EXAMINING THE SOIL LEGACY EFFECTS OF SPOTTED KNAPWEED (*Centaurea stoebe*)

by

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Abstract

Spotted knapweed (*Centaurea stoebe*) is the most aggressive invasive forb in North American grasslands. Since its arrival, it has spread from the west coast of North America and reached far east. It has been able to accomplish this via the production of many small seeds per plant, and by altering soil conditions making it difficult for native plants to grow in. Control efforts have been extensive. Broadcast chemical controls have been applied, as well as biological and physical controls; however, despite these interventions, spotted knapweed continues to have negative effects on ecosystems and their functions. Spotted knapweed may have a negative legacy effect in the soils they inhabit, which perpetuates even after removal of this plant. To test for potential soil legacy effects, a greenhouse experiment was devised in which *C. stoebe* and rough fescue (*Festuca campestris*) were grown in different soil types. Activated carbon and pulp mill fly ash were used as soil amendments in each soil type in an attempt to return soils to a pristine state, and we found that *F. campestris* grew best in unamended invaded soils during a 90-day growing period. Pre-growing conditions of this soil displayed lower levels of both carbon and nitrogen compared to other soil types, indicating that *F. campestris* grew best in less hospitable conditions. As this was an unexpected result, a field experiment was designed in which different concentrations of ash were applied to transplanted rough fescue plugs; however, plug viability tapered off quickly after transplanting. Conclusions drawn from this study indicate ash as a potential soil amendment for knapweed-affected soils with directions for use in future research. Further investigation into the use of ash as a broadscale solution to negative soil legacy effects is warranted. Ash, an industrial waste product, could be potentially useful in areas heavily invaded by spotted knapweed in order to deter the spread of these noxious weeds.

Keywords: invasive, spotted knapweed, fly ash, activated carbon, rough fescue, greenhouse

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Chapter 1: Introduction

Invasive species are defined by three elements: 1) the species is introduced to a new area with human assistance, 2) the species can establish and spread without human assistance, and 3) the species is found far beyond its point of arrival (Simberloff, 2013). Introduced species, in comparison are defined only by the first element in the invasive species definition. Throughout the kingdom of life, there are many examples of both invasive and introduced plants, animals, fungi, and microorganisms (Lowe et al., 2000). Introductions of species may be intentional. For example, in re-introduction efforts to bolster wild populations of wolves in Yellowstone National Park (Phillips & Smith, 1997), or for aesthetic purposes to make a garden appear more vibrant or exotic. Introductions may also be unintentional, for example during an exchange of ballast water on ships dinoflagellates may be taken in at one international port and transported to another port where they would be released during the next ballast exchange (Hallegraeff, 1998). No matter the intent, humans are left to manage the consequences of these introductions, including a full-scale invasion by an introduced species. Once introduced, invasive species increase in abundance, often at the expense of native species (Simberloff et al., 2013). Without proper management and in more extreme cases, invasive species may devastate the biodiversity of an area by removing the local species to establish dominance (Simberloff et al., 2013; Vilà et al., 2011).

Plants may have traits that allow for successful establishment into new areas. This is especially true for invasive plants. For example, small seeded invasive plants may go unnoticed and become attached to animals, our clothing or vehicles, allowing them to be transported long distances (Hodkinson et al., 1998). Other invasive plants can alter their surroundings by negatively altering soil conditions, thereby impacting available forage for grazing animals (Vilà et al., 2011; Watson & Renney, 1974). In British Columbia (BC), Canada, there are approximately 229 plant species that exhibit invasive traits (Ministry of Forests and Range - Range Branch, 2020). As a result, researching and developing strategies for managing these ecological threats should be prioritized. This is especially important in ecosystems under severe threat of collapse due to pressure from invasive species (Klinkenberg, 2012). In BC, grasslands account for less than 1% of the total land base but provide habitat for approximately 30% of the provinces' species at risk, making grasslands

one of the most critical ecosystems to protect from the threat of invasion (Wikeem & Wikeem, 2004). Current management solutions in place for many invasive species are reactive (i.e., invasive plants are managed after they become problematic), though proactive approaches could allow us to prevent invasions altogether. In this way, land managers can prevent the spread of invasives before they negatively impact ecosystems. To achieve this, we first need a complete understanding of the invasive species in question.

Lac Du Bois Grasslands Protected Area, a provincial park located north of Kamloops, BC, is a complex mosaic of both pristine grassland and disturbed, invaded landscape. The undisturbed areas of the upper Lac Du Bois park are described as rough fescue (*Festuca campestris*) grassland woven with layers of shrubs, forbs, grasses, and biological crusts throughout (Delesalle et al., 2009). Many invasive plants have made their way into this region, some forming large patches that lie adjacent to native plant communities (Invasive Alien Plant Program, 2017). Spotted knapweed (*Centaurea stoebe* L.) is an invasive plant readily found in this park despite the control efforts that have been applied since 1970 (Fraser & Carlyle, 2011; Gayton & Miller, 2012). The impact spotted knapweed may have on ecosystem function, its mechanisms of invasion, and its susceptibility to control methods deserve further attention.

SPOTTED KNAPWEED

In B.C., spotted knapweed (*Centaurea stoebe* L.) is considered an invasive, noxious forb (Klinkenberg, 2012). It is hypothesized to have arrived in North America in the late 1800s, likely as a contaminant in alfalfa seed shipments from Europe (Moore, 1972; Moore et al., 1974). Spotted knapweed inhabits upper grassland regions near forest interfaces where it roots in rich soils. Since its arrival in North America, spotted knapweed has genetically diverged from its European ancestry, thereby becoming an invasive plant in two key ways: 1) it is diploid at the chromosome level when found in Europe but in North America it is tetraploid, and 2) it is a biennial plant in Europe, whereas it is a more aggressive and short-lived perennial in North America (Jacobs, 2012). As such, these changes warrant that spotted knapweed be placed into two subspecies based on location. The native, European, diploid

biennial is classified into subspecies *Centaurea stoebe* spp. *stoebe*, and the invasive, North American, tetraploid perennial is subspecies *Centaurea stoebe* spp. *micranthos* (Integrated Taxonomic Information System, 2017; Jacobs, 2012; Keil & Ochsmann, 1993). However, there is still a lack of uniformity of the scientific name applied to North American spotted knapweed. For example, the Encyclopedia of Life (EOL), The Plant List, and the Pan-European Species directories Infrastructure (PESI) list North American spotted knapweed as *Centaurea stoebe* ssp. *australis* (Pančić ex A. Kern.) Greuter, but the Integrated Taxonomic Information System (ITIS), United States Department of Agriculture (USDA) PLANTS Database, and E-Flora BC refer to spotted knapweed as *Centaurea stoebe* ssp. *micranthos* L. (Gugler) Hayek (Carpinelli, 2013; Greuter, 2007; Integrated Taxonomic Information System, 2017; Klinkenberg, 2017; The Plant List, 2013; USDA NRCS, 2017). Generic and scholarly search tools return more results for the term "*Centaurea stoebe* ssp. *micranthos*" compared to the term "*Centaurea stoebe* ssp. *australis*". For this reason and for simplicity, *C. stoebe* will refer to *Centaurea stoebe* spp. *micranthos* throughout this thesis, unless otherwise specified.

Centaurea stoebe reproduces only by seed production and may produce tens of thousands of seeds per square meter in a single growing season (Jacobs, 2012; Schirman, 1981). This method of reproduction allows *C. stoebe* to continually expand within existing stands as many of the seeds will drop in the immediate vicinity. When mature, seeds disperse via attachment to animals or vehicles, wind, or water (Sheley et al., 1998). Seeds have a high chance of germinating once they are established in an area, and can remain viable in soils for eight or more years (Davis et al., 1993). Combined, these traits allow for rapid dispersal. In North America, spotted knapweed is now found in 46 states within the United States and eight provinces and territories of Canada (Desmet & Brouilet, 2013; EDDMapS, 2017). Seedlings emerge during the first year of growth and share physical similarities with some other forb species (Figure 1.1). The next growth stage is the rosette, characterized by pinnatifid leaves with many narrow teeth. The rosette may develop directly from the seedling within the same growing season depending on when the seedling first emerged. Finally, flowering plants are upright with highly branched stems and pink to purple flowering heads on the branch ends. When *C. stoebe* is flowering, it can be distinguished from other forbs and knapweeds by the black-tipped involucre bracts beneath the flowering head, giving it a

spotted appearance and lending to its name. There may be up to 30 flowers in each composite flowering head, all producing one seed each. With large, branching individuals, it is thus reasonable to observe a single plant producing over 10,000 seeds.

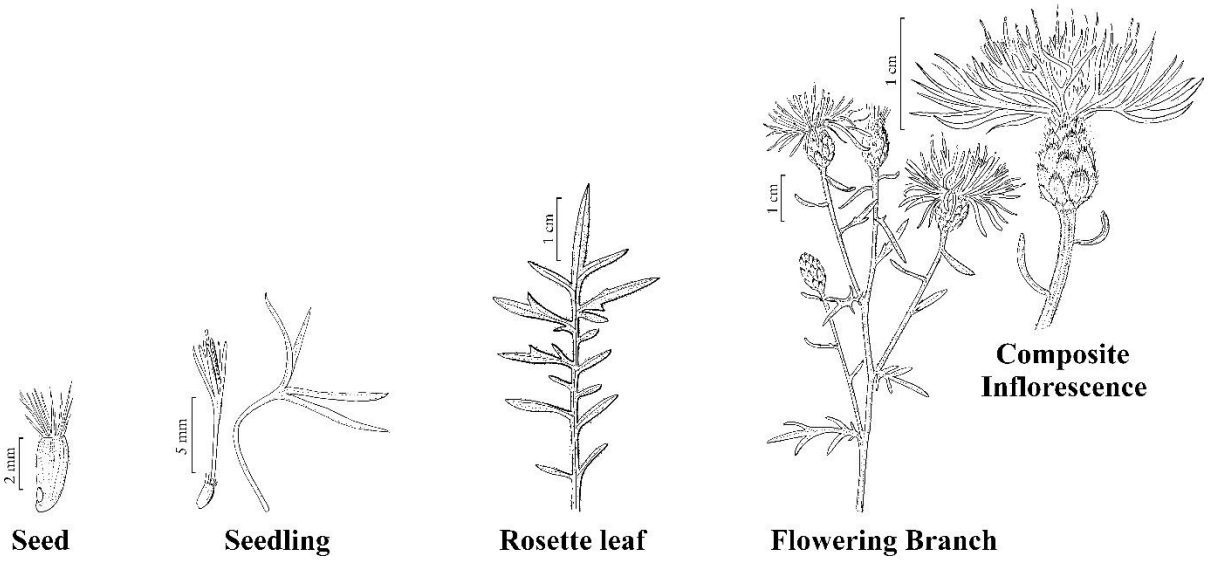

Figure 1.1: Aboveground development of *Centaurea stoebe* from seed (left) to an adult flowering plant (right). In Keil & Ochsmann (1993), courtesy of the Flora of North America Administration, illustrated by John Myers.

Belowground, *C. stoebe* has a deep taproot which presents another challenge for restoration and management of this weed (MacDonald et al., 2013). The lateral root system is moderately sized in relation to the taproot and forms larger mats in older individuals, making it more difficult to remove. On top of that, the root system as a whole has the ability to negatively influence surface runoff, soil erosion, plant communities, and ecosystem processes compared to pristine areas (Lacey et al., 1989). *C. stoebe*, when established, will drain soils of available nutrients and water faster than native plants. This induces a cascade where *C. stoebe* outcompetes native plants, reduces biodiversity, and increases the amount of runoff and soil erosion that a given area receives. Furthermore, this plant is suspected to release catechin into the soil, a phytotoxic chemical that inhibits the growth of nearby plants (Bais et al., 2003; Reinhart & Rinella, 2011; Ridenour & Callaway, 2001). To sum up, *C.*

stoebe produces numerous seeds which remain viable for years and can outcompete other plants for resources and space.

Currently, there are a few methods in use to eradicate *C. stoebe* in North America. One method is physical removal by hand pulling (Jacobs, 2012; Sheley et al., 1998). This method works well in certain scenarios if a few conditions are met: 1) when the soil is wet as this allows for the entire plant with taproot to be removed, 2) individuals are immediately placed in sealed plastic bags to prevent the spread of seeds or pollen during transportation, and 3) the site is revisited in later years as multiple hand removal events are required. This method is often avoided due to the reliance on suitable weather and the need for repeated visits. Thus, biocontrol agents and herbicide treatments have been attempted for controlling this weed. In BC, there are at least 12 biocontrol insects that have been used to target *C. stoebe* (Gayton & Miller, 2012; Invasive Species Council of British Columbia, 2014; Province of British Columbia, 2002). Some of these insects will feed on the root system of the knapweed, which can disrupt the belowground functioning, and structural integrity of the plant itself (e.g., *Cyphocleonus achates*, a wood boring weevil). Other biocontrol insects forage the seeds and seed heads of the knapweed plant which ultimately decreases the level of spread and invasion (ex: *Larinus minutus*, a seed-feeding beetle). It is also possible to reduce *C. stoebe* biomass through grazing with sheep and goats, as well as cattle though it is less palatable to them (DiTomaso, 2000; Olson et al., 1997). Targeted grazing on invasive plants has had some positive effects in the ranching industry (Voth, 2010). Although grazing by livestock is a form of biological control, it may not be feasible to graze all invaded areas due to accessibility and terrain (Olson et al., 1997).

In addition to mechanical or grazing controls, chemical controls have shown to reduce the spread of *C. stoebe*. Herbicide use is the most common form of control of *C. stoebe*, though some precautions should be considered: 1) picloram is the most effective active ingredient in herbicide treatments against both *C. stoebe* and *C. diffusa*, but should avoid being used on coarse soils since it can negatively impact neighboring broadleaf herbs, and 2) it is best to apply herbicide treatments to the rosette stage of a plants development (Mackay, 2008). The broad use of herbicides may bring forth challenges into the future with regards to chemical resistance. This has been first documented in 2016 in an invaded range in

East Kootenay, BC. This range had been historically treated with various herbicides to deal with the spread of *C. stoebe*, and when individuals from this range were grown in a greenhouse alongside plants from a historically untreated range, applications of picloram and clopyralid did not have as pronounced of a response in plants from treated areas as opposed to plants from untreated areas (Mangin & Hall, 2016). The implication of resistance demonstrates that further action is required to mitigate the invasive action of *C. stoebe* and perhaps long-term management methods should be developed to limit the impact of *C. stoebe* on the ecosystems it invades.

Restoration efforts associated with the spread of *C. stoebe* are mostly tailored towards revegetation of an affected area after treatment, usually with a native seed mix (Jacobs, 2017). Obviously, there is support for this method because it re-establishes past communities, increases biodiversity, and improves soil conditions and ecosystem health. However, this ignores the existing seed bank as well as the physical and chemical makeup of the affected soils. While combinations of mechanical, biocontrol, and chemical treatments can address the seed bank issue, these treatments do not address changes of soil structure and composition. Specifically, the accumulation of phytotoxins in invaded soils can leave a long term or legacy effect that persists into the future, inhibiting reseeding efforts (Del Fabbro & Prati, 2015; Grove, 2014; Grove et al., 2012). Such legacy effects have yet to be studied in detail, and it is unknown with *C. stoebe* how important these effects are or how to effectively treat them. A better understanding of these legacy effects is thus necessary for land managers to strategize restoration plans.

ROUGH FESCUE

Grasslands in BC can be divided based on elevation, plant communities, and climate patterns. From valley bottoms to about 600 m above sea level, lower grassland communities are generally dominated by drought resistant shrubs of big sagebrush (*Artemisia tridentata*) and groups of bluebunch wheatgrass (*Pseudoroegneria spicata*) (Lee et al., 2014; van Ryswyk et al., 1966). These regions of the grasslands are typically hotter and drier than other grassland regions. As a result, moisture is limiting and can lead to large gaps of bare ground

between individual plants. A transition zone between lower and upper grassland communities occurs between 600-900 m above sea level. These middle grasslands receive more moisture than in the lower grasslands causing a reduction of *A. tridentata*, but other dryland plants common to the lower grasslands, including *P. spicata*, remain present in this zone. Upper grasslands tend to occur near Interior Douglas Fir forests between 900-1200 m above sea level. There are smaller gaps between individual plants at this level and thus a higher total cover of vegetation. Mild temperatures and increased precipitation ultimately allows for greater plant diversity compared to lower elevation grasslands as well (Wikeem & Wikeem, 2004). The upper grassland communities are commonly dominated by rough fescue (*Festuca campestris* Rydb.), a cool season, native, perennial bunchgrass (Fleenor, 2011). Biological crusts and a heavy litter layer assist in moisture retention in these grasslands which assist in the development of rich soils; however, these favorable growing conditions also allow for invasive plant encroachment and subsequent ecosystem damage (Delesalle et al., 2009; Fraser & Carlyle, 2011).

Festuca campestris forms large bunches from the older leaves which aid in moisture retention. It forms a fibrous root system but does not penetrate deep soils. Leaves are generally 10-60 cm long, 1-2 mm wide, and rough on the lower surface (Fleenor, 2011). Stems stand 30-90 cm tall without nodes and, when mature, the inflorescence is paniculate measuring 5-18 cm in length with 2 branches per node (Figure 1.2). Spikelets are 8-12 mm long housing between 3-7 florets and are purplish (Douglas et al., 2001). The glumes of the spikelet are shorter than the floret, and the lemmas, which are either sharply pointed or awned, measure between 7-8.5 mm long. *F. campestris* reproduces primarily via vegetative regeneration or seed production, though the amount of seed produced can vary by year (Hook et al., 1994; Johnston & MacDonald, 1967). This presents further opportunity for invasive plants to establish in years that fewer seeds are produced simply by germinant number. Fortunately, germination rates are relatively high for this species (86-97%), thus in habitable areas not affected by invasive plants *F. campestris* can successfully establish and become forage for herbivores (Fleenor, 2011; Johnston & MacDonald, 1967).

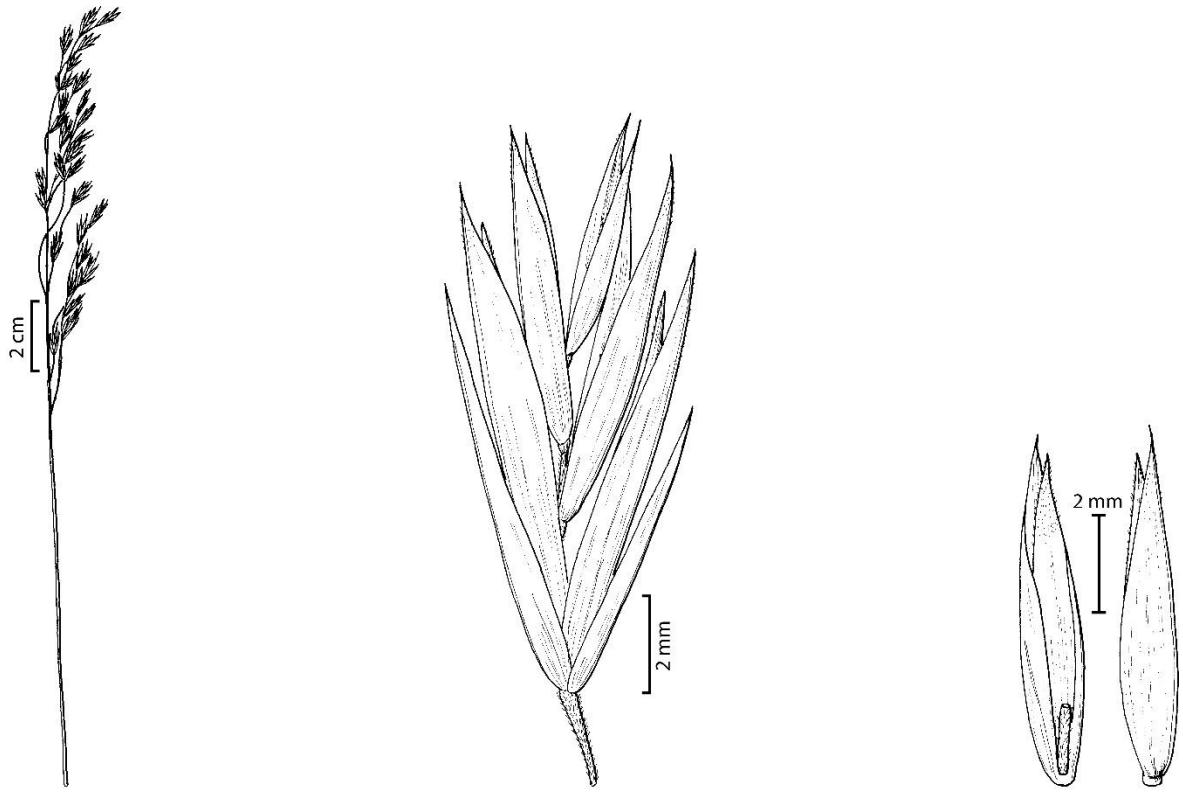

Figure 1.2: Flowering structures of *Festuca campestris* with approximate measurements. In Darbyshire & Pavlickf (1993), courtesy of the Flora of North America Administration, copyright Utah State University, illustrated by Cindy Roché.

Festuca campestris is often preferred by grazing livestock (Fleenor, 2011). Incidentally, it has high protein content which is retained even when hayed for winter grazing, thus it has a high forage value (Alberta Prairie Conservation Forum, 2017). In turn, livestock improve grassland conditions by providing a fertilizer (nitrogen) through their waste which can continue to be useful even after the year it was applied (Forge et al., 2005; Schröder et al., 2007). Despite this reciprocal relationship, research in Alberta has shown that over-stocking a rangeland can have negative consequences including decreases in *F. campestris* cover, increases in less valuable forage, and deterioration of the grasslands (Willms et al., 1985). Overgrazing may also be a precursor to plant invasion as well, therefore it is imperative that ranchers manage their livestock effectively to avoid these impacts and ensure more productive grasslands in future growing seasons for their livestock (Mack et al., 2000). With such high importance in ecosystem function and within the

ranching industry, rough fescue is an ideal and important plant to study alongside the invasive, noxious spotted knapweed.

ACTIVATED CARBON

Activated carbon (AC) is a fine, porous material known for its binding properties and adsorption abilities (Bansal & Goyal, 2005; Chiang et al., 2001; Hameed et al., 2007). More technically, AC contains spaces enclosed by carbon atoms in between graphene layers, and during an activation process AC will develop adsorption sites which will vary in size and shape (Marsh & Rodríguez-Reinoso, 2006c). Activation of carbon molecules can happen through thermal, physical, or chemical means, thus AC is always a synthetic product (Marsh & Rodríguez-Reinoso, 2006a). The final product has an incredibly large surface area per unit volume due to the atomic porosity of the material. AC has a wide variety of uses. Industrially, it has uses in water purification, air pollution reduction, removal of dyes from wastewater, and methane storage, all accomplished by adsorption processes (Marsh & Rodríguez-Reinoso, 2006b; Pereira et al., 2003; Rodríguez-Reinoso, 2002). AC also has its use as a consumer product where it is commonly used as an aquarium water purifier. These adsorptive properties have spawned research on the potential removal of allelopathic compounds from invasive plants using AC as a soil amendment (Nilsson, 1994; Prati & Bossdorf, 2004; Ridenour & Callaway, 2001). Catechin, the suspected allelopathic compound of *C. stoebe*, is also readily adsorbed by AC when added to soils (Callaway & Aschehoug, 2000). The use of AC thus is important in studies of allelopathy; however, its use in studies of soil legacy effects are unclear. For instance, Grove et al. (2012) studied the effects that the invasive *Cytisus scoparius* (allelopathic shrub) had on Douglas-fir in the western US. They found that *Cytisus* invaded soils negatively affected the growth of Douglas-fir in comparison to soils from the nearby non-invaded forest. The invaded soils continued to have a negative effect on Douglas-fir growth in the presence of either AC or *Cytisus* litter alone, but the combination of AC and *Cytisus* litter had a positive effect on Douglas-fir growth possibly caused by a fertilizing effect of the litter. These results suggested that hand pulling an invasive plant was not enough to encourage native plant

growth in the area; rather, a soil amendment was required to overcome the soil legacy effects of *Cytisus scoparius*. A separate study has shown that soil legacy effects can influence native plant growth in an opposite manner as well. Del Fabbro and Prati (2015) examined the role of soil legacy in comparison to immediate allelopathy on native plants in pairwise monocultures and mixtures with invasive plants. They did not find evidence that invasive soil legacies affect the growth of native plants in monocultures, but in competitive mixtures with invasive plants the results varied: the addition of AC positively affected the growth of native plants, while no soil amendment (i.e.; the invaded control soil condition) negatively affected native plants. These findings are contradictory to those by Grove et al. (2012), but still illustrate that AC can play a role in invasion ecology and that its role may vary from one invasive species or community to the next. Results like this require further investigation using other invasive and native plants from other areas of the world.

If AC can be an effective tool for restoring native plant communities impacted by aggressive invasive plants, then it is important that we find solutions that are economically feasible and environmentally beneficial. At the time of writing, laboratory grade AC from Fisher Scientific was available at a price of $1493 for a total of 2,100 g of the product (Fisher Scientific, 2021). For laboratory experiments, this may suffice but in terms of field studies, it is important we find less expensive alternatives. Pulp mill fly ash, a free industrial waste generated by the pulping process, contains some amount of carbon in the activated form and should thus behave similarly to laboratory grade activated carbon with regards to adsorptive properties (Ahmaruzzaman, 2010; Wang & Wu, 2006). There have not been many studies conducted using pulp mill fly ash for adsorbing biochemical products; however, bagasse fly ash, the waste product from sugarcane mills, has been used extensively in research due to its ability to adsorb various chemical and biochemical compounds (Mall et al., 2005; Rafatullah et al., 2010; Srivastava et al., 2006). The pulping processes that yield bagasse fly ash and pulp fly ash are very similar when Kraft pulping is involved (Biermann, 1996; Rainey & Covey, 2016), therefore I proposed the use of pulp fly ash from Domtar Pulp Mill in Kamloops, BC, to be used as a soil amendment for treating the biochemical release of phytotoxins by *C. stoebe*. The adsorption effectiveness of fly ash was compared alongside laboratory grade AC purchased from Fisher Scientific.

FLY ASH

Fly ash in this experiment was sourced from the pulping process occurring at Domtar Pulp Mill in Kamloops, British Columbia. Starting from wood chips, this mill produces northern bleached softwood kraft (NBSK) paper products, cellulose fibres, and other specialty pulp grades (Domtar Corporation, 2017). One of the by-products of the pulping process is fly ash, which is generally stored on site or relocated to a landfill (Ahmaruzzaman, 2010; Pöykiö et al., 2004). Alternative uses have been found for this waste material including being used in asphalt surfacing (Naik et al., 1994), removal of various toxic compounds and waste materials from wastewater (Burgess et al., 2009; Rafatullah et al., 2010; Wang & Wu, 2006), and as a fertilizing soil amendment (Basu et al., 2009; Bi et al., 2009; Pöykiö et al., 2004). For the current study, it will be used as a soil amendment with the goal of adsorbing inhibitory biochemicals left behind by *C. stoebe*. We may find that it acts as a fertilizing agent and positively affects growth, though this would be a secondary effect of the material.

It is important to note that the application rate of fly ash can have varying effects on the soil chemistry and plant growth. Lower application rates have been associated with greater seed germination and may also stimulate sugar production in plant tissues, whereas higher rates may inhibit plant growth and sugar production (Singh et al., 1994, 1997). The addition of fly ash may directly or indirectly affect soil microbial activity which could be the reason for the varying growth effects of fly ash application rate (Gupta et al., 2002). Nitrogen fixing bacteria can convert nitrogen gas and organic nitrogen to ammonium which is then used by nitrifying bacteria (Fowler et al., 2013). In the absence of plants, nitrifying bacteria have the opportunity to convert ammonium to nitrate, which is then used by denitrifying bacteria to convert it back to nitrogen gas. Nitrate may also be assimilated in plant roots to be used by plants during growth; however, there is a point where plants can no longer absorb any more nitrate. At this point, denitrifying bacteria would convert the excess nitrate to nitrogen gas. When fly ash is applied, a small amount of organic nitrogen is also added to the soil which would stimulate the production of nitrate via the nitrification process, resulting in the greater germination. Higher application rates of fly ash may cause denitrifying bacteria to

rapidly reproduce, thereby causing the negative effects towards plants since they would be converting abundant soil nitrate to nitrogen gas. Low application rates of fly ash appear to have greater benefits for the resulting plants (Gupta et al., 2002; Singh et al., 1994, 1997). This should be a strong consideration for usage in research.

CURRENT STUDY

The soil legacy effects of *Centaurea stoebe* on *Festuca campestris* was characterized in a controlled greenhouse environment. *Festuca campestris* is commonly found throughout the upper grasslands in BC and has a clear negative response to invasive weed presence (Kuang, 2015). Field collected soils were amended either with fly ash from the local Domtar Pulp Mill in Kamloops, BC, or Fisher brand AC, or not amended at all. The growth of *C. stoebe* and *F. campestris* was compared under these soil treatments and the following questions were asked:

1. Are soil legacy effects from *C. stoebe* dominated sites impacting the establishment and growth of rough fescue seedlings? And
2. Can activated carbon and pulp mill fly ash be used as mitigating factors on potential allelopathic chemicals residual in soils collected from spotted knapweed-dominated sites?

The results of the first question will allow native grassland restoration efforts following spotted knapweed removal to become more detailed and focussed. Results from the second question will be used to determine the feasibility of adding fly ash to the restoration tool kit for spotted knapweed affected areas. Additionally, a field study was conducted to determine the applicability of these amendments in a field setting. The application of fly ash would provide further use to this waste product and would be relatively inexpensive for restoration purposes.

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Chapter 2: Examining soil legacy effects of *Centaurea stoebe* in the context of the Lac Du Bois Grasslands Protected Area

INTRODUCTION

Invasive plants can exploit new areas through its reproductive biology, a lack of competitors or predators in the new area, or disturbance in the area they will inhabit (Mack et al., 2000). *Centaurea stoebe* is a good example of an invasive plant, with its ability to rapidly produce thousands of viable, small seeds that readily spread to new areas, root, develop into new adult plants, reproduce and begin the process anew (Jacobs, 2012; Jacobs & Sheley, 1998). Furthermore, it is suspected that *C. stoebe* can release catechin into the soils, a phytotoxin thought to be responsible for its invasive success (May & Baldwin, 2011; Perry et al., 2007; Pollock et al., 2011). There is debate in the literature as to whether catechin release occurs and whether catechin has an effect on native plant communities (Bais et al., 2003; Blair et al., 2006; Callaway & Ridenour, 2004; Duke et al., 2009; Stermitz et al., 2009). While it is difficult to confirm whether *C. stoebe* actively releases catechin into the soils, it deserves a review of the research that has been done in the past that assesses catechin and, more specifically, the mode in which catechin inhibits native plant growth. Finally, a discussion on soil legacy effects is necessary to establish the grounds for this study and to determine whether this plant does exhibit these traits.

Immediate Soil Effects and Catechin

Since arriving in North America in the late 1800s, *C. stoebe* has made its way across most of the Canadian provinces and American states making it a destructive invasive weed. Efforts to study its soil invasion properties have been met with mixed results. In 2003, Bais et al. founded the “novel weapons hypothesis”, which states that an invaders success is due to that invader possessing a “novel weapon”, or biochemical, against the native species it is infringing upon (Bais et al., 2003; Callaway & Ridenour, 2004). The researchers developed this hypothesis in an experiment using *C. stoebe* as their model invasive species, where they provided evidence of *C. stoebe* releasing catechin through its roots into the soils. When a

susceptible species encounters it, it triggers root death via the generation of reactive oxygen species at the target species' root meristem (Bais et al., 2003). It is thought that *C. stoebe* may be successful due to this release of catechin into the soils. Since this discovery, several studies have attempted to replicate this design but have shown contradictory results (Blair et al., 2005, 2006; Perry et al., 2007). In fact, this was addressed in 2010 when a correction for the Bais et al. paper (2003) was published stating that only one other report (see: Perry et al., 2007) found catechin in invaded soils at similar concentrations ("Corrections and Clarifications," 2010). Other labs that have attempted to replicate their design have not been able to detect catechin in such high concentrations, or at all (Blair et al., 2005, 2006; Tharayil et al., 2008).

The debates regarding the nature of catechin with regards to *C. stoebe* and its soils continue, yet there are still vast amounts of evidence that through some means *C. stoebe* is negatively impacting native plant communities (Gayton & Miller, 2012; May & Baldwin, 2011; Story, 1976; Story et al., 2008). Perhaps, the prevailing ecological conditions are all that is driving the competitive nature of *C. stoebe*. It has been suggested that animal herbivory can account for the loss of native species in areas adjacent to shrubs due to preferential grazing (Bartholomew, 1970). Indeed, *C. stoebe* is naturally unpalatable to grazing mammals and requires biological control or targeted grazing efforts for herbivory to occur (Story, 1976; Watson & Renney, 1974). Another explanation for *C. stoebe*'s competitive advantage argues that the changing soil conditions following exotic plant invasion lead to a reduction in native plant biodiversity. A study examining *C. stoebe* patch size found that the larger an invaded area is, the less available N and C are within the soils (Fraser & Carlyle, 2011). These elements are crucial to the growth and establishment of most plants and when they are less available, the resulting grassland will appear less productive and healthy. The lack of vegetation on the landscape can also influence other soil properties as well. Fraser and Carlyle (2011) found this when comparing knapweed communities to native grassland communities: soil temperature, total phosphate, and total potassium within the knapweed invaded soils increased while water content, litter biomass, species richness and diversity decreased. It is plausible to assume that the removal of a large knapweed patch

would not result in the plant community returning to a pre-invaded state due to the altered properties incurred from invasion.

Soil Legacy Effects

In certain cases where an invasive plant has established a large colony and existed for an extended period of time, alterations to the soil may have occurred which make long-term restoration plans more difficult to achieve (D'Antonio & Meyerson, 2002). One alteration may be the addition of invaded seed to the seed bank over time compared to recently invaded, or non-invaded areas. Holmes and Cowling (1997b, 1997a) studied this and found that species richness, cover, and frequency of standing vegetation were inversely correlated with timing of invasion by *Acacia saligna* (Labill) Wendl. They found a similar pattern when it came to native seedling recruitment after clearing the area of the invasive *Acacia* (i.e.: less seedlings established in areas that were invaded for a longer period of time compared to areas that were invaded for shorter time periods and non-invaded areas). Ultimately, they provided evidence of persistence of an invasive plant even after its removal from an area. Other work has shown evidence of altered soil chemistry persisting after plant invasion. Maron and Jefferies (2001) describe the invasive bush lupine (*Lupinus arboreus*) as a N-fixing shrub that enriches the soil with N, and an increase in N has been shown to decrease diversity in grasslands (Foster & Gross, 1998). The experimental removal of bush lupine allowed other plants to establish, including early successional perennial forbs that were both native and exotic. These forbs retain plant-available N in their roots and very slowly uptake N from the soils, whereas later successional species will uptake soil N more quickly. With high amounts of soil N, the shift back to a fully native plant community is slowed or even halted due to the lingering presence of early successional forbs, even after a 5-year study period. In this way, bush lupine is still affecting the resulting ecosystem in its absence. Conversely, other invasive plants can deteriorate soils by leaving behind a nutrient deficit. Using N again as an example, soils in the patches of *C. stoebe* have exhibited consistently less total N than their native soil counterparts, which influences future restoration strategies and plant communities (Fraser & Carlyle, 2011; Kuang, 2015). In all these examples, invasive plants are continuing

to negatively affect plant communities that they no longer inhabit, leaving behind soil legacy effects which make long-term restoration more difficult to achieve.

Few studies have evaluated the hypothesis that soil legacy effects are, in fact, present in recently invaded areas. One novel study uses a comparative approach to estimate immediate allelopathy and soil legacy effects of eleven different invasive species on two native species (Del Fabbro & Prati, 2015). Specifically, soil was collected from eleven sites where an invasive species was found, and at sites adjacent to the edge of invasion (within 2-5 m). Both soil types were split in half: one half received an AC treatment to neutralize allelochemicals, whereas the other half did not receive this treatment. Then, pairwise treatments were set up in each soil type. Each native species was grown by themselves, the invasive species was grown by itself, and then there were competition trials as well for a total of 5 treatments in 4 different soil types for each invasive plant. Their results suggested that native plant performance was determined by both immediate allelopathy and soil legacy effects of an invasive plant, and that the physical removal of an invasive plant should trigger the restoration of a native plant. The study did not use invasive plant members from the genus *Centaurea*; however, this study provides a great model on which to base a similar study evaluating the soil legacy effects of *C. stoebe* in a greenhouse setting.

Thus, in a similar manner to Del Fabbro and Prati's work (2015), I performed a comparative study growing *Festuca campestris* and *Centaurea stoebe* in six different soil treatments in a controlled greenhouse environment. The role of *C. stoebe*'s soil legacy effects on *F. campestris* was studied, and the role of ash as a tool for restoration was explored. The following research questions were addressed throughout this study:

1. Are lingering soil legacies from *Centaurea stoebe*-invaded soils affecting the germination, survival, mortality, and biomass of the native grass, *Festuca campestris*? And,
2. Can pulp mill ash be used to treat *Centaurea stoebe*-invaded soils and foster better growth of *Festuca campestris*?

Additionally, plant invasion in the Lac Du Bois Grasslands Protected Area is compared to B.C. using publicly available datasets and freely open-sourced geographic information system (GIS) tools.

METHODS

Site Selection

Prior to the greenhouse study, sites needed to be located for soil collection and, to ensure that soils came from "invaded" or "pristine" areas. Plant surveys needed to be conducted to objectively classify these areas. The area under investigation resides in the upper elevation grasslands of Lac Du Bois Grasslands Protected Area (GPA), north of Kamloops, B.C. This park has been a focal point for much research in the past due to the interesting ecological gradient that appears with subtle changes in elevation. The Lac Du Bois GPA spans an elevation gradient from approximately 400-1200 m a.s.l., and encompasses an area approximately 15,000 ha (Delesalle et al., 2009). Lower elevations are dominated by dryland shrubs and patches of bluebunch wheatgrass amidst the bare ground and tend to occur in the bunchgrass biogeoclimatic zone where there are higher temperatures and lower amounts of precipitation. These lower grasslands occur up to approximately 600 m a.s.l. and due to the climatic restrictions results in lower overall vegetative cover. Middle grasslands are generally found between 600-900 m a.s.l. and are also dominated by bluebunch wheatgrass, though the occurrence of dryland shrubs decreases significantly. There is a greater amount of vegetation cover in these grasslands compared to lower grasslands, and there is also a significant presence of spotted knapweed. Upper grasslands span an elevation gradient of approximately 900-1200 m a.s.l. and are dominated by fescues and needlegrasses. Depending on the location, upper grasslands may be parts of the Interior Douglas Fir, or Ponderosa Pine biogeoclimatic zones where there are cooler temperatures and higher precipitation compared to lower grasslands in the bunchgrass zone. Additionally, the middle and upper grassland areas contain the invasive plant under study, *C. stoebe* (Figure 2.1). It occurs in varying patch sizes, with larger negative effects having been noted in larger patch sizes (Fraser & Carlyle, 2011). A pair of sites from the upper grasslands were

used in this project: an area heavily invaded by *C. stoebe* for at least a decade, and a nearby area within 20 m of the edge of invasion that is free of spotted knapweed. The actual invaded area was delineated by walking along the edge of invasion using a Garmin GPS (model GPSMAP 76CSx) and pristine sites were selected outside of the delineated area. Within the invaded area, 10 sites were selected for plant surveys and soil collection. Invaded sites were required to have > 80% *C. stoebe* cover for plant survey and soil collection purposes. Ten pristine sites were then randomly selected within a 20 m buffer of the edge of invasion (Appendix Table B.1). Proximity of invaded and pristine areas was important to ensure that soil type and climatic measurements were similar between them. The point data for each sampling site and the tracklog for the invaded area was imported into Garmin MapSource (v. 6.16.3) and exported as a shapefile for use in R for Statistical Computing (R Core Team, 2020). Permission to use the area for research purposes was granted by the BC Ministry of Environment under Park Use Permit No. 102724.

Soil temperature monitors (Thermochrons, iButtonLink Technology) were inserted into soils at two locations within both invaded and pristine areas on June 12, 2017 and collected on August 30, 2017. These temperature monitors recorded the soil surface temperature every 2 hours while they were in the field, on every even hour (i.e.: 12:00 AM, 2:00 AM, 4:00 AM, etc.). This was recorded to further distinguish invaded and pristine sites. In July 2017, plant surveys were carried out at each of the 20 sites to quantify the total top-down percent cover and richness of all species in invaded and pristine areas (Appendix Table B.3). To do this, 1 m × 1 m quadrat surveys were performed in each of the 20 sites and the top-down percent cover of each species was estimated to nearest 1%. The specific location for invaded quadrats within the invaded site were randomly chosen based on the cover of *C. stoebe* when a quadrat was placed down: if there was 80% or greater top-down cover of *C. stoebe*, the area within the quadrat was surveyed. Similarly, pristine areas were randomly chosen within 20 m of the main knapweed patch in order to keep environmental variables as constant as possible. GPS coordinates were recorded at each of the sample locations as well as at each of the locations where soil temperature monitors were inserted using a cell phone's map application (Google Maps, Android version 9.57.1) (Appendix Table B.2).

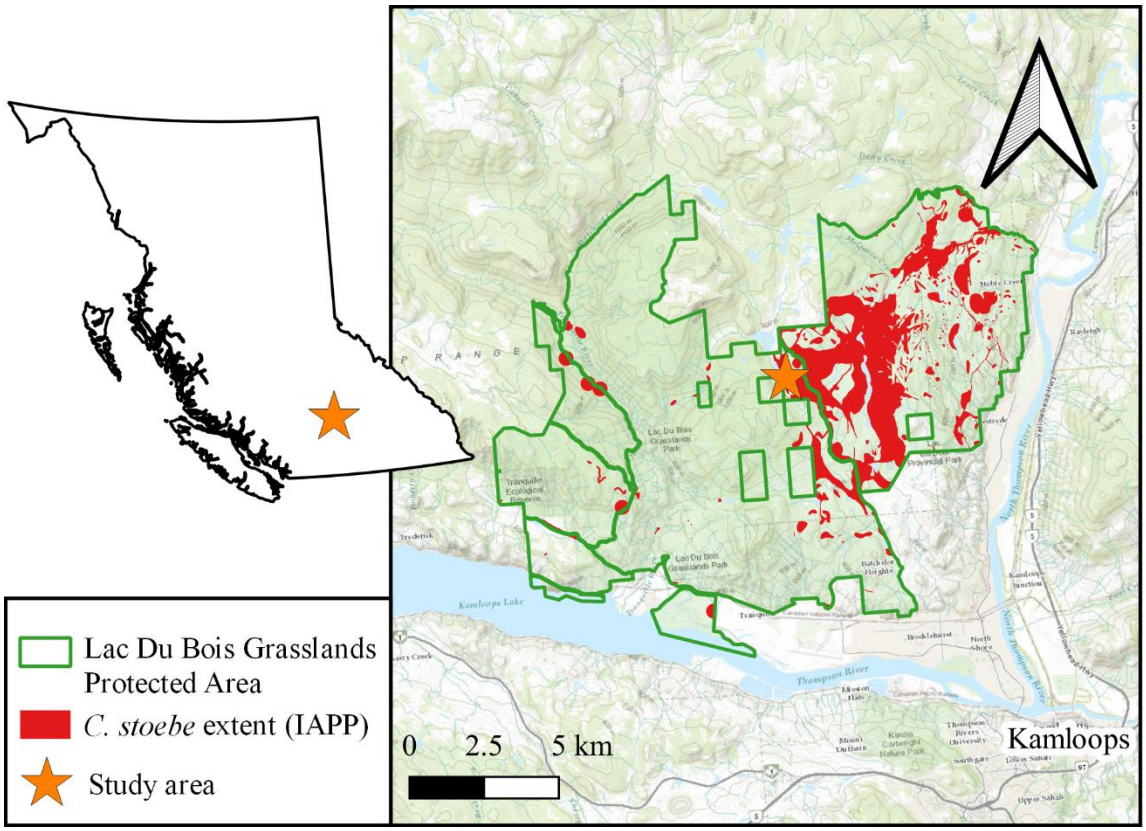

Figure 2.1: Provincial context of the Lac Du Bois Grasslands Protected Area and the associated *C. stoebe* extent of invasion throughout the park. Further spatial context is given for the location of the study area within the Lac Du Bois Grasslands Protected Area. The map was created using QGIS version 3.16.0 (Hannover).

GIS Considerations

Recently, a new R package was released that allowed for simple downloading of spatial data from the BC Data Catalogue called "bcdata" (Teucher et al., 2020). There is a separate package, "bcmaps", that contains some commonly used spatial layers including biogeoclimatic ecosystem classification (BEC) information (Teucher et al., 2021). These packages were used to download the following spatial layers:

- BC Parks, Ecological Reserves, and Protected Areas, for the boundary of the Lac Du Bois GPA (Erlandson & MacPhail, 2020);

- Invasive Alien Plant Site, for information on invasive plant sites throughout BC (Miller & Osborne, 2020); and
- Biogeoclimatic Ecosystem Classification (BEC) Map, for information at the subzone level (Salkeld et al., 2020).

Additionally, the B.C. Ministry of Forests, Lands, Natural Resource Operations and Rural Development have made a gridded digital elevation model (DEM) of the entire province publicly available at an approximately 25 m^2 resolution (2020). This data is made available through the Terrain Resource Information Management (TRIM) base map collection through individual tiles. The appropriate tiles for the Lac Du Bois GPA were downloaded to gather information about the subzones within it. ClimateBC was used to generate seasonal temperature raster images of the Lac Du Bois GPA to compare summer mean temperatures throughout the area (Wang et al., 2016). A smaller clip of the study area was also used to compare the elevation, slope, and aspect between invaded and pristine sites using parametric T-tests. Slope and aspect layers were generated using the "terrain" function in the "terra" package, utilizing bilinear interpolation of those values at each site (Hijmans, 2021).

Using R for Statistical Computing (R Core Team, 2020), the Lac Du Bois GPA was extracted from the BC Parks layer. This extraction served as a clipping extent for the Invasive Alien Plant Site and BEC Map layers. The Invasive Alien Plant Site layer was then filtered to retrieve all records of *C. stoebe* within the Lac Du Bois GPA.

Spatial analyses included computations of the area of all invasive species within the Lac Du Bois GPA compared to the area covered by *C. stoebe* only, and *C. stoebe* extent by BEC subzones to determine potential trends of its location. Additional province wide analyses were completed to compare the Lac Du Bois GPA to trends across the province. All spatial calculations were performed using the "terra" and "sf" packages in R (Hijmans, 2021; Pebesma, 2018).

Soil, Ash, and AC Collection and Characterization

Soil collection took place between October 6-9, 2017 at the same locations that plant surveys took place earlier in the season. In other words, invaded soils came from areas that are approximately 80-100% covered by *C. stoebe*, and pristine soils came from pristine areas free of *C. stoebe* within 20 m of the edge of invasion. The soils in this study area are classified as orthic black chernozems (Canadian Soil Information Service, 2013; Filatow, 2019). The collected soil was used to grow the plants from seed. In order to eliminate the wild seed bank and mitigate the chance of other seeds sprouting the top 5 cm was scraped away and discarded in both invaded and pristine soils (Bonis & Lepart, 1994). Sanitized trowels were used to collect soil to a depth of approximately 20 cm, the depth at which spotted knapweed produces most of its roots, and a total of approximately 200 L of each invaded and pristine soils were gathered (i.e.: approximately 20 L from each site) (Story et al., 2000). Soils were transported from the field sites directly to the Thompson Rivers University Research Greenhouse in 4 L Ziplock freezer bags. Once the soils arrived in the greenhouse, the bags were opened and allowed to air dry for a minimum of 30 days. After this period, soil was sieved (1 cm mesh size) to remove stones and roots, and sieved soils were collected in 189 L Rubbermaid Jumbo Storage tote bins (1 bin for invaded soil, and 1 bin for pristine soil). The drying and sieving steps here were based on the soil preparation methods presented by Del Fabbro and Prati (2015). All collected soils from invaded sites were thoroughly mixed inside this bin using a sanitized trenching shovel and motorized auger with a sanitized 10 cm × 80 cm bit attached. These same methods were applied separately to pristine soils. The mixing step ensures that soils are homogenous for each soil type, thus when plants are potted, they will be using the same soils throughout. Approximately 1 L of each soil type was collected at this stage to be sent to the BC Government's Analytical Laboratory in Victoria, BC for chemical analysis of major elements. Additionally, approximately 1 L of soil was set aside for CNH analysis using a Thermo Scientific FlashSmart CHNS/CHNS Elemental Analyzer unit at the TRU campus.

Ash was obtained from Domtar Pulp Mill in Kamloops, BC on November 27, 2017. The bulk ash was transported from the mill in large 5-gallon (~20 L) buckets, and then combined into a single 102 L tote bin. The bulk ash was stored until it was required for

preparing soils for the greenhouse trials. Again, approximately 1 L was set aside to be sent for chemical analysis. CHN data was obtained from a concurrent students thesis (Antonelli, 2018).

Activated carbon was purchased from Fisher Scientific in September 2017. According to its certificate of analysis, the exact substance was activated coconut charcoal. Having arrived in a granulated form, it was ground to a finer size for homogenization later using a cyclone mill (UDY Corporation, model no. 3010-030, mesh size 0.4 mm). Finely ground AC was collected and stored in the airtight jars they were shipped in until they were ready for mixing. Approximately 100 mL of AC was set aside to be sent for chemical analysis. CHN data was gathered from multiple literature sources characterizing activated coconut charcoal and averaged (Astuti et al., 2021; Hidayu & Muda, 2016; Iqbaldin et al., 2013; Phan et al., 2006).

Soil Preparation and Greenhouse Trials

Both bulk pristine and bulk invaded soil types required separation and soil amendments added. In order to create the 1% ash and 1% AC treatments, 49.5 L of the bulk soils was removed and placed into smaller 102 L tote bins. This was done using 6 L and 500 mL Erlenmeyer flasks to accurately measure soils and amendments. Once 49.5 L of bulk pristine or invaded soil was placed in a sanitized tote bin, 500 mL of either ash or AC was added to create the 1% amendments. Each soil type was further mixed with a sanitized trenching shovel and motorized auger to ensure that the amendment was spread equally throughout the entire soil volume. This resulted in 6 soil treatment combinations:

1. Invaded soil (control)
2. Invaded soil + Ash
3. Invaded soil + AC
4. Pristine soil (control)
5. Pristine soil + Ash
6. Pristine soil + AC

Either *C. stoebe* or *F. campestris* was grown in each soil treatment separately for a total of 12 treatments: thus a 2 × 3 factorial design with 2 soil types (pristine and knapweed-affected), and 3 soil treatments (control, AC and ash), repeated for 2 plant species. These treatments were replicated 10 times and potted into 8 cm × 8 cm × 8 cm (i.e.: 512 mL) square growing pots (Greenhouse Megastore, model SVT-400). To prevent soil from spilling through the drainage holes, measured and cut weed barrier was inserted into the base of each pot. Enough soil was added to each pot so that the surface of the soil was approximately 2 cm from the top of the pot. Prior to planting any seeds, the soils were moistened with distilled water. The pots were arranged in the research greenhouse using a randomized block design, and 5 seeds of a species were planted in each pot (one in each corner, and one in the middle). Individual seeds of *C. stoebe* were hand collected from the invaded field site on September 7, 2017 and were used in this experiment, whereas individual seeds of *F. campestris* needed to be sourced from Splitrock Environmental out of Lillooet, B.C. Purchased *F. campestris* seeds were found to have a much higher germination rate than field collected seeds (~80% for purchased vs. ~15% for field collected), hence their use in this experiment. Pots were then watered every 2-3 days using distilled water. Greenhouse conditions were kept constant for the duration of the experiment and were controlled using Argus Controls software (Table 2.1). Individuals were grown for a period of 90 days, after which the number of successful individuals were counted, height measurements were taken, and aboveground biomass was clipped and placed in paper bags. Belowground biomass (i.e.: roots) was collected through washing the soil off of the roots and was also placed in paper bags. All biomass collections were dried in a drying oven at 70℃ for 72 hours, then weighed on a Fisher Scientific Accu-Series 4102 model scale measuring to the nearest hundredth of a gram.

Based on existing literature, the expectations of the growth response of each species is outlined and explained (Table 2.2), and through this experiment we aim to address two major concerns (Del Fabbro & Prati, 2015). First, the determination of whether soil legacy effects exist in these soils is of major import. This was accomplished by comparing the biomass of rough fescue in invaded and pristine soils. Comparing the biomass of spotted knapweed in each soil should indicate if soil legacies from knapweed invasion improve, inhibit, or have no effect on its growth. Second, we can determine whether ash may be used

as an alternative soil amendment to AC in spotted knapweed-invaded sites. The growth of *F. campestris* will be compared in ash and AC treated soils to answer whether ash can be added to the knapweed restoration toolkit. Comparing the growth of spotted knapweed in those soils should provide valuable information as to the effects of ash on spotted knapweed, and if it is indeed an appropriate soil amendment for knapweed infested sites.

Table 2.1: Environmental variables and values pertaining to optimum growth of many plants. These conditions were replicated in the research greenhouse for the growth experiment. Retrieved from Hendry & Grime (1993).

	Day	**Night**
Photoperiod	08:00-22:00 (Growing lights on)	22:00-08:00 (Growing lights off)
Temperature	22°C	15°C
Humidity	40% R.H.	40% R.H.

Table 2.2: Expectations of the impacts to the growth of *Centaurea stoebe* and *Festuca campestris* in each soil type based on existing literature.

Soil type	**Species**	**Expected impacts to growth**
Invaded (control)	***C. stoebe***	None: *C. stoebe* is growing in soils it came from
Invaded + Ash	***C. stoebe***	Increase in growth due to ash acting as a fertilizer
Invaded + AC	***C. stoebe***	None: AC should not inhibit invasive plant growth
Pristine (control)	***C. stoebe***	Slight increase from invaded (control) soils due to higher nutrient content
Pristine + Ash	***C. stoebe***	Increase in growth compared to pristine (control) due to ash acting as a fertilizer
Pristine + AC	***C. stoebe***	None: AC should not inhibit invasive plant growth
Invaded (control)	***F. campestris***	Decrease in growth compared to pristine (control) due to *C. stoebe* soil legacy
Invaded + Ash	***F. campestris***	None: Soil legacy effects mitigated by ash treatment
Invaded + AC	***F. campestris***	None: Soil legacy effects mitigated by AC treatment
Pristine (control)	***F. campestris***	None: *F. campestris* is growing in native soils
Pristine + Ash	***F. campestris***	Increase in growth due to ash acting as a fertilizer
Pristine + AC	***F. campestris***	None: AC should not inhibit native plant growth

Elemental Analysis

Soils from the homogenized bulk collections were analyzed for total carbon, nitrogen, hydrogen, and sulphur using a Thermo Scientific FlashSmart CHNS/CHNS elemental analyzer. Specifically, sieved soils were dried in a Yamato drying oven (model DKN812) at 80°C for approximately 12 hours to remove any potential moisture. Next, approximately 10-15 mg of soil were weighed and placed in small tin capsules and loaded sequentially into the elemental analyzer sample wheel. This was repeated for each soil type five times for a total of 30 soil samples. Values generated from the analysis were in percentage values of the total sample. Additionally, homogenized soil samples were sent to the B.C. Ministry of Environment analytical laboratory in Victoria, B.C., for further elemental analysis of the major elements, including aluminum, boron, calcium, copper, iron, potassium, magnesium, manganese, molybdenum, sodium, phosphorus, sulfur, and zinc. These elements were detected using inductively coupled plasma optical emission spectrometers (ICP-OES) after preparation using microwave acid digestion. Resulting values were returned in either mg/kg, or total percentage depending on the element in question.

Data Analysis

FIELD DATA

Plant cover data from invaded and pristine sites was compared using Shannon and Simpson diversity indices, along with a comparison of overall species richness using parametric T-tests. Species richness was further explored by splitting the species into two separate types: invasive species, and native species. Native species richness data was made normal by a square root transformation for use in a T-test, but invasive species richness required the use of a non-parametric Wilcoxon rank sum test. Invasive species were identified using the most up to date invasive species list published from the Invasive Alien Plant Program (IAPP) (Ministry of Forests and Range - Range Branch, 2020). This program acts as a publicly accessible repository housing invasive plant information, including location

and control efforts. The IAPP is managed by the B.C. Ministry of Environment, but the tools to report weeds are not restricted to government users.

Litter cover was compared between sites using a parametric T-test. Bare ground cover was compared using a Wilcoxon rank sum test as raw and transformed data violated tests of normality. Raw surface temperatures were compared between sites using a Wilcoxon rank sum test. Elevation, slope, and aspect were all compared using parametric T-tests. Those results are tabulated separately to focus on the terrain component from those sites.

GREENHOUSE DATA

Biomass

Two-factor analyses of variance (ANOVA) were performed separately for each *C. stoebe* and *F. campestris* to compare the effect of 2 soil types (invaded soils and pristine soils) and 3 soil amendments (control, activated carbon, and fly ash) on total biomass. Assumptions of this test include normality of the input data, as well as homogeneity of variances. The raw data violated normality assumptions for both species, so a square root transformation was applied thereby fitting a normal distribution and passing a homogeneity of variance test. By performing the same transformation to both species, the results and effects of soil type and treatment were comparable. An analysis of the main effects of soil types and soil treatments was then conducted for both species, and pairwise comparisons were made between the pairings of the soil types and soil treatments. A Tukey Honest Significant Difference (HSD) test was finally used to assess the interactions of soil type and soil treatments.

Borrowing from Del Fabbro and Prati (2015), a continuous index of soil legacy was measured using the raw biomass data. Essentially, it is expected that if *C. stoebe* exhibits legacy effects, the biomass of *F. campestris* should increase more in AC soil with legacy effects than without legacy effects. The index was calculated as:

Allelopathic legacy in monocultures =
(biomass in invaded soil without AC − biomass in invaded soil with AC) −
(biomass in native soil without AC − biomass in native soil with AC)

It should be noted that this is a strict measure of the strength of the soil legacy effects exhibited by *C. stoebe*, and the measurement is conducted ignoring trials using ash. Additionally, measures of immediate allelopathy are not able to be calculated since this requires a competitive experiment which was omitted from this study. Thus, the values given will only be a vague indication of the direction of any legacy effects. That is, any positive result indicates a positive legacy effect, whereas negative results indicate a negative legacy effect. All statistical analyses and figures were produced using R for Statistical Computing (R Core Team, 2020).

Plant Survival

Various metrics were used to assess the survival of individuals through the duration of the greenhouse trials. These included germination rate, death rate, survival rate of the germinated seeds, and overall trial survival rate (Table 2.3). A similar application of the two-factor ANOVA was used to assess differences in these metrics between soil types and treatments. Normality was violated in all metrics. Using a square root transformation on the germination rate values validated the normality and homogeneity of variance assumptions of the two-factor ANOVA. In order to properly use the other metrics in a two-factor ANOVA, an aligned rank transformation was applied to the data in order to fit a normal distribution (Wobbrock et al., 2011). All statistical analyses and figures were produced using R for Statistical Computing (R Core Team, 2020).

Table 2.3: Description of plant survival metrics to be applied to each individual pot. In this case, the number of seeds planted equates to 5.

Metric	Description	Equation
Germination Rate	Ratio of seeds that germinated after 30 days	$\frac{\#\text{ Seeds Germinated}}{\#\text{ Seeds Planted}}$
Death Rate	Ratio of seedlings that died between 30 and 90 days	$\frac{\left(\#\text{ Survived Individuals} - \#\text{ Seeds Germinated}\right)}{\#\text{ Seeds Germinated}}$
Germination Survival Rate	Ratio of seedlings that lived between 30 and 90 days	$\frac{\#\text{ Survived Individuals}}{\#\text{ Seeds Germinated}}$
Trial Survival Rate	Ratio of seedlings that lived to the end of the experiment compared to the number of planted seeds	$\frac{\#\text{ Survived Individuals}}{\#\text{ Seeds Planted}}$

Elemental Analysis

Soil carbon, nitrogen, and hydrogen results from each of the soil treatments were compared using a parametric two-way ANOVA for each element, in which assumptions of normality and homogeneity of variance were satisfied. Results from the major elemental analysis were tabulated. A carbon-to-nitrogen ratio (C:N) was calculated from the C and N data, respectively. Normality was violated on the C:N data and was unable to be transformed to fit a normal distribution, thus an aligned rank transformation was applied to validate the assumptions of a two-way ANOVA (Wobbrock et al., 2011).

RESULTS

GIS Considerations

The Lac Du Bois GPA encompasses an approximately 159.37 km^2 area and is broken down into six subzones and spanning an elevation gradient of approximately 334 – 1410 m above sea level (Table 2.4). Each of the subzones are classified either as “dry” or “very dry”,

and most of the subzones are located on warmer aspect slopes. The range of summer temperatures also appear to show warmer temperatures at lower elevation subzones.

Table 2.4: Biogeoclimatic Subzones of the Lac Du Bois Grasslands Protected Area. Detailed for each subzone are descriptions of the subzone codes, the area in km^2 that the subzone takes up in the Lac Du Bois GPA, and associated elevation descriptions gathered from TRIM.

BGC Subzone	Description	Area (km^2)	Elevation Range (m)	Elevation Mean (m)	Summer Temperature Range (°C)	Summer Temperature Mean (°C)
BGxh2	Bunchgrass: Thompson Very Dry Hot	21.76	334.0 – 710.1	481.8	11.6 – 29.8	20.7
BGxw1	Bunchgrass: Nicola Very Dry Warm	43.83	408.9 – 912.8	731.3	10.2 – 28.9	19.0
IDFdk1	Interior Douglas Fir: Thompson Dry Cool	4.33	842.2 – 1312.7	1117.7	8.9 – 25.8	16.8
IDFdk2	Interior Douglas Fir: Cascade Dry Cool	10.14	870.3 – 1410.5	1218.7	8.2 – 25.4	16.1
IDFxh2	Interior Douglas Fir: Thompson Very Dry Hot	50.85	522.7 – 1301.4	924.7	8.9 – 28.3	17.8
PPxh2	Ponderosa Pine: Thompson Very Dry Hot	28.46	365.8 – 1024.1	703.3	9.8 – 29.3	19.3

The IAPP found 795 total invasive plant records throughout the entire Lac Du Bois GPA spanning 26.30 km^2 (~16.5%). These records show that 32 of the 229 invasive species listed by the IPCC have been recorded throughout the area. *C. stoebe* was identified in 356

records and covered an approximate area of 21.99 km^2 (~13.8%) within the Lac Du Bois GPA. As literature suggested, a majority of the sites invaded by *C. stoebe* were located in higher elevation, cooler grasslands and forest interfaces (Table 2.5). This confirms that the location for the study area (the IDFxh2) was appropriate. The observed invaded area at this study site was 11,659 m^2, and this area was within the bounds of an identified *C. stoebe* patch by the IAPP. While the declared pristine sites appear to fall within an invaded area identified by the IAPP, ground observations at the time of sampling showed that *C. stoebe* was absent at those sites.

Table 2.5: Details of plant invasion in the Lac Du Bois Grasslands Protected Area broken down by BGC subzone. "Total" values indicate the total number of records, total physical area, and invaded ratio of all invasive plants combined throughout the Lac Du Bois GPA in order to highlight the same metrics for *C. stoebe* only. Numbers of records sum to be greater than the total number of records throughout the Lac Du Bois GPA due to splitting of polygons during spatial intersections.

BGC Subzone	Total IAPP records	Total Invaded area (km^2)	Total Invaded ratio[1]	*C. stoebe* IAPP records	*C. stoebe* Invaded area (km^2)	*C. stoebe* Invaded ratio[1]
BGxh2	43	1.27	5.83%	18	0.39	1.78%
BGxw1	514	18.03	41.15%	230	15.53	35.43%
IDFdk1	6	0.010	0.24%	1	0.006	0.15%
IDFdk2	0	0	0%	0	0	0%
IDFxh2	236	5.96	11.71%	120	5.37	10.57%
PPxh2	52	1.03	3.63%	27	0.69	2.44%

It is important to note that a given polygon representing an invaded area may encompass multiple invaded species, thus while *C. stoebe* occupies 35.43% of the entire BGxw1 subzone it should not be interpreted that all other invasive species within the subzone occupy the remaining 5.72% of total invaded space. For example, *C. diffusa*, the next most invasive plant in the Lac Du Bois GPA, occupies 29.47% of the same subzone due to its close relation to *C. stoebe*. Indeed, *C. stoebe* is the most invasive plant throughout the Lac

[1] Invaded ratio is calculated as the invaded area divided by total physical area of the subzone.

Du Bois GPA by total area and by subzone, except in the BGxh2 subzone where *C. diffusa* is the most invasive plant.

Throughout B.C., there were 153,799 total invasive species recordings made through the IAPP and 35,593 of those contained records for the presence of *C. stoebe*. Invasive species make up a total of 1,369.76 km^2 in B.C. (0.144%), and by itself *C. stoebe* covers a total area of 521.81 km^2 (0.055%). Comparatively speaking, this is greater than the city area of Winnipeg, Manitoba (~464.33 km^2).

An exploratory analysis of the same BGC subzones as the Lac Du Bois GPA indicated that these subzones are among the most invaded of those types throughout the province (Table 2.6). For example, the BGxh2 subzone is a comparatively smaller subzone throughout the province being only the 171st largest; however, it is 4th most invaded subzone when accounting for all invasive species throughout the province and contains the greatest amount of *C. stoebe* cover compared to all other subzones. Comparing the invaded ratio of the subzones within the province to within the Lac Du Bois GPA, we start to notice that the larger subzones in the park are more heavily invaded compared to the province wide results. For instance, the BGxw1 subzone accounts for 43.83 km^2 within Lac Du Bois and was found to be 35.43% covered by *C. stoebe*; however, across the province, *C. stoebe* invades only 5.76% of that entire subzone (Table 2.7). The same trend holds true for the IDFxh2 subzone, but the opposite is true for the BGxh2, IDFdk2, and PPxh2 subzones where smaller proportions are invaded in the Lac Du Bois GPA compared to provincially reported proportions.

Table 2.6: Province wide BGC details, only including the BGC subzones that were also within the Lac Du Bois Grasslands Protected Area. Rank values refer to the entire list of 210 subzones with a rank of 1 being the highest rank.

BGC Subzone	Total Subzone area (km^2)	Provincial Proportion of area	Subzone Area Rank	Overall Invasive Rank	*C. stoebe* Invasive Rank
BGxh2	678.98	0.072%	171	4	1
BGxw1	684.02	0.072%	169	5	2
IDFdk1	5394.87	0.569%	39	18	11
IDFdk2	2440.03	0.257%	89	13	7
IDFxh2	4423.58	0.467%	54	11	4
PPxh2	1364.74	0.144%	126	6	3

Table 2.7: Details of plant invasion throughout B.C. broken down by BGC subzone. "Total" values indicate the total number of records, total physical area, and invaded ratio of all invasive plants combined throughout B.C. in order to highlight the same metrics for *C. stoebe* only.

BGC Subzone	Total IAPP records	Total Invaded area (km^2)	Total Invaded ratio	*C. stoebe* IAPP records	*C. stoebe* Invaded area (km^2)	*C. stoebe* Invaded ratio
BGxh2	1932	53.73	7.91%	1046	40.17	5.91%
BGxw1	1745	45.68	6.68%	1067	39.41	5.76%
IDFdk1	4297	78.99	1.46%	2185	53.33	0.989%
IDFdk2	2127	67.01	2.75%	850	35.35	1.45%
IDFxh2	7711	167.69	3.79%	4685	140.28	3.17%
PPxh2	2950	85.39	6.26%	1570	69.04	5.06%

Field Collections

The results of multiple T-test and Wilcoxon rank sum tests are tabulated (Table 2.8). An independent samples T-test did not show any statistical difference in overall species richness between invaded and pristine sites; however, a trend was noted. When species richness was broken down between native and invasive plant types, more clear results emerged: a Wilcox rank sum test showed that pristine sites had significantly lower invasive species richness than invaded sites. An independent samples T-test comparing the native

species richness between sites showed significantly higher native species richness in pristine sites than in invaded sites. An independent samples T-test comparing the litter cover between sites showed significantly higher cover in pristine sites than in invasive sites. Conversely, a Wilcoxon rank sum test showed significantly lower bare ground cover in pristine sites compared to invaded sites. T-tests on both Shannon-Wiener and Simpson diversity metrics showed that pristine sites were significantly more diverse than invaded sites. Finally, a Wilcoxon rank sum test showed significantly lower temperatures in pristine sites compared to invaded sites (Figure 2.2).

Table 2.8: Summarized comparisons of field metrics between pristine and invaded sites, and the respective T-test or Wilcox rank sum test results. Asterisks on P-values indicate significant differences at the 0.05 level for that metric between pristine and invaded sites.

	Mean ± *SE*			
Metric	**Pristine**	**Invaded**	**Statistic**	***p***
Overall Species Richness	7.8 ± 0.83	5.8 ± 0.57	T = -1.99	0.064
Invasive Richness	0.2 ± 0.13	1.5 ± 0.17	W = 98	**<0.001***
Native Richness	7.6 ± 0.85	4.3 ± 0.52	T = -3.70	**0.005***
Ground Litter Cover (%)	40.9 ± 4.69	16.3 ± 2.75	T = -4.52	**<0.001***
Bare Ground Cover (%)	0.7 ± 0.52	11.3 ± 1.92	W = 99	**<0.001***
Shannon-Weiner Diversity (H)	1.275 ± 0.14	0.569 ± 0.08	T = -4.54	**<0.001***
Simpson Diversity (D)	0.585 ± 0.06	0.244 ± 0.03	T = -5.25	**0.008***
Average Daily Ground Temperature (°C)	17.77 ± 0.20	20.86 ± 0.18	W = 322083	**<0.001***

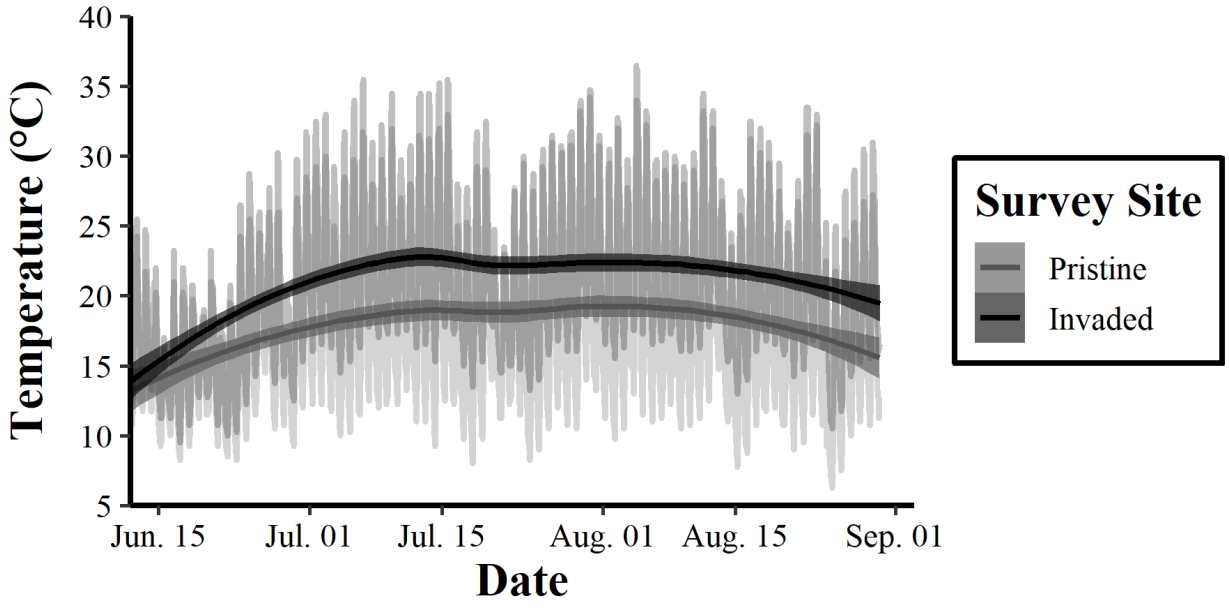

Figure 2.2: Ground temperature from invaded (red) and pristine (black) sites in the Lac Du Bois GPA from June 12 - August 30, 2017. Extended vertical bars represent actual ground temperature readings at 2-hour intervals, and the smoothed lines represent mean daily ground temperatures and a standard error at invaded and pristine sites.

Comparisons of elevation, slope, and aspect between invaded and pristine sites are tabulated (Table 2.9). Independent samples T-tests revealed no significant differences in elevation (T = 0.005, p = 0.996), slope (T = 0.465, p = 0.647), or aspect (T = – 0.893, p = 0.384) between sites.

Table 2.9: Summarized comparisons of elevation, slope, and aspect between pristine and invaded sites, and their associated T-test results. N = 10 for each site.

	Mean ± *SE*			
Variable	**Pristine**	**Invaded**	**T**	***p***
Elevation (m)	918.55 ± 1.01	918.56 ± 0.30	0.005	0.996
Slope (radians)	0.071 ± 0.009	0.077 ± 0.008	0.465	0.647
Aspect (radians)	4.23 ± 0.22	4.01 ± 0.11	– 0.893	0.384

Greenhouse Trials

Since *F. campestris* and *C. stoebe* were grown separately and not together in any competitive scenario, the interaction between their results was not explored; however, interactions between soil type and soil treatment were explored within each species.

BIOMASS

A two-way ANOVA was used to examine the effects of soil type and soil treatment on biomass. A square root transformation of the biomass data was applied in order to pass the Shapiro-Wilks normality test ($p_{(RF)} = 0.36$; $p_{(SK)} = 0.14$) and Levene's test of homogeneity of variances ($p_{(RF)} = 0.49$; $p_{(SK)} = 0.61$) for both species. For both *C. stoebe* and *F. campestris*, a significant effect of the soil treatment was found (RF: $F = 24.553$, $p = < 0.001$; SK: $F = 34.195$, $p < 0.001$; Table 2.10). There was no significant effect of soil type found (RF: $F = 1.734$, $p = 0.193$; SK: $F = 0.879$, $p = 0.353$). A slight but nonsignificant interaction of soil type and soil treatment was noted as well (RF: $F = 2.761$, $p = 0.072$; SK: $F = 2.583$, $p = 0.085$).

An analysis of the main effects of soil treatment was performed using one-way ANOVA's grouped by soil type. It was very apparent that the significant effect of soil treatment on biomass occurred in both invaded and pristine soil types for both *C. stoebe* and *F. campestris*, where the addition of AC caused a reduction in biomass (Figure 2.3). Analyses of variance were also carried out to explore the effect of soil type on biomass by grouping data by soil treatments. A significant effect of soil type was noted in control soils; all other soil treatments showed no effect of soil type on biomass (Table 2.11). Pairwise comparisons between soil treatments when grouped by soil type showed biomass in soils treated with AC was significantly lower than either a control or ash treated soils for both *F. campestris* and *C. stoebe*.

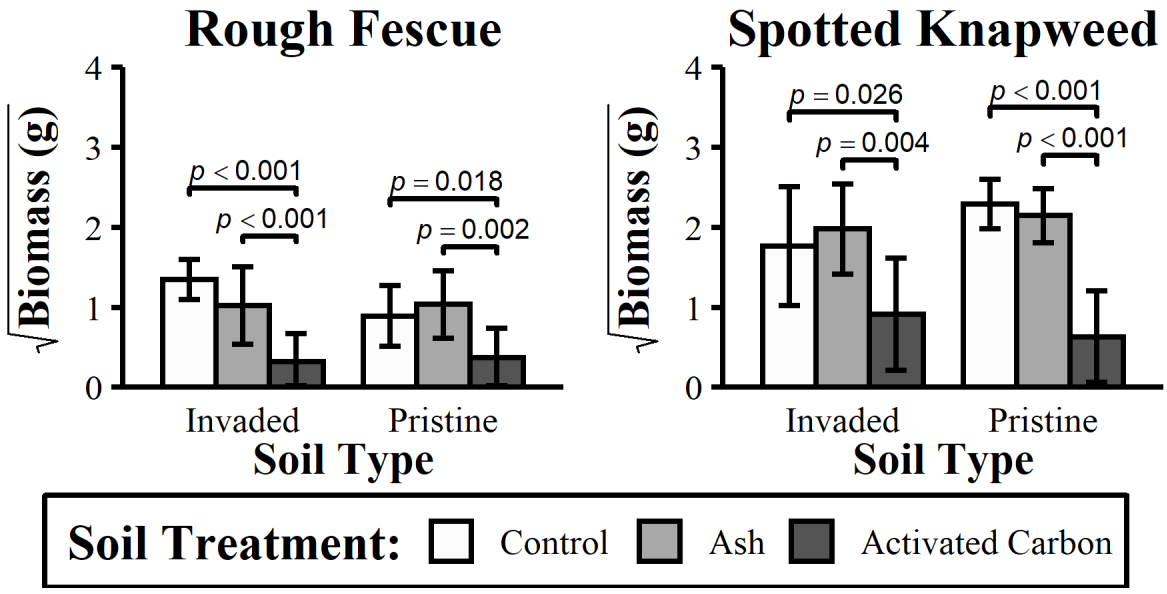

Figure 2.3: Square root of biomass (mean ± *SD*) of *Festuca campestris* (left) and *Centaurea stoebe* (right) when grown in different soil types and soil treatments. Comparisons of biomass are made between control, ash, and activated carbon soil treatments for each soil type. P-values above brackets denote significant pairwise comparisons between soil treatments as determined by pairwise t-tests.

Table 2.10: Results of a two-way ANOVA, performed to discover differences in the square root of plant biomass with respect to each factor and their interactions. F and p values are reported for both *Festuca campestris* and *Centaurea stoebe* as subscripts "RF" and "SK", respectively. Significant values are bold faced.

Effect	df_{effect}	df_{error}	F_{RF}	F_{SK}	p_{RF}	p_{SK}
Soil Type	1	54	1.734	0.879	0.193	0.353
Soil Treatment	2	54	24.553	34.195	**<0.001**	**<0.001**
Soil Type × Soil Treatment	2	54	2.761	2.583	0.072	0.085

Table 2.11: Exploration of the main effects of soil treatment and soil type on the square root of biomass for each species. F and p values are reported for both *Festuca campestris* and *Centaurea stoebe* as subscripts "RF" and "SK", respectively. Significant values are bold faced.

Soil Type	**Main Effect**	df_{effect}	df_{error}	F_{RF}	F_{SK}	p_{RF}	p_{SK}
Pristine	Soil Treatment	2	54	8.411	26.697	**<0.001**	**<0.001**
Invaded	Soil Treatment	2	54	18.903	10.082	**<0.001**	**<0.001**
Soil Treatment	**Main Effect**	df_{effect}	df_{error}	F_{RF}	F_{SK}	p_{RF}	p_{SK}
Control	Soil Type	1	54	7.157	4.359	**0.01**	**0.042**
Ash	Soil Type	1	54	0.009	0.432	0.925	0.514
AC	Soil Type	1	54	0.09	1.256	0.766	0.267

Interestingly, the index of allelopathic legacy was positive when *F. campestris* was grown in invaded soils (0.947). This result occurred due to greater biomasses in invaded soils compared to pristine soils. In comparison, the index was negative for *C. stoebe* when planted in invaded soils (–1.712), indicating that soil legacies from *C. stoebe* do not allow it to grow better in its own soils compared to when grown in pristine soils. This index further shows that *C. stoebe* performed best in pristine (newly invaded) soils, though is only intended to show relative trends of allelopathic legacy. Finally, observations of chlorosis were made in 40% of the *C. stoebe* pots during the growing period, but no significant trends were noted between treatments.

PLANT SURVIVAL

Germination Rate

The germination rate for this experiment was defined as the ratio of seeds that germinated after 30 days when 5 seeds were planted in a single pot. A two-way ANOVA was carried out to determine any effects of soil type and soil treatment on the germination rate. A square root transformation was applied to the germination rate data to pass the Shapiro-Wilks normality test ($p_{(RF)} = 0.144$; $p_{(SK)} = 0.149$) and Levene's test of homogeneity of variances ($p_{(RF)} = 0.223$; $p_{(SK)} = 0.082$). Soil type and soil treatment were found to significantly affect the germination of *F. campestris* on their own without any interacting effects (Table 2.12). The

germination rate of *C. stoebe* did not appear affected by either soil type or soil treatment by contrast.

An analysis of the main effects of soil treatment on germination rate showed that the germination rate of *F. campestris* varies significantly across soil treatments in both pristine and invaded soils (Table 2.13). Germination rate of *C. stoebe* was only significantly different across soil treatments in invaded soil types. Exploring the main effects of soil type on the germination rate, it is clear to see that the germination rates of both *F. campestris* and *C. stoebe* are significantly affected by soil type in ash treated soils only. In other soil treatments, this significant effect is absent. Pairwise comparisons between soil treatments grouped by soil type showed that the square root of *F. campestris*' germination rate is significantly lower when soils are treated with AC in both invaded and pristine soils (Figure 2.4). An ash treatment did not appear to have any significant effects on the germination rates of either species in either soil type when compared to a control.

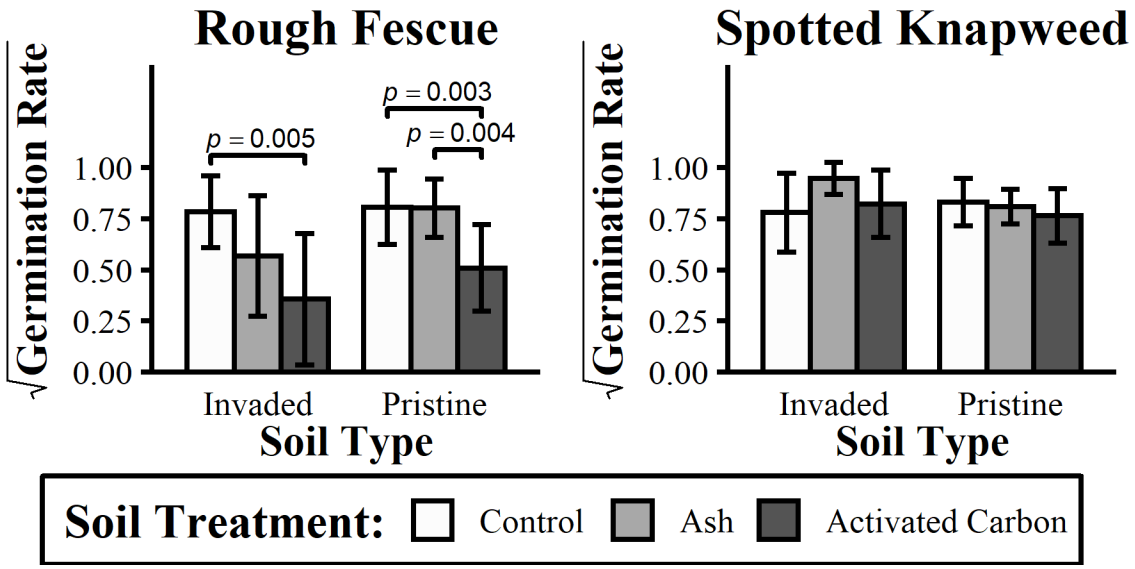

Figure 2.4: The square root of germination rates (means ± *SD*) of *Festuca campestris* and *Centaurea stoebe* when grown in different soil treatments and soil types. Comparisons of the germination rates are made between control, ash, and activated carbon treated soils for each soil type. P-values above brackets denote significant pairwise comparisons between soil treatments as determined by pairwise t-tests.

Table 2.12: Results of a two-way ANOVA exploring the effects of soil type and soil treatment on the square root of the germination rate. F and p values are reported for both *Festuca campestris* and *Centaurea stoebe* as subscripts "RF" and "SK", respectively. Significant values are bold faced.

Effect	df_{effect}	df_{error}	F_{RF}	F_{SK}	p_{RF}	p_{SK}
Soil Type	1	54	5.271	1.931	**0.026**	0.17
Soil Treatment	2	54	12.94	2.299	**<0.001**	0.11
Soil Type × Soil Treatment	2	54	1.048	2.464	0.358	0.095

Table 2.13: Exploration of the main effects of soil treatment and soil type on the square root of the germination rate of each species. F and p values are reported for both *Festuca campestris* and *Centaurea stoebe* as subscripts "RF" and "SK", respectively. Significant values are bold faced.

Soil Type	**Main Effect**	df_{effect}	df_{error}	F_{RF}	F_{SK}	p_{RF}	p_{SK}
Pristine	Soil Treatment	2	54	5.445	0.612	**0.007**	0.546
Invaded	Soil Treatment	2	54	8.542	4.152	**<0.001**	**0.021**

Soil Treatment	**Main Effect**	df_{effect}	df_{error}	F_{RF}	F_{SK}	p_{RF}	p_{SK}
Control	Soil Type	1	54	0.054	0.708	0.817	0.404
Ash	Soil Type	1	54	5.116	5.225	**0.028**	**0.026**
AC	Soil Type	1	54	2.196	0.926	0.144	0.34

Death Rate

The death rate for this experiment was defined as the ratio of seedlings that died between the 30- and 90-day mark of the greenhouse trial. A two-way ANOVA was used to determine the effects of soil type and soil treatment on the death rates of *F. campestris* and *C. stoebe*. Prior to analysis, the death rate data was not normal and could not be transformed to fit a normal distribution. To overcome this issue, an aligned rank transformation was used to force the statistical tests to run a two-way ANOVA on the non-parametric data (Wobbrock et al., 2011). Post-hoc analyses were then completed in a similar manner to the parametric two-way ANOVA post-hoc analyses. Soil type, soil treatment, and the interaction of both factors

all played a significant role on the death rate of *F. campestris* (Table 2.14). A significant effect of soil treatment on the death rate was found in *C. stoebe* individuals.

An analysis of the main effects of soil treatment on the death rate revealed that the death rate of both species is significantly affected in invaded soils but not pristine soils (Table 2.14). When analyzing the main effects of soil type among the soil treatments, a significant difference in the death rates of *F. campestris* was found in each soil treatment (Table 2.15). For *C. stoebe*, soil type only affected the death rates in control treated soils. Pairwise comparisons between soil treatments grouped by soil type showed that the death rate of *F. campestris* was significantly higher in AC treated soils in invaded soils types when compared to a control. The death rate of *C. stoebe* was also significantly higher in AC treated soils in invaded soil types when compared to either a control or ash treatments (Figure 2.5). The addition of ash did not appear to influence death rates compared to a control in any soil treatment for either species, though the death rates were always lower in ash treated soils when compared to AC treated soils.

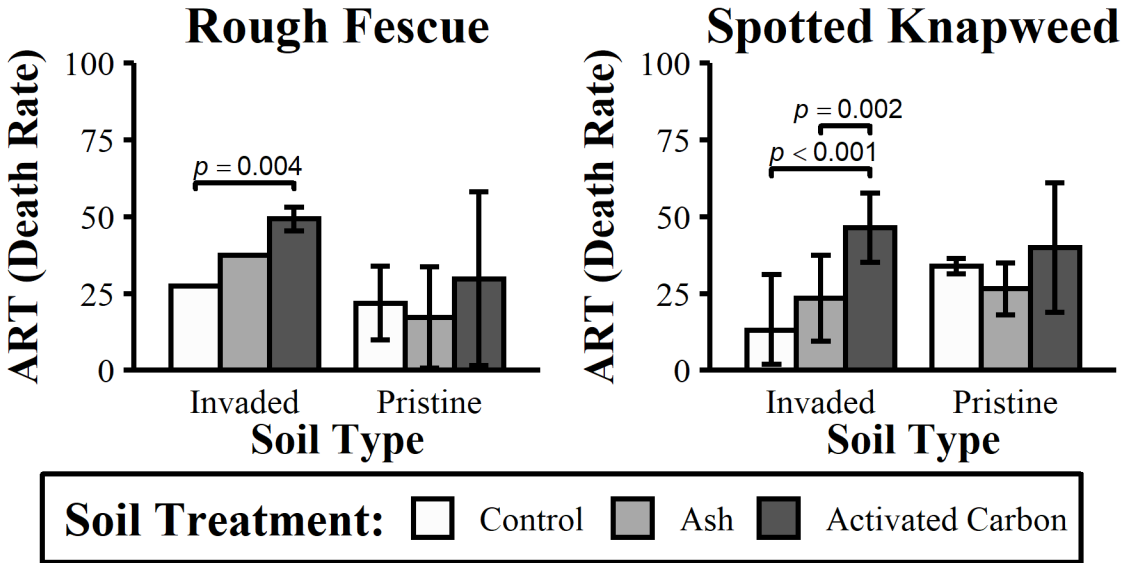

Figure 2.5: The aligned rank transformed death rates (means ± *SD*) of *Festuca campestris* and *Centaurea stoebe* when grown in different soil treatments and soil types. Comparisons of the death rates are made between control, ash, and activated carbon treated soils in each soil type. P-values above brackets denote significant pairwise comparisons between soil treatments as determined by pairwise t-tests.

Table 2.14: Results of a two-way ANOVA exploring the effects of soil type and soil treatment on the aligned rank transformation of the death rate. F and p values are reported for both *Festuca campestris* and *Centaurea stoebe* as subscripts "RF" and "SK", respectively. Significant values are bold faced.

Effect	df_{effect}	df_{error}	F_{RF}	F_{SK}	p_{RF}	p_{SK}
Soil Type	1	54	29.148	2.332	**<0.001**	0.133
Soil Treatment	2	54	6.117	12.328	**0.004**	**<0.001**
Soil Type × Soil Treatment	2	54	6.067	0.050	**0.004**	0.952

Table 2.15: Exploration of the main effects of soil treatment and soil type on the aligned rank transformation of the death rate of each species. F and p values are reported for both Festuca campestris and Centaurea stoebe as subscripts "RF" and "SK", respectively.

Soil Type	**Main Effect**	df_{effect}	df_{error}	F_{RF}	F_{SK}	p_{RF}	p_{SK}
Pristine	Soil Treatment	2	54	1.987	2.3	0.147	0.110
Invaded	Soil Treatment	2	54	5.766	14.928	**0.005**	**<0.001**

Soil Treatment	**Main Effect**	df_{effect}	df_{error}	F_{RF}	F_{SK}	p_{RF}	p_{SK}
Control	Soil Type	1	54	17.069	5.508	**<0.001**	**0.023**
Ash	Soil Type	1	54	8.552	0.297	**0.005**	0.588
AC	Soil Type	1	54	5.268	0.061	**0.026**	0.806

Germination Survival Rate

The germination survival rate was defined as the ratio of seedlings that lived between the 30- and 90-day mark during the greenhouse trial. Like the death rate data, the germination survival rate was not normal and could not be transformed to fit a normal distribution, so an aligned rank transformation was used to be able to calculate statistical measures for this data. Soil treatment appeared to have significant effects on the germination survival rate for both *F. campestris* and *C. stoebe* (RF: $F = 9.093$, $p < 0.001$; SK: $F = 13.098$, $p < 0.001$; Table 2.16). The different soil types and interacting effects of soil type and

treatment did not have any significant impact on the germination survival rate for either species.

An analysis of the main effects of soil treatment showed that the germination survival rate is significantly affected in both invaded and pristine soil types across all levels of soil treatment for both species (Table 2.17). Conversely, there was no significant main effects of soil type on the germination survival rate across any of the soil treatments for both species.

For *F. campestris*, pairwise comparisons of the germination survival rate between soil treatments when grouped by soil type revealed that invaded soils treated with ash had a significantly greater germination survival rate when compared to either control or AC treated soils (Figure 2.6). In pristine soils, the germination survival rate was significantly greater in control soils when compared to either ash or AC treated soils. For *C. stoebe*, the germination survival rate was significantly lower in AC treated soils in invaded soil types. An ash treatment in pristine soil types increased the germination survival rate of *C. stoebe* slightly compared to control soils, but was significantly greater than the rate when treated with AC.

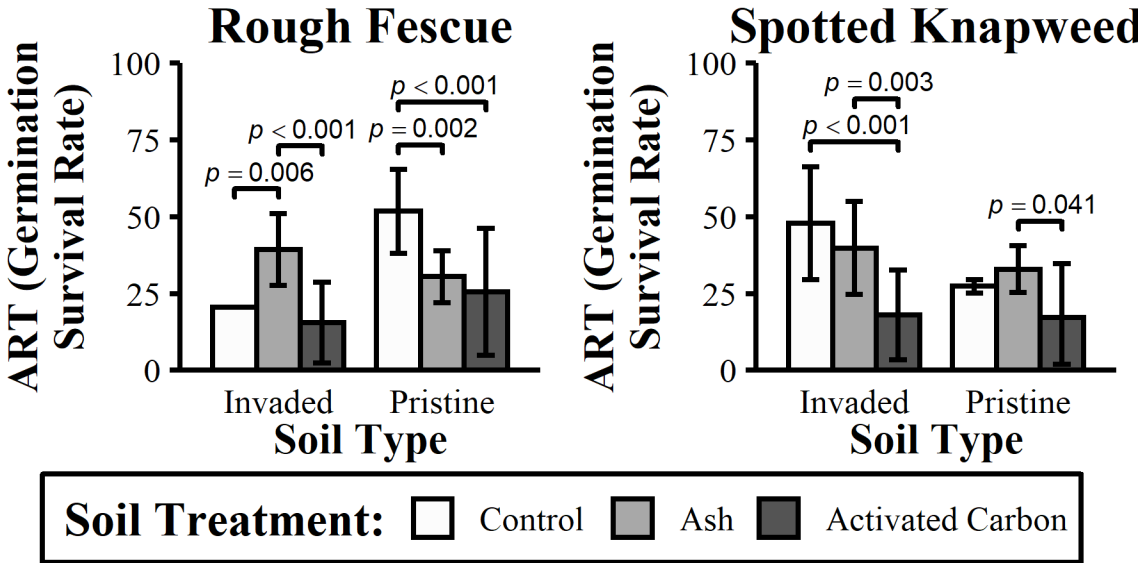

Figure 2.6: The aligned rank transformed germination survival rates (means ± *SD*) of *Festuca campestris* (left) and *Centaurea stoebe* (right) when grown in different soil types and soil treatments. Comparisons of the germination survival rates are made between control, ash, and activated carbon treated soils in each soil type. P-values above brackets denote significant pairwise comparisons between soil treatments as determined by pairwise t-tests.

Table 2.16: Results of a two-way ANOVA exploring the effects of soil type and soil treatment on the aligned rank transformation of the germination survival rate. F and p values are reported for both *Festuca campestris* and *Centaurea stoebe* as subscripts "RF" and "SK", respectively. Significant values are bold faced.

Effect	df_{effect}	df_{error}	F_{RF}	F_{SK}	p_{RF}	p_{SK}
Soil Type	1	54	0.019	0.537	0.890	0.467
Soil Treatment	2	54	9.093	13.098	**<0.001**	**<0.001**
Soil Type × Soil Treatment	2	54	0.179	0.377	0.836	0.688

Table 2.17: Exploration of the main effects of soil treatment and soil type on the aligned rank transformation of the germination survival rate of each species. F and p values are reported for both *Festuca campestris* and *Centaurea stoebe* as subscripts "RF" and "SK", respectively. Significant values are bold faced.

Soil Type	**Main Effect**	df_{effect}	df_{error}	F_{RF}	F_{SK}	p_{RF}	p_{SK}
Pristine	Soil Treatment	2	54	11.672	3.331	**<0.001**	**0.043**
Invaded	Soil Treatment	2	54	9.5	12.413	**<0.001**	**<0.001**

Soil Treatment	**Main Effect**	df_{effect}	df_{error}	F_{RF}	F_{SK}	p_{RF}	p_{SK}
Control	Soil Type	1	54	0.802	0.179	0.374	0.674
Ash	Soil Type	1	54	1.48	1.323	0.229	0.255
AC	Soil Type	1	54	0.006	0.294	0.936	0.590

Trial Survival Rate

The trial survival rate was defined as the ratio of seedlings that lived to the end of the experiment compared to the number of planted seeds in a pot (i.e.: 5). Again, the two-way ANOVA required an aligned ranks transformation to bypass the tests of normality and homogeneity of variances in order to be properly calculated. Significant differences in trial survival rate were found to be due to soil treatments for both *F. campestris* and *C. stoebe* (RF: $F = 23.237$, $p < 0.001$; SK: $F = 8.913$, $p < 0.001$; Table 2.18). Soil type and the interactions between soil type and soil treatment did not have any significant effects on the trial survival rate of either species.

An analysis of the main effects of the soil treatments showed significant effects of soil treatment on trial survival rate in both invaded and pristine soil types for both *F. campestris* and *C. stoebe* (Table 2.19). There were no main effects of soil type on the trial survival rate of either species under any soil treatment.

For *F. campestris*, pairwise comparisons between soil treatments of the trial survival rate revealed significantly lower rates in soils treated with AC compared to control and ash treated soils in both pristine and invaded soil types (Figure 2.7). The greatest trial survival rates were found in control soils, though this rate is not significantly greater when compared to an ash treatment. For *C. stoebe*, pairwise comparisons showed significantly lower trial survival rates in soils treated with AC when compared to the rates in ash treated soils for both soil types. Ash treated soils had the highest trial survival rates for *C. stoebe*, though not significantly greater compared to a control.

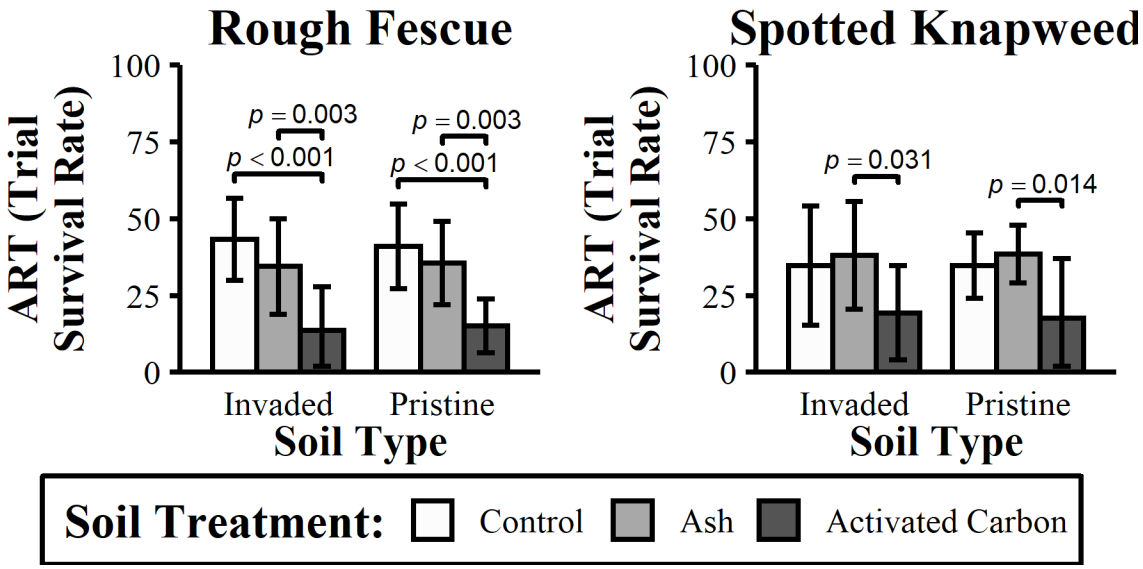

Figure 2.7: The aligned rank transformed trial survival rates (means ± *SD*) rates of *Festuca campestris* and *Centaurea stoebe* when grown in different soil treatments and soil types. Comparisons of the trial survival rates are made between control, ash, and activated carbon treated soils in each soil type. P-values above brackets denote significant pairwise comparisons between soil treatments as determined by pairwise t-tests.

Table 2.18: Results of a two-way ANOVA exploring the effects of soil type and soil treatment on the aligned rank transformation of the trial survival rate. F and p values are reported for both *Festuca campestris* and *Centaurea stoebe* as subscripts "RF" and "SK", respectively. Significant values are bold faced.

Effect	df_{effect}	df_{error}	F_{RF}	F_{SK}	p_{RF}	p_{SK}
Soil Type	1	54	1.046	1.112	0.311	0.296
Soil Treatment	2	54	23.237	8.913	**<0.001**	**<0.001**
Soil Type × Soil Treatment	2	54	1.259	1.990	0.292	0.147

Table 2.19: Exploration of the main effects of soil treatment and soil type on the aligned rank transformation of the trial survival rate of each species. F and p values are reported for both *Festuca campestris* and *Centaurea stoebe* as subscripts "RF" and "SK", respectively. Significant values are bold faced.

Soil Type	**Main Effect**	df_{effect}	df_{error}	F_{RF}	F_{SK}	p_{RF}	p_{SK}
Pristine	Soil Treatment	2	54	10.479	4.915	**<0.001**	**0.011**
Invaded	Soil Treatment	2	54	12.873	4.02	**<0.001**	**0.024**
Soil Treatment	**Main Effect**	df_{effect}	df_{error}	F_{RF}	F_{SK}	p_{RF}	p_{SK}
Control	Soil Type	1	54	0.09	0.031	0.766	0.862
Ash	Soil Type	1	54	0.807	0.214	0.373	0.645
AC	Soil Type	1	54	0.329	1.413	0.528	0.240

Elemental Analysis

The FlashSmart Elemental Analyzer returns concentrations for carbon, nitrogen, and hydrogen. Two-way ANOVA's were performed for each of elemental contents to determine the effects of soil type and soil treatment on the respective elemental concentrations as well as the ratio of carbon to nitrogen (C:N). Assumptions of a two-way ANOVA were validated using a Shapiro-Wilks test of normality ($p_{Carbon} = 0.973$; $p_{Nitrogen} = 0.298$; $p_{Hydrogen} = 0.786$) and a Levene's test of homogeneity of variances ($p_{Carbon} = 0.378$; $p_{Nitrogen} = 0.839$; $p_{Hydrogen} = 0.365$) for each of the elemental concentrations. C:N data was not normal and was unable to be transformed to fit a normal distribution, thus an aligned rank transformation was used to force the statistical tests to run a two-way ANOVA on the non-parametric data. Soil type

significantly affected carbon and nitrogen concentrations, whereas soil treatment had a more pronounced effect on hydrogen concentrations and the C:N ratio (Table 2.20). The interaction of soil type and soil treatment also had a significant effect on carbon concentrations and the C:N ratio, and a nearly significant effect on nitrogen concentrations as well.

Analyzing the main effects of soil treatments shows that the differences in carbon concentrations can be found in both invaded and pristine soil types (Table 2.21). The effects of soil treatment are more pronounced in pristine soil types when it comes to the differences in nitrogen concentrations and the C:N ratio. No effects of soil treatment on hydrogen concentrations were found in either soil type. The effects of soil type on elemental concentrations were significant in each soil treatment for each element.

Pairwise comparisons between soil treatments among the soil types show significantly greater carbon concentrations in soils treated with AC in invaded soil types (Figure 2.8). In pristine soil types, soils treated with AC had the lowest amount of carbon reported and had a significantly lower concentration compared to a control. Trends with respect to nitrogen were less apparent, though concentrations were significantly lower in pristine soils treated with AC compared to a control. Pairwise comparisons of hydrogen concentrations were all insignificant. A significantly greater C:N ratio was found in pristine soils treated with AC, and there was a trend towards a greater C:N ratio in invaded soils as well. Concentrations of all elements were found to be greater in pristine soils compared to invaded soil types.

Table 2.20: Results of a two-way ANOVA exploring the effects of soil type and soil treatment on carbon, nitrogen, and hydrogen concentrations, as well as the ratio of carbon to nitrogen. Significant values are bold faced.

	df	***df***	**Carbon**		**Nitrogen**		**Hydrogen**		**C:N Ratio**	
Effect	**effect**	**error**	**F**	***p***	**F**	***p***	**F**	***p***	**F**	***p***
Soil Type	1	18	191.6	**<0.001**	107.1	**<0.001**	1.734	0.205	1.128	0.302
Soil Treat.	2	18	1.469	0.256	2.356	0.123	62.99	**<0.001**	8.146	**0.003**
Soil Type × Soil Treat.	2	18	16.81	**<0.001**	3.53	*0.051	0.945	0.407	7.553	**0.004**

Table 2.21: Exploration of the main effects of soil treatment and soil type on carbon, nitrogen, and hydrogen concentrations, as well as the ratio of carbon to nitrogen. Significant values are bold faced.

Main effects of Soil Treatment										
Soil Type	***df***	***df***	**Carbon**		**Nitrogen**		**Hydrogen**		**C:N Ratio**	
	effect	**error**	**F**	***p***	**F**	***p***	**F**	***p***	**F**	***p***
Pristine	2	18	7.479	**0.004**	5.705	**0.012**	2.519	0.108	5.209	**0.016**
Invaded	2	18	10.80	**<0.001**	0.181	0.836	0.160	0.854	3.247	0.062

Main effects of Soil Type										
Soil	***df***	***df***	**Carbon**		**Nitrogen**		**Hydrogen**		**C:N Ratio**	
Treatment	**effect**	**error**	**F**	***p***	**F**	***p***	**F**	***p***	**F**	***p***
Control	1	18	128.2	**<0.001**	59.21	**<0.001**	21.13	**<0.001**	0.611	0.444
Ash	1	18	85.31	**<0.001**	39.20	**<0.001**	30.77	**<0.001**	0.764	0.394
AC	1	18	11.65	**0.003**	15.76	**<0.001**	12.98	**0.002**	0.034	0.856

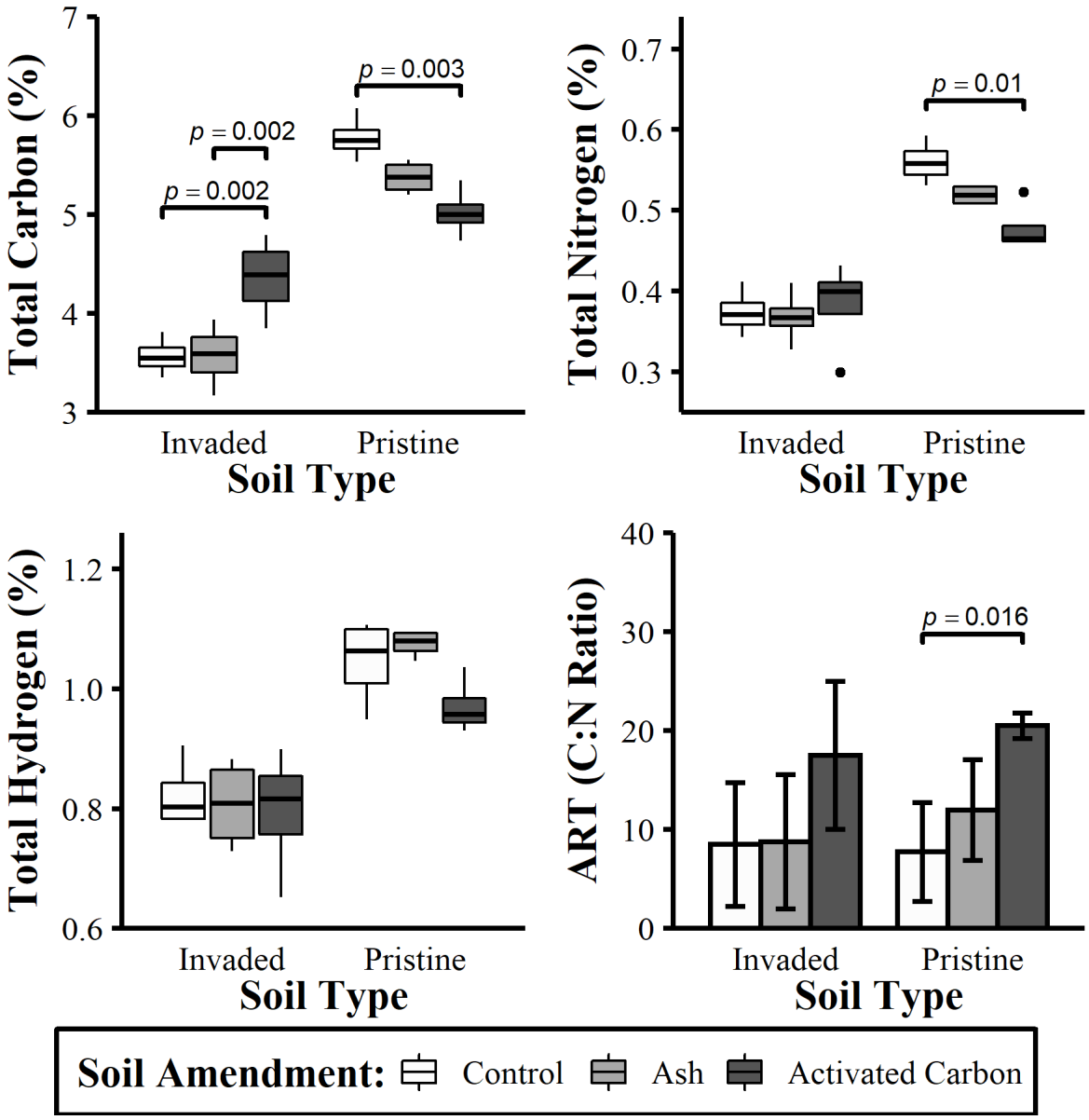

Figure 2.8: Total carbon, nitrogen, and hydrogen concentrations, as well as the aligned rank transformation of the carbon to nitrogen ratio in each soil type (N = 4 for each group). P-values above brackets denote significant pairwise comparisons between soil treatments as determined by pairwise t-tests.

Results from the major elements analysis highlight the effects of adding either ash or AC (Table 2.22). Interestingly, the addition of AC had differential effects on pristine and invaded soils. For pristine soils, this addition caused decreased concentrations of aluminum, boron, copper, potassium, manganese, sodium, phosphorus, sulfur, and zinc. Increased

concentrations were observed in invaded soils for those elements, as well as increases in calcium, iron, magnesium, and sulfur. The addition of ash appeared to increase concentrations for all elements in invaded soils except for magnesium, molybdenum, and phosphorus, and slightly increased calcium, potassium, magnesium, and sulfur concentrations in pristine soil types.

Table 2.22: Results from the major elements analysis. Concentration values reported are separated by reported units for simplicity. C, H, and N values for soil blends are mean values gathered from analysis using the FlashSmart CHNS/CHNS Elemental Analyzer whereas pure ash and AC values were gathered from literature (see footnotes).

Element (mg/Kg)	**Pristine Control**	**Pristine + AC**	**Pristine + Ash**	**Invaded Control**	**Invaded + AC**	**Invaded + Ash**	**Pure Ash**	**Pure AC**
Al	28000	27000	28000	26000	28000	27000	20000	22000
B	6.1	5.9	6.3	<5	6.7	6.1	52	7.1
Cu	45	43	44	47	50	48	42	100
Fe	32000	33000	32000	35000	36000	36000	17000	390
Mn	970	920	970	920	950	960	2600	11
Mo	<1	<1	<1	<1	<1	<1	2.5	<1
Na	1200	1000	1200	820	950	920	3200	610
Zn	82	75	78	75	74	77	150	16
Element (%)	**Pristine Control**	**Pristine + AC**	**Pristine + Ash**	**Invaded Control**	**Invaded + AC**	**Invaded + Ash**	**Pure Ash**	**Pure AC**
C	5.8	5.0	5.4	3.6	4.4	3.6	22.5[2]	80.4[3]
Ca	1.3	1.3	1.4	1.3	1.4	1.4	7.8	0.06
H	1.0	0.97	1.1	0.82	0.80	0.81	*NA*[2]	1.6[3]
K	0.50	0.49	0.52	0.47	0.51	0.50	2	2.4
Mg	0.87	0.90	0.88	1.0	1.1	1.0	1	0.03
N	0.56	0.48	0.52	0.37	0.38	0.37	0.05[2]	0.36[3]
P	0.15	0.14	0.15	0.13	0.13	0.13	0.44	0.02
S	0.090	0.083	0.091	0.077	0.080	0.080	0.36	0.09

[2] Data from Antonelli (2018).
[3] Data from Astuti et al. (2021), Hidayu & Muda (2016), Iqbaldin et al. (2013), and Phan et al. (2006).

DISCUSSION

Preferential Invasion based on Biogeoclimatic Subzone

The GIS analyses completed show that *C. stoebe* preferentially invades higher elevation grasslands with cooler temperatures and forest interfaces within the Lac Du Bois GPA. The most affected subzone in the Lac Du Bois GPA is the BGxw1, where over a third of the area is now covered by *C. stoebe*. This is an important finding as it may allow directed management of the invasive plant, as well as provide predictions for future invasion. The publicly available IAPP database is an exciting tool for extracting invasive plant information for both small study areas and province wide analyses. Its use is likely not harnessed as well as it could be, and studies of plant invasions within B.C. should utilize its power in making spatial predictions of future spread. Future studies could focus on the modelling required to make those predictions and define what factors are important in an individual plants spread.

The finding that the BGxw1 and IDFxh2 subzones were the most negatively affected of the subzones throughout the Lac Du Bois GPA is also important for species at risk. Indeed, B.C. only contains only a small portion of grasslands, but they also provide important niches for an abundance of endangered animals. The protection of grasslands should therefore be a high priority, including invasive species management. Across B.C., *C. stoebe* is the most invasive plant along with *C. diffusa*. The threat of habitat infringement for species at risk by invasive species should be more closely studied and managed in order to more directly impact the spread of invasive species.

Finally, the difference in amount of invasion by *C. stoebe* in the Lac Du Bois GPA compared to similar BGC subzones throughout B.C. shows just how necessary some type of intervention and protection is in our local grasslands. The bunchgrass zone throughout B.C. is already at risk from climate change, woody encroachment, and anthropogenic impacts, and the Lac Du Bois GPA is not immune to those factors either. With a high level of plant invasion in this park compared to other areas in B.C., it is very important that this park receives a focussed effort regarding the removal of noxious species. Such efforts may include further biological and chemical controls that have been used in the past, as well as targeted grazing efforts that may have a positive impact on the health of the grasslands overall.

Positive Soil Legacies Detected

This study aimed to answer the following questions:

- Can we detect soil legacy effects in invaded soils?
- Can potential legacy effects be lessened or mitigated using pulp mill fly ash as a soil amendment?

By comparing *F. campestris* biomass from pristine and invaded soils with no amendments, we may answer the first question. These greenhouse trials lead us to the conclusion that positive soil legacy effects are found in invaded soils which aid in the growth of a native plant. This result was unexpected: literature indicates that soil legacies in invasive soils harbor negative effects on native plant biomass (Del Fabbro & Prati, 2015; Elgersma et al., 2011). In certain cases, this can be visually confirmed in the very grasslands that soils were collected from, thus this result from the greenhouse is peculiar. Moreover, results from the AC treatments indicate that AC effectively kills most individuals. This is contradictory to literature as well, where most studies using similar amounts of this material show that when added to an invaded soil type, the soil condition improves for native plants, perhaps due to the adsorption of potential allelochemicals (Del Fabbro & Prati, 2015; Nolan et al., 2015; Perry et al., 2007). Perhaps the homogenized invaded soils did not contain potential allelochemicals, in which case the AC addition was essentially an increase in soil carbon. As part of the nitrogen cycle, soil microorganisms take in nitrogen to make use of available carbon, thus depleting soils of nitrogen (Vitousek & Howarth, 1991). The apparent lack of nitrogen therefore limits the growth of plants in that soil, which helps to explain the lack of growth witnessed in pristine soils with AC. This can be confirmed by viewing the carbon to nitrogen ratio analysis, where AC addition in pristine soils caused a significant increase in the C:N ratio compared to control soils, indicating that there was more carbon left in the soils and that nitrogen was depleted upon AC treatment. While significance didn't transfer over to invaded soils, there is a trend showing a greater C:N ratio in AC treated soils compared to control and ash treated soils, which further assists in the interpretation of this result. Other research suggests that a combination of AC and invasive plant litter is required to create soils

that are more hospitable for native plants with litter use being analogous to applying N fertilizer (Grove et al., 2012). The present study made use of soil materials only and omitted litter as part of the substrate. The results obtained from the AC treatment require further exploration, and perhaps an investigation of the soil microbial community is necessary to garner more conclusive results.

In order to answer the second question, we must compare the biomass of *F. campestris* in invaded soils that were ash treated soils to invaded control soils. Indeed, a legacy effect was noted in this experiment; however, the question implies that the soil legacy effects are negative towards native plants. The addition of ash in each soil type had opposite effects: in invaded soils, biomass decreased while biomass increased in pristine soils. While these are not significant trends, they are worth noting since this goes against what the expectations of this experiment were. Evidence of positive soil legacies from invasive soils do exist (Del Fabbro & Prati, 2015; Inderjit et al., 2011), and perhaps the most logical explanation would be that the chemicals released by the invasive plant (i.e.: the suspected cause of legacy effects) have been degraded by soil microorganisms, and are no longer affecting the native plants harbored within (Kaur et al., 2009; Zackrisson & Nilsson, 1992). Additionally, Barto and Cipollini (2009) have shown that the secondary metabolites produced by *Alliaria petiolata* have a very short half life even in sterile soil (~45 hours). The metabolites produced by *A. petiolata* are flavonoids, which bears close resemblance to the chemical structure of catechin, thus it may be that legacy effects are not witnessed in these invaded soils due to the natural degradation of the suspected allelopathic compound (Muhamad et al., 2015; van't Slot & Humpf, 2009). This information may also help to answer why conflicting results have occurred in past studies leading to the eventual retractions of certain articles. If such temporal differences create these discrepancies, perhaps firm methodology should be created to assist collecting data relevant to studying allelopathy and soil legacy effects from invasive plants.

Impacts of Ash on Biomass

The addition of ash in this experiment did not have any significant effect on the biomass of either species in either soil type when compared to a control; however, the direction of biomass change is important to note for both species. Specifically, using ash seemed to have a more neutralizing effect on the biomass of *F. campestris*, where it appears to have increased biomass in pristine soils and decreased biomass in invaded soils compared to a control. Biomass of *F. campestris* was equal in ash treated pristine soils and ash treated invaded soils in the end, hence the "neutralizing" effect. A similar, yet opposite trend was witnessed with *C. stoebe* where biomass was brought closer together in both soil types with the addition of ash; however, biomass decreased in pristine soils and increased in invaded soils with an ash amendment. In other words, it appears that adding ash in pristine soil types incurs a benefit to native plants and not *C. stoebe* and adding ash in invaded soils is beneficial to *C. stoebe* but not *F. campestris*. That addition may have altered soil conditions such that it was no longer preferable for growing in those soil types. Regardless, this differential effect is important to note: in the field, pristine soils are defined by the absence of invaders, thus adding ash to a site may act to increase native biomass through a fertilizing effect (Basu et al., 2009; Kishor et al., 2010). Indeed, ash acted to marginally increase certain element concentrations in pristine and invaded soils, which likely accounted for the increased biomass seen in some of the soils (Table 2.22). Interestingly, the addition of the ash essentially had no effect on soil carbon or nitrogen values in this case (Figure 2.8). From a management perspective, this can be positive since trends in biomass were witnessed upon the addition of ash, but underlying soil characteristics were not largely affected. Conversely, the results here indicate that we might wish to avoid using ash to remediate invaded soils since it tends to aid in the growth of *C. stoebe* but hinders *F. campestris* (Figure 2.3). Further investigation into methods of application, amounts of ash, and levels of *C. stoebe* invasion should be explored to confirm these results in a field scenario.

Effects of Ash and AC on Survival and Mortality

This study examined four different metrics associated with seedling survival and mortality: germination rate, mortality rate, the entire 90-day survival rate, and the survival rate of germinated seedlings between 30- and 90-days to fully understand how longevity was affected in each soil combination. The first observation that can be made is that activated carbon had an overall negative impact on the survival of both *F. campestris*: germination rates and both survival rates were lowest in activated carbon treated soils, and mortality rates were highest in these treatments. This suggests that the use of activated carbon here was not an appropriate measure against ash treatments since the results were very different. Likely, this is an outcome of the activated carbon processing that occurred. The purchase of granulated activated carbon was an oversight and closer results would be more likely found if a finer form was used. Despite this, the seeds of *C. stoebe* appeared to have no difficulty germinating in the presence of activated carbon. Beyond that, *C. stoebe* exhibited similar outcomes to *F. campestris* with respect to activated carbon addition: survival rates were lower and death rates were higher, further supporting the result that the use of granulated activated carbon was inappropriate.

The addition of ash had interesting effects on the survival metrics of *F. campestris* and *C. stoebe*. Mostly, there were no significant differences compared to a control; however, the long-term survival rate of *F. campestris* (i.e.: the germination survival rate) was significantly greater in invaded, ash treated soils compared to a control and AC treated soils. This effect was not seen in pristine soil types. Moreover, *C. stoebe* had a slightly lower long-term survival rate in the same soils compared to a control. This implies that *F. campestris* individuals that survive to the 30-day period are more likely to survive the entire duration of the trial when grown in invaded soils treated with ash. This leads to further implications with management: according to these results, if an invaded area is treated with ash and seeded with *F. campestris* we might see limited success in the first 30 days but after that point it is likely that those that are left are what is going to withstand. While interactions between *F. campestris* and *C. stoebe* were not examined in this experiment, it still may be necessary to remove *C. stoebe* seedlings at that 30-day period to maintain similar results in a field application. In invaded soils that did not have any treatment, the greatest mortality of *F.*

campestris happened between the 30- and 90-day periods, suggesting that the ash may be alleviating a negative soil effect that would otherwise cause mortality after 30-days. This effect is not witnessed in pristine soils where the greatest survival is seen in control soils, hence the possible alleviatory effect.

Invasive Boundary Movement

By comparing the currently invaded boundary that was walked to the old boundary mapped by the IAPP, we are witnessing a change over time in the sizes of invaded boundaries and their relative locations. The IAPP polygon overlapping the current study area was created in 2006, thus it may be that the IAPP polygon is out of date. In fact, there have been at least 1,287 recordings of either mechanical, chemical, or biological treatments applied within the park, and a release of *Cyphocleonus achates*, the root boring weevil, was applied in 2016 within 200 m of the study area. This could account for the apparently shrunken boundary of the study area from what the IAPP had previously declared in 2006. Another consideration may be that the IAPP identified both *C. stoebe* and *C. diffusa* in this polygon, and that *C. diffusa* may be present outside of the observed polygon; however, *C. diffusa* was not present in or near the study site. A final consideration may be that the IAPP over-generalizes an invaded area, and a contiguous polygon was created where it should not have been. Methods of the original sampling are not publicly available; thus, it is unknown how the original polygon was created. Regardless, it is widely known that plant communities can be drastically altered in the face of climate change, thus it is important to view future areas of invasive expansion as well as current ones (Settele et al., 2014).

This apparent shift in the invasive site boundary also points to the need for improved sampling of invaded areas in B.C. If the IAPP is to be the sole resource for invasive plant information, it stands to reason that invasive areas should be monitored on a frequent basis. Remote sensing advances in recent years has shown incredible potential for collecting a wealth of data at a fine scale with little effort (Hung & Wu, 2018). In the future, there may be ways to identify species from a simple image taken by unmanned aerial vehicles (UAV's), which would be immensely useful for more than just invasive species identification and

location. Indeed, efforts are underway to solve this task, though at the moment it seems this accomplishment is limited to forest canopies (Baena et al., 2017; Santos & Ustin, 2018). With invasive species threatening to alter landscapes, technologies should be developed for modelling future expansions and identify areas of concern, allowing for preventative management of invasive spread.

Limitations

GREENHOUSE CONDITIONS

As with most greenhouse studies, the climate-controlled environment within the greenhouse is meant to promote the growth and establishment of the plants under study. As such, these are not exactly reflective of what occurs in a field condition. In general, greenhouse studies allow for simpler statistics and allows for better experimental manipulation on selected variables; however, they might fail to capture the full complement of soil and environmental processes (May & Baldwin, 2011). Consistent watering schedules allow for plants to stay healthy throughout the duration of the study, but it may change belowground processes and even certain aspects of plant growth which are not indicative of field scenarios. Ideally, a field study would be conducted alongside greenhouse trials in order to verify the applicability of the greenhouse study.

Additionally, certain aspects of the greenhouse climate are beyond human control. The greenhouse does its best to keep within the set climatic parameters but may encounter errors if there are external factors such as a power outage or equipment failure. In these scenarios, climates are not being monitored or controlled and thus the greenhouse experiment could be at risk. Even slight variations like these have been reported to have effects on the final outcome of a greenhouse experiment on plants grown from seed (Hammer & Hopper, 1997).

SOIL CONDITIONS

Soils were collected in the late fall of 2017, just before snowfall. At this point, most of the vegetation on the landscape had gone dormant, including spotted knapweed. Perhaps at this time of year, it is not legacy effects that are impacting the growth of vegetation in the environment, but climatic and temporal factors are playing a role. Soils in this study were collected at one point in time, but perhaps if soils had been collected at various points throughout the year, spotted knapweeds soil legacies would be more pronounced. In one study, multiple samplings found varying concentrations of soil catechin throughout the year at one study site (Perry et al., 2007). That result was fairly suspect since there was only a single record of catechin occurrence during the entire sampling period at only a single site, but it does indicate that some temporal sampling may need to be incorporated into future studies of soil legacy effects on neighboring native plant species. Furthermore, litter was discarded in this study from both invaded and pristine soils during collection, which Grove et al. (2012) suggest is an important constituent in studies using AC.

Another consideration of the soil collection would include the depth of soil collected as well. In this study, the top 5 cm was discarded with the intent to omit any lasting seeds in the field seed bank (Bonis & Lepart, 1994). In doing so, however, much of the organic material was removed as well. The top most layer of any soil horizon contains the most mineral nutrients that a plant will draw from (Watson, 2014); however, the soils in this study area are classified as orthic black chernozems which are reported to have an A horizon from 0 - 25 cm just below the soil surface (Canadian Soil Information Service, 2013; Filatow, 2019). Regardless, soil microorganisms can change with soil depth, and these microorganisms can have a pronounced effect on the aboveground plant communities (Bhattarai, 2015; Vitousek & Howarth, 1991). This removal may create a misrepresentation of the field soils in the greenhouse study, thus the results should be taken cautiously. The organic substances within the top 5 cm of the soils could have been more suitable for a greenhouse trial, or oppositely, contained allelopathic catechins that would inhibit native plant growth more effectively. The over-arching purpose of taking soils from a deeper source was to capture potential legacy effects which would be found in older (deeper) soils; however, in the lens of plant growth, this may not have been an optimal goal.

POTTING

The pots used in the greenhouse trial were 512 cm^3, which are generally smaller than what is normally used in greenhouse trials. Though crowding of individuals was not an issue in this present study, the depth of soil (or lack thereof) may have been influential in the results here. In nature, *C. stoebe* produces a large taproot, and with the limited depth of pots there may have been a physical constraint on its growth. Having only reached a rosette life history stage, however, the individuals did not grow large enough to produce a taproot. Additionally, volume of soil in these pots could have influenced results as well. If 1,000 cm^3 pots were used, a total of 1 L of soil could have been used in each pot. Instead, only 512 mL could be used in the pots here. This is where restrictions may become more prevalent: the limited volume of soil also contains a limited volume of nutrients which may be problematic for plant growth. Initial errors in sample design were the cause of this, as well as having a limited batch of soils to draw from.

ELEMENTAL ANALYSIS

Chemical composition of a soil can provide a wealth of information on the soils in question, including salinity, nutrient composition, pH, and overall quality. The data that I obtained in my study for elemental analysis represents a snapshot in time for pre-treatment soil conditions (i.e.: amended bulk soils with no plants yet); however, it would have been beneficial to observe changes in these nutrient compositions after the 90-day growing period. Doing so may have provided insight into the belowground processes and aided in explanations for why treatments using activated carbon performed differently than described in literature.

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Chapter 3: Research Conclusions

This study showed that the removal of an invasive species promotes the growth of a seeded native species, and the addition of pulp mill fly ash did not harbor any significant effects to the growth of either *Festuca campestris* or *Centaurea stoebe* (though certain trends were noted). While removal and reseeding seems like a simple solution to dealing with the threat of invasive species spread, there are many factors that need to be considered. Soil legacy effects from the invasive spotted knapweed were determined to be positive in this greenhouse study; however, field experience demonstrates that left alone, recently invaded areas do not typically regrow native vegetation naturally or when seeded (May & Baldwin, 2011; Rand et al., 2015). Taking this into account, we must explore the research and management implications that this study has brought forward.

Another item to further discuss is the limitations that were brought on by the project design. Standard methods for comparing the growth of various plants with respect to soil legacy effects have been established in previous works but this project marks the first examination of soil legacy effects of the invasive *C. stoebe* on a native bunchgrass (Del Fabbro & Prati, 2015).

RESEARCH IMPLICATIONS

This study serves as a solid steppingstone for future research with invasive plants and the use of ash as a soil amendment to alleviate the potential negative allelopathic or soil legacy effects. Further studies should focus on a field component, where ash is broadcast applied to an invaded site much like a chemical control. It would be interesting to see this method alongside hand-pulling the weed when it isn't seeding, and then observe the native plant community in these trial areas. Other aspects to a study involving these parameters could include the concentration of ash applied, comparisons to areas free of invasive plants, comparisons to different BEC zones or wider regions (if applicable), and comparisons at different times of the year. While the present study was able to control these aspects, a robust field component could be helpful to provide a broader context for the results.

While the present study brought about some confounding results, we should not immediately discount fly ash as a potential tool for invasive species management. It has been shown to promote growth of various plants in other forms, much like that of a fertilizer (Emilson et al., 2018; He et al., 2017). Presently, we found that adding the ash component assisted in increasing nutrient values of B, Ca, K, Mg, and S in pristine soils and increasing Al, B, Ca, Cu, Fe, K, Mn, Na, S, and Zn in invaded soils, albeit slightly. Perhaps with the addition of more ash, clearer results from the ash addition could have emerged. He et al. (2017) studied the growth of alfalfa in loessial soil and added differing concentrations of fly ash (5%, 10%, 20%, and 40% by weight), and found that the addition of these nutrients promoted the growth of alfalfa. Here, the researchers also noted chlorosis in their plants, though this was at application rates of 10% and over. It is important that this study highlighted that: with the chlorosis mentioned, we might confirm that too much AC was added in our soils, even at a rate of 1% by volume and further tuning on the amount of AC addition is required.

The addition of ash may also be of importance in soils with low pH: industrial fly ash is consistently characterized as having high pH (in the range of 8 – 12), thus its application into acidic soils would assist in increasing pH to a more suitable level (Domes et al., 2018; Magiera et al., 2013). Studies on the remediation of Canadian forest soils using ash have recently been of great interest. A federal working group, AshNet, is actively exploring the potential of industrial wood ash application into forest soils and the associated publications have shown great promise with benefits to forest health, including increased soil pH (Brais et al., 2015; Emilson et al., 2018). As more details emerge from this group, it is likely that ash application rates will become more refined and better characterized based on local conditions of a given site. While invasive species management is beyond the scope of this working group, it still points to ash being a useful remediation tool in forested soils. Future work within this group with regards to ash's effects on invasive species would prove useful. In B.C., the BGC zone with the greatest amount of plant invasion by square kilometer is the Interior Douglas Fir zone which holds a great amount of timber value within the province. Preserving this zone from invasive species is therefore important and it would be interesting

to see research into the use of ash within this zone for the dual purpose of invasive species management and forest soil remediation.

This study provided a basis for further experimentation in both the field and the greenhouse, though the limitations addressed in Chapter 2 should be acknowledged. While the ash additions may not have yielded the expected results, further studies on the control of invasive plant species should consider its use as a potential control agent. In the present study, there was no significant trend in decreasing *C. stoebe* biomass; however, this was a trial using a 1% by volume ash addition. Perhaps further manipulations could be made on this value to produce more significant results. This type of work is currently being performed in the grasslands near Merritt, B.C. (Hampton, 2020).

There are also remote sensing and GIS considerations that can be taken into consideration for future research as well. This study noted certain provincial BGC subzones to have a greater proportion of *C. stoebe* found within them than others. While beyond the scope of this study, it would be possible to generate spatial models that predict the presence and potential of *C. stoebe*, along with other invasive species based on the existing input data (Cutler et al., 2007). With the maps of the species' potential, we may be able to locate areas of rampant spread before they occur and implement management strategies that prevent further environmental deterioration.

MANAGEMENT IMPLICATIONS

This study aimed to explore the soil legacy effects of *C. stoebe* and assess the use of pulp mill fly ash as a restoration tool for invaded areas. While we found no significant trends to indicate that using fly ash was a promising amendment, it should be noted that the data presented are representative of a small-scale greenhouse experiment. Indeed, further efforts to propagate the use of ash are in place on a much larger scale and show promising results in both agricultural and forestry applications (Emilsson, 2006; Hannam et al., 2016). In many cases, its use in agriculture may contribute to (or replace, depending on the reasoning for application) the soil macronutrients and thus produce a healthier ecosystem. Forestry applications of ash to bolster the soil nutrients appear to be more case specific and have many

factors that influence their results, including the chemical composition of the ash, soil type, and dominant tree species (Augusto et al., 2008; Emilson et al., 2018; Pitman, 2006). The method of application is likely a factor in this as well. In many studies of ash use, the ash is broadcast applied whereas it was thoroughly mixed into the soils in this project. Where successes have been noted, it is important to acknowledge the method of application in order to have similar results in our own work. Drawing on methods from agricultural projects may be more suitable to attempt to characterize the soil legacy effects of *C. stoebe* rather than a greenhouse experiment, and further research should have a strong field component to accomplish that goal.

The world of GIS and spatial analysis is advancing quickly, and it is important to understand the impact that may have on the future of invasive species research. In terms of management, we can monitor and model the advance of invasive species based on publicly available data. It is therefore important that the dataset is updated as new data becomes available. Additionally, investments into remote sensing hardware and software should become more relevant since we can interpret more data from remote sensing equipment than we could doing a standard field survey or walkabout. Innovations in remote sensing equipment may one day allow us to differentiate invasive from native species at multiple life stages based on imagery. If that potential could be harnessed, it would be immensely useful for the detection of invasive species in order to mitigate their spread. For now, we must focus on the modelling and responding to the invasive impact that is occurring on our landscapes and especially in the grasslands.

The threat of invasive species should be taken seriously since they have an inherent ability to cause lasting damage to an ecosystem. In BC, the grasslands account for less than 1% of the land base but provide important habitat for over 30% of the province's species at risk. Protecting these grasslands should be prioritized, and strategies for invasive species mitigation should continue to be studied. The present study attempted to highlight a phenomenon witnessed in the field, where an invasive plant may have lasting soil effects after its removal. While this was unable to be replicated in the greenhouse trials, it is obvious that there is something going on preventing native plant species from repopulating recently invaded areas. There are many explanations for these results: perhaps the amount of soil in

the pots was not appropriate in this study, or the removal of the first 5 cm of soil prior to collection is not standard for studies of this nature. Whatever the case, these sources of error allow future studies to build on this and perhaps more rigorous designs will be implicated to determine whether spotted knapweed has lasting negative effects in the grasslands of B.C.

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Appendix A: Characterizing immediate and legacy soil effects in the field: A lesson in transplanting

INTRODUCTION

Often, greenhouse trials do not correspond directly to field conditions. Field conditions contain much variability in climate, moisture availability, soil conditions and nutrients, herbivory, slope, and much more, thus results gained from greenhouse trials may not always represent field conditions exactly since these types of factors are controlled for. An obvious way to address this issue is to perform a similar study in the field, observe growth through time, and extrapolate the information gained from the experimental process.

The native *Festuca campestris* and the invasive *Centaurea stoebe* are both found commonly in upper elevation grasslands in the interior of British Columbia. To reiterate, *C. stoebe* is an aggressive invasive forb that has caused much damage to pristine grassland areas in North America in recent history, and its spread continues to this day despite various control efforts. Negative effects of its invasion have been noted, including a decrease of plant biodiversity, decrease in available forage for grazing animals, and alteration of soil properties. Further intervention is required to diminish the presence of this invasive plant, and with the interesting results from the greenhouse experiment, it is prudent that field methods be developed which not only mimic the greenhouse experiment, but also have practicality in mind on larger scales. Overall, the field trials were implemented with the following questions in mind:

- Are the results of the greenhouse trials representative of what would happen in the field?
- What concentration(s) of ash will work to mitigate the potential legacy effects of *C. stoebe*?

Once again, *F. campestris* was chosen as the experimental plant since it is the most common native plant found in the upper grasslands. Planting sites were located in a heavily invaded patch of the upper grasslands of the Lac Du Bois Protected Area, North of

Kamloops, British Columbia where moisture is more readily available, and temperatures are cooler relative to lower elevation grasslands.

The goal of the field trial was to support the conclusions drawn from the greenhouse trials, and to discover the effect that ash concentration had on the growth of individual *F. campestris* seedlings.

METHODS

Seedling Germination

Purchased *F. campestris* seeds were planted in small plug trays at the Thompson Rivers University Research Greenhouse on March 5, 2018 with each plug measuring 4 cm in diameter and 6 cm tall. A single seed was planted in potting soil (Sunshine Mix 4, Canadian sphagnum peat moss) housed in each plug. A total of 1,160 seeds were planted. Growing conditions in the greenhouse were set to follow the ISP conditions laid out by Hendry and Grime (1993). Seedlings were allowed to grow under these constant conditions until May 2, 2018 when field planting commenced.

Field Design

This study took place in the upper grasslands of the Lac Du Bois Protected Area, North of Kamloops, British Columbia. Specifically, the same 10 heavily invaded locations from the greenhouse component of this project (i.e.: Chapter 2) were chosen, and grids were formed no farther than 2 m away from the soil sampling locations. *C. stoebe* was physically removed from a 2 m × 2 m square in the heavily invaded areas. Within the 4 m^2 grid, 9 sample points were established in a 3 × 3 fashion, and sample points were located 0.5 m from the edge of the 4 m^2 grid, and 0.5 m from each other (Figure A.1). The 9 sample points served as the different treatment locations for this project and were arranged in a randomized block design. The treatments used in the field included a range of ash concentrations, a single Activated Carbon (AC) treatment, and a control condition where no ash or AC was applied.

Concentrations of ash and AC were based on a 500 mL volume of soil which was the anticipated volume that these roots would take up in a 90-day growing period, and the individual treatments are as follows:

1. Control (no AC or Ash addition)
2. 1% (5 mL) AC addition
3. 1% (5 mL) Ash addition
4. 2% (10 mL) Ash addition
5. 5% (25 mL) Ash addition
6. 10% (50 mL) Ash addition
7. 20% (100 mL) Ash addition

Since there were only a total of 7 treatments, there were 2 "blank" locations where no planting occurred to account for the 9 total sample points. Transplanting of the individual *F. campestris* plugs occurred on May 2, 2018 and the study lasted 90 days. At each sample point where treatments were present, a spade-type shovel was used to open the ground. The soil treatments were then poured into the opening in the ground, and the *F. campestris* plugs were planted in the openings afterwards. This method was chosen for simplicity on a larger scale, should it be applicable. At the time of planting, the number of leaves and the height of the tallest leaf of each *F. campestris* individual was recorded. Over the course of the growing period, individuals were hand watered every 2-4 days using tap water, depending on the weather. At the end of the growing period, number of leaves and height were once again recorded, and aboveground biomass samples were taken from each individual. Biomass samples were brought back to the Thompson Rivers University Research Greenhouse where they were dried in a drying oven (Yamato DKN8132) at 70°C for 72 hours prior to weighing. Biomass was recorded to the nearest hundredth of a gram using a Fisher Scientific top loading scale (accu-4102).

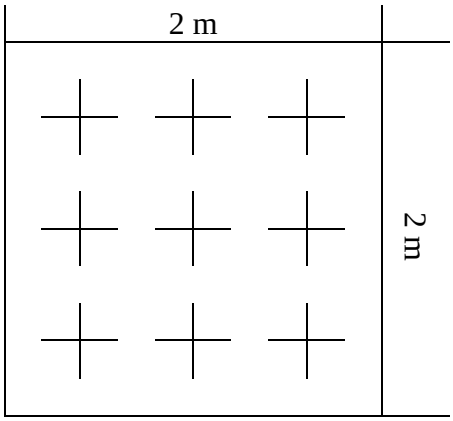

Figure A.1: Design of the field grids. The crosshatches represent the 9 sample points within the 2 m × 2 m grid.

Statistical Analyses

A one-way analysis of variance (ANOVA) was used to determine if soil treatments had any effect on biomass. Assumptions of this test include normality of the input data as well as homogeneity of variances. The raw data violated the normality assumptions; thus, a square root transformation was applied to fit a normal distribution and pass a homogeneity of variance test. A Tukey post-hoc analysis was completed to view the differences between each pairing.

Two-way repeated measures ANOVA's were used to see the differences in the number of leaves and leaf height between the soil treatments at each time point. Normality testing for this dataset was applied; however, the repeated measures test was carried out regardless of the results since this test is robust against departures from normality. An analysis of the main effects of soil type and time was conducted, and pairwise comparisons were made between the pairings of soil treatments and time. P-values were adjusted using the Bonferroni method to account for the unique pairwise comparisons involved in a two-way repeated measures ANOVA. All calculations and computations were performed using R for Statistical Computing (R Core Team, 2020).

RESULTS

Perhaps due to inherent issues with transplanting, many of the individual plugs did not survive the duration of the experiment. Out of the 70 individuals planted, only 22 (31.4%) survived to the end of the 90-day growing period. The subsequent results will not carry any real significance. Nevertheless, statistical measures were applied to examine the plants throughout their growing period.

Trends with Survived Plugs

Both the 1% ash and control treatments had the greatest number of plugs survive to the end of the experiment, each having 5 survivors out of 10 planted. The least successful trial was the 1% AC treatment, which resulted in 0 survivors (Table A.1). While this compliments the results from the greenhouse trials, we cannot rule out the possibility that local conditions or issues with transplanting aided in this result. All survived plugs resulted in a net loss of leaf height, with the greatest height loss occurring in 20% ash treatments. Conversely, all trials resulted in a net gain of number of leaves with 5% ash treatments having the greatest increase. Finally, average aboveground biomass of each living plug was always below 0.5 g. This is not likely indicative of how *F. campestris* grows in the field, thus these results should be taken lightly.

Table A.1: Summarized field data captured from both the planting date (May 2, 2018) and the harvest date (July 30, 2018). The values in this table are only representative from plants which survived the full 90-day growing period, hence the different values for N. Mean values are appended with standard errors to indicate the spread of the data.

Soil Treatment	Mean Planting Height (cm)	Mean Harvest Height (cm)	Mean Δ Height (cm)	Mean Planting Leaves (n)	Mean Harvest Leaves (n)	Mean Δ Leaves (n)	Mean Biomass (g)	N
AC-01	NA	NA	NA	NA	NA	NA	NA	0
Ash-01	9.26 ± 0.73	4.24 ± 0.89	-5.02 ± 0.46	18 ± 2.14	20.6 ± 5.31	2.6 ± 6.96	0.174 ± 0.019	5
Ash-02	9.25 ± 0.85	3.8 ± 1.1	-5.45 ± 0.25	15.5 ± 0.5	34 ± 5.0	18.5 ± 5.5	0.175 ± 0.035	2
Ash-05	8.95 ± 2.35	4.75 ± 2.75	-4.2 ± 0.4	19 ± 5.0	51.5 ± 19.5	32.5 ± 14.5	0.185 ± 0.065	2
Ash-10	10.05 ± 1.82	4.925 ± 1.01	-5.13 ± 1.56	15.75 ± 3.90	28.5 ± 10.90	12.75± 7.54	0.145 ± 0.079	4
Ash-20	11.025 ± 1.02	3.875 ± 0.98	-7.15 ± 0.97	12.5 ± 2.53	20.75 ± 4.21	8.25 ± 3.57	0.145 ± 0.060	4
Control	7.82 ± 0.63	3.58 ± 0.91	-4.24 ± 1.47	17 ± 3.08	23.8 ± 4.26	6.8 ± 5.83	0.214 ± 0.018	5

Biomass

A one-way ANOVA was performed to find any differences in biomass between the soil treatments. A square root transformation was applied to the biomass data to pass a Shapiro-Wilks normality test ($p = 0.075$) and Levene's test of homogeneity ($p = 0.234$) to allow the use of the parametric one-way ANOVA. No significant differences in biomass were found between the treatments ($F_{(6, 63)} = 0.962$, $p = 0.458$). The mean biomass of *F. campestris* when grown in soils treated with activated carbon was slightly lower compared to when grown in other soil treatments (Table A.2). A Tukey post-hoc analysis revealed no significant differences between any pairing of groups (Table A.3).

Table A.2: Summarized mean and standard deviation of biomass of *F. campestris* grown in each soil treatment.

Treatment	√Biomass ± SD	N
AC_01	0.180 ± 0.104	10
ASH_01	0.231 ± 0.201	10
ASH_02	0.248 ± 0.139	10
ASH_05	0.227 ± 0.151	10
ASH_10	0.281 ± 0.141	10
ASH_20	0.245 ± 0.149	10
CONTROL	0.330 ± 0.154	10

Table A.3: Tukey HSD pairwise comparisons of biomass, change in number of leaves, and leaf height change between each treatment grouping.

Group 1	Group 2	p
AC_01	ASH_01	0.987
AC_01	ASH_02	0.949
AC_01	ASH_05	0.992
AC_01	ASH_10	0.743
AC_01	ASH_20	0.96
AC_01	CONTROL	0.3
ASH_01	ASH_02	1
ASH_01	ASH_05	1
ASH_01	ASH_10	0.99
ASH_01	ASH_20	1
ASH_01	CONTROL	0.771
ASH_02	ASH_05	1
ASH_02	ASH_10	0.999
ASH_02	ASH_20	1
ASH_02	CONTROL	0.889
ASH_05	ASH_10	0.984
ASH_05	ASH_20	1
ASH_05	CONTROL	0.729
ASH_10	ASH_20	0.998
ASH_10	CONTROL	0.991
ASH_20	CONTROL	0.869

Leaf Height and Number of Leaves

A two-way repeated measures ANOVA was used to determine the effects of soil treatments over time on the maximum leaf height and number of leaves of the planted *F. campestris* individuals. Normality was violated for the raw data ($p < 0.001$), though the test was still used as it is robust against departures from normality. Moreover, these departures can be explained by the fact that only 22 individuals survived to the end of the experiment, thus biasing calculations of normality to tend towards zero. The decision was made to proceed with a two-way repeated measures ANOVA to test for differences in leaf height and number of leaves.

There was a significant effect of timing on the leaf heights of *F. campestris* between the treatment groups ($F_{(1, 9)} = 68.49$, $p < 0.001$, Figure A.2). There was no significant effect of treatment on leaf height of *F. campestris* ($F_{(6, 54)} = 0.419$, $p = 0.864$); however, the interaction of treatment and timing had a slight but non-significant effect ($F_{(6, 54)} = 2.11$, $p = 0.067$). An analysis of the main effects of soil treatment supported the conclusion that there were no significant differences between the treatments at either time of planting or harvesting (Table A.4). Conversely, a significant main effect of timing was found within each soil treatment on leaf height. Pairwise comparisons of soil treatments showed no significant difference between any two treatment groups (Table A.5). Oppositely, significant differences were found in the pairwise comparisons of timing for each soil treatment group (Table A.6)

No significant effects on the number of leaves were found between soil treatments ($F_{(6, 54)} = 1.136$, $p = 0.354$) or timing ($F_{(1, 9)} = 2.879$, $p = 0.124$, Figure A.2). Additionally, there was no significant interaction of soil treatments or timing of sampling on the number of leaves ($F_{(6, 54)} = 1.34$, $p = 0.256$). There were no significant main effects of soil treatment on the number of leaves when grouped by timing of sampling (Table A.4). A significant main effect of timing on the number of leaves was found in soils treated with AC ($F_{(1, 9)} = 108.07$, $p < 0.001$). Pairwise comparisons between each soil type showed no significant differences between any soil treatments (Table A.5), and the only significant comparison between timing of sampling occurred in the soils treated with AC (Table A.6).

Table A.4: Results from a two-way repeated measures ANOVA on leaf height and number of leaves separately. Effect of time and treatments are reported for each group. Significant p values are bold faced. P-values on the treatment and timing effects have been adjusted using the Bonferroni method accounting for the different number of groupings.

Two-way repeated measures ANOVA

			Leaf Height		**No. Leaves**	
Effect	df_{effect}	df_{error}	**F**	***p***	**F**	***p***
Treatment	6	54	0.419	0.864	1.136	0.354
Timing	1	9	68.486	**<0.001**	2.879	0.124
Treatment × Timing	6	54	2.111	0.067	1.34	0.256

Effect of soil treatment by time

			Leaf Height		**No. Leaves**	
Timing	df_{effect}	df_{error}	**F**	***p***	**F**	***p***
Planting	6	54	0.583	1	1.23	0.612
Harvest	6	54	1.966	0.174	1.21	0.63

Effect of timing by soil treatments

			Leaf Height		**No. Leaves**	
Treatment	df_{effect}	df_{error}	**F**	***p***	**F**	***p***
AC_01	1	9	86.822	**<0.001**	108.07	**<0.001**
ASH_01	1	9	38.203	**0.001**	0.868	1
ASH_02	1	9	44.361	**0.001**	1.442	1
ASH_05	1	9	48.968	**<0.001**	0.584	1
ASH_10	1	9	41.834	**0.001**	0.515	1
ASH_20	1	9	67.953	**<0.001**	0.72	1
CONTROL	1	9	24.121	**0.006**	0.16	1

Table A.5: Results of pairwise T-tests of leaf height and number of leaves between each treatment group, separated by planting and harvest timing. T statistics and Bonferroni adjusted p values are reported.

		Leaf Height				No. Leaves			
		Planting		Harvest		Planting		Harvest	
Group 1	**Group 2**	**T**	***p***	**T**	***p***	**T**	**p**	**T**	***p***
AC_01	ASH_01	0.696	1	-2.579	0.624	-0.090	1	-2.425	0.804
AC_01	ASH_02	0.544	1	-1.427	1	1.994	1	-1.480	1
AC_01	ASH_05	1.331	1	-1.259	1	-0.703	1	-1.381	1
AC_01	ASH_10	1.641	1	-2.227	1	-0.733	1	-1.861	1
AC_01	ASH_20	0.176	1	-2.134	1	1.681	1	-2.231	1
AC_01	CONTROL	1.107	1	-2.435	0.792	0.182	1	-2.677	0.531
ASH_01	ASH_02	-0.125	1	2.368	0.882	1.432	1	1.496	1
ASH_01	ASH_05	0.964	1	2.072	1	-0.613	1	0	1
ASH_01	ASH_10	1.282	1	0.135	1	-0.529	1	-0.192	1
ASH_01	ASH_20	-0.508	1	0.775	1	1.510	1	0.442	1
ASH_01	CONTROL	0.328	1	0.677	1	0.218	1	-0.544	1
ASH_02	ASH_05	0.607	1	-0.253	1	-1.986	1	-0.631	1
ASH_02	ASH_10	0.979	1	-1.198	1	-2.231	1	-0.881	1
ASH_02	ASH_20	-0.303	1	-0.902	1	0.285	1	-0.286	1
ASH_02	CONTROL	0.315	1	-1.661	1	-0.929	1	-1.589	1
ASH_05	ASH_10	0.210	1	-1.204	1	0.082	1	-0.198	1
ASH_05	ASH_20	-0.845	1	-1.067	1	1.775	1	0.331	1
ASH_05	CONTROL	-0.311	1	-2.060	1	0.942	1	-0.240	1
ASH_10	ASH_20	-1.479	1	0.387	1	2.059	1	0.481	1
ASH_10	CONTROL	-0.674	1	0.181	1	0.594	1	-0.073	1
ASH_20	CONTROL	0.669	1	-0.370	1	-0.857	1	-0.654	1

Table A.6: Results of pairwise T-tests of leaf height and number of leaves between planting and harvesting, separated by treatment group. T statistics and Bonferroni adjusted p values are reported. Significant p values are bold faced.

			Leaf Height		No. Leaves	
Treatment	**Group 1**	**Group 2**	**T**	***p***	**T**	***p***
AC_01	Planting	Harvest	9.318	**<0.001**	10.396	**<0.001**
ASH_01	Planting	Harvest	6.181	**<0.001**	0.931	0.376
ASH_02	Planting	Harvest	6.660	**<0.001**	1.201	0.261
ASH_05	Planting	Harvest	6.998	**<0.001**	0.764	0.464
ASH_10	Planting	Harvest	6.468	**<0.001**	0.718	0.491
ASH_20	Planting	Harvest	8.243	**<0.001**	0.849	0.418
CONTROL	Planting	Harvest	4.911	**0.001**	0.400	0.699

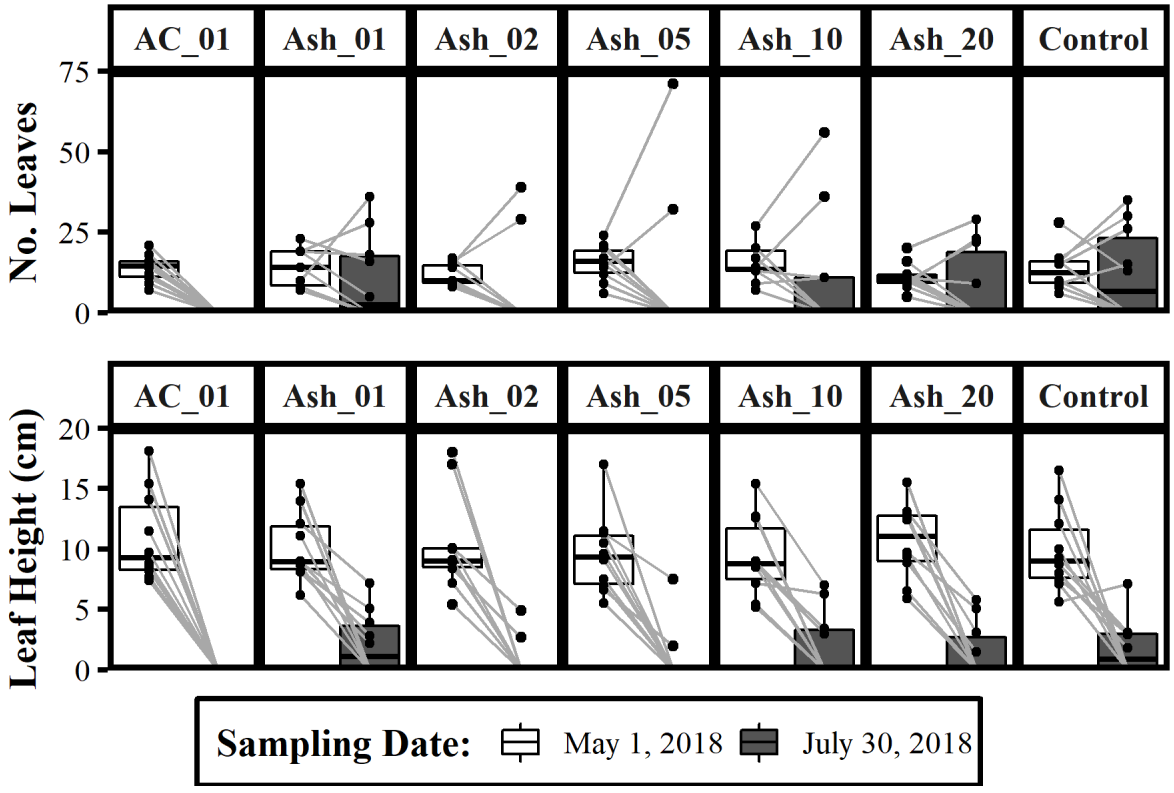

Figure A.2: Measurements of number of leaves (top) and leaf height (bottom) for each treatment taken on the planting date and the harvest date (N = 10 for each treatment). Points connected by lines are indicative of individuals change in either leaf height or number of leaves.

DISCUSSION

This experiment acted as a showcase for the difficulty involved in maintaining a field trial with transplanted grasses. Only 22 of the 70 original transplanted individuals survived to the end of the experiment despite efforts to promote establishment in the field. When the plugs were grown in the greenhouse, they were subjected to constant and ideal growing conditions. Likely, the temperature fluctuation observed in the field played a critical role in the survival of the individuals. Official climate records indicate that the lowest temperature reached between May 1 and July 30, 2018 was on May 10, 2018 at 4:00 AM where the temperature dropped to 5.3°C, and the highest temperature was on July 30, 2018 at 3:00 PM where the temperature reached 37.8°C at the Kamloops Airport (LaZerte & Albers, 2018). The greenhouse germinated *F. campestris* plugs may not have exhibited phenotypic plasticity due to their controlled greenhouse climate resulting in many of the plugs initially dying. The soils that the plugs were grown in initially was a nutrient rich potting soil and there was no competition for resources from other plant species. This further extends the argument that the plugs were not well suited for the environment that they would be planted in: soil nutrients are likely to change and competition is introduced. These variables were not considered in the initial design and lead to the poor results of this experiment.

There were no significant differences found in biomass between the different treatment groups. In this field application, this indicates that there were no detrimental effects of any of the soil amendments. Due to the very small individuals that managed to survive to the end of this experiment, it was impossible to recover any belowground biomass. Perhaps the soil amendments used in this trial impacted the root networks of the *F. campestris* individuals. Future studies applying a field use of ash or activated carbon should attempt to create a design using established natural plants as opposed to transplanting individuals. In this manner, such an experiment could potentially highlight some effectiveness of ash as a tool for restoration purposes. Indeed, this is being done currently in the Laurie Guichon Memorial Grasslands by another student of the Fraser Lab (Hampton, 2020). In work being completed by Whitehouse, attempts to limit the spread of *C. stoebe* are being made by applying varying concentrations of ash and herbicides to a heavily invaded area. Other

studies on the remedial effects of ash are being conducted in many parts in Canada as well through a program called AshNet (Emilson et al., 2018).

No significant differences in leaf height or number of leaves were found between treatments at either the time of planting or harvesting. During planting, this was expected as it shows that there was no bias towards any treatment for those variables. The harvesting data was not significantly different between treatments either, though this is not likely what was expected. The data shows that compared to a control, none of the soil amendments help to create either a beneficial or harmful environment for *F. campestris*. This is very contradictory to what is generally described in literature, as well as contradictory to the results of the greenhouse study. It is very likely that the individuals that were planted became stressed and had limited capacity to thrive in a shockingly different environment from standard greenhouse conditions, especially at such a young life stage. Given more time and resources, the application of ash and AC would have been better completed on a broader scale on established plants in the field.

The significant differences in leaf height were all within treatments between the time at planting and the time of harvest, where the leaf height decreased over time. In a more rigorous design, we might have expected other results; however, the fact that all treatments including the control experienced decreases in leaf height over time indicate that there were flaws in the implementation of the field experiment.

The only significant difference in the number of leaves was found within the AC treatments between planting and harvest. This may indicate some effect of AC on the number of leaves; however, the data that exists for the 22 individuals that survived across the 7 treatments show sporadic results in all other treatments. The reason we see a significant difference in the number of leaves of the AC treatment is because all individuals that received the AC treatment died in the field. Unfortunately, since many other individuals of other treatments also died, I cannot rule out the possibility that the reason that all individuals of the AC treatment died was random.

Field implementations of greenhouse projects are difficult to carry out; however, when executed properly they can have some powerful results should they share similar

results to the greenhouse portion. This experiment was an attempt to follow that process; however, the transplanting procedure incurred too much stress to the planted individuals. Due to the low number of survived individuals, there is no true significance to the data. Rather, this portion serves as a record of my attempt at a field study and the statistical methods that could be applied had it been successful.

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Appendix B: Data Tables

Table B.1: X, Y coordinates for the sampling locations of each site in the Lac Du Bois GPA. The coordinate reference system used is NAD 1983 BC Environment Albers (EPSG 3005).

Invaded Site ID	X	Y	Pristine Site ID	X	Y
Inv_01	1390732.1578	656644.9089	**Pri_01**	1390786.1589	656640.2280
Inv_02	1390740.7013	656647.7801	**Pri_02**	1390812.8783	656610.1828
Inv_03	1390748.3212	656654.0467	**Pri_03**	1390822.1066	656593.3971
Inv_04	1390775.4742	656631.4404	**Pri_04**	1390835.5155	656556.3348
Inv_05	1390776.6700	656620.5521	**Pri_05**	1390819.5016	656549.8116
Inv_06	1390793.4424	656611.4967	**Pri_06**	1390784.4188	656543.2845
Inv_07	1390791.3640	656600.2051	**Pri_07**	1390764.6073	656562.8504
Inv_08	1390786.2598	656595.1902	**Pri_08**	1390761.4072	656574.9540
Inv_09	1390778.7992	656591.8181	**Pri_09**	1390743.3708	656594.0062
Inv_10	1390761.0764	656609.7199	**Pri_10**	1390706.4964	656620.5365

Table B.2: X, Y coordinates of the soil temperature monitors used to gather ground temperature data. The coordinate reference system used in NAD 1983 BC Environment Albers (EPSG 3005).

Site ID	Site Association	X	Y
Site 17	Pristine	1390886.9596	656607.2115
Site 18	Pristine	1390877.8708	656516.0344
Site 19	Invaded	1390792.3790	656569.6235
Site 20	Invaded	1390750.6190	656633.3511

Table B.3: Plant inventory for the sampled pristine and invaded areas, and respective mean cover values in top-down percent (N = 10). A value of *NULL* indicates that the plant was not found in any of the plots. Bare ground and litter covers were estimated via ground cover measurements rather than top-down. Invasive species are denoted with an asterisk prepended to their scientific names. All scientific and common names were retrieved from Antos et al. (2018).

Scientific Name	**Common Name**	**Mean cover in pristine soils (%)**	**Mean cover in invaded soils (%)**
Achillea millefolium	Yarrow	4	1
Agropyron spicata	Bluebunch wheatgrass	0.4	*NULL*
Androsace septentrionalis	Fairy candelabra	*NULL*	0.3
Antennaria neglecta	Field pussytoes	0.3	0.2
Arnica fulgens	Orange arnica	6.3	0.6
Astragalus miser	Timber milk-vetch	5	2.2
Calochortus macrocarpus	Sagebrush mariposa lily	0.4	0.3
Campanula rotundifolia	Common harebell	2.4	*NULL*
Carex praegracilis	Field sedge	0.1	*NULL*
**Centaurea stoebe*	Spotted knapweed	*NULL*	86.1
Elymus glaucus	Blue wildrye	8.6	*NULL*
Erigeron corymbosus	Long-leaved daisy	0.1	*NULL*
Eriogonum heracleoides	Parsnip-flowered buckwheat	0.4	*NULL*
Festuca campestris	Rough fescue	21.7	*NULL*
Fritillaria lanceolata	Chocolate lily	*NULL*	0.1
Geranium viscosissimum	Sticky geranium	1.4	*NULL*
Hackelia micrantha	Blue stickseed	*NULL*	1.3
Heuchera cylindrica	Round-leaved alumroot	0.1	*NULL*
Juncus balticus	Baltic rush	1	*NULL*
Koeleria macrantha	Junegrass	0.4	1.5
Lathyrus nevadensis	Purple peavine	1.2	*NULL*
Lithophragma parviflorum	Small-flowered woodland star	1.3	0.8
Lithospermum ruderale	Lemonweed	0.2	0.8
Poa pratensis	Kentucky bluegrass	34.8	3.5
Sisyrinchium idahoense	Idaho blue-eyed-grass	0.3	*NULL*
Stipa comata	Needle-and-thread grass	0.1	*NULL*
Stipa occidentalis	Stiff needlegrass	0.7	*NULL*
Stipa richardsonii	Spreading needlegrass	5.9	*NULL*
Taraxacum officinale	Common dandelion	0.2	*NULL*
**Tragopogon dubius*	Yellow salsify	0.5	1.4
Zigadenus venenosus	Meadow death-camas	0.3	*NULL*
	Unknown	0.1	*NULL*
	Bare Ground	0.7	11.3
	Litter	40.9	16.3