The fate of ^{15}N-labelled fertiliser applied in autumn or winter to white clover (*Trifolium repens* L.) or perennial ryegrass (*Lolium perenne* L.)

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Abstract

While increasing amounts of nitrogen (N) are being applied to white clover and perennial ryegrass in autumn and winter when low temperatures prevail, the uptake of fertiliser N at low temperatures, and consequent possible effects on leaching, have not been quantified. A lysimeter study to investigate the fate of ^{15}N applied in May or August to white clover (*Trifolium repens* L.) or perennial ryegrass (*Lolium perenne* L.) was established in the autumn of 1998. The objective was to determine the extent to which differences in agronomic features between the two species would affect the uptake and losses of N. The composition of the leachate collected during winter was determined. Plant uptake and the distribution of ^{15}N in soil were analysed after destructive harvesting in September 1998. Leaching losses of mineral N were greater under white clover than perennial ryegrass ($P<0.05$). White clover took up less ^{15}N than perennial ryegrass ($P<0.05$). This was due to the slower growth of white clover, which may have been a consequence of biological N fixation. Sixty five percent of fertiliser N remaining in the soil in white clover was present in the top 100 mm; 65% of both white clover and perennial ryegrass roots were in the top 100 mm suggesting that N was available in the rooting zone for subsequent uptake and growth.

Additional key words: *lysimeter, leachate, nitrogen uptake*

Introduction

In New Zealand, pastoral agriculture is a major industry, contributing 54% of total export dollars (Edlin, 1999). White clover (*Trifolium repens* L.) has been described as the key to New Zealand's pastoral industry, due to its N fixing potential and feed value (Caradus *et al.*, 1996). White clover is commonly grown in conjunction with perennial ryegrass (*Lolium perenne* L.); mixed white clover/perennial ryegrass swards occupy more than 6.4 million hectares of improved pasture land in New Zealand (Newsome, 1987). Due to their importance in improved pastures, white clover and perennial ryegrass are also grown for seed: in the 1998/99 growing season, 15,217 ha of white clover and 19,756 ha of perennial ryegrass were entered for certification (Seed Certification Statistics, 1998/1999).

To maximise seed and pasture production, and minimise N inputs, all aspects of the growth of white clover and perennial ryegrass must be well understood (Evans, 1977). Differnces in the physiological and morphological characteristics of the two species may influence their relative response to applied N at low temperatures (Woledge and Dennis, 1982). White clover is particularly vulnerable during winter and spring when its lower temperature threshold of growth in comparison with perennial ryegrass leads to differences in performance (Rhodes, 1981). The relative growth rate of perennial ryegrass at 8°C has been reported to be 43% greater than white clover (Glendining and Mytton, 1989). The rate of N uptake is related to shoot growth (Grindlay, 1997), and at low temperatures demand may exceed supply (Macduff *et al.*, 1989; Hatch and Macduff, 1991) as mineralisation of soil-N is slow (Ball and Field, 1982). N stress in pasture is common in late autumn and late winter (Ball and Field, 1982), and increasing amounts of N fertiliser are being applied at these times to improve growth. Annual N fertiliser use has trebled in New Zealand in the past decade (Furness *et al.*, 1997).

There are, however, many economic, environmental, and social problems associated with fertiliser N use, resulting in calls for new approaches to agricultural research as well as practices at the farm level (Magdoff *et al.*, 1997). Of major concern over the years has been the leaching of nitrates into groundwater (Macdonald *et al.*, 1989; Magdoff *et al.*, 1997). In order to devise strategies to minimise nitrate leaching, it is necessary to quantify autumn/winter N losses, as it is autumn applied N that is at risk to leaching because winter precipitation exceeds evapotranspiration (Macdonald *et al.*, 1989).

Lysimeters allow controlled experiments to be conducted on undisturbed soil profiles and are therefore useful for studying leaching losses (Fraser *et al.*, 1994). By using ^{15}N labelled fertiliser it is possible to quantify the losses of applied N. The objectives of this study were to determine the fate of ^{15}N applied to white clover and perennial ryegrass in autumn/winter, and to determine the agronomic features that may contribute to the differences in recovery of applied N.

Materials and Methods

Lysimeter establishment

Twenty four monoliths of a Templeton silt loam (Udic ustochrept, USDA) were extracted from 9 year old pasture located at the Sheep Breeding Unit, Lincoln University, Canterbury, New Zealand in February 1998. Lysimeters (180 mm internal diameter and 250 mm depth) were extracted by the method devised by Clough *et al.* (1996), and were placed randomly in a trench, with the top of the lysimeter 20 mm above ground level to avoid surface runoff.

Treatments

Lysimeters were hand cultivated to a depth of 50 mm on 19 March 1998. Twelve lysimeters were sown (five seeds per lysimeter) with white clover cv. Grasslands Huia. The remaining twelve lysimeters were sown with perennial ryegrass cv. Grasslands Nui. After six weeks, and before the first application of N, seedlings were thinned to one plant per lysimeter to avoid plant competition. The equivalent of 23 kg N/ha was applied as ^{15}N-labelled urea at 10 atom percent at two application dates (1) 4 May (autumn, 47 days after planting, when soil temperatures at 100 mm were above 8°C), or (2) 13 August (winter, 148 days after planting, when soil temperatures had risen above 4.5°C (Hoglund, 1980); a third of the lysimeters received no added N. Sixteen millimetres of simulated rainfall was applied immediately after urea application to suppress volatilisation (Black *et al.*, 1987). Treatments were replicated four times in a completely randomised design.

Leachate Analysis

Leachate was collected within 2 days of any drainage to minimise N losses. Total volumes were measured on site and a sub-sample (100 ml) from each lysimeter was taken for N analysis. Nitrate concentrations were determined by ion exchange chromatography (Waters, USA) and ammonium concentrations were determined by automated flow injection analysis (Tecator, Sweden). The total N $^{14}N/^{15}N$ ratio in the leachates were determined by isotope ratio mass spectrometry. The leachates were prepared for analysis by the method described by Cookson *et al.* (2000b).

The percent recovery of applied urea ^{15}N was calculated using the following formula (Cabrera and Kissel, 1989):

$$\%^{15}N\ recovered = \frac{p(c-b)}{f(a-b)} \times 100$$

where p = moles of N in samples; f = moles of N in urea applied; c = atom % ^{15}N abundance in the sample; a = atom % ^{15}N abundance in the urea; b = atom % ^{15}N abundance in the sample without applied urea.

A risk:benefit analysis was used to evaluate the balance between the amount of above ground N recovery up by the shoots, roots and soil and, that reflecting fertiliser N lost by leaching and denitrification:

$$\frac{\underline{Total\ N\ losses\ (leaching\ and\ denitrification)}}{Above\ ground\ recovery\ (shoot)}$$

Plant and Soil Analysis

The lysimeters were harvested on 21 September 1998. Plant material was dried in paper bags at 20°C for 3 weeks (subsequent analysis indicated no change in N concentration between plants dried at 20°C or 70°C suggesting a rapid halt to enzyme activity and hence minimal loss of carbohydrate). White clover, due to its small size, was ground in a mortar and pestle; perennial ryegrass was ground (<250 μm) using a "Cyclotec 1092" sample mill (Tecator, Sweden). Dried plant material was weighed into aluminium foil capsules and analysed for total N and $^{14}N/^{15}N$ ratio as described above.

The lysimeters were sectioned into 0-50, 50-100 and 100-250 mm depths after plant harvest. Soil was sectioned into quarters and opposing quarters were taken for soil or root analysis. The roots were washed using a rotating drum, with water sprayed via teejet nozzles. A sub sample was taken at each depth, dried at 70°C, weighed, sieved (2 mm), ground in a mortar and pestle and analysed for total N and $^{15}N/^{14}N$ ratio. Total N and $^{15}N/^{14}N$ ratio of the soil was determined on a subsample from each depth after grinding to a particle size <150 μm using a Tema mill grinder (N.V. Tema, Netherlands).

Meteorological data

Rainfall was recorded automatically on-site using a tipping bucket measurement system connected to a data logger (Campbell Scientific, USA). Soil temperature at 50, 100 and 300 mm was also recorded automatically on-site using temperature probes (RS Component Ltd., model no. LM 35CZ) connected to a data logger (Campbell Scientific USA).

Statistical analysis

Leachate data were analysed using Genstat 5 4.1. All other data were analysed using the Minitab (1994) computer program. Analysis of variance was performed with least significant differences at 0.05, calculated from the error mean sum of squares.

Results and Discussion

Rainfall and temperature

Total rainfall during 1998 was less than that of the long term average for this area, although approximately 30 mm more rain than the 10 year mean fell during May (Table 1). Rainfall was unevenly distributed, with intense, sporadic periods of rainfall occurring (Fig. 1). Average monthly soil temperatures were 3°C warmer in March and April and 3°C cooler in August than the 10 year mean (Table 1).

Total N leached

Total N leaching losses were greatest following major rainfall events (Fig. 1). Significantly ($P<0.05$) more N was leached under white clover than perennial ryegrass (32 kg N/ha versus 21 kg N/ha) respectively; this is consistent with results obtained by McLenaghen *et al.* (1996). The increased leaching loss of N under white clover is thought to reflect the agronomic differences (discussed in a later section), and hence N demand, between the species. N applications in excess of plant demand, in association with water inputs in excess of evapotranspiration, will favour the loss of N (Fraser *et al.*, 1994; Freeney *et al.*, 1995; Cookson *et al.*, 2000a).

Table 1. Mean monthly 100mm soil temperature (°C) measured daily at 9 AM, and monthly rainfall (mm) for the duration of the lysimeter trial in 1998. Long term (25 year) monthly means are also shown.

	Soil temperature @100 mm		Rainfall	
Month	1998	Long term average	1998	Long term mean
March	17.5	14.8	31.4	60.0
April	13.4	10.2	26.8	51.8
May	7.0	7.0	74.7	50.4
June	3.7	4.2	41.8	63.0
July	4.0	3.8	48.5	73.7
August	3.0	4.9	52.0	68.1
September	9.6	10.2	24.3	40.1

^{15}N Balance

There were no significant differences in quantities of ^{15}N leached between the white clover and perennial ryegrass treatments, despite the large apparent differences. This may reflect the large inter-lysimeter variability. Although small lysimeters have been used for leaching experiments before (Clough *et al.*, 1996; Cookson *et al.*, 2000a), the current results suggest that a large number of replicates is needed, because of the non-uniform nature of the soil in terms of drainage (i.e., preferential flow due to differing flow channels).

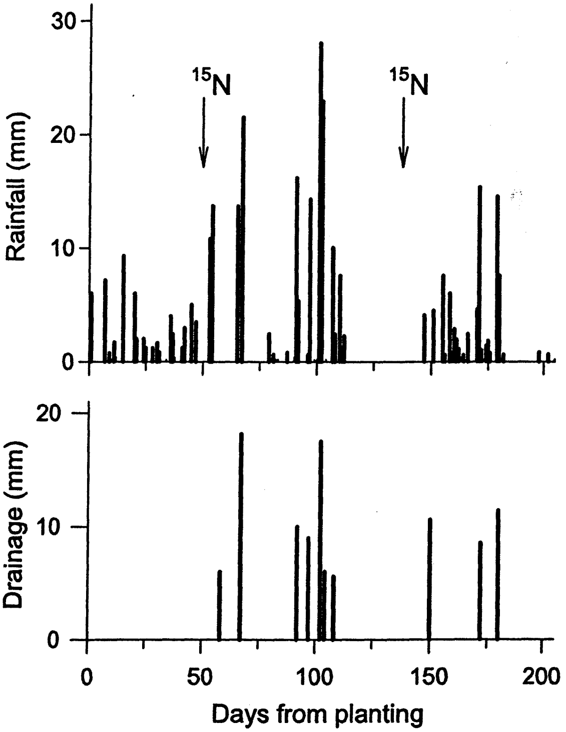

Figure 1. Rainfall (mm) and drainage (mm) over the duration of the lysimeter trial.

Significantly more ^{15}N leached from the May treatment compared with the August treatment, possibly because after the August application of fertiliser N, plant demand for N increased, and rainfall was insufficient to move fertiliser through the profile.

Species had a significant ($P<0.05$) effect on ^{15}N uptake in the shoots at both ^{15}N application dates: perennial ryegrass took up approximately 8.5 times more ^{15}N than white clover, and both species took up almost 6 times more ^{15}N in the August treatment compared with the May treatment (Table 2). The uptake of August ^{15}N in perennial ryegrass is consistent with similar work by Cookson *et al.* (2000b), although the latter measured recovery between August and December, while the study period reported here was August to September. Increased uptake of ^{15}N in May in comparison with the August application is thought to reflect the increases in plant growth rates which were observed between August and final harvest in comparison with growth rates between May and August, and which occurred when ^{15}N was still available for plant uptake (i.e., it had not been leached or immobilised).

There were no significant differences between species in ^{15}N recovered in roots after the May application, but perennial ryegrass recovered 4.5 times more ^{15}N than white clover after the August application (Table 2). There were no significant differences between species in the amount of ^{15}N found in the soil after the May application of N fertiliser. In contrast, significantly ($P<0.05$) more ^{15}N was found in the soil after the August fertiliser application in white clover than perennial ryegrass. This reflects the slower growth rate of white clover compared with perennial ryegrass, which is reflected in the different ^{15}N recovery in the shoots of the two species, and differences in leaching.

In both species, approximately 35% of ^{15}N remained unaccounted for in the balance for the May application (Table 2). Simulated rainfall followed ^{15}N application in an attempt to minimise volatilisation (Black *et al.*, 1987). However, volatilisation can occur due to repeated wetting and drying of the soil when cover is minimal and there is a high concentration of fertiliser N in the soil surface (Black *et al.*, 1987). Furthermore, under the conditions when ^{15}N was applied (high soil moisture, high nitrate and a readily available source of carbon), losses due to denitrification have been reported to reach 30% (Von Rheinbaben, 1990). Hence, gaseous losses are thought to be the major source of N losses in this study.

Table 2. September recovery of ^{15}N (%) in shoots, roots, soil and leachate from white clover and perennial ryegrass following nitrogen application in May and August.

	May		August		
	Clover	Ryegrass	Clover	Ryegrass	$LSD_{P<0.05}$
Shoots	0.8	6.8	4.5	39.0	4
Roots	2.5	3.8	2.5	11.6	2
Soil	39.5	46.4	67.5	42.7	12
Leachate	22.5	10.1	0.2	0.0	NS
Total	65.3	67.1	74.7	93.3	

There was four times as much ^{15}N unaccounted for after the August application in white clover than perennial ryegrass (Table 2). This may reflect volatilisation due to wetting and drying which would have been exacerbated by the lack of plant cover (Black *et al.*, 1987) under white clover compared with perennial ryegrass.

Agronomic factors

White clover produced significantly less dry matter ($P<0.05$) in all N treatments (0.024, 0.16 or 0.18 g dry matter) than perennial ryegrass (2.39, 2.42 or 3.78 g dry matter) in the control, May and August treatments, respectively.

N applied in May or August increased white clover dry matter by 85% relative to the control. In other research the decrease in growth of white clover plants relying upon N fixation as the predominant source of N has been attributed to a decrease in available carbon (Haystead *et al.*, 1980). Slower growth rates and smaller leaf area, and a decrease in partitioning of assimilates to the shoots, reflect an increase in assimilates partitioned to the roots for maintenance of nodule activity (Ryle *et al.*, 1981; Arnott and Ryle, 1982; Woledge and Calleja Suarez, 1983). In addition, although there were no significant differences in root distribution (%) between species (Table 3), white clover had only a third of the biomass in the top 50 mm of the soil profile in comparison with perennial ryegrass, which may have resulted in reduced N uptake.

^{15}N application in August increased dry matter production in perennial ryegrass by 35% compared with the control and May applications. This is consistent with the total amount of ^{15}N recovered, which was higher in the August treatment than the May treatment (Table 2). Although few differences in the maximum photosynthetic rates between perennial ryegrass and white clover have been reported (Davidson and Robson, 1986; Woledge and Dennis, 1982), at low temperatures the maximum photosynthetic rates are reached more slowly in white clover compared with perennial ryegrass (Davidson and Robson, 1986). Furthermore, supplying N has been shown to increase the photosynthetic rates in perennial ryegrass but not in white clover (Davidson and Robson 1986). It is possible that, for white clover, temperature is more limiting than the supply of N (Kessler *et al.*, 1990), whereas in perennial ryegrass when N is limiting, there is a reduction in dry matter production at low temperatures (Davidson *et al.*, 1986).

Risk: benefit analysis

The ratio between fertiliser N losses and shoot N uptake indicated losses were higher in the May treatment than the August treatment, and under white clover compared with perennial ryegrass (Table 4). Recent work in perennial ryegrass by Cookson *et al.* (2000b) has also indicated that a larger environmental risk is associated with applying fertiliser in autumn in comparison with winter, highlighting the need to match plant N demand with N supply.

Further investigation is needed in order to increase the understanding of factors that may contribute to the losses of fertiliser N in white clover; such factors include biological N fixation, N uptake, root and shoot growth at low temperatures.

Table 3. The relationship between soil depth and ^{15}N distribution (%) and root distribution (%) in white clover and perennial ryegrass.

	^{15}N distribution		Root distribution	
Depth (mm)	Clover	Ryegrass	Clover	Ryegrass
0-50	32.6	36.5	48.3	58.1
50-100	6.0	5.4	18.9	17.1
100-150	2.3	2.7	17.0	14.0
LSD $_{P<0.05}$	12.0	12.0	17.0	17.0

Table 4. The effect of fertiliser treatment on the ratio of total N loss (leaching and denitrification) to above ground (shoot) recovery of N.

Fertiliser treatment	N loss : Above ground recovery
White clover (May)	72.00
Perennial ryegrass (May)	6.32
White clover (August)	5.60
Perennial ryegrass (August)	0.17

Implications

White clover produced less dry matter compared with perennial ryegrass for all treatments. However, the relative response of white clover to an application of N was higher than perennial ryegrass. Applying N in May or August to white clover resulted in 85% greater dry matter production than in the control treatment. In comparison, applying N in May had no effect on dry matter production in perennial ryegrass and applying N in August increased dry matter production by 35%. However, even though white clover had a greater relative response to N compared with perennial ryegrass, overall dry matter production of white clover in comparison with perennial ryegrass was small. Research into physiological factors which may influence dry matter production in white clover is required in order to identify the best time to apply N to maximise white clover dry matter production.

Conclusions

- White clover grew more slowly than perennial ryegrass
- Uptake of ^{15}N was less in white clover than perennial ryegrass ($P<0.05$)
- Total N losses were greater under white clover compared with perennial ryegrass ($P<0.05$)
- Leaching losses of ^{15}N were greater from the May treatments than from the August treatments ($P<0.05$)

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