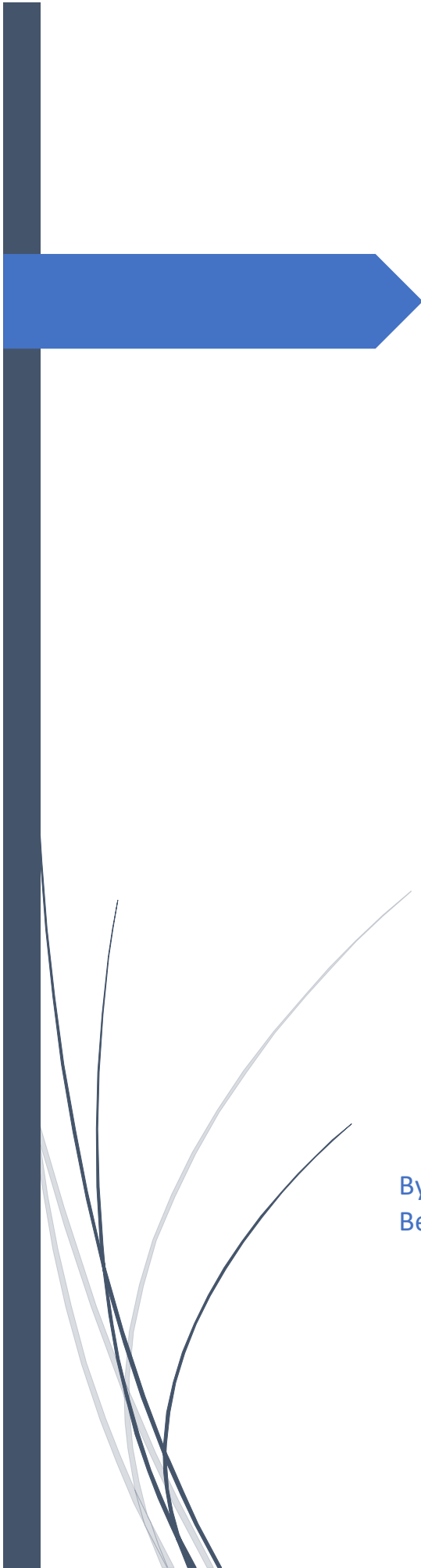




Impacts of the invasive species *Linaria vulgaris* on soil ecology and native plant biodiversity

Rocky Mountain Biological Laboratory
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Abstract

Invasive species are a major threat to biodiversity. Invasive plant species can alter ecosystems by outcompeting natives, lowering biodiversity, and change soil ecology. Additionally, invasive species can be costly to manage. *Linaria vulgaris*, a non-native noxious weed from Europe and Asia, was used as a study organism to better understand how invasive plant species impact biodiversity and soil ecology. I hypothesized that *Linaria vulgaris* would lower native plant biodiversity and increase litter and total nitrogen levels in invaded areas. However, there was no significant difference found between plots with *L. vulgaris* present or absent or with plots where *L. vulgaris* has been experimentally pulled for 19 years. Additionally, I hypothesized that the soil ecology was negatively affecting *L. vulgaris* growth. However, a potted-plant experiment found no significant difference in the growth of *L. vulgaris* between soils where it was locally abundant vs. declining. Taken together, these results suggest that *L. vulgaris* may surpass its own carrying capacity and decline its own population, allowing native plant species to rebound in abundance in the presence of *L. vulgaris*.

Introduction

The presence of invasive species can alter ecosystems (Wilke and Irwin 2010), threaten native species, and incur costs in damage and management to landowners and stakeholders (McDermott et al. 2013). Biological invasions are recognized one of the major threats to biodiversity and threaten species extinctions (di Castri 1990). Invasive plant species can change ecosystems by outcompeting native species, altering soil composition, and introduce poisonous secondary compounds or allelochemical which disrupt trophic levels. Management of invasive species is estimated to incur over \$100 billion dollars annually in ecological damages and economic losses (Pimentel et al. 2005). One of the leading threats to native plants on the endangered species lists is the presence of invasive plants and competition between the two (di Castri 1990).

Our experiment studies the on-going effects of the invasive species *L. vulgaris* on native plant biodiversity, soil composition, and removal strategies to help inform management of noxious weeds. *L. vulgaris*, also known by its common name of Toadflax, is native to Europe and Asia and was introduced to the United States in the mid-19th century (Kadrmaz and Johnson 2002) for its ornamental and medicinal properties. *L. vulgaris* grows throughout the United States and is prolific in the Rocky Mountains, especially in disturbed areas (Lajeunesse 1999; Pauchard et al. 2003). In the Rocky Mountains, *L. vulgaris* is now considered a noxious pest in natural areas and rangelands (Lajeunesse 1999; Pauchard et al. 2003), and *L. vulgaris* may threaten existing native plants by changing soil compositions and outcompeting natives for space and nutrients.

The native plants in the Rocky Mountains are adapted to nitrogen poor soil (Bowman & Fisk 2001). *L. vulgaris* is thought to add nitrogen to the soil through increased litter at senescence which creates a source of nitrogen for microbes in soil, releasing available nitrogen (N) during litter breakdown. This increase in litter and available N may alter native plant biodiversity, but no research on *L. vulgaris*' impacts on soil and biodiversity have been done. I will study how *L. vulgaris* impact carbon and nitrogen compositions in the soil. Our experimental plots were established in 2001 and treated with plant removal to better understand and inform management and removal decisions. Amongst our plots, I have observed a reduction in *L. vulgaris* in invaded plots, including plots where *L. vulgaris* has and has not been pulled (Figure 1). The experiments in 2019 are occurring a time when *L. vulgaris* is at lower abundance than earlier in the invasion process (Figure 1).

To study the impacts of *L. vulgaris* I will use existing data from soil and litter samples to test soil ecology and will use the same plots to assess biodiversity. I setup an additional potted plant experiment using two soil conditions (*L. vulgaris* abundant and *L. vulgaris* reduced) to grow *L. vulgaris* from both root and ramet to understand if soil ecology contributes to the growth success of this invasive species. Our research explored three specific questions:

- 1) How does *Linaria vulgaris* change soil ecology?
- 2) How does *Linaria vulgaris* impact plant biodiversity?
- 3) How does soil environment affect growth rate of *Linaria vulgaris*?

I hypothesized that *L. vulgaris* increases soil nitrogen through litter decomposition creating a positive feedback loop for continued invasion success, and that *L. vulgaris* negatively impacts biodiversity by outcompeting native plants, especially for space. Additionally, I hypothesized that differences in soil environments cause *L. vulgaris* populations to naturally decline.

Methods

Study subject

L. vulgaris is a perennial plant that reproduces both sexually, producing seeds, and through rhizomes that produce shoots from the main and lateral roots below ground (Arnold 1982; Docherty 1982).

L. vulgaris vegetation can be seen starting in early June and produces bee pollinated flowers from early July to September. Due to high seed production, 0 to 6,000 seeds per ramet per year and up to 30,000 seeds per genets annually, and the ability to spread by rhizomes, *L. vulgaris* has the potential to spread (Wilke and Irwin 2010). *L. vulgaris* is currently on Colorado's Noxious Weeds List B, which states that its continued spread must be halted by private landowners and government officials.

Experimental Setup

Our research used existing plots established in 2000 and 2001 in the Gothic Townsite, Gothic, Colorado, Gunnison County, USA. There are 22 existing 2x2 meter (4 m²) plots. Plot treatments were setup as a 2x2 factorial design; 11 plots with *L. vulgaris* present and 11 plots with *L. vulgaris* absent (control) crossed by an additional treatment with *L. vulgaris* removed (*L.*

vulgaris pull) and control biomass removed (control pull), resulting in four possible treatments (*L. vulgaris* pull, *L. vulgaris* no pull, control pull, and control no pull); The hand pulling treatment is applied every year in August.

Soil Ecology

In mid-June, soil cores were collected from each of the 22 plots. Four soil core samples were collected from quadrants of each plot. Each soil core was 6 cm in depth. Litter samples were collected from each plot using a 25 cm² quadrant haphazardly placed within each plot. Soil and litter samples were then analyzed for total nitrogen and total carbon at a stable isotope lab at the University of Georgia (UGA). I will compare changes in total nitrogen and carbon levels across our historical data to assess for trends in nitrogen and carbon in soil in the presence and absence of *L. vulgaris* and where it is removed when data becomes available. I used dry litter weight (g/cm²) as a preliminary indicator of potential changes in soil biogeochemistry. At the time of statistical analysis for this paper, I only included litter because the soil samples were still being analyzed for total nitrogen and carbon by UGA.

Additionally, I tested if differences in soil environment were the cause of *L. vulgaris*' reduced abundance in our plots even in the absence of pulling (hereafter referred to as the potted plant experiment). Our experiment used homogenized soil from two sites where *L. vulgaris* was abundant and homogenized soil from two sites where *L. vulgaris* was naturally declining. I grew *L. vulgaris* from both root segments and ramets in each soil treatment in four-inch square pots within a weather port (20 root segments and 20 ramets per soil treatment, n=80 pots). Pot placement was randomized within the weatherport.

I measured the pots weekly. For the root pots, I measured germination date and plant height (cm). For the ramet pots, I measured plant height weekly. Prior to starting the experiment, I measured wet weight (g) of all of the ramets and root cuttings. At the end of the experiment, I harvested the above and below-ground biomass, dried them for 48 hours at 60C, and measured dry weight.

Biodiversity

I measured plant species diversity by analyzing species richness and evenness across plots. I measured the plots for biodiversity for two census months (June and July). August biodiversity was also measured but not included in this paper. Biodiversity was measured using a 25 x 25 cm quadrat haphazardly thrown into four separate areas within each plot. Species percent cover and stem count were recorded. Grass and bare ground percent cover were also recorded. Species within the plot but outside of the quadrat were recorded for richness but percent cover was not measured.

Statistics

I calculated our statistical results in the program R. For our analysis of biodiversity species richness, evenness, and litter weight I used a two-way ANOVA with census month as a random effect. Additionally, I used the Shannon-Weiner index to calculate species evenness. For our potted plant experiment I analyzed growth and biomass using a one-way ANOVA and used start weights as a covariant.

Results

I analyzed species richness and evenness as a measurement of *L. vulgaris*' impacts on biodiversity. I found no significant difference of species richness across treatments (Table 1 and Figure 3). ($F_{1,42} = 0.0481$, $p < 0.8241$ *L. vulgaris* present (+); $F_{1,42} = 0.0014$, $p < 0.9697$ pull (+), and $F_{1,42} = 0.2626$, $p < 0.7186$ *L. vulgaris* present (+)/pull (+) respectively).

I used the Shannon-Weiner diversity index to measure species evenness. I found no significant difference of species evenness across treatments (Table 2 and Figure 4). ($F_{1,42} = 0.2616$, $p < 0.5669$ *L. vulgaris* present (+); $F_{1,42} = 0.3598$, $p < 0.5486$ pull (+), and $F_{1,42} = 0.1298$, $p < 0.7186$ *L. vulgaris* present (+)/pull (+) respectively).

For litter, I found no significant difference of litter weight (g/cm^2) across treatments (Table 3 and Figure 5). ($F_{1,42} = 4.1144$, $p < 0.06371$ *L. vulgaris* present (+); $F_{1,42} = 2.6910$, $p < 0.10092$ pull (+), and $F_{1,42} = 1.5667$, $p < 0.21069$ *L. vulgaris* present (+)/pull (+) respectively).

For our potted plant experiment, there was not enough above ground sprouting of root segments for analysis so only ramets were measured for growth in abundant and reduced soil treatments. Our analysis of above ground growth rate indicated no significant difference amongst treatments ($F_{1,35} = 0.487$, $p = 0.490$; Figure 6). In the analysis, I included in initial ramet size as a covariate, and initial ramet size was not associated with ramet growth rate ($F_{1,35} = 0.421$, $p < .521$). I also found no significant difference amongst above ground biomass ($F_{1,36} = 0.19$, $p < 0.67$; Figure 7) or below ground biomass ($F_{1,36} = 1.75$, $p < 0.19$; Figure 8) in the two soil treatments. Ramets in both soil treatments increased their root below biomass. Equal numbers of ramets in each soil treatment produced flowers (four each).

Discussion

I hypothesized that *L. vulgaris* decreased biodiversity because it was able to outcompete native plants for resources and space. I found no significant difference amongst biodiversity (species richness and evenness) across treatment plots. It is important to note that *L. vulgaris*'s abundance was lower in 2019 than when the plots were established in 2001. The plots were heavily invaded in 2001 and averaged 150-200 ramets of *L. vulgaris* per m^2 . *L. vulgaris* continued to invade these plots and averaged between 250 ramets (*L. vulgaris* (+)/ pull (-)) to 400 ramets (*L. vulgaris* (+)/ pull (+)) in 2005 before there was a natural decline of *L. vulgaris* in invaded plots (Figure 1). At the time of this experiment *L. vulgaris* averaged less than 100 ramets per m^2 (Figure 1). It is possible that because *L. vulgaris* is a successful invader, it overshot its own carrying capacity and caused its own natural decline due to density dependence. My data suggest that native plants are able to naturally rebound in species biodiversity after *L. vulgaris* has invaded an area. It is possible that in previous years, during higher occurrences of *L. vulgaris* invasion, biodiversity was negatively impacted. Further analysis of available data in previous years should be done to assess impacts of *L. vulgaris* during times of heavy invasion. It seems overall native species richness was able to rebound, but rebound may not apply across all species and some species may still be at risk in *L. vulgaris* invaded areas.

I also hypothesized that *L. vulgaris* created a positive feedback loop in invaded areas by creating increased litter at senescence, thus increasing available nitrogen for microbes and allowing *L. vulgaris* to outcompete native plants with increased nitrogen availability in soil. I did not find any significant difference in litter weight collected across treatments; thus, *L. vulgaris* does not increase available litter and potential nitrogen availability in invaded areas. It should be noted at times of heightened invasion that *L. vulgaris* may have increased litter in invaded areas. Additional analysis should be considered for future studies. I am still awaiting analysis from the lab for total nitrogen and carbon levels of soil and litter and historical total nitrogen and carbon levels should be considered for future studies.

I hypothesized there was a difference in soil environments which caused *L. vulgaris* to proliferate in some areas and decline in others. I tested this by conducting a potted plant experiment in our weatherport in two soil treatments; abundant and reduced. I did not see significant above ground or below ground growth of ramets in varying soil treatments. It should be noted that ramets may have been near mature height at the time of planting thus above ground height may not be a good indicator of growth. Above ground and below ground biomass (g) increased in both treatments but there was no significant difference between the two treatments. It is possible that *L. vulgaris* is declining in some areas because it has surpassed its carrying capacity.

Invasive species cause threats to ecosystems by outcompeting native plants for resources. However, some native plants may be able to rebound from invasions of certain invasives, such as *L. vulgaris*. Such considerations should be made when making future management decisions.

Acknowledgments

I would like to acknowledge Dr. Becky Irwin for facilitating my research this summer. Thank you to Rachel Dickson, Becky Dalton, Michael Stemkovski, Connor Morozumi, Andrea Keller, and Loy Xingwen for your overall support and help with statistics. Thank you Ian Breckheimer and Rick Williams for all of the plants identified for this research. Thank you to the Rocky Mountain Biological Laboratory for hosting this program. Special thank you to my family.

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Tables and Figures

Tables

Table 1

Treatment	F-value	p-value	Interpretation
<i>L. vulgaris</i> present (+)	$F_{1,42} = 0.0481$	$p < 0.8241$	Not significant
pull (+)	$F_{1,42} = 0.0014$	$p < 0.9697$	Not significant
<i>L. vulgaris</i> present (+)/pull (+)	$F_{1,42} = 0.2626$	$p < 0.7186$	Not significant

Table 2

Treatment	F-value	p-value	Interpretation
<i>L. vulgaris</i> present (+)	$F_{1,42} = 0.2616$	$p < 0.5669$	Not significant
pull (+)	$F_{1,42} = 0.3598$	$p < 0.5486$	Not significant
<i>L. vulgaris</i> present (+)/pull (+)	$F_{1,42} = 0.1298$	$p < 0.7186$	Not significant

Table 3

Treatment	F-value	p-value	Interpretation
<i>L. vulgaris</i> present (+)	$F_{1,42} = 4.1144$	$p < 0.06371$	Not significant
pull (+)	$F_{1,42} = 2.6910$	$p < 0.10092$	Not significant
<i>L. vulgaris</i> present (+)/pull (+)	$F_{1,42} = 1.5667$	$p < 0.21069$	Not significant

Figures

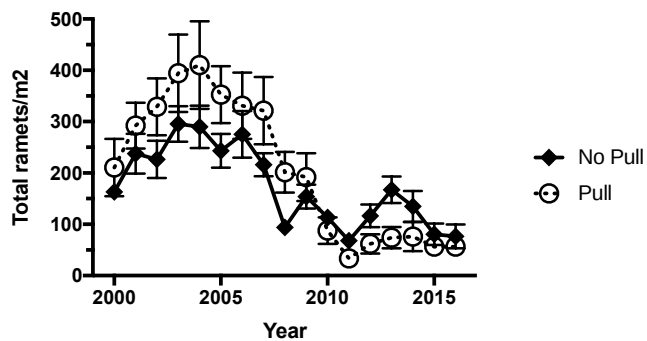


Figure 1: *Linaria vulgaris* ramets per m² in pull and no pull plots from 2001-2016. Plot shows decline of *L. vulgaris* regardless of treatment.

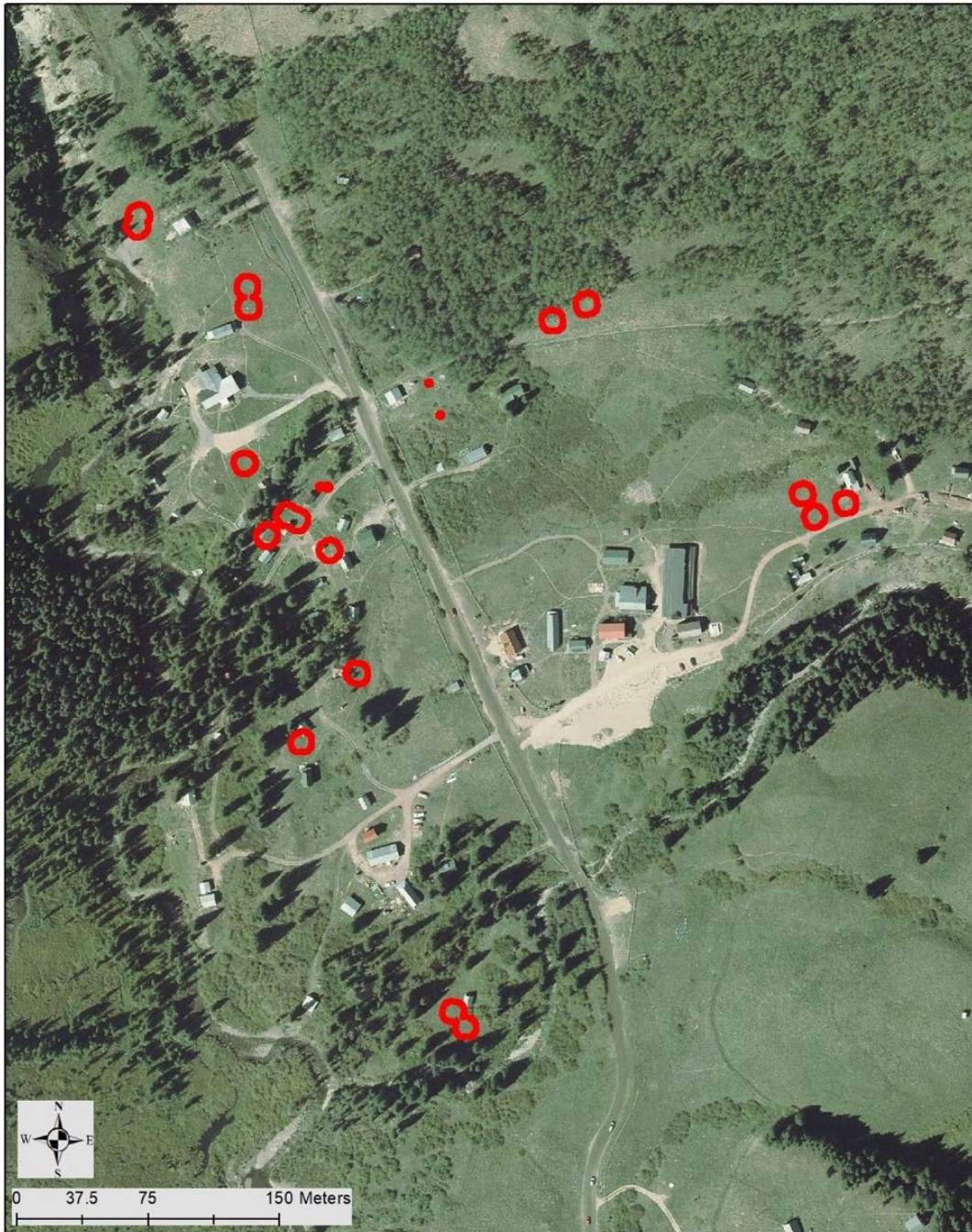


Figure 2: Map of Gothic Townsite, Colorado, USA experimental plots.

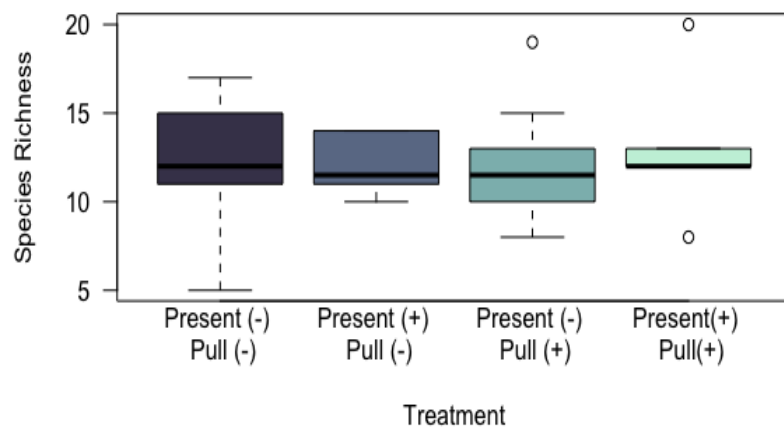


Figure 3: Species richness with standard deviation error bars shows no significant difference amongst treatments. Species richness ranges between 10-15.

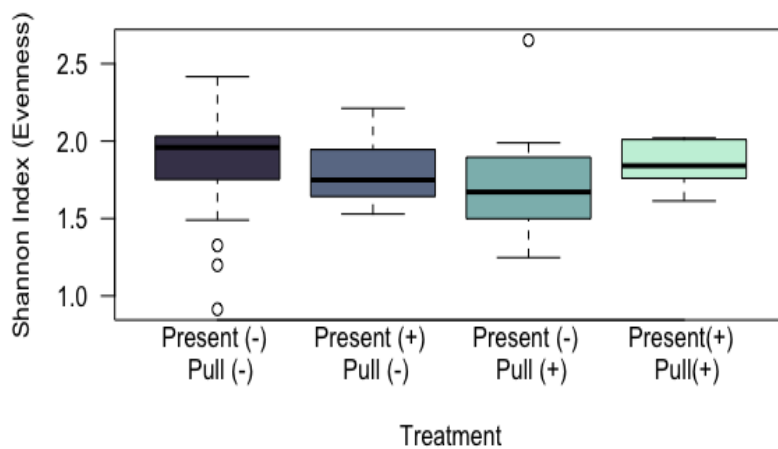


Figure 4: Shannon Index measures species diversity across treatments with standard deviation error bars. The index is between 1.5-2.0 for all treatments.

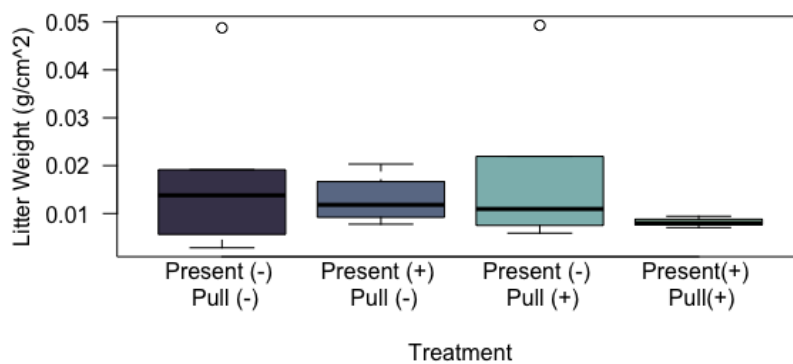


Figure 5: Litter weight (g/cm^2) as an indicator for *L. vulgaris* impacts on soil ecology. No significant difference of litter added by *L. vulgaris* across plots.

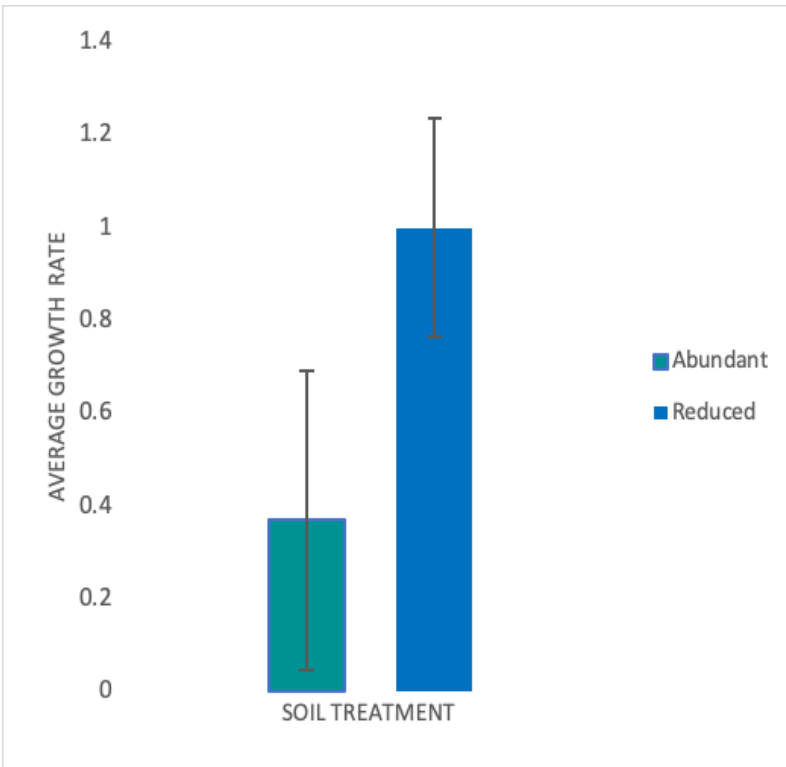


Figure 6: Average growth rate (cm) of above ground biomass of ramets in abundant and reduced soil treatments show no significant difference of growth.

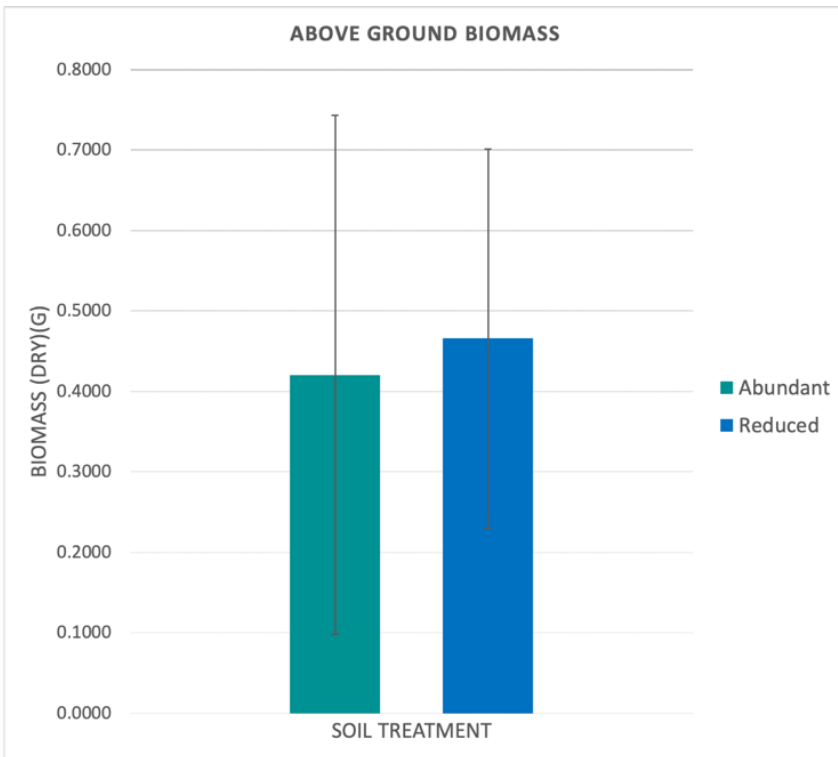


Figure 7: Average above ground biomass dry weight (g) of ramets in abundant and reduced soil treatments show no significant difference of growth.

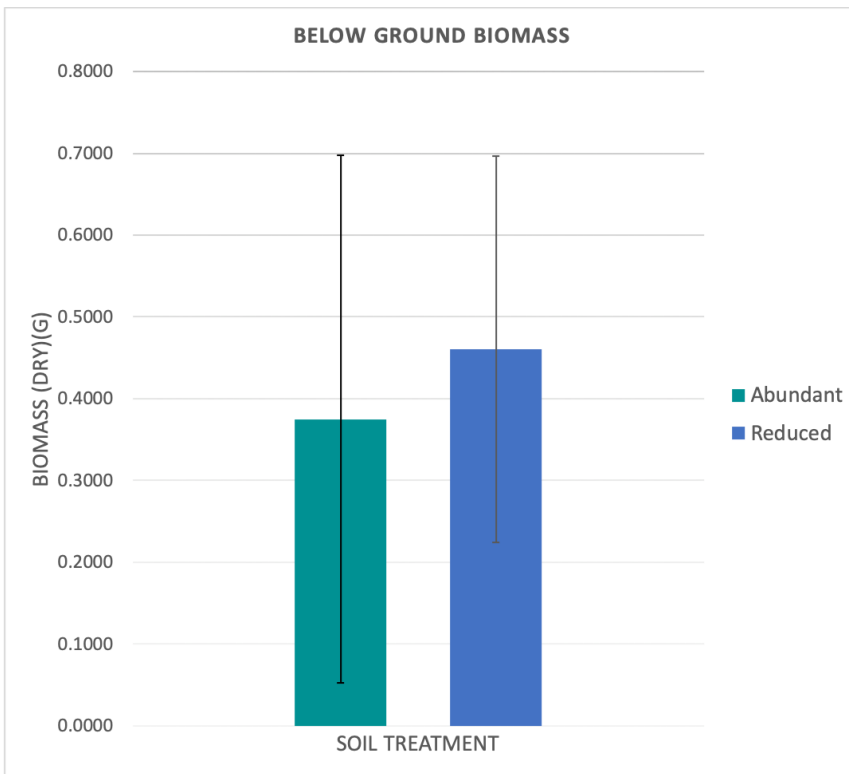


Figure 8: Average below ground biomass dry weight (g) of ramets in abundant and reduced soil treatments show no significant difference of growth.