Evaluating Lupin's Agricultural Potential as a Cover Crop in Vermont

College of Agriculture and Life Sciences

Distinguished Undergraduate Research Thesis Cover Page

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Sean Pease

Abstract

Vermont's intense seasonality and short, highly variable growing season can make it difficult for farmers to implement cover crops in crop rotations. Cover cropping is an important practice for improving soil quality, increasing the soil's capacity to hold nutrients, and reducing run-off of fertilizers into rivers, streams, and lakes. Legumes with their nitrogen-fixing capacity and taproot structure are important as farmers look to reduce synthetic fertilizer inputs, soil compaction and increased environmental and fertilizer costs. The narrow leaf blue lupin (Lupinius augustifolius), evaluated in this field trial, is an annual variety grown extensively for green manure and cover cropping practices. It's cold-hardiness, special taproot system, ability to mine phosphorus and fix nitrogen offer many potential uses in Vermont's agriculture. It has potential for being a substitute for the less cold-tolerant soybean in maize systems and as a new source of highly digestible protein feed for dairy and livestock systems. Field trials were conducted to evaluate the potential of lupins as a cover crop here in Vermont by measuring yields and effect on nutrient status and soil properties across five varieties of narrowleaf lupin. Narrowleaf blue lupins were found to be viable cover crops. The lupin variety Roland showed the most promising performance by increasing macronutrient and micronutrient profile, having the earliest flowering time, and performing strongly for other important agronomic traits.

Introduction

Lupins (*Lupinus* spp.) are an agronomically important genus of legumes well adapted to acidic, sandy, and low fertility soils. With a history of being cultivated for thousands of years as a forage and green manure crop and bred to create sweet varieties that lack alkylating agents (Gladstones, 1970), lupins are now grown worldwide for a wide range of agricultural purposes. The four annual species (*L. albus, L. augustifolius, L. luteus, L. mutabilis*) have been grown as a cool season crop for cover cropping, green manuring, fresh forage, silage, haying, and seed production (Stoddard et al., 2015; Allen et al., 1978). The use of legumes in cover cropping has become an increasingly important method for improving soil quality because they decrease synthetic fertilizer inputs and reduce soil erosion and compaction (SARE, 2019). Legume-based cover crops are further known to improve upon soil quality by fixing nitrogen, reducing pathogens, and increasing subsequent cash crop yields (Marques et al., 2020).

There is an increased interest in the role of cover crops in facilitating improved cash crop yields through soil conditioning. This conditioning can result from plants fostering specialized communities of microbes, improving nutrient availability and soil properties within its local rhizosphere (Hallama et al., 2018). This interest has culminated in a standardized plant-soil feedback framework that can be used to quantify the effects of a plant on its immediate rhizosphere (Mariotte et al., 2018). Studies have since been able to draw conclusions between the relationship of plants, their functional traits, and impact on rhizosphere through soil conditioning (Ingerslew and Kaplan, 2018). There is currently no information regarding lupins' soil conditioning effects in a cover cropping context.

Lupinus augustifolius, the narrow leaf or the blue lupin, is a widely used annual crop species with many agronomically and economically important traits. Its high bulk density, carbohydrate, fat and protein content make it an ideal, highly digestible food crop for both livestock and humans. Lupins are especially suited for in-dairy feeding systems because milk yields increase significantly as a result of the higher metabolisable energy content of lupins when compared with cereal grains (White, Staines & Staines, 2007).

Like other legume species, lupins form relationships with nitrogen-fixing diazotrophs, known as rhizobia. The narrowleaf species, specifically, can mineralize upwards of 116 kg of nitrogen per acre (White, French & Mclarty, 2008). Lupins are distinguished from other legume species in that they can form a specialized cluster root structure shown to secrete copious amounts of carboxylates capable of mining and mobilising previously soil-bound phosphorus, a nutrient where availability is correlated positively with aggregate stability in soils, an important proxy in assessing soil quality (Lambers, 2006; Hallama et al., 2018; Sanchez-Novarro, 2019). Its root system is further characterized by a deep and aggressive taproot with non-annuals able to reach depths of 2.5 meters (Clements et al. 1993). Root depth is an important component in nitrogen and potassium cycling in soils, as it prevents soil compaction and further improves aggregate stability through increased soil organic carbon inputs (Garcia et al., 2018; Mpeketula and Snapp, 2019).

Despite being touted as having multifaceted benefits, cover cropping has yet to be adopted extensively. Increased management requirements, implementation costs, performance variability, and the lack of determined savings from reduced synthetic inputs are all cited as key barriers to its adoption (Daryanto et al, 2019). Farmers in Vermont are additionally challenged by a harsh climate and short growing season. Only a limited number of viable cover crops are available to farmers in the northeast, and with a short growing season many farmers need to maximize their production in the summer. Alternative summer crops that enrich the soil and have market value may fill this need. Soybeans, a well-established crop in corn and field crop rotation, are poorly adapted to the northern climate despite targeted breeding programs for improved cold tolerance (van Heerden & Kruger, 2000; Cober et al., 2013). This lack of regionally viable cover crop options (i.e., "tools in the toolbox") decreases the adoption and efficacy of cover cropping by increasing seed and operation costs while decreasing potential benefits. Lupins are an excitingly innovative cold hardy management tool. Vermont farmers could exploit the crop for both its commercial and nutritional value as a forage and feed with significant pod yields and its cover cropping value with its cold hardiness, ease of termination, and improvement of soil fertility. Nonetheless, the viability of annual lupins as a cover cropping option in Vermont has not been evaluated agronomically.

The purpose of the study is to evaluate the viability of narrowleaf blue lupin as a multifaceted cover crop in Vermont. It was hypothesized that there are certain varieties of cultivated annual *Lupinus* variety that will be highly effective as a cover crop by providing vigorous biomass, yields, and taproot growth while improving the nutrient availability and wet aggregate stability of resident soils. The objective of this project is to evaluate five varieties of the narrowleaf blue lupin species as a cover crop based on performance measurements of important agronomic traits: biomass and pod weight for forage value, growth, height, stem diameter, taproot length and mass, flowering time, and effects on soil wet aggregate stability and soil nutrient status (i.e., soil conditioning).

MATERIALS AND METHODS

Trial Overview

In Summer 2019, a lupin cover crop variety trial was conducted at the UVM Horticulture Research and Education Center (HREC) in South Burlington, VT. The soil type at the HREC location was an extremely sandy Adams and Windsor loamy sand. The experimental design for the variety trial was a randomized complete block design with four blocks using five different varieties of *L. augustifolius* received from the Czech Ministry of Agriculture Crop Research Institute (CRI) genebank (Table 1). Czech varieties were chosen as potentially matching the climate of Vermont.

Table 1. Varieties evaluated for variety trial 2019, South Burlington, VT.

Species	Variety Name
Scientific Name: *Lupinus augustifolius*	1. Lo4 - ROLAND 2. Lo4 - REGENT 3. Lo4 - TYTAN 4. Lo4 - WARS 5. Lo4 - DALBOR
Common Name(s),: narrowleaf blue lupin, narrowleaf lupines, blue lupines	

Varieties were made available by the Czech Ministry of Agriculture Crop Research Institute (CRI) genebank in collaboration with the UVM Crop Genetics Lab.

Seeding rates were adjusted according to seed availability, extension recommendations, and available space at the HREC facility. These considerations led to a desired seeding rate of 15 grams per two meters squared for each demonstration plot within the randomized block design. Three rows were planted within each plot using a hand-pushed Jang seeder to simulate a seed drill (see Table 2). The seeds were inoculated with the appropriate *Bradyrhizobium* species specifically for lupins (see Table 2). The seeds were weighed out to the desired seeding rate prior to being inoculated to prevent change in planting density from increased weight of added inoculant.

Table 2. Lupin variety trial specifics 2019, South Burlington, VT.

Location	UVM Horticulture Research Center		
Timeline	**Planting**	**Emergence**	**Harvest**
	May 24	May 31	August 12
Seeding Specs	**Field Size (meters)**	**Row Spacing (cm)**	**Density (grams/meter ^{2})**
	0.9 x 42.6	18 with 3 rows	7.5g
Soil Type	Adams and Windsor loamy sands, 0-5% slope		
Seeder	Jang JP- 1Seeder, (Johnny's Seed Company, Maine)		
Weather Station	Rainwise AgroMET, (RainWise Inc., Maine)		
Seed Inoculant	H Type Inoculant, (Hancock Seed & Company, Florida)		

UVM Horticulture Research Center is a farm in South Burlington, VT operated by the University of Vermont.

The experiment was irrigated throughout the trial with surface dripline for one hour per week to prevent physiological plant stress. The establishment rates of the lupin varieties were recorded two weeks after planting to assess stand uniformity across plots and maintain records of potential sources of variability in performance measurements. An on-farm weather station (Rainwise AgroMET) with sensors for temperature, leaf wetness, precipitation, relative humidity, and solar radiation was used to track seasonal weather data.

Sampling Procedure for Agronomic Traits

Three individual plants per plot were selected randomly as subsamples by using a random number generator to define the sequence at which plants would be picked (e.g.. 3, 5, 7… pull 3rd plant, pull 5th plant from 3rd, pull 7th plant from 5th). The subsamples for each of the plant measurements were then averaged by plot to represent field replicates within the experiment. In summary, measurements were made for five varieties that were replicated four times for a total of 20 experimental units. The central most of the three planted rows was used for all subsample collections in both plant and soil measurements. The outermost rows were left as buffer zones along with a spacing of 0.3m between plots. The most central area of each of these buffer zones was used as the sampling area of control plots for the soil measurements (i.e., no lupin treatment). The individual plots were used as subsamples for the soil measurements with a replicate representing the mean of four plots of a treatment from the four different blocks. This resulted in only one replicate for each variety used as a treatment or four in total for evaluating the general lupin soil conditioning effect on soil (i.e., only 4 experimental units).

Statistical Analysis

Statistical analysis of the data collected was done using R statistical language (R Core Team, 2019). The package LmerTest was used to perform mixed linear ANOVA tests with random effects and Satterwaite’s degrees of freedom for assessing significance (Kuznetsova, Brockhoff & Christensen, 2017). The compatible packages multcompView and emmeans were used to compare, assess, and visualize significant differences between varieties using Tukey’s post hoc tests (Piepho and Hans-Peter, 2004, Lenth, 2019). The Tukey’s post hoc tests were coded using the base package already available within R. Visuals and graphics were created primarily using the ggplot and ggpubr packages (Whickham, H., 2016 and Kassambara, 2019). The main focus was to analyze the mean performance of all varieties evaluated to determine the magnitude of potential that narrowleaf blue lupins in general, or varieties of the species specifically, have for being used as a cover crop in Vermont.

Plant-Soil-Feedback Framework:

A plant-soil feedback framework was used to evaluate the soil conditioning effect of lupines on soil quality by using soil nutrient status and wet aggregate stability as proxies. The following formula was used to determine the impact lupines had on these proxies:

$$PSF = ln\left(\frac{measurement}{control\ \mu}\right)$$

where measurement is the single replicate value from a measurement of a soil nutrient (e.g. phosphorous, boron, magnesium etc.) or soil property (e.g., pH, wet aggregate stability, % organic matter, etc.) at each treatment plot and the control mean (μ) is the mean of all four blocks with blocks being used as subsamples. The natural log in this equation gives direction and magnitude (e.g. $0 = \ln \frac{1}{1}$, $0.7 = \ln \frac{2}{1}$, $-0.7 = \ln \frac{1}{2}$) for estimating cascading effects temporally. This standardized PSF value allows for a valuable and straightforward interpretation of treatment effects. If PSF < 0, a negative or decrease in the observed value was found under the lupin treatment at a rate or magnitude less than what was found under control conditions. If PSF > 0, a positive or increase in the observed value was found under the lupin treatment at a rate or magnitude greater than what was found under control conditions.

Plant Agronomic Traits: Flowering, Height, Stem, Taproot, Biomass and Yield

Plots were observed daily for occurrence of the first flower and when half of the plot was observed to be flowering. Time to half-plot flowering was assumed to represent variation within individuals of plants across blocks. First flower date was defined as the potential of individuals within the populations tested. The height of each variety for each block was recorded after initial flowering. Four plants from the central row of each plot were selected randomly for measurement. A ruler was used to measure height from the soil surface to the youngest meristem of the plant to the nearest tenth of a millimeter (Niels et al., 2009). The basal stem diameter was recorded once at flowering using the same plant. The basal stem diameter is defined as being 2 cm above the soil surface (Spetich et al. 2002). Stem diameter was measured with digital calipers and recorded to the nearest hundredth of a millimeter. Biomass and taproot lengths for each variety was recorded after being harvested and dried. Four plants within the plot were selected randomly, dried at 105°F until a stable weight was attained, and then the individual weights of the entire plant including roots were recorded (Darby, 2018). Subsequently, the pods were removed, counted, and weighed for each individual plant to evaluate yields. Finally, roots were separated from the plant for length measurement and mass weights to assess root mass and lengths.

Soil Effects: Plant-Soil Feedback, Wet Aggregate Stability, Nutrient Status

Wet aggregate stability and nutrient status were evaluated as proxies for lupin treatment effects on soil quality. Aggregate stability is an important parameter for soil quality representing the soil's physical structure. Nutrient status is an important parameter representing the chemical structure of soil and a basis for fertility that supports subsequent plant growth. These effects were quantified using a plant-soil feedback approach.The order and spatial differences of sampling used were as follows; control samples were taken per block right before planting and immediately after plant harvest within designated buffer zones between treatment plots, Treatment samples were taken for each plot, prioritizing soil area that had at least three plants in close proximity, effective shade from those plants, and that occupied the most central area of the most central row (see supplemental figure 1). Samples were taken with an intact core sampler to minimize disturbance in sample extractions while using a modified core cup that was split down the middle to allow splitting of samples for subsequent analysis (see supplemental figure 2). Samples for soil analysis were bagged, labeled, and sent to the Agricultural & Environmental Testing Laboratory for a soil analysis of pH, organic matter, available aluminum, boron, calcium, copper, iron, magnesium, manganese, phosphorous, potassium, sulfur and zinc; % base saturation, and effective CEC. Wet aggregate stability was evaluated using a modified version of Yoder's (1936) procedure with a wet sieving machine using a non-vacuum, pre-wetting technique and 2mm, 1mm, and 0.5mm sieve cans (see supplemental figure 1). Briefly, soil samples were allowed to air dry for one week before 25 grams of air dried soil was pre-wetted for three minutes and sieved for 10 minutes. The sieve cans with the distributed soil were then carefully removed and allowed to dry in an oven for 48 hours at 90°F. The weights of the sieve cans were weighed and subtracted from the post-sieved soil weight to determine the fractionation of aggregate size across the sieve cans (see supplemental figure 3).

Results

The overall growing season of 2019 was relatively cold and wet in the spring and hot and dry through the summer months (Table 3). There was a strong decrease in rainfall and increase in temperature in July, specifically (Table 3). Overall, the season in which the lupins were trialed was cool for Burlington, VT accumulating a low of 1793 Growing Degree Days (GDDs) (Table 3). The higher amount of precipitation and cooler temperatures provided ideal conditions for good germination and establishment of the blue narrow leaf lupin varieties that lead to uniform stands in the plot (Table 3).

Table 3. Seasonal weather data collected in Burlington, VT, 2019

Burlington, VT	May	June	July	August
Average temperature (°F)	54.9	66	74.9	70.2
Departure from normal	-1.4	0.2	4.3	1.4
Precipitation (inches)	5.15	4.99	1.91	2.77
Departure from normal	3.45	1.3	-2.2	-1.14
Growing Degree Days (base 50°F)	150	413	684	546
Departure from normal (# of days)	-62	-110	-45	-58

Based on weather data from Rainwise AgroMET weather station, and the newa.cornell.edu website: http://newa.cornell.edu/index.php?page=weather-station-page&WeatherStation=kbtv. s
The 2019 growing season in Burlington, VT was characterized by a cold wet spring and hot, dry summer.
Historical averages(normal) are for 30 years of NOAA data (1981-2010) from Burlington, VT.

Soil samples from the experimental site were collected prior to any planting treatments to gauge initial fertility issues and afterwards to quantify seasonal control and lupin plant effects.The field had initially high phosphorus, magnesium and calcium saturation % and low initial potassium, sodium, magnesium saturation %, and potassium saturation % (Figure 1). There were substantially large increases in soil sodium content that occured for both treatment plots over the season (Figure 1). There was another very large increase in calcium saturation % that occured over the season for the lupin treatment plots. In the post-planting, control plot values showed large increases in initial sodium and magnesium values and decreases in initial potassium values. The post-planting lupin treatment plots showed a similar decrease in potassium to control treatment plots and a similar but smaller increase in sodium.

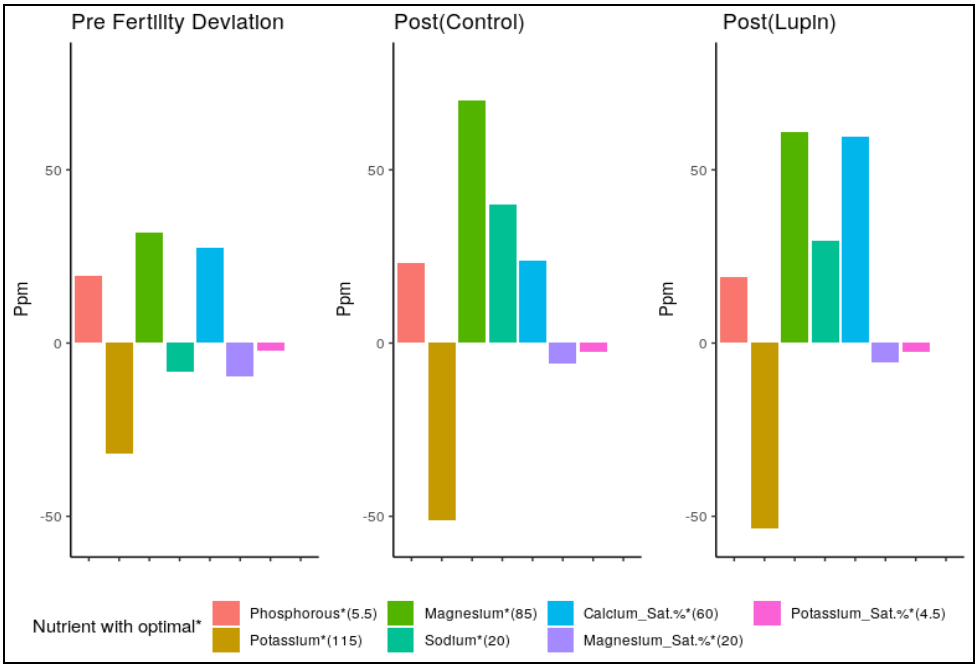

Figure 1: **Initial and Post-Planting Fertility Deviations** Initial and post-planting fertility deviations. Optimal values for field crops are located on column titles after * (e.g.: (*5.5) is the optimal ppm value for phosphorus). These optimal values are determined for field crops by Agricultural & Environmental Testing Laboratory. Nutrients measured in this study, but not showing large differences, are not illustrated (i.e., sodium, calcium).

Narrowleaf Blue Lupin: Soil Conditioning Effects Overview

Soil conditioning effects from the variety trial were averaged out to assess the mean performance of the species as a singular whole (i.e., narrowleaf blue lupin). The narrowleaf blue lupin varieties tested in this trial had mild soil conditioning not exceeding either -0.25 or +0.25 for Plant-Soil Feedback (PSF) values (Figure 2). This was not the case for lupins' soil conditioning effects on cation exchange capacity (Effective CEC) with large negative PSF values being observed (Figure 2).

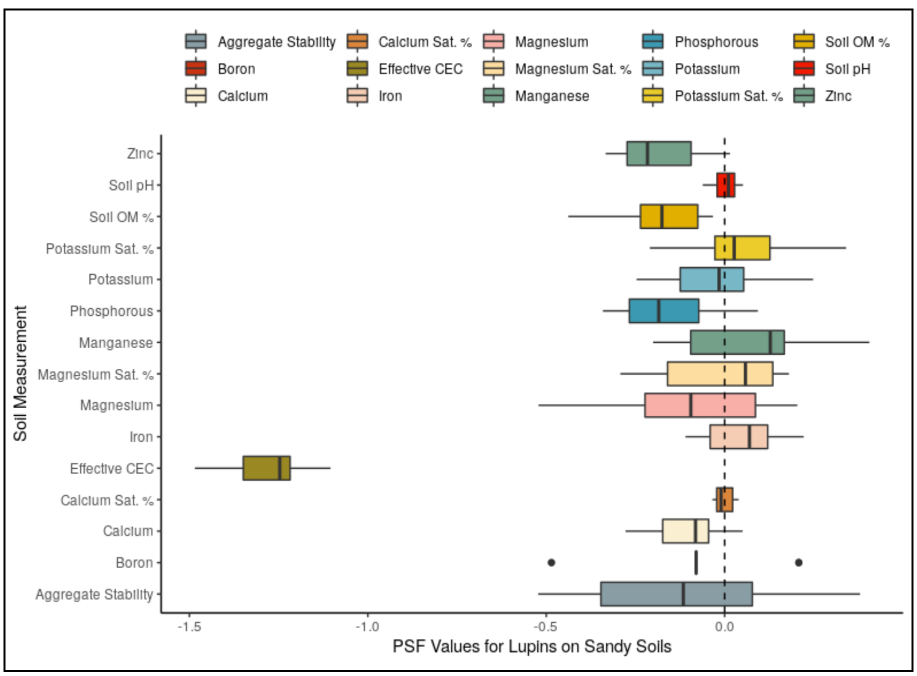

Figure 2: **PSF Values for Lupins on Sandy Soils.** Illustrated are means (n = 80) of lupin plants to assess the mean performance of the species as a singular whole (i.e., Blue Narrowleaf Lupin). The majority of soil conditioning effects were indistinguishable staying within a range -0.25 to +0.25. Plant-Soil Feedback (PSF) values, representing a standardized effect that a plant has on the feedback loops present in soils The largest soil conditioning effect was a negative effect on Effective CEC. A 95% confidence level was used for plot ranges. The dotted line at 0 indicates no effect. A positive effect for this figure would indicate there was an increase for a value in soil grown under lupins than soil grown under control or bare ground conditions.

Effective cation exchange capacity saw a markedly large increase from initial values under control conditions but a small decrease under lupin conditions (Figure 3). This is accounted for by a large increase in sodium between the initial and final values, which was greater under control than lupin treatments (Figure 3). Moreover, there was an additional increase in calcium saturation % under lupin but not control treatments (Figure 3).

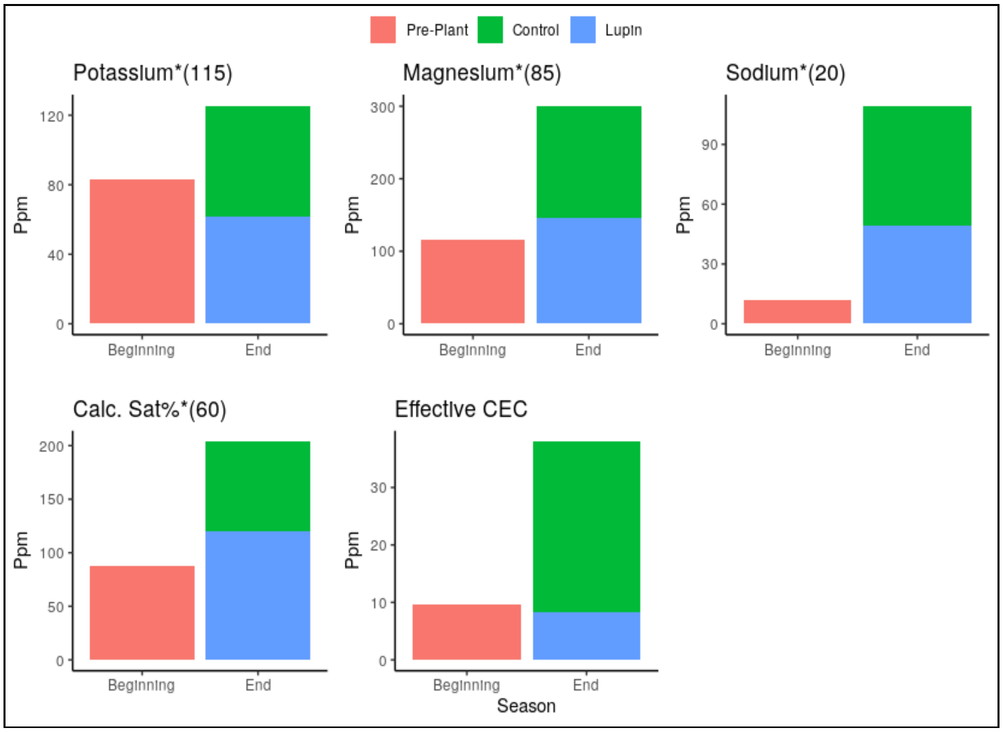

Figure 3: **Initial and Post-Planting Fertility Values affecting CEC**. Illustrated are means (n = 4) of soil sampled across treatments prior to any planting treatments. * (e.g.: (*5.5) is the optimal ppm value for phosphorus). Optimal values are determined for field crops by Agricultural & Environmental Testing Laboratory.

Soil Conditioning and Wet aggregate stability Effects for Varieties

Wet aggregate stability and nutrient status among lupin and control treatments were similar (Table, 4). There was a non-significant trend for all varieties to exhibit negative soil conditioning. Tytan and Roland both had the most positive soil conditioning effects on increasing aggregate stability (Figure 4). Wars and Dalbor both showed consistent negative or decreasing effects on aggregate stability (Figure 4).

Table 4: Analysis of Variance for Soil Conditioning Effects: Plant- Soil Feedback

All ANOVAs were mixed linear tests with blocks used as the random effect. Type III Analysis of plant traits using Satterwaithe's Degrees of Freedom. Significance at Pr(>F) < 0.05 in bold type.

	Sum Sq	Mean Sq	NumDF	DenDF	F value	Pr(>F)
Aggregate Stability	0.397	0.099	4	12.000	2.602	0.089
Macronutrients	0.100	0.025	4	60.339	1.314	0.275
Micronutrients	0.107	0.027	4	54.085	0.879	0.483
Soil Properties	0.044	0.011	4	89.000	0.043	0.996

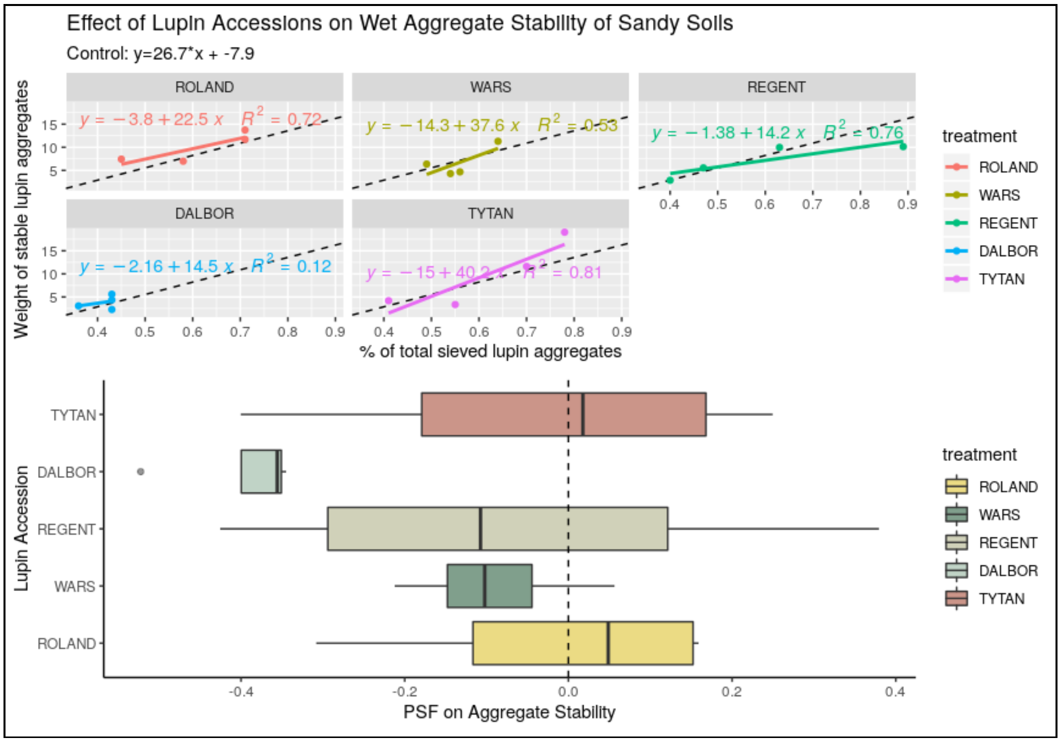

Figure 4: **PSF Soil Conditioning effects on Wet Aggregate Stability**
The regression equations represent the slope of change for aggregate stability under lupin conditions contrasted to that of regular seasonal change. The box plots illustrate the median, range and 95% confidence levels of the Plant-Soil Feedback values for each lupin variety. The dotted black lines represent the control or seasonal conditioning effects of the production of stable aggregates (P = 0.089) under the soil conditions that were kept bare. A positive value indicates an increased proportion of stable aggregates.

Lupin Varietal effect on Soil Nutrient Status

Roland and Tytan had the most consistent positive effects for both micro- and macronutrient status. Soil conditioning effects on the macronutrient status of sandy soils were not significantly different among lupin varieties ((Pr>F) = 0.275) (Table 4). All varieties showed some range of negative effects, besides the variety Roland which had an overall positive effect on increased potassium content, but showed at least some range of negative effects for other macronutrients (Figure 5). Dalbor had the largest positive effect on magnesium with Tytan and Roland also showing some positive effects (Figure 5). There were no significantly different (Pr>F) <0.05 soil conditioning effects for lupin variety on the micronutrient status of sandy soils (Pr>F) = 0.483 (Table 4). Roland, along with Tytan, were observed as having entirely positive effects on iron and manganese content (Figure 6). All varieties had negative effects on the boron status with Dalbor and Regent having large negative effects on boron status(Figure 6). There were no significant ((Pr>F) <0.05) soil conditioning effects for lupin variety on the various soil properties ((Pr>F) = 0.966) of sandy soils (Table, 4). The largest observed effects on soil properties were from Roland, Wars, and Regent on potassium saturation.

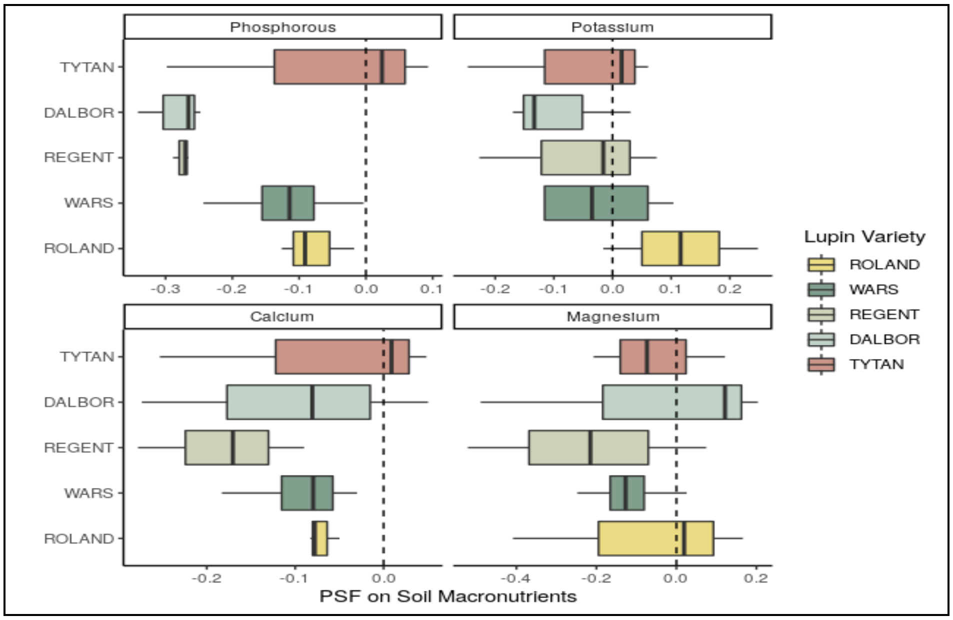

Figure 5: **Standardized Plant-soil-feedback effects on macronutrients.**
The dotted black lines represent the control or seasonal conditioning effects on macronutrient status under the soil conditions that were kept bare. A 95% confidence level was used for plot ranges. A positive effect for this figure would indicate an increased value of the nutrient.

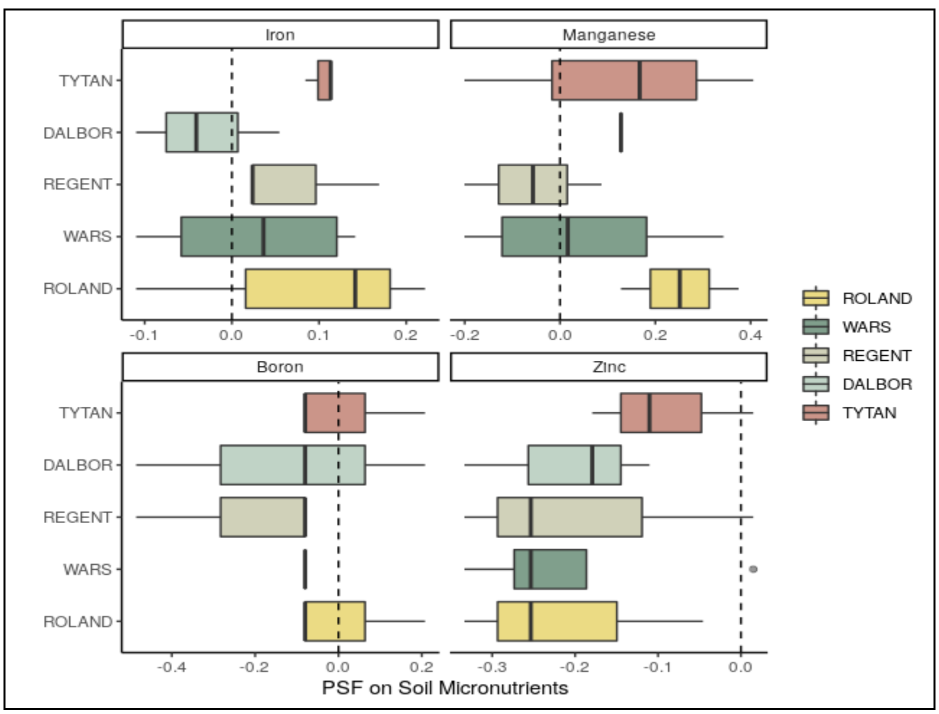

Figure 6: **PSF Soil Conditioning effects on Macronutrients**
The dotted black lines represent the control or seasonal conditioning effects on micronutrient status under the soil conditions that were kept bare. A 95% confidence level was used for plot ranges. A positive effect for this figure would indicate an increased value of the nutrient.

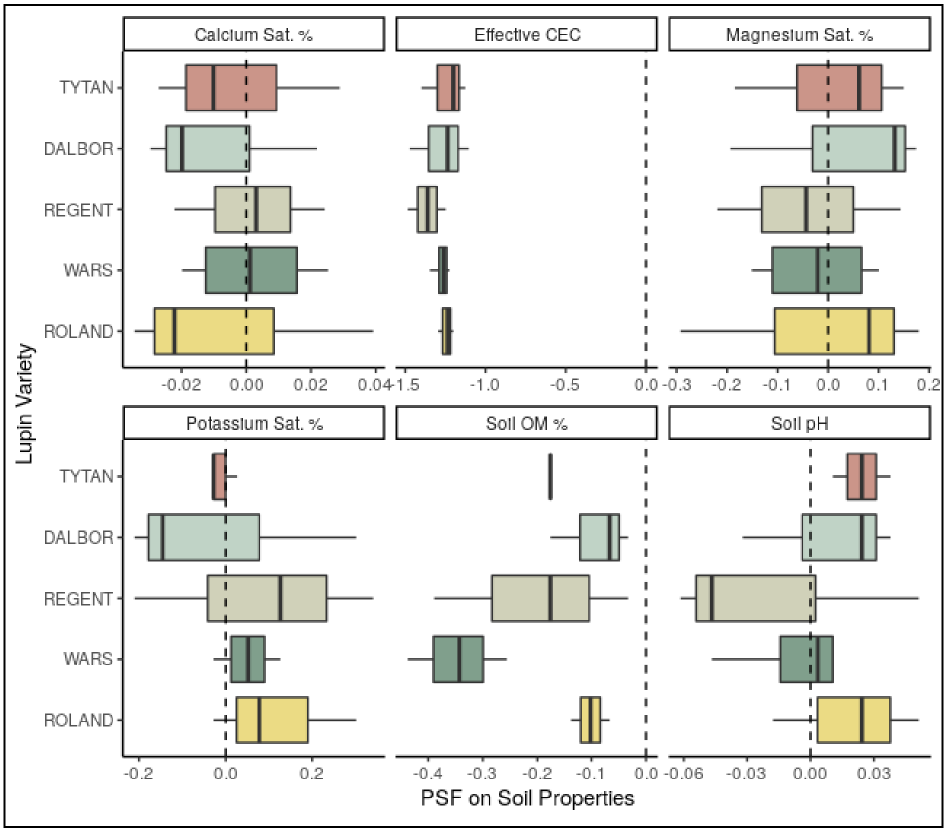

Figure 7: **PSF Soil Conditioning effects on Soil Properties**
The dotted black lines represent the control or seasonal conditioning effects on various soil properties under the soil conditions that were kept bare. A 95% confidence level was used for plot ranges. PSF refers to Plant-Soil Feedback; A conceptual framework that helps estimate and standardize the effect that a plant has on the feedback loops present in soils. A positive effect for this figure would indicate an increased value of the property.

Plant Agronomic Traits

Narrowleaf Blue Lupin: Plant Trait Overview

Values from the variety trial were averaged out to assess the mean performance of the species as a whole. The narrowleaf blue lupin varieties tested in this trial had the species flowering after approximately 30 days from emergence, accumulating an average of 13.6 grams of biomass, yielding 7.8 grams in pod weight and having a root mass of 0.9 grams. The species grew to an average of 19.9 cm with a basal stem diameter of 3.1 cm. The rooting depth of the species averaged to 19 cm.

Narrowleaf Blue Lupin: Plant Trait Overview for Varieties

Five varieties of the narrowleaf blue lupin species, *L. augustifolius*, were evaluated for important agronomic traits and then compared. There were significant differences ((Pr>F) <0.05) between lupin varieties for the traits; height, flowering, root length, root mass, above and below-ground biomass accumulation, and pod weight (Table, 5). There were no statistically significant differences between varieties for basal stem diameter (Table, 5).

Table 5: Analysis of Variance for Plant Traits between Varieties

	Sum Sq	Mean Sq	NumDF	DenDF	F value	Pr(>F)
Height	68.765	17.191	4	16	6.909	**0.002**
Stem	0.311	0.078	4	16	1.338	0.299
1st Flower	18.500	4.625	4	20	3.978	**0.016**
50% Flower	18.300	4.575	4	20	4.112	**0.014**
Root Length	39.069	9.767	4	16	3.461	**0.032**
Biomass	63.190	15.797	4	16	5.302	**0.006**
Pod Weight	31.665	7.916	4	20	4.698	**0.008**
Root Mass	0.171	0.043	4	16	3.383	**0.035**

Significance at Pr(>F) < 0.05 in bold type. Type III Analysis of plant traits using Satterwaithe's Degrees of Freedom. All ANOVAs were mixed linear tests with blocks used as the random effect in R, a programming language and statistical computing software. The package used was LmerTest (Kuznetsova, Brockhoff & Christensen, 2017) . 50% Flower refers to the time at which a plot for a particular accession had at least half of it's plants flowering.

Varieties were significantly different ((Pr>F) <0.05) in their flowering times for first flower and half plot flowering, ((Pr>F) = 0.016, 0.015) (Table, 5). Using Tukey's Honest Significant Test for ANOVA contrasts, Roland's early flowering time was only significantly different from the Dalbor variety (Figure 8). The variety Roland had the earliest flowering times with the first flower and 50% flower being observed at 25 and 29 days after emergence, respectively (Figure 8). The variety Dalbor had the latest flowering time at 28 and 34 days after emergence (Figure 8).

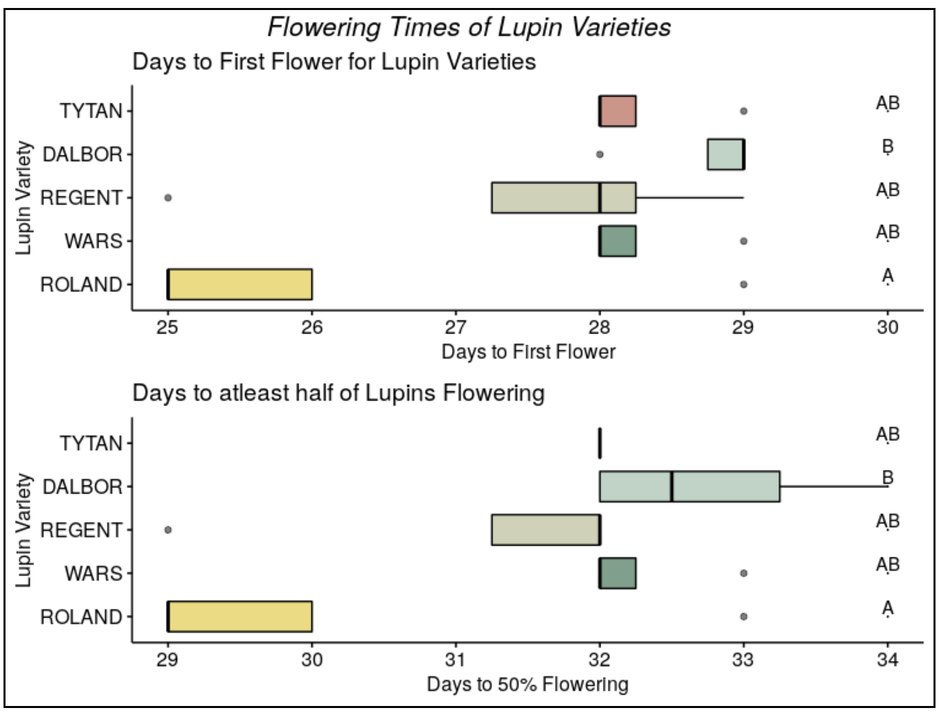

Figure 8: **Flowering** The lupin variety Roland had the earliest flowering times, Dalbor had the latest flowering times. There was a statistically significant difference between the Dalbor and Roland flowering times. The letters represent significant differences between varieties using Tukey's Honest Significance Test(HSD). Tukey's HSD uses a consevative p-value to test if means are significantly different from each other. Varieties with the same letter are not statistically different from each other. A 95% confidence level used for plot ranges.

Lupin varieties were significantly different ((Pr>F) <0.05) in their height ((Pr>F) = 0.002) (Table 5). Roland had the highest observed heights between 25.4 cm and 20.15 cm (Figure 9). Roland had observed heights that were significantly taller than those of the Dalbor, Regent, and Wars varieties (Figure 9). Tytan, the second tallest variety, was not significantly different in height to any varieties (Figure 9). Lupin varieties were not significantly different in their basal stem diameters (Table 5). Tytan had the largest observed basal stem diameter of 3.6 cm. The other varieties; Dalbor, Regent, and Wars all had variable basal stem diameters ranging between 2.8 and 3.4 cm, approximately (Figure 9).

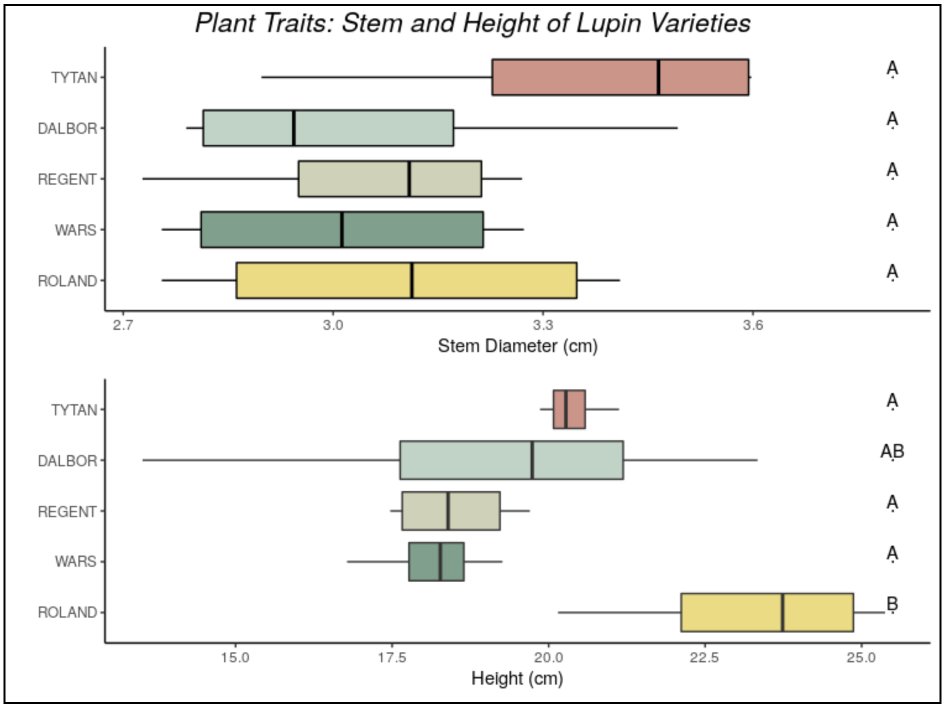

Figure 9: **Stem and Height of Lupin Varieties**
The lupin variety Tytan had the largest observed basal stem diameters. There were no statistically significant (Pr>F) <0.05 differences between varieties for basal stem diameter (Pr>F) = 0.002. The lupin variety Roland had the tallest observed heights. Roland was significantly taller than all of the other varieties other than Dalbor. The letters represent significant differences between varieties using Tukey's Honest Significance Test(HSD). Tukey's HSD uses a consevative p-value to test if means are significantly different from each other. Varieties with the same letter are not statistically different from each other. A 95% confidence level was used for plot ranges.

Although there was a statistically significant effect ((Pr>F) <0.05) of variety on the difference in root length and root mass using an analysis of variance ((Pr>F) = 0.032, 0.035), Tukey's more conservative Post-hoc HSD did not confirm this in the contrast testing (Table 5, Figure 10). Roland and Dalbor had the longest observed root length with lengths ranging between 19 and 22 cm, approximately (Figure 10). Tytan and Regent had the shortest observed root lengths with lengths ranging between 16.5 and 18.5 cm, approximately (Figure 10). Roland had the largest observed root mass values. Roland and Dalbor both had an observed root mass value of 1.1 grams (Figure 10). All other varieties had observed root mass ranges between 0.57 grams and 0.95 grams, approximately (Figure 10).

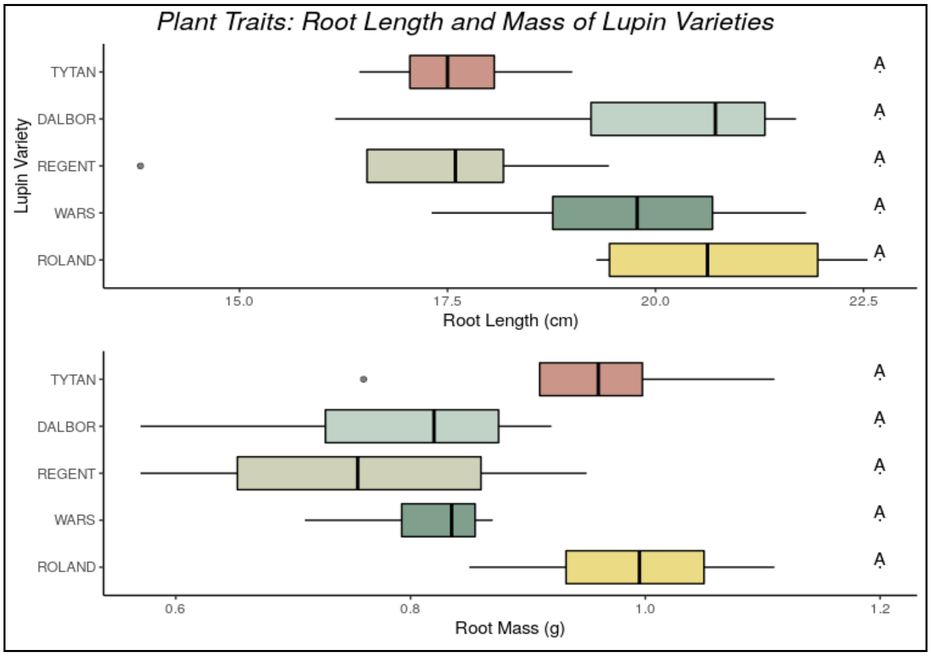

Figure 10: **Root Length and Mass of Lupin Varieties** The lupin varieties Roland and Dalbor had the longest observed root lengths, respectively. Roland and Tytan had the greatest root masses.The letters represent significant differences between varieties using Tukey's Honest Significance Test(HSD). Tukey's HSD uses a consevative p-value to test if means are significantly different from each other. Varieties with the same letter are not statistically different from each other. A 95% confidence level was used for plot ranges.

There was a significant effect ((Pr>F) <0.05) of variety on biomass accumulation and pod weight for lupins ((Pr>F) = 0.006, 0.008) (Table 5). The lupin variety Tytan had the greatest biomass (Figure 11). Tytan, Regent and Roland had the largest observed pod weights, respectively (Figure 11). Tytan, with the largest observed pod weight values was only statistically larger than Wars which showed the smallest observed values for pod weight (Figure 11).

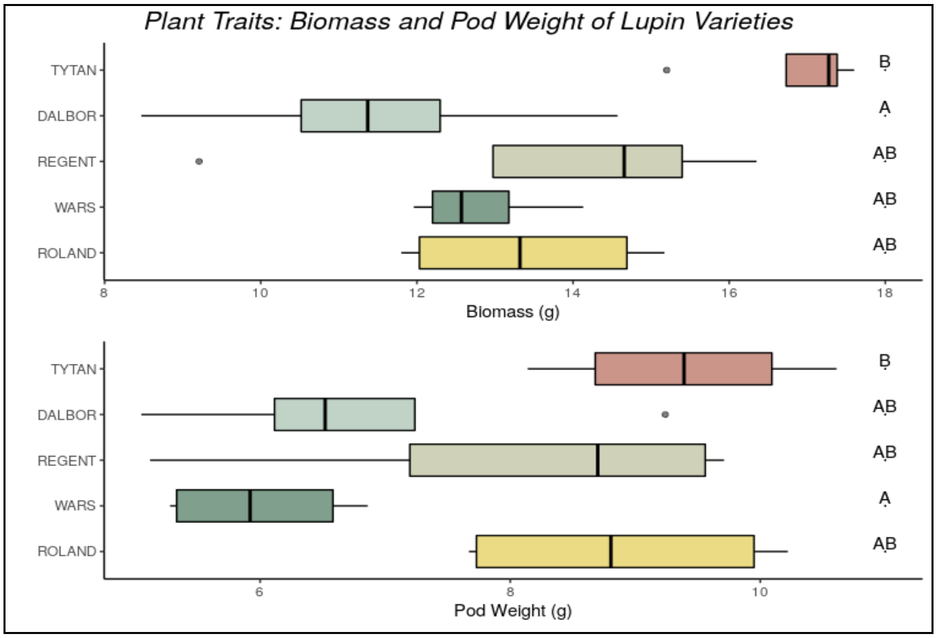

Figure 11: **Biomass and Pod Weights of Lupin Varieties** The letters represent significant differences between varieties using Tukey's Honest Significance Test(HSD). Tukey's HSD uses a consevative p-value to test if means are significantly different from each other. Varieties with the same letter are not statistically different from each other. A 95% confidence level was used for plot ranges.

DISCUSSION

The spring was cold and wet, ideal for germination and emergence of lupins, with an especially hot and dry July, the period when the lupins were flowering that resulted in minimal leaf scorch but may also have affected pod set. The initial field soil status showed excess phosphorus, magnesium and calcium saturation. Initial field soil status also showed deficiencies in potassium and sodium.

Nutrient excesses have been shown to have either neutral or negative effects on root biomass and PSF as there is less drive for root growth with adequate nutrient sinks and larger competition from antagonistic soil biota (Zandt et al., 2019). This may have shifted both root measurements and PSF soil conditioning effects more negative for this trial. Lupins also have exceptional phosphorus mining capacity and may generate additional negative PSF effects. Species with high phosphorus content have been shown to harbour more pathogenic organisms which can in turn result in greater negative feedback on plant growth (Kong, Song, & Ryu, 2019). It is important to consider that a classic example of a positive PSF is N-fixation done in poor soils. At first the build up of nutrients from fixation in poor soils can lead to pathogen competition and result in an initially observed negative psf but in later stages as competition dynamics stabilize can contribute to increased fertility and improve the growth of subsequent plants (van der Putten, et al., 2013). The general negative PSF values observed in lupin's effects on soil conditioning may be an example of this initial negative PSF effect that has been observed from N-fixation in poor soils and thus requiring longer term studies for clarification.

The large negative PSF on cation exchange capacity under the lupin plots corresponds strongly with a large decrease in sodium when compared to control plots. The large increase in excess sodium observed in control conditions over the season seemed to be mitigated under lupin treated soils. This suggests that lupins mitigated the excess sodium accumulation in soils. This may have resulted from lupin's functioning either as a source of shade, decreasing the evaporation and subsequent salinization of top soil, as a sink, accumulating sodium within plant tissues or as an interaction of both functions. Additionally there was a large increase in calcium saturation under lupin conditions. In mitigating excess sodium levels, a monovalent cation and allowing a larger saturation of divalent calcium cations to saturate soil surfaces, the lupin treated plots could have mitigated the large increase in effective CEC that was observed for the control plots, registering a large negative PSF effect for the effective cation exchange capacity. There were no statistically significant PSF soil conditioning effects that were observed in this trial. This may be a result of low sample size and sampling techniques that were not discrete enough for observing the minute but important cascading changes that can occur in soils.

In assessing wet aggregate stability there were only three sieve sizes, with the largest sieve segregating aggregates either greater than or less than 2mm. This large segregation may not have been discrete enough for observing changes in soil aggregation. This suggests that more discrete sieve sizes (e.g. 0.5mm, 0.75mm, 1mm, 1.25, 1.50, 1.75 mm, and 2mm sized sieves) could be useful for estimating cover crops' effects on wet aggregate stability.

The Roland lupin variety performed the best when evaluated for performance as a cover crop in Vermont. This variety showed the most promising PSF soil conditioning effects with the observed effects of increased Wet Aggregate Stability, manganese, potassium, and iron. This variety's promising soil conditioning effects were coupled with promising observations in performance as measured by having the earliest flowering time (μ = 25.5 days), flowering largest root length values (μ = 22 cm), tallest height values (μ = 24 cm), high pod weight values (μ = 9 grams), and large root mass values (μ =0.10 grams).

Narrowleaf blue lupins performed well in field trials at the HREC site, in sandy soils, in Burlington, Vermont. This trial suggests that blue lupins are viable cover cropping options with high biomass, yield and taproot measurements. Narrowleaf blue lupins effectively reduced sodium accumulation over the season. There were no significant conditioning effects but improved aggregate stability and nutrient status were observed under the Roland variety. Major limitations to this study include trials being run with relatively low sample size, in only one site, under only one type of soil. Despite these limitations Narrowleaf blue lupins showed promising performances when evaluated for its potential as a cover crop for use in Vermont.

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Supplemental Material

Supplemental materials include pictures that help clarify methods used for measuring aggregate stability in the variety trials.

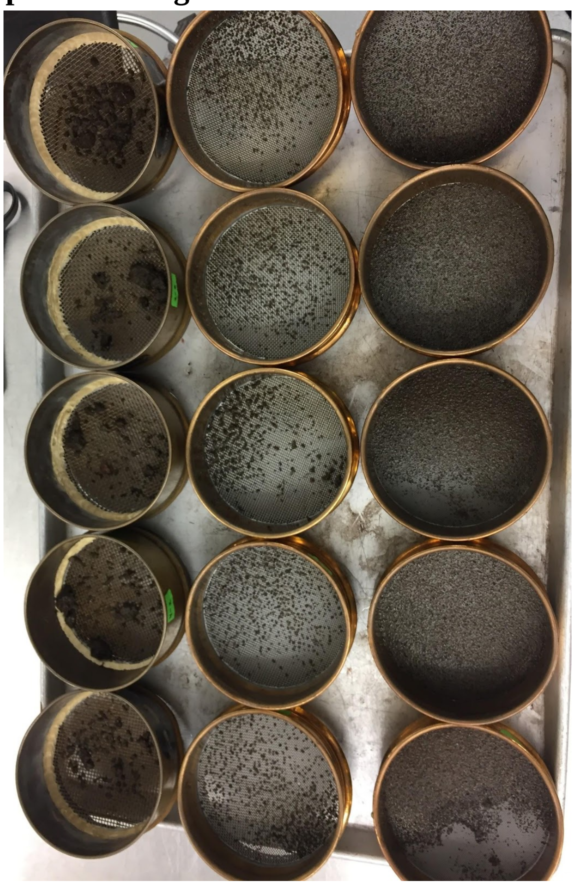
Supplemental Figure 1:

Supp. Figure 1: Shows the method where the central of the three rows was used for sampling. The two outermost rows were left as buffer zones.

Supplemental Figure 2:

Supp. Figure 2: Shows the modified soil core that was pre-cut and then taped together, used and then a razor was used to separate the two halves.

Supplemental Figure 3:



Supp. Figure 3: Shows the three sieve cans (2mm, 1mm, 0.5mm, right to left) with segregated aggregates

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