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# LIME, PHOSPHORUS AND SULPHUR RESPONSE OF FRENCH SERRADELLA

## (Ornithopus sativus) GROWN IN AN ACID UPLAND SOIL

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### Abstract

Soil acidity severely restricts legume persistence and growth in grazed upland agriculture in New Zealand. An alternative and potentially acid tolerant forage legume, French serradella (Ornithopus sativus), was examined in a climate controlled experiment. Plants were grown for 48 weeks in an acid ( $pH_{H20}$  4.9) upland soil and shoot yield measured every 8 weeks. Treatments were fully replicated combinations of lime (CaCO<sub>3</sub>; 0, 2, 4 or 8 t ha<sup>-1</sup>), phosphorus (P; 0, 50, 150 or 500 mg P L soil<sup>-1</sup>) plus various controls. Shoot yield varied significantly between lime treatments (P < 0.001), but were not strongly affected by P rate. Importantly, yields on the unlimed control treatments were 85% of maximum yield, suggesting that high yields are potentially achievable on even very acid soils. French serradella grew 16.9 g DM pot<sup>-1</sup> compared to 5.3 g DM pot<sup>-1</sup> for the commonly grown reference species, subterranean clover (Trifolium subterraneum). French serradella showed significant potential as a new pasture legume suitable for acidic upland soils.

Key words: French Serradella, Soil acidity, Yield, Lime, Phosphorus.

## **1. INTRODUCTION**

Pasture legumes are critical to New Zealand (NZ) upland systems as the provider and sole source of nitrogen for sward growth, through the process of biological atmospheric nitrogen ( $N_2$ ) fixation. Addition of nitrogen into the system stimulates resident grasses and increases both the quantity and quality of herbage produced from the grasses and introduced legumes (Matthews et al., 1999). Improved feed quality, from increased sward legume content, results in substantial increases in animal production, increasing the productivity and profitability of the overall farm system. The extensiveness of upland farming means it is uneconomic to apply expensive nitrogen fertilisers, hence legumes are vital for the addition of nitrogen to this habitually N-deficient soil/plant/animal system.

The upland soils of NZ are typically acidic (pH 4.8 - 5.5) and have low fertility with deficiencies of nitrogen (N), phosphorus (P), sulphur (S) and molybdenum (Mo). Associated with low soil pH, these acidic soils have high levels of soluble aluminium (Al). Many pasture legumes are strongly affected by Al toxicity (Moir and Moot, 2010). The upland environment is also characterized by low winter temperatures and seasonal moisture deficits making it an extremely challenging environment for pasture growth (Wangdi et al., 1990; Matthews et al., 1999). As such, the growth and persistence of traditional pasture legumes such white clover (*Trifolium repens L.*) and lucerne (*Medicago sativa L.*) are often very limited by the soil acidity, fertility and climatic conditions in this environment (Scott et al., 1995). Therefore there is a critical need for alternative legume species which are better adapted to acid soils with low soil P and that can survive summer dry environments.

Legumes identified as potentially being suitable for upland pastures include French serradella (*Ornithopus sativus*) and Russell lupin (*Lupinus polyphyllus*), as they anecdotally have the ability to grow on acidic soils lacking phosphate (Wangdi et al., 1990; Hill, 1993). There have been some reports of French serradella performing well in deep, infertile acid sandy soils in South Australia (Loi et al., 2005; Nichols et al., 2007). This suggests some potential to grow in the low fertility, upland acid soils of NZ. It is known that the establishment, growth and persistence of traditionally sown subterranean clover (*Trifolium subterraneum*) in upland NZ soils country is strongly limited by soil pH, fertility and climate. Comparing subterranean clove to a 'new generation' pasture legume species would therefore provide a suitable relative growth comparison.

The objective of this study was to quantify how lime, P and S inputs affect the yield of French serradella. The same responses were also investigated for subterranean clover, included as a reference species. The species were grown in a typical acid upland soil from 'Armidale Station' in Central Otago, NZ. It was hypothesised that yield would differ within and between species as they would exhibit different yield responses to variable soil pH available P and S status.

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# 2. MATERIALS AND METHODS

## 2.1 Soil collection and preparation

A total of 2.5 tonnes of soil was collected from 'Armidale Station', an upland farm in Central Otago, New Zealand ( $45^{\circ}09'24.39''S$ ,  $169^{\circ}51'29.30''E$ ). The soil is a Pukerangi moderately deep silt loam, classified as an Argillic soil (Immature Pallic soil, Hewitt, 1998; Udic Haplustepts, Soil Survey Staff 1998). The altitude is 850 masl with a mean annual rainfall of 450 - 500 mm. Previous fertiliser history on the site includes an application of 300 kg sulphur super in 1998, there were no further fertiliser applications until December 2011 when 250 kg of Mainland Minerals 'Mainphos' was applied to the bottom third of the block. Soil tests were performed in July 2011 which indicated that pH and Olsen P were low, at 5.2 and 13, respectively.

The field site was sampled on October 16 2012 (Figure 1). Bulk soil was collected (0-0.2 m depth) at several locations on the hill block. The soil was transported back to Lincoln and then passed through a 4 mm sieve (field moist) to remove all plant material. Ten sub-samples of soil were collected from the now homogenised bulk sample of field moist soil and then air dried at 30°C. Soil chemical analyses were conducted on the soil samples prior to treatments being added and seed sowing.



Figure 1. Collecting the soil from an upland pasture site, Central Otago, New Zealand.

# 2.2 Soil chemical analysis

Soil fertility analyses were conducted on the field soil and the results are presented in Table 1. Soil pH was measured using a water: soil ratio of 2.5:1 (Blackmore et al., 1987). The amount of available soil P was determined using the method of Olsen et al. (1954), and phosphate retention was measured using the methods of Blackmore et al. (1987) and Saunders (1965). The level of extractable sulphate in the soil was measured using the method of Schollenberger and Simon (1945), and cation exchange capacity was determined using the method of Hesse (1971). The Dumas method of combustion (Horneck and Miller, 1998) was used to determine the total carbon and nitrogen contents,

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using as Elementar 'Vario' MAX CN Analyzer (Elementar Analysensystane, GmbH). Exchangeable aluminium was measured using the 0.02 CaCl<sub>2</sub> extraction method (Edmeades et al., 1983) then measured by ICP-OES (Varian 720-ES ICP-OES; Varian Inc., Victoria, Australia). Reserve magnesium was determined using the method of Metson and Brooks (1975) and reserve potassium was measured using the methods of Blackmore et al. (1987) and Metson et al. (1956; 1968). Anaerobic mineralisable N was measured using a modified method of Waring and Bremner (1964) and Keeney and Bremner (1966).

Soil Property	Value
рН	4.9
Olsen P	$20 \text{ mg L}^{-1}$
Sulphate S	$5 \text{ mg kg}^{-1}$
Organic S	$1 \text{ mg kg}^{-1}$
Exchangeable Ca	2.7 me 100 g <sup>-1</sup>
Exchangeable Mg	$0.96 \text{ me } 100 \text{ g}^{-1}$
Exchangeable K	$0.65 \text{ me } 100 \text{ g}^{-1}$
Exchangeable Na	0.22 me 100 g <sup>-1</sup>
CEC	$16 \text{ me } 100 \text{ g}^{-1}$
Base Saturation (Total)	29.2 %
Exchangeable Al	15.7 mg kg <sup>-1</sup>
Total C	4.2 % w/w
Total N	0.27 % w/w
C:N Ratio	15:1
Organic Matter	7.1 % w/w
Mineralisable N	130 kg ha <sup>-1</sup>
Anion Storage Capacity	41 %
Reserve K	4.7 me 100 g <sup>-1</sup>

Table 1. Initial soil test values for the upland Argillic Pallic soil used in this experiment

### 2.3 Experimental design and management

The pot trial was conducted at the glasshouse facility, Lincoln University, New Zealand. During the plant establishment period (November 2012 to March 2013) the pots were kept in glasshouse conditions (Figure 2), before being moved to tables outside the glasshouse in late March 2013 (Figure 3). The pot trial examined two species of pastoral legumes, with the main focus being French serradella (*Ornithopus sativus* cv. 'Grasslands Koha'). Subterranean clover (*Trifolium subterraneum*, cv. 'Denmark') was included as reference legume species.

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Figure 2. Pot trial set up at Lincoln University, February 2013. Species are left, French serradella (*Ornithopus sativus* cv. 'Grasslands Koha') and right, Subterraneum clover (*Trifolium subterraneum*; cv. 'Denmark').



Figure 3. Moving the pot trial to tables outside the glasshouse, Lincoln University March 2013.

A full factorial design was used as the basis of the experiment. Lime treatment rates were 0 (L0), 2 (L1), 4 (L2) or 8 (L3) t lime ha<sup>-1</sup> (as CaCO<sub>3</sub>) and P rates were 0 (P0), 50 (P1), 150 (P2) or 500 (P3) mg P kg<sup>-1</sup> soil (as Ca(H<sub>2</sub>PO<sub>4</sub>)<sub>2</sub>). Sulphur was applied to all treatments (excluding the 0S treatment) at a rate of 120 kg S ha<sup>-1</sup> (as CaSO<sub>4</sub>). An 'All' nutrient treatment was also included, which received a semi-complete (nil N) nutrient solution (North Carolina State University: Booking, 1976; Caradus and Snaydon, 1986). The key legume at the focus of the experiment, French serradella had 22 treatment combinations, as presented in Table 2. The reference species, Subterranean clover, had very similar but fewer treatments (13 treatments). All treatments were replicated four times for French serradella and three time for subterranean clover.

	PO	P1	P2	P3
LO	LOPO (+/- S)	LOP1	LOP2	LOP3
L1	L1P0	L1P1	L1P2	L1P3
L2	L2P0	L2P1	L2P2	L2P3
L3	L3P0	L3P1	L3P2	L3P3
- S	L2P0	L2P1	L2P2	L2P3
ALL	Complete + T.E. (L2)			

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Table 2. Experimental design outlining different treatment level combinations for French serradella.

Large pots, 5 L in volume were used. Field moist soil (4 L) was measured for each pot and the appropriate lime, phosphorus and sulphur treatment was weighed and added to the bag. The treated soils were then emptied into the pots, with saucers to catch any leachate, preventing nutrients from being lost from the system. The pots were placed in the glasshouse in November 2012.

The pot trial was initially established in the glasshouse to enable the plants to establish successfully and to allow for rapid initial growth. The glasshouse was heated, with the temperature constantly monitored inside the glasshouse during the trial period. The mean daily temperature for the duration the pot trial was contained within the glasshouse was 20.3°C, with the minimum and maximum recorded temperatures being 10.6 and 37.1°C, respectively (Figure 4).

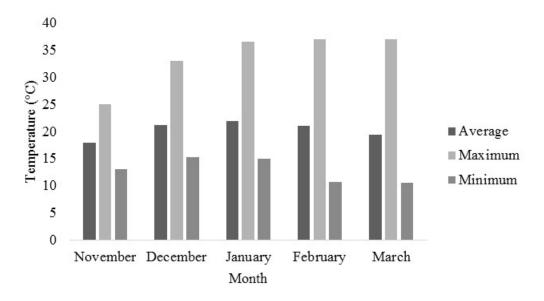


Figure 4. Average, maximum and minimum monthly temperature (°C) at Craddock Glasshouse, Lincoln University for the period November 2012 to March 2013.

All pots were moved outside of the glasshouse on the 26 March 2013. Moving the plants outside allowed them to be exposed to more realistic environmental conditions over autumn/winter/spring, giving a better indication of their ability to survive under field conditions. The pots were placed on tables to protect them from pests such as rabbits which could be an issue at ground level.

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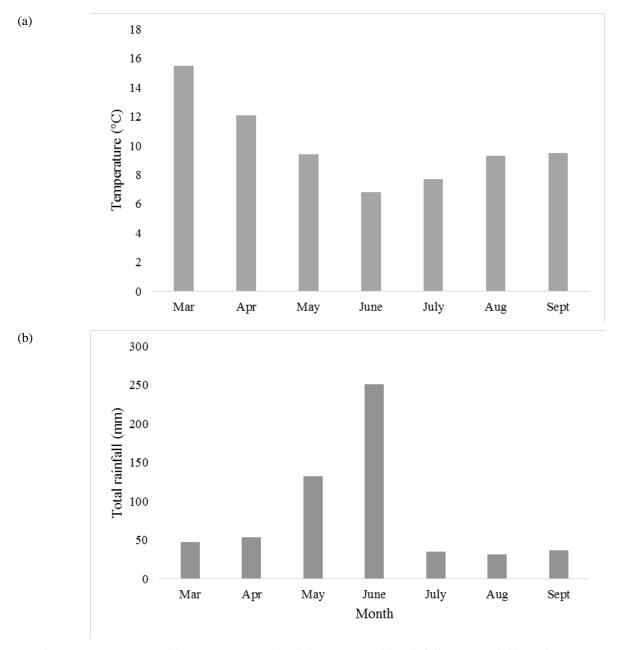


Figure 5. (a) Mean monthly temperature (°C) and (b) Total monthly rainfall (mm) at Field Service Centre, Lincoln University during the outside trial period.

French serradella was grown in this experiment to be investigated for its potential to grow in the NZ upland environment. The reference species, subterranean clover, is commonly (commercially) grown across New Zealand, but has been found to have limited persistence. Both species were sown at 20 seeds per pot in late November 2012 at a depth of 1-2 mm. The plants were arranged in their species and were watered regularly during the establishment phase.

The pots were inoculated with commercial rhizobia strains ('Nodulaid', Becker Underwood Ltd, Australia) when the seedlings were 3-4 weeks old. A peat inoculum was mixed to form a slurry, the appropriate rhizobia strain was added to the slurry and then applied to the different legume species in December 2012. The strains of inoculums applied to the legume species were 'G' (French serradella) and 'C' (Subterranean clover).

Throughout the duration of the experiment the pots were weeded to ensure the plants under investigation were free from inter-specific competition. Plant counts were carried out throughout the experiment, ensuring that 15

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plants were growing in all French serradella and subterranean clover pots. Where pots had missing plants, new seeds were sown.

Basal nutrient solution was applied to the 'ALL' treatment pots weekly in the initial phases of the experiment; 14 March, 4 April, 11 April and 18 April. Application ceased in late April as heavy rainfall and low temperatures in late autumn meant that pots were saturated and plant growth was limited, therefore nutrients were no longer required over that period. Once temperatures began to increase at the start of spring, application of nutrient solution resumed, and was applied on the 29 August and 17 September. The nutrient solution applied was a 'complete treatment', containing all macro and trace elements required for plant growth.

Pots were watered to weight as required to 35-40% volumetric water capacity. This represented a soil which was neither waterlogged nor dry. During the period the plants were in the glasshouse watering took place several times a week, with watering daily throughout the hotter months. Once the pots were moved outside both natural rainfall events and the lower temperatures, resulting in slower plant growth, meant that watering was required less often. Virtually no watering was required over the winter months due to regular rainfall.

## 2.4 Measurements

Plants were harvested during periods of rapid growth. This allowed maximum shoot yield prior to plants becoming reproductive. French serradella was cut at 40 mm above ground level, and subterranean clover at 20 mm. Two harvests were done before winter. All species were harvested on the 18/19 March 2013. The second harvest took place on 13 May. During the winter period plants were left to recover green leaf material, and limited growth over this time meant that no harvests were performed. The warmer temperatures of spring increased the growth rate of the plants and allowed for two more harvests to be conducted on the 19 August and 12 October. Following harvests all herbage samples were oven dried at 70°C for 72 hours. All herbage samples were then weighed to obtain the shoot dry weights (g DM pot<sup>-1</sup>) of each pot. This represented the shoot DM yield for the given growth period.

For soil sampling at the completion of the experiment, one soil core (25 mm diameter x 75 mm long) was taken from each pot on the 3 September and cores bulked on a treatment basis. The soil was dried at 30°C for 7 days and then underwent basic analysis (Olsen P, pH, exchangeable Al,  $SO_4^{2^-}$ -S). The soil analyses carried out were similar to those described in Section 2.2.

## 2.5 Statistical analysis

All data were statistically analysed using Genstat version 14.0 (VSN International), analysis of variance (ANOVA) tests were carried out to investigate treatment effects. The model included treatments lime rate, phosphorus rate and sulphur as fixed components and shoot yield as a variable. Where statistically significant interactions between species and treatments occurred then regression analysis (curve fitting) using Sigmaplot 11.0 (Systat Software Inc. GmbH. 2008) was used to better understand these relationships.

## **3. RESULTS**

## 3.1 Main effects

The two species differed significantly (P<0.001) in the total shoot DM yield, with French serradella yielding 16.3 g DM pot<sup>-1</sup> and subterranean clover yielding 6.8 g DM pot<sup>-1</sup> (Table 3). Across the two species there was no significant interaction between species and lime rate for total yield. Yet, the interaction between species and phosphorus rate was highly significant (P<0.001), as French serradella and subterranean clover responded differently to the phosphorus rates.

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	Y	rield (g DM pot <sup>-1</sup> )	
	Summer/Autumn	Spring	Total
Species:			
French serradella	8.1	8.2	16.3
Subterranean clover	5.4	1.4	6.8
SEM	0.45	0.82	0.82
LSD (5%)	1.28	2.34	2.34
Species	***	***	***
Species x Lime	***	*	ns
Species x P	*	***	***

ns = not significant; \* = P < 0.05; \*\*\* = P < 0.001.

# Table 3. Treatment mean values of summer/autumn, spring and total yields for two annual legume species grown in a NZ upland soil.

French serradella yielded significantly higher (P<0.001) than subterranean clover during the summer/autumn growth period, achieving 8.1 g DM pot<sup>-1</sup> compared to 5.4 g DM pot<sup>-1</sup> produced by subterranean clover (Table 3). The interaction between the species and lime rate was highly significant (P<0.001) for summer/autumn as a result of the two species responding differently to the various lime application rates. Across the species, there was also a significant (P<0.05) interaction with phosphorus rate for summer/autumn. A similar trend occurred for the winter/spring yield, with French serradella significantly out yielding (8.2 g DM pot<sup>-1</sup>, P<0.001) subterranean clover (1.4 g DM pot<sup>-1</sup>, Table 4). These annual species had a significant (P<0.05) interaction with phosphorus rate.

### 3.2 *Lime response*

Total shoot dry matter (DM) yield varied significantly (P<0.001) with increasing rates of lime across all species. French serradella had the highest observed total DM yield of 20.1 g DM pot<sup>-1</sup> at 2000 kg ha<sup>-1</sup> of lime, with yields decreasing significantly (P<0.001) the further the lime rate was from this optimum (Table 4). Subterranean clover was observed to have the highest total DM yields below 4000 kg ha<sup>-1</sup> of lime. The highest total DM yield of 7.7 g DM pot<sup>-1</sup> was achieved at 2000 kg ha<sup>-1</sup> lime, this was statistically similar to the yield achieved at 0 kg ha<sup>-1</sup> of lime, indicating that subterranean clover does not have a high requirement for lime (Table 4).

French serradella had the highest summer/autumn DM yield of 9.7 g DM pot<sup>-1</sup> at 4000 kg ha<sup>-1</sup> of lime, however this was not statistically different from the yield achieved at 8000 kg ha<sup>-1</sup> (Table 4). Subterranean clover had yields being highest below 4000 kg ha<sup>-1</sup> of lime. The highest summer/autumn DM yield of 6.4 g DM pot<sup>-1</sup> was achieved at 2000 kg ha<sup>-1</sup> lime, this was statistically similar to the yield achieved at 0 kg ha<sup>-1</sup> of lime, indicating that subterranean clover does not have a high requirement for lime (Table 4).

Subterranean clover did not show a significant spring DM yield response (P<0.01) to increasing lime rates. French serradella had the highest spring DM yield of 13.8 g DM pot<sup>-1</sup> at 2000 kg ha<sup>-1</sup> of lime, with yields significantly decreasing (P<0.001) the further the lime rate was from this optimum (Table 4).

### 3.3 P response

French serradella did not respond significantly (P<0.05) in terms of total DM yield to increasing phosphorus rate. However, subterranean clover did show an increased total DM yield as phosphorus application rates increased. The highest total subterranean clover yield was 6.6 g DM pot<sup>-1</sup>, achieved at a phosphorus rate of 500 mg kg<sup>-1</sup> (Table 4). The summer/autumn DM yield response to phosphorus application rate varied across species, with Subterranean clover showing a significant difference (P<0.05) in yield with increasing rates of phosphorus.

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French serradella had the highest observed summer/autumn DM yield of 9.2 g DM pot<sup>-1</sup> at 150 mg kg<sup>-1</sup> P rate, with significantly lower (P<0.01) yields at 0 and 50 mg P kg<sup>-1</sup> (Table 4). Subterranean clover did not show any difference (P>0.05) in summer/autumn DM yield responses at the different application rates of phosphorus (Table 4). Subterranean clover was the only species to show a significant difference (P<0.05) in spring DM yield in response to increasing phosphorus rates. Subterranean clover had an observed increase in spring DM yield as the phosphorus application rate increased, with the highest yield of 1.8 g DM pot<sup>-1</sup> achieved at phosphorus application rate of 500 mg kg<sup>-1</sup> (Table 4).

	French serradella (g DM pot <sup>-1</sup> )		Subterranean clover (g DM pot <sup>-1</sup> )			
	Summer / Autumn	Spring	Total	Summer / Autumn	Spring	Total
Lime rate (kg ha <sup>-1</sup> ):						
0	8.1	8.9	17.0	6.2	1.6	7.7
2000	6.3	13.8	20.1	6.4	1.3	7.7
4000	9.7	7.4	17.1	2.1	0.0	2.1
8000	8.7	4.7	13.4	2.8	1.0	3.8
SEM	0.43	1.07	1.02	0.44	0.62	0.70
LSD (5%)	1.23	3.05	2.91	1.35	1.87	2.11
Significance	***	***	***	***	ns	***
P rate (mg kg <sup>-1</sup> ):						
0	7.5	10.4	17.8	4.0	0.1	4.1
50	7.3	9.2	16.5			
150	9.2	8.9	18.0			
500	8.9	6.4	15.3	4.8	1.8	6.6
SEM	0.43	1.07	1.02	0.31	0.44	0.49
LSD (5%)	1.23	3.05	2.91	0.95	1.32	1.49
Significance	**	ns	ns	ns	*	**
Grand mean	8.2	8.7	16.9	4.4	1.0	5.3
SEM	0.86	2.14	2.05	0.63	0.87	0.99
LSD (5%)	2.46	6.09	5.83	1.90	2.65	2.99
P x Lime	ns	ns	ns	***	ns	***
L0P0 x L0P0-S	***	*	ns			
L0P0 x ALL	ns	ns	ns	ns	ns	ns
L2P3 x ALL	**	**	ns	ns	ns	ns

ns = not significant; \* = P < 0.05; \*\* = P < 0.01; \*\*\* = P < 0.001.

Table 4. Shoot yield (g DM pot<sup>-1</sup>) of French serradella and Subterranean clover grown in a NZ upland soil supplied with different rates of lime (kg ha<sup>-1</sup>) and phosphorus (mg kg<sup>-1</sup>).

# 3.4 Lime x P response surfaces

The only species to show a significant P x lime interaction (P<0.001) for total shoot DM yield was subterranean clover. This interaction was consistent for the summer/autumn shoot DM yield. French serradella appeared to

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yield well across a wide range of treatment combinations, with the maximum total DM yield occurring at 2000 kg lime ha<sup>-1</sup> and 50 mg P kg<sup>-1</sup> (Figure 6). Subterranean clover had total DM yields highest at 0 and 2000 kg lime ha<sup>-1</sup> and 500 mg P kg<sup>-1</sup> (Figure 7).

French serradella showed no significant differences (P>0.05) in total DM yield achieved with and without sulphur (Table 5). However, French serradella did show a highly significant difference (P<0.001) in summer/autumn DM yield when sulphur was applied (Table 5). Also when sulphur was applied to French serradella plants there was a significant difference (P<0.05) in the spring DM yield achieved (Table 4).

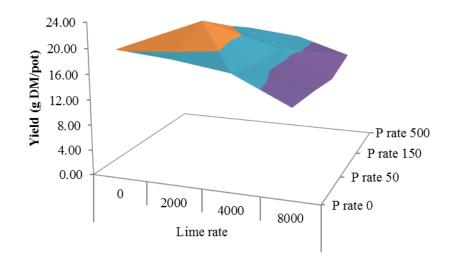


Figure 6. Total accumulated shoot dry matter (DM) yield response of French serradella, grown in a NZ high country soil supplied with increasing rates of lime (kg ha<sup>-1</sup>) and phosphorus (mg kg<sup>-1</sup>).

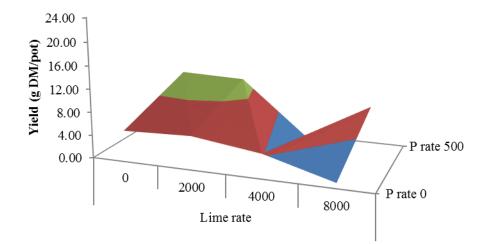


Figure 7. Total accumulated shoot dry matter (DM) yield response of subterranean clover, grown in a NZ high country soil supplied with increasing rates of lime (kg ha<sup>-1</sup>) and phosphorus (mg kg<sup>-1</sup>).

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## 3.2 Soil fertility and pH

As expected, the variable lime rates applied in this experiment significantly increased the pH of the soil (Table 5). The pH increased from 4.8 at 0 kg ha<sup>-1</sup> to 7.0 at 8000 kg ha<sup>-1</sup>. On average, the increase in pH of 4.8 to 7.0, meant the pH increased by  $2.75 \times 10^{-4}$  pH unit for every kg lime ha<sup>-1</sup> applied.

Increasing phosphorus input levels increased the soil Olsen P values of the soil (Table 5). There was a linear increase in Olsen P from 23.5  $\mu$ g ml<sup>-1</sup> at 0 mg P kg<sup>-1</sup>, to 147.2  $\mu$ g ml<sup>-1</sup> at 500 mg P kg<sup>-1</sup>. This is an average increase in Olsen P of 0.25  $\mu$ g ml<sup>-1</sup> per mg P applied kg<sup>-1</sup> soil. At increasing lime rates, the exchangeable aluminium values of the soil decreased (Table 5). Exchangeable aluminium remained high (8.7 mg kg<sup>-1</sup>) where no lime was applied, but decreased to 1.3 mg kg<sup>-1</sup> at lime application rates of 2000 kg ha<sup>-1</sup>. Above applications of 4000 kg ha<sup>-1</sup> of lime, exchangeable aluminium levels dropped below 0.5 mg kg<sup>-1</sup>.

Lime Rate (kg ha <sup>-1</sup> )	pН	Exchangeable Al (mg kg <sup>-1</sup> )	
0	4.8	8.7	
2000	5.4	1.3	
4000	6.2	0.0	
8000	7.0	0.0	
P Rate	Olsen P (mg L <sup>-1</sup> )		
0	23		
50	42		
150	68		
500	147		

Table 5. pH, exchangeable Al and Olsen P values of a NZ upland soil supplied with different levels of lime and P. Analyses conducted at the completion of the experiment.

# 4. DISCUSSION

The objective of this study was to investigate the effects of lime, phosphorus and sulphur on the yield of a new potential dryland legume, French serradella when grown in an acid high country soil from Central Otago, NZ. The key results and potential implications are discussed below.

### 4.1 Differences between species

Across all growth periods, French serradella yielded significantly higher (P<0.001) than subterranean clover. Annual species generally have a shorter growth period than perennials, as annuals go to seed, die and re-establish from seed each year (Evans et al., 2003). However, during the trial period French serradella did not exhibit this behaviour. The French serradella had a longer growth period, and subsequently yielded higher than subterranean clover. These two annual species showed no species by lime rate interaction at the conclusion of the total growth period, indicating that both species responded to these lime rates in a similar manner. Yet, over this same period the interaction between species and phosphorus rate was highly significant (P<0.001), with French serradella and subterranean clover responding to phosphorus in a different way. French serradella had no significant yield response to increasing phosphorus inputs, possibly due to the medium starting Olsen P level (20 mg P L<sup>-1</sup>), and

possibly indicating a low P fertility requirement. Whereas, subterranean clover yield increased (P<0.01) as phosphorus inputs increased from 0 to 500 mg kg<sup>-1</sup>.

Although information is limited, some studies have found that French serradella is one of the most efficient species at utilising phosphorus, which explains why French serradella and subterranean clover responded to phosphorus differently, as indicated by the interaction term. Deruiter (1981) examined the phosphate (P) fertiliser response of eight legumes, including French serradella and subterranean clover. The yield of subterranean clover was increased two to three fold by the application of P, whereas French serradella did not show statistically significant growth increases with added P. However, French serradella accumulated the highest levels of foliar P, especially at low or zero P additions. These results indicated that French serradella is adapted to low P levels, as it was the most efficient legume species at using P to maximise growth. Blair and Cordeo (1978) also looked at the response of French serradella to varying P levels. They found that French serradella was the most efficient species at utilising P, with efficiency of P utilization defined as the ability to produce dry matter with a given amount of applied P. Our results expand upon and reinforce the findings of those studies.

## 4.2 Treatment effects within species

Lime addition strongly and significantly influenced the mean yield of both legume species (P<0.001), and this effect differed between species. At the most acidic soil pH (pH 4.8, lime rate 0 kg ha<sup>-1</sup>), French serradella was the most productive legume species with a maximum total DM yield of 17 g DM pot<sup>-1</sup>. Subterranean clover more tolerant of acidic soil pH levels. At the highest lime rate of 8000 kg ha<sup>-1</sup>, both species showed a significant reduction in yield, producing poor total DM yields at the highest pH level of 7.0.

Lime inputs increased the pH of the soil in the experiment. For this acidic Pallic soil from N.Z. it was found that pH increased by 0.3 pH units for every 1000 kg lime ha<sup>-1</sup> applied. This correlates to 364 kg lime ha<sup>-1</sup> required to raise the soil pH by 0.1 unit, indicating that the soil has a low pH buffering capacity. The buffering capacity is ability of the soil to resist change in pH, these soils are predicted to have a low buffering capacity due to the low organic matter content of the soil. A low organic matter content means there are fewer cation exchange sites, therefore the addition of lime (CaCO<sub>3</sub>) bumps acidic cations such as H<sup>+</sup> and Al<sup>3+</sup> off exchange sites, replacing them with base cations such as Ca<sup>2+</sup> (Menzies, 2003). The removal of acidic cations when lime is applied results in a rapid increase in soil pH.

Soil pH had a large and highly significant (P<0.001) effect on the yield of the annual species. The increase in yield up to pH of 6.1 was likely strongly driven by increasing P and Mo availability, as a result of lime application up to 4000 kg ha<sup>-1</sup>. Literature suggests that lime additions reduce soil exchangeable Al levels (Widdowson and Walker, 1971). Generally there is a decrease in Al levels when lime additions promote a pH above 5.8 in brown soils, common to NZ upland soils (Moir and Moot, 2010). As well as amelioration of Al toxicity, lime additions also increase P and Mo availability in the soil, increasing the plants ability to use available P (Wheeler and O'Connor, 1998). All of these factors, acting either individually or collectively, would be expected to result in a positive yield response when lime additions raised pH to within a range of 5.2 - 6.0 (Maxwell et al., 2012).

All species showed a positive yield increase up to lime application rate of 4000 kg ha<sup>-1</sup>, however, beyond this both species showed a yield decrease. This is can be attributed to a depression in available phosphorus and Boron (B) at high pH (>6.0) due to formation calcium phosphate at a rate that increases with soil pH (Larsen et al., 1965). Other trials have found similar results where legume species have exhibited a negative yield response at high lime rates. Maxwell et al. (2012) found that all four clover species used in their glasshouse experiment had decreased yield at lime rates above 2000 kg ha<sup>-1</sup> equivalent, where the soil pH level rose above 5.7. In a field trial, Lambert and Grant (1980) found that in NZ upland pastures, both legume vigour and soil P showed a positive response to low lime application (0 – 3500 kg ha<sup>-1</sup>). However, above applications of 3500 kg ha<sup>-1</sup>, pasture legume yield decreased below that of the control. Again, these findings are thought to result from a decreased availability of phosphorus at pH levels above 6.0. Furthermore, Maxwell et al. (2012) found that while Mo concentration increased in response to lime addition, boron (B) and copper (Cu) became deficient in clover shoots when grown at a pH of 7.2 (8000 kg lime ha<sup>-1</sup> equivalent). Therefore, not only does a high lime rate induce P deficiency, but at applications of 8000 kg lime ha<sup>-1</sup> trace elements such as B and Cu had an induced deficiency.

While the species within the trial showed a positive yield response to lime addition up to 4000 kg ha<sup>-1</sup>, subterranean clover yielded highest at 0 - 2000 kg lime ha<sup>-1</sup> (pH 4.8 – 5.4). These results are relatively consistent with findings by Hayes et al. (2008) and also Maxwell et al. (2012), who found that subterranean clover showed highest total DM yields between base soil pH 5.2 and 5.5; i.e. below a pH of 6.1 achieved at the 4000 kg ha<sup>-1</sup>

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lime rate. Furthermore, Cordero and Blair (1978) found that subterranean clover yielded equally as well at pH 5.9 and pH 4.4 (measured in CaCl which is typically 0.5 units lower than measuring pH in  $H_2O$ , as was done in this experiment). The combination of these results provide evidence that subterranean clover are tolerant of acid soil conditions.

Total shoot DM yield of subterranean clover was also influenced by phosphorus rate (P<0.05), but not French serradella. However, French serradella yielded equally as well over all four phosphorus treatments. As to be expected, subterranean clover had an observed increase in total shoot yield when phosphorus application rate increased from 0 to 500 mg kg<sup>-1</sup>. The difference in P response for the novel legume illustrates that some species have better P utilisation than others.

Phosphorus is one of the most important macro-nutrients for legume growth (Wynn-Williams, 1982; Khumalo, 2012), therefore the yield response of French serradella to an increased P input above 0 mg kg<sup>-1</sup> is in accordance with other literature. Dodd and Orr (1995) looked at the phosphate response of 18 annual legumes grown in hill-country soil, including French serradella and subterranean clover. All species had enhanced growth with added phosphate. A high P response ratio indicated that species were adapted to variable soil P conditions, but the smaller response ratios shown by species such as French serradella and subterranean clover were an indicator of their adaptability to low P levels. These species were able to maintain high yields at low P levels relative to their potential yield at unlimited P availability. In our experiment, French serradella did not show statistically different yield responses as phosphorus rate increased above 0 mg kg<sup>-1</sup>, probably due to adaptations allowing it to maintain growth at the 'medium' background soil P levels. Therefore our results concur with those of Dodd and Orr (1995).

Deruiter (1981) also found that French serradella did not show statistically significant growth increases with added P, in accordance with both this experiment and the findings by Dodd and Orr (1995). However, at the highest levels of applied P, Deruiter (1981) noted a decreased yield in French serradella and subterranean clover. This effect became more pronounced over time which was explained by ion immobilisation and precipitation on root and soil surfaces prevented P uptake. A decreased yield was not noted at high P inputs (500 mg P kg<sup>-1</sup>) in this experiment, even though this may occur with a longer experimentation period.

It would be expected that where no phosphorus was applied, increasing lime rates would induce a phosphorus deficiency due to the formation of calcium phosphate at soil pH levels above 6.0 (Larsen et al., 1965; Haynes, 1982). However, the Olsen P of the soil used in this experiment was already at medium levels (Olsen P of 20 mg P  $L^{-1}$ ) so the interaction between lime and phosphorus was not significant. The plant yield response to increasing P inputs may have been greater if the initial Olsen P values had been lower. Therefore it is hard to gauge a true yield response to P inputs for the legumes under investigation in this experiment, as the control (0 mg P kg<sup>-1</sup> applied) already had medium levels of available P. Further research to investigate legume response to P should be focused on a high country soil where Olsen P values are 'typically' low.

Total shoot DM yield of French serradella did not differ (P>0.05) with the application of sulphur. Other studies done by Maxwell et al. (2012) also found that the four annual clover species used in their experiment showed a low response or were unresponsive to added sulphur. These results indicate that available S, may not be the key macro-nutrient limiting the growth of these legumes. Field experiments are required to confirm this result.

# **5. CONCLUSIONS**

The objective of this study was to determine the effects of lime, phosphorus and sulphur inputs on the yield and nutrient uptake of French serradella on an acidic upland soil from Central Otago, NZ. Treatment effects were identified in a pot trial under a combination of glasshouse and controlled external conditions at Lincoln University, NZ.

The highest yielding species was French serradella with a grand mean of 16.9 g DM pot<sup>-1</sup>. Surprisingly, this annual species did not exhibit behaviour typical of annual pasture species, continuing to grow when other annuals such as subterranean clover went to seed and died.

Lime inputs significantly increased (P<0.001) the yields of all species up to a maximum and was the key driver of yield response in this experiment. Beyond this maximum species showed a decrease in yield with further lime additions. The lime rate, and subsequent pH value, at which plants reached maximum yield varied between the two species. French serradella achieved maximum total DM yields between lime application rates of 2000-4000 kg ha<sup>-1</sup>, representing a soil pH of 5.4 - 6.1, but still yielded very well in the unlimed treatment. This was driven

by a substantial decrease in soil exchangeable Al levels and probably greater Mo and P availability. At lime application rates of 8000 kg ha<sup>-1</sup> the total DM yield became depressed, probably as a result of decreased B and P availability above a pH of 7. Subterranean clover was the most acid tolerant species, achieving maximum yield at a lime rate of 0-2000 kg ha<sup>-1</sup> (pH 4.8 – 5.4).

French serradella was found to be efficient at utilising low levels of P, yielding equally as well over all four phosphorus treatments. Overall, French serradella showed a high potential as an alternative legume for acidic upland soils. To validate the findings of this experiment French serradella should be examined in a field experiment in an upland environment.

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