Nitrate effects on N_2 fixation, growth and feed quality of lucerne and perennial lupin

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Abstract

The effects of NO_3^- supply (0–500 kg N/ha) on total plant dry weight (DW), shoot N content and nutritional quality, and the proportion of plant N derived from the atmosphere (%Ndfa) were determined for lucerne and perennial lupin using $^{15}NO_3^-$ under glasshouse conditions. Fodder beet was used as a non-legume reference plant. In both the initial and repeat experiments, total plant DW, shoot N% and shoot nutritional quality for lucerne and perennial lupin were unaffected by NO_3^- supply. Total plant DW increased 10-fold and shoot N% tripled for fodder beet with increased N supply. In the initial experiment, the %Ndfa for lucerne decreased from 89 to 37% with increasing N supply from 0 to 500 kg N/ha, where comparable values for perennial lupin were 96 to 64%. In the repeat experiment, %Ndfa decreased from 90 to 49% and 93 to 65% for lucerne and perennial lupin, respectively, with increasing NO_3^- supply from 0 to 500 kg N/ha. Both legumes showed an increased reliance on NO_3^- with increased soil NO_3^- level, but even at 500 kg N/ha (similar to amount of N in sheep urine patch), perennial lupin obtained much of its N from N_2 fixation.

Keywords: legume, plant N, nitrate assimilation, ^{15}N isotope

Introduction

Most legumes (Fabaceae) can fix atmospheric nitrogen (N_2) *via* symbiotic bacteria (rhizobia) in root nodules and also utilise soil inorganic N (nitrate (NO_3^-) and ammonium (NH_4^+)) when available (Andrews *et al.,* 2013). There are many reports for legumes having increased reliance on soil N in comparison with N_2 fixation as soil N levels increase, but the ability of legumes to use soil N is species dependent (Barron *et al.,* 2011; Menge *et al.,* 2015).

Lucerne (*Medicago sativa*) and perennial ('Russell') lupin (*Lupinus polyphyllus*) can fix substantial levels of N_2 under suitable conditions in high-country farming systems on the South Island of New Zealand (NZ) (Black *et al.,* 2014; Berenji *et al.,* 2018). Annual yield and nutritive values are greater for lucerne than perennial lupin under optimal conditions, but the latter can grow in acidic soils with high levels of aluminium, which lucerne cannot tolerate. In grazed crops of these legumes, substantial N will be returned to the soil as animal excreta that potentially (after transformation to NO_3^-) could be leached from the soil into waterways (Andrews *et al.,* 2007; Che *et al.,* 2018). The ability of lucerne and perennial lupin to utilise soil N, and the effect of soil N on N_2 fixation could be important factors determining inputs and losses from the system. For example, if legumes can utilise substantial soil NO_3^- and, as a result, their N_2 fixation decreases, this would reduce N input into the system and should be a factor considered in nutrient budgeting models of the system. On-farm surveys of the proportion of plant N derived from the atmosphere (%Ndfa) for lucerne in Australia ranged from 17–90% with averages of 60–65% (Yang *et al.,* 2011; Peoples *et al.,* 2012). The sources of soil N taken up were not identified. The ability of perennial lupin to utilise NO_3^- and the impact of soil N/NO_3^- on its %Ndfa has not been tested.

Here, the effects of NO_3^- supply (0–500 kg N/ha) on total plant dry weight (DW), shoot N content and nutritional quality, and the proportion of plant N derived from the atmosphere (%Ndfa) were determined for lucerne and perennial lupin using $^{15}NO_3^-$ under glasshouse conditions. Fodder beet (*Beta vulgaris*) was used as a non-legume reference plant. The objectives of the following study were to determine the ability of perennial lupin and lucerne to utilise soil NO_3^-, and the impact of soil NO_3^- on growth, N_2 fixation and nutritional quality of two legume species.

Materials and Methods

Experimental procedures

Seed of lucerne *cv.* Force 4 and fodder beet *cv.* Brigadier was obtained from Seed Force Ltd, Christchurch, NZ. Perennial lupin seed was obtained from Gavin Loxton, Sawdon Station, Lake Tekapo, South Island, NZ.

The initial and repeat experiments were carried out in separate parts of the same glasshouse, and the setup, design and measurements taken were the same. Seed was sown from 13–15 March 2019 into 45 cm tall × 15 cm diameter tubes ('pots'; 20 seeds/pot) with mesh at the base containing 5 l of N-free potting mix comprised of 80% composted bark and 20% pumice (1–4 mm) to which was added 1 g/l agricultural lime (primarily

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calcium carbonate), 0.3 g/l superphosphate (9% P, 11% S, 20% Ca; Ravensdown, NZ) and 0.3 g/l Osmocote (6 months, 0% N, 0% P, 37% K), 0.3 g/l Micromax trace elements and 1 g/l Hydraflo, all three obtained from Everris International, Geldermalsen, the Netherlands. The pH of the medium was 5.8. All lucerne and perennial lupin pots were watered by weight to field capacity every 3 days with a low NO_3^- supply (0.5 mM KNO_3) until the first cut on 14 May. Commercial peat-based rhizobia inoculum for lucerne and perennial lupin (Nodulaid BASF, Canberra, Australia) was mixed with water into a slurry and applied at 5 ml/pot for the first three waterings. All legumes were nodulated at harvest. Fodder beet received 0.5 mM NO_3^- until 4 April then 2 mM KNO_3 until 14 May, due to the plants showing N deficiency symptoms. Saucers were placed under each pot to collect leachate which was returned to the pots throughout the experiment. Plants were thinned out to 10 per pot for lucerne and five per pot for perennial lupin and fodder beet two weeks after sowing.

Lucerne and perennial lupin were cut to 4 cm in height using scissors on 14 May to simulate grazing. After this, the different rates of N were applied: 0, 25, 50, 100, 200 and 500 kg N/ha as $K^{15}NO_3$ labelled at 10 atom% in 100 ml of water for all treatments. The 500 kg N/ha was assumed to be similar to that within a sheep urine patch (Monaghan *et al.,* 1989; Marsden *et al.,* 2016). Thereafter, all pots were watered (tap water) by weight to field capacity every 3 days until harvest on 12 June and 20–21 June for the initial and repeat experiments, respectively. The temperature in the glasshouse ranged from 14 to 28°C during the experiments.

At harvest, plants from all pots were divided into shoot and root, dried at 60°C for 7 days then weighed. Shoot and root material was then ground, and total N content of 0.2 g samples of roots was determined using a CN elemental analyser (Elementar VarioMax CN Elemental Analyser, GmbH, Hanau, Germany). The ground shoot material was analysed for $^{15}N/^{14}N$ with a Sercon (Crewe, UK) GSL (gas, liquid, solid) elemental analyser attached to a Sercon 20–22 isotope ratio mass spectrometer. The %Ndfa was determined via the ^{15}N isotope dilution method (Unkovich *et al.,* 2008) as:

%Ndfa legume = (1 – atom%^{15}N excess N_2 legume/atom%^{15}N excess reference plant) x 100.

The nutritional quality of the lucerne and perennial lupin (dry matter digestibility (DMD), crude protein (CP) and metabolizable energy (ME)) was determined on ground shoot material using near infrared spectroscopy (NIRS; FOSS NIRSystems 5000, FOSS NIRSystems Inc., Laurel, MD, USA).

Experimental design and data analysis

The initial and repeat experiments were conducted as a fully randomised design with three replicate pots per N treatment for all three species. A two-way analysis of variance (ANOVA) was carried out on all data with plant species and N rate as fixed factors. All effects discussed had an F ratio with a probability $P<0.01$. Regression analysis was carried out on the data for fodder beet, and a quadratic model used with R^2 given. For legumes, exponential models were fitted to the %Ndfa data.

Results

The main effects were the same for the initial and repeat experiments. Results for the initial experiment are presented in Figures 1 and 2. Total plant DW was greater and shoot to root DW ratio (S:R) was lower for perennial lupin than lucerne regardless of N supply (Figure 1a, b). For both legume species, neither total plant DW nor S:R were affected by N supply. For fodder beet, total plant DW increased 14-fold and S:R three-fold with increased N supply from 0 to 500 kg N/ha. Total plant DW was less for fodder beet than for perennial lupin or lucerne at 0–25 kg N/ha, but greater for fodder beet than the two legumes at 200–500 kg N/ha. S:R was greater for fodder beet than the two legumes, regardless of N supply.

Shoot %N was greater for lucerne than perennial lupin at all N levels, but for both species was unaffected by N supply (Figure 2a). Shoot %N for fodder beet increased four-fold with higher N supply from 0 to 500 kg N/ha. Values were lower for fodder beet than perennial lupin or lucerne at 0–100 kg N/ha, but greater for fodder beet than perennial lupin and similar for fodder beet and lucerne at 500 kg N/ha. In the initial experiment, the %Ndfa for lucerne decreased from 89 to 37% with increasing N supply from 0 to 500 kg N/ha: comparable values for perennial lupin were 96 to 64% (Figure 2b). In the repeat experiment, %Ndfa decreased from 90 to 49% for lucerne and 93 to 65% for perennial lupin with increasing NO_3^- supply from 0 to 500 kg N/ha.

Nutritional quality of the legumes was unaffected by N supply. Across both experiments, values were 71–73% DMD, 29–30% CP and 11–12 MJ/kg DM ME for lucerne and 76–78% DMD, 23–25% CP and 11 MJ/kg DM ME for perennial lupin. This represented high nutritional quality for both species (Machado *et al.,* 2007; Ryan-Salter 2019) and indicated that increased reliance on NO_3^- with decreased N_2 fixation did not affect the nutritional quality of the shoots.

Discussion

The results indicated that both lucerne and perennial lupin have increased reliance on NO_3^- nutrition with increased soil NO_3^- level. Increased uptake of soil NO_3^-

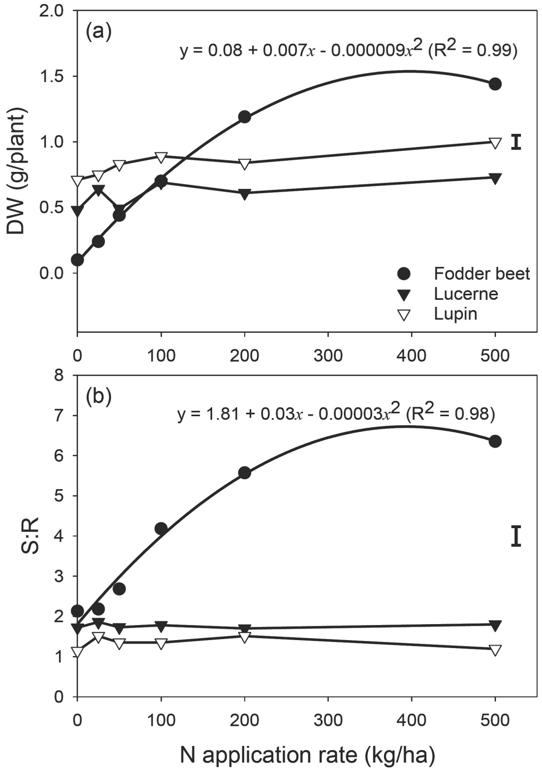

Figure 1 The effect of nitrogen (N) supply as nitrate on (a) total plant dry weight (DW) and (b) shoot:root DW ratio (S:R) for lucerne, perennial lupin and fodder beet. Vertical bars indicate SE obtained from the ANOVA.

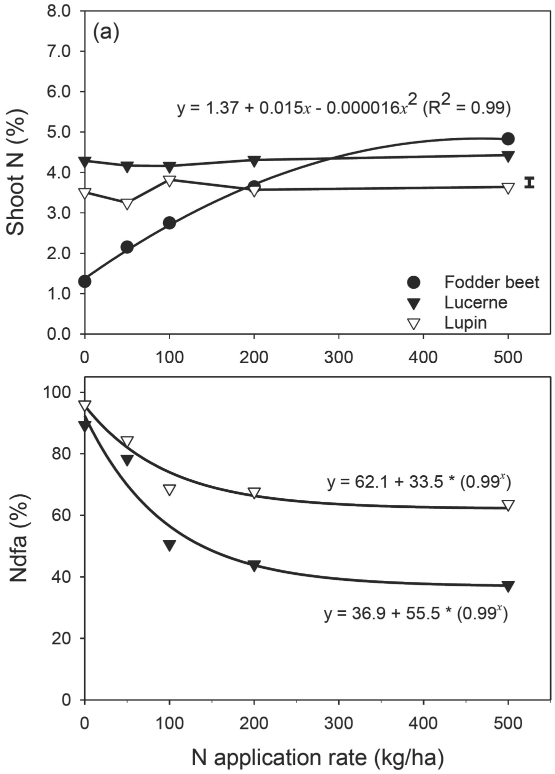

Figure 2 The effect of nitrogen (N) supply as nitrate on shoot N content of lucerne, perennial lupin and fodder beet, and the proportion of total plant N derived from the atmosphere (%Ndfa) for the two legumes. Vertical bar indicates SE obtained from the ANOVA

was matched by a similar size decrease in N_2 fixation, such that total plant N and DW changed little with N supply. In contrast, fodder beet, the control plant, showed substantial increases in total plant DW, S:R and tissue N content with increased supply, as would be expected for most non-legume species (Andrews *et al.,* 2013). At comparable NO_3^- supply, the %Ndfa was greater for perennial lupin than lucerne and, even at 500 kg N/ha (similar to a sheep urine patch), perennial lupin derived the major proportion of its N from N_2 fixation. This indicated that, under grazing in the field, perennial lupin will still maintain high levels of N_2 fixation, but this needs to be further tested on mature plants under field conditions.

Ryan-Salter (2019) carried out a $^{15}NO_3^-$ experiment similar to that described here, and reported that %Ndfa was 38% for perennial lupin and 26% for lucerne at 600 kg NO_3^--N/ha. These results were lower than predicted from the values obtained in the current study, but can be explained, at least in part, by the use of a non-legume reference plant in the current experiment, but not in the Ryan-Salter (2019) study.

On a per pot basis (≡ area), dry matter growth was greater for lucerne than perennial lupin as there was twice as many plants per pot (10) with lucerne. This was more indicative of the field situation in high-country farming systems on the South Island of NZ, where dry matter yields/area/annum are likely to be greater for lucerne than perennial lupin.

At pot level, the amount of N_2 fixed was greater with lucerne than perennial lupin at 0–100 kg N/ha, but similar for the two species at 200 and 500 kg N/ha, due to a greater decrease in N_2 fixation associated with a higher increase in NO_3^- assimilation for lucerne. Scaling this to field level, the range of values for %Ndfa for lucerne in pots, 90–37%, was in the range quoted for lucerne on farms in Australia (Yang *et al.,* 2011; Peoples *et al.,* 2012). Yang *et al.* (2011) estimated annual average N_2 fixation of 322 kg N/ha/annum with uptake of 181 kg N/ha/annum from the soil. The sources of soil N were not identified, but nitrate reductase (a substrate (NO_3^-) induced enzyme) was substantially greater in lucerne shoots than in associated species, which indicated that it assimilated substantial NO_3^- in the shoot.

Overall, the current results obtained, and in the literature, indicated that lucerne has a high capacity to utilise soil NO_3^-. This uptake and its assimilation resulted in decreased N_2 fixation, and these responses were of a magnitude that should be considered when modelling N inputs and losses from lucerne systems. The results indicated that perennial lupin has a lower ability to utilise soil NO_3^- and the effect of soil NO_3^- on its N_2 fixation is less. Further studies are required to confirm this in the field.

Conclusions

Both lucerne and perennial lupin showed more reliance on soil NO_3^- with increased soil NO_3^- level. Higher uptake of soil NO_3^- was matched by a similarly sized decrease in N_2 fixation, such that total plant N and DW changed little with N supply. At comparable NO_3^- supply levels, perennial lupin utilised less soil NO_3^- than lucerne. Even at 500 kg N/ha (about equal to N in sheep urine patches) perennial lupin obtained the major proportion of its N from N_2 fixation. Nutritional quality of the legumes was unaffected by N supply.

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