Lucerne (*Medicago sativa* L.) dry matter, root growth and nodulation in response to lime and phosphorus in an acid, high country soil in New Zealand

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Abstract
Soil acidity is the main constraint for lucerne establishment and growth in high country regions of New Zealand. Root growth is suppressed in these aluminium-rich acid soils. In this experiment, lucerne was grown in PVC columns (0.8 m long, 0.15 m diameter), filled with soil collected from the high country overlaying gravel at 0.3 m, to mimic the actual soil profile. Nitrogen (rhizobium peat inoculant, 50 kg N/ha, and control), lime (0, 0.5, 1, 2 and 4 t/ha), and phosphorus (0 and 250 mg P/kg soil) responses were measured from a split-split plot experiment at Lincoln University. The soil pH (H_2O) was elevated from 5.4 to 5.5, 5.6, 6.1, and 6.7 by the lime rates, respectively. Shoot dry matter (DM) was increased from 27 to 551 g/m^2 by lime application and from 127 to 464 g/m^2 by rhizobium inoculant. Root DM was increased by the three way interaction effect of lime, phosphorus and nitrogen. Root volume and total length were higher, with added lime, in the rhizobium treatment than with inorganic N. There was no difference between applying 50 kg N/ha and control on shoot or root DM yield. The number of nodules was increased by lime in the inoculated treatment from 11 to 150 per column. The applied phosphorus without lime, was not used by plants and was fixed by Al in soil. The application of half a tonne of lime with P reduced Al to 1.8 mg/kg, elevated the soil pH (H_2O), and more P became available. Results indicate the importance of elevating soil pH for phosphorus availability and nitrogen fixation rather than applying P and N fertilizer, to overcome soil constraints.
Key Words
alfalfa, soil acidity, aluminium toxicity, lime, nodulation, phosphorus

Introduction
Aluminium toxicity is a major factor limiting crop production and yield in acid soils worldwide (Foy, 1988). In New Zealand low soil pH coupled with toxic levels of soil aluminium and low phosphorus limit establishment and maintenance of legumes on about 500 000 ha of farmed high country (Moir and Moot, 2010). Reduction in nodulation and root growth combine to severely inhibit lucerne survival, nitrogen fixation and production in these regions (Humphries *et al.*, 2008). Liming is often recommended for these low pH soils (Edmeades *et al.*, 1983). Most of the lime response by lucerne has been attributed to improved nodulation (Munns, 1965).
Most research has focused on aboveground traits with relatively little attention being paid to below ground processes such as root dynamics. In this experiment, Lucerne plants were grown in 0.8 by 0.15 m PVC columns to focus on the impact of different lime and phosphorus rates on lucerne dry matter, root growth and nodulation in an acid high country soil.

Methods
Collecting soil
Soils were collected from the high country overlaying gravel at 0.3 m of a paddock in Mt Pember Station in the Lees Valley (high country marginal site, 43˚, 07', 15.60″ S and, 172˚, 11′, 48.27″ E), North Canterbury. The soil is a high country Brown stony soil (USDA classification: Inceptisol) where no fertilizers had been added. The texture of the surface soil is silt loam. Soils were passed through a 4 mm sieve and homogenized by mixing separately and plant residues separated. Few stones remained after sieving the soil. Soil analysis for macro and micro-elements showed phosphorus and calcium deficits (12 mg/l, and 1.3 Mol/kg/100g soil, respectively), low pH= 5.4 (1:2 (v/v) soil: water), and high aluminium levels (15.1 mg/kg soil).

Fertilizer and treatments
Lime treatments (five levels as lab-grade $CaCO_3$ equivalent to 0, 0.5, 1, 2 and 4 t/ha) were added to plastic bags containing 5.1 kg soil for each tube and mixed, four weeks before sowing the seeds. Phosphorus was applied as [Ca $(H_2PO_4)_2.H_2O$], two levels (0 and 250 mg P/kg soil) containing 24.6% P (w/w), was added to the soil in the plastic bags and mixed one day before sowing.

Preparing P.V.C columns

The design of the columns was adapted from (LeNoble *et al*., 1996) and (Vaughan *et al*., 2002). Columns were cut uniformly from polyvinylchloride (P.V.C) pipe. Each column was 0.15 m diameter and 0.8 m long, with a wall thickness of 4 mm. A plastic pot (0.2×0.15 m) was put in the bottom of each column to allow drainage. The columns then filled with 0.3 m of stone from commercial source with same parent material, and the soil from Lees Valley (5.1 kg soil provided as lime and P treatments, in plastic bags) in top 0.3 m above, to mimic the actual Lees Valley soil profile.

Lucerne seeds were sown on 29th of March 2011, equivalent to 14 kg/ha. Standard peat-based inoculants for the field trials were prepared. A specific rhizobium strain for lucerne (*Ensifer meliloti*) was used for inoculation. This experiment was conducted in the clover cage facility at the Nursery, Lincoln University. After 15 days, seedlings were thinned to 20 plants per column. The columns were watered after measuring moisture in the top 20 cm of soil with a TDR (Trace system, Soil Moisture Equipment, Santa Barbara, CA, USA) weekly, to keep the soil moisture at least at 50% of field capacity.

Harvesting

Plants from each column were completely removed from the pot with the soil and stones separated carefully from the roots. Plants were carefully washed to prevent root damage and losses. Nodulation was assessed on the plants of each column according to the criteria modified from Rice *et al*., (1977). The roots were subsequently separated from shoots and stored in the refrigerator at 4°C for later root analysis with the commercial software package WinRHIZO (Regent Instruments Inc, Quebec, Canada). Root and shoot dry matter were determined after drying at 70°C for 48 h.

Results and discussion

Shoot and Root DM

The soil pH (H_2O) was elevated from 5.4 to 5.5, 5.6, 6.1, and 6.7 by the 0.5, 1, 2 and 4 t/ha lime rates, respectively. Shoot dry matter (DM) was increased from 27 to 551 g/m^2 with lime application (P<0.001), (Fig.1). Shoot DM was also increased from 127 in control to 464 g/m^2 by rhizobium inoculation ($P\leq$ 0.009). Shoot DM for the control was 202 g/m^2 compared with ($P\leq$ 0.064) 319 g/m^2 where P (250 mg/kg soil) was applied.

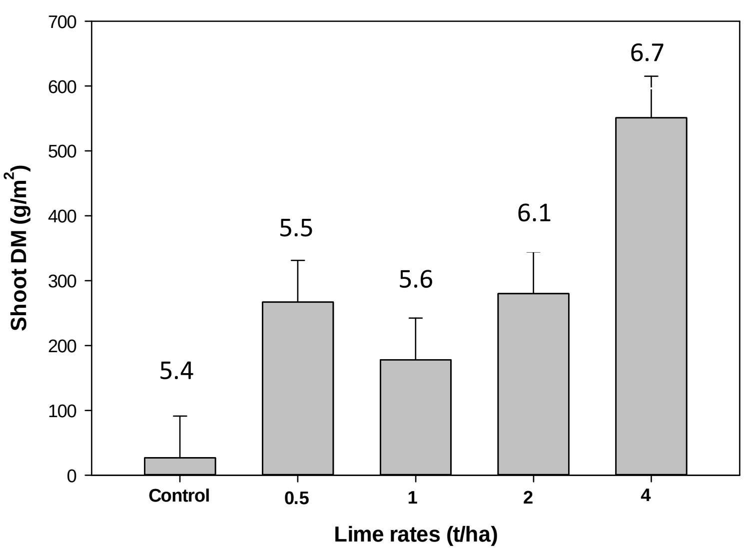

Figure 1. The effect of lime rates on shoot DM of lucerne. Number on bars shows the soil pH.

Root DM was affected by the three way interaction effect of lime, phosphorus and nitrogen treatments ($P\leq$ 0.017), increasing from 0 in control to 1170 g/m^2 in 2 t/ha lime+rhizobia inoculation+P treatment. Root DM was also increased by lime application and rhizobia inoculant (P<0.001) from 40 g/m^2 in control to 1025

g/m^2 in 2 t/ha lime treatment (Fig. 2). There was no difference between applying 50 kg N/ha and the control on shoot or root DM of lucerne. Total root length was increased from 0.44 m in the control to 39.8 m, by lime rates and rhizobia inoculant ($P \leq 0.011$). Root volume was increased from 0 in control to 41 cm^3, with lime rates and rhizobia inoculant ($P < 0.001$). Shoot DM had a positive relationship ($R^2 = 0.625$) with root hair growth (root diameter < 0.5 mm). The positive relationship between lucerne root branching and higher forage yield has been reported (Saindon *et al.*, 1991).

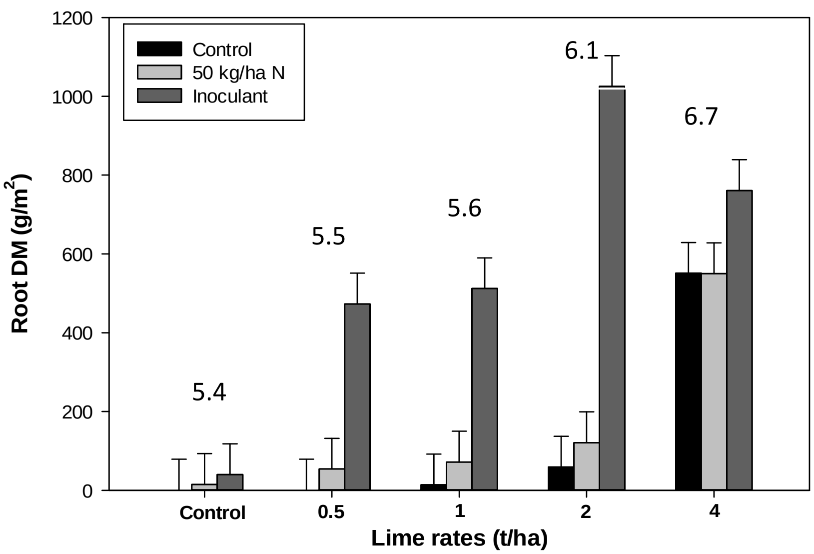

Figure 2. The effect of lime rates on Root DM of lucerne. Number on bars shows the soil pH.

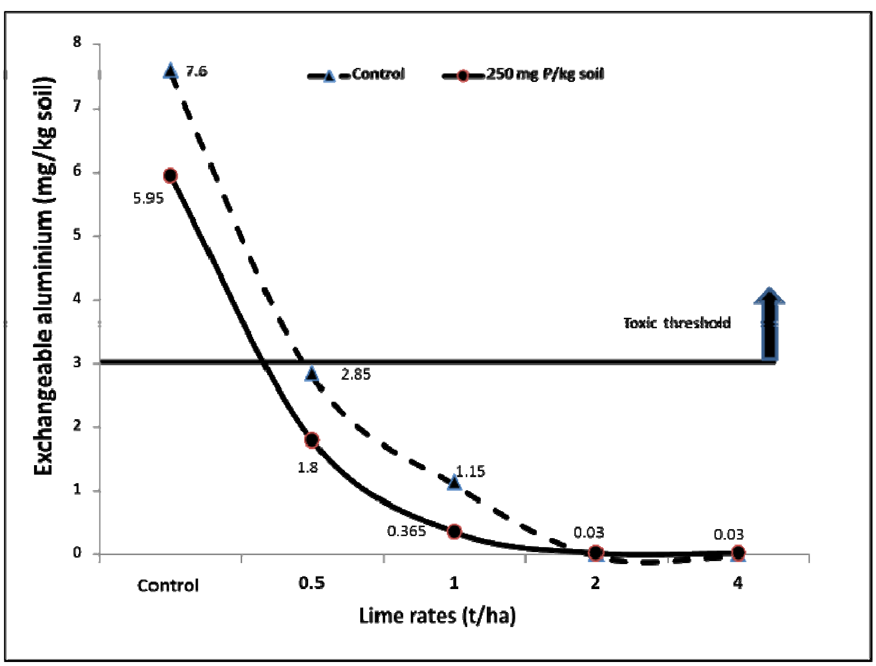

Figure 3. The relationship between exchangeable soil aluminium, phosphorus and lime treatments. The soil pH (H_2O) was elevated from 5.4 to 5.5, 5.6, 6.1, and 6.7 by the lime rates, respectively.

Nodulation

Number of nodules in rhizobia inoculation treatment was increased with lime rates (*P*$<$ 0.001), from 11 (pH 5.4) in control to 39 (pH 5.5), 41 (pH 5.6), 137 (pH 6.1) and 150 (pH 6.7) nodules/pot respectively. Positive relationship (R^2=0.84) was found between the number of nodules/pot and the soil pH. Most of the nodules were found on fine roots of lucerne in our experiment. Viands et al., (1981), also reported that nodulation primarily occurs on fibrous roots of Lucerne. *Ensifer meliloti*, has been reported as the most acid sensitive microbial symbiant, within the genus Rhizobium (Vincent, 1970). Its numbers decline sharply with increasing soil acidity (Rice et al., 1977) which is a major factor contributing to the nodulation failure.

Soil test results of different lime rates showed that exchangeable Al levels was associated (R^2= 0.54) with soil pH (H_2O). Soil exchangeable Al was low within the soil pH (H_2O) range of 5.6 - 6.7. Below this range Al levels increased sharply to 7.6 mg/kg soil at soil pH (H_2O) of 5.4 (Fig. 3). Similar results have been reported from a field trial at the Lees Valley (Moir and Moot, 2010). At soil pH (H_2O) of 5.4, Olsen P was 36 mg/l and increased to 51 at pH (H_2O) of 5.5. This suggests that added P was partially fixed by Al at pH (H_2O) =5.4, resulted in reduced exchangeable Al from 7.6 to 5.9 mg/kg soil (Fig. 3). Phosphorus was directly affected by soil pH. The applied phosphorus without lime was not used by plants and was fixed by Al in soil. The application of half a tonne of lime with P, reduced Al to 1.8 mg/kg (Fig.3), elevated the soil pH (H_2O), and more P became available. Results indicate the importance of elevating soil pH for phosphorus availability and nitrogen fixation rather than applying P and N fertilizer, to overcome soil constraints.

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