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**EFFECTS OF N FIXATION BY UNDERSTOREY  
ON NITROGEN AVAILABILITY AFTER THREE  
YEARS OF INTERSPECIFIC COMPETITION**

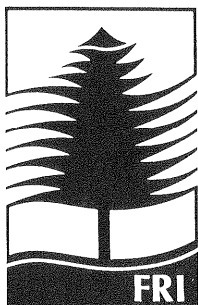
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**NEW ZEALAND FOREST SITE MANAGEMENT  
COOPERATIVE**



**NEW ZEALAND  
FOREST RESEARCH INSTITUTE  
LIMITED**

**Effects of N fixation by understorey on nitrogen availability after three years of interspecific competition**

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## Executive Summary

Foliage samples of radiata pine and legumes growing together under contrasting conditions of water and nutrient supply were collected in February 1995 and analysed for their total nitrogen content and  $^{15}\text{N}$  natural abundance ( $^{15}\text{N}$ ). Values of  $^{15}\text{N}$  for radiata pine growing with either herbaceous grasses or Maku lotus did not increase when fertiliser was applied, but did increase significantly following fertiliser application in the presence of a broom or gorse understorey or where there was no understorey. The lack of change in  $^{15}\text{N}$  values of radiata pine growing with herbaceous understorey species is attributed to reduction in soil N availability to the tree caused by competition with the understorey for N. This effect was greatest for grasses and occurred to a lesser extent for Maku lotus.

Legumes differed in their capacity to fix N, but this aspect of the study needs further investigation. Differences in  $^{15}\text{N}$  between N fixers and pine were small and  $^{15}\text{N}$  values were negative. The resulting estimates of the proportion of N fixed by legumes are therefore considered inaccurate.

Legumes (gorse, broom, and Maku lotus) did not increase transformations of N and N availability as much as the fertiliser N application because the legume inputs of N had less effect on radiata pine foliage nitrogen content and  $^{15}\text{N}$  natural abundance ( $^{15}\text{N}$ ) values than fertiliser N.

## Introduction

Within New Zealand plantation forests it is common to find in the understorey either woody N-fixers or herbaceous N-fixers amongst other herbs, grasses and woody species. While the effect of these competing understorey plants on tree growth is obvious (Clinton *et al.* 1996), little is known about the biological nature of these interactions. Studies of physiological performance, water use efficiency, nutrient status, and/or nutrient availability should help to explain differences in growth due to competition, especially differences due to the presence of particular understorey types (Nambiar, 1991). Of particular interest to forest managers is the contribution to radiata pine of N fixed by legumes and how this might compare with N fertiliser application.

N transformations and availability in forests have been assessed by measuring differences in the natural abundance of the stable isotope of N ( $^{15}\text{N}$ ) in the foliage of non N-fixing plants (Vitousek *et al.*, 1989; Garten 1993; Garten and Van Miegroet 1994; Schulze *et al.* 1994). Although the technique has not been applied to competition studies before, the application of  $^{15}\text{N}$  natural abundance techniques to studying the interactions between understorey species and crop trees in plantation forests should provide useful information to managers, particularly if the interactions influence N availability and cycling.

The theory behind the use of  $^{15}\text{N}$  in competition studies relies on the phenomenon of isotopic fractionation of N into  $^{14}\text{N}$  and  $^{15}\text{N}$  which commonly occurs during N transformations such as immobilisation/mineralisation, nitrification, and denitrification. The extent of these processes are likely to be affected by the availability of N and by competition for N between plant species (as well as between plants, microbes and the soil processes themselves). The net effect of this fractionation between the two N isotopes is detected as a small increase in the  $^{15}\text{N}$  natural abundance ( $^{15}\text{N}$ ) of soil compared with the natural abundance of atmospheric  $\text{N}_2$  (0.3663 atom %  $^{15}\text{N}$ ) (Shearer and Kohl 1986). The increase in ( $^{15}\text{N}$ ) is due to discrimination between  $^{14}\text{N}$  and  $^{15}\text{N}$  during N transformations with  $^{14}\text{N}$  being favoured during N transformations such as immobilisation/mineralisation, nitrification, and denitrification. Changes in  $^{15}\text{N}$  of soil are measured relative to the natural abundance of atmospheric  $\text{N}_2$  which is zero.

In studies of N fixation, the proportion of legume N derived from biological fixation of N from the atmosphere (P) versus that derived from soil N pool can be determined using natural abundance techniques (Peoples and Herridge 1990). This relies on measuring the  $^{15}\text{N}$  levels in the legume of interest and the  $^{15}\text{N}$  of the plant-available N pool, i.e.,  $^{15}\text{N}$  of soil, or alternatively it is obtained from a non  $\text{N}_2$ -fixing reference plant, which should reflect the  $^{15}\text{N}$  of the bulk soil. The  $^{15}\text{N}$  of the reference plant may also reflect the predominant pool of soil

N available to the plant, i.e., inorganic N (ammonium or nitrate), or alternatively that of N fertiliser.

Because isotopic fractionation during N fixation is not zero, the estimation of P needs to be adjusted accordingly and ideally should be determined for each new species of legumes for which P is determined. Isotopic fractionation during N fixation (B) is defined as the  $^{15}\text{N}$  of total plant N accumulated by a nodulated legume grown in N-free media.

The objective of this study was to first assess interspecific competition for N between radiata pine and contrasting understorey vegetation (woody N fixers, herbaceous N fixers and non N fixers) by measuring variation in foliage  $^{15}\text{N}$  in current year pine needles and gorse, broom, Maku lotus and grass samples. Secondly, to calculate the proportion of N fixed by legumes using the differences in the natural abundance of  $^{15}\text{N}$  between N-fixers and non N-fixers, in this case using the legumes broom, gorse, and Maku lotus with radiata pine as the reference plant.

Our hypotheses were that there would be differences in  $^{15}\text{N}$  values in the foliage of radiata pine growing with different understorey species and this would reflect different soil N availability due to their presence. Secondly, it was hypothesised that N fixation by legumes would increase soil N content and lead to increased N availability and consequently elevating  $^{15}\text{N}$  values of trees growing with legumes.

## Methods

### *Site*

The site at Eyrewell Forest (altitude 160 m, lat. 43°25'S, long. 172°, 16'E (long-term mean annual rainfall of 849 mm) is typical of the low rainfall area of the Canterbury Plains, New Zealand. Summer maximum temperatures may exceed 30°C, and dry conditions are enhanced by Fohn-type winds which result in periodic droughts. Soils are free draining, infertile, stony or very stony Lismore silt loams (Kear *et al.* 1967).

### *Experimental design*

The factors in the replicated (n=3) complete factorial design were

- (1) understorey vegetation (n=5) i.e. woody legumes *Cytisus scoparius* (broom) or *Ulex europaeus* (gorse), mixed herbaceous grasses (*Dactylis glomerata* (cocksfoot "Wana"), *Agrostis capillaris* (browntop "Massey Basyn"), *Holcus lanatus*. (yorkshire fog), *Lolium perenne* (ryegrass "Concord")), mixed herbaceous legumes (*Trifolium repens* (white

clover "Tahora), *Lotus pedunculatus* (Grasslands Maku), *T. subterraneum* (sub clover), *T. hybridum* (alsike clover), *Lupinus polyphyllus* x *L. arboreus* (Russell lupin)), complete control of vegetation using herbicide,

- (2) plus or minus fertiliser (Table 1),
- (3) plus or minus irrigation applied as a split plot (Table 2).

Pine foliage (current year) from the upper part of the crown of 3-year-old trees was collected from all treatment plots except the voluntary treatment. This was not included in this study because the vegetation in this treatment varied greatly depending on the combination of fertiliser and irrigation treatment. Samples of current fully sunlit leaf and stems were collected from the legume plants in all broom, gorse, and herbaceous legume plots (only Maku lotus sampled as dominant).

**Table 1**      **Total fertiliser additions (kg element/ha) until February 1995.**

N	P	Mg	B	K	S	Ca
350	74	132	8.6	311	138	172

**Table 2**      **Summary of annual rainfall and irrigation (mm) 1992-95.**

	1992-93	1993-94	1994-95
annual rainfall	835	693	658
summer irrigation	412	221	537
Total	1247	914	1195

### ***Stable isotope measurements***

Samples were oven dried at 70°C prior to weighing, fine grinding and homogenising using a Rechst ball mill. Total N and  $^{15}\text{N}$  were determined using a Dumas Elemental Analyser (Carlo Erba-NA1500, manufactured by Carlo Erba Strumentazione, Rodano, Milan, Italy) interfaced to a stable isotope mass spectrometer (Europa Scientific Tracermass, manufactured by Europa Scientific Ltd, Crewe, U.K.). The machine was calibrated against a urea standard calibrated against atmospheric N.

The proportion of N fixed (P) was calculated using the  $^{15}\text{N}$  in the respective legumes and in the pine trees growing with the sampled legumes. This approach assumes that the  $^{15}\text{N}$  of radiata pine is similar to that of the bulk soil  $^{15}\text{N}$ . No allowance was made for discrimination against  $^{15}\text{N}$  by the legume during N fixation.

### ***Statistical analysis***

Unless specified, treatment effects on legume and pine N concentration and  $^{15}\text{N}$  were analysed using the GLM procedure (SAS Institute) using a factorial model with a split plot design. Scheffes test was used to determine differences between means when treatment effects were significant ( $P < 0.05$ ).

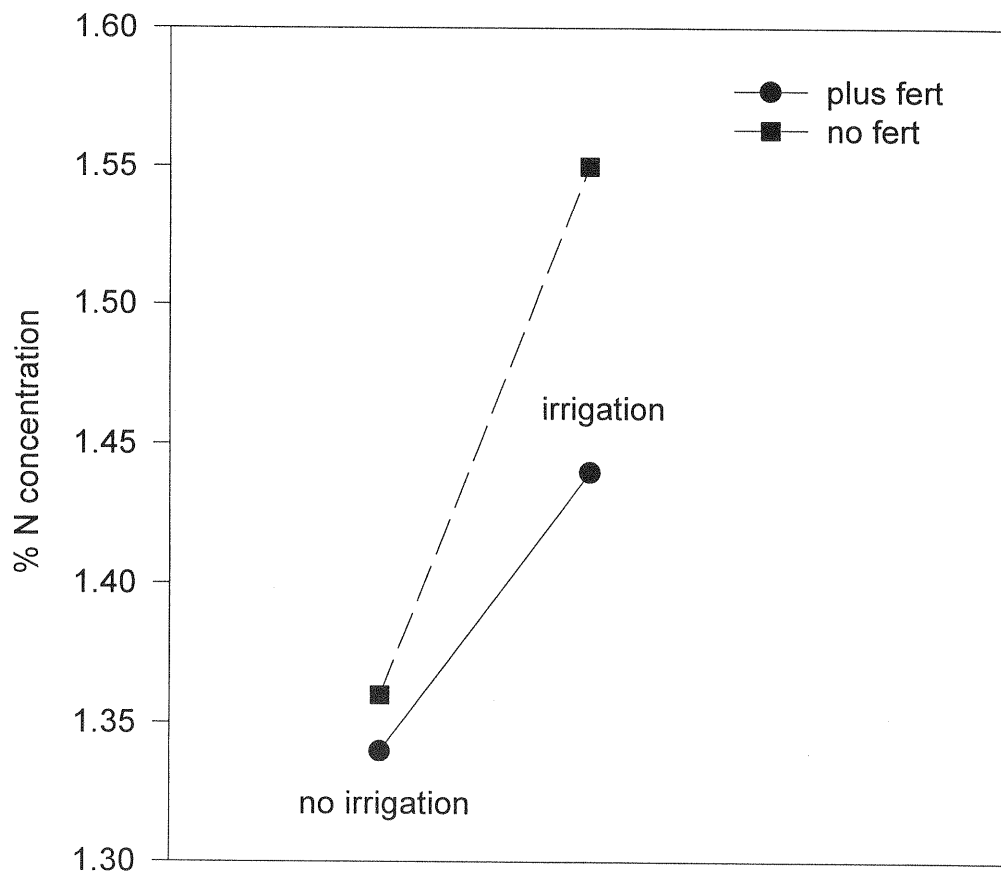
## Results

### *Radiata pine foliar N*

The addition of fertilisers significantly reduced pine foliar N concentrations in all understorey types ( $P < 0.01$ ) although the decrease was less than 5%. Similarly when applied in combination with irrigation, fertiliser again reduced pine foliar N concentrations ( $P < 0.05$ ; Fig 2). This response is due to the stimulation of the understorey by fertiliser. Pine trees growing with grass had significantly lower foliar N concentrations than those growing with broom and gorse. However, pine foliar N concentrations were higher with broom than in the control and Maku lotus treatments ( $P < 0.05$ ; Table 3).

**Table 3** Foliage N concentrations of pine trees growing in mixture with various understorey species.

Broom	Gorse	Maku Lotus	Control	Grasses
% N				
1.53a	1.47ab	1.37bc	1.42bc	1.32c



**Figure 1.** The effect of irrigation and fertiliser on foliar N concentration in radiata pine.

**\* $^{15}\text{N}$  in radiata pine foliage**

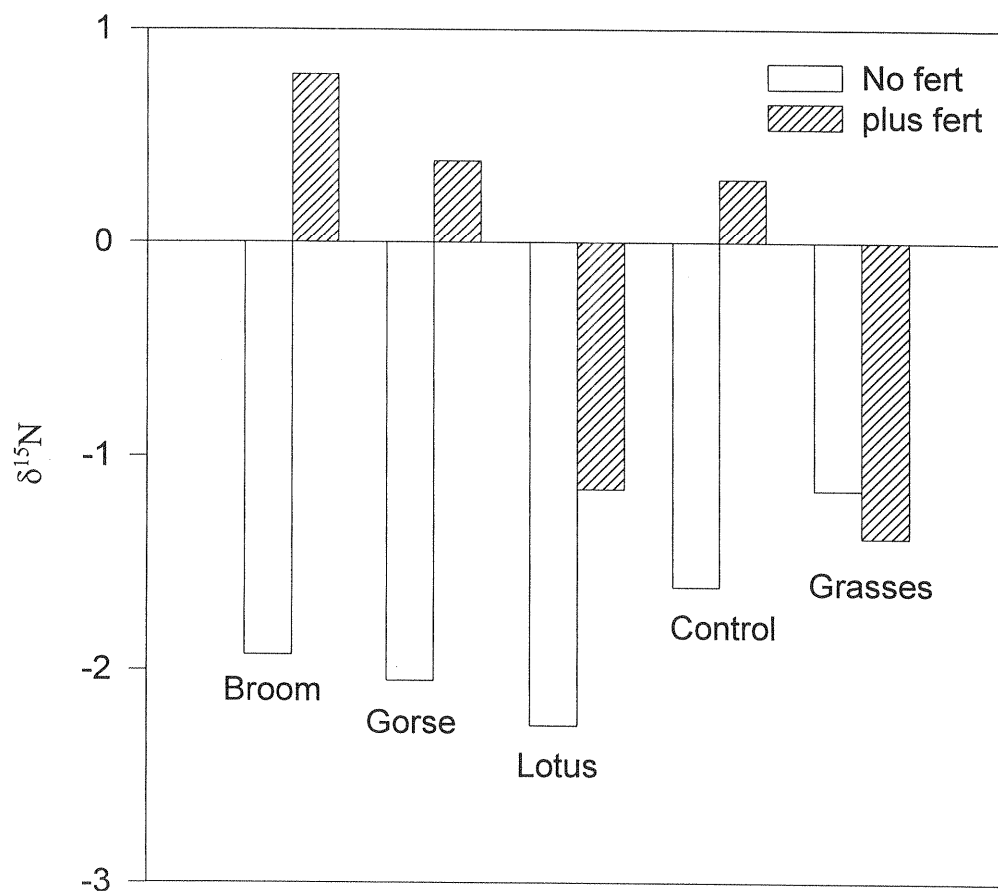
There was a strong interaction between fertiliser treatment and type of understorey in the effect on radiata pine  $^{15}\text{N}$  ( $P < 0.01$ ; Fig. 2). The application of fertiliser N ( $^{15}\text{N}$  approximately = 0) resulted in positive  $^{15}\text{N}$  values (i.e., enriched in  $^{15}\text{N}$ ) in all treatments



except where Maku Lotus dominated or where grasses were present. The largest increase in radiata pine  $^{15}\text{N}$  was with broom which was greater than gorse. There was no interaction between the irrigation and fertiliser treatments ( $P>0.05$ ). In the absence of irrigation or fertiliser there was no significant difference in  $^{15}\text{N}$  values in radiata pine foliage between understorey treatments (Table 4).

**Table 4**      \* $^{15}\text{N}$  of pine tree foliage growing with various understorey species without irrigation or fertilisers.

Broom	Gorse	Maku Lotus	Control	Grasses
$^{15}\text{N}$				
-1.40	-2.12	-1.44	-1.23	-1.20



**Figure 1.** Effect of understorey type and fertiliser treatment on <sup>15</sup>N natural abundance in radiata pine

**\*<sup>15</sup>N in legumes**

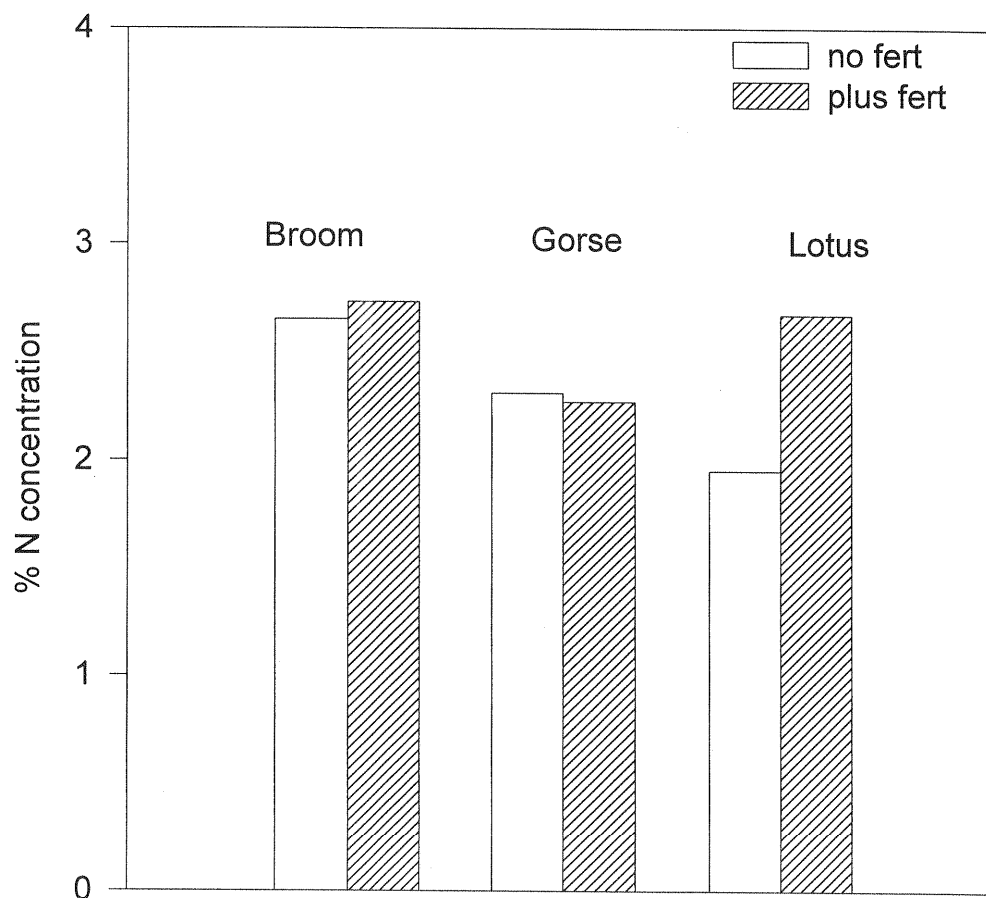
Although the fertiliser treatment had no effect on <sup>15</sup>N values in either herbaceous and woody legumes ( $P > 0.05$ ), data were subsequently analysed only for the non-fertilised plots. Values of <sup>15</sup>N in Maku lotus tissue from non-fertilised plots were significantly lower than in broom and gorse tissue ( $P < 0.01$ ; Table 5).

**Table 5**      **\*<sup>15</sup>N in legumes and pine trees from fertilised and non fertilised plots.**

	no fertiliser		Plus fertiliser	
	irrigation		irrigation	
	minus	plus	minus	plus
Broom	-1.28	-1.60	-1.23	-1.39
Pine	-1.40	-2.45	0.58	0.99
Gorse	-1.79	-0.92	-1.65	-1.39
Pine	-2.12	-1.98	0.40	0.37
Maku Lotus	-2.64	-2.83	-2.16	-2.21
Pine	-1.44	-3.07	-0.83	-1.47

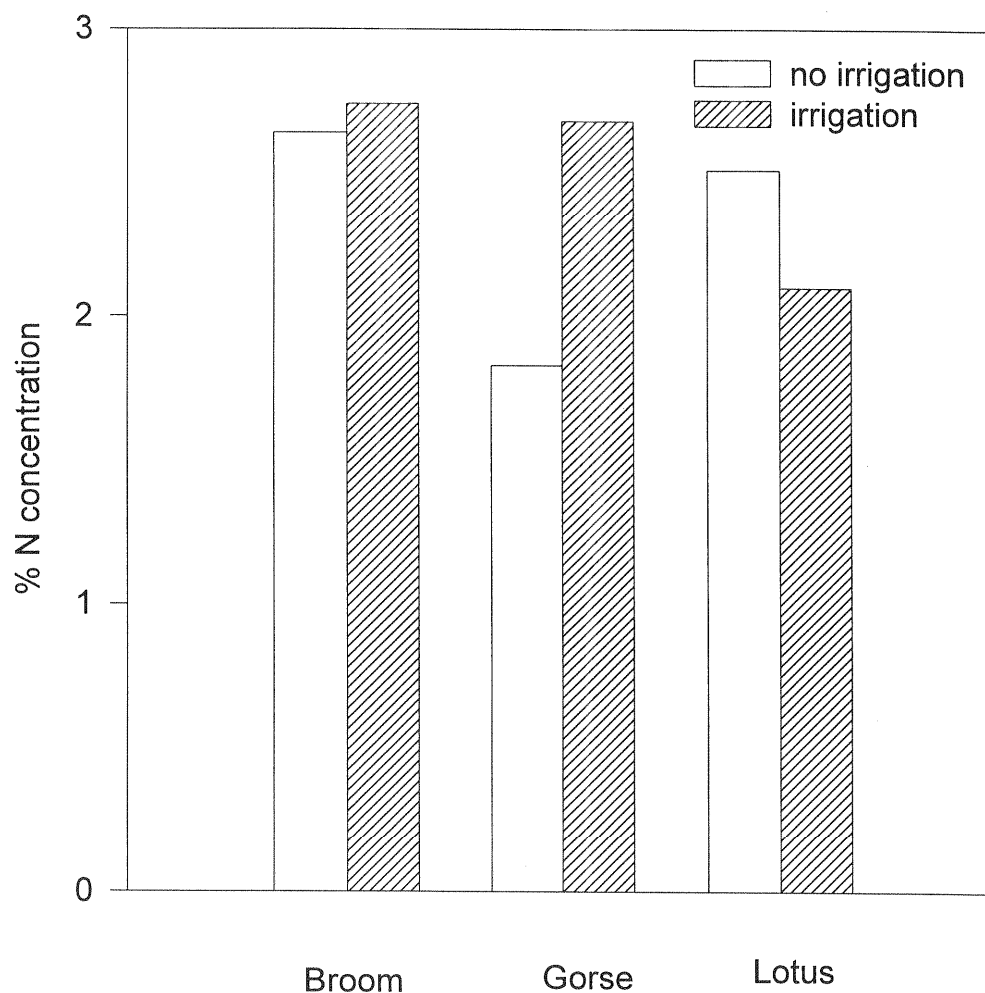
***Legume foliar N***

Maku Lotus N concentrations were increased from 1.9% to 2.6% by fertiliser (P<0.05) but broom and gorse N concentrations did not change (Fig 3).



**Figure 3. Effect of fertiliser on foliar N concentrations in legumes.**

However, there was a significant interaction between irrigation treatment and the type of understorey ( $P < 0.01$ ; Fig. 4). Irrigation increased N concentration in both broom and gorse and reduced it in lotus.



**Figure 4. Effect of irrigation on legume foliar N concentrations.**

***Proportion of legume N derived from fixation of atmospheric N.***

In all except one case the  $^{15}\text{N}$  level of the legumes was closer to that of atmospheric  $\text{N}_2$  than that of pine foliage in all non-fertilised treatments (Table 5). However, the difference between  $^{15}\text{N}$  values of legumes and pines was very small, much less than that ideally used in other studies that have estimated P. This has resulted in low estimates of the proportion of N fixed by the broom and gorse in the non-fertilised plots (Table 6). The negative values of  $^{15}\text{N}$  for lotus do not allow an estimate of P to be calculated.

**Table 6** Estimates of the proportion of N fixed by legumes (P(%)).

	Broom		Gorse	
	plus irrigation	minus irrigation	plus irrigation	minus irrigation
P (%)	34	8	53	15

## Discussion

The application of fertiliser increased the N concentration of Maku lotus substantially in contrast to having only a very minor effect on broom and gorse N concentrations. This large increase in Maku lotus N concentration supports the notion that Maku lotus was competing with radiata pine for the fertiliser N, or at least significantly reducing the availability of N.

The positive  $^{15}\text{N}$  values of pine trees in all fertilised treatments except with the herbaceous grasses and Maku lotus. This suggests that along with direct uptake by the pine trees of fertiliser N (which had a  $^{15}\text{N}$  value of nearly 0), that the application of fertiliser N resulted in increased discrimination between the isotopes of N, (i.e.  $^{15}\text{N}$  and  $^{14}\text{N}$ ) in the soil N cycle, probably during nitrification of fertiliser N. This discrimination between the N isotopes would have resulted in the soil N pool becoming enriched in  $^{15}\text{N}$ , and, subsequent tree uptake from this pool would have resulted in trees becoming further enriched in  $^{15}\text{N}$ . For example, where there was no understorey present,  $^{15}\text{N}$  values suggest that N transformations and N availability were greatly increased by the fertiliser treatment. In contrast, in the grass and to a lesser extent in the Maku lotus-dominated plots,  $^{15}\text{N}$  values of pine trees do not suggest this pattern of increased discrimination against  $^{15}\text{N}$  and uptake of  $^{15}\text{N}$ -enriched fertiliser. This variation in  $^{15}\text{N}$  values of pine trees growing in combination with different understorey species further suggests that herbaceous grasses and Maku lotus were competing for fertiliser N whereas broom and gorse were not.

Most studies that have examined the  $^{15}\text{N}$  natural abundance technique in natural systems or non agricultural systems have found that it is not suitable for estimating N fixation (Binkley *et al.* 1985; 1992). In most cases, low and or variable soil or reference plant  $^{15}\text{N}$  values have been given as the reason (Handley *et al.*, 1994). This is because Shearer and Kohl's 1986 model of N fixation assumes that non N fixing reference plants obtain their N from what is an isotopically single source. Other interactions between the legume and the reference plant have rarely been considered (Handley *et al.*, 1994). This needs further examination with respect to competition for water.

It has been suggested that negative  $^{15}\text{N}$  values occur in some soils, particularly forest soils, due to low inputs of N with negative  $^{15}\text{N}$  values combined with low outputs of N, i.e., no N lost via mechanisms such as nitrification, denitrification or volatilisation that would discriminate against  $^{15}\text{N}$  (Vitousek *et al.* 1989). Transformations such as ammonia volatilisation and denitrification discriminate against  $^{15}\text{N}$  directly whereas nitrate leaching does not result in discrimination between  $^{15}\text{N}$  and  $^{14}\text{N}$ . However, nitrification does. Nitrate lost from the system by leaching can be depleted in  $^{15}\text{N}$  resulting in elevated  $^{15}\text{N}$  levels in plants.

In this study, trees that were irrigated but not fertilised had more negative  $^{15}\text{N}$  values than unirrigated trees ( $P < 0.1$ ) suggesting either that no nitrate leaching occurred or alternatively  $^{15}\text{N}$  values were negative because of N inputs with low values, i.e., N in irrigation water. This is likely to be the case as the irrigation water used in this study was sourced from rainwater which generally has negative  $^{15}\text{N}$  values (Peterson and Fry 1987).

Although leaf  $^{15}\text{N}$  values have been used to estimate the plant N derived from atmospheric  $\text{N}_2$  fixation in agricultural systems (Peoples and Herridge 1990), the approach does not appear to hold for this study. Estimates of P for broom and gorse were low and highly variable compared to estimates published for some woody N-fixers (Domenach *et al.*, 1989). Determining the proportion of plant N derived by legumes grown with atmospheric N as their only N source may improve the estimates for this study. However, differences in  $^{15}\text{N}$  between pine and N fixers were small and  $^{15}\text{N}$  values were negative (from -1.93 to -2.26 and -1.44 to -2.73 for pines and legumes respectively). This is in contrast to agricultural studies where values may be as high as 3 to 13 and P has been successfully determined (Shearer and Kohl 1991).

The  $^{15}\text{N}$  isotope dilution technique may be more appropriate in situations where  $^{15}\text{N}$  values are negative and differences between N fixers and reference plants are small. This technique requires application of  $^{15}\text{N}$  to both a non N fixing reference plant and a N fixer. The difference in  $^{15}\text{N}$  enrichment between the N fixer and the non N fixing plant is used to determine the proportion of N in the N-fixer derived from the atmosphere. This method is based on the following assumptions: (1) all N in the reference plant is derived from the soil, (2) the legume and reference plant explore the same soil N pool, and (3) the isotope applied to the soil is equally available to both the N-fixer and reference plant. Estimates of P have been made for a variety of herbaceous legumes in agroforestry systems and plantations in New Zealand using the isotope dilution technique (Steel and Percival 1984; Mansur 1994; Wanjiku 1996) and range between 56-92%, 71-92% and 81-95%, respectively.

To use  $^{15}\text{N}$  techniques to trace legume N to pine requires mineralisation of legume material (either roots or shoots) containing fixed N. The results of this study show that the amount of biologically fixed N mineralised from any of the legumes tested had less effect on the soil pool of plant-available N and plant uptake than the addition of 350 kg N/ha applied in the fertiliser treatment. This suggests that either the amount of N fixed is small or that only a small amount of biologically fixed N has been mineralised after 3 years of legume growth. This is not to say that the legume contributes no N to the site, but rather that the contribution of N is small in the early years and likely to be of no consequence to trees. Further study of N fixation rates and breakdown and mineralisation of legume biomass is required over a longer period of a forest rotation to determine fully the benefits of legumes to forest sites.

## Conclusions

- The natural abundance of the stable isotope of N ( $^{15}\text{N}$ ) in foliage of radiata pine, a non N-fixing plant reflected differences in N transformations and N availability brought about by differing levels of understorey competition for N.
- Inputs of N by legumes did not appear to increase N transformations and N availability to the same extent as 350 kg N/ha applied over a 3-year period.
- Legumes differed in their capacity to fix N, but this aspect of the study needs further investigation.

## Implications for Forest management

- Forest managers should ensure that they use optimal weed control when N fertiliser is applied to sites where the understorey is dominated by grass species because significant amounts of N will be taken up by grasses and whose growth will be substantially improved, resulting in increased competition for water.
- Although, broom and gorse and to a lesser extent, Maku lotus did not appear to be competitive for N, they may well be capable of competing for other nutrients such as P and this requires further investigation.

## Acknowledgments

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