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Estimates of Nitrogen Fixations by Legumes in Alternate Cropping Systems at Warra, Queensland, using Enriched -^{15}N Dilution and Natural ^{15}N Abundance Techniques

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Abstract

Nitrogen fixation was measured using two isotopic techniques over 2 years as part of a long-term field experiment established to test alternative management strategies for restoring fertility in a vertisol at Warra, Southern Queensland. Treatments containing legumes were: grass- legume ley (purple pigeon grass and Rhodes grass, lucerne and annual medics) for 4 years followed by 4 years of wheat; a 2-year rotation of lucerne and wheat; a 2-year rotation of medic and wheat; and a 2-year rotation of chickpea and wheat.

For the enriched-^{15}N procedure, the proportion of N derived from air (% Ndfa) for the grass-legume and lucerne and medic leys ranged from 67 to 97%, and averaged 85%, with little evidence for effects of season, pasture establishment, time or species. The % Ndfa for chickpea was significantly lower (62%). Values for the natural abundance ^{15}N procedure were mostly lower and more variable than for the enriched method, ranging from 62 to 91% for the grass-legume, lucerne and medic leys, and averaged 76%. It was concluded that the enriched procedure provided more reliable estimates of N_2 fixed by the legumes.

N_2 fixation measured by the enriched-^{15}N dilution method in the grass-legume ley averaged 80 kg N ha^{-1} year^{-1} during 2 years. A similar amount of N was fixed by the lucerne ley during 1 year (83 kg N ha^{-1} year^{-1}), but medic ley fixed less (56 kg N ha^{-1} year^{-1}). The amount of N_2 fixed by chickpea was 72 kg N ha^{-1}year^{-1}. The dry matter yield of the legumes in leys, mainly lucerne, was closely associated with the amount of N_2 fixed, with a value of 28 kg of N_2 fixed for each tonne of dry matter produced.

Keywords: nitrogen, fixation, legumes, cropping systems.

Introduction

In the major cereal-growing areas of southern Queensland, farming systems, in particular continuous cropping, have caused severe depletion of organic carbon and nitrogen on a range of vertisols (Dalal and Mayer 1986). This has led to decreasing grain protein concentrations and yield (Dalal *et al.* 1991). As a result, nitrogen fertilizer use has increased, but there is currently much interest in rotational cropping systems involving legumes as a source of nitrogen.

In southern Australia, cropping systems with legumes have been successfully developed and applied (Donald 1965), but in the cereal-growing areas of southern Queensland much less attention has been given to this approach because of initial high soil fertility and a shorter history of cropping. The benefits of pasture

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legumes, particularly lucerne, have been shown by workers such as Littler and Whitehouse (1987) and of grain legumes by Doughton (1988). However, there has been no comparison made of the nitrogen fixation by legumes in rotation at the one site.

There are few quantitative data available for nitrogen fixation in southern Queensland apart from the work of Doughton (1988) with grain legumes. Traditionally, N_2 fixation in the field has been estimated using the N difference procedure in which a comparison is made of the N yields of fixing and non-fixing plants or the crop assay methods. Recently, more quantitative isotopic procedures such as the enriched-^{15}N dilution and ^{15}N natural abundance methods (Ledgard and Peoples 1988) have been applied to measure N_2 fixation over time in the field. Since no method is clearly superior, there is an advantage in using more than one procedure.

The experiments reported in this paper are part of a wider study, established by Queensland Department of Primary Industries (Dalal *et al.* 1991, 1994), to test alternate management strategies for their effectiveness in restoring or maintaining soil fertility in a fertility-depleted vertisol at Warra, southern Queensland. The objective of work reported in this paper was to estimate N_2 fixation, using the enriched-^{15}N dilution method and the ^{15}N natural abundance method, in four rotations containing legumes.

Materials and Methods

Experimental Site

The site is located at Warra in southern Queensland and it originally carried brigalow (*Acacia harpophylla*) vegetation. The climate is semi-arid and subtropical with a mean annual rainfall of 630 mm and mean annual temperature of 19·4°C. The site had been continuously cropped for 50 years at the start of the experiment in 1986, mainly for cereal grains, and it was selected because soil fertility had been depleted especially in regard to nitrogen and organic matter (Dalal and Mayer 1986).

The soil is a deep and uniformly textured clay (Typic Chromustert, Soil Survey Staff 1975). Soil is alkaline (pH 8·2) at the surface (0–10 cm) and to a depth of about 1 m, but beyond this becomes strongly acid (pH 5·0). Organic carbon and total nitrogen in the soil surface are now very low, 0·68% and 0·077% respectively (Dalal *et al* 1991).

A Summary of the Warra Experiments

A description of the Warra experiment including full treatment details is given by Dalal *et al.* (1991, 1994). Treatments reported in this study are shown in Table 1. There were four rotations containing legumes and continuous wheat cropping: (1) grass-legume ley (purple pigeon grass and Rhodes grass, lucerne and annual medics) for 4 years followed by 4 years of wheat; (2) a 2-year rotation of wheat (lucerne undersown) and lucerne; (3) a 2-year rotation of wheat (medics undersown first year) and medic; (4) chickpea and wheat in a 2-year rotation; and (5) continuous wheat (control).

Procedure for the Natural Abundance ^{15}N Method

Field Study

Five microplots (1m × 1 m) were established in June 1988 in each plot and fenced to exclude grazing animals. Plant samples from each microplot were harvested at a height of 10 cm in September and December 1988 and March and June 1989. Samples were separated into different species and bulked for determination of plant N concentration and N isotope ratio. Total N was determined by a procedure similar to that described by Bremner (1965), but with a stainless steel modification of the traditional glass assembly, using procedures to minimize cross-contamination during both distillation and titration (Saffigna and Waring 1977). The

Table 1. Some treatment sequences from the Warra Experiment

Treatment	Calendar years						
	1986	1987	1988	1989	1990	1991	1992
Grass-legume 86[C]	GL[A]	GL	GL	GL	W	[B]	W
Grass-legume 87		GL	GL	GL	GL	–	W
Grass-legume 88		W	GL	GL	GL	GL	W
Lucerne 88		W_L	L	W_L	L	–	L
Lucerne 89		W	W_L	L	W_L	L	W_L
Medic 88		W_M	M	W	M	–	M
Medic 89		W	W_M	M	W	M	W
Chickpea 88		W	CP	W	CP	–	W
Continuous wheat		W	W	W	W	–	W

[A] GL, grass-legume pasture consisting of purple pigeon grass (*Setaria incrassata* Stapf cv. Inverell); Rhodes grass (*Chloris guayana.* Kunth cv. Katambora); medic (a mixture of snail, *Medicago scutellata* L. Mill. cvv. Sava and Kelson, barrel-*M. truncatula* Gaertn cvv. Jemalong, Cyprus, Paraggio and Sephi); lucerne, *M. sativa* L. cv. Trifecta; W_L, lucerne undersown with wheat; W_M, medic undersown with wheat; self-regenerating medic; L, lucerne ley; M, medic ley; CP, chickpea; W, wheat (cv. Hartog).
[B] No wheat crop was sown in 1986 and 1991 due to drought.
[C] Treatments are identified with the first year of the ley or chickpea crop.

distillate was acidified to pH 3·0, dried at 90°C and nitrogen isotope ratio analysis performed on a mass spectrometer (Micromass 602E or AE1 Isotope MS-20) using the procedures described by Ross and Martin (1970). Nitrogen fixation was calculated as described by Ledgard and Peoples (1988). For the calculation of the proportion of legume N derived from air (% Ndfa), the reference plants used were grass for the legumes in grass-legume leys, wheat for lucerne and medic where legumes were undersown with wheat, and also for chickpeas and milk thistle (*Sonchus oleraceous*) for lucerne and medic swards. To allow consistent comparisons of N_2 fixed between the isotopic methods, yield data from the enriched-^{15}N microplots were used in calculating the amount of N_2 fixed.

Glasshouse determination of the $\delta^{15}N$ of fixed N_2 in the legumes (B)

Seeds of lucerne (cv. Trifecta), snail medic (cv. Sava) and barrel medic (cv. Paraggio) were surface-sterilized, washed and germinated on 1% water agar (sterile and N-free) and transplanted into Leonard jars containing 900 mL of N-free medium. For the inoculated treatment, a water suspension of *Rhizobium meliloti* strain CC169 at 2 mL jar^{-1} (approximately 10^8 cells mL^{-1}) was used. Inoculated jars contained 4 plants and uninoculated 6–8 plants, maintained in a controlled temperature glasshouse between 15 and 20°C (mean 17°C).

Plants were harvested after 45 days, separated into shoots and roots (including nodules), dried and ground to <1 mm. The $\delta^{15}N$ was calculated as parts per thousand with reference to atmospheric N_2 (Shearer and Kohl 1986). B values were calculated as described in Table 2.

Procedure for the Enriched-^{15}N Dilution Method

Microplots (1 m × 1 m) were confined by a 15 cm galvanized steel sheet forced to a depth of 10 cm into the soil (4 per treatment). Fences were placed around the microplots to prevent grazing. Where necessary, pasture plots were trimmed to ground level, and on 28 June 1988, a single application of 50 mg ^{15}N m^{-2} as KNO_3 (99 atom % excess ^{15}N) and 5 g sucrose m^{-2} in 100 mL of water was applied to the soil surface. To ensure a uniform application, a grid with a cell of 20 cm x 20 cm was superimposed and the solution applied with a syringe using 4 mL applications to each cell. The ^{15}N application was followed by a watering can wash equivalent to 5 mm of rainfall.

Microplots were harvested at ground level from September 1988 to September 1990 at approximately 3-monthly intervals in the grass-legume leys and at varying intervals to October 1989 in the remaining plots. Plant samples were separated into different species, dried at 60

to 70°C and ground to < 1 mm. Analysis for ^{15}N in the plant materials was done using a Micromass 602E isotope ratio mass spectrometer. For the calculation of % Ndfa, the reference plants used were the same species as for the natural abundance ^{15}N method.

Results

δ^{15} N of Fixed N_2 in Legumes

For the inoculated plants, the shoot N yields were much higher than for the uninoculated plants (Table 2). Shoot tissues of each legume showed more negative $\delta^{15}N$ values for the inoculated than for the uninoculated plants. B values of shoots were also negative with lucerne more negative than the medics (Table 2).

Table 2. Nitrogen accumulation and $\delta^{15}N$ of fixed N_2 (B) in lucerne and medics

Plant species	Inoculated N (mg/4P[B])	Inoculated $\delta^{15}N$ (‰)	Uninoculated N (mg/4P)	Uninoculated $\delta^{15}N$ (‰)	B[A] value
Lucerne	3·66	−3·01	0·36	−1·46	−3·18
Snail medic	13·67	−1·72	1·86	−0·82	−1·86
Barrel medic	6·03	−2·27	0·60	−1·57	−2·25
Mean	–	–	–	–	−2·43

[A] $B = [\text{Total N} \times \delta^{15}N_{\text{Inoculated plant}}] - [\text{Total N} \times \delta^{15}N_{\text{Uninoculated plant}}]/[\text{Total N}_{\text{Inoculated plant}} - \text{Total N}_{\text{Uninoculatedplant}}]$.
[B] 4P = 4 plants.

Field Studies on N_2 Fixation

Dry matter and nitrogen yield

Dry matter and N yields of mixed pastures for the enriched ^{15}N microplots over two seasons are given in Table 3. Rainfall was the main factor causing high variability in yields of individual cuts and accounted for the lower dry matter yields in the second season. Grass yields tended to be higher overall than for legumes, particularly in the autumn, whereas legumes were dominant in the spring. The age of the pasture did not substantially affect yields except in the first season when grass yield was lower for pasture established in 1987 than for pastures established in 1986 or 1988. Seasonal nitrogen yields showed a similar pattern to dry matter yields, but values were mostly higher for legumes due to their higher N concentration.

In 1988 legume dry matter yields were similar for the lucerne and medic leys, but N yield was higher for lucerne (Table 4). In 1989 lucerne dry matter and N yields were similar to those of 1988, but medic yields were much lower. Dry matter yields for legume undersown with wheat in 1988 were higher for medic (Medic 89) than lucerne (Lucerne 89) and this was reflected in N yields. The N yield for chickpea in 1988 was similar to that for lucerne.

Proportion of N derived from air (% Ndfa)

For the enriched-^{15}N dilution method, the % Ndfa for the legume in grass-legume leys established in 1986, 1987 and 1988 ranged from 67 to 97% (Fig. 1*a*). There was no consistent seasonal trend, although the mean values for these three leys were lowest in March 1989 and highest in September 1989. Differences between

Table 3. Total dry matter and N yields of legumes and grasses grown in grass-legume leys from 1988 to 1990

Treatment	Total dry matter (kg ha^{-1} year^{-1})		Total N (kg ha^{-1} year^{-1})	
	Legume	Grass	Legume	Grass
	September 1988–June 1989			
Grass-legume 86	3169	6340	80	73
Grass-legume 87	3681	4674	108	52
Grass-legume 88	2942	7797	80	102
l.s.d. ($P < 0{\cdot}05$)	*n.s.*	1433	23	25
	September 1989–June 1990			
Grass-legume 87	3441	2873	101	27
Grass-legume 88	2426	3817	69	36
l.s.d. ($P < 0{\cdot}05$)	*n.s.*	*n.s.*	*n.s.*	*n.s.*

Table 4. Total dry matter and N yields in rotation containing lucerne, medics, chickpeas and continuous wheat in 1988 and 1989

Treatment	Dry matter yield (kg ha^{-1} year^{-1})		Nitrogen yield (kg ha^{-1} year^{-1})	
	Legume	Non-legume	Legume	Non-legume
		1988		
Lucerne 88	3591	49[A]	103	1
Medic 88	3348	30[A]	72	1
Lucerne 89	296	5703[B]	9	38
Medic 89	1256	6422[B]	28	49
Chickpea 88	5272	–	102	–
Continuous what	–	7270[B]	–	56
l.s.d. ($P < 0{\cdot}05$)	799	1838	17	20
		1989		
Lucerne 89	2922	102[A]	99	3
Medic 89	993	1178[A]	31	32
l.s.d. ($P < 0{\cdot}05$)	847	1046	25	27

[A] Weed. [B] Wheat.

leys were usually relatively small except in March 1989 when grass-legume 86 and grass-legume 88 were at their lowest (67% and 71%) and grass-legume 87 remained at a relatively high level. Values of % Ndfa using the enriched-^{15}N dilution method ranged from 78% to 96% for the lucerne and medic leys (Fig. 1*b*). Values for chickpea were much lower (62%) and were similar when determined at flowering and maturity.

δ^{15}N values of the reference plant (available soil N) ranged from 7·4‰ to 11‰ and those of the legume from 0·6‰ to 1·9‰ (Table 5). Using the natural abundance ^{15}N method and a mean B value of −2·43 (Table 2), the % Ndfa for the legumes in grass-legume leys for September 1988 cuts ranged from 62% to 71% (Table 5) and showed little effects of sampling time or pasture age during 1988 and 1989 samplings (data not shown). Values for lucerne and medic were

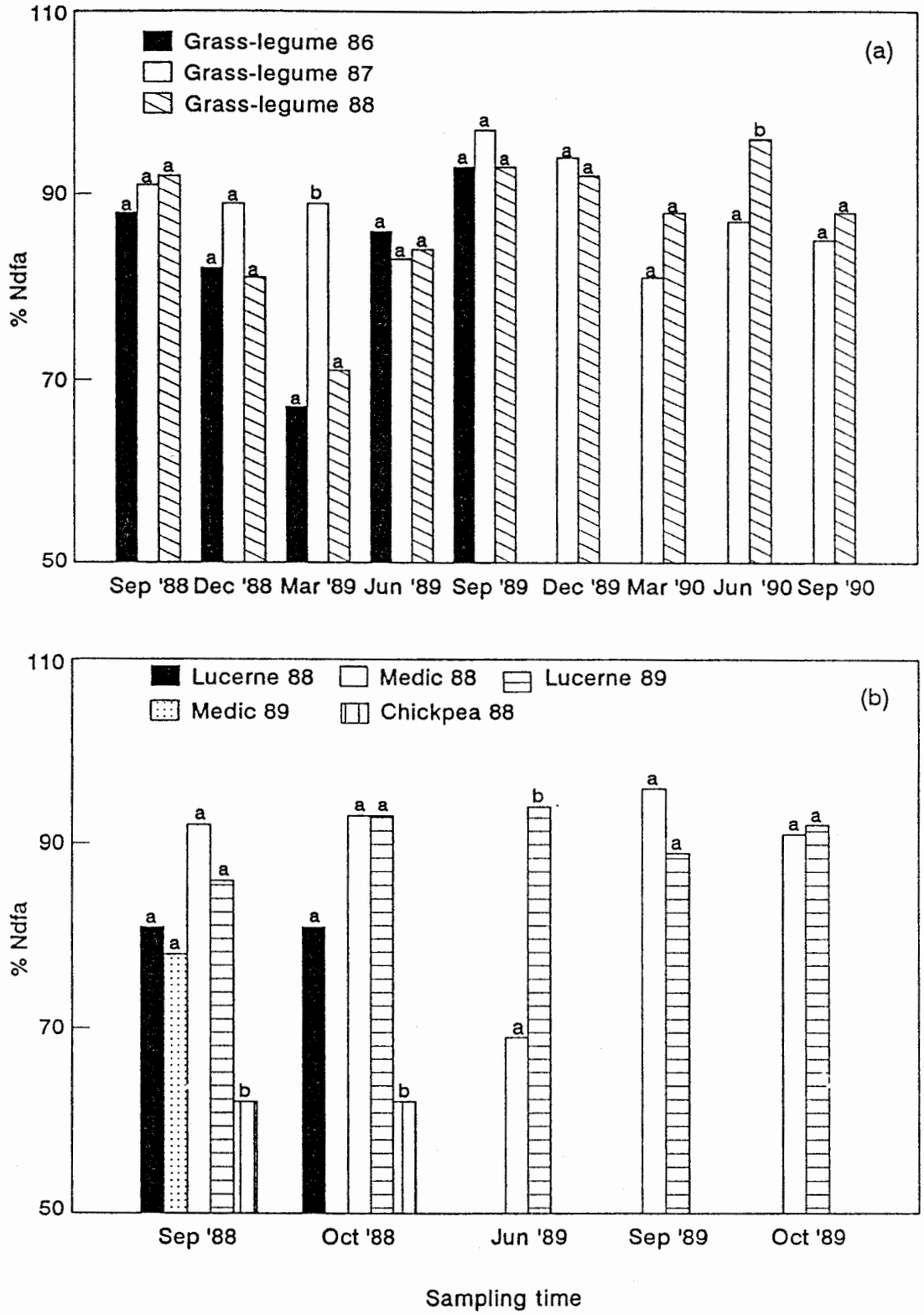

Fig. 1. The percentage of N derived from air (% Ndfa) for (*a*) legumes grown in grass-legume leys and (*b*) lucerne, medic and chickpeas grown in rotations at different sampling times using the enriched-^{15}N dilution method. The letters not in common denote significant difference ($P < 0{\cdot}05$) within any sampling period.

69% (Table 5) and ranged from 72% to 82% during 1988 and 1989 period (data not shown). Comparing the two isotopic methods (Fig. 2), values for % Ndfa were mostly lower for the natural abundance method. Mean values ($n = 16$) for comparable sampling times and species were 85% for the enriched dilution method and 76% for the natural abundance method. For the September 1988 sampling of grass-legume leys, mean values of % Ndfa were 90% and 67% respectively; but % Ndfa by the latter method was 85% if a B value of zero was used.

Table 5. δ^{15}N values of reference plant (available soil N) and legumes and the percentage of nitrogen derived from air (% Ndfa) by legumes using natural ^{15}N abundance method in September 1988

Treatment	δ^{15}N (‰)		%Ndfa
	Reference	Legume	
Grass-legume 86	9·159	0·997	70·4
Grass-legume 87	8·995	1·925	61·9
Grass-legume 88	11·070	1·529	70·7
Lucerne 88	9·664	1·379	68·5
Medic 88	7·371	0·628	68·8

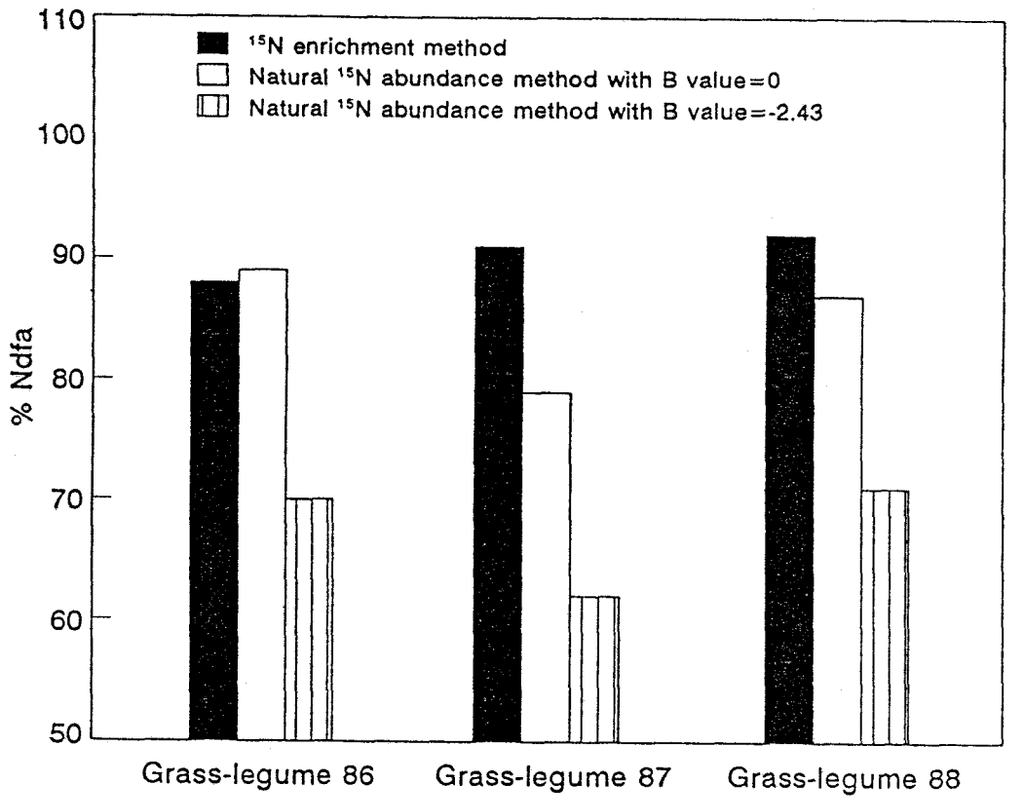

Fig. 2. Comparison of ^{15}N-enrichment and natural ^{15}N abundance methods to estimate N derived from air (% Ndfa) by legumes in grass-legume leys at September 1988 sampling.

Quantity of N_2 Fixed

Seasonal values for N_2 fixed in the grass-legume leys using the enriched-^{15}N dilution method were extremely variable (Fig. 3*a*), reflecting the different conditions affecting legume growth, prior to each cut. The quantity of N_2 fixed was almost

always highest for the pasture established in 1987. Annual N_2 fixation ranged from 64 to 98 kg ha^{-1} N $year^{-1}$ (Fig. 3a) with higher values in both years for the pasture established in 1987 and similar values for pastures established in 1986 and 1988. For the lucerne and medic leys in September 1988, similar amounts of N_2 were fixed with much lower values where the legume was undersown with wheat (Fig. 3*b*). Cumulative fixation by lucerne was significantly higher than for medic in both years (Fig. 3*b*, Table 6). Fixation by chickpea was intermediate between that for lucerne and medic (Table 6) and higher at maturity than flowering, although the difference was not significant.

Annual values for N_2 fixed using the natural abundance ^{15}N method were consistently lower than for the enriched-^{15}N dilution method (Table 6). The best comparison of the two procedures is probably that for the grass-legume leys for the September 1988 to June 1989 period where the mean fixation over the three leys was estimated to be 80 kg ha^{-1} for the enriched procedure and 62 kg ha^{-1} for the natural abundance procedure. The two methods could not be rigorously compared for lucerne or medic leys because of incomplete comparisons across sampling times. The N difference method could only be used to estimate N_2 fixation by chickpea which gave a low value of 46 kg ha^{-1} (chickpea N yield, 102 kg N ha^{-1}; wheat N yield, 56 kg N ha^{-1}; Table 4) compared with 72 kg ha^{-1} for the enriched-^{15}N dilution method.

Discussion

Knowledge of the $\delta^{15}N$ of fixed N_2 in legume (B) is an essential requirement for quantitative assessment of N_2 fixation using the natural ^{15}N abundance method. The negative values obtained (Table 2) are expected as a result of isotopic discrimination during fixation (Shearer and Kohl 1986). There do not appear to be reports in the literature regarding isotopic fractionation in the medics. For lucerne, values for $\delta^{15}N$ of fixed N_2 were more negative in the present study than others have reported: $-0{\cdot}92‰$ by Yoneyama *et al.* (1986) for the whole plant and $+3{\cdot}56‰$ by Turner and Bergersen (1983) for shoots. Steele *et al.* (1983) and Yoneyama *et al.* (1986) concluded that host plants and rhizobium strains can influence isotopic fractionation during N_2-fixation. Ledgard (1989) found for white clover much larger fractionation for a field isolate than for the common inoculate strain PDD2668, so that the difference between our B value and those reported by others for lucerne may be associated with different rhizobial strains.

Comparison of % Ndfa for the two isotopic methods at the same sampling time showed consistently lower values for the natural ^{15}N abundance procedure (Fig. 2), although $\delta^{15}N$ values of the reference plant exceeded 6‰ (Table 5), a value below which standard error of % Ndfa rises sharply (Ledgard and Peoples 1988). Conversely, Bergersen and Turner (1983) obtained good agreement between these methods in a clover-perennial rye grass sward, although the initial two harvests gave lower values by the natural ^{15}N abundance method. Ledgard *et al.* (1985) evaluated the two methods for lucerne and clover in grass-legume pastures and found for lucerne lower but not significantly different values for the natural ^{15}N abundance procedure. For clover an opposite trend was observed. The difference between the two methods found in the present study is unrelated to the reference plant since the same plant species was used for both methods. It seems likely that the B values determined in the glasshouse may not be appropriate for the

field conditions, especially if there was a difference in rhizobial strain. This was apparent when % Ndfa values were essentially similar by both methods when B value was taken as zero. Shearer and Kohl (1986) have found B values can be either negative or positive. Another difficulty is the extreme precision required

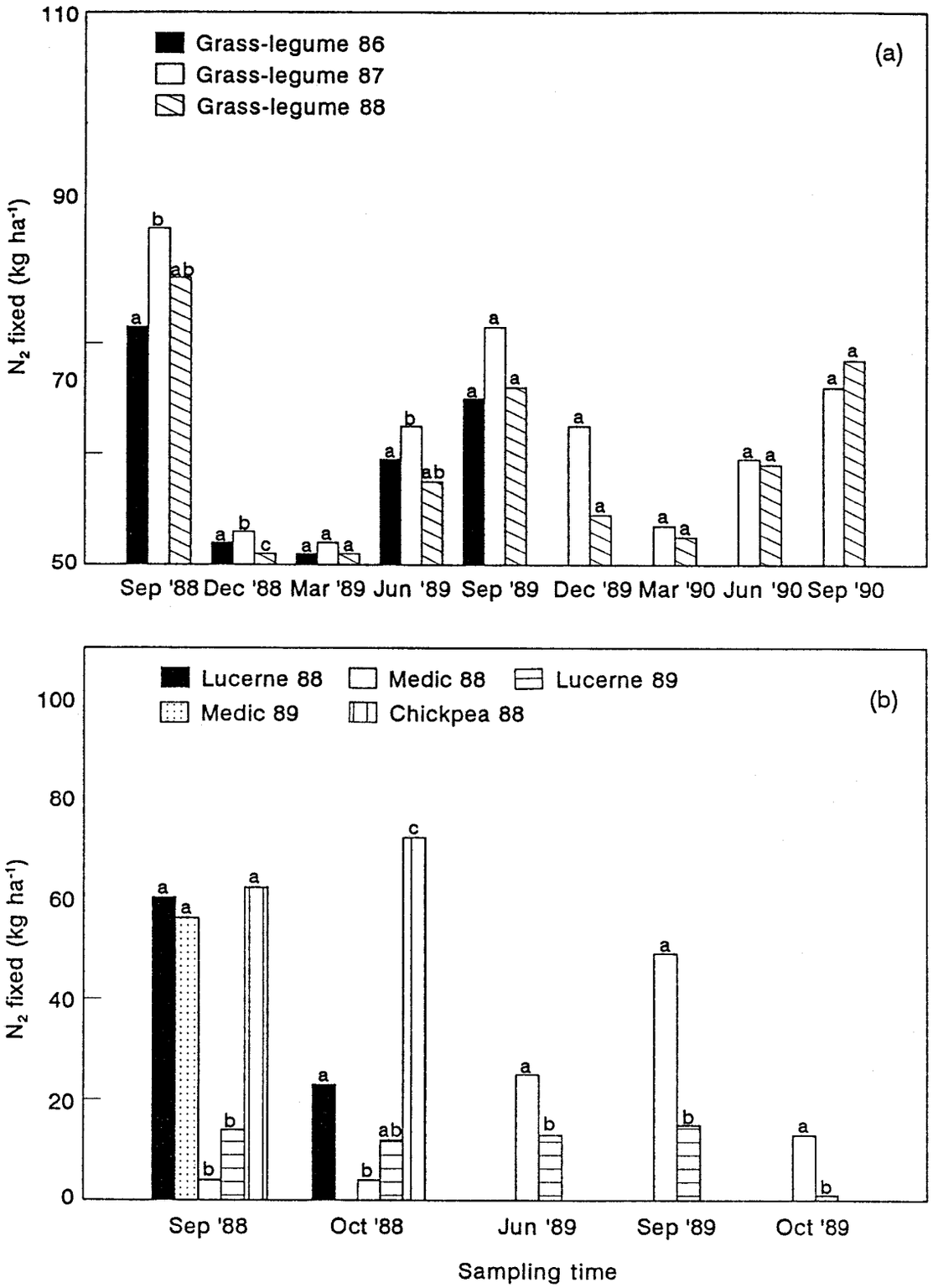

Fig. 3. The amount of N_2 fixed by legumes in (*a*) grass-legume leys, and (*b*) lucerne, medic and chickpeas at different sampling times using the enriched-^{15}N dilution method. The letters not in common denote significant difference ($P < 0{\cdot}05$) within any sampling period.

in measuring isotopic enrichment with the natural ^{15}N abundance method. It therefore seems probable that the enriched-^{15}N dilution method provides better estimates of % Ndfa than the natural ^{15}N abundance method, a view supported by Shearer and Kohl (1989) who indicated that the natural abundance method gave only semi-quantitative estimates of % Ndfa.

Table 6. Total amount of N_2 fixed by grass-legume leys, lucerne and medic leys and chickpea at different sampling periods using the enriched-^{15}N dilution and natural abundance methods

Treatment	Sampling period	N_2 fixed (kg ha^{-1} year^{-1})	
		Enriched-^{15}N method	Natural abundance ^{15}N method
Grass-legume 86	Sept. '88–June '89	69	61
Grass-legume 87	Sept. '88–June '89	98 (94)[A]	69
Grass-legume 88	Sept. '88–June '89	72 (64)	57
Lucerne 88	Sept. '88–Oct. '88	83	60
Lucerne 89	June '89–Oct. '89	91·5	n.d.[B]
Medic 88	Sept. '88	56	50
Medic 89	June '89–Oct. '89	28·4	n.d.[B]
Chickpea 88			
Flowering	Sept. '88	62	
Maturity	Oct. '88	72	
l.s.d. ($P < 0·05$)		23	16

[A] Figures in parentheses are corresponding values for Sept. '89–June '90 period.
[B] n.d., not determined.

Accepting that the enriched-^{15}N dilution method gives more realistic values for % Ndfa, the estimates obtained in this study for legumes in grass-legume leys or for single legume swards (Fig. 1), are generally of a similar order to those obtained by other workers, for example Gault *et al.* (1991) for lucerne at Gininderra in the ACT. There was no consistent seasonal effect evident for the legumes in the grass-legume leys, although there were lower values in March 1989 for pasture established in 1986 and 1988 (Fig. 1). These results differed from those of Heichel *et al.* (1984) who found a decline in % Ndfa in autumn for lucerne. The much lower % Ndfa for chickpea than for the other legumes and legume systems is consistent with results from other workers (Giller *et al.* 1988) and is probably due to the inhibitory effect of fallow-accumulated nitrate-nitrogen (Doughton *et al.* 1993). It is also possible that differences in the rooting characteristics of wheat, the reference plant used, may have affected the calculation of % Ndfa since such effects have been shown by Witty (1983). Similar estimates of N_2 fixation by lucerne and medic were obtained using grass, wheat, or milk thistle as the reference plant, supporting the results of Boller and Nosberger (1988) who observed less effect of reference plant on % Ndfa than earlier believed.

The quantity of N_2 fixed by leys, using the enriched-^{15}N dilution method, varied considerably for individual harvests and also annually (Fig. 3, Table 5). In view of the relatively consistent seasonal values for % Ndfa, the main determinant of the amount of N_2 fixed was the yield of legume dry matter (Tables 3 and 4), evidenced by the high correlation ($r = 0·98$, Fig. 4) between legume dry matter and N_2 fixed by legumes, mainly lucerne over the 2 year period. The amount of N_2 fixed by the legumes was 28·2 kg N for each tonne of dry matter produced. The much higher legume fixation in the mixed grass-legume leys for the June

and September harvests (a mean of 64·7 kg ha^{-1} $year^{-1}$ compared with 15·0 kg ha^{-1} $year^{-1}$ for the December and March harvests) is clearly due to the better legume growth during these periods. The unexpectedly low value for medic in the September 1989 harvest (Fig. 3*b*) is probably due to severe defoliation at sampling in June 1989 which retarded subsequent regrowth.

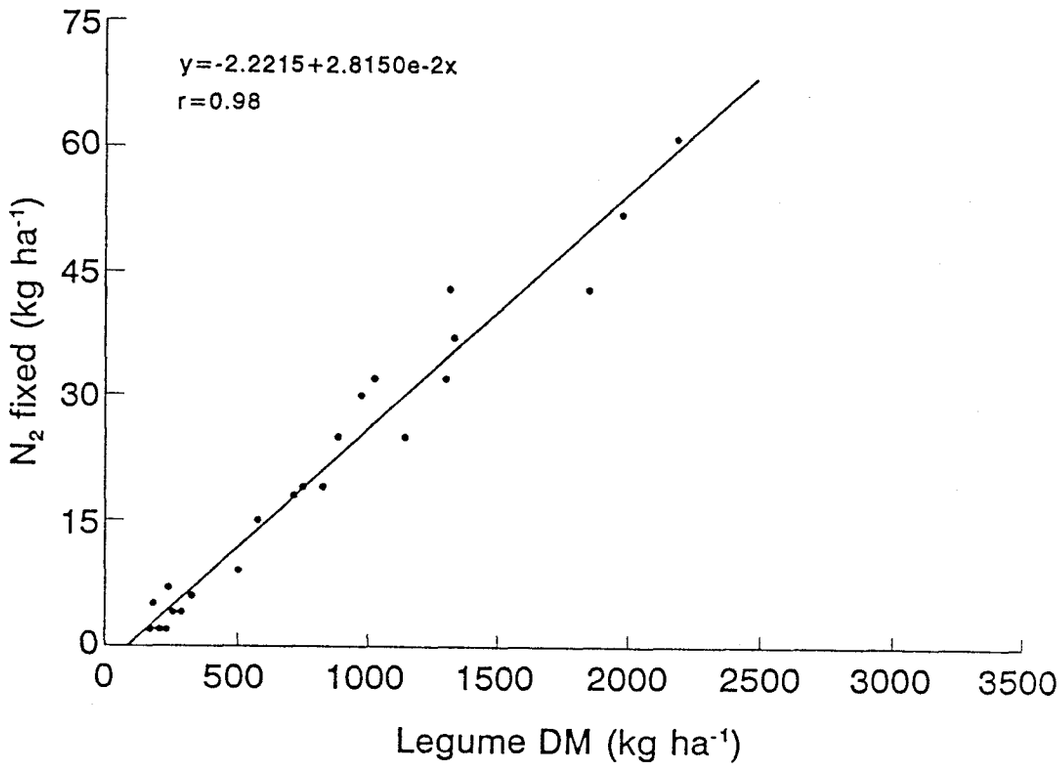

Fig. 4. Relationship between dry matter yields and N_2 fixed by legumes, mainly lucerne in grass-legume leys, at different sampling times over 2 years using the enriched-^{15}N dilution method.

Annual values of N_2 fixation (Table 5), using the enriched ^{15}N dilution method, show for the grass-legume leys higher fixation in both years for the pasture established in 1987 which was clearly related to the higher legume yields for that pasture (Table 3). Annual N_2 fixation was higher for lucerne than for medic most probably due to a longer growth period throughout the year for the perennial species. A valid comparison of annual N_2 fixation for lucerne compared with legumes in a grass-legume ley is difficult, but results indicate similar levels of fixation because lucerne was the dominant legume in the mixed pasture. The amounts of N_2 fixation obtained in this study for lucerne and for legumes in grass-legume leys are in a similar range to those reported elsewhere (West and Wedin 1985), but valid comparisons require similar environmental conditions in view of the over-riding effect of legume yield on N_2 fixation. Fixation by chickpea at maturity was of the same order as that for the other legume treatments and within the range of 33–97 kg ha^{-1} reported by Doughton *et al.* (1993).

The technique of using successive cuts of legume tops in the measurement of N_2 fixation is commonly used, but may give an underestimate due to fixed nitrogen occurring in legume roots, stubble or litter and a possible slow transfer to associated non-legume plants (Simpson 1976).

The levels of N_2 fixation observed in this study indicate that a sustainable crop rotation based on either lucerne or grass-legume leys, may be developed at Warra in southern Queensland. These may provide more nitrogen for the succeeding crops than medic or chickpeas.

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