wool Research & Development Corporation*. pp 64.

Ledgard, S.F. and Steele, K.W. (1992). Biological nitrogen fixation in mixed legume/grass pastures. *Plant and Soil*, **141**: 137-153.

Ledgard, S.F., Steele, K.W. and Saunders, W.M.H. (1982). Effects of cow urine and its major constituents on pasture properties. *New Zealand Journal of Agricultural Research*, **25**: 61-68.

McCown, R.L., Cogle, A.L., Ockwell, A.P. and Reeves, T.G. (1988). Nitrogen supply to cereals in legume ley systems under pressure. *In* 'Advances in Nitrogen Cycling in Agricultural Ecosystems' (Ed J.R.Wilson) pp. 292-314. (C.A.B. International, Wallingford, UK.).

McNeill, A. M., Chunya Zhu and Fillery, I. R. P. (1997). Use of *in situ* ^{15}N-labelling to estimate the total below-ground nitrogen of pasture legumes in intact soil-plant systems. *Australian Journal of Agricultural Research*, **48**, 295-304.

Peoples, M.B., Herridge, D.F. and Ladha, J.K. (1995a). Biological nitrogen fixation: an efficient source of nitrogen for sustainable agricultural production? *Plant and Soil*, **174**: 3-28.

Peoples, M.B., Ladha, J.K. and Herridge, D.F. (1995b). Enhancing legume N2 fixation through plant and soil management. *Plant and Soil*, **174**: 83-101.

Peoples, M.B., Lilley, D.M., Burnett, V.F., Ridley, A.M. and Garden, D.L. (1995c). Effects of surface application of lime and superphosphate to acid soils on growth and N2 fixation by subterranean clover in mixed pasture swards. *Soil Biology & Biochemistry*, **27**, 663-671.

Peoples, M.B., Gault, R.R., Scammell, G.J., Dear, B.S., Virgona, J., Sandral, G.A., Paul, J., Wolfe, E.C. and Angus, J.F. (1997). The effect of pasture management on the contributions of fixed N to the N-economy of ley-farming systems. *Australian Journal of Agricultural Research (in press)*.

Quigley, P., Riffkin, P. and Peoples, M (1996) Limitations to nitrogen fixation in white clover dairy pastures. *In* : 'Proceedings of the 8th Australian Agronomy Conference'. (Ed. M Asghar). Toowoomba. Australian Society of Agronomy, pp 702.

Richardson, A.E. and Simpson, R.J. (1988) Enumeration and distribution of *Rhizobium trifoli* under a subterranean clover-based pasture growing in an acid soil. *Soil Biology & Biochemistry*, **20**: 431-438.

Rossiter, R.C. (1966). Ecology of the Mediterranean annual-type pasture. *Advances of Agronomy*, **18**:1-56.

Sanford, P., Pate, J.S., Unkovich, M.J. (1994). A survey of proportional dependence of subterranean clover and other pasture legumes on N2 fixation in south-west Australia utilizing 15N natural abundance. *Australian Journal of Agricultural Research*, **45**: 165-181.

Sanford, P., Pate, J.S., Unkovich, M.J. and Thompson, A.N. (1995). Nitrogen fixation in grazed and ungrazed subterranean clover pasture in south-west Australia assessed by the 15N natural abundance technique. *Australian Journal of Agricultural Research*, **46**: 1427-1443.

Simpson, J.R., Bromfield, S.M. and McKinney, G.T. (1974). Effects of management on soil fertility under pasture. I. Influence of experimental grazing and fertilizer systems on growth, composition and nutrient status of the pasture. *Australian Journal of Experimental Agriculture and Animal Husbandry*, **14**: 470-478.

Thomas, R.J. (1995). Role of legumes in providing N for sustainable tropical pasture systems. *Plant and Soil*, **174**: 103-118.

Unkovich, M. J., Pate, J. S., and Sanford P. (1997). Nitrogen fixation by annual legumes in Australian mediterranean agriculture. *Australian Journal of Agricultural Research*, **48**, 267-293.

Wilson, A.D. and Simpson, R.J. (1993). The pasture resource base: status and issues. *In* 'Pasture Management: Technology for the 21st Century.' (Eds D.R. Kemp and D.L. Michalk) pp. 1-25 (*CSIRO*: Melbourne).