

**SOIL LEGACY EFFECTS ON GROWTH OF NATIVE AND NON-  
NATIVE SPECIES IN TEMPERATE GRASSLANDS IN THE  
SOUTHERN INTERIOR OF BC**

by

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## ABSTRACT

Southern and Central British Columbia are predicted to have less precipitation in the summer and more in the winter, but not in the form of snowfall. The combination of reduced summer precipitation and reduced spring snowmelt can lead to more frequent, intense and prolonged droughts. Drought may have immediate or long-lasting legacy effects on soils. Soil legacy effects may influence plant communities directly through recovery time after a drought, or indirectly through carbon and nitrogen availability. If an ecosystem is unable to recover after a drought period, competing invasive species may take advantage of the available space and colonize. Due to their competitive adaptability, invasive species are one of the greatest threats to natural grassland communities and can severely decrease plant diversity and affect ecosystem functioning. The objectives of this study were (1) to determine if rain-out shelters simulate drought and influence species composition, soil moisture and soil temperature; and (2) investigate potential soil drought legacy effects on biomass growth and competitive effect, and (3) to investigate the effects of fertilizer on invasive competitiveness. To address these objectives, rain-out shelters were established in the Lac du Bois grasslands and monitored over a 4-year period. Using soils collected from under these rain-out shelters and controls without rain-out shelters, a greenhouse experiment was conducted to investigate the effects of drought, fertilizer and soil on growth and competitive ability of spotted knapweed (*Centaurea stoebe*) against rough fescue (*Festuca campestris*).

The results of this study indicated some problems with the rain-out shelter design. Higher soil moisture and lower soil temperature were observed in rain-out shelter plots compared to the control plots. These observations influenced the interpretation of results from the greenhouse experiment. The field soils under the rain-out shelters, referred to as drought field soils, had a higher aboveground biomass than the control soils after 90 days of growth. This result may suggest a positive soil legacy effect where a higher biomass is observed in drought soils after a re-wetting event. However, caution is recommended regarding these results as it is unclear if the field soils are considered drought, or not. Further results found increased competitiveness in spotted knapweed over rough fescue

which was increased with the absence of fertilizer.

This study provides some insight on grassland soils as multiple differences were found between the drought and control field soils in biomass growth, competitive effect, and root:shoot ratios. Further research is needed to understand the mechanisms involved with positive or negative legacies, and to investigate other soil mechanism, such as microbial activity.

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## CHAPTER 1 – INTRODUCTION

Fossil fuel combustion and various types of land use, such as deforestation, have caused atmospheric concentrations of carbon dioxide (CO<sub>2</sub>) and other greenhouse gases (GHGs) to rise (Shukla et al. 2019). By 2011, atmospheric CO<sub>2</sub>, nitrous oxide and methane had increased by 40, 40 and 150 (%) respectively since 1750 (Hartmann et al. 2013). These increased emissions have altered our global climate, resulting in warmer land-surface air temperatures in recent years. Average land-surface air temperature (LSAT) between 1880-2012 was recorded at  $0.094^{\circ}\text{C} \pm 0.013$ . Between 1979-2012 LSAT had increased to  $0.254^{\circ}\text{C} \pm 0.049$  (Hartmann et al. 2013). Other indicators of climate change include increased ocean temperature and sea level, reduced sea ice, reduced snow cover, and large-scale changes in precipitation (Cubasch et al. 2013). Extreme weather events such as droughts are predicted to increase in frequency and magnitude over the years (Yusa et al. 2015). Southern and Central British Columbia are predicted to experience less precipitation in the summer and more in the winter, but not in the form of snowfall (Spittlehouse 2008; Briceño et al. 2014). The combination of reduced summer precipitation and spring snowmelt can lead to more frequent, intense and prolonged droughts (Shukla et al. 2019).

### *The Impacts of Drought on Grasslands*

Drought, defined as a period of abnormally low precipitation, varies in length and has many negative social, economic and environmental impacts (Schiraldi and Roundy 2016; Slette et al. 2019). Drought reduces water availability by lowering reservoir levels, groundwater supplies and overall soil moisture (Yusa et al. 2015). This can create environmental hazards such as reduced water quality, wetland degradation, soil erosion and overall habitat destruction (Bonsal et al. 2011). This ultimately impacts needed services such as agriculture and forestry, hydro- electricity production and various recreational activities (Georgakakos et al. 2014). The social impacts of drought can devastate the social and economic wellbeing of a community as unemployment rates rise and anxiety increases, causing mental health issues (Alston et al. 2004).

In grasslands, the negative effects of drought can be compounded by other threats including land conversion, land degradation, over-grazing and the introduction of invasive species (Asner et al. 2004; Densmore-McCulloch 2013). Grasslands in British Columbia occupy approximately 1% of the provincial land base but provide habitat for over 30% of the province's species at risk (Grassland Conservation Council of BC 2017). These are crucial ecosystems to maintain high species diversity and overall ecosystem health. Lac du Bois Grasslands Protected Area (GPA) is a temperate grassland near Kamloops, British Columbia, Canada that provides a wide range of ecosystem goods and services to the community (Ministry of Environment 2004). There is an elevational gradient within the park that corresponds with changes in grassland plant communities and soil moisture availability (Carlyle et al. 2014; Lee et al. 2014).

Precipitation is an important driver of plant community dynamics (Carlyle et al. 2014; Densmore-McCulloch et al. 2016). Three grassland types have been categorized along the elevational gradient found in Lac du Bois: the lower, middle and upper grasslands. These areas have distinctive soil types and species composition, which contributes to high species diversity (van Ryswyk et al. 1996). In grasslands, water availability is a major limiting factor for above-ground biomass production (Naudts et al. 2013; Reichmann and Sala 2014). When exposed to drought, some plants respond by increasing stomatal density, thereby controlling stomatal conductance (Fraser et al. 2009). Reducing the size, or altering the density, of the stomata can limit the amount of carbon uptake during photosynthesis, thus reducing water loss (Chaves et al. 2002; Klaus et al. 2016). However, with the limitation of the uptake and fixation of atmospheric carbon, plants must rely on stored reserves to meet their energy requirements (Chapin et al. 2002). This can increase plant susceptibility to mortality after long periods of time, create gaps in the plant community, and shift spatial distributions of plant species. Invasive species may colonize gaps, which can lead to increased competition with native species (Fraser and Carlyle 2011; Holzmueller and José 2013; van Kleunen et al. 2015). Invasive species are one of the greatest threats to natural grassland communities; they can severely decrease plant diversity and affect ecosystem functioning, which in turn may increase grassland vulnerability to climate change (Klaus et al. 2016).

### *Invasive Species*

Invasive species are an increasing worldwide threat economically and environmentally. Once established, these species often dominate and are difficult to control due to their possession of life history traits such as rapid growth, high seed production, efficient seed dispersal and the ability to colonize areas where they have few to no natural enemies (van Kleunen et al. 2010; Kuang 2015). High abundance of invasive species can reduce plant diversity, decrease forage availability, and alter soil communities (Reid et al. 2009). This can cause negative economic impacts by reducing the productivity of forests, rangelands, crops, and animal production (Greer et al. 2014). Invasive species can compete directly and indirectly with native plant communities. They can directly compete through superior seed dispersal methods, deeper roots to survive low water availability, and more rapid or robust growth (Holzmueller and Jose 2013). Some invasive species are avoided by ungulates due to defense mechanisms of the plant or lower palatability and nutritional quality (Gong and Zhang 2014). This can indirectly result in native species loss as invaders may promote native species herbivory (Radtke and Wilson 2015). By outcompeting native plant communities, invasive species may grow in large monocultures reducing plant diversity and increasing the susceptibility to drought and further invasion (Han and Young 2014; Klaus et al. 2016). Overall, invasive species are detrimental to grasslands through the reduction of forage quality and biodiversity (Reid et al. 2009). Invasive species, combined with changing climate and drought, can severely impact grassland functioning, eventually resulting in land degradation.

Soil communities may be heavily influenced by drought and invasive species even after these stressors have been removed. Species such as *Centaurea stoebe* (spotted knapweed) may directly invade areas, via superior competitive ability, or indirectly invade through the production and release of allelochemicals (Meiman et al. 2006). Released from roots, these chemicals can suppress the growth and establishment of other plants (Inderjit et al. 2011). These allelopathic compounds may persist in the soil and have long-term impacts known as legacy effects (Kulmatiski and Beard 2008). However, the concept of long-term soil effects is debated; some studies have suggested that invasive species do not create negative soil conditions through allelopathic chemicals (Del Fabbro and Prati 2015).

Spotted knapweed may influence the availability of nitrogen and other soil nutrients or alter microbial communities in ways that are disadvantageous for the natural plant communities (Fraser and Carlyle 2011; Del Fabbro and Prati 2015; Kuang 2015). The relationship between soil and plants can be visualized by positive or negative feedback loops where a change in one can alter the other. The negative feedback process may slow the rate of population growth for a species and stabilize diversity whereas positive feedback may increase the growth rate of a plant community, leading to the development of monocultures (Greer et al. 2014). There are many studies based on the aboveground perspective of invasiveness, but belowground processes in soil are less understood, but equally important (Schrama and Bardgett 2016).

Disturbances such as climate events, and anthropogenic activities, such as cattle grazing can alter ecosystems and allow invasive species to readily invade. For example, when exposed to drought, soil resources may be added in pulses instead of at a consistent level (Schrama and Bardgett 2016). These nutrient pulses may increase the “invasibility” of those ecosystems if invasive species are more resilient to these pulses than the native community. Depending on the duration and severity of the drought, a plant or soil community may not fully recover, resulting in loss soil processes and ecosystem functioning (Bérard et al. 2011). Similar to the effect of invasive species, drought may alter soil nitrogen, carbon and other nutrients, thus increasing ecosystem vulnerability and reducing resistance to further disturbance (Bloor and Bardgett 2012). Some studies have found that drought stress may be reduced with higher levels of plant diversity in grasslands (Klaus et al. 2016). This highlights the importance of maintaining biodiversity within our grasslands to ensure long term ecosystem functioning and health through above and belowground processes to combat drought and the threat of invasive species (Cowles et al. 2016).

#### *Competitive Ability and Root:Shoot Ratios*

The ability to compete for light or soil resources is advantageous for survival when

resources are limited (Wang et al. 2010). There are two main components to competitive ability, competitive effect and competitive response. Competitive effect is the ability to suppress the growth of another species and competitive response is the ability to avoid being suppressed (Fraser and Milette 2008). Previous effort to link species traits, such as growth rate, to competitive effect has been done to help predict species distribution over time (Keddy et al. 1998). Species that have strong competitive effects may cause the reduction of species diversity in natural communities (Moyer and Brewer 2018). My study investigates the competitive effect of the invasive plant, spotted knapweed and the native bunchgrass, rough fescue. Spotted knapweed is a highly competitive plant that has strong competitive effects due to its potential ability to suppress the growth of other species (Meiman et al. 2006). Spotted knapweed is able to survive in low nutrient soils and through drought and is often avoided by herbivores, furthering its competitive capabilities (Fraser and Carlyle 2011; Gruntman et al. 2013). Rough fescue is an important native bunchgrass that has less of a competitive ability compared to spotted knapweed (Carlyle et al. 2010). These two species may be an example of asymmetric competition: spotted knapweed has a large effect on rough fescue and rough fescue has little effect on spotted knapweed (Mariotte et al. 2012).

Dominant competitive plant traits are commonly linked to size and height. Larger and taller plants can shade and suppress the growth of smaller neighbors and utilize resources unavailable to them (Wang et al. 2010). However, in many natural plant communities, root biomass takes up a large portion of plant biomass and provides another way to compete effectively (Lamb and Cahill 2008). Before a plant can grow above ground, below ground resources such as water and nutrients must be established to support shoot growth (Sainju et al. 2017). In response to nutrient availability, resource allocation between roots and shoots is essential (Ågren and Franklin 2003). Nutrient uptake is heavily influenced by the size of the root system and a high root:shoot ratio can help with drought tolerance (Crush et al. 2009; Ma et al. 2009). When nutrients are readily available, less resources are used to grow roots and more are used to grow aboveground biomass. Root competition is believed to be more important at lower productivity sites and aboveground competition important in high productivity sites (Kiær et al. 2013). Thus, the

addition of soil resources, in the form of fertilizer, should reduce root competition (Lamb et al. 2007). Root:shoot competition reduces shoot growth by allocating more reserves towards root production. Ultimately, this impacts aboveground growth and competition for resources such as light (Mariotte et al. 2012). Invasions are predicted to become more likely and difficult to reverse as invasive species compete and increase pressure on native plants through above and below ground processes (Orrock et al. 2015). Once again, species rich communities are the greatest defense against invasive and can reduce the establishment or abundance of invaders (Moyer and Brewer 2018).

### *Fertilization*

Grasslands are important components for terrestrial land-cover and biodiversity and make up over 40% of the earth's surface (White et al. 2012; Iravani et al. 2019). It is estimated that grasslands can mitigate 10-40% of greenhouse gas emissions every year (Boval and Dixon 2012, Sun et al. 2015). Grasslands can act as carbon (C) and nitrogen (N) sinks through soil sequestration. This may influence the world's carbon and nitrogen storage and aid against the effects of global warming (Du et al. 2014). However, the extent of this ecosystem is decreasing due to the effects of climate change and conversion to farmland for larger livestock herds (White et al. 2012). Grassland management and fertilizer application can have large impacts on carbon and nitrogen sequestration, making good management critical to improve soil quality and crop production (Sainju et al. 2008). When exposed to environmental changes, nutrient cycling, productivity and decomposition may be negatively impacted due to changes in precipitation and nitrogen deposition (Alster et al. 2013). However, with increased soil nutrient availability and nitrogen fertilization, above and belowground processes of an ecosystem may be stabilized. This concept still needs to be investigated as Bloor and Bardgett (2012) found that soil nitrogen availability had little effect on short-term stability when exposed to drought; they suggested that, high plant species diversity was a better indicator for ecosystem stability. Additionally, application of organic amendments may play an important part to mitigate climate change effects by stabilizing soil carbon (Canarini et al. 2016). However, Canarini et al.'s (2016) study of a one-year drought showed little effect on carbon but found some increase in soil



carbon stability with surface compost application.

Not all fertilizer applications have positive interactions on stability and productivity. A study by Khalili et al. (2016) found that drought and nitrogen addition had negative additive effects on microbial biomass, which can influence soil cycling and other processes. They also suggest that during dry years, nitrogen plant demand is reduced allowing for the buildup of soil nitrogen. If a rewetting event occurs, this accumulated nitrogen could potentially promote productivity and soil quality if enough moisture is available (Khalili et al. 2016). Studies by Vink et al. (2015) and Schrama and Bardgett (2016) suggest that soil legacies (from previous cultivation) and fertilization may impact growth and establishment of native and non-native species and may increase the risk of invasion. Invasive species may benefit more from increased nitrogen, however Vink et al. (2015) found that field nitrogen addition had little effect on plant biomass, but greenhouse nitrogen addition increased the biomass of native species over that of exotics. Schrama and Bardgett (2016) looked at soil functioning during drought and compared intensively (fertilizer, heavily grazed) and extensively (no fertilizer, low grazing) managed grasslands. During their experiment, they found a higher growth and survival rate of invasive species compared to native species in both types of grasslands when exposed to drought. This may suggest that invasive species invasion may rise in the upcoming years due to increased extreme climatic events combined with higher nutrient availability found with intensively managed grasslands (Schrama and Bardgett 2016). Overall, there are many conflicting studies on the benefits and costs to using a fertilizer. Furthermore, few studies have looked at long-term drought and how it may influence above or below-ground processes with the addition of a fertilizer.

### *Objectives*

The short-term effects of drought, of a year or less, on plant communities have been well researched, with respect to invasiveness and soil processes (Klaus et al. 2016; Schrama and Bardgett 2016; Burri et al. 2018). The project described in my thesis investigated drought over a 4-year span. This project will look at prolonged drought and

how it may influence native and invasive growth or survival, the invasiveness of exotic plant species, and the positive or negative effects of fertilizer. With the increasing amount of literature support for more frequent and intense droughts in the upcoming years (Shukla et al. 2019), understanding the potential impacts on grasslands as valuable, sensitive and endangered ecosystems is critical. This project aims to provide a better understanding of the effect of drought on temperate grasslands that can be used to predict potential impacts on grassland health and biodiversity in the future.

This project was a part of a global network designed to assess terrestrial ecosystem sensitivity to drought (International Drought Experiment 2015). This experiment may help expand previous drought experiments by providing information on B.C.'s ecosystems that can be compared to that from different regions (Fraser et al. 2013). The experiments that are part of this network use standardized protocols to ensure consistency regarding rain-out shelter construction, plant survey data and soil sampling. This methodology is designed to increase the robustness of analysis for this experiment by using the collaborative data from this network. Although some parts of the protocol for this experiment have been standardized, my project is unique where I investigate drought over a 4-year period and remove soil from the field to analyze post-drought growth. Chapter 2 is a field experiment that addresses the question: will rain-out shelters simulate drought and will this influence species composition, soil moisture and soil temperature? Chapter 3 is a greenhouse experiment that asks the questions (1) Will soil legacies from drought impact the growth of native or invasive species? (2) Will drought increase the competitive effect of invasive plants? (3) How will the effects of fertilizer application influence competition? Chapter 4 places my work in a broader context and discusses potential implications or recommendations based on observations from Chapters 2 and 3.

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## CHAPTER 2 – INVESTIGATING THE EFFECTS OF DROUGHT ON GRASSLAND COMMUNITIES

### INTRODUCTION

Grasslands cover about a quarter of the Earth's ice-free land surface and have high soil carbon content, and are thus an important factor in the global carbon balance (Jérôme et al. 2014). Agriculture, forestry and other land use (AFOLU) contribute about 12% of greenhouse gas (GHG) emissions and fossil fuel combustion contributes about 78% of carbon emissions (Shukla et al. 2019). Carbon, from fossil fuels, is released into the atmosphere but forests and grasslands can mitigate 10-40% of emissions every year (Sun et al. 2015). Not only are grasslands important for biodiversity and livestock production, but they are crucial for reducing the carbon imprint of greenhouse gases to help mitigate the effects of climate change (Batalla et al. 2015). Temperature changes, due to increased greenhouse gas emissions, are predicted to cause shifts in precipitation patterns, seasonal runoff and in evapotranspiration (Nalley et al. 2013). Specifically, in Southern and Central British Columbia, there is predicted to be less precipitation in the summer and more in the winter (Spittlehouse 2008).

Lac du Bois Grasslands Protected Area (GPA) is important for conservation as it represents the vast biodiversity of the Thompson Basin and Northern Thompson Upland Ecosections. In Lac du Bois, there are lakes, ponds, wetlands, forests and glacial landscape features which provide a diverse environment for species to inhabit (Ministry of Environment 2004). According to the Conservation Data Centre (COSEWIC 2001), about 45 wildlife species, 11 plant species and 13 plant communities in the Lac du Bois ecosystem are Red or Blue-listed (Ministry of Environment 2004). There is an elevational gradient within the park that corresponds with changes in grassland plant communities and increasing moisture (Lee et al. 2014). These communities are known as the lower, middle and upper grassland; each of which has a distinctive soil type and species composition, which contributes to high species diversity (van Ryswyk et al. 1996).

Precipitation is important in Lac du Bois GPA due to the elevational gradient of plant



communities. With the prediction of warmer and drier growing seasons, grassland communities may change drastically and expand northward or into forested areas (Lee et al. 2014). Extreme weather events, such as droughts, are predicted to increase in frequency and magnitude (Yusa et al. 2015). Long term exposure to drought can increase plant susceptibility to mortality and shift spatial distributions of plant species (Smith 2011). Studies investigating change in community composition after drought typically look at species richness. There are few that address change in the abundance of species and species turnover over time (Ploughe et al. 2019). The importance of plant-plant interactions and how they may influence community level change should be acknowledged as they may affect future response to drought (Smith 2011; Grant et al. 2014 Ploughe et al. 2019). A study by Hoover et al. (2014), found no change in overall species richness, but a dramatic reduction in the abundance of the dominant forb species, which was compensated for by an increase in the dominant grass species. This caused a significant species composition shift and reordering of species in terms of their abundance.

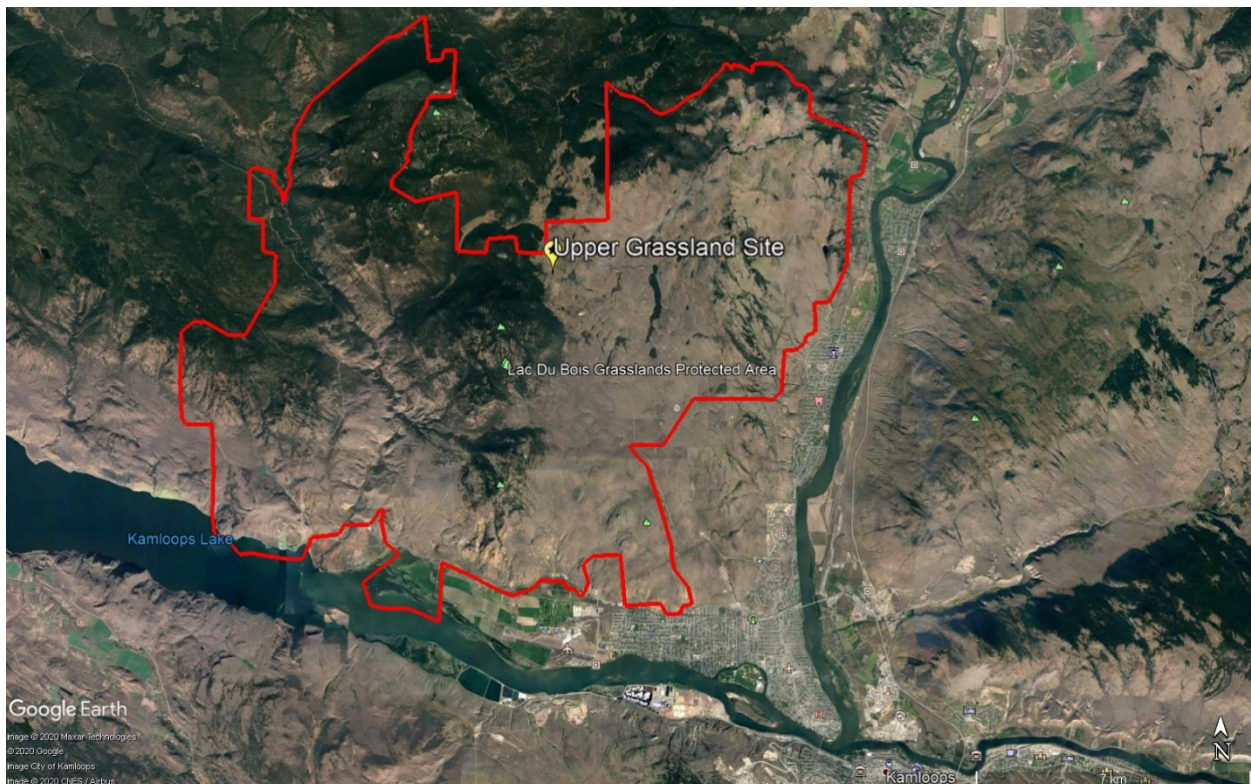
My study investigated multi-year drought and how it may influence species composition, soil moisture and soil temperature in temperate grasslands. In this chapter, I ask (1) whether species composition changes over time in rain-out shelter plots compared to control plots? (2) if rainout shelters alter soil moisture, and (3) if rainout shelters alter soil temperature. I hypothesized that differences would be found between the rain-out shelter and control plots. Specifically, I hypothesized that rainout plots would have a lower soil moisture content and plant species diversity compared to the controls. These hypotheses were tested in the Lac du Bois grasslands using rain-out shelters constructed to simulate drought conditions over the span of 4 years. Soil moisture and temperature were monitored and plant surveys were done yearly to evaluate species diversity.

## METHODS

### *Study site*

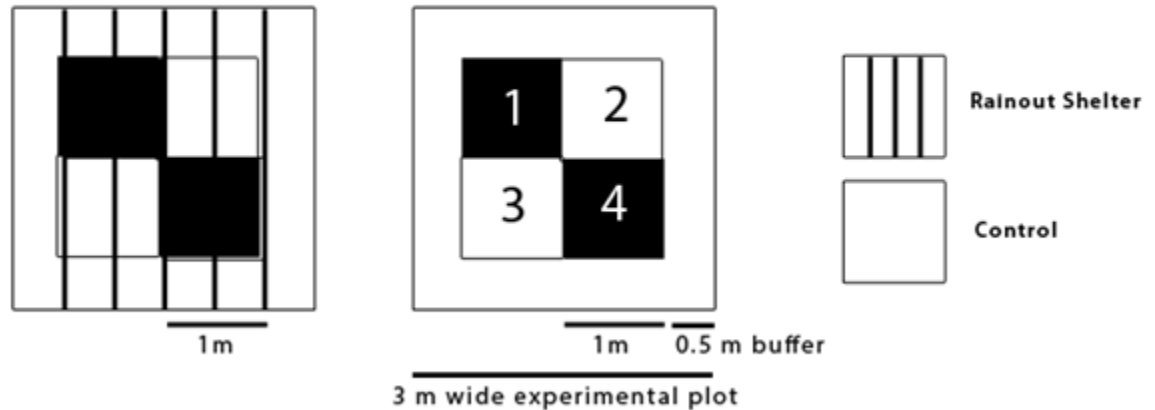
Cattle exclosures (30 m x 30 m) were erected throughout Lac du Bois by the Agriculture and Agri-Food Canada Kamloops office in April 2010 (Densmore-McCulloch

2013). In 2015, study sites were established in the upper grasslands (Figure 2.1) within the Bunchgrass zone (BG) of Lac du Bois (Ministry of Environment 2004). The study site located at 900 m asl (50°47'20.4"N 120°26'53.2"W) has Black Chernozem, sandy loam soils and the dominate species is rough fescue (*Festuca campestris*) (van Ryswyk et al. 1965; Densmore-McCulloch 2009). This region experiences a semi-arid climate with low annual precipitation (<350mm) and hot, dry summers.



**Figure 2.1.** Lac du Bois Grassland Provincial Park boundary outlined in red. The study site is indicated by the yellow pin.

HOBO® Micro Station Data Loggers were set up in November 2014 to record pre-experiment soil temperature and soil moisture. There were 12 experimental plots in total, six with a rainout shelter and six without (controls). There were four subplots of 1 m x 1 m placed in a square formation in each of the 12 plots. There was a 0.5 m buffer around this square formation of subplots, resulting in a 3 m wide experimental plot (Figure 2.2). The rain-out shelters were finished within the exclosures in August 2015.



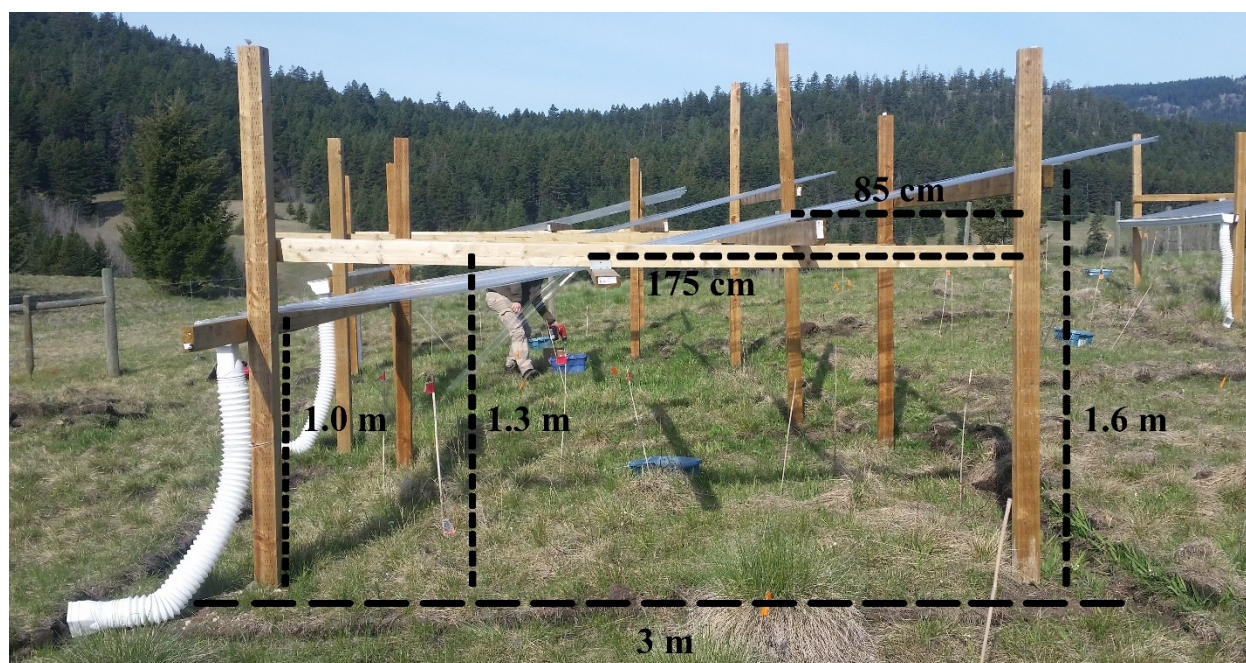
**Figure 2.2.** Experimental plot set up. There were 6 control plots without a rain-out shelter and 6 plots with a rain-out shelter. Within each experimental plot, there were four 1 m wide quadrats in the middle of the plot. This allowed for a 0.5 m buffer zone around the quadrats resulting in a 3 m wide experimental plot.

### *Rain-out shelter construction*

The Drought-Net protocol was used as a baseline during the construction of the rainout shelters (International Drought Experiment 2015). The shelters were constructed on south facing slopes to control for the prevailing winds, which were north. Post holes were dug and 0.089 x 0.089 x 2.438 m pieces of commercially treated wood were secured with concrete in each corner of the plot. The supports of the structure consisted of 0.038 x 0.089 x 3.048 m pieces of wood with the side pieces placed at 1.3 m above grade. The upper support of the shelter was placed at 1.6 m above grade and the lower support was placed at 1 m above grade. Two support beams across the plot were screwed on the side supports at 80 cm and 175 cm from the top. These cross beams provided additional support for SUNTUF® Corrugated Polycarbonate strips to gently rest on (Figure 2.3). All supports and posts were leveled and straightened. The polycarbonate was cut into strips containing two troughs (roughly 14 cm wide) then screwed onto the supports. Glue was used on the screws in the polycarbonate to prevent any leaks. The strips were spaced 14 cm apart to reduce rainfall by about 50% to simulate drought. The intensity of rainfall reduction was aimed to be between the 1<sup>st</sup> and 5<sup>th</sup> percentile of the Kamloops historical record for total

precipitation. The percentiles were calculated based on the average total precipitation from 1951 to 2012. The average precipitation for this period was 200 mm. To get between the 1<sup>st</sup> and 5<sup>th</sup> percentile, rainfall would need to be reduced between 96 mm and 115 mm, thus we targeted a 50% rainfall reduction in the experimental drought plots.

The unmanipulated (control) plots were not covered by rain-out shelters. Construction of the shelters was completed in late August. Trenching around all of the plots occurred in September 2015 to hydrologically isolate them from each other. Other rainout shelters (3 shelters) were constructed in the lower grasslands of Lac du Bois, but will not be discussed in this chapter.



**Figure 2.3.** Rain-out shelter dimensions and construction blueprint

### *Plant Surveys, Soil Temperature and Soil Moisture Measurements*

Yearly plant surveys were conducted in the spring and plant species composition was estimated to the nearest 1% using a modified Daubenmire method with 1 x 1 m quadrats. Initially, in 2015 and 2016, between one and two quadrats were randomly placed. In 2017, four quadrats were placed per plot and covered the entire experimental plot. Soil was removed from one random quadrat in the fall of 2017, so only 3 quadrats could be analyzed during plant surveys in 2018. The change in surveys was partly due to

the transition between the International Drought Experiment and the start of this experiment. Slope and aspect were determined using a clinometer. Moisture content and temperature were recorded by 10HS Soil Moisture Smart Sensors and 12-Bit Temperature Smart Sensors every fifteen minutes throughout the year, starting from November 2014. Two soil moisture and two temperature sensors were used for each experimental plot. The sensors were placed in opposite quadrants from each other. For example, referring to Figure 2.2, soil sensors would be placed in quadrat 1 and 4 and the temperature sensors would be placed in 2 and 3. To install the sensors, a spade was inserted into the soil to make space to place the sensors. The soil and temperature sensors were inserted into the contact soil horizontally beyond the cut of the blade, roughly 10 cm deep. The spade was removed and the soil was tapped down. This process was repeated to bury 0.5 m of the sensor cables at the same depth. The data loggers, to monitor the sensors, were placed in a plastic container in the center of each plot to prevent water damage and animal interference. Data logger data was retrieved biannually, once in the spring and once in the late summer or fall.

### *Statistical Analysis*

The data were downloaded twice a year from the Hobo® devices using HOBOWare software. The 15-minute interval data was summarized into mean daily soil temperature and mean daily soil moisture. Species richness, Shannon ( $H'$ ) and Simpson's ( $D_2$ ) indices of plant community diversity was calculated with the species cover data for each plot using the following equations:

**Table 2.1.** Species diversity index and the corresponding equation. Equations were obtained and referenced from Madonsela et al. (2017).

Species Diversity Index	Equation
<i>Species Richness</i>	$S=N$
<i>Shannon Index</i>	$H' = - \sum_{i=1}^s p_i \ln(p_i)$
<i>Simpson Index</i>	$D_2 = 1 / \sum_{i=1}^s p_i^2$

Where N is the total number of species in a sample,  $p_i$  is the proportion of one particular species (i) relative to the total abundance of all species (S) in a plot.  $\ln(p_i)$  is the natural logarithm of the proportion of  $p_i$ .

Aboveground soil moisture, soil temperature, percent cover and diversity indices were analyzed in R version 3.4.3 "Kite-Eating Tree" (The R Foundation for Statistical Computing). Significant differences between control and drought soil moisture and temperature were determined using the Welch's two sample t-test.

A non-metric multidimensional scaling (NMDS) ordination using Bray-Curtis distance measures was done to visualize changes in species composition over time and treatment types. Analyses were performed using the meta-MDS function in the vegan package in R. P values were estimated from 1000 random permutations of the relative cover data in the drought and control experimental field plots in each year. The relative vegetation cover for each experimental plot was calculated by summing the percentage cover of each species per plot in each treatment (control/drought), then dividing by the total cover of all species.

## RESULTS

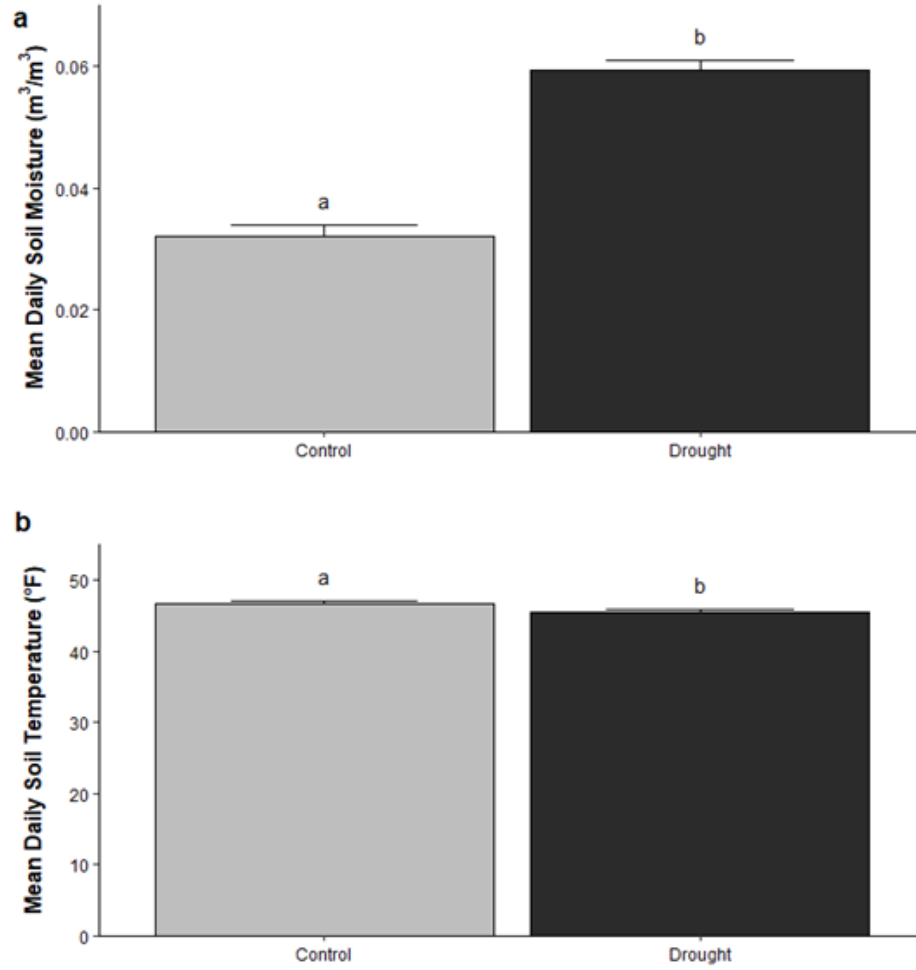
### *Soil Moisture and Temperature*

Over the 4 years, soil moisture content averaged ( $0.032 \pm 0.002 \text{ m}^3 \text{ m}^{-3}$ ) and soil temperature averaged ( $46.70 \pm 0.35 \text{ F}^\circ$ ) in control plots. In the drought plots, soil moisture content averaged higher at ( $0.059 \pm 0.002 \text{ m}^3 \text{ m}^{-3}$ ) and had a lower soil temperature at ( $45.48 \pm 0.33 \text{ F}^\circ$ ) (Figure 2.4). T-tests were conducted to compare average soil moisture of the control and drought plots over the entire 4-year period. In Figure 2.5, the soil moisture content of the drought and control plots unexpectedly changed near the summer of 2016. The soil temperature data shows the average temperature of the control plots increasing over time.

The target precipitation reduction from the rain-out shelters was between 96 mm and 115 mm. This represents the 1<sup>st</sup> and 5<sup>th</sup> percentile of the Kamloops's historical record for total precipitation between 1951-2012. Looking at the total precipitation and mean yearly temperature for each year between 2014-2018, only one year (2017) reached this target (Table 2.2). This suggests the rain-out shelters produced a drought in 1 out of 4 years.

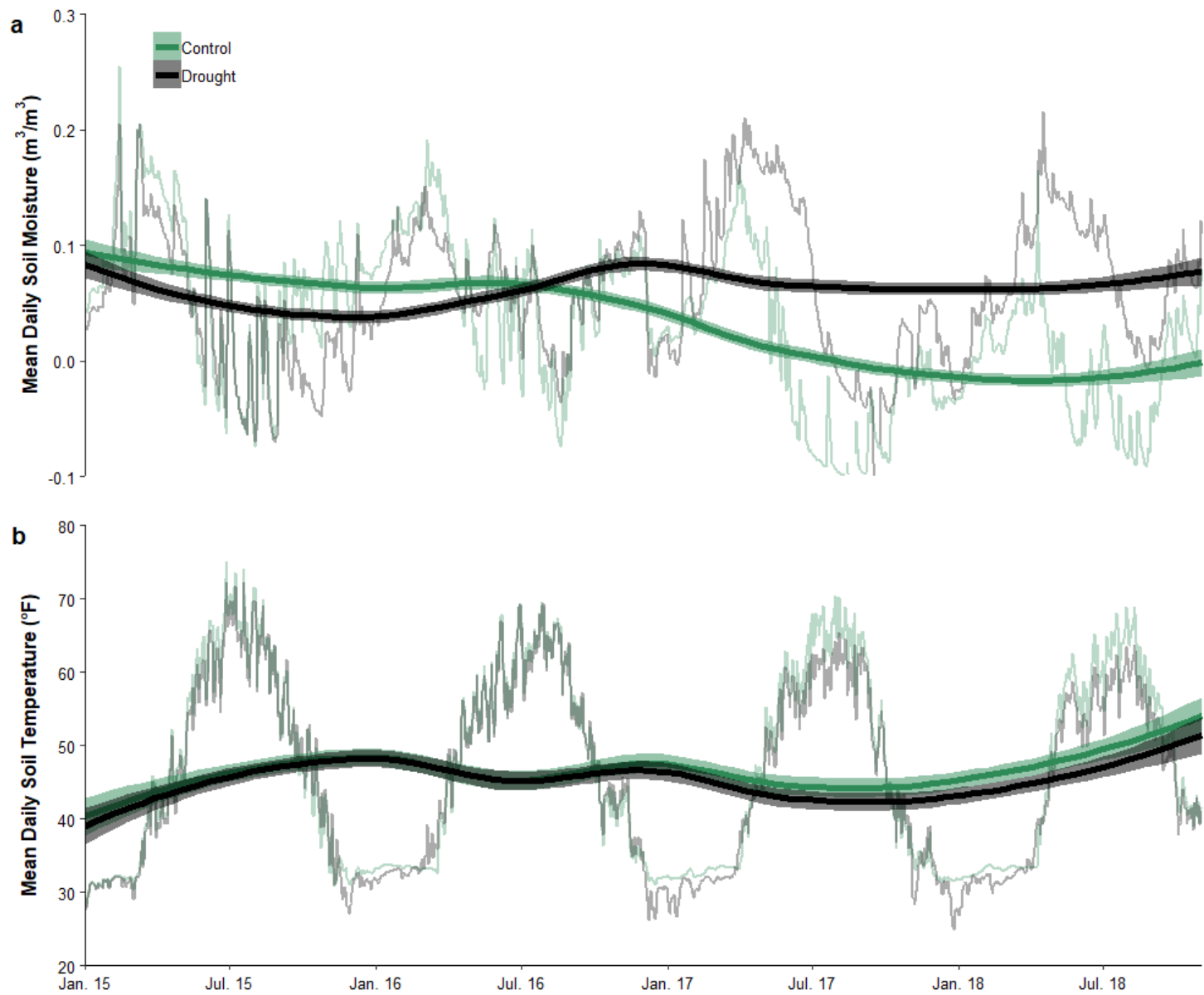
**Table 2.2.** Mean yearly temperature, total precipitation, calculated precipitation reduction between 2014- 2018. Percentiles are based on historical data from 1951-2012 for Kamloops, BC.

<b>Year</b>	<b>Mean Yearly Temperature (°C)</b>	<b>Sum of Total Precipitation (mm)</b>	<b>Amount of Precipitation Reduced by Shelters (mm)</b>	<b>Percentile Based on Historical Data</b>
2014	9.53	277.60	138.80	13
2015	10.78	313.10	156.55	24
2016	10.47	335.10	167.55	27
2017	9.07	200.30	100.15	1
2018	9.25	363.80	181.90	40



**Figure 2.4.** Mean soil moisture and soil temperature of the control and drought field plots from 2015-2018. Bars are standard error of the mean and any bar with different letters (Welch's t-test) are significantly different ( $P < 0.05$ ).





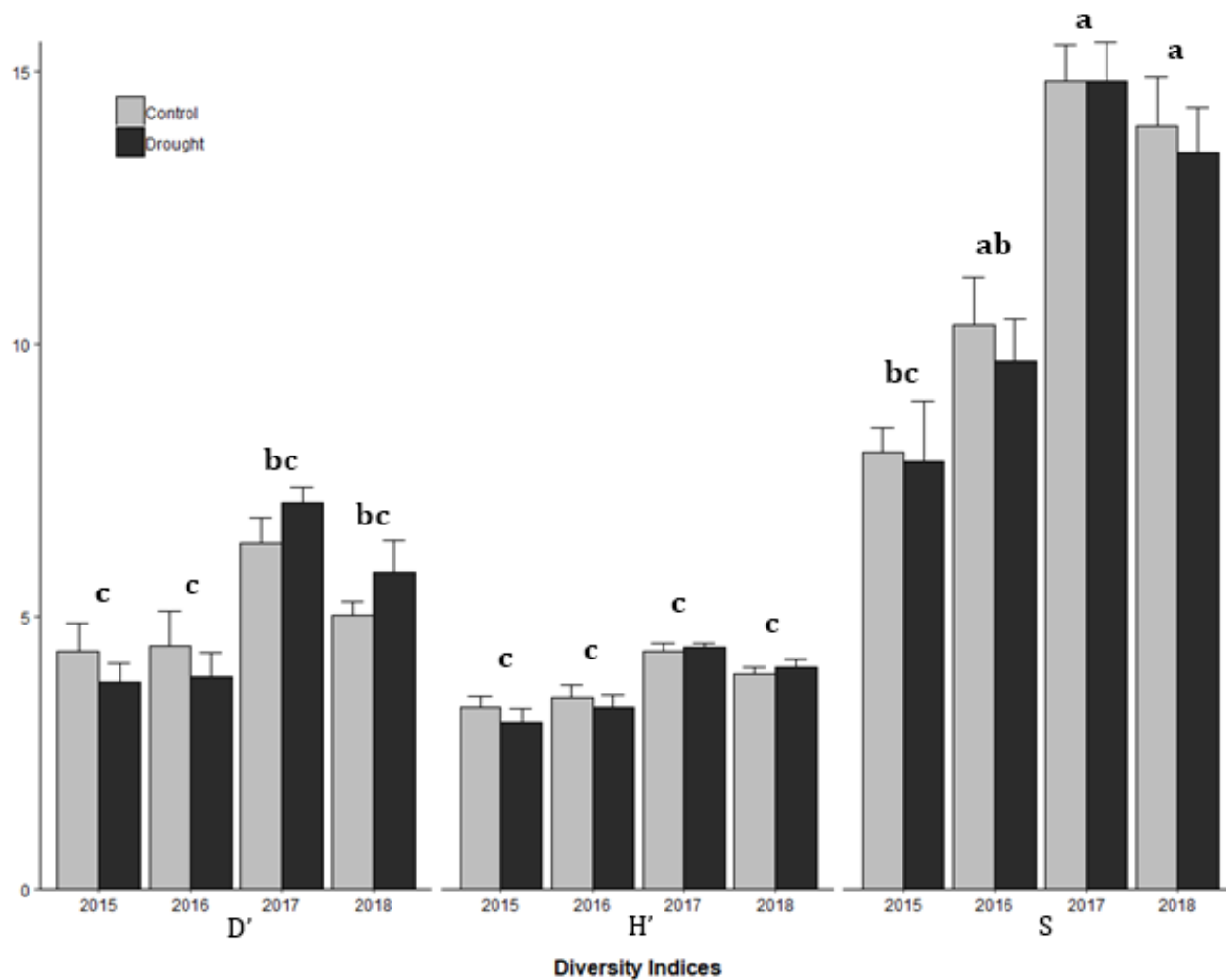
**Figure 2.5.** Mean daily soil moisture and soil temperature between January 2015 and November 2018. Lines represent the moving mean average.

### *Species Diversity Indices and Species Richness*

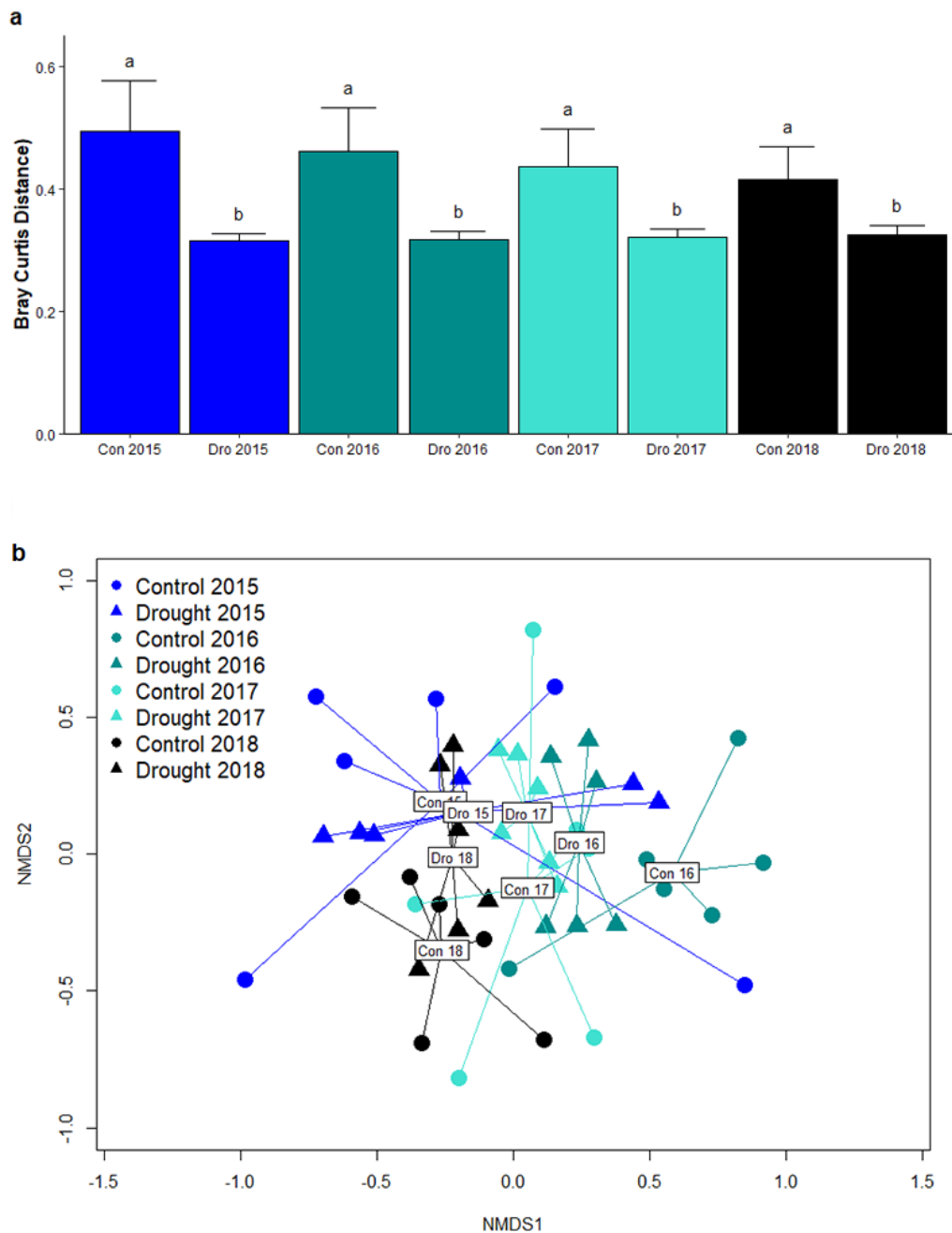
Overall, there were no differences between the drought and control plots with respect to species richness or diversity ( $p=0.85$ ). However, differences were found between the years where 2017 and 2018 had the highest species richness ( $p<0.05$ ) (Figure 2.6). This might be attributed to the increase in plant surveys quadrats used in 2017 and 2018.

To visualize changes in plant species composition with drought over the years and to determine how closely plots are related, a non-metric multidimensional scaling (NMDS) ordination was done using Bray-Curtis distance. Treatment (drought vs. control) had a significant effect on vegetation and was significantly correlated with the NMDS ordination structure ( $r^2 = 0.33$ ,  $P < 0.001$ ) (Figure 2.7). The ordination analysis confirmed a difference between plant communities in control and drought plots.

Plant surveys were done yearly in the spring. In 2017, *Achnatherum richardsonii* (spreading needlegrass) became the dominant species in the drought shelter plots. In 2018, the sudden emergence of *Galium boreale* (northern bedstraw) became apparent. Average percent cover of spreading needlegrass and northern bedstraw was determined to show changes in cover of these species over the years (Figure 2.9). Visual differences can be observed in Plot D2 over the years (Figure 2.8).



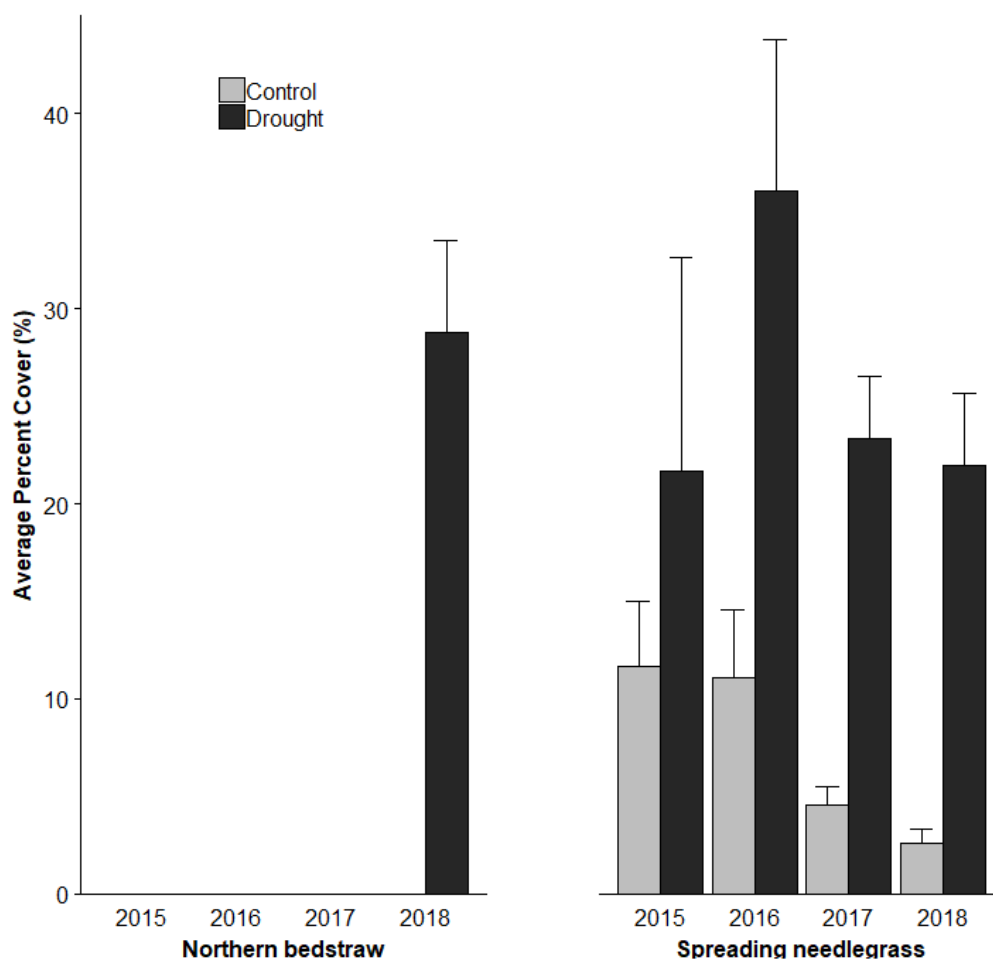
**Figure 2.6.** Mean diversity indices and species richness of control and drought plots over 4 years.  $D'$  represents Simpson Diversity Index,  $H'$  represents Shannon's Diversity Index and  $S$  represents species richness. Bars are standard error and different letters (Tukey HSD) indicates a significance difference ( $p < 0.05$ ) ( $n=6$ ). No significance differences were found between the drought and control plots in any year or indices. Significant differences were found in species richness where 2017 and 2018 had a higher overall species richness compared to 2015.



**Figure 2.7.** a) Average Bray-Curtis distance of all species in each plot. A difference was found between the control and drought treatment plots in species composition ( $n = 6$ ,  $p < 0.001$ ). On average, control plots had a more diverse composition of species in the plots compared to the drought plots. Bars are standard error of the mean and any bar with different letters (Tukey HSD) are significantly different ( $P < 0.05$ ). b) Non-metric multidimensional scaling (NMDS) ordination using Bray-Curtis distance measure of drought and control plots after 4 years of altered precipitation. Triangles represent drought plots and circles represent control plots. Colors represent years. Ordination is based on the relative cover of species data. There were 6 plots in each treatment. Text shows the centroids for each year and treatment and lines connect points to the centroids. The location of ordination indicates the degree of similarity between each one. The closer together points are, the more similar they are.



**Figure 2.8.** Drought shelter in the spring of 2016 (top), 2017 (middle) and 2018 (bottom). 2017 shows the sudden emergence of spreading needlegrass and 2018 shows the emergence of Northern bedstraw.



**Figure 2.9.** Mean percent cover of Northern bedstraw and spreading needlegrass in 2015, 2016, 2017 and 2018. Bars are standard error (n=6).

## DISCUSSION

This study explored the long-term effects of severe drought in semi-arid grasslands of the Southern Interior of BC over a 4-year period. Unexpectedly, soil moisture was higher and soil temperature was lower in drought plots compared to the control. Higher soil moisture in drought plots may have occurred due to errors in the soil sensors, increased condensation, or the combination of lower temperature and increased shading due to the rain-out shelters. Another possibility is that the slight slope ( $2^\circ$ ) of the landscape led to pooling of water towards one end of the enclosure, suggesting the trenching of the plots

was insufficient. Future studies should incorporate control shelters with improved trenching to avoid any interactions of shading, temperature alteration or runoff.

It was found that 2015, 2016, and 2018 had a higher than average total precipitation compared the historical record (Table 2.2). The increase in total precipitation resulted in the failure of the rain-out shelters to simulate a severe drought in 3 out of 4 years. The increased precipitation in 2015, 2016 and 2018 may have contributed to increased plant growth and subsequently higher plant litter in the following year. Plant litter is important for decomposition and is critical to carbon cycling, soil formation and nutrient availability (Fraser and Hockin 2013). The change in soil moisture and soil temperature that was observed in the experimental plots may have altered decomposition rates. Specifically, higher soil moisture and lower soil temperature found in the drought experimental plots may have experienced higher decomposition rates than the control plots. Some studies suggest that decomposition rates are higher with elevated temperature (Shaw and Harte 2001; Burke et al. 2003; Davidson & Janssens 2006; Conant et al. 2011). However, Shaw and Harte (2001) concluded that elevated temperature had a higher decomposition rate when soil moisture was not limiting, and that increased temperature may promote soil drying. Alternatively, higher soil temperature combined with the higher than average precipitation in 3 out of 4 years, may have resulted in elevated decomposition rates in the control plots. However, other studies have found that moisture had a greater impact on decomposition than temperature (Fraser and Hockin 2013; Palmer et al. 2019).

Higher decomposition rates can alter nutrient cycling and soil resources. More nutrients and resources may lead to higher plant growth in those years and in turn, higher plant litter in the following year. During drought, decomposition is lowered, nutrient cycling is reduced, and plant nutrient uptake is decreased (Davidson & Janssens 2006; Buttler et al. 2019). Soil resources, such as carbon and nitrogen, may have accumulated in 2017, due to drought conditions. The higher than average precipitation in the following year (2018), may have released the accumulated soil resources and promoted productivity and soil quality. This could be a contributing factor to the change in species composition that was found between the drought and control plots (Khalili et al. 2016; Griffin-Nolan et

al. 2018; Meisner et al. 2018).

A change in species composition was observed in the experimental plots over the 3-year span. The drought and control plots differed from each other and had a higher overall diversity in more recent years (2017,2018). However, species richness was the same in the drought and control plots. A species reordering was observed where the sudden growth of a forb species (northern bedstraw) occurred in 2018, exclusively under the drought shelters. This result may resemble the study by Hoover et al. (2014) where they found a reduction of their dominant forb that was compensated for by an increase of their dominant grass. The authors suggest their results may be due to the greater sensitivity to drought of their forb species compared to their dominant grass species. For this study, it may have been the cumulative effects of multi-year drought that drove the shift in species composition. However, it is difficult to ascribe drought as a driver due to the increase in soil moisture under the rain-out shelters, and the historical precipitation data indicating that severe drought conditions were not simulated in every year. It may be possible that northern bedstraw benefitted more from the increased soil moisture under the rain-out shelters compared to spreading needlegrass. As shown in Figure 2.4, there was a lower temperature and higher soil moisture observed under these plots. These conditions may have favored Northern bedstraw more than spreading needlegrass as the years continued. This could also indicate that the drought plots may increase the vulnerability of a monoculture as the control plots tended to have a more diverse species composition as indicated by the Bray-Curtis dissimilarity and overall higher species richness. The resistance to species turnover may prevent the increase of invasive or rare species (Ploughe et al. 2019). Control plots, although warmer and with less moisture, had a higher species diversity, which may help them resist the growth of a monoculture species.

Although the goal of the rain-out shelters was to simulate a 1 in 100-year drought, the experimental treatment might not have succeeded. In the beginning of the experiment, the drought plots were lower in soil moisture content. Over time, there was a switch, and the drought plots ended up with a higher soil moisture content than the control plots after the 4 year period (Figure 2.5). The total precipitation data for 2014-2018 (Table 2.2) may



have attributed to this result, where it was found that the rain-out shelters produced a drought in one of the four years. The higher than average total precipitation in 2015, 2016 and 2018, resulted in the rain-out shelters failing to create extreme drought conditions. These conditions combined with the potential increase in shading, condensation, and plant litter may have contributed to the higher soil moisture content and lower soil temperature found in the drought plots. Higher soil moisture may promote decomposition rates and contributed to the changes in species composition between the drought and control plots.

If the data loggers are accurate, this would mean that the “drought plots” were not lower in soil moisture and thus were not exposed to drought conditions compared to the control plots. This has many implications for the results of the field and the greenhouse component of this study (Chapter 3). An important question is whether the data loggers were accurate. The results of Chapter 2 should be considered in Chapter 3, and the question of if this should be considered drought or not, should be explored. If the plots were not in drought conditions, and the data loggers were correct, which we have little reason to question, then changing species composition could be the result of increased soil moisture and lower temperature found in the “drought” experimental plots. If these were drought conditions, and the data loggers were incorrect, then it is possible that the change in plant composition is due to an increase in drought hardy plants and their ability to persevere and outcompete species when faced with multi-year drought. For now, both of these explanations are plausible. These results and how they may affect the results of the greenhouse experiment in Chapter 3, will be explored. Specifically, I will explore if the soils are drought field soils or not, and how that may affect the interpretation of the results.

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## CHAPTER 3 – INVESTIGATING SOIL DROUGHT LEGACY EFFECTS ON BIOMASS GROWTH OF NATIVE AND NON-NATIVE SPECIES

Indicators of global climate change such as increases in temperature and large-scale changes in precipitation have been well documented (Shukla et al. 2019). Extreme weather events such as drought, are expected to become more variable, influencing frequency, intensity and duration (Warren and Lemmen 2014). Southern and Central British Columbia are predicted to experience less precipitation in the summer and more in the winter, but not in the form of snowfall (Spittlehouse 2008; Briceño et al. 2014). The combination of reduced summer precipitation and spring snowmelt can lead to more intense and prolonged droughts. When exposed to drought, some plants respond by decreasing stomatal conductance (Fraser et al. 2009). That is, they reduce water loss by restricting the size, or altering the density, of the stomata which then limits the amount of carbon uptake during photosynthesis (Chaves et al. 2002; Klaus et al. 2016). If drought conditions remain, carbon reserves may become exhausted due to prolonged stomatal closure, resulting in the death of the plant (Baudis et al. 2014). Increased mortality may create gaps in the plant community and shift spatial distributions of plant species (Verlinden et al. 2013).

If native species are unable to recover after a period of drought, competing invasive species may take advantage of the available light and space resources and colonize (Manea et al. 2016). In contrast, during a drought water availability is a major limiting factor for aboveground biomass production suggesting that the invasion risk may be reduced during extreme drought. (Diez et al. 2012; Naudts et al. 2013; Reichmann and Sala 2014). Due to their competitive ability, invasive species are one of the greatest threats to natural grassland communities; they can severely decrease plant diversity and affect ecosystem functioning (Holzmueller and Jose 2009; Klaus et al. 2016). Ultimately, the combination of climate change and invasive species is detrimental to biological processes and biodiversity and may be synergistic or additive (Côté et al. 2016).

Soils provide crucial ecosystem processes for plant growth such as soil moisture

retention, nutrient cycling and carbon and nitrogen storage (Power 2010). Drought may have immediate or long-lasting legacy effects on soils. Immediate effects such as plant or soil microbe death, changes in soil structure and loss of carbon and nitrogen have been well documented (Schimel et al. 2007; Bérard et al. 2011; Zhang et al. 2013; Sun et al. 2018). Soil legacy effects may influence plant communities directly through recovery time after a drought, or indirectly through carbon and nitrogen availability (Borken and Matzener 2009; de Vries et al. 2012). The connection between above and belowground processes is important for understanding invasion, especially for species that are able to suppress the growth of other plants through mechanical processes such as shading, or via allelochemical effects (Bohlen 2006). Plant-soil feedback is the process in which a plant may influence soil characteristics and in turn the soil may influence growth of the plant (Floríanová and Münzbergová 2018; Mariotte et al. 2018). The relationship between soil and plants can be visualized by neutral, positive or negative feedback loops in which a change in one can alter the other (Dostálek et al 2016). A negative feedback process may alter the soil to slow the rate of population growth for a species and stabilize diversity whereas positive feedback may increase the growth rate of the plant community leading to the development of monocultures (Greer et al. 2014). This may be problematic if invasive species modify the soil to increase its performance to outcompete native species (Crawford 2017).

Plant-soil feedback can differ depending on the range or location of the species being studied; a stronger negative feedback may be found in a species' native range than in their invasive range (Dostálek et al 2016). For example, studies by Kilronomos (2002) and Kulmatiski et al. (2008) found that the invasive species they investigated had stronger positive, or less negative, feedback compared to native species. Furthermore, a study by Andonian and Hierro (2011) found that the invasive *Centaurea solstitialis* grew significantly larger in soils from introduced regions than in soils from their native range. A more species relevant study by Callaway et al. (2011), found that when *Centaurea stoebe* was grown in its non-native range with neighboring species, it had minimal impacts on growth and reproduction compared to when grown in its native range. However, there are few studies investigating plant soil feedback experiments and environmental conditions. A recent study by Floríanová and Münzbergová (2018) investigated soils that were watered,

shaded or held at ambient environmental conditions. They found that watering and shading influenced plant-soil feedback and that most of their species had a positive or neutral feedback. Another study by Kaisermann et al. (2017), looked at legacy effects of drought on plant-soil feedbacks and found that drought displayed negative feedback in soil. Contrary to this study, Griffin-Nolan et al. (2018) found a positive drought legacy and a higher aboveground net primary production. The authors attribute these results due to an increased nitrogen availability post drought.

Soil microbial communities also play an important part in plant-plant or plant-soil interactions during disturbance. Drought has been shown to negatively impact the activity or alter the composition of soil microbial communities and influence nutrient cycling, decomposition or primary production (Davidson and Janssen 2006; Kaisermann et al. 2017, Legay et al. 2018). Past droughts may influence the microbial community or increase nitrogen availability during drying and re-wetting events in lab conditions (Meisner et al. 2018). A study by Meisner et al. (2018) found legacies in drying and re-wetting history that influence soil microbial communities and may affect plant-soil feedback. These findings combined with their previous work (Meisner et al. 2013) suggest that not only do legacy effects influence soil composition, but they may also boost soil fertility through increased nitrogen availability and soil respiration rates. These changes in the soil nitrogen can directly improve the performance of plant growth post drought during a re-wetting event. Their results suggest that legacy effects may remain in the soil through changes in microbial composition and may explain why plant communities change even when extreme weather events such as drought, end (Meisner et al. 2018).

Two common grassland plant species were used in this greenhouse experiment, the invasive spotted knapweed (*Centaurea stoebe*) and native bunchgrass rough fescue (*Festuca campestris*). These species were selected because they are commonly found in the temperate grasslands near Kamloops, British Columbia. Spotted knapweed is a perennial forb from Europe with deep taproots that can tolerate low nutrient soils, drought and may alter soil properties to reduce native plant diversity (Fraser and Carlyle 2011). Rough fescue is a native perennial bunchgrass found in higher elevations and is less tolerant to

drought conditions than spotted knapweed. It is one of the highest yielding native grasses and it is palatable and provides good nutritional value to foragers (Dobb and Burton 2012). Investigating these species may provide insight on the potential outcomes or changes in plant composition that may be brought on in this area as more frequent droughts become apparent.

While the effects of drought less than two years in duration have previously looked (Klaus et al. 2016; Schrama and Bardgett 2016; Burri et al. 2018), the aim of this study was to investigate how drought over multiple years may influence the soil to alter plant growth, plant-plant interactions or invasiveness and their response to further drought. Fertilizer application was investigated for further plant-plant interactions. Specifically, we tested three hypotheses: first, we hypothesized that potential soil drought legacies would reduce plant growth; second, we hypothesized that drought would increase the competitive effect of the invasive plant on the native plant; and third, we hypothesized that fertilizer would increase invasive competitiveness. These hypotheses were tested using a greenhouse experiment utilizing collected field soils exposed to two years of severe drought (1 in 100-year drought). Spotted knapweed and rough fescue were grown in monoculture or in pair-wise competitive pots with a randomized block design that was used to evaluate the different combinations of watering, fertilizer, and legacy soil.

The results from Chapter 2 should be noted in this experiment involving the drought and control field soils. Based on the results in Chapter 2, the “drought” soils had a higher soil moisture content than the control soils. Furthermore, according to the historical total precipitation data, the rain-out shelters produced a drought in 1 of 4 years. This suggests the drought field soils used in this experiment were not exposed to multi-year drought. It is unclear if the rain-out shelters were working correctly, or not due to the soil moisture data. The intent of the experiment was to create drought exposed soils using rain-out shelters, so going forward it will be referred to, and assumed to be, “drought field soils”. The implications and alternative hypotheses regarding if the field soils are drought or not, are acknowledged in the discussion, conclusions and in Chapter 4.

## METHODS

### *Experimental Design and Study Sites*

The study site for soil removal was located in Lac du Bois Grasslands Protected Area, British Columbia, Canada (see Chapter 2). The upper grasslands study site is located at 900 m asl (50°47'20.4"N 120°26'53.2"W). Soils are classified as Black Chernozems with a sandy loam soil texture (van Ryswyk et al. 1966).

Soil was removed from control and drought experimental plots in the upper grassland site in September 2017. Soil was removed from a 1 m x 1 m quadrat in each plot. Plant material and roots were removed, or peeled away in a sheet, from the surface of the soil and as much soil as possible was salvaged (Figure 3.1). The top 5-10 cm were removed evenly from the quadrat area until 16 liters were removed and placed in freezer bags. All equipment was cleaned and sanitized between each plot using disinfecting alcohol wipes. Collected soil was stored in a freezer until the start of the experiment in January 2018. Soil was defrosted and passed through a 4 mm sieve and mixed to form a homogenous mixture and then placed into 1-litre pots. Soil samples were prepared in February 2019 to be sent for analysis (for minor elements) at the British Columbia Ministry of Environment Analytical Laboratory (BCMEAL) (Victoria, BC). Leftover soil was placed into freezer bags and re-frozen until April 2019 when it was defrosted and analyzed for total carbon (C) and total nitrogen (N) at Thompson Rivers University.





**Figure 3.1.** The vegetation was peeled back and removed. Soil was salvaged from the root mass and removed evenly from the surface.

### *Greenhouse Experiment*

The greenhouse experiment was conducted from January to April 2018 at the Thompson Rivers University (TRU) Research Greenhouse, Kamloops, B.C. The goal of this experiment was to investigate the effects of drought, fertilizer and soil on growth and competitive ability of spotted knapweed (*Centaurea stoebe*) and rough fescue (*Festuca campestris*). A randomized block design was used to evaluate a total of 24 combinations of watering (well-watered/drought), fertilizer (presence/absence), soil (control/drought field soils), and plant combination (single rough fescue, single spotted knapweed, or paired combination of rough fescue and spotted knapweed) replicated 5 times for a total number of 120 pots (Figure 3.2).



**Figure 3.2.** Randomized block design showing two replicates. Start of the experiment (left) and growth after 90 days (right).

One-litre nursery pots with drainage (13 cm top x 10 cm base x 11 cm height) were filled with soil taken from Lac du Bois. Slow release fertilizer (5.7 g) of 14-14-14 was placed on the soil surface of fertilizer treatment pots. Spotted knapweed (collected from Lac du Bois) and rough fescue (from Pickseed Canada Inc., Abbotsford, B.C) seeds were placed on the soil surface of the pots. In monoculture pots 10 seeds were placed near the middle of the pot about 1-2 cm apart. In mixed, competitive pots, 5 seeds of each species were placed in a line about 2-3 cm apart from the other species (Figure 3.3). The greenhouse was under controlled conditions of natural and artificial light, day/night 18 h/6 h, temperature: day/night 21 °C /15 °C, and humidity 40%. All pots were sprayed with water every other day and wrapped with thin plastic with holes to increase humidity but still allow for air movement. This was done until seed germination was complete and seeds began to establish (9 days). Saranwrap was removed and room temperature water was used to water pots to saturation. Watering was done every other day or every two days to maintain soil moisture above 10% soil moisture content. After 28 days of allowing the plants to establish, the drought component to this experiment was started. Water was severely restricted and drought pots were maintained under 10% soil moisture content. Weeding was done throughout the experiment to prevent unwanted species from growing and competing. After 90 days, plant root and shoot tissue were harvested. Prior to harvesting, survival was determined of the remaining species. Plant shoots were clipped at

the soil surface and roots were washed of soil. Plant samples were dried at 65°C for 48 hours then weighed to determine root and shoot biomass.



**Figure 3.3.** View of seedling set up in mixed pots (left) and saran wrapped pots of one replicate (right).

### *Statistical Analysis*

Aboveground biomass, root biomass and survival were analyzed in R version 3.4.3 "Kite-Eating Tree" (The R Foundation for Statistical Computing). All data were assessed through boxplots for normality and homogeneity of variance was checked using the Fligner-Killeen test. Data were transformed using a logarithm +1 function. Significant differences between species in competition and in monoculture were determined using the Welch's two sample t-test. Analysis of variance tests (ANOVA) were conducted to look for differences between treatment effects or interactions. Post-HOC analysis was done on all ANOVA analysis.

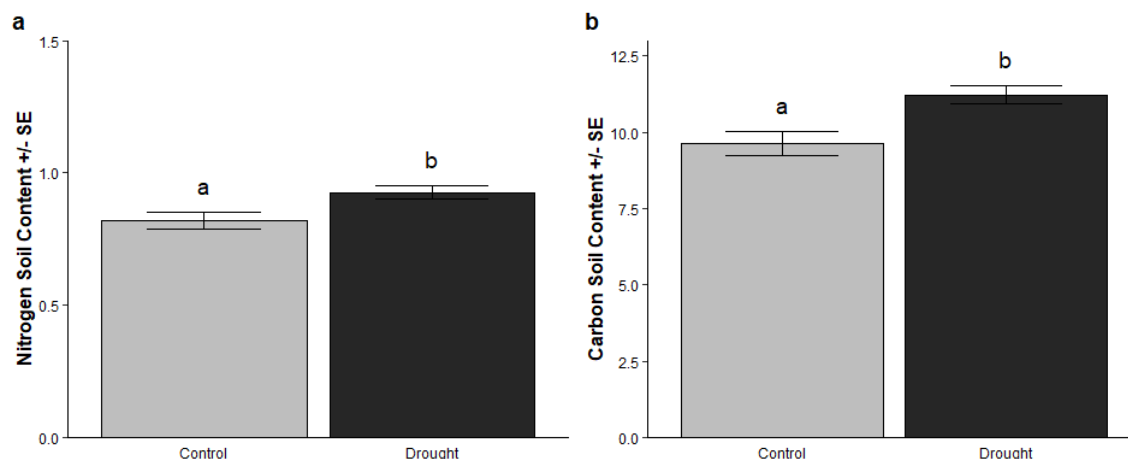
Survival, competitive effect and root/shoot ratio were analyzed using ANOVA and Welch's two sample t-test to analyze differences between species in monoculture or in competition and to check for treatment effects.

## RESULTS

### *Soil Analysis*

Soil was analyzed for nitrogen and carbon at TRU, Kamloops, BC. A significant difference was found between the control and drought field soils in nitrogen and carbon

content ( $P=0.029$ ,  $P=0.013$ ) where drought field soils had a higher nitrogen and carbon content (Figure 3.4; Table 3.1). Minor soil elements were also analyzed from Victoria (Table 3.2).



**Figure 3.4.** Soil elemental analysis of nitrogen and carbon in drought and control field soils. (a) Mean soil nitrogen (b) mean soil carbon. Soil was analyzed using an elemental analyzer at Thompson Rivers University. Bars are standard error of the mean and any bar with different letters (Tukey HSD) are significantly different ( $P < 0.05$ ),  $n=5$

**Table 3.1.** Soil content analysis of nitrogen and carbon. P values were obtained from t-tests,  $n=5$ . Bolded values indicate a statistical significance at the 5% probability level.

Soil Content	Control	Drought	P
Nitrogen	$0.819 \pm 0.032$	$0.926 \pm 0.023$	<b>0.029</b>
Carbon	$9.634 \pm 0.388$	$11.219 \pm 0.292$	<b>0.013</b>

**Table 3.2.** Minor soil content analysis. Soil was pooled from 6 field plots (control,  $n=6$ , drought,  $n=6$ ) and mixed to form a homogenous mixture into one sample.

Metals via Acid, Microwave Digestion, ICP-OES Analysis

Soil	Al mg/kg	B mg/kg	Ca %	Cu mg/kg	Fe mg/kg	K %	Mg %	Mn mg/kg	Mo mg/kg	Na mg/kg	P %	S %	Zn mg/kg
Control	20000	10	1.1	41	24000	0.46	0.67	980	<1	630	0.13	0.12	88
Drought	22000	7.7	1.1	41	26000	0.48	0.72	990	<1	760	0.13	0.11	89

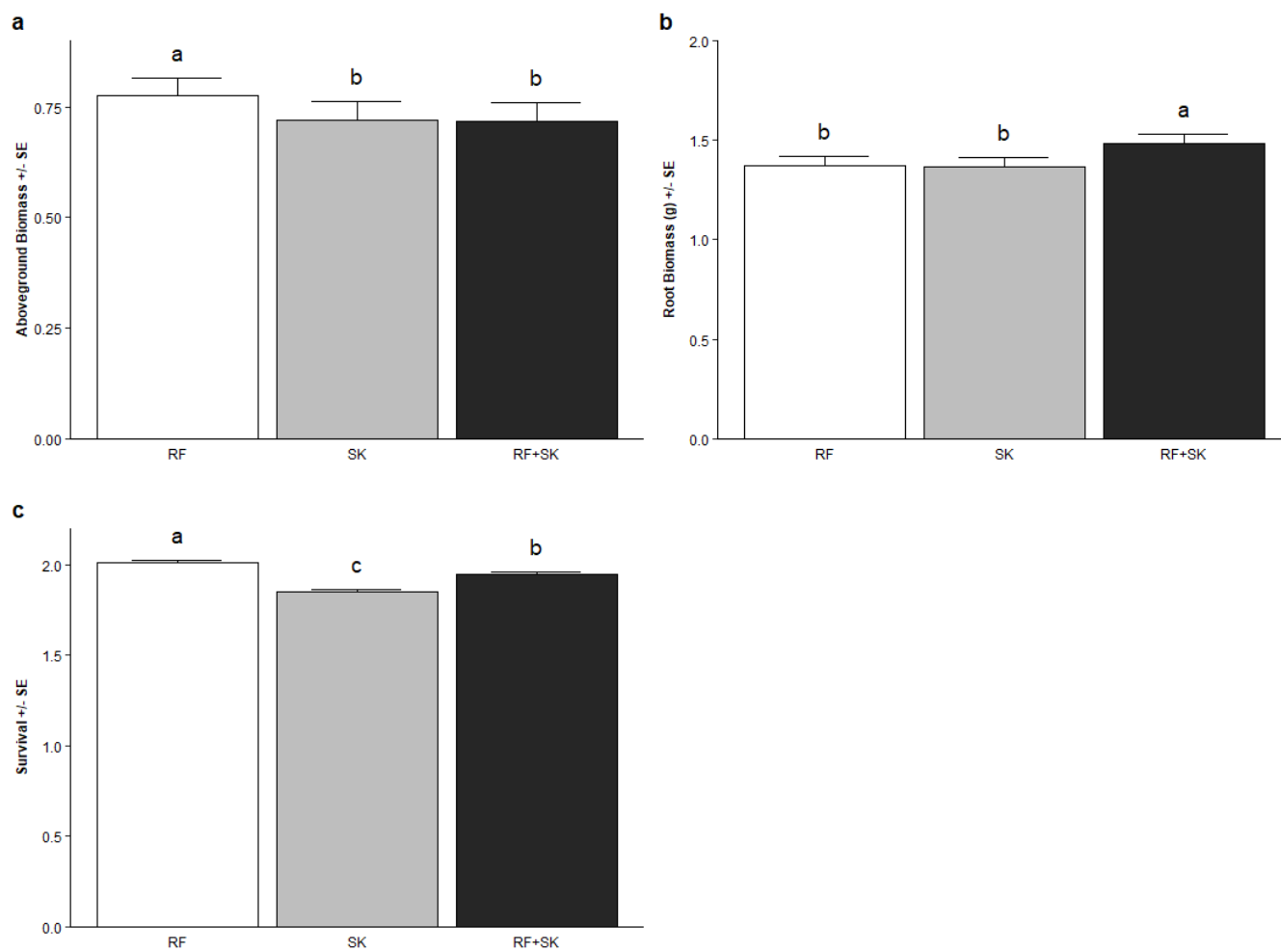
### *Treatment Response and Interactions*

Survival was significantly higher in rough fescue ( $2.01 \pm 0.01$  g) than spotted knapweed ( $1.84 \pm 0.01$  g) ( $P < 0.001$ ) (Figure 3.5). Treatment responses and interactions

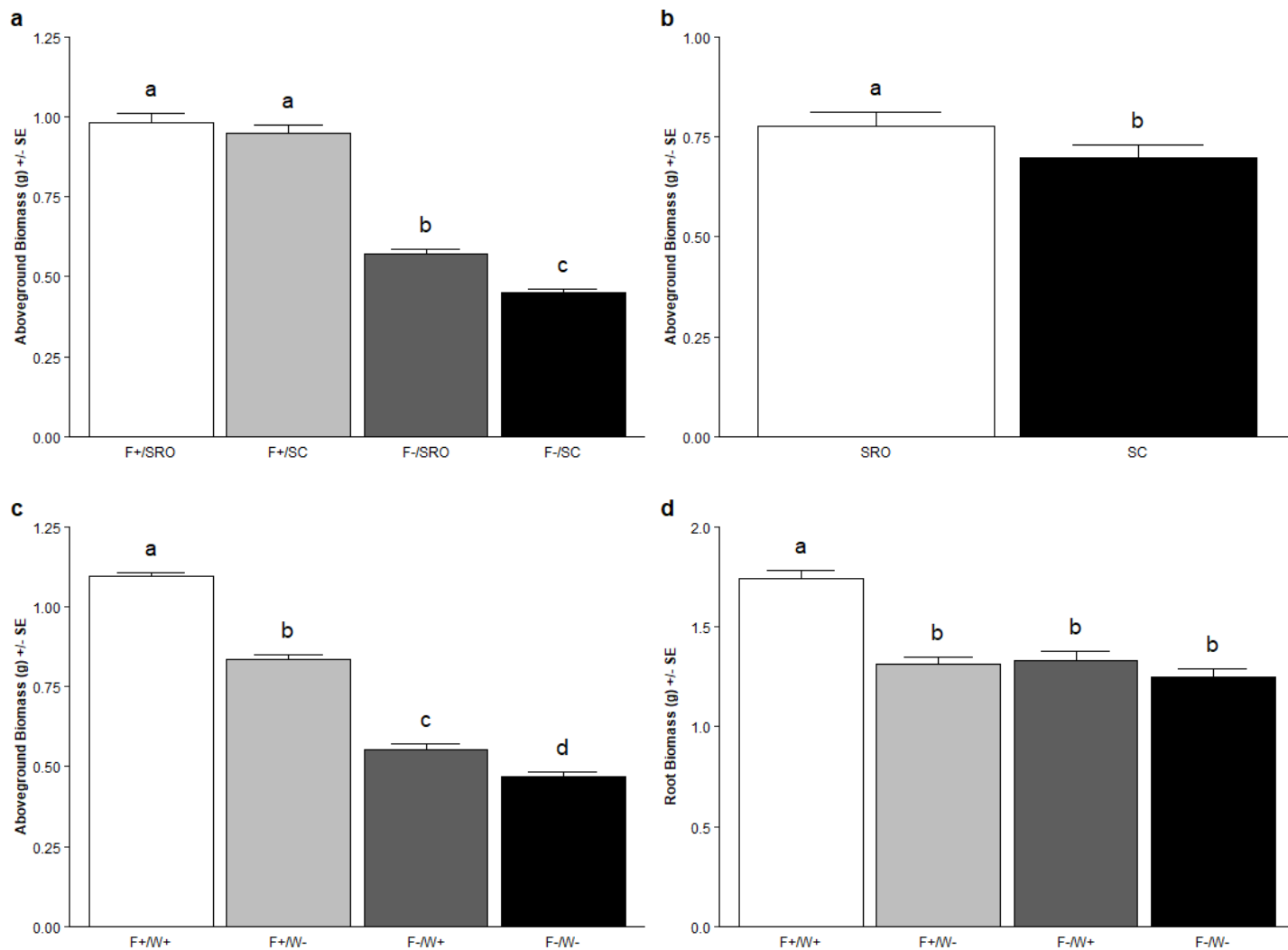
were found where fertilizer application, plant combination and the combination of the two treatments differed in survival (Table 3.3). Fertilizer significantly reduced survival of spotted knapweed ( $P < 0.001$ ) but did not influence the survival of rough fescue in monoculture. Rough fescue had the highest survival followed by mixed pots and spotted knapweed alone ( $P < 0.001$ ).

Significant differences were found in aboveground biomass in the watering, fertilizer, soil and plant combination treatments ( $P < 0.001$ ) (Table 3.3). Total aboveground biomass was highest in well-watered, fertilized pots ( $P < 0.001$ ) (Figure 3.6). Rough fescue had the highest biomass ( $0.77 \pm 0.04$  g) compared to spotted knapweed ( $0.71 \pm 0.04$  g) and the combination of the two ( $0.071 \pm 0.04$  g) (Figure 3.5). A significance difference was found in field soils where drought field soils ( $0.77 \pm 0.04$  g) had a higher total biomass than control field soils ( $0.70 \pm 0.4$  g) ( $P < 0.001$ ) (Figure 3.6). Significant interactions between watering and fertilizer and interactions between soil and fertilizer were found ( $P < 0.001$ ). An in depth look at all treatment interactions and its influence on aboveground biomass was visualized in Figure 3.7.

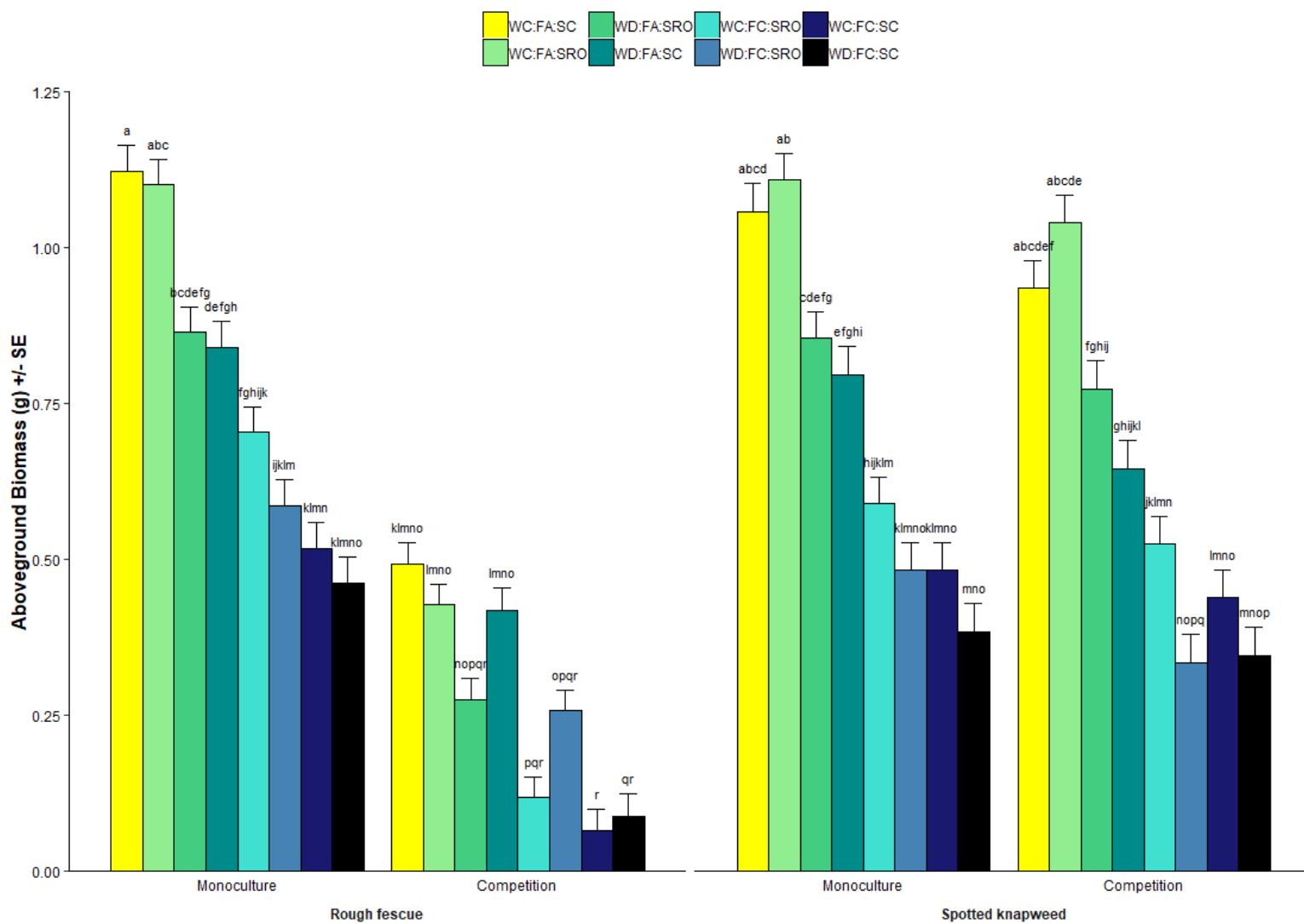
Significant differences in root biomass were found in the well-watered, fertilized pots (Table 3.3). Interactions between the watering and fertilizer treatments were found ( $P < 0.001$ ) (Figure 3.6). Differences were found in plant combination where the combination pots ( $1.49 \pm 0.04$  g) had a higher root biomass than the species alone ( $1.37 \pm 0.05$  g for rough fescue and  $1.36 \pm 0.04$  g for spotted knapweed monocultures)(Figure 3.5). Significant differences were found in blocking of the pots where block 4 had the highest root biomass and block 5 had the lowest root mass ( $P < 0.001$ ). Slight interactions between soil and plant combination were found where control field soils with monoculture plants had a lower root biomass than the other combinations ( $P = 0.059$ ).



**Figure 3.5.** Mean aboveground biomass, root biomass and survival after 90 d growth of all plant combinations of rough fescue (RF), spotted knapweed (SK) or the combination of the two (RF + SK). (a) Mean aboveground biomass. (b) Mean root biomass. (c) Mean survival. All data have been  $\log(x+1)$  transformed. Bars are standard error of the mean and any bar with different letters (Tukey HSD) are significantly different ( $P < 0.05$ ),  $n=40$ .



**Figure 3.6.** Mean aboveground and root biomass showing significant treatment interactions after 90 d growth. Pots were grown with (F+) or without (F-) fertilizer, well-watered (W+) or drought (W-) and using field control soils (SC) or field drought soils (SRO). (a) Mean aboveground biomass with fertilizer and field soil type interactions, n=30. (b) Mean aboveground biomass using drought or control field soils, n=60 (c) Mean aboveground biomass and fertilizer and watering type interactions, n=30. (d) Mean root biomass and fertilizer and watering type interactions. Bars are standard error of the mean and any bar with different letters (Tukey HSD) are significantly different ( $P < 0.05$ ), n=30.



**Figure 3.7.** Mean aboveground biomass of rough fescue and spotted knapweed in monoculture or competition with every combination of treatment. WC represents well-watered, WD is drought, FA is fertilizer application, FC is no fertilizer, SC is control field soils and SRO is drought field soils. Bars are standard error and any bar with different letters (Tukey HSD) are significantly different ( $p < 0.05$ )  $n=5$ .



**Table 3.3.** Results of 4-way ANOVA looking at the effects of watering (well-watered/drought), fertilizer (present/absent), soil (control/drought field soils), and plant combination (rough fescue, spotted knapweed, rough fescue/spotted knapweed together) on total aboveground biomass, root biomass and survival.

Treatment	Aboveground		Root		Survival	
	<i>F</i>	<i>P - value</i>	<i>F</i>	<i>P - value</i>	<i>F</i>	<i>P - value</i>
Watering	240.50	<b>&lt;0.001</b>	46.35	<b>&lt;0.001</b>	0.17	0.68
Fertilizer	1647.37	<b>&lt;0.001</b>	41.40	<b>&lt;0.001</b>	12.94	<b>&lt;0.001</b>
Soil	49.22	<b>&lt;0.001</b>	0.68	0.41	0.00	1.00
Plant Combo	11.30	<b>&lt;0.001</b>	4.40	<b>&lt;0.001</b>	40.05	<b>&lt;0.001</b>
Block	1.18	0.33	5.44	<b>&lt;0.001</b>	1.06	0.38
Watering x Fertilizer	60.56	<b>&lt;0.001</b>	21.56	<b>&lt;0.001</b>	0.91	0.34
Watering x Soil	0.05	0.83	0.41	0.52	0.00	0.98
Watering x Plant Combo	0.16	0.85	0.45	0.64	0.41	0.66
Fertilizer x Soil	15.47	<b>&lt;0.001</b>	0.35	0.56	0.00	0.98
Fertilizer x Plant Combo	2.95	<i>0.06</i>	0.26	0.77	6.31	<b>&lt;0.01</b>
Soil x Plant Combo	0.00	1.00	2.91	<i>0.06</i>	0.43	0.65

\* Bolded values indicate a statistical significance at the 1% probability level or lower and italicized values at the 10% probability level.

### *Competitive Effect, Root:shoot Ratio, and Treatment Effects*

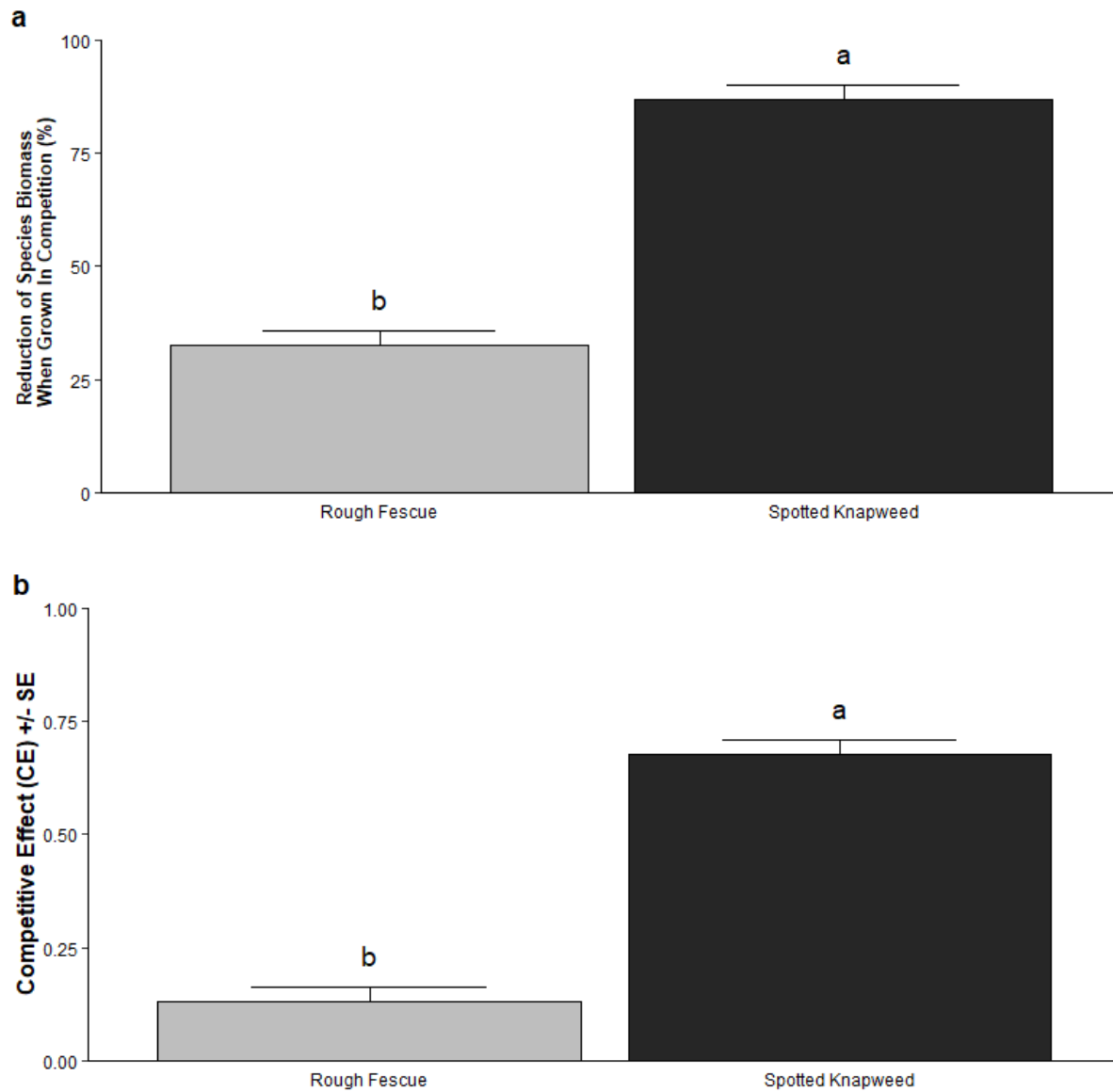
Competitive effect (CE) is the ability to suppress the growth of another species due to plant traits such as growth rate (Carlyle et al. 2010). Higher CE values indicate the greater ability to suppress the growth of another species. The formula used from Carlyle et al. (2010) is  $C_e = 1 - N_+ \div N_-$  where  $C_e$  is the competitive effect of the target species,  $N$  is the biomass of the neighbor species, and competitors are present (+) or absent (-). The biomass of each species when grown with a neighbor was determined by dividing the biomass of the species grown in competition, by the biomass of the species grown without competition (Figure 3.8). This value was converted to a percentage. Rough fescue is  $32.38 \pm 3.28 \%$  of its biomass when grown with spotted knapweed and spotted knapweed is  $86.95 \pm 3.08 \%$  of its biomass with grown with rough fescue (Figure 3.8).

There was a significant difference in competitive effect between rough fescue and spotted knapweed ( $P < 0.001$ ). On average, rough fescue had a competitive effect of  $0.131 \pm 0.03 C_e$  and spotted knapweed had  $0.676 \pm 0.03 C_e$  indicating that spotted knapweed has the greater ability to suppress the growth of rough fescue (Figure 3.9). To investigate if drought or drought legacies had an effect on competitive effect, interactions between field soil type and fertilizer were analyzed (Figure 3.9). Competitive effect in rough fescue was significantly lower than spotted knapweed, but it did not differ in the fertilizer application or field soil type. Drought field soils and the reduced watering treatment did not differ in competitive effect compared to the controls (Figure 3.9). Looking at fertilizer application alone, spotted knapweed had a higher CE value without fertilizer compared to with fertilizer application (Figure 3.10).

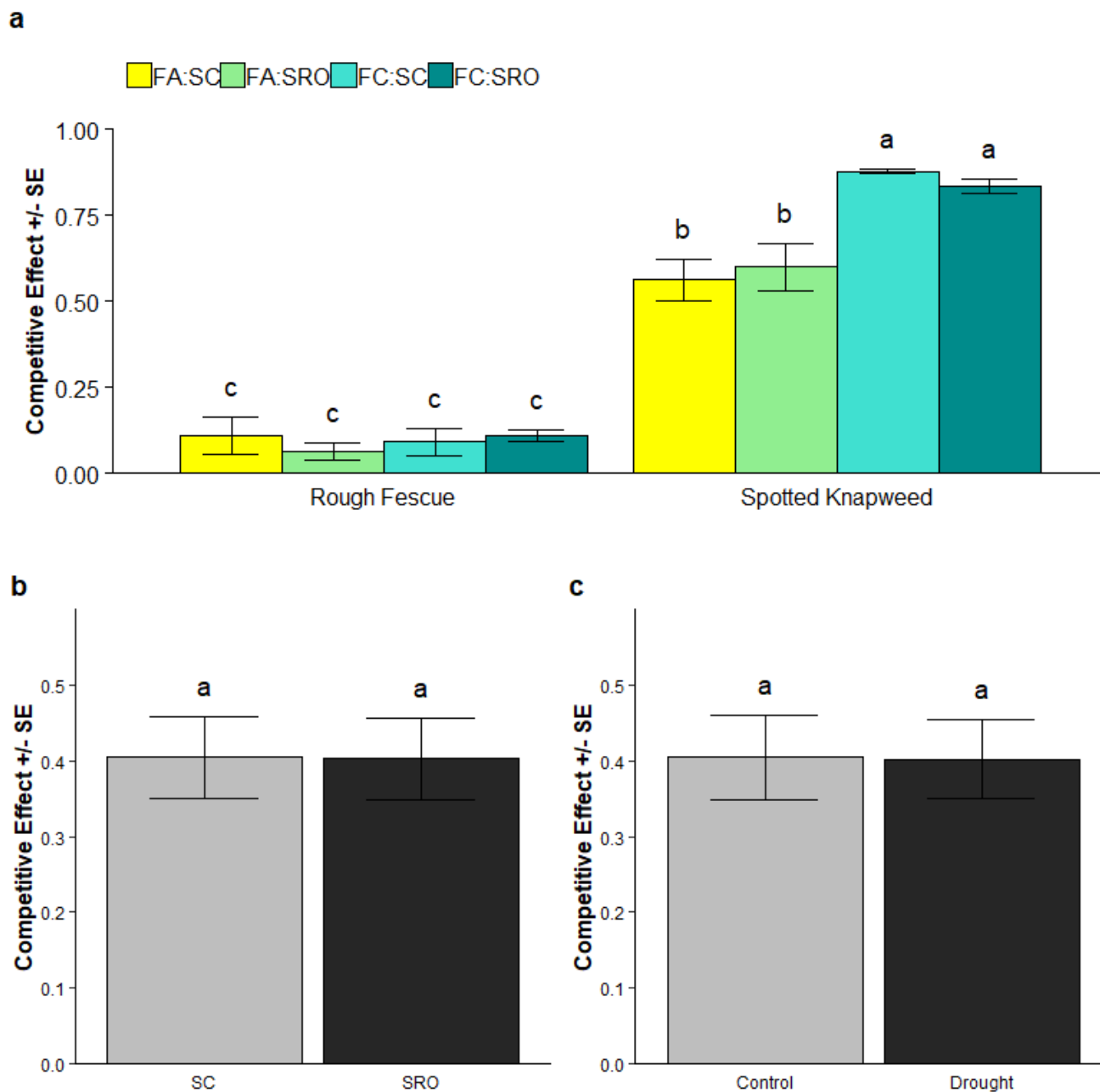
The root:shoot ratio of rough fescue ( $1.67 \pm 0.04$  g) and spotted knapweed ( $1.74 \pm 0.05$  g) in monoculture pots did not differ between each other ( $P = 2449$ ). The root:shoot ratio in competitive pots were highest for rough fescue ( $2.67 \pm 0.07$  g)( $P < 0.001$ ) in mixed pots followed by spotted knapweed in mixed pots ( $1.92 \pm 0.07$  g)( $P < 0.001$ )(Figure 3.11). Some treatment responses and interactions were found in root:shoot ratio when plants were grown alone or in competition (Table 3.4). Well-watered rough fescue in competition

had a higher root:shoot ratio ( $P > 0.001$ ). The absence of fertilizer had a higher root:shoot ratio in rough fescue and spotted knapweed when grown alone or in competition ( $P < 0.05$ ). Control field soil had a higher root:shoot ratio than the drought field soils in rough fescue and spotted knapweed in competition ( $P = 0.014$ ,  $P = 0.0023$ ). A treatment interaction was found in rough fescue in competitive pots between fertilizer and soil ( $P < 0.001$ ). A higher root:shoot ratio indicates that a plant is using their resources to grow their roots, increasing the ratio (Ågren and Franklin, 2003).

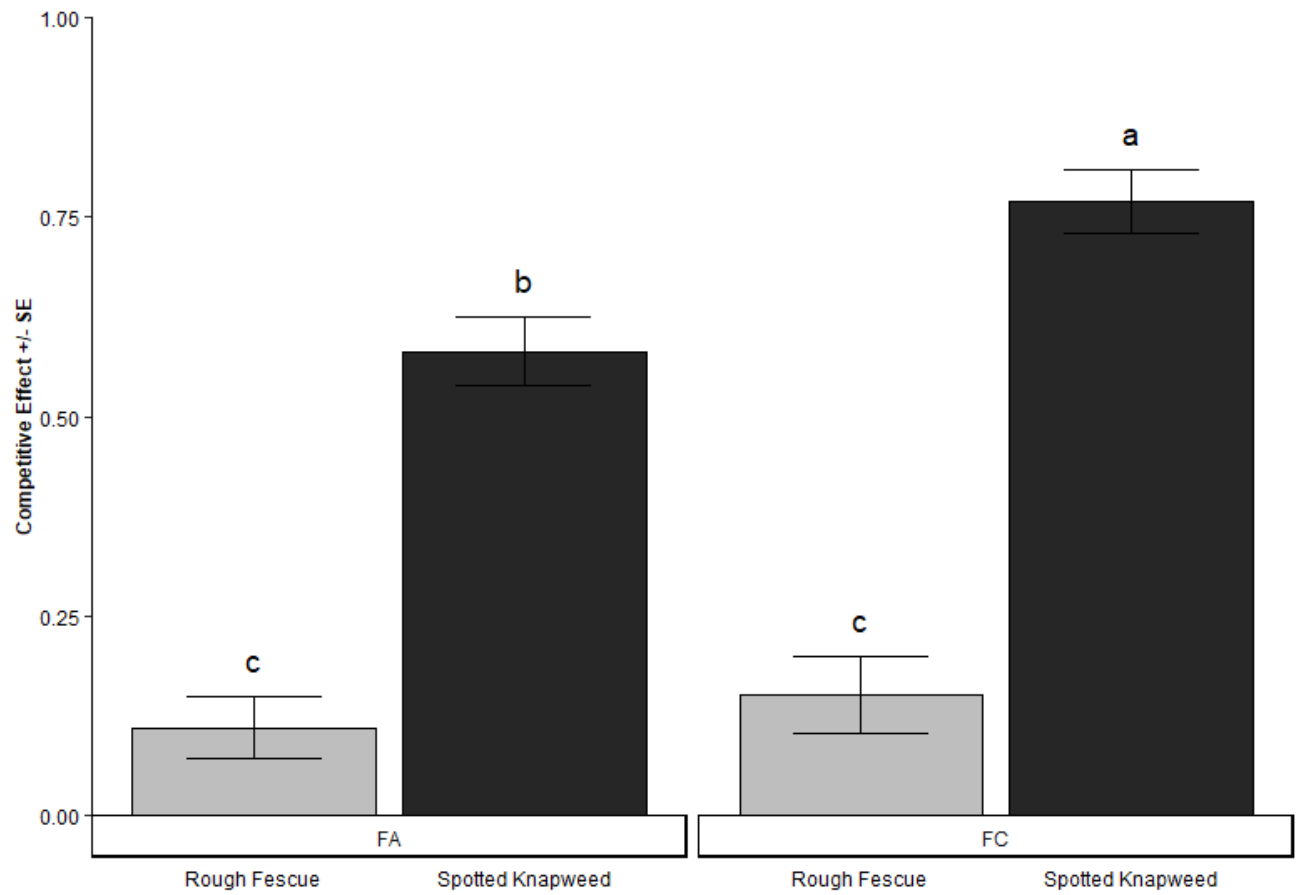
Overall, differences in aboveground biomass, root biomass and root:shoot ratio were found when species were grown alone or in competition (Figure 3.11). Rough fescue had a significant reduction in aboveground biomass when grown with spotted knapweed suggesting competitive effects. Root biomass was highest in competitive pots and root:shoot ratio was highest in rough fescue in competitive pots. Survival was lower in spotted knapweed regardless if grown alone or with rough fescue. Total biomass was investigated, but no significant difference was found between rough fescue and spotted knapweed.



**Figure 3.8.** (a) Species biomass when grown in competition with a neighbor (%) (b) Mean competitive effect (CE) of rough fescue and spotted knapweed. Bars are standard error of the mean and any bar with different letters (Tukey HSD) are significantly different ( $P < 0.05$ )  $n=40$ .



**Figure 3.9.** (a) Mean competitive effect (CE) of rough fescue and spotted knapweed investigating the effects of fertilizer application and field soil type. FA indicates fertilizer application, FC indicates no fertilizer, SRO indicates drought field soils and SC indicate control field soils. (b) No differences in competitive effect were found between field soils. (c) No differences were found in the control or drought watering treatment in competitive effect. Bars are standard error and any bar with different letters (Tukey HSD) are significantly different ( $p < 0.05$ ).

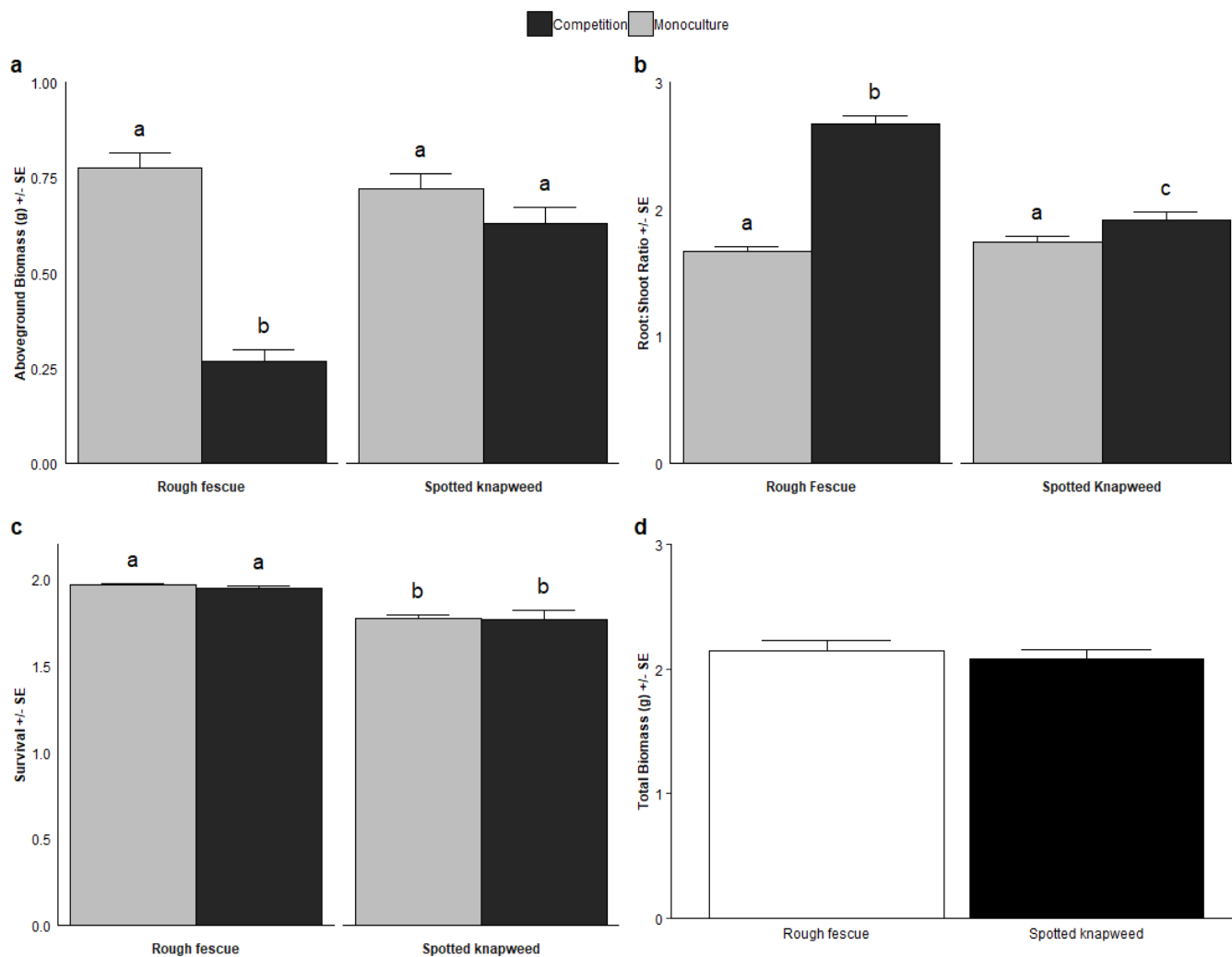


**Figure 3.10.** Mean competitive effect (CE) of rough fescue and spotted knapweed with fertilizer application (FA) or the control (FC). Bars are standard error of the mean and any bar with different letters (Tukey HSD) are significantly different ( $P < 0.05$ ),  $n = 20$ .

**Table 3.4.** Summary of three-way ANOVA investigating the effects of the treatments watering, fertilizer, and soil on the root:shoot ratio of rough fescue (RF) and spotted knapweed (SK) when they are grown in monoculture (RF/SK Alone) or in competition (RF/SK Competition).

Treatment	RF Monoculture		RF Competition		SK Monoculture		SK Competition	
	<i>F</i>	<i>P - value</i>	<i>F</i>	<i>P - value</i>	<i>F</i>	<i>P - value</i>	<i>F</i>	<i>P - value</i>
<i>Aboveground biomass</i>								
Watering	52.72	<b>&lt;0.001</b>	0.14	0.71	131.70	<b>&lt;0.001</b>	29.61	<b>&lt;0.001</b>
Fertilizer	301.78	<b>&lt;0.001</b>	39.30	<b>&lt;0.001</b>	889.53	<b>&lt;0.001</b>	128.40	<b>&lt;0.001</b>
Soil	10.91	<b>&lt;0.01</b>	0.01	0.92	25.23	<b>&lt;0.001</b>	3.95	<i>0.06</i>
Watering x Fertilizer	13.23	<b>&lt;0.01</b>	5.05	<b>0.03</b>	24.31	<b>&lt;0.001</b>	3.13	<i>0.09</i>
Watering x Soil	0.04	0.84	0.04	0.84	0.00	0.99	0.22	0.64
Fertilizer x Soil	10.42	<b>&lt;0.01</b>	6.14	<b>0.02</b>	2.43	0.13	1.05	0.31
<i>Root:shoot ratio</i>								
Watering	0.05	0.82	15.94	<b>&lt;0.001</b>	0.04	0.84	2.01	0.17
Fertilizer	11.92	<b>&lt;0.01</b>	24.29	<b>&lt;0.001</b>	24.73	<b>&lt;0.001</b>	4.82	<b>0.04</b>
Soil	0.05	823214.00	6.67	<b>0.01</b>	0.27	0.61	10.97	<b>&lt;0.01</b>
Watering x Fertilizer	3.05	<i>0.09</i>	1.16	0.29	2.13	0.15	0.06	0.81
Watering x Soil	0.01	0.91	0.01	0.93	1.82	0.19	0.22	0.64
Fertilizer x Soil	0.07	0.79	16.75	<b>&lt;0.001</b>	0.61	0.44	1.64	0.21

\* Bolded values indicate a statistical significance at the 1% probability level or lower and italicized values at the 10% probability level. Bolded and italicized values indicate a statistical significance at the 5% probability level.



**Figure 3.11.** (a) Mean aboveground biomass, (b) root:shoot ratio, (c) survival of rough fescue and spotted knapweed in competition or alone after 90 d growth, and (d) total biomass (above and below ground growth). Bars are standard error of the mean and any bar with different letters (Tukey HSD) are significantly different ( $P < 0.05$ ).

## DISCUSSION

### *Survival*

Survival was significantly higher in rough fescue than in spotted knapweed when grown alone. This may suggest that intraspecific competition lowered spotted knapweed survival due to a stronger competitive effect on itself. Fertilizer significantly reduced survival. This may be due to fertilizer being applied too early or too close to the seeds, resulting in damage to the seed and failure to germinate (Dobb and Burton 2012). Future



studies should be careful with fertilizer placement and proximity to seeds.

### *Aboveground Biomass*

The treatment combination of watering and fertilizer resulted in higher total biomass. A lower total biomass was found in the reduced watering, or drought, treatment. This result aligns with Grime's hypothesis of lower primary productivity with increased abiotic stress (Grime 1979). Rough fescue had a higher biomass than spotted knapweed when grown alone but had a lower growth in competition with spotted knapweed, suggesting the competitive effect of spotted knapweed on rough fescue. The pots using drought field soils collected from Lac du Bois had a higher total biomass than pots using the control field soils. This could indicate positive legacy effects where a higher biomass was found in field soils exposed to multi-year drought. However, it should be noted that the field soils were not actually "drought" soils as found in Chapter 2. If the soils are not drought, it may suggest negative feedback soil legacies as were found by Kaisermann et al. (2017). If the soils are considered as drought soils, there may be positive legacy effects comparable to those found by Griffin-Nolan et al. (2018). Sala et al. (2012), developed models based on aboveground net primary production (ANPP) and found legacies where previous year precipitation had a significant influence on the current year production. They found that dry legacies had a lower production in the current year and wet legacies had a higher production. Negative drought legacies may be due constraints in growth due to loss of carbon resources or prolonged stomata closures (Chaves et al. 2002; Klaus et al. 2016). Positive drought legacies may occur due to a higher soil N availability or accumulation of nitrogen due to reduced uptake during drought (Griffin-Nolan et al. 2018). If a rewetting event occurs, this accumulated nitrogen could potentially promote productivity and soil quality if enough moisture is available (Khalili et al. 2016). This may be the case in this study, as drought field soils had a higher carbon and nitrogen content and were well watered for 30 days prior to the simulated drought treatment of the experiment.

A significant interaction between field soil and fertilizer was also found where there was a higher aboveground biomass in the fertilized field soils. Furthermore, unfertilized

drought field soils had a higher aboveground biomass than unfertilized control field soils (Figure 3.6). This may suggest that 1) productivity was promoted due the sudden availability of resources that were unused during drought periods and 2) fertilizer supported control field soils with nutrients that would be otherwise missing.

### *Root Biomass*

A higher root biomass was found in well-watered, fertilized pots. Competitive pots had a higher root biomass than monoculture pots, indicating some belowground competition. Before a plant is able to allocate resources to grow above ground, roots and below ground resources must be established first to support shoot growth (Sainju et al. 2017). A higher root biomass in competitive pots may suggest the two plants are competing for belowground resources at a higher rate than in monoculture pots. Some interactions between field soils and plant combination were found where control field soils with monoculture plants had a lower root biomass than any other combination. The drought field soils and the control field soils in competitive pots had a higher biomass. These results may be due to the combination of drought soils being rich in now available resources and the competitive pots allocating more resources to root growth. Root biomass differed in the replicate pots of the experiment. Block 4 had the highest biomass and block 5 had the lowest. This was a surprising result due to these blocks being next to each other on the same bench. However, this may be due to differences in the greenhouse pod such as lighting or air moisture. Block 4 was next to the greenhouse siding on two sides and block 5 had one side allowing for more ambient lighting. Overall, it is uncertain why these two blocks differed in root biomass from each other when they were in such close proximity to each other.

### *Root:Shoot Ratio and Competitive Effect*

A high root:shoot ratio indicates that a plant is allocating resources to grow their roots, thus increasing the ratio and root biomass (Ågren and Franklin, 2003). The root:shoot ratio did not differ in monoculture pots. The root:shoot ratio was highest in rough fescue grown in competition. This may indicate that rough fescue allocated more

resources to its roots over aboveground growth. Well-watered pots with rough fescue in competition with spotted knapweed had a higher root:shoot ratio. Once again, this indicates that rough fescue is competing belowground for resources before it can grow aboveground. The absence of fertilizer resulted in a higher root:shoot ratio in both rough fescue and spotted knapweed when grown alone or in competition. Or alternatively, the presence of fertilizer provided ample resources for aboveground growth, reducing root competition and thus, lowering the root:shoot ratio (Pødenphant Kiær et al. 2013). These results align with Lamb et al. (2007) who hypothesized that the addition of limiting soil resources, in the form of fertilizer, should reduce root competition. However, it should be noted that contrary to their expectations, their study found that root competition increased with added nitrogen. Control field soils had a higher root:shoot ratio in competition compared to drought field soils. This is another interesting result that may further suggest that drought field soils have resources that are stored or unused until a rewetting event, providing enough resources for aboveground growth.

Competitive effect (CE) is the ability to suppress the growth of a neighboring plant. A higher competitive effect indicates greater ability to suppress. Spotted knapweed had a significantly higher competitive effect, indicating a higher potential to suppress the growth of rough fescue. The results of aboveground biomass, root biomass and root:shoot ratio indicate some competitive effects between rough fescue and spotted knapweed. Rough fescue had a significantly reduced biomass when grown in competition and had a higher root biomass and root:shoot ratio when grown with spotted knapweed. These are indicators of competition where rough fescue must allocate resources belowground at the cost of aboveground growth. The absence of fertilizer increased the competitiveness of spotted knapweed when grown with rough fescue. This may suggest that without the addition of soil resources, rough fescue growth is negatively impacted allowing for spotted knapweed to outcompete. No differences were found in competitive effect in the field soils and the watering treatment. Competition may be reduced depending on the severity of abiotic stress. Stress, such as drought, may limit plants ability to rapidly acquire soil resources or water, thus limiting primary productivity and competition between species (Callaway et al. 1997).

### *Drought Field Soils vs. Control Field Soils*

Referring to the results of Chapter 2, the question of: are these drought field soils, or not, needs to be addressed. Starting the experiment, it was predicted that the rain-out shelters would simulate drought conditions and that the drought plots would have less soil moisture compared to the control plots. At the end of Chapter 2, data loggers indicated that the drought plots had higher soil moisture than the control. Additionally, the total precipitation data showed that the rain-out shelters failed to simulate severe drought in 3 out of 4 years. If the field soils are not under drought conditions, it changes how the results are interpreted. If we assume these are drought soils, the results of this experiment may indicate positive legacy effects, as previously described (Griffin-Nolan et al. 2018). If the soils are not drought soils, it may be indicative of negative legacy effects, where moist soils found in the “drought” plots had a higher aboveground biomass growth in the greenhouse experiment than control soils (Kaisermann et al. 2017). The literature on drought legacies has shown both positive and negative legacy effects, so it is unclear which is the case for this study. Caution should be exercised with respect to whether the legacy effects of the soil exist due to drought conditions. It is clear that some difference was found between the drought and control field soils in element content and aboveground biomass growth. However, there is uncertainty as to why these differences exist.

## CONCLUSIONS

When grown alone, rough fescue had an overall higher biomass production. When in competition with spotted knapweed, rough fescue biomass growth was limited, indicating some competitive effects. It is unknown if allelochemicals were part of this competitive effect or if the competition was mostly physical such as shading and overall growth. Regarding field soils, the drought field soils produced a higher aboveground and root biomass compared to the control field soils. This may be due to the build-up of soil resources over the years which was released and available when rewet. Soil resources may have been higher in the drought plots due to higher plant litter and increased decomposition rates. Soil analysis results support this hypothesis where it was indicated that the drought field soils had a higher content of carbon and nitrogen. The soil resource

storage may have later been released during watering, allowing for the drought field soils to have a higher aboveground biomass. Previous studies that have found positive drought legacies (Griffin-Nolen et al. 2018) but there are studies that have found negative drought legacies (Kaisermann et al. 2017). Our results may be further supported by the fertilizer treatment on aboveground biomass results. No difference in biomass was found in the drought and control field soils when fertilizer was added, but in the absence of fertilizer, drought field soils had a higher biomass (Figure 3.6). In other words, fertilizer may have provided limiting soil resources to allow for optimal growth. Without fertilizer, drought field soils may have a potential resource pool ready to be utilized once enough moisture was available. A similar finding was found in the root biomass results that may suggest the same as the aboveground biomass. A higher root biomass was found in drought field soils in competition or monoculture, compared to control field soils in monoculture. With an increase in soil resources, the plants may be able to grow at a higher rate than in soils without.

If we consider the field soils to be in drought conditions, the results of this study may suggest positive drought legacies in aboveground biomass growth after a re-wetting event (Griffin-Nolan et al. 2018). However, if the soils are not in drought conditions compared to the control, it may suggest negative drought legacies (Sala et al. 2012; Kaisermann et al. 2017). Caution is recommended regarding these results as it is unclear if the field soils are considered drought, or not. Although the interpretation of these results is uncertain, this study provides some insight on grassland soils as differences were found between the drought and control field soils. Further research is needed to understand the mechanisms involved with positive or negative legacies, or to investigate other mechanisms that could have been missed.

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## CHAPTER 4 – MANAGEMENT IMPLICATIONS

In this study, the directionality of drought legacy effects on growth of invasive and native species is unknown. Due to the soil moisture data in Chapter 2, it is unclear if the results should be interpreted as positive or negative drought legacies in Chapter 3. Additionally, it is unclear if the results should be considered drought, at all. Positive or negative legacies may be dependent on the length of the drought. However, Griffin-Nolan et al. (2018) acknowledge that although it may be easy to assume short-term droughts are more likely to lead to positive legacies compared to long-term droughts, there are studies which counteract this observation (Sala et al. 2012). It is difficult to discuss management implications when it is unclear on what mechanisms are present for this experiment. Legacy effects will be taken into account, but other management considerations will be discussed involving experimental design and climate change in grasslands.

### *Experimental Design*

Based on the results from Chapter 2, the rain-out shelter design used in this experiment may not have been optimal to simulate multi-year drought. According to the data loggers, the experimental design was working as expected for the first year (2015 to 2016). After 2016, the drought experimental plots increased in soil moisture while the control plots decreased. Soil temperature was also influenced where the drought plots had a lower temperature compared to the control at the end of the experiment. The rain-out shelter design was based on the requirements of the International Drought Experiment (2015). Prior to the start of this experiment, in the winter of 2014 snow was removed randomly from one 1 m x 1m subplot in each experimental plot. The data from the snow removal and subsequent monitoring was used to contribute to a globally coordinated field experiment (Hugh et al. 2018). After the snow removal, in the summer of 2015 the rain-out shelters were constructed. Half of the experimental plots were covered with a rain-out shelter using a wooden frame and transparent plastic troughs to reduce rainfall, as described in Chapter 2 (Backhaus et al. 2014; Kaiserman et al. 2017; Hugh et al. 2018). Gutters were mounted to divert water away and trenches were dug to hydrologically isolate the plots from each other.

In Chapter 2, it was found that the soil moisture and temperature were different between the drought and control plots. This observation may have occurred due to shading, shelter effects, microenvironments or UV light interception from the plastic troughs or wooden structures. To control for these potential differences, Canarini et al. (2016) built the same shelters for all of experimental plots but mounted the frame upside down in the control plots to allow precipitation through. Hoover et al. (2014) used deer netting in their control plots to reduce for light radiation so that it would be equivalent to the drought shelters. To help account for microclimatic effects of the shelters, Schrama and Bardgett (2016) utilized drought shelters, a control with no shelter, and a microclimatic control that uses the same shelter as the drought plots, but had holes drilled throughout. To prevent runoff or lateral flow, Canarini et al. (2016) buried plastic barriers into the ground facing the slope while Luo et al. (2018) buried aluminum flashing. Another design element to consider would be to intercept precipitation from gutters and divert it to a storage tank (Yahjian and Sala 2002). Other designs include tunnel-shaped rainout shelters using plastic foil that are built above ground level, open at both ends and positioned towards the main wind direction (Bollig and Feller 2014; Grant et al. 2014; Hasibeder et al 2015; Burri et al. 2018).

There are many different examples of rainout shelters that can be used and implemented. The rainout shelters for this experiment were built and designed to last between 5-10 years, so changing or altering these shelters would be difficult. To improve this experiment, I would recommend the construction of control shelters of similar design and to implement further trenching or use of a physical barrier to prevent run off or lateral flow. I would suggest data loggers be re-calibrated and that species composition continue to be monitored via surveys to observe any shifts in the control and drought plots.

### *Climate Change*

Climate around the world is experiencing warming and changing precipitation patterns (Shukla et al. 2019; Eze et al. 2018). Southern and Central British Columbia are predicted to have less precipitation in the summer which may lead to more frequent and intense drought (Spittlehouse 2008; Briceño et al. 2014). Changes in climate is expected to

have an effect on terrestrial ecosystems and their nutrient pools, plant biomass and microbial biomass, potentially leading to feedback loops (Eze et al. 2018; Florianová and Münzbergová 2018; Mariotte et al. 2018). Experimental manipulation of precipitation and temperature is important to provide insight of potential impacts on ecosystems in the future (Eze et al. 2018; Knapp et al. 2018). However, do climate change experiments reflect real world predictions or impacts? Knapp et al. (2018) caution that results from climate experiments may be inconsistent with natural conditions. The authors compiled and compared experimental data to 33-year precipitation and aboveground net primary production (ANPP) records to determine if the experiments were comparable to observational data. They determined that the experimental data did not deviate from the observations in that ecosystem and historical range. The authors recommend continued experiments investigating extreme precipitation manipulation (extreme wet and dry years) to provide insight on future precipitation patterns (Knapp et al. 2018). Grasslands are sensitive to changes in precipitation that can lead to desertification or driven to woodland ecosystems with increased rainfall and tree invasion (Thébault et al. 2014). Due to the results of Chapter 2, I would recommend increasing the severity of drought by altering the number of plastic troughs. Furthermore, I recommend that the rainout shelters that were constructed continue to be monitored to collect long-term data. This data may be compared over the years to gain better insight on long-term trends in temperate grasslands in British Columbia.

### *Positive or Negative Drought Legacies*

As explored in Chapter 2 and Chapter 3, it is unclear if true drought legacies, positive or negative, were observed. Drought legacy effects show varying results from improved growth after climatic stress (de Vries et al. 2012; Griffin-Nolan et al. 2018; Legay et al. 2018) to negative growth after drought (Sala et al. 2012, Kaisermann et al. 2017). A study by Meisner et al. (2013) found that drought legacies may promote exotic species and suppress native species. This experiment could not confirm if positive or negative drought legacies exist due to contradicting soil moisture results and historical total precipitation data. However, differences were found between the experimental field soils in growth and

elemental content and further research is recommended. Further soil analyses should be conducted on the field soils and the microbial communities should be analyzed and compared. Perhaps with that knowledge, we could answer the questions of how did the rainout shelters alter the soil communities to influence growth post-climatic stress?

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