

Agronomical and physiological responses of white clover (*Trifolium repens*) and perennial ryegrass (*Lolium perenne*) to nitrogen fertiliser applied in autumn and winter

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Abstract Mixed pastures of white clover and ryegrass are profitable and widely used. However, loss of clover is common with time due to N fertiliser applications in late winter. The factors that cause this loss have yet to be determined. This work investigated reasons for the competitive disadvantage suffered by white clover relative to ryegrass in autumn and winter. White clover (*Trifolium repens* cv. 'Grasslands Huia') and ryegrass (*Lolium perenne* cv. 'Grasslands Nui') were sown in March in Canterbury, New Zealand, and grown as single plants in silica sand. Nitrogen (N; 0.5 mol m⁻³ N); was applied daily simulating

an unfertilised soil; solution N concentration was increased to 5.0 mol m⁻³ N for treatments in autumn (May) or winter (August), simulating fertiliser treatment. Net photosynthesis was measured before each harvest and over a diurnal period at 123 days after sowing. Plants were destructively harvested six times (33–185 days after sowing) and plant dry weight and N concentration (%) measured. White clover had a significantly ($P < 0.05$) lower dry weight than ryegrass. Extra N applied from May significantly increased ($P < 0.05$) dry weight of ryegrass. No significant changes in shoot N concentration were found. Except at the last harvest, net photosynthesis was significantly lower ($P < 0.05$) in white clover than in ryegrass; this was also apparent for diurnal net photosynthetic rates 123 days after sowing. Results indicated that white clover growth was limited by temperature, whereas ryegrass growth was limited by N supply.

Keywords apparent nitrogenase activity; *Lolium perenne*; nitrate; nitrogen fertiliser; perennial ryegrass; photosynthesis; *Trifolium repens*; white clover

INTRODUCTION

The use of nitrogen (N) fertilisers in autumn and winter is common practice in many temperate agricultural areas and frequently results in increased pasture growth rates (Ball & Field 1982; Thompson et al. 1991). However, the application of N fertiliser to mixed swards can decrease the proportion of white clover (Laidlaw et al. 1992), particularly in spring following a late winter N application (Laidlaw 1985; Rowarth et al. 1996). This is because low temperatures decrease the growth rate of white clover more than ryegrass, and hence white clover is less responsive to N than ryegrass (Arnott & Ryle 1982; Davidson et al. 1986). However, the physiological limiting factor in white clover growth has not yet been identified.

Lower root temperatures affect carbon (C) and N partitioning in white clover. Whether this is due to a C or N limitation or surplus has not been confirmed (Wilson 1988). This reduction in N_2 fixation reported at low temperatures (Hatch & Macduff 1991) may be a response to a decrease in C supply, reflecting a reduction in photosynthesis at low temperatures (Woledge & Dennis 1982). The result is a decrease in leaf growth and area of leaves available to intercept radiation (Wilson 1988; Glendining & Mytton 1989; Ericsson 1995). This has been proposed as a key determinant of N demand because the photosynthetic function of leaves requires a large content of reduced N (Grindlay 1997). An understanding of whether temperature affects either C or N uptake may be a key factor in determining why the growth of white clover is reduced at low temperatures.

Leaf growth is also influenced by the source from which white clover plants obtain their N. Plants receiving N from the soil have a greater leaf area than those which depend on rhizobially fixed N (Ryle et al. 1979; Arnott & Ryle 1982). Again, this may be a direct result of C partitioning.

As light interception controls plant growth rates (Monteith 1977), the slow early growth of white clover relative to ryegrass at low temperatures reduces the ability of the white clover to compete with ryegrass in a mixed-species sward (Davidson et al. 1986). This effect is exaggerated if N fertilisers are applied because the poor light interception by white clover prevents response to the extra N.

In the short term appropriate management protocols allowing the efficient use of N fertiliser must be developed; in the longer term, plant breeders will select for characteristics in white clover that will improve competitiveness at low temperatures, thus leading to the development of sustainable pastures. Both of these initiatives require information on factors that influence the uptake and assimilation of N over the autumn and winter months. This research was established to quantify the agronomical and physiological differences influencing N uptake and assimilation in white clover and ryegrass.

MATERIALS AND METHODS

Growing conditions

Eighty-eight plastic pipes, 300 mm long and 190 mm diam., were covered at one end with plastic netting (<2 mm mesh). The pipes were placed in

trenches (netting end down) and filled with silica sand (0.5–5 mm) at a field site at Lincoln University, Canterbury, New Zealand. On 19 March 1999, 44 pipes were sown (five seeds per pipe) with white clover (*Trifolium repens* cv. 'Grasslands Huia'); the remaining 44 were sown (five seeds per pipe) with perennial ryegrass (*Lolium perenne* cv. 'Grasslands Nui'). One week after emergence the seedlings were thinned to one plant per pipe to avoid interplant competition. Plants were supplied with a nutrient solution (Smith et al. 1983), which was modified to contain either 0.5 mol m^{-3} N (simulating an unfertilised soil) or 5.0 mol m^{-3} N (simulating a soil which has recently received fertiliser N) (Andrews et al. 1992). Fifty ml of solution per pot were applied on alternate days. Timing of treatments was based on an autumn application of N fertiliser (Ball & Field 1982) or a winter application (Thompson et al. 1991), and were: (1) control, 0.5 mol m^{-3} N throughout; (2) designated May, plants received 0.5 mol m^{-3} N solution before 5 May 1999, and 5.0 mol m^{-3} N from 5 May 1999 (58 days after sowing onwards); (3) designated August, plants received a 0.5 mol m^{-3} N solution before 13 August 1999, and 5.0 mol m^{-3} N from 13 August 1999 (157 days after sowing onwards). The trial had a fully randomised design.

Photosynthesis measurements

Leaf area was measured using a LI-3100 (LI-COR Inc., Lincoln, Nebraska, United States) leaf area machine. Dry weights of shoots and roots (60°C for 48 h) were recorded (g) and average daily growth rate ($g\ day^{-1}$), leaf expansion ($m^2\ day^{-1}$), and the ratios of shoot to root dry weight were calculated.

The rate of net photosynthesis was measured on six occasions during the growing period, 2 days before each harvest, using a LI-6400 (LI-COR Inc., Lincoln, Nebraska, United States) portable infrared gas analyser. Single cuvettes were used on two young fully expanded leaves per plant. The LI-COR allowed the light intensity for photosynthesis measurements to be kept at 1000 $\mu\text{mol photons } m^{-2}\ s^{-1}$ (80% of saturation (Woledge & Dennis 1982)), to avoid the confounding effects of different light intensities (Woledge & Leafe 1976). The block temperature (i.e., the temperature to which the leaf was exposed) was set at the average grass minimum of the week before the measurement date, except at 88, 123, and 157 days after sowing when the mean air temperature of the previous week was used, because the grass minimum temperature was less

than $0 \pm 1^\circ\text{C}$. Grass minimum temperatures were measured and recorded by temperature probes (RS Component Ltd., model no. LM 35CZ) connected to a data logger (Campbell Scientific).

Diurnal change in net photosynthesis was measured (10.00–16.00 h) on white clover and ryegrass (four control and four May N-treated plants) on 6 July 1999, 123 days after planting. Light intensity was measured using a LI-188B (LI-COR Inc., Lincoln, Nebraska, United States) integrating quantum photometer. The light intensity was between 50 and 180 $\mu\text{mol photons m}^{-2}\text{s}^{-1}$, and block temperature was between 7 and 15°C; measurements were made at hourly intervals at the ambient light intensity and temperature for that hour.

Plant analysis

At each harvest, four whole plants per treatment were removed and destructively analysed. As treatments were not applied until May and August, plants analysed increased from 4 per species at harvest 1, to 8 at harvest 3 and 12 at harvest 6.

White clover and ryegrass plants were too small to analyse for shoot or root N concentration at 33 and 58 days after sowing. For subsequent harvests, plant material was ground (<250 μm) using a Cyclotec 1092 sample mill (Tecator, Sweden). Total N concentration was analysed using a total carbon, nitrogen, and sulphur analyser (LECO CNS-2000 elemental analyser, Australia). Sap nitrate (NO_3^- -N) was extracted using the method described by Walinga et al. (1995), and NO_3^- -N concentration was determined by ion exchange chromatography (Waters, United States), followed by automated flow injection analysis (Tecator, Sweden).

Apparent nitrogenase activity

Three 1000-ml pots of silica sand (0.5–5 mm) were sown with white clover (*Trifolium repens* cv. 'Grassland Huia'). Plants were grown in a glasshouse, where temperatures averaged 15/5°C day/night, for 8 weeks. Once germinated, plants were flushed once daily with 50 ml of a modified Hoagland's nutrient solution (Qubit Systems Inc.). A low (0.5 mM) concentration of KNO_3 was added for 10 days to encourage nodulation (Qubit Systems Inc.). Diurnal (10.00–16.00 h) apparent nitrogenase activity was measured on three pots of white clover on 6 July 1999. Apparent nitrogenase activity was measured at hourly intervals at the ambient light

intensity and temperature for the hour using a flow-through hydrogen sensor (Model NF1, Qubit Systems Inc., Kingston, Ontario, Canada) until enzyme activity had stabilised or until a maximum of 10 min had elapsed.

Statistical analysis

Data were analysed (general linear model, correlation, and regression), using the Minitab 11.12 (1996) computer program.

A model of the form:

$$y = \frac{b}{(1 + \exp\{-c(x - m_1)\}) (1 + \exp\{c(x - m_2)\})}$$

was fitted to the photosynthetic rate data for the means for each treatment group and time period.

This model can be described as a back-to-back logistic response, where: m_1 is the time of the mid point of the increasing response; m_2 is the time of the mid point of the declining response; b is a function of the maximum, which occurs where $x = \frac{1}{2}(m_1 + m_2)$; c is a measure of the rate of change during the increasing and decreasing periods (assumed to be the same); x is a measure of time of the maximum response.

RESULTS

Shoots

Dry weight increased significantly ($P < 0.05$) over time in both species (Fig. 1). White clover had a significantly lower ($P < 0.05$) dry weight than ryegrass from 123 days after sowing onwards (Fig. 1). Treatments simulating fertiliser N had no significant effect on white clover dry weight. In contrast, applying extra N to ryegrass in May caused a significant ($P < 0.05$) increase in dry weight from 157 days after sowing onwards (Fig. 1). The shoot growth rate (g day^{-1}) of white clover was significantly ($P < 0.05$) lower than ryegrass from 105 days after sowing. The greatest growth of white clover plants occurred between 140 and 172 days after sowing. In comparison, the greatest increase in growth rates occurred in May treated ryegrass plants between 105 and 140 days after sowing, whereas in control plants the growth rate increased between 140 and 180 days after sowing.

Leaf area

Leaf area in both species increased significantly ($P < 0.05$) with time (Fig. 2). Extra N did not have

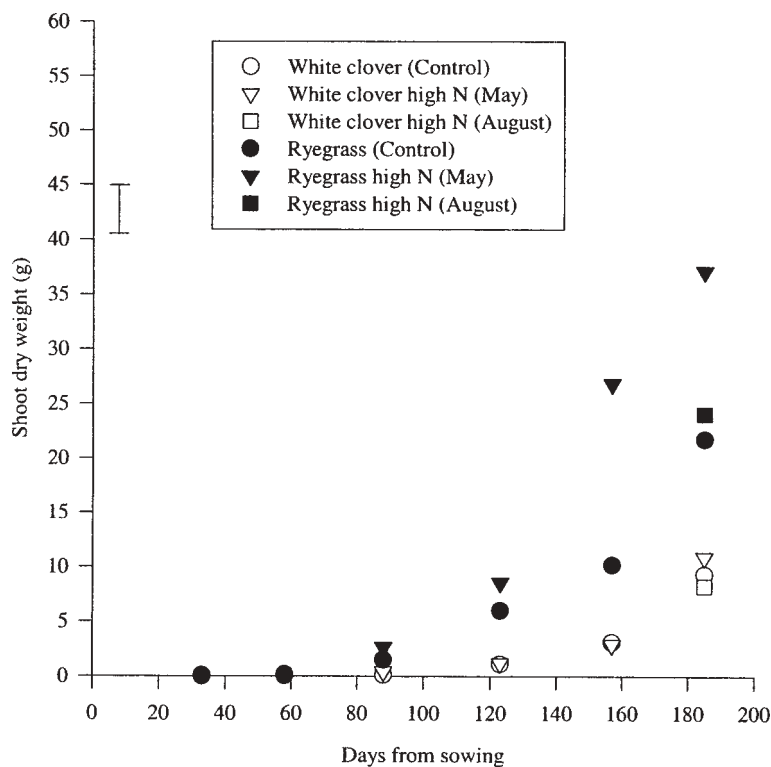


Fig. 1 Shoot weight (g) of white clover and ryegrass under three N regimes: control, 0.5 mol m⁻³ N; high N, 5.0 mol m⁻³ N from May; or high N, 5.0 mol m⁻³ N from August (LSD $P < 0.05 = 4.8$) over time (days).

a significant effect on the leaf area of white clover plants. In contrast, a May treatment to ryegrass caused a significant ($P < 0.05$) increase in leaf area compared with the control or August treatments (Fig. 2). By 123 days after sowing, May-treated ryegrass plants had a significantly ($P < 0.05$) greater leaf area than May-treated white clover. Leaf expansion followed a similar pattern to that of leaf area. However, between 157 and 185 days after sowing the daily leaf expansion of May-treated ryegrass decreased by two thirds from 0.0030 m² day⁻¹ to 0.0010 m² day⁻¹.

Shoot N

Sap NO₃⁻-N concentration in white clover was significantly ($P < 0.05$) less than in ryegrass (11 versus 21 µg g⁻¹ DW, respectively; SEM = 3.8). At the last two harvests the control white clover plants had, on average, 20% of the sap NO₃⁻-N concentration of the May-treated plants (2.26 versus 12.17 µg g⁻¹ DW, respectively; SEM = 2.6).

The average N concentration (%) was significantly ($P < 0.05$) greater for white clover than ryegrass (4.2 versus 3.8%, respectively; SEM =

0.19), but was not affected by time or treatment (data not presented).

The total amount of shoot N uptake increased significantly ($P < 0.05$) with time (Fig. 3). Total shoot N uptake in white clover was not significantly different between N treatments (Fig. 3). However, from 157 days after sowing, May-treated ryegrass plants had a significantly ($P < 0.05$) greater total shoot N uptake than the control plants (Fig. 3). A cubic regression fitted to the individual response between N uptake and shoot dry matter gave a significant ($R^2 = 96.9$, $P = 0.00$ and $R^2 = 100.0$, $P = 0.00$) relationship for white clover and ryegrass, respectively (Table 1).

Roots

The pattern of root dry weight was similar to that of the shoot dry weight (data not presented). By the end of the experiment white clover had, on average, a significantly ($P < 0.05$) lower root dry weight than ryegrass (1.86 compared with 17.25 g DW; SEM = 1.40). N treatments had no effect on root weight in white clover. Total root N followed a pattern similar to that of total shoot N, except for

Fig. 2 Leaf area (m^2) of white clover and ryegrass under three N regimes: control, $0.5 \text{ mol m}^{-3} \text{ N}$; high N, $5.0 \text{ mol m}^{-3} \text{ N}$ from May; or high N, $5.0 \text{ mol m}^{-3} \text{ N}$ from August (LSD $P < 0.05 = 0.029$) over time (days).

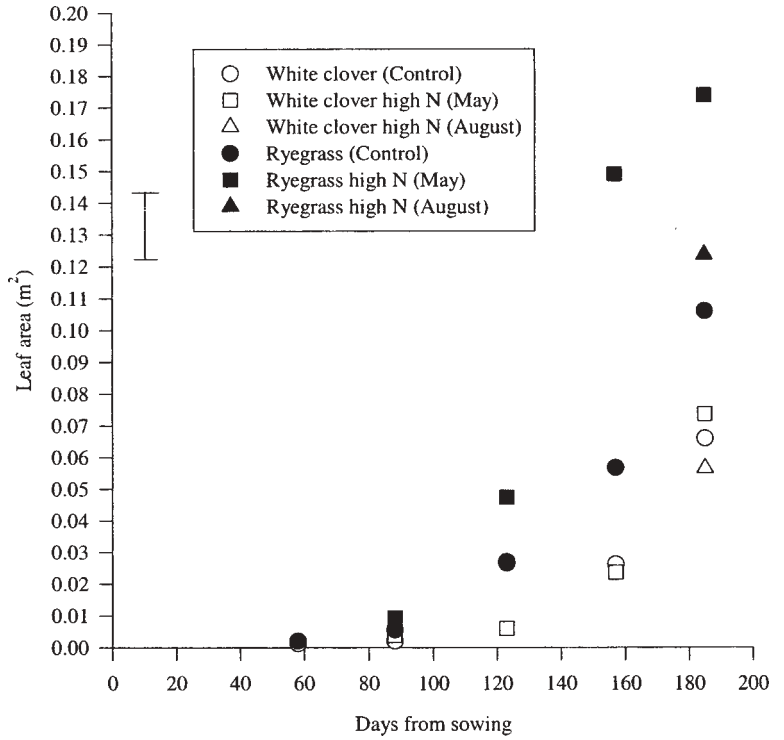
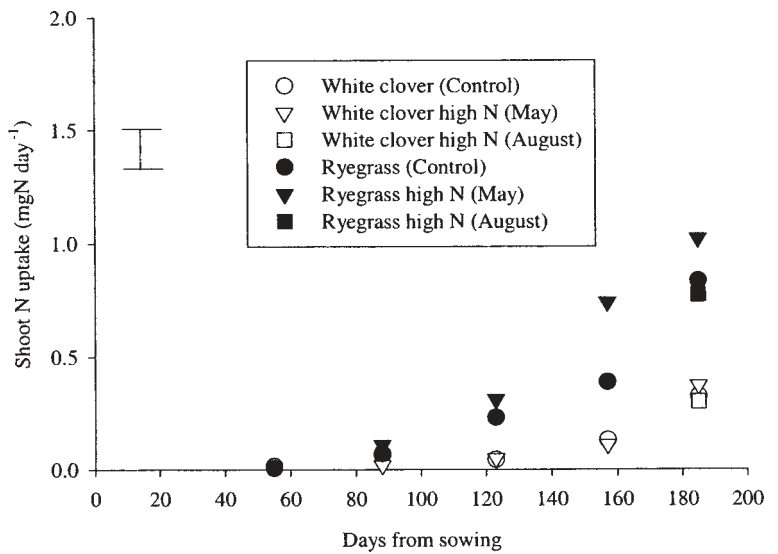


Fig. 3 Shoot N uptake (mg N day^{-1}) of white clover and ryegrass under three N regimes: control, $0.5 \text{ mol m}^{-3} \text{ N}$; high N, $5.0 \text{ mol m}^{-3} \text{ N}$ from May; or high N, $5.0 \text{ mol m}^{-3} \text{ N}$ from August (LSD $P < 0.05 = 0.07$) over time (days).



a decrease from 1.1–0.4 mg N day⁻¹ between 157 and 185 days after sowing, in the amount of total root N in May-treated ryegrass plants.

Shoot:root ratios

The shoot to root ratios of white clover showed a trend towards increased partitioning of dry weight to the shoot over time, whereas in ryegrass the reverse trend was apparent. A third order polynomial fitted to the response of shoot to root ratio to days from sowing revealed a significant regression ($R^2 > 0.97$): white clover ratio = $-1.1 + 0.08b - 0.0008b^2 + 0.0000026b^3$, and ryegrass ratio = $5.6 + 0.8b - 0.0005b^2 + 0.0000007b^3$; this cubic effect varied significantly ($P < 0.05$) between species.

Net photosynthesis

Field net photosynthetic rates

White clover tended to have a lower net photosynthetic rate than ryegrass until 185 days after sowing (Fig. 4), when net photosynthesis in white clover was greatest and showed an effect of N treatment: plants on extra N were photosynthesising significantly ($P < 0.05$) faster than control plants. In contrast, the greatest net photosynthesis rate in

ryegrass occurred at 157 days after sowing; at 185 days after sowing net photosynthesis had decreased. There was no significant effect of N treatment on net photosynthesis in ryegrass at any stage.

Diurnal variation of net photosynthesis

The net photosynthetic rate increased from 5 to 20 $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ in the control clover, and from 5 to 26 $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ in the extra N clover (Fig. 5). In contrast, the increase in ryegrass was from 5 to 42 $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$, regardless of N concentration (Fig. 5). White clover and ryegrass at both N concentrations were at their highest net photosynthetic rates between 12.00 and 15.00 h (Fig. 5). In the control N white clover, the plateau occurred an hour earlier. Estimates of model parameters (Table 2) showed no significant difference between the c values (intrinsic rate of increase), but simply in the difference in maximum potential.

Apparent nitrogenase activity

Hydrogen production (N_2 fixation) increased steadily with time until 14.00 h, after which a decrease occurred (Fig. 6). A fitted cubic curve revealed a significant ($P < 0.05$) regression

Table 1 Regressions of shoot N content with dry weight (DW), shoot growth rate (SGR), leaf area (LA), leaf expansion (LE), and photosynthesis (P/S) in white clover and ryegrass plants.

Treatment	Regression equations	R^2	P
White clover			
Control	N Uptake = $-0.830 + 31.4\text{DW}$	96.9	0.00
	N Uptake = $-0.0185 + 0.696\text{SGR}$	95.3	0.00
	N Uptake = $0.00297 + 0.208\text{LA}$	76.1	0.03
	N Uptake = $-0.000080 + 0.00478\text{LE}$	87.9	0.03
	N Uptake = $-0.148 + 0.0209\text{P/S}$	16.8	0.01
May	N Uptake = $0.302 + 30.2\text{DW}$	99.9	0.00
	N Uptake = $0.0047 + 0.71\text{SGR}$	92.9	0.02
	N Uptake = $-0.000029 + 0.189\text{LA}$	99.9	0.00
	N Uptake = $0.000164 + 0.00327\text{LE}$	91.7	0.01
	N Uptake = $-0.284 + 0.0238\text{P/S}$	99.6	0.00
Ryegrass			
Control	N Uptake = $-0.176 + 26.5\text{DW}$	100.0	0.00
	N Uptake = $-0.0022 + 0.47\text{SGR}$	99.1	0.02
	N Uptake = $0.00160 + 0.133\text{LA}$	98.9	0.00
	N Uptake = $0.000085 + 0.00215\text{LE}$	89.3	0.03
	N Uptake = $27.6 - 17.6\text{P/S}$	27.8	0.23
May	N Uptake = $-2.37 + 38.8\text{DW}$	99.6	0.01
	N Uptake = $0.043 + 0.467\text{SGR}$	95.4	0.00
	N Uptake = $-0.0057 + 0.185\text{LA}$	39.7	0.23
	N Uptake = $0.250 + 224\text{LE}$	7.5	0.38
	N Uptake = $27.8 - 4.4\text{P/S}$	0.0	0.89

Fig. 4 Net photosynthetic rate ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) of white clover and ryegrass under three N regimes: control, $0.5 \text{ mol m}^{-3} \text{ N}$; high N, $5.0 \text{ mol m}^{-3} \text{ N}$ from May; or high N, $5.0 \text{ mol m}^{-3} \text{ N}$ from August (LSD $P < 0.05 = 7.4$) over time (days).

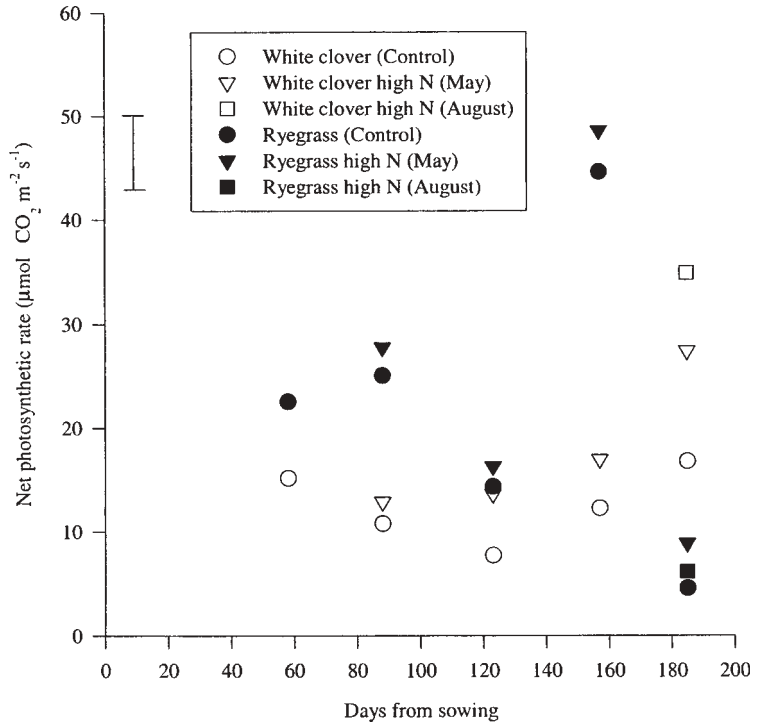


Fig. 5 Diurnal net photosynthetic rate ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) of white clover and ryegrass under two N regimes: control $0.5 \text{ mol m}^{-3} \text{ N}$; or high N, $5.0 \text{ mol m}^{-3} \text{ N}$, ($R^2 = 97$) (Table 3).

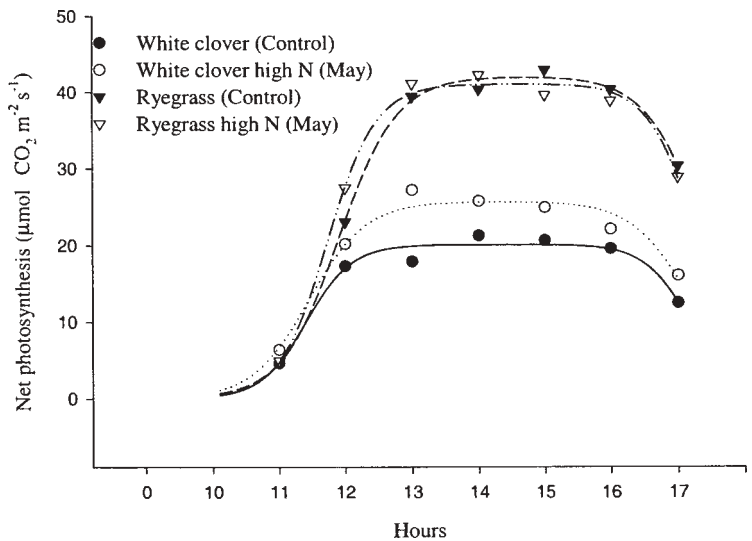


Table 2 Parameter estimates (\pm SE) for the model fitted to the means for the four treatment groups. Values sharing a common letter are not significantly different ($P < 0.05$). (b = function of the maximum, which occurs where $x = y^2 (m_1 + m_2)$; c = measure of the rate of change during the decreasing periods (assumed to be the same).

Group	b	c	m_1	m_2
White clover (Control)	20.02 (0.79) a	2.90 (0.72) a	1.408 (0.121) a	7.171 (0.111) a
White clover high N (May)	25.68 (1.17) b	2.31 (0.58) a	1.445 (0.133) a	7.183 (0.138) a
Ryegrass (Control)	42.05 (0.72) c	2.29 (0.24) a	1.908 (0.045) b	7.413 (0.067) b
Ryegrass high N (May)	41.05 (0.86) c	2.73 (0.38) a	1.834 (0.058) b	7.308 (0.075) b

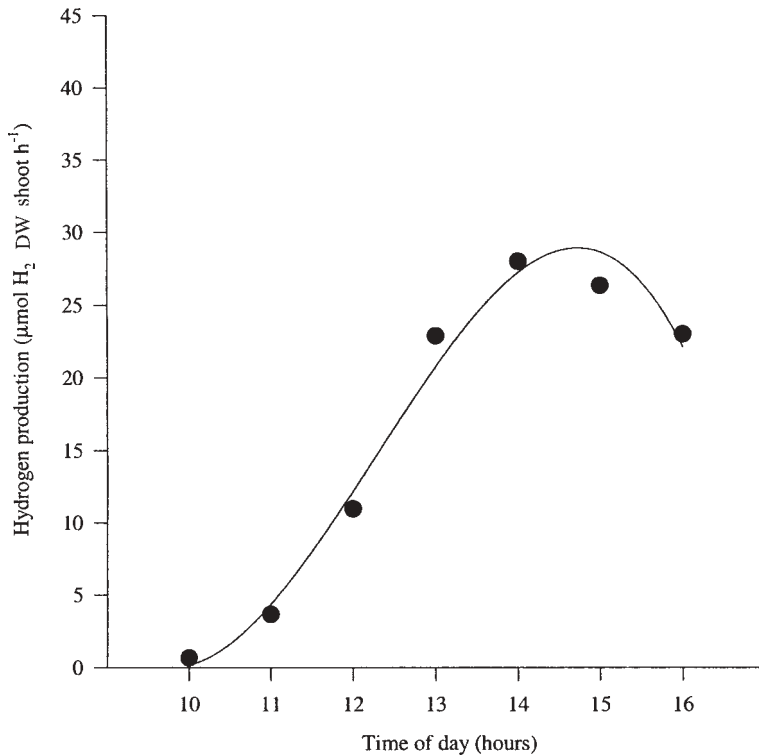


Fig. 6 Apparent nitrogenase activity ($\mu\text{mol H}_2 \text{ DW shoot h}^{-1}$) of white clover.

($R^2 = 0.98$) between time of day and the amount of hydrogen produced: (hydrogen production, $\text{H}_2 = 795.2 - 208.6b + 17.7b^2 - 0.5b^3$). Correlation analysis showed apparent nitrogenase activity was more closely related to light intensity ($r = 0.80$) than to temperature ($r = 0.50$).

DISCUSSION

Early in the trial period, when temperatures were low, white clover transferred a large proportion of newly combined C to the roots, resulting in a low shoot to root ratio, and slow dry weight gain. Furthermore, white clover dry weight did not respond to an application of N, and total shoot and root N concentrations did not differ with N treatment in the white clover plants. This suggests that N supply was able to keep up with N demand and supports the theories (Kessler et al. 1990) that N_2 fixation is able to satisfy the shoot demand for N and that N supply does not affect the growth of white clover at low temperatures.

In contrast, ryegrass retained a larger proportion of fixed C in the leaves during the early stages of growth. As temperatures increased, the balance

shifted and more C was transferred to the roots. At the last harvest, leaf expansion rates in ryegrass had decreased; a decrease in the photosynthetic rate was also recorded. A decrease in the total amount of root N was found, but total shoot N continued to increase. The decrease in total root N may have been a consequence of a change in the root metabolic activity; as soils warm up after winter root growth increases, but root death increases more (Matthew 1996). Therefore, a larger proportion of less metabolically active roots may have been present in the sample at the end of winter.

During autumn and winter, white clover invested proportionally more assimilate in the roots than shoots. Reduced investment in above-ground dry weight was reflected in a smaller leaf area in clover than ryegrass plants. White clover has been reported to have a slower leaf expansion rate than ryegrass at low temperatures (Peacock 1975; Parsons & Robson 1980). Similar results were reported by Davidson et al. (1986) and Woledge et al. (1989), working in winter and on mixed white clover/ryegrass swards. Net photosynthetic rates in the present investigation were higher than those reported by Davidson et al. (1986) and Woledge et

al. (1989), possibly because light interception by the canopy was increased in the single plants used in the present research. This suggestion is supported by results comparing spaced plants with dense white clover stands; the spaced plants exhibited a higher net photosynthetic rate than the dense stands due to increased light interception (Faurie & Soussana 1993).

Regression analysis was used in an attempt to identify the factors driving N uptake (Table 1). Although shoot dry weight had the greatest regression coefficient in both species, the relationship is confounded as dry weight is used in the calculation of shoot N content. The closest relationship which was not confounded appeared to be leaf area expansion, suggesting that leaf expansion is a controlling factor in the uptake of shoot N; this supports Davidson et al. (1986). If leaf expansion is a key determinant of plant N demand (Grindlay 1997), this study supports the view that early in the growing period, leaf expansion is likely to be a limiting factor in shoot N uptake by white clover. However, because leaf expansion is dependent upon many variables, e.g., temperature, light intensity, plant age, and N nutrition, and given that photosynthesis is the overall driver of leaf expansion, a change in one or more of the above factors may result in a decrease in the leaf expansion and photosynthetic rate by affecting photosynthesis either directly or indirectly, resulting in an imbalance between N demand and N supply. For example, in May-treated ryegrass plants, shoot total N continued to increase, but root total N decreased. Given that shoot and root N uptake continued to increase in the control and August-treated ryegrass plants but a decrease in the photosynthetic rate still occurred, there may have been an overriding environmental or physiological factor affecting photosynthesis. The decrease in photosynthetic rate of all the ryegrass plants may have been due to the amount of light received by the canopy. Early in the growing period a greater amount of light would have been able to be intercepted per unit area of the canopy and, therefore, leaf expansion would have been the main factor governing shoot N uptake and shoot growth (Woledge & Dennis 1982; Davidson et al. 1986). However, with increased tiller production, developing tillers would be shaded, and tillers produced later in the season under low light intensities have a lower photosynthetic rate (Dennis & Woledge 1982). This may be why there was no significant relationship found between leaf expansion and photosynthesis.

Research has indicated that N₂ fixation is costly to the plant as the nodules' requirement for carbohydrate for growth limits the amount of carbohydrate available for shoot growth (Marriott et al. 1988). However, at low temperatures N₂ fixation has been reported to have no effect on the overall growth of white clover (Kessler et al. 1990), although white clover plants supplied with N have shoot to root ratios higher than those in plants reliant upon N₂ fixation (Woledge & Calleja Suarez 1983; Hatch & Macduff 1991). This indicates that more carbohydrate is being partitioned to the shoot than the root in the N-supplied plants (Haystead et al. 1980).

The net photosynthetic rates obtained for white clover over a diurnal period were similar to those reported by Ryle et al. (1992), who observed that, regardless of N treatment, there was no detrimental effect of N₂ fixation on the net photosynthetic potential of white clover.

Apparent nitrogenase activity showed a diurnal response that may have been related to a change in net photosynthesis. This supports the view that N₂ fixation is primarily controlled by the availability of photosynthate in the nodules (Hardy & Havelka 1976), therefore, diurnal fluctuations in either light intensity or temperature affecting photosynthate supply will influence N₂ fixation (Eckart & Raguse 1980).

These results suggest that in white clover temperature rather than N is a limiting factor in dry weight gain. In contrast, the N response exhibited by ryegrass indicated that N supply rather than temperature affected dry weight gain. The difference in responses means that it will be difficult to develop an N fertiliser protocol that will suit mixed pastures which contain both species. In white clover the shoot to root ratio indicated an investment in the roots, not shoots, therefore, early in the season this may have resulted in white clover being disadvantaged. As photosynthesis drives leaf expansion (Grindlay 1997), the reduced photosynthetic rate of white clover over the growing season, combined with the lower photosynthetic rate exhibited by white clover compared with ryegrass over a diurnal period, resulted in white clover having a poorer leaf area expansion compared with ryegrass, and, hence, a reduced shoot N uptake and demand.

Improving the net photosynthetic rate of white clover, and hence leaf expansion and therefore N demand, may improve the competitiveness of white clover at low temperatures. This might be achieved

by increasing gross photosynthesis or reducing respiration (plant and nodules) or a combination of both. Increasing the net photosynthetic capacity of white clover and selecting for increased N₂ fixation at low temperatures would be an important step towards the development of ecologically sustainable pastures.

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