Clover and grass foliar nutrient responses to phosphorus and molybdenum fertilisers, and herbicide application on a summer dry hill pasture

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Abstract

Pastures on the Port Hills, Canterbury, are typically grass-dominant growing on low Olsen P soils and enduring summer-dry conditions. Subterranean (sub) clover is the preferred legume on steeper dryland hill slopes, but clovers struggle to compete with resident grasses for soil moisture and nutrients. Nutrient concentrations in sub clover and grass foliage were sampled each spring, from 2021 to 2023, in an experiment with factorial combinations of phosphorous (0 or 60 kg P/ha, -P or +P), sulphur (0 or 50 kg S/ha, -S or +S), and molybdenum (0 or 0.06 kg Mo/ha, -Mo or +Mo) fertilisers, and plus or minus autumn grass-supressing herbicide. In the unfertilised controls, sub clover had higher foliar concentrations of N, Ca, Mg, B, Cu and Zn than the grasses which had higher concentrations of P, K, S and Mo, which are the nutrients commonly applied in fertilisers to support clover growth. Applying P increased foliar P from 0.16 to 0.27% in sub clover and 0.21 to 0.34% in grass in 2023. Sub clover and grass foliar S concentrations were mainly affected by sampling year. The +Mo treatment only elevated sub clover Mo in 2022 from 0.05 to 0.11 mg/kg. However, each year +Mo increased sub clover foliar N% from 4.1 to 4.5% in 2021, 3.7 to 4.0% in 2022, and 4.3 to 4.8% in 2023. Grass foliar Mo was elevated by +Mo from ~0.45 to 1.42 mg/kg in 2021 but then concentrations declined to 0.99 and 0.67 mg/kg in 2022 and 2023 respectively. Grass Mo values were, on average, 10 times higher than in the sub clover. Grass N% was increased where herbicide was applied, increasing from 2.6 to 3.0% in 2022 and 3.6 to 3.9% in 2023. In 2023, the +P+S+Mo fertiliser treatment increased N, P, S, Mg and B in fertilised sub clover compared with control. To maximise clover nutrition and growth on this low P site the regular addition of superphosphate, with molybdenum every four years, is recommended in conjunction with grass control in autumn.

Keywords: competition, grass suppression, sub clover, sulphur, *Trifolium subterraneum* L.

Introduction

Clovers are an important legume component of grazed mixed-pastures in New Zealand because they fix nitrogen, which improves the grazing preference of associated grasses (Edwards et al. 1993) and provide high quality feed that increases animal performance (Nicol & Edwards 2011). To optimise legume and pasture growth, soil and foliar nutrient levels should be routinely monitored and managed by applying fertilisers when required. This is particularly important in hill and high country areas where soils typically have low plant available phosphorus (P) and sulphur (S) (Morton et al. 2021). Molybdenum (Mo), which plays a key role in N-fixation, is also deficient in many soils (Sherrell & Metherell 1986).

According to Sherrell and Metherell (1986) both Mo and N must be below optimum concentrations, i.e., 0.1 mg/kg and 4% N respectively in white clover (*Trifolium repens* L.) foliage (lamina+petiole[1]), before a growth response to applied Mo will occur. For white clover lamina, Askew et al. (1958) reported 4.6% N and 0.02 mg Mo/kg in the control plots of a Mo responsive site. In a review of Mo in New Zealand pastures by Morton (2023), only three references provided white clover N and Mo foliar nutrient values and no values were presented for subterranean (sub) clover (T*rifolium subterraneum* L.). Morton (2023) noted that yield responses to Mo addition were more likely on Pallic and some brown soils, and on soils with an initial pH < 5.5.

For sub clover, there are no unique foliar nutrient critical values so analysis results are compared with those of white clover. Reuter and Robinson (1997) suggested that sub clover had lower critical values for foliar N at 3.0 to 3.2% compared with white clover at 4.4 to 4.7% (lamina+petiole).

The availability of nutrients to clover is influenced by the competitive nature of grass. This was highlighted by Jackman and Mouat (1970) who found that 580 kg/

1 Lamina are the clover leaflets, and the petiole is the clover leaf stem.

ISSN 2463-2872 (Print) ISSN 2463-2880 (Online) https://doi.org/10.33584/jnzg.2024.86.3702

ha more superphosphate was needed to produce 800 kg of white clover DM/ha when grown with browntop (*Agrostis capillaris* L.) than when grown alone. Scott (1973) discussed this 'depressive effect' of grass on clover growth, and therefore N fixation, and concluded that the grass P demand needed to be fulfilled before the clover. Caradus (1980) supported this conclusion after screening grasses and legumes for P use and suggested grasses were more tolerant of soils low in P because of their more intensive root systems. Thus competition for P is an important inefficiency in the grass/clover relationship which depends on the N fixed by clovers.

Individual plant species have specific optimum foliar nutrient concentrations associated with the plant part sampled and the season samples are taken (e.g., Reuter & Robinson 1997). There are few published studies where the foliar nutrient concentrations of legumes and grasses growing in mixed pastures have been analysed separately. McNaught (1970) described the relative nutrient concentrations in perennial ryegrass (*Lolium perenne* L.) and white clover. The perennial ryegrass usually had higher concentrations of K and Mo, lower N, Ca, and B, and P and S were not different to the associated white clover (lamina + petiole)(no statistical analysis was provided to confirm this). McNaught (1970) stated that separated species analyses, particularly legumes, were a more useful indicator plants for nutrient analysis. Sampling clover in spring is recommended because plants are vegetative, fast growing and herbage is available pre-grazing (Olykan et al. 2021). Samples of clover lamina-only eliminates errors caused by the variable proportion of petiole, which differs in N, P, K and S concentration (Olykan et al. 2019). Olykan et al. (2019) concluded that fertiliser recommendations should be based on clover lamina-only samples. However, the research required to define the critical nutrient concentrations in white or sub clover lamina has not been undertaken.

This experiment investigated the addition of phosphorous (P), sulphur (S) and molybdenum (Mo) fertilisers on the nutrition of sub clover and the associated grass species on a dryland hill country site on the Port Hills, Canterbury, that had low initial Olsen P soils. The fertiliser treatments were applied ± grass-suppressing herbicide. The effect of these treatments on pasture and clover yields and feed quality were reported by Olykan et al. (2024). They found that applying herbicide in autumn increased sub clover% and decreased grass%, without affecting total pasture yield, but had no effect on the resident white clover. The application of herbicide without fertiliser (+H) increased ($P<0.001$) total clover yield from 1460 (-H) to 2930 kg DM/ha. Then applying P and Mo fertilisers (i.e., +H+P+Mo) further increased ($P<0.05$) the total clover yield to 4010 kg DM/ha. There were no yield responses to +S or +Mo unless added with P.

The responses of sub clover and grass foliar nutrient concentrations to applications of P, S and Mo fertilisers and the herbicide treatment that reduced the grass competition are described in this paper.

Materials and Methods

Location and experimental details

The location and experimental details were described in detail by Olykan et al. (2024). In brief, the experiment was located on the Port Hills to the south of Christchurch (GPS -43.6252, 172.5836) on a northwest facing slope (~10°) at ~200 m a.s.l. The existing pasture was dominated by grasses, mainly browntop, with sweet vernal (*Anthoxanthum odoratum* L.) and clumps of cocksfoot (*Dactylis glomerata* L.), with low populations of resident white and sub clovers. Historically, the pasture has received minimal fertiliser inputs because it is only intermittently grazed and not farmed commercially.

The soils are predominantly a Timaru stoneless silt (Mottled Fragic Pallic Soil) characterised as moderately deep (50-70 cm) and imperfectly drained, with 93 mm of plant available water (PAW to 60 cm) and low P retention (21%) (Manaaki Whenua 2021).

Full soil analysis results were described by Olykan et al. (2024). Soil samples taken in October 2020 measured an Olsen P of 8 mg/L, sulphate-sulphur S of 4 mg/kg, and organic-S of 7 mg/kg. Olykan et al. (2024) also found an analysis of sub clover foliage indicated low P, S and Mo. The addition of phosphorous (P) fertiliser in May 2021 lifted the soil Olsen P from 8 to 12 mg/L and the addition of S increased sulphate-S from 3 to ~5.5 mg/kg when measured in November 2022. The P and S fertiliser treatments were reapplied in May 2023. By November 2023, the Olsen P was 24 mg/L and sulphate-S was 15 mg/kg.

On 14 April 2021 the site was mown to ~30 mm residual herbage and the clippings removed. Then a 50:50 mix, by weight, of 'Woogenellup' and 'Denmark' sub clover cultivars at 120 kg seed/ha was oversown.

The experimental design was a 2^4 factorial, based on additions of 0 or 60 kg P/ha (-P or +P) x 0 or 50 kg S/ha (-S or +S) x 0 or 0.06 kg Mo/ha (-Mo or +Mo) x minus or plus herbicide (-H or +H). These 16 treatments were allocated randomly to plots (1.5 m wide x 5 m long) in three replicates. On 11 May 2021 the P (triple super, P 19) and S (gypsum granules, S 16) fertiliser treatments were applied to each plot. On 20 May 2021 the Mo treatment was applied as a dilute sodium molybdate ($Na_2MoO_4.2H_2O$, Unilab) solution in ~4 L water using a watering can. On 2 May 2023 the same P and S fertiliser treatments were reapplied, but no Mo.

No herbicide application was needed to suppress grass growth prior to winter in 2021. The herbicide Centurion® Xtra (a.i. 360 g clethodim/L) was applied at 330 ml /ha + oil using a knapsack sprayer (two overlapping passes per plot) on 15 March 2022 and was reapplied on 24 March 2023. The experiment was mown (cut and carry) to 50 mm stubble height on 1 November 2021, 22 February 2022, and 16 June 2023, and to 100 mm stubble height on 2 March 2023. The site was opened up for grazing through January and February 2023.

Foliage sampling and analysis

Foliage sampling of the plots was carried out once each year during the spring, on 19 October 2021, 3 November 2022, and 5 October 2023, and was associated with a pasture harvest cut as the site was subsequently mown to simulate grazing.

From each plot ~10 'grab' samples of pasture were taken from adjacent to the 0.2m^2 harvested site and centre line of each plot. The samples were bulked by treatment to give 16 samples. From each sample, fully expanded healthy sub clover lamina (leaflets) were manually separated from the petioles and counted. Between, 50 and 100 laminae were required to provide the minimum 0.4 g of dry matter for chemical analyses. White clover laminae were also separated from the samples in 2022. From each bulk sample, a mixed grass subsample was selected based on the healthy young grass blades from vegetative tillers of the species present. Clover lamina samples were dried at 65°C for 48 hours, weighed, and ground. Grass samples were only dried and ground. Clover lamina weights were taken as they may indicate a growth response to the treatments.

Foliar N concentrations were measured using near-infrared (NIR) spectroscopy (FOSS NIRSystems 5000). Analytical Services at Lincoln University measured a range of nutrient concentrations, including P, K, S, Ca, Mg, B, Cu, Zn and Mo, by adding 2.0 ml of trace element grade nitric acid (69%) and 2.0 ml of 30% hydrogen peroxide to 0.2 g of sample. This was digested by microwave (CEM MARS Xpress; 4.6°/min to 90°, then held for 5 min, followed by 9°/min to 180° then held for 15 min before cooling) and analysed with an ICP-OES (Varian 720).

Climate

The long term mean (LTM) and actual climate during the experiment were described in detail by Olykan et al. (2024) using data accessed from NIWA Virtual Climate Station (VCS) agent number 19952 located at -43.635 172.575 (NZGD1949 positioning system) (NIWA 2024).

The soil was at field capacity by 1 July in 2021 and 2023, and the 12 July in 2022. In October and November of 2022, up to the foliage sampling date, the soil moisture deficit (SMD) was >46.5 mm which equates to 50% of plant available water (Table 1). The SMD in 2023 was lower than the LTM throughout spring.

Table 1 Average soil moisture deficit from 1 July to sampling date from 2021 to 2023, Port Hills, Canterbury. *Partial month to sampling date.

	Average soil moisture deficit (mm)				
Sampled	**Jul**	**Aug**	**Sep**	**Oct**	**Nov**
19/10/2021	3.4	7.7	38.1	44.7*	
3/11/2022	12.8	8.3	31.0	64.0	79.5*
5/10/2023	2.4	4.3	33.5	32.6*	
LTM	5.7	10.0	25.3	45.7	70.0

Data analysis

Genstat statistical software (22nd edition) was used for all statistical analyses. For the 2021 foliar lamina weights and nutrient concentration data, the effect of fertiliser was assessed with orthogonal contrasts (based on applied nutrients P, S and Mo) in a one-way ANOVA. For the 2022 and 2023 foliar lamina weights and nutrient concentrations, a two-way ANOVA of fertiliser (with orthogonal contrasts) by herbicide (no randomised blocks) as main effects only. A two-way ANOVA analysed the effect of year and fertiliser (with orthogonal contrasts) on sub clover lamina weight.

All the sub clover and grass nutrient concentrations were analysed by ANOVA to investigate the main effects of species (sub clover or grass), year (2021, 2022, and 2023), and fertiliser with orthogonal contrasts. The effect of clover or grass on the control nutrient concentrations was investigated with a one-way ANOVA. Differences in 2022 sub and white clover laminar weights and foliar nutrient concentrations were analysed with a one-way ANOVA.

When significant, fertiliser means were separated by Tukey's HSD test at the α=0.05 level.

The sub clover foliar Mo data were skewed in 2021 and 2023 because of many low values, with some close to the detection limit, and the high level of variability in the dataset. Standard data transformations of square root and Log(n+1) (Ireland 2010) did not alleviate the skewness. Therefore, untransformed means are presented.

Table 2 Sub clover lamina and grass foliar nutrient concentrations in the unfertilised, no herbicide control plots, based on mean of 2021 to 2023 values ± standard error of the mean, Port Hills, Canterbury. Probability (P) value for sub clover or grass species effect. Bolded means were higher (α=0.05).

Macro %	N	P	K	S	Ca	Mg
Sub clover	**4.0±0.2**	0.15±0.01	1.9±0.1	0.26±0.03	**1.15±0.02**	**0.25±0.01**
Grass	2.9±0.2	**0.20±0.01**	**2.7±0.1**	**0.31±0.04**	0.30±0.02	0.21±0.02
P value	0.035	0.001	0.004	0.048	<0.001	0.028
Micro mg/kg	**B**	**Mo**	**Cu**	**Zn**		
Sub clover	**21.9±1.7**	0.08±0.01	**12.2±0.6**	**40.6±1.7**		
Grass	9.8±1.7	**0.45±0.08**	7.2±0.6	19.0±0.9		
P value	<0.001	0.041	0.039	0.005		

Results

Sub clover and grass foliar nutrients in the control

Across the major plant macro- and micro-nutrients, there were differences (P<0.05) between sub clover and grass foliar nutrient concentrations in the unfertilised control treatment. Sub clover had higher concentrations of N, Ca, Mg, B, Cu and Zn while the grasses had higher P, K, S, and Mo (Table 2). The biggest differences were found for Ca and B (P<0.001), P (P=0.001) and Zn (P=0.005). Sub clover and grass foliar Mo concentrations were 0.08 and 0.45 mg/kg, respectively.

Sub clover lamina weights

There were differences in sub clover lamina weights across the three sampling years. Mean lamina weights in 2023 were heavier (P<0.05) than in 2021 and 2022 (0.022 vs. ~0.015 g/lamina).

There was no fertiliser effect (*P*=0.168) on sub clover lamina weights on 19 October 2021, however with P lamina were heavier (*P*=0.013) than no P (+P = 0.0156 g *vs*. -P = 0.0138 g/lamina). Sub clover lamina weight on 3 November 2022 was increased by Mo (P=0.003) from 0.0137 g to 0.0182 g/lamina. There was also a trend (P=0.061) of increased sub clover lamina weights with fertiliser on 5 October 2023 due to the P contrast (P=0.009, -P 0.020 g *vs*. +P 0.025 g/lamina).

Foliar nutrients 2021 to 2023

The sub clover and the grass foliar nutrients of particular interest were P, S and Mo, which were applied in the fertiliser treatments and can be expected to influence N concentration.

Nitrogen

In each year, sub clover foliar N% was higher (P<0.001) than in the grasses (Figure 1A). In both sub clover and grasses, foliar N% differed (P<0.001) between the years being highest (P<0.05) in 2023 and lowest in 2022.

Sub clover foliar N% was mainly elevated by the addition of Mo (Figure 1A). Adding Mo increased sub clover N from 4.1 to 4.5% in 2021 (P=0.004), 3.7 to 4.0% in 2022 (P<0.001), and 4.3 to 4.8% in 2023 (P<0.001). In 2021, the addition of P increased (P=0.015) sub clover N% from 4.2 to 4.4%. In 2023 sub clover N% was increased (P<0.001) by herbicide (-H 4.3% *vs*. +H 4.8%).

The grass N% was higher where herbicide was applied in 2022 (P=0.007, -H 2.64% *vs*. +H 3.01%) and 2023 (P=0.004, -H 3.55% *vs*. +H 3.90%) (Figure 1A). In both years the increase in grass foliar N was ~0.36% units. In 2021 the mean value was 3.11±0.04%.

Phosphorous

Foliar P% was higher (P<0.05) in grass than sub clover in each year (Figure 1B). Comparing averages across three years, grass foliage contained an average of 0.29±0.02% P in 2021, which was higher (P<0.05) than the 0.26±0.01% in 2022. For sub clover, P was highest (P<0.05) in 2023 at 0.21±0.02% and lowest in 2022 at 0.15±0.01%.

Sub clover and grass foliar P% values were affected by the addition of P fertiliser. The largest increases were for grass foliar P% from ~0.22% (-P) to 0.35% in 2021 (P<0.001), 0.31% in 2022 (P=0.002), and 0.34% in 2023 (P<0.001) (Figure 1B). Adding P increased sub clover foliar P% from 0.16 to 0.27% in 2023 (P<0.001). Herbicide increased (P=0.003) sub clover P% in 2023 from 0.20 to 0.23%.

Sulphur

Foliar S was consistently higher (P<0.05) in grass than sub clover. There were year differences (i.e., 2023 > 2021 > 2022; P<0.05). Foliar S% in the sub clover and grass was affected by the fertiliser treatments but increases due to +P, +S or +Mo were only 0.02 to 0.04% units (Figure 1C). Herbicide addition increased sub clover S% in 2023.

Molybdenum

In the -Mo treatments, foliar Mo was ~5 times higher in the grasses than sub clover (Figure 1D). Adding Mo increased (P<0.001) foliar Mo in the grasses from ~0.45 mg/kg (-Mo) to 1.42 mg/kg in 2021, 0.99 mg/kg in 2022, and 0.67 mg/kg in 2023.

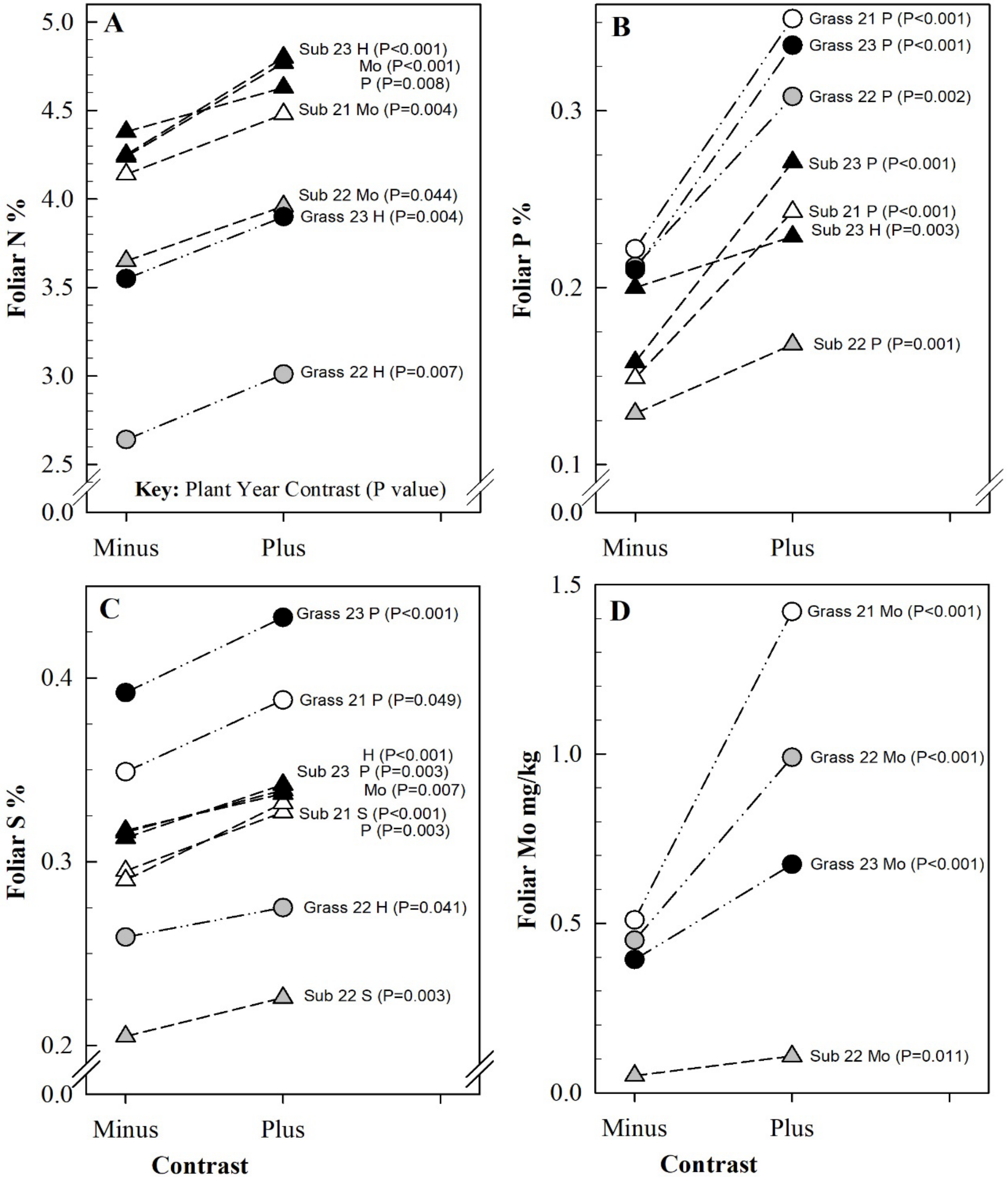

Figure 1 Foliar nutrient concentrations of subterranean clover (sub,△) or grass (○) on 19/10/2021 (open symbols), 1/11/2022 (grey) or 5/10/2023 (black), after application of fertiliser (P = phosphorous, S = Sulphur, Mo = Molybdenum) or herbicide (H) (minus or plus) on Port Hills, Canterbury. Treatment effects with P values <0.01 shown unless effect only one for the year. See key in Graph A.

In 2021, sub clover Mo was not affected (P=0.47) by Mo fertilizer (mean of 0.13±0.06 mg/kg). However, comparing the control with the Mo-alone treatments, sub clover lamina Mo was increased (P<0.10) from 0.047 to 0.524 mg/kg. When Mo was added with P and/or S, the concentrations were ~0.135 mg/kg and not different from Mo-alone or where no Mo was applied. In 2022, +Mo elevated (P=0.011) sub clover lamina Mo from 0.05 to 0.11 mg/kg (Figure 1D). In 2023, Mo addition had no affect (P=0.83) on sub clover Mo (mean of 0.019±0.007 mg/kg).

Table 3 Sub clover foliar nutrient concentrations in response to fertiliser treatments, Port Hills, Canterbury. Samples taken 5/10/2023. P values for fertiliser treatment. Bolded means were higher (α=0.05).

	N	P	S	Mg	B	Cu
Treatment		**%**			**mg/kg**	
Control	4.37	0.164	0.306	0.235	20.3	**13.1**
+P+S+Mo	**5.04**	**0.284**	**0.365**	**0.267**	**23.7**	8.4
P value	0.001	<0.001	0.015	0.003	0.001	0.020

Table 4 Mean sub clover and white clover lamina weights and foliar nutrient concentrations, Port Hills, Canterbury. Samples taken 3/11/2022. Effect of clover species was P<0.001. SEM = standard error of means. Bolded means were higher (α=0.05).

	Lamina wt	N	P	K	S	Zn	B
	g		**%**			**mg/kg**	
Sub	0.016	3.80	0.149	1.48	0.216	**37.8**	26.8
White	**0.024**	**4.56**	**0.195**	**2.02**	**0.243**	18.9	**32.9**
SEM	0.001	0.05	0.004	0.05	0.003	1.0	0.6

Treatment effects on sub clover foliar nutrients in 2023

Compared with the unfertilised control in 2023, the addition of the +P+S+Mo resulted in higher (P<0.05) foliar concentrations of N, P, S, Mg and B, but lower Cu (Table 3). Of the other fertiliser treatments, those including P increased sub clover P% (-P = 0.16 vs. +P = 0.27%, see Figure 1), while the +P+S treatment had the highest Mg at 0.286%. There was no fertiliser effect on foliar K, Ca, Mo, or Zn.

Comparison of sub clover and white clover in 2022

Lamina weights and foliar nutrient concentrations differed between the two clover species in 2022 (Table 4). White clover had heavier (P<0.05) lamina and higher (P<0.05) concentrations of N, P, K, S and B while sub clover had higher foliar Zn (Table 4). There were no differences in foliar concentrations of Ca (~1.3%), Mg (0.29%), Mo (~0.07 mg/kg), or Cu (10.7 mg/kg).

Discussion

The differences in foliar nutrient concentrations for sub clover and grasses found in this study were consistent with those reported previously between ryegrass and white clover (McNaught 1970). Sub clover had higher concentrations of N, Ca, Mg, B, Cu and Zn while the grasses had higher P, K, S and Mo (Table 2). At this site, grasses were the dominant vegetation being 55% of the total herbage in 2023 compared with 22% total clover (Olykan et al. 2024). The combination of grass dominance, their higher requirement for P, K, S and Mo (Table 2), and a root system more efficiently able to forage for nutrients, e.g., P (Caradus 1980), means the clovers were under intense competition for light, water, and nutrient resources.

Two options to optimise sub clover yields were explored in this experiment – herbicide application to suppress the grasses and P, S, and/or Mo fertiliser additions.

Herbicide application

The herbicide treatment was consistent with ‘chemical topping’. Olykan et al. (2024) reported that in 2023, which had the longest spring growing season, herbicide had a greater effect on sub clover yield than the fertiliser treatments because it suppressed grass and therefore reduced the competition for resources including light and the applied nutrients. Applying herbicide increased sub clover foliar N, P, and S% in 2023 (Figure 1). For increasing foliar P, however, the addition of P was more significant.

The application of herbicide increased grass foliar N% in 2022 and 2023 (Figure 1A). As herbicide addition increased (P<0.001) accumulated sub clover DM in both years (Olykan et al. 2024), it is likely the increased fixed N was available for the grasses. This implies a plant (sub clover) to soil transfer of N. Within the soil, root exudation and decomposition are major N transfer pathways from clover to grass (Reay et al. 2022). Ledgard (1991) estimated that below-ground transfer of N, fixed by white clover, to the grasses was ~70 kg N/ha/yr in a dairy pasture but no values have been reported for sub clover. Higher foliar N% in grasses because of improved clover yields was also found by Scott (1963).

Fertiliser additions

Olykan et al. (2024) found that, within the +H treatment in 2023, applying P and Mo fertilisers (i.e., +H+P+Mo) further increased sub clover yield, and therefore total clover yield, to 4010 kg DM/ha. However, S was in the optimum +P+S+Mo fertiliser combination that increased sub clover lamina N, P, S, Mg and B concentrations, compared with the control plots (Table 3), also noting that foliar P was increased by any fertiliser combination that included P. On these Pallic hill country soils, P addition was primarily needed and there were no clover yield responses to +S or +Mo unless added with P (Olykan et al. 2024). These responses to P addition were expected based on the initially low Olsen P value of 8 mg/kg that was increased to ~24 mg/kg in November 2023 after two applications of 50 kg P/ha in March 2021 and 2023.

Of the three nutrients applied, P had the most impact on clover and grass foliar nutrient concentrations – primarily increasing foliar P but also increasing sub clover and grass S% (Figure 1B). These results highlight the strong demand for P by the grass, as shown by Jackman and Mouat (1970), but also that sub clover foliar P% increased and was therefore also benefitting from the added P.

In contrast, sub clover and grass foliar S% was most affected by the year as shown by fluctuations in the minus S% values (Figure 1C). While the initial soil and sub clover foliar S concentrations were less than current recommendations, Olykan et al. (2024) reported no sub clover yield response to S when applied alone. The lack of a yield response to sulphur addition, despite low soil S, was also found by Smith et al. (2004). They suggested that sub clover was tolerant of soil S levels <3 mg/kg in dryland hill and high country and these results support that.

During this experiment, sub clover lamina Mo in the control treatment averaged 0.08 mg/kg and was associated with an N% of 4.0 (Table 2). Perhaps these values can be considered less than optimal because of the clover response to Mo. The greatest effect of +Mo occurred in 2021 in the Mo-alone treatment, i.e., no added P and/or S, that lifted the concentration to 0.52 mg/kg. It is evident from Olykan et al. (2024) that adding Mo-alone did not increase clover yields in 2023 but was instrumental in increasing yields where herbicide and P were also applied. When P and/or S were applied with Mo, the foliar concentrations were ~0.14 mg Mo/kg. This suggests that foliar Mo declined, compared with Mo-alone, because the sub clover yield increased and the Mo demand for N-fixation in the roots increased. While the addition of Mo had little effect on sub clover foliar Mo (Figure 1D), it increased sub clover N% each year (Figure 1A). Sherrell and Metherell (1986) stated that in situations where shoot Mo was low, but N was adequate, the N-fixation process must be working satisfactorily and shoot Mo concentration increases in proportion to the surplus Mo not required by the nodule bacteria.

The difference in foliar Mo between the grasses and sub clover was large in the -Mo treatments and the addition of Mo had the most effect increasing grass foliar Mo (Figure 1D). The trend over the three years was a rapid decline in the effectiveness of the +Mo to elevate grass Mo. McNaught (1970) recommended that the analysis of separate pasture species was required for fertiliser recommendations. Specifically, our results support their recommendation that legumes should be the preferred indicator plants for nutrient applications. The exception was that a response to added Mo was not apparent in subsequent clover foliage samples but was identified in the grasses each year (Figure 1D). Mo in grass may be a more reliable measure given that Mo content in clovers is often close to the detection limit.

There were differences between the three years of foliar data. Two factors could explain the lower nutrient concentrations in 2022 (Figure 1). Firstly, P and S fertilisers were applied in autumn of 2021 and again in 2023 with elevated foliar P and S recorded in the following spring of these years. However, 18 months had passed before the 2022 foliage samples were taken. Secondly, from August 2022, the monthly effective rainfall was less than normal resulting in an average soil moisture deficit of 64 mm (~30% PAW) in October, which increased into November (Table 1). In contrast, in 2023 the soil moisture deficits were lower than normal.

Changes in sub clover lamina weights appeared to be seasonal with the highest weight of 0.022 g/lamina occurring in 2023, the longest growing season and the most productive for sub clover growth (Olykan et al. 2024). Lamina weights increased with P or Mo in different years but not the herbicide application in 2022 or 2023. Therefore, changes in clover lamina weight do not necessarily reflect clover DM responses to herbicide or fertiliser additions.

The fertiliser nutrients applied to support sub clover growth in mixed pastures include P, S, K, and Mo (e.g., see Olykan et al. 2022). Their requirements are confirmed by clover foliage analysis. At the Port Hills site, the priority is to maintain an Olsen P of 12 to 15 mg/L with regular P applications as superphosphate, for example that also includes S, with molybdenum included every four years.

The sub and white clover lamina results from 2022 (Table 4) highlight the nutrient concentration differences, with white clover having higher N, P, K and S, underlining the need for separate foliar nutrient critical levels for these two species as advocated by Olykan et al. (2019).

This experiment highlighted the difference in sub clover and grass foliar nutrients and therefore the competitive need of grasses for P, K, S and Mo in the pasture system. Of the fertilisers applied on the Port Hills, P and Mo had the greatest effect on foliar nutrients. Applying herbicide had little effect on foliar nutrients but a large impact on clover yields (Olykan et al. 2024) and, as a consequence, resulted in elevated grass N%.

On these dryland hills, an alternative strategy to maximise legume pasture content on drillable slopes is to sow a lucerne monoculture, as demonstrated by Smith et al. (2022), or clover-herb mixtures (Macfarlane et al. 2015). Undrillable steeper hill slopes would be suitable for oversowing the resident pastures with sub clover.

Conclusions

Sub clover and grasses had different foliar nutrient concentrations with grasses having higher P, K, S and Mo. Controlling the grass component, using supressing herbicide or rotational grazing, will increase sub clover yields, increase the N content of sub clover and the associated grasses, and thereby improve the feed quality of the pasture. On this low P site, the priority is to apply P fertiliser, such as superphosphate, with molybdenum included every four years to maximise clover growth and nutrition.

Sub clover, white clover and grasses have different foliar nutrient concentrations, and these results reinforce the need for precise pasture foliar nutrient data to make decisions about fertiliser nutrient applications. If fertilisers are not applied when they should be, then there is less than optimal plant growth and reduced pasture productivity, but if applied when not needed then this is an unnecessary cost and a waste of resources.

ACKNOWLEDGEMENTS

Special thanks to the property owners Professor David Schiel and Dr Barb Hayward for hosting the experiment and their ongoing support of Dryland Pastures Research projects. We thank Emily Huang for mineral nutrient analysis.

Funding for this project was provided by Beef + Lamb New Zealand, MBIE, RAGT New Zealand, and PGG Wrightson Seeds under the "Hill Country Futures" research programme (BLNZT1701).

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