Unexpected Responses in Ecologically Based Weed Management

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Abstract

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Spotted knapweed (*Centaurea stoebe L.*) infestations have threatened western rangelands and grasslands since the late 1800s when it was introduced to North America in ship ballast and alfalfa seed. Traditional invasive plant management has relied on eradicating the plants with herbicide only, rather than addressing the fundamental ecological principles that allow these invasions to establish and re-establish. Field trials were established in the Marquart West pasture at the Laurie Guichon memorial grassland in the Lundbom Commonage approximately 10 km east of Merritt, British Columbia (BC), Canada. Our study used a split-plot experiment design to test combinations of herbicide, wood ash soil amendment and grass seeding to control knapweed and restore desirable grasses in a highly, knapweed invaded rangeland. Herbicide was the main plot, wood ash (0 Mg ha^{-1}, 1 Mg ha^{-1} and 10 Mg ha^{-1}) were the sub-plots and seeded species were *Pseudoroegneria spicata*, *Thinopyrum intermedium* and *Agropyron cristatum* in sub-sub-plots.

Results after two years determined that herbicide and ash interactions significantly increased *Bromus tectorum, Poa pratensis* and *Koeleria macrantha.* Herbicide significantly decreased spotted knapweed cover, and there were no significant differences in any treatments where herbicide was not present. Herbicide application significantly increased soil nitrate but those results diminished after time. Herbicide treated plots with ash had significantly less plant available nitrogen than the herbicide and no ash plot, suggesting ash did lead to immobilization of nitrate. Seeded grass species did not have any significant establishment during this study; we did see a noticeable increase in *P. pratensis* and *K. macrantha* which were not seeded. This increase was significant only in the herbicide/high ash treatment which suggests that suppression of knapweed was necessary and that wood ash has fertilization properties. Herbicide was the only treatment that significantly decreased spotted knapweed but it lead to secondary invasion of *B. tectorum*. One explanation is the increased soil nitrogen from the herbicide treated knapweed as annual grasses are able to rapidly take up soil nitrogen. This study provided important insight into managing highly invaded semi-arid rangeland ecosystems.

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1 Introduction

With the marvels of a globalized world came the ability for invasive species to travel around the planet at record speeds, calling for the International Union of Conservation of Species to list invasive species as the second greatest threat to biodiversity (International Union for Conservation of Nature, 2021) Spotted knapweed (*Centaurea stoebe L.*) infestations have threatened western rangelands and grasslands since the late 1800s (Watson and Renney, 1974; Strang et al., 1979), when it was introduced to North America in ship ballast and alfalfa seed. The lack of natural predators in North America combined with its deep taproot, extensive seed production and allelopathic chemicals have allowed it to proliferate across the north west, displacing native vegetation and threatening biodiversity (Jacobs and Sheley, 2013). Traditional invasive plant management has relied on eradicating the plants with herbicide only, rather than addressing the fundamental ecological principles that allow these invasions to establish and re-establish (Krueger-Mangold et al., 2006).

1.1 Invasion Ecology and a New Framework

Invasions by invasive plants have been commonly viewed through a reductionist paradigm which aims to determine the single key mechanism that will unlock our understanding of the invasion process (Foxcroft et al., 2011). Several attempts have been made to generalize invasion ecology to establish a model in which to categorize and understand the invasion process, (Richardson et al., 2000; Cadotte et al., 2006; Richardson and Pysek, 2006). Foxcroft et al. (2011) explored these and proposed a new framework using three conceptual tools to expose the mechanisms of invasion and to integrate across species, ecosystems and scales. They argued that complex systems have multiple drivers, different spatial scales, variation over time and using a framework or model that is insensitive to these components will not be able to generate conclusions. Their proposed model addresses these needs by dividing invasions into three contributing processes: (1) species characteristics or traits, (2) system context and (3) habitat susceptibility. Species characteristics are the plant traits of a potential invader that can regulate how invasive it may be. System context focuses on influences arising outside the habitat, such as the proximity of transportation corridors that can easily move invaders from one location to another. Lastly, habitat susceptibility (e.g., soil properties, climate) refers to the aspects of the receiving environment that either promote or impede the invasion process and are limited to the actual location of the potentially invadable area (Foxcroft et al., 2011). The next section will further explain the parts of the framework while applying it to a spotted knapweed site in the interior of British Columbia, Canada.

Applying the framework to *Centaurea stoebe* in a BC grassland.

The Laurie Guichon Memorial Grassland Interpretive Site (LGMGIS) in the Lundbom Commonage is an ecologically, culturally and historically important grassland in the southern interior of British Columbia, Canada (Figure 1.1) that has been continually invaded by spotted knapweed since the early 1990s. The framework proposed by Foxcroft et al. (2011) is useful in displaying the interrelatedness among invasion factors and to structure a list of the mechanisms contributing to this invasion.

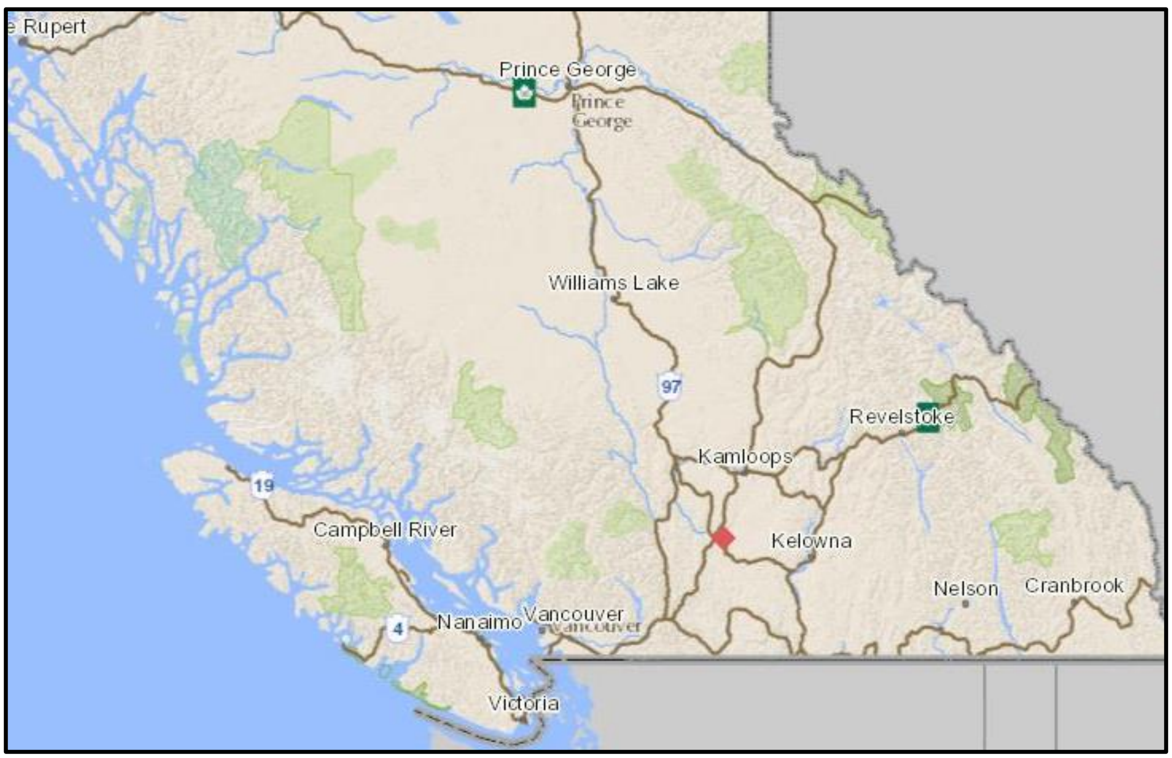

Figure 1.1. Map of Southern BC showing geographic location of study area (Represented by red diamond). Map source: iMapBC 2021.

Species traits: Spotted knapweed is a tap-rooted short-lived perennial, with a potential lifespan of up to nine years. It is dependent on seeds for spread, which can remain viable in the soil for eight or more years (NRCS, 2007). Seed production of spotted knapweed on a western Montana, Idaho fescue habitat type ranged from 1,000 to 7,800/m^2 and was reported as high as 40,000/m^2 in Washington (USDA, 2015). In addition to seed, radial expansion of populations through peripheral enlargement of stands can be as much as a few meters per year (USDA, 2015). Seeds can be transported by animals, birds, the undercarriage of vehicles, mud and humans (Sheley et al., 1998). The final major weapon employed by spotted knapweed is its production of the allelopathic chemical catechin which has been found to suppress the growth of some plants (USDA, 2015).

Systems context: The LGMGIS is located adjacent to a main highway from the coast to the interior of the province. Not only is it a popular place to stop for commuters travelling north, it is also a common recreational destination for recreational off-road vehicles, bike riding, horseback riding, fishing and camping. There are multiple roads leading into and through the site making travel easy and extensive. It has been a historical spot for community grazing, with multiple ranchers moving cattle through with high stocking rates.

Habitat susceptibility: Historic livestock overstocking of this range has resulted in long term overgrazing and therefore degradation of an already sensitive grassland ecosystem (Grassland Conservation Council, 2019). Overgrazing has been shown to result in low native species diversity, and increasing potential for invasion by non-native plant species (Maron and Marler, 2007). Spotted knapweed is native to central Europe and east to central Russia and is most prolific in the forest steppe and mesic soils (Sheley et al., 1998) which may be a similar habitat type to the LGMGIS. The absence of natural predators or grazing animals that prefer forbs on the LGMGIS likely accelerate the invasion potential as cattle and deer select for grasses, and accelerate knapweeds ability to move into those openings.

The practical outcome of a framework is to develop a management plan by listing all the possible mechanisms and the roles they play to ensure that the plan is multidimensional and sustainable.

1.2 Herbicide Safety and Limitations

Invasive plant control remained a relatively minor phase of agrology until the 1950s despite research involving inorganic herbicides beginning in the 1890s (Timmons, 2005). The 1960s saw the release of many new herbicides and their use has remained steady until now (Timmons, 2005). Despite the consistent use of herbicides for almost 60 years, there is recent questioning of their safety. In 2018, Monsanto was ordered by a judge in California to pay $289 Million to a man who alleged that his cancer was caused by using the popular glyphosate product Roundup™. Despite numerous studies concluding that glyphosate is not carcinogenic, highly publicised cases like this add to the growing public perception that “chemical” is synonymous with dangerous. Unfortunately this can then restrict the use of herbicides, a key method for invasive plant control.

Although herbicides are needed in the invasive plant management toolbox, they present several limitations that have not been addressed in typical invasive species management plans. First, in natural systems, simply lowering pest abundance does not directly translate to ecosystem recovery as numerous factors can inhibit ecosystem recovery following the reduction or removal of a dominant invader (Pearson et al., 2016). Legacy effects such as alteration of soil chemical properties, disturbance regimes or reduced native propagules can all hinder native plant establishment and overall ecosystem recovery in natural systems (D'Antonio and Vitousek, 1992). Another limitation to the reliance of herbicides is the phenomenon of secondary invasion. The most relevant to our location is the large-scale suppression of spotted knapweed which has resulted in secondary invasion of cheatgrass (*Bromus tectorum*), an introduced invader with an even greater threat to native ecosystems than spotted knapweed (Pearson et al., 2016). Currently, public land managers in British Columbia are hesitant to address large knapweed population due to the threat of secondary invasion (Personal communication Val Miller, 2018).

Resistance of plants to chemical herbicides is a concerning reality of this century. Much like over-prescription of antibiotics to humans and animals creating “superbugs” in humans and animals, a consistent regime of herbicides has lead to the first reports of herbicide resistance. Mangin and Hall (2016) reported the first documented instance of spotted knapweed resistance to auxinic herbicides in a managed rangeland in East Kootenay, BC. They conducted greenhouse studies with the resistant population and found that it took 32 times the recommended label rate of clopyralid to control spotted knapweed. Resistance to auxinic herbicides will further limit control options for spotted knapweed and leave up to 10 million hectares of western Canada at risk (USDA, 2015). Herbicide resistance is caused from repeated use of the same active ingredient in a herbicide on the same population. While it is often suggested in the best practices to implement a rotation of herbicide types, there is no regulation to ensure it.

A new issue that has surfaced is the efficacy of herbicide with current and future climate change projections. Only a fraction of the thousands of global noxious weeds have been studied, but there is sufficient evidence that increasing atmospheric CO_2 will lower the efficacy of herbicides (Waryszak et al., 2018); however, this level is species-specific and so far has only been studied for glyphosate. This increase in herbicide tolerance is thought to be due to biochemical changes; plants might reduce stomatal conductance and decrease their stomata number, lowering the uptake of glyphosate (Ainsworth and Long, 2005). It can also be attributed to an increase in leaf and wax thickness (Hikosaka and Shigeno, 2009). As climate change predictions include increases of weed populations and these same changes include less overall efficacy of herbicide control - either through application rates or through plant biology - it is crucial to understand how to adapt to these changes in a world that has over relied on herbicides for invasive plant control (Ziska, 2016).

1.3 Carbon Additions and Their Effect on Soil Chemistry

One of the factors thought to be attributed to spotted knapweeds' invasion success is its production of the allelopathic chemical (+-)-catechin secreted through the plant roots. Catechin is highly variable in its effects on surrounding plants, showing great site to site variability, plant species variability and production rates in plants, and studying these differences is complicated further as catechin is difficult to quantify in studies (Pollock et al., 2009). Many have argued that studies looking at catechin are unrepeatable and a uniform scientific opinion has yet to be formed. That has not prevented many scientists from looking at ways to mitigate the effects of catechin. Thorp et al. (2009) determined that catechin had strong negative effects on North

American native plants (*Pseudoroegneria spicata* and *Zigadenus elegans*) compared to plants from its host range. Ridenour and Callaway (2000) studied the allelopathic relationship between spotted knapweed and *Festuca idahoensis* (Idaho fescue), a common bunchgrass in the Interior Douglas Fir zone, and found that Idaho fescue decreased dramatically with increases of spotted knapweed and its allelochemical, but found that these effects were ameriolated with the addition of activated carbon (AC) in the growing medium. Several other studies have used activated carbon to neutralize the effects of allelopathy in soils (Schreiner and Reed, 1907; Mahall and Callaway, 1992; Ridenour and Callaway, 2001) while others argue that these studies could not be repeated and therefore Idaho fescue was not as sensitive to catechin as previously reported (Blair et al., 2006). Nevertheless, despite the inconsistencies in research it is still likely that spotted knapweed displays some form of allelopathy which can be neutralized by the addition of AC.

Activated carbon can also decrease soil nitrogen (N) and phosphorus mineralization rates and reduce the availability of those nutrients to plants (Kulmatiski and Beard, 2006). This can remove the advantage of fast growth rates that exotics use to overcome natives. Biochar is a similar material to AC formed by thermochemical conversion of sustainably sourced biomass, generally used for agricultural applications (Hagemann et al., 2018). Biochar studies have found other positive effects on soil such as increased water holding capacity and bulk density, improved hydraulic conductivity and liming effects (Karim et al., 2020); however, the economics of the biochar soil amendment is usually a prohibiting factor in making its use widespread practice. In 2020, 1 cubic ft of landscape grade biochar was listed at $52.00 through the Canadian supplier AgriChar, which makes it cost prohibitive to land managers on a large scale. Other properties of ash are determined by the species of tree, amount of bark, conditions of growth, contamination and conditions of the burn (Park et al., 2005). Due to the variability of physical, chemical and biological properties of biochar and wood ash, it is difficult to predict plant and ecosystem response to application of either of these materials (Blackwell et al., 2009).

Some studies have attributed soil N availability to the performance of non-indigenous (i.e. invasive species) relative to indigenous species (Huenneke et al., 1990; Milchunas and Lauenroth, 1995). Blicker et al. (2001) attributed the success of spotted knapweed to its greater or more rapid use of soil N compared to native grasses. Their greenhouse experiment found that spotted knapweed removed more N from the soil compared to bluebunch wheatgrass and *Agropyrum smithii* (western wheatgrass). An explanation for this result is that these grasses typically evolved in N limited ecosystems, suggesting that they have a lower rate of growth as an adaptation to lower nitrogen concentrations in the soil. Mandre et al. (2006) and Gomez-Rey and Madeira (2012) showed a decrease in N with ash application; therefore, wood ash may be an effective way to reduce N availability in the soil, thus favouring slow-growing native grasses

1.4 Seedbanks and Reseeding for Weed Management

Two of the limitations in herbicide dominated weed control regimes are a seedbank without native propagules and, secondary invasions. Both of these problems can be mitigated by including seeding activities in an invasive plant management plan. It has long since been accepted that rangeland invasive plant management goals need to include the establishment of an ecologically diverse plant community that is relatively resistant to invasion by non-native plants (Krueger-Mangold et al., 2006) but land managers are often reluctant to attempt revegetation because of the high costs and probability of failure (Sheley et al., 1999). Therefore, a crucial element of research in the field of invasive plant management is the need for more locally adapted methods for increasing the success of reseeding activities in a range of soil and climatic conditions.

Sheley et al. (1999) looked at the success of *Thinopyrum intermedium* (intermediate wheatgrass) establishment in spotted knapweed infestations seeded at much higher rates than those typically recommended. As the recommended seeding rates for intermediate wheatgrass range from 10 to 12 pounds pure live seed (PLS) or approximately 200 to 250 seeds m^{-2} (Hybner and Jacobs, 2012), their study found that no intermediate wheatgrass established at rates under 500 seeds m^{-2}. The high failure rates of reseeding activities may be attributed to using the currently recommended seeding rates which are most likely based around a bare or weed-free seed bed. Establishment success could be improved in other species by increasing seeding density. A study by Jacobs et al. (1996) found that increasing the density of bluebunch wheatgrass seedlings from 200 plants m^{-2} to 1000 plants m^{-2} allowed it to be more competitive than spotted knapweed.

Several examples of success have been recorded when reseeding with *Agropyron cristatum* (crested wheatgrass) in invaded rangelands (Hubbard, 1975; Benz et al., 1999; Cox, 2009), however the results were often a monoculture and therefore not in line with the usual goals of ecological restoration. Carpinelli et al. (2004) looked at ways to manipulate the 3 basic components of succession (site availability, species availability and species performance) to "capture" a site from cheatgrass using crested wheatgrass. They then studied the emergence of seeded desirable natives on these captured sites and found that they had much greater success of establishment in these crested wheatgrass areas than the cheatgrass dominated areas. This suggests a two-step approach to restoring native diversity using assisted ecological succession where one first converts a site from annual to perennial domination and then inserts native species into the stable perennial matrix (Carpinelli et al., 2004). This shows that using crested wheatgrass as a reclamation species can be warranted if native species are part of the future prescription.

Another key piece of a holistic approach to weed management is choosing reclamation species that have some natural ability to resist the characteristics of invasions. It is possible to prescribe a seeding regime to mitigate some of the previously discussed residual effects of long-term invasive species infestations. Perry et al. (2005) looked at grassland species for revegetation in spotted knapweed invaded areas, specifically those that were resistant to catechin. They examined 23 common grassland species used in reclamation or found growing within spotted knapweed populations and sought to identify a suite of species most likely to be resistant to catechin. *Bromus carinatus*, *Grindellia squarrosa* and *Hesperostipa comata* were found to be highly resistant, while *Astralagus cicer* and *Leymus cinereus* were resistant (based on length of root elongation). They also found a positive correlation between seed size and catechin resistance. This was one of the few studies done to determine species specific resistance to catechin and it is worth pursuing future studies due to the high variability in field conditions and catechin concentrations in real world situations.

My thesis is organized into four chapters; Chapter 1 is the introduction, Chapter 2 investigates the vegetation response to the experimental control of spotted knapweed, Chapter 3 investigates the soil chemistry response, and Chapter 4 explores the management implications and recommendations I have drawn from my study. Chapters 2 and 3 are based on a field experiment in LGMG, where I tested herbicide use, wood ash additions, and seed sowing. Since Chapters 2 and 3 are results from the same experimental design, I will simply refer to the Chapter 2 methodology in Chapter 3.

1.5 References

Ainsworth, E., & Long, S. 2005. What Have We Learned from 15 Years of Free-Air CO2 Enrichment (FACE)? A Meta-Analytic Review of the Responses of Photosynthesis, Canopy Properties and Plant Production to Rising CO2. The New Phytologist, 165(2), 351-371.

Benz, L. J., K. G. Beck, T. D. Whitson, and D. W. Koch. 1999. Reclaiming Russian knapweed infested rangeland. Journal of Range Management 52:351–356.

Blackwell, P., G. Riethmuller and Collins. M. 2009. Biochar Application to Soil pp.207- 226 in Lehmann, J. and S. Joseph. (ed.) Biochar for Environmental Management Science and Technology. Earthscan, London, Sterling. 416 pp.

Blicker, P. S., Olson, B. E., and Engel, R. 2002. Traits of the invasive *Centaurea maculosa* and two native grasses: Effect of N supply. Plant and Soil, 247(2), 261–269. http://doi.org/10.1023/A:1021596627967

Cadotte, M.W., McMahon, S.M., Fukami, T., 2006. Conceptual Ecology and Invasion Biology: Reciprocal Approaches to Nature. Springer, Dortrecht, The Netherlands.

Richardson, D.M., Pyˇsek, P., 2006. Plant invasions: Merging the Concepts of Species Invasiveness and Community Invasibility. Prog. Phys. Geog. 30, 409–431

Carpinelli, M. F., Sheley, R. L., and Maxwell, B. D. 2004. Revegetating Weed-Infested Rangeland with Niche-Differentiated Desirable Species. Journal Of Range Management, 57(1), 97–105. http://doi.org/10.2111/1551-5028(2004)057

Cox, R. D. 2009. Increasing Native Diversity of Cheatgrass-Dominated Rangeland through Assisted Succession. Society for Range Management 57(2), 203–210.

D'Antonia, C.M and Vitousek, P.M 1992. Biological Invasions by Exotic Grasses, the Grass/Fire Cycle, and Global Change. Annual Review of Ecology and Systematics, 23.1, 63-87

Foxcroft, L. C., Pickett, S. T. A., and Cadenasso, M. L. 2011. Expanding the Conceptual Frameworks of Plant Invasion Ecology. Perspectives in Plant Ecology, Evolution and Systematics, 13(2), 89–100. http://doi.org/10.1016/j.ppees.2011.03.004

Grassland Conservation Council. 2019. Laurie Guichon Memorial Grassland Interpretive Site Baseline Inventory. https://bcgrasslands.org/wp-content/uploads/2019/07/lgmgis-baseline-inventory-july-19-2019-1.pdf

Hagemann, N., Subdiaga, E., Orsetti, S., María de la Rosa, J., Knicker, H., Schmidt, H., Kappler, A., Behrens, S. 2018. Effect of biochar amendment on compost organic matter composition following aerobic composting of manure. Science of The Total Environment 613–614, 20-29. https://doi.org/10.1016/j.scitotenv.2017.08.161.

Hikosaka, K. and Shigeno, A. 2009. The Role of Rubisco and Cell Walls in the Interspecific Variation in Photosynthetic Capacity. Oecologia, 160(3), 443-451. Retrieved from http://www.jstor.org/stable/40310091

Hubbard, W. A. 1975. Increased Range Forage Production by Reseeding and the Chemical Control of Knapweed. Journal of Range Management, 28(5), 406–407.

Huenneke, L. F., Hamburg, S. P., Koide, R., Mooney, H. A., and Vitousek, P. M. 1990. Effects of Soil Resources on Plant Invasion and Community Structure in Californian Serpentine Grassland. Ecology, 71(2), 478–491. http://www.jstor.org/stable/1940302

Hybner, RM and Jacobs, J. 2012 Intermediate Wheatgrass (*Thinopyrum intermedium L*.): An Introduced Conservation Grass for Use in Montana and Wyoming. United Sates Department of Agriculture. Plant Material Technical Notes.

International Union for Conservation of Nature. 2021. IUCN Brief. Invasive Species and Climate Change. https://www.iucn.org/sites/dev/files/ias_and_climate_change_issues_brief_2021.pdf

Jacobs, J. S., Sheley, R. L., and Maxwell, B. D. 1996. Effect of Sclerotinia sclerotiorum on the Interference between Bluebunch Wheatgrass (*Agropyron spicatum*) and Spotted Knapweed (*Centaurea maculosa*). Weed Technology, 10(1), 13–21.

Jacobs, J. S., and Sheley, R. L. 2013. Observation : Life History of Spotted Knapweed Observation. Society for Range Management, 51(6), 665–673.

Karim, R., Halim, A., Gale, N.V., Thomas, S.C. 2020. Biochar Effects on Soil Physiochemical Properties in Degraded Managed Ecosystems in Northeastern Bangladesh. Soil Systems. 4(69); http://doi:10.3390/soilsystems4040069.

Krueger-Mangold, J. M., Sheley, R. L., & Svejcar, T. J. 2006. Toward ecologically-based invasive plant management on rangeland. Weed Science, 54(3), 597–605. https://doi.org/10.1614/WS-05-049R3.1

Kulmatiski, A., & Beard, K. H. 2006. Activated carbon as a restoration tool: Potential for control of invasive plants in abandoned agricultural fields. Restoration Ecology, 14(2), 251–257. http://doi.org/10.1111/j.1526-100X.2006.00127.

Mahall B. E. and Callaway R. M. 1992. Root Communication Mechanisms and Intracommunity Distributions of two Mojave Desert Shrubs. Ecology 73, 2145–2151.

Mandre, M., Pärn, H., Ots, K., 2006. Short-term effects of wood ash on the soil and the lignin concentration and growth of Pinus sylvestris L. Forest Ecology and Management, 223, 1–3. https://doi.org/10.1016/j.foreco.2005.11.017.

Mangin, A. R., and Hall, L. M. 2016. First report: spotted knapweed (*Centaurea stoebe*) Resistance to Auxinic Herbicides. Canadian Journal of Plant Science, 96(6), 928–931. http://doi.org/10.1139/cjps-2016-0008

Mangold, J. M. and Sheley, R. L. 2008. Controlling Performance of Bluebunch Wheatgrass and Spotted Knapweed Using Nitrogen and Sucrose Amendments. Western North American Naturalist, 68(2), 129–137. http://doi.org/10.3398/1527-0904(2008)68

María Gómez-Rey, Madeira, Coutinho. 2012. Wood ash effects on nutrient dynamics and soil properties under Mediterranean climate. Annals of Forest Science, Springer Nature (since 2011)/EDP Science. 69 (5), pp.569-579. ff10.1007/s13595-011-0175-yff. ffhal-00930746f

Maron, J., and Marler, M. 2007. Native Plant Diversity Resists Invasion at Both Low and High Resource Levels. Ecology, 88(10), 2651–2661. https://doi.org/10.1890/06-1993.1

Milchunas D.G and Lauenroth, W. 1995. Inertia in Plant Community Structure: State Changes after Cessation of Nutrient Enrichment Stress. Ecological Applications, 69(1), 40–45.

Natural Resource Conservation Services (NRCS). 2007. Brush Management – Invasive Plant Control Spotted Knapweed. https://www.nrcs.usda.gov. Accessed 04/20/2021.

Park, B. B., Yanai, R. D., Sahm, J. M., Lee D. K. and Abrahamson, L. P. 2005. Wood Ash Effects on Plant and Soil in a Willow Bioenergy Plantation. Biomass & Bioenergy 28 (4): 355-365.

Pearson, D. E., Ortega, Y. K., Runyon, J. B., & Butler, J. L. 2016. Secondary invasion: The bane of Weed Management. Biological Conservation, 197(May), 8–17. http://doi.org/10.1016/j.biocon.2016.02.029

Perry, L. G., Johnson, C., Alford, É. R., Vivanco, J. M., & Paschke, M. W. 2005. Screening of Grassland Plants for Restoration after Spotted Knapweed Invasion. Restoration Ecology, 13(4), 725–735. http://doi.org/10.1111/j.1526-100X.2005.00092.x

Pollock, J. L., Callaway, R. M., Thelen, G. C., & Holben, W. E. 2009. Catechin-Metal Interactions as a Mechanism for Conditional Allelopathy by the Invasive Plant *Centaurea maculosa*. Journal of Ecology, 97(6), 1234–1242. http://doi.org/10.1111/j.1365-2745.2009.01553.

Richardson, D. M. and P. Pysek. Plant invasions: merging the concepts of species invasiveness and community invasibility. Progress in Physical Geography30(3):409-431. http://doi.org/10.1191/0309133306pp490pr

Richardson, D. M., Pysek, P., Rejmánek, M., Barbour, M. G., Dane, F., & West, C. J. 2000. Naturalization and Invasion of Alien Plants : Concepts and Definitions. Diversity and Distribution, 6(2), 93–107. http://doi.org/10.1046/j.1472-4642.2000.00083.

Ridenour, W. M., & Callaway, R. M. 2001. The relative importance of allelopathy in interference: The effects of an invasive weed on a native bunchgrass. Oecologia, 126(3), 444–450. http://doi.org/10.1007/s004420000533

Schreiner O. and Reed H. S. 1907. The Production of Deleterious Excretions by Roots. Bull Torrey Botany Club 34, 279–303

Sheley, R. L., Jacobs, J. S., and Velagala, R. P. 1999. Enhancing intermediate wheatgrass establishment in spotted knapweed infested rangeland. Journal of Range Management, 52(1), 68–74. http://doi.org/10.2307/4003494

Sheley, R. L., Jacobs, J. S., Carpinelli, M. F., and Technology, W. 1998. Distribution , Biology , and Management of Diffuse Knapweed (*Centaurea diffusa*) and Spotted Knapweed (*Centaurea maculosa*). Weed Technology, 12(2), 353–362. http://doi.org/10.1007/s10021-001-0101-5

Strang, R. M., Lindsay, K. M. and Price R.S. 1979. Knapweed: British Columbia's Undesirable Aliens. Rangelands. 1:141-143

Thorpe, A.S., Thelen, G.C., Diaconu, A. and Callaway, R.M. 2009. Root exudate is allelopathic in invaded community but not in native community: field evidence for the novel weapons hypothesis. Journal of Ecology, 97: 641-645. https://doi.org/10.1111/j.1365-2745.2009.01520.x

Timmons, F. 2005. A History of Weed Control in the United States and Canada. Weed Science, 53(6), 748-761. doi:10.1614/0043-1745(2005)053[0748:AHOWCI]2.0.CO;2

Jacobs, J. 2006. Ecology and Management of Spotted knapweed. USDA-NRCS Montana State Office, 1.15p.
https://www.nrcs.usda.gov/Internet/FSE_PLANTMATERIALS/publications/mtpmctn13098.pdf

USDA. 2015. Species profile:spotted knapweed (Centaurea maculosa L.).
http://www.invasivespeciesinfo. gov/plants/spotknapweed.shtml

Waryszak, P., Lenz, T. I., Leishman, M. R., and Downey, P. O. 2018. Herbicide Effectiveness in Controlling Invasive Plants under Elevated CO2: Sufficient Evidence to Rethink Weeds management. Journal of Environmental Management, 226(July), 400–407.
http://doi.org/10.1016/j.jenvman.2018.08.050

Watson, A.K and A.J. Renney. 1974. The biology of Canadian weeds. 6. *Centurea diffusa* and *Centaurea maculosa*. Canadian Journal of Plant Science. 54:687-701.

Ziska, L. H. 2016. The Role of Climate Change and Increasing Atmospheric Carbon Dioxide on Weed Management: Herbicide Efficacy. Agriculture, Ecosystems and Environment, 231, 304–309. http://doi.org/10.1016/j.agee.2016.07.014

2 Rangeland Plant Responses to Herbicides, Seeding and Soil Amendment

2.1 Introduction

Invasive plants are a global threat to agricultural and natural ecosystems (Pimentel et al., 2005) and must be a significant consideration in efforts to enhance global food security, maintain biodiversity and reduce environmental degradation (Murray et al., 2012). The impacts of invasive plants on our ecosystems and our lives are widespread and unique to location but range from decreasing bird populations (Grzędzicka and Reif, 2020), preventing forest regeneration (Langmaier and Laplin, 2020) to potentially causing cancer and liver damage in human (Luchetti et al., 2016). *Centaurea stoebe* (Spotted knapweed) is of particular concern to Pacific Northwest rangelands and is heavily distributed across southern BC (Invasive Alien Plant Program, Government of BC). Coordinated efforts have been in place in British Columbia to control spotted knapweed since 1970's (Maxwell et al., 1992) but in the present day we are still seeking new ways to control it. Spotted knapweed invasions lead to severe forage losses that are detrimental to livestock productions and critical wildlife habitat (Maxwell et al., 1992).

Spotted knapweed is a tap-rooted perennial native to Europe and Russia with an average lifespan of 3-4 years, but can live up to nine years and reports on seed production have ranged from 1000 to 40,000 m^2 ((Natural Resources Conservation Service, 2006). The life cycle can be quite variable where plants can persist for an entire growing season as a seedling, and plants that flower one year can then remain as a rosette the following year (Natural Resource Conservation Service, 2006). While seed is the main method of spread, spotted knapweed can also produce lateral roots that sprout a new seedling up to 3 cm away (Watson and Renny, 1974). The earliest known North American collection of spotted knapweed is from Victoria, BC in 1893 (Groh, 1944) and it can now be found in most Canadian provinces (Brouillet et al., 2016). Infestations are often correlated to level of disturbance (Watson and Renny, 1974). The lack of any natural predators in North America combined with its deep taproot, extensive seed production and allelopathic chemicals have allowed it to proliferate across the north-west of North America, displacing native vegetation and threatening biodiversity (Jacobs and Sheley, 2013).

There are numerous control methods for every weed, however none of them are a silver bullet. Each year billions of dollars are spent in the United States to control invasive plant species (Westbrooks, 1998; Pimentel et al., 2005). A 2008 report for the Invasive Species Council of BC estimated the cost of damage for six combined invasive species in BC to total $65 million (ESSA Technologies et al., 2008). The main categories of weed control include chemical (herbicides), mechanical (hand pulling and mowing), cultural (targeting grazing, cover cropping) and biological (use of biocontrol agents to effect plant growth or seed production), and within each category exists entire fields of research. A meta-analysis done by Kettenring and Adams (2011) stated that despite their large sample size of literature on invasive plant control experiments (355 papers), many only had moderate restoration success. The main limitations of the studies were a lack of focus on revegetation, limited spatial and temporal scope and an incomplete evaluation of costs and benefits with frequent mentions of re-invasion or secondary invasion. Herbicide control was used for 55% of the studies, 34% investigated mechanical control and 24% studied burning. Many years before this review, Sheley and Krueger-Mangold (2003) stated, “it is becoming increasingly clear that the prescriptions for rangeland weed control are not sustainable because they treat the symptoms of the weeds rather than their cause”.

The goal of an integrated management plan is to address all the ecological factors resulting in persisting invasions in one season to reduce the burden and complications associated with retreating a site year after year. On highly degraded rangelands weed control is often short-lived because desirable species are not available to occupy niches left open by successful weed control (Kedzie-Webb et al., 2002) and therefore introducing desirable competitive plants should be a component of a management plan. In my study I looked at the interaction effects between herbicide/wood ash amendment and three separate seed mixes. Based off of relevant studies by Blumenthal et al. (2001) and Mitchel and Baker (2011) which used carbon additions to immobilize plant-available soil N necessary for invasive plants rapid growth, I tested if wood ash amendment be a viable control option.

Establishing competitive perennial grasses is another important component of restoration in spotted knapweed infested rangelands. Would grass seeding in a highly invaded rangeland be effective without the use of herbicides? Species were selected based on their potential ability to establish and persist despite a high cover of spotted knapweed . Sheley et al. (1999) suggested that revegetation with *Thinopyrum intermedium* (intermediate wheatgrass) at high density is possible and can control spotted knapweed. *Agropyron cristatum* or crested wheatgrass can be a contentious species. It is thought to be a desirable species for reclamation because it establishes quickly and can persist through disturbance and is highly productive; however, it has been criticized for having detrimental effects (Dormaar et al., 1978; Lesica and Deluca, 1996). For the third seed treatment, native species were introduced. *Pseudoroegneria spicata* or bluebunch wheatgrass was the keystone graminoid at the study site, pre-invasion, and would therefore be the most ideal species to re-establish on the site as part of a long term restoration plan. *Poa secunda* or Sandberg's bluegrass is another native species commonly found in this area where invasive cover is lower, is adapted to a range of habitat types and is considered one of the six most important grasses of the Intermountain and Pacific Northwest region (Winslow, 2013). It has not been widely studied for use in reclamation but it emerges early in the spring (Majeurs et al., 2009) giving it potential to compete with early season invasive plants. In the herbicide treated plots I wanted to quantify the effects of herbicide on spotted knapweed and non-target species, see if ash had any positive responses when used in conjunction with herbicide and which grass species would establish most aggressively.

My study aims to establish desirable perennial grasses to a long-term spotted knapweed (*Cenareau stoebe L.*) infested rangeland by addressing and manipulating soil chemistry and the seed bank to create a healthy community that is invasion resistant. Results will be measured by the increase of desirable grasses and a reduction in spotted knapweed at the site which will determine what treatment combinations were ideal for the site. There are several other questions that will be examined reductively to answer the broader research question. These are: 1) what is the effect of wood ash on soil chemical properties; 2) which grass species are ideal for withstanding knapweed invasion and allowing natural succession; and, 3) what is the quantitative role in using herbicides with these other management techniques?

2.2 Methods

Field trials were established in the Marquart West pasture at the Laurie Guichon memorial grassland in the Lundbom Commonage approximately 10 km east of Merritt, British Columbia (BC), Canada (Figure 2.1). The blocks chosen were traditionally Bluebunch wheatgrass - Idaho fescue - June grass grasslands in the Interior Douglas Fir biogeoclimatic zone which had become dominated with spotted knapweed and other invasive or introduced weeds (Llyod et al., 1990). The B.C. Soil Survey Map (BC SIFT BC Government, 2020) describes the site as dominantly well-drained orthic black chernozems developed from morainal till. They are calcareous in nature and 55% silt, 35% sand and 10% clay.

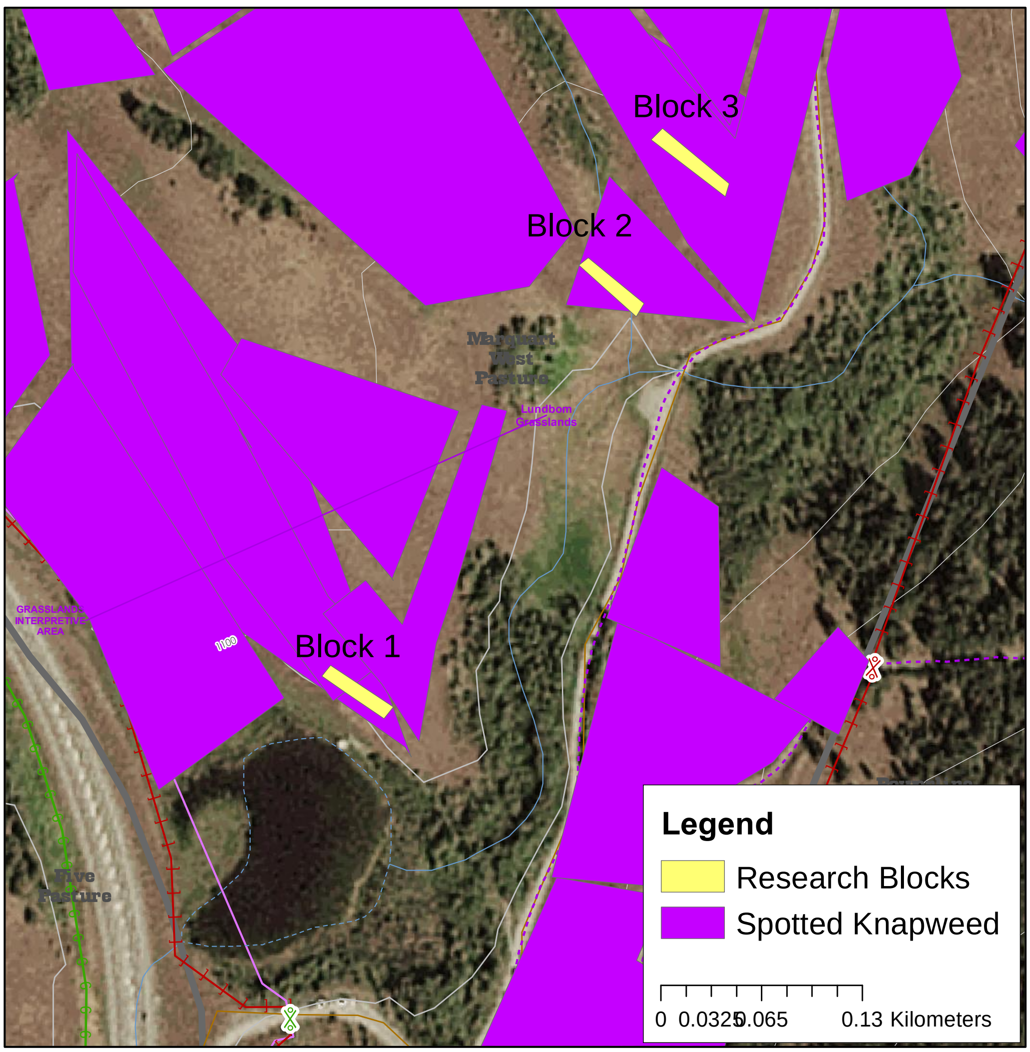

Figure 2.1 Map of research plots. The three research blocks are shown in yellow. Purple polygons indicates >50% spotted knapweed cover.

2.3 Experimental Design

The cumulative effects of several common invasive plant management methods were evaluated: herbicide, soil amendment and reseeding with different combinations of each. The research design was replicated over three locations within the site, with similar slopes, aspects and community types. A split-plot design was used with herbicide treatment being the whole plot, ash application rate being the sub-plot and seeding treatment being the sub-sub-plot. This design was chosen to prevent herbicide drift as it is difficult to accurately apply a spray to a small location. A 2 m buffer was used between the herbicide treatment plots to further prevent herbicide drift. Ash was pre-measured and applied to each plot separately by buckets and then evenly raked across the blocks. Seed treatments were randomly assigned and replicated in each sup-plot. The sub-sub-plots of each herbicide, ash and seed combination were 2x5 m.

Picloram was applied at the recommended label rate of 2.25L/ha on the sprayed plots (Figure 2.2). Soil wood ash amendments were applied at rates of 0 g/m^2 (no), 100 g/m^2 (low) and 1000 g/m^2 (high). Seed treatments consisted of 33.33% bluebunch wheatgrass, 66.66% Sandberg's bluegrass (1), 100% crested wheatgrass (2), 100% intermediate wheatgrass (3) and a control (4).

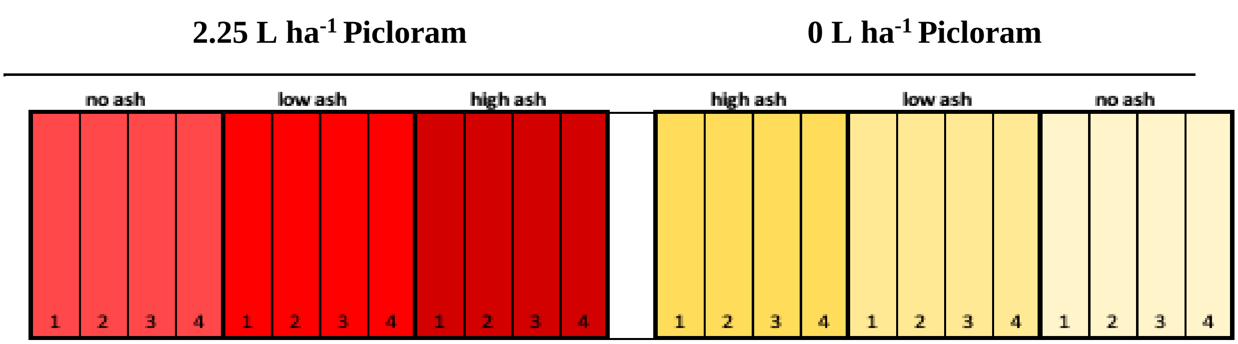

Figure 2.2 Replicated split-plot experimental design

Herbicide treatment was applied in July 2018. Wood ash was applied in October 2018 and hand spread during a cool, wind-free day. Plots were broadcast seeded by hand with a mixture of pure sand to aid in even distribution, in late November of the same year. Seeding rates for crested wheatgrass, intermediate wheatgrass, bluebunch wheatgrass and Sandberg's bluegrass were applied at rates of 2000, 2000, 1000 and 2000 seeds/m^2, respectively. Electric fencing was placed around plots to coincide with cattle turnout. Fencing was removed after cattle were taken off the range.

2.4 Sampling

At the start of the growing season of both years after treatment, 2019 and 2020 a HOBO Data Logging Rain Gauge (RG3) was installed and activated to record rainfall and temperature over the growing season. Vegetation sampling was done in mid-June, once a year starting in 2018 Baseline plant species and soil conditions of the site were sampled in the first year before any treatments were applied. Species composition was estimated by absolute foliar cover using a 0.5 m quadrat in a randomly stratified approach throughout each plot at three locations (bottom of slope, mid slope and top of slope). Visual percent cover was estimated for every identifiable species, bare ground, litter and rock within the plot three times and was then averaged to get relative cover per species per plot. Plots were allowed to add up to more than or less than 100%. The same vegetation sampling procedure was used for the two years post treatment.

2.5 Statistical Analysis

Vegetation data was analyzed for each year using SPSS statistical software. A multivariate ANOVA with Tukey post hoc was used to compare mean differences for each treatment combination. A repeated measures analysis was completed to determine if treatment effects were consistent over the entire study, or varied by year. A Speakman's rank order correlation was used to compare the relationship between knapweed and cheatgrass. Diversity calculations and NMDS computed with R version 4.0.0 using the vegan package (Oksanen et al., 2019) and visualized with ggplot2 (Wickham, 2016).

2.6 Results

Baseline vegetation cover was recorded for every identifiable species. Between all blocks, 20 forb species were recorded of which 13 were native and 7 were introduced or invasive. There were 7 different species of grass of which 3 were native species (Appendix 1). As there were many forbs recorded in small numbers a summary of the grasses and combined forbs is presented in table 2.6.1. Spotted knapweed cover deviated greatly but had a mean of 29.4% ±18.2 and cheatgrass cover averaged 3.6% ±3.8. A multivariate ANOVA test revealed no significant differences between species cover between plots before treatment (p >457), however block 1 had some significant differences from 2 and 3. Spotted knapweed was significantly lower in block 1 ($p<0.001$) and cheatgrass was significantly higher ($p<0.001$). June grass was significantly higher in block 3 ($p<0.001$).

Table 2.1 Baseline species composition of sites in 2018 showing minimum, maximum, mean and standard deviation. Other forbs includes all forbs recorded other than spotted knapweed. A list of all species can be found in Appendix 1.

Species	**N**	**Minimum**	**Maximum**	**Mean**	**Std. Deviation**
Centaurea stoebe	72	2	73	29.4	18.2
Other Forbs	72	2	48	15.1	9.2
Bromus tectorum	72	0	23.3	3.6	3.8
Pascopyrum smithii	71	0	3	0.1	0.5
Poa pratensis	72	0	30	2.3	3.8
Festuca idahoensis	72	0	11	1.0	1.9
Agropyron cristatum	72	0	4	0.1	0.6
Koeleria macrantha	72	0	5	0.3	0.8
Poa secunda	72	0	0	0.0	0.0
Juncus balticus	72	0	4	0.2	0.7

Changes by Year

A repeated measures ANOVA was conducted to determine if there was significant variation between years. Spotted knapweed, cheatgrass, Kentucky bluegrass and Junegrass were all significantly different over the 3 years; however, Tukey post-hocs determined that 2019 and

2020 were not significantly different from each other, and unsprayed sites did not have any significant differences between treatments.

Table 2.2 Repeated Measures ANOVA showing F and P values for treatment differences by year. P value <0.05 denotes significance.

Treatment	Species	df	Mean Square	F	Sig.
year	***Centaurea stoebe***	2	70.32	60.18	<0.0001
	Bromus tectorum	2	239.28	132.63	<0.0001
	Poa pratensis	2	104.11	76.78	<0.0001
	Koeleria macrantha	2	7.70	7.43	0.0009
herbicide	***Centaurea stoebe***	1	346.77	296.76	<0.0001
	Bromus tectorum	1	276.48	153.25	<0.0001
	Poa pratensis	1	4.18	4.41	0.04
	Koeleria macrantha	1	0.57	1.09	0.3
ash	*Centaurea stoebe*	2	2.99	1.28	0.28
	Bromus tectorum	2	17.01	2.75	0.07
	Poa pratensis	2	17.95	6.27	0.00
	Koeleria macrantha	2	2.51	2.42	0.1
herbicide X year	***Centaurea stoebe***	2	437.67	187.28	<0.0001
	Bromus tectorum	2	204.94	56.80	<0.0001
	Poa pratensis	2	20.87	7.70	0.0007
	Koeleria macrantha	2	1.87	1.80	0.17

First Year Species Composition

One year after treatment, vegetation composition was recorded at a similar time as the previous year. Seeded grass establishment was so low treatment effects will not be discussed. Species with low covers (<1%) were determined to be negligible and were not analyzed with ANOVA. Species of interest were determined to be spotted knapweed and all grasses. There were no outliers present and data was distributed normal; no transformations were done. The spray X ash interaction was significant for cheatgrass and Kentucky bluegrass while herbicide on its own had significant effects on spotted knapweed, and Sandberg’s bluegrass cover (Table 2.3).

Table 2.3 F and P values of species of interest from Multivariate ANOVA test on effects of herbicide, ash and herbicide X ash in year one (2019). P value 0.05 denotes significance.

Treatment	Species	df	Mean Square	F	Sig.
Herbicide	***Centaurea stoebe***	**1**	**26285.4**	**164.875**	**0.0001**
	Bromus tectorum	**1**	**40328.0**	**124.077**	**0.0001**
	Pascopyrum smithii	1	0.6	0.287	0.594
	Poa pratensis	**1**	**216.7**	**4.896**	**0.03**
	Agropyron cristatum	1	1.1	0.138	0.712
	Koeleria macrantha	1	1.5	0.501	0.482
	Poa secunda	1	83.9	8.712	0.004
ash	*Centaurea stoebe*	2	141.6	0.888	0.416
	Bromus tectorum	**2**	**1822.1**	**5.606**	**0.006**
	Pascopyrum smitii	2	5.6	2.836	0.066
	Poa pratensis	**2**	**357.8**	**8.086**	**0.0001**
	Agropyron cristatum	2	15.7	2.057	0.136
	Koeleria macrantha	2	8.1	2.688	0.075
	Poa secunda	2	7.5	0.779	0.463
herbicide X ash	*Centaurea stoebe*	2	415.1	2.604	0.082
	Bromus tectorum	**2**	**2819.3**	**8.674**	**0.0001**
	Pascopyrum smitii	2	0.2	0.083	0.921
	Poa pratensis	**2**	**144.8**	**3.272**	**0.044**
	Agropyron cristatum	2	1.4	0.188	0.829
	Koeleria macrantha	2	0.1	0.029	0.972
	Poa secunda	2	10.6	1.099	0.339

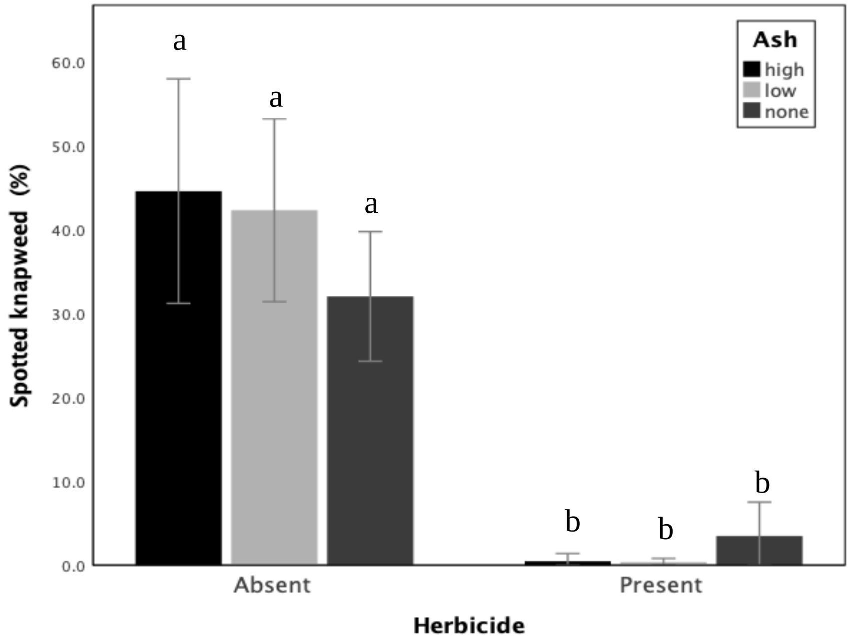

Figure 2.3 Mean percent cover of *Centaurea stoebe* in 2019 at high, low and no ash when herbicide is present or absent. Error bars are standard error (multiplier of 2). Bars sharing the same letters are not statistically different.

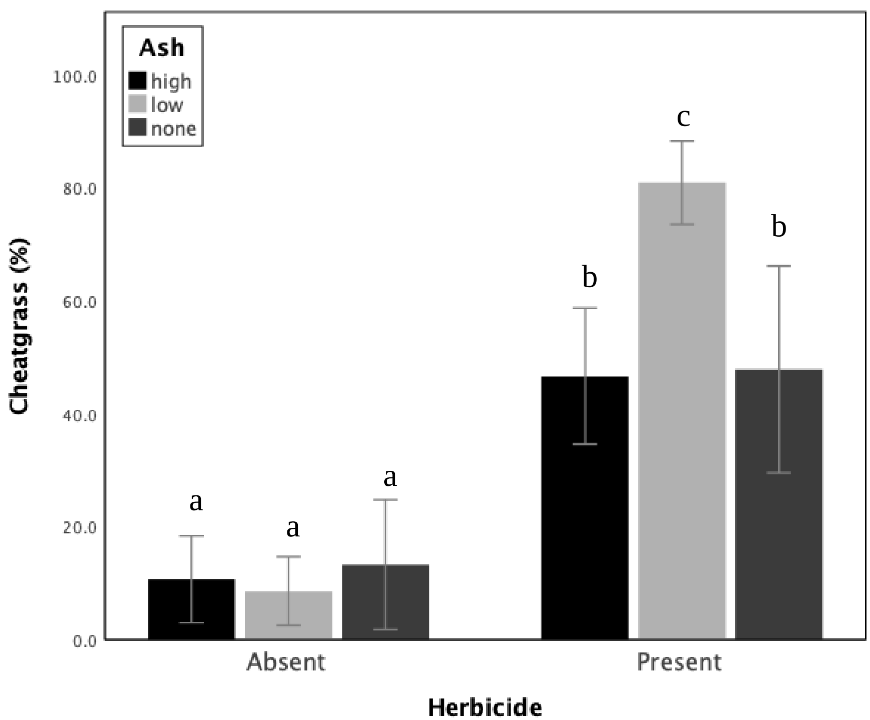

Figure 2.4 Mean percent cover of *Bromus tectorum* in 2019 at high, low and no ash when herbicide is present or absent. Error bars are standard error (multiplier of 2). Bars sharing the same letters are not statistically different.

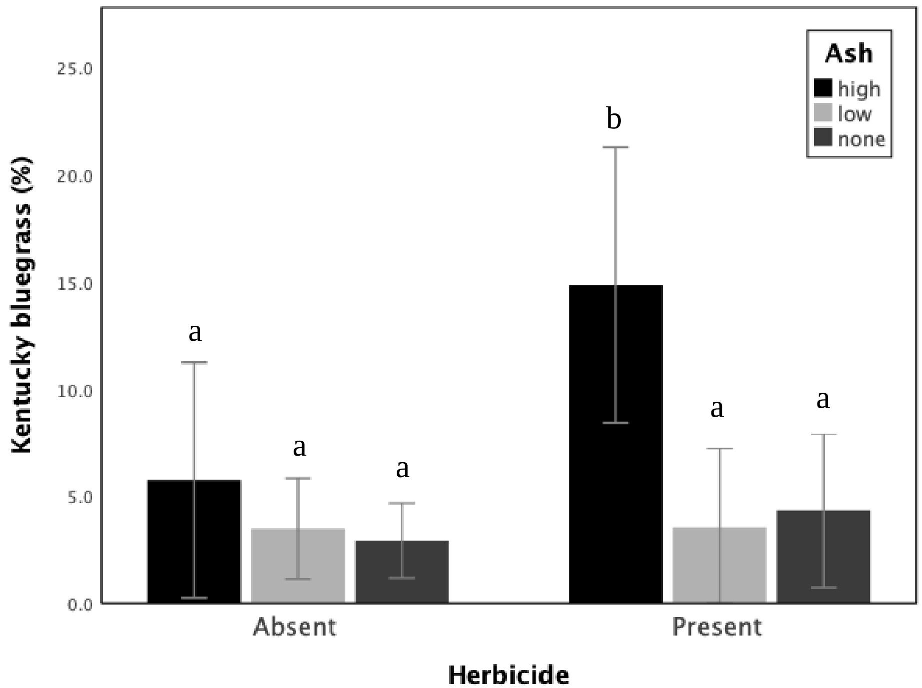

Figure 2.5 Mean percent cover of *Poa pratensis* in 2019 at high, low and no ash when herbicide is present or absent. Error bars are standard error (multiplier of 2). Bars sharing the same letters are not statistically different.

A Spearmans Rank Order correlation was run to assess if there was a relationship between cheatgrass and spotted knapweed cover 1-year post treatment. The scatterplot had a linear, monotonic relationship (Figure 2.6). Cheatgrass had a significant, strong, negative correlation to spotted knapweed cover (r_s = -.684, p<0.001). The greatest range in cheatgrass was measured when spotted knapweed was very low or absent.

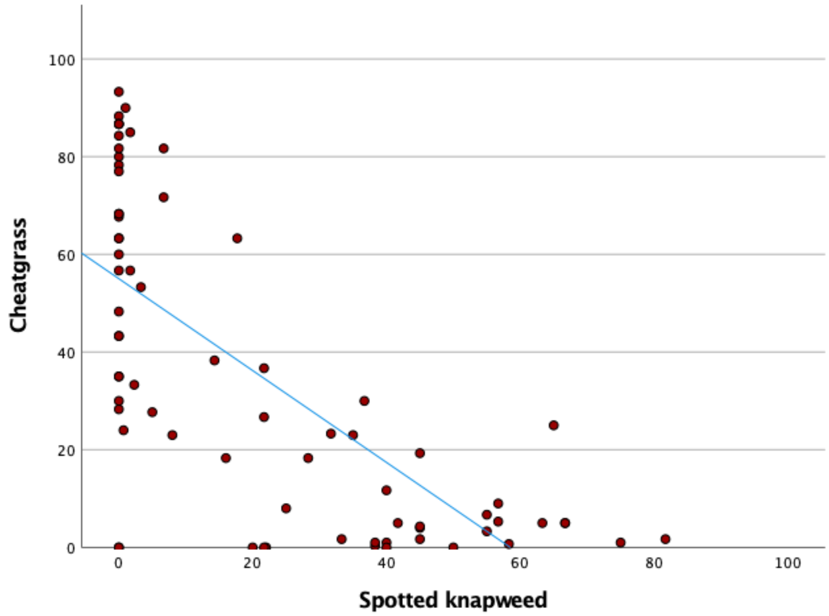

Figure 2.6 Scatterplot of cheatgrass and spotted knapweed cover one year after treatment.

Second Year Species Composition

Two years after treatment (2020), vegetation composition was recorded and analyzed. Results were similar to the first year however the herbicide X ash interaction was not quite significant for Kentucky bluegrass and was significant for Junegrass (p=0.02). It should be noted that the data for Junegrass was non-normal and could not be transformed. Tukey post-hoc indicated that Junegrass cover was significant only in the high ash and herbicide treatment.

Table 2.4 F and P values from percent cover of dominant species from Multivariate ANOVA on effects of herbicide, ash and herbicide X ash. P value 0.05 denotes significance.

Treatment	Dependent Variable	df	Mean Square	F	Sig.
herbicide	***Centaurea stoebe***	**1**	**29188.1**	**121.723**	**0.0001**
	Bromus tectorum	**1**	**20010.3**	**38.289**	**0.0001**
	Poa pratensis	**1**	**2346.5**	**12.08**	**0.001**
	Koeleria macrantha	1	39.5	3.677	0.059

ash	*Centaurea stoebe*	2	112.1	0.467	0.629
	Bromus tectorum	2	745.1	1.426	0.248
	Poa pratensis	2	569.4	2.932	0.06
	Koeleria macrantha	**2**	**45.0**	**4.191**	**0.02**
herbicide X ash	*Centaurea stoebe*	2	112.1	0.467	0.63
	Bromus tectorum	**2**	**1765.2**	**3.378**	**0.04**
	Poa pratensis	2	547.5	2.819	0.07
	Koeleria macrantha	**2**	**33.1**	**3.085**	**0.05**

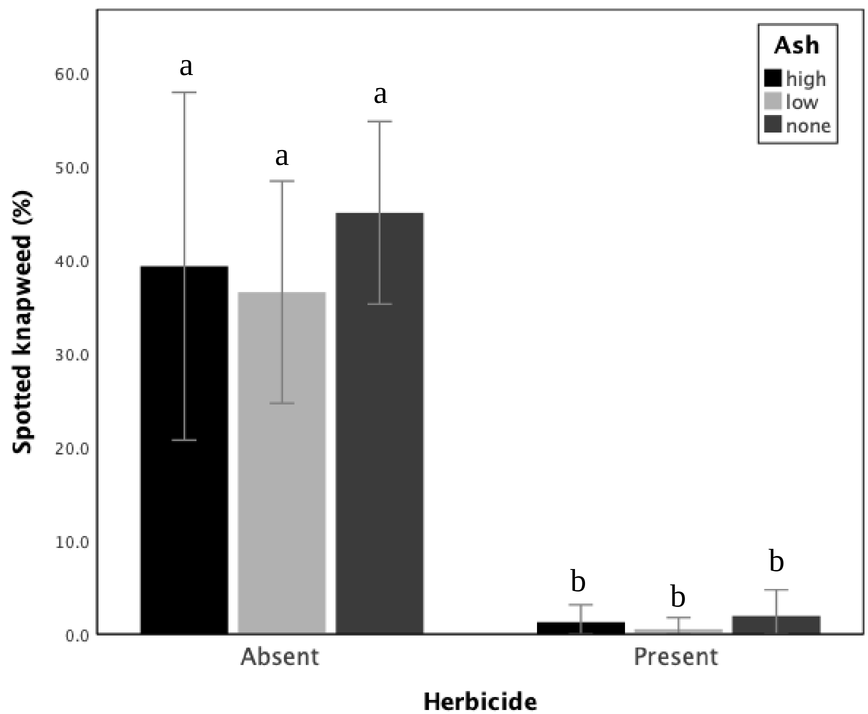

Figure 2.7 Mean percent cover of *Centaurea stoebe* in 2020 at high, low and no ash when herbicide is present or absent. Error bars are standard error (multiplier of 2). Bars sharing the same letters are not statistically different.

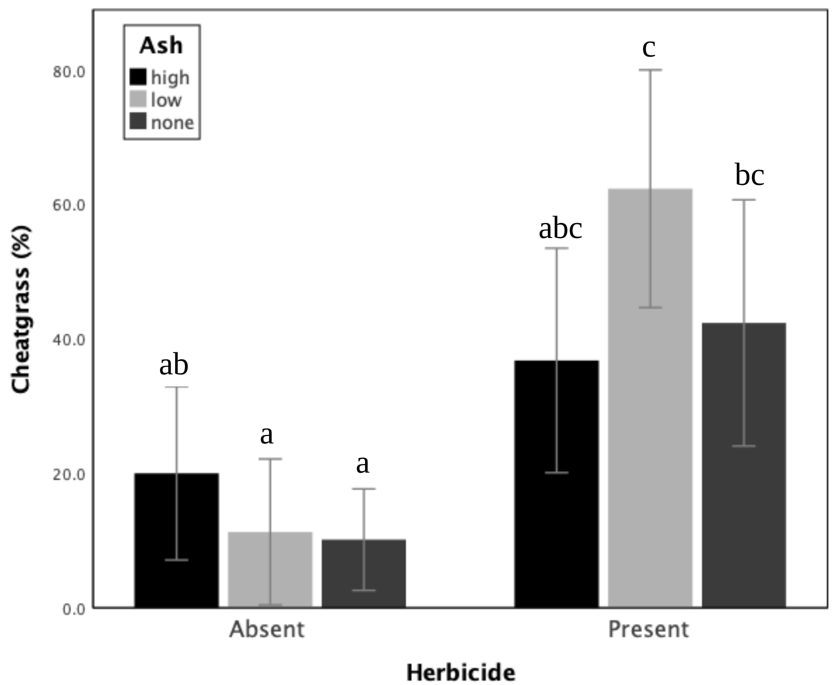

Figure 2.8 Mean percent cover of *Bromus tectorum* in 2020 at high, low and no ash when herbicide is present or absent. Error bars are standard error (multiplier of 2). Bars sharing the same letters are not statistically different.

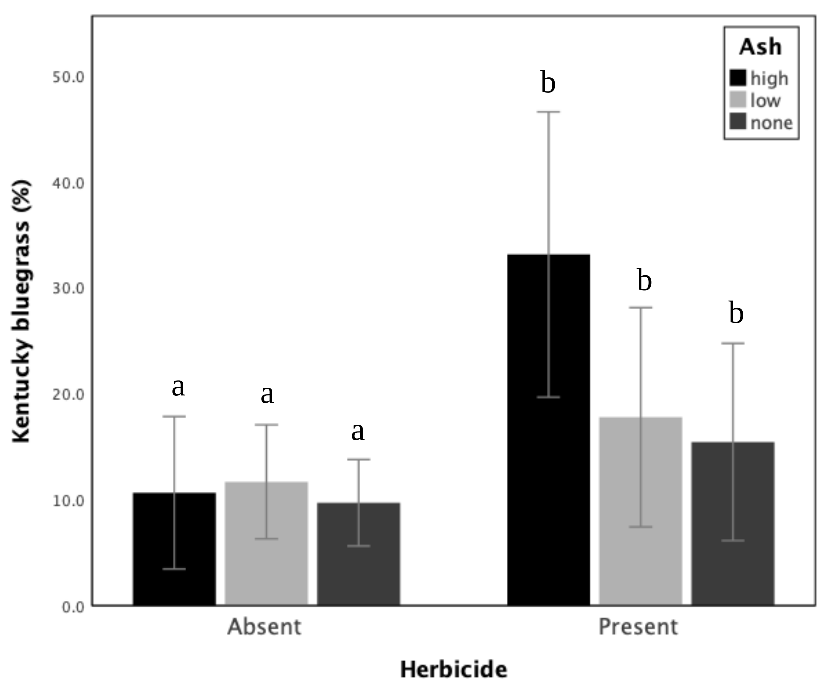

Figure 2.9 Mean percent cover of *Poa pratensis* in 2020 at high, low and no ash when herbicide is present or absent. Error bars are standard error (multiplier of 2). Bars sharing the same letters are not statistically different.

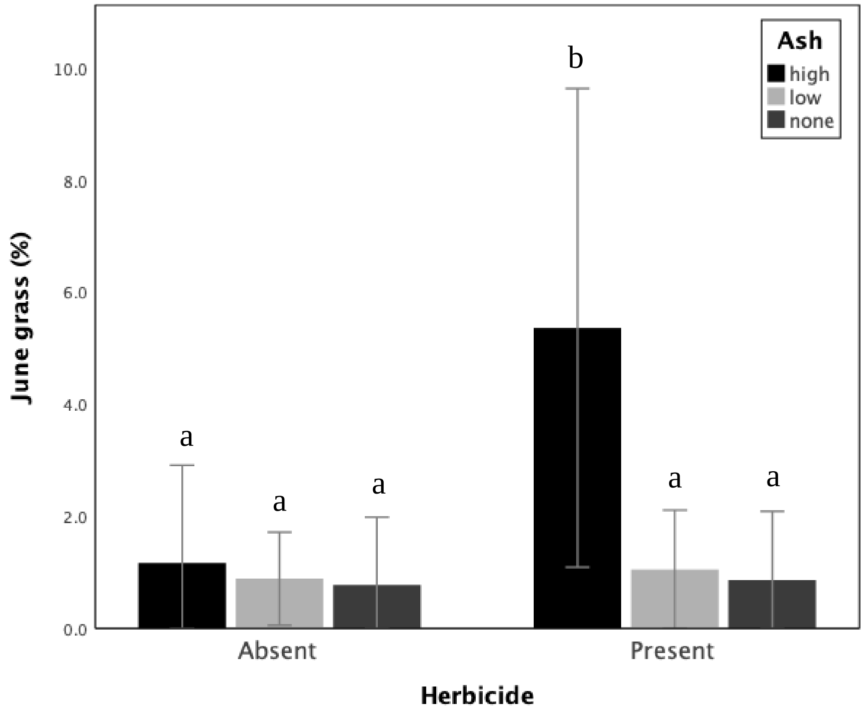

Figure 2.10 Mean percent cover of *Koeleria macrantha* in 2020 at high, low and no ash when herbicide is present or absent. Error bars are standard error (multiplier of 2). Bars sharing the same letters are not statistically different.

A Spearmans Rank Order correlation was run to assess if there was a relationship between cheatgrass and spotted knapweed cover two years post treatment. The scatterplot had a non linear, monotonic relationship (Figure 2.11). Cheatgrass had a significant, strong, negative correlation to spotted knapweed cover (r_s = -.593, p<0.001).

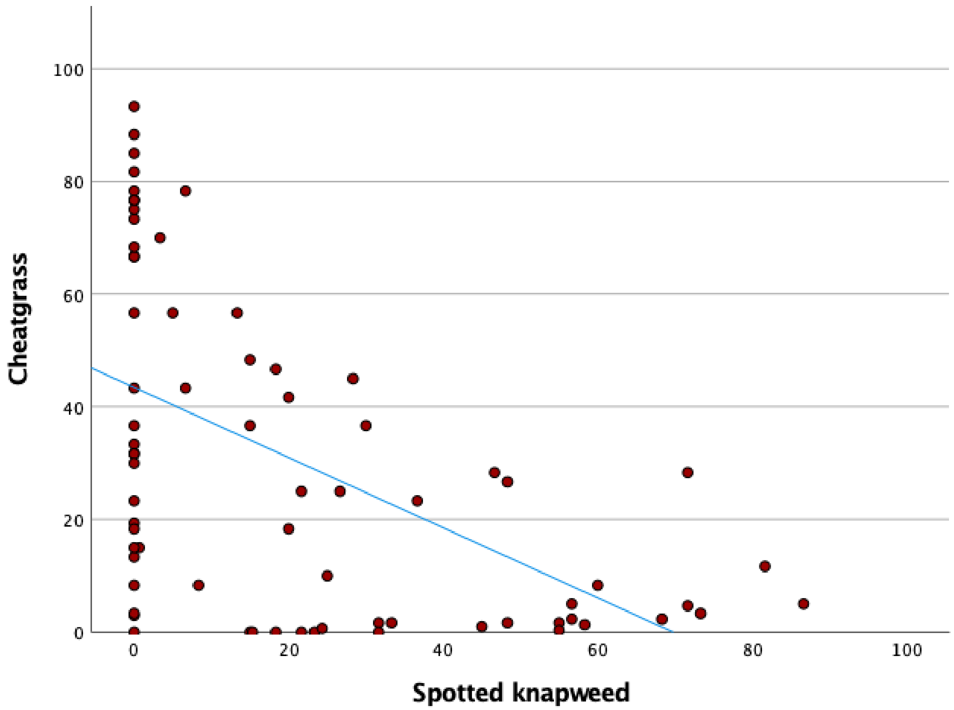

Figure 2.11 Scatter plot of cheatgrass and spotted knapweed cover two years after treatment.

Diversity

Shannon's diversity indices and richness were analyzed for differences between years and treatments. Shannon-Weiner indices (H) were calculated using relative cover and species richness (R) was calculated as the total number of species present in each plot. Data was normal and there were no outliers. Diversity significantly varied between years and between herbicide treatments. Ash had no effect on the diversity of plots, nor did seeding (Table 2.5). Tukey post hoc tests determined that there were no differences between the two years post treatment ($p>0.05$). Mean H pre-treatment was 1.7±0.38. One year after treatment, H in herbicide treated plots decreased to 0.79 ±0.48 but two years after treatment this increased up to 1.15 ±0.49. In untreated plots H stayed relatively the same both years after treatment (1.66 ±0.44 and 1.57 ±0.36, respectively).

Richness followed a similar pattern with a mean of 11.15 (± 2.2) species pre-treament. Richness decreased to 6.22 ±1.92 one year after herbicide treatment and then increased to 7.72 ±2.37 two years after treatment. In the unsprayed treatment richness showed no change after one year (11.78 ±2.41) and decreased slightly to 10.28 ±2.37 after two years.

Table 2.5 F and P values for Shannon's Diversity Indices and Species Richness. P value 0.05 denotes significance.

Treatment	Index	df	Mean Square	F	Sig.
year (pre and post treatment)	**Shannon's Index**	**2**	**4.345**	**24.6**	**0.000**
	Richness	**2**	**111.227**	**21.9**	**0.000**
sprayed (post-treatment)	**Shannon's Index**	**1**	**15.176**	**79.2**	**0.000**
	Richness	**1**	**592.111**	**115.9**	**0.000**
ash (post-treatment)	Shannon's Index	2	0.115	0.6	0.549
	Richness	2	3.083	0.6	0.548
year X sprayed (post-treatment)	**Shannon's Index**	**1**	**1.796**	**9.4**	**0.003**
	Richness	**1**	**81**	**15.9**	**0.000**
year X ash (post treatment)	Shannon's Index	2	0.053	0.3	0.761
	Richness	2	1.333	0.3	0.771

The change in H and R was calculated in each plot from 2018 (baseline) to 2020 (two years after treatment) to help conceptualize the results in terms of community change. Shannon's diversity decreased by 0.16±0.43 in the unsprayed plots and decreased 0.53±0.66 in the sprayed plots which species richness 0.97±2.4 and 3.33±2.2 in the unsprayed and sprayed plots, respectively. Figure 2.12 and 2.13 visualize Shannon's diversity in 2019 and 2020 showing that despite the lowering of diversity in the spray treatment, by 2020 diversity indices were becoming more similar and had the same value for the mode.

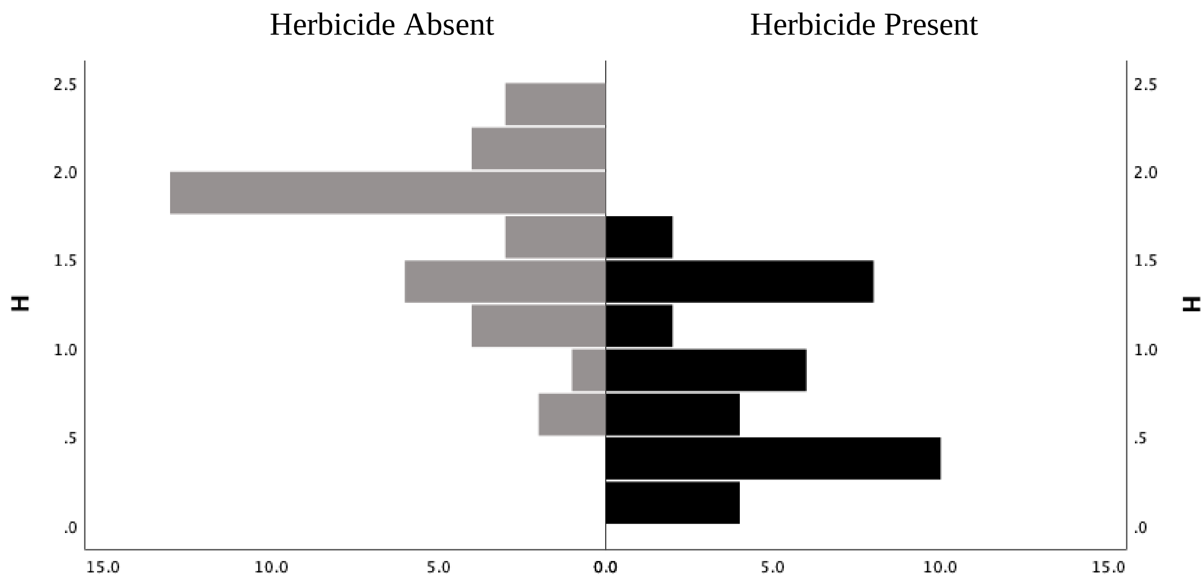

Figure 2.12 Population pyramid plot of Shannon's (H) Diversity indices by herbicide treatment in 2019.Chart shows the number of observations at each level of diversity index value for each treatment. In the unsprayed treatment, an H indice of 1.8 was the most common observation.

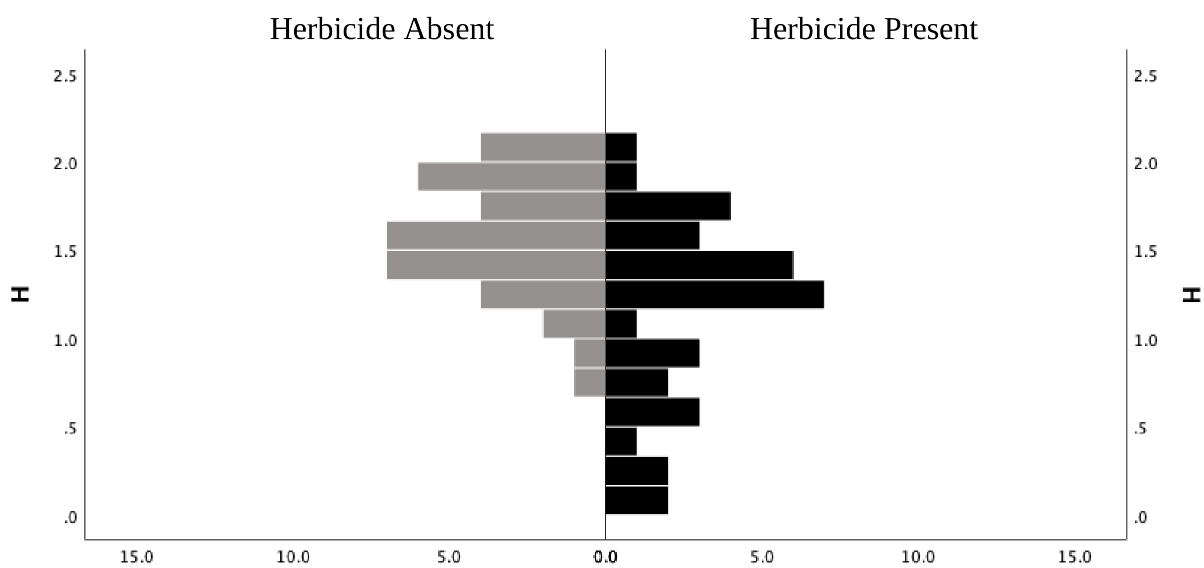

Figure 2.13 Population pyramid plot of Shannon's (H) Diversity indices by herbicide treatment in 2020. Chart shows the number of observations at each level of diversity index value for each treatment. In 2020, both treatments showed a similar number of observations around 1.5. The sprayed treatment had more observations below 0.5 than the unsprayed, however no treatment had any observations above 2.4.

Community Composition

Differences between community by year and treatment were visually assessed using non-metric multidimensional scaling (NMDS) using vegetation cover by plot computed to Bray-Curtis distances, eigen values set to 3 and run with 20 iterations. Plant communities post-treatment were similar to each other but different to the baseline community (Figure 2.14). Plant communities after herbicide treatment converged and became quite dissimilar with some overlap. They were very similar in 2019 and 2020 (Figure 2.15).

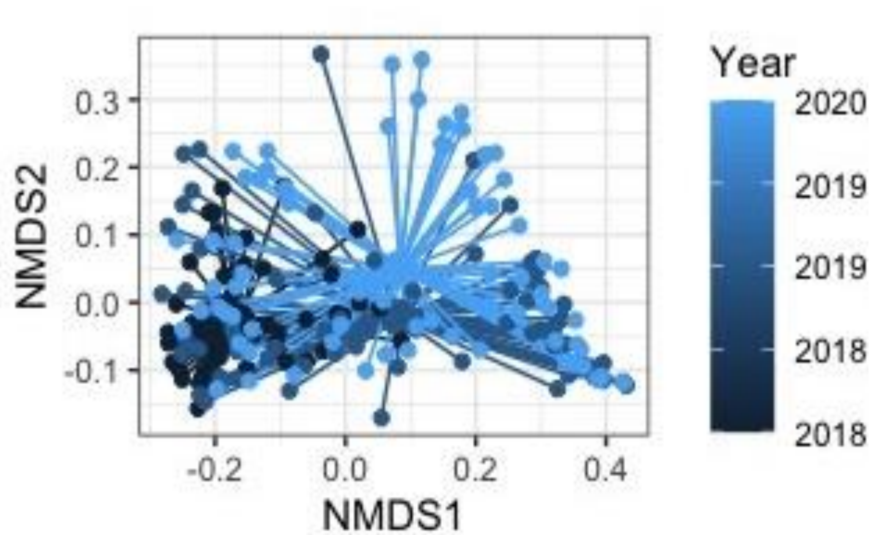

Figure 2.14 NMDS graph of differences of community composition by year. 2018 represents pre-treatment data and 2019 and 2020 represent post-treatment. Stress=0.0903

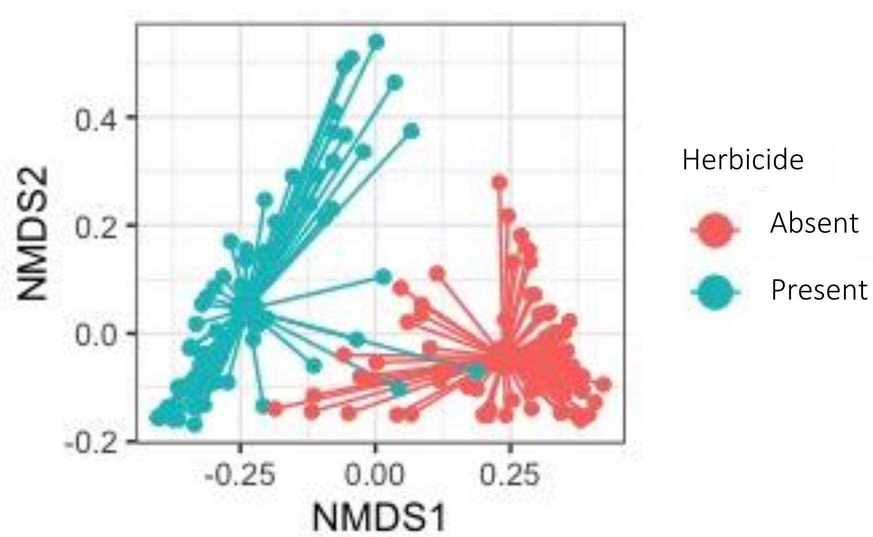

Figure 2.15 NMDS graph showing differences in community composition by herbicide treatments. Stress=0.0586.

Differences between functional group were compared from baseline to the 2020 (second year) by ANOVA and visualized by clustered boxplots (Figure 2.16). Functional groups were classified as non-native forbs (including spotted knapweed), native forbs, perennial grasses (*Poa pratensis, Bromus commutatus* and *Thinopyrum intermedium*, predominantly*)*, native grasses (*Poa secunda, Koeleria macrantha* and *Festuca idahoensis*, predominantly) and cheatgrass as it was the only invasive annual grass. Non-native forbs were significantly lower and non-native, perennial grasses (mostly Kentucky bluegrass) and cheatgrass were significantly higher in sprayed plots ($p<0.0001$) and there were no significant differences between native forbs and native grasses despite herbicide treatment (p-value=0.087 and 0.804, respectively).

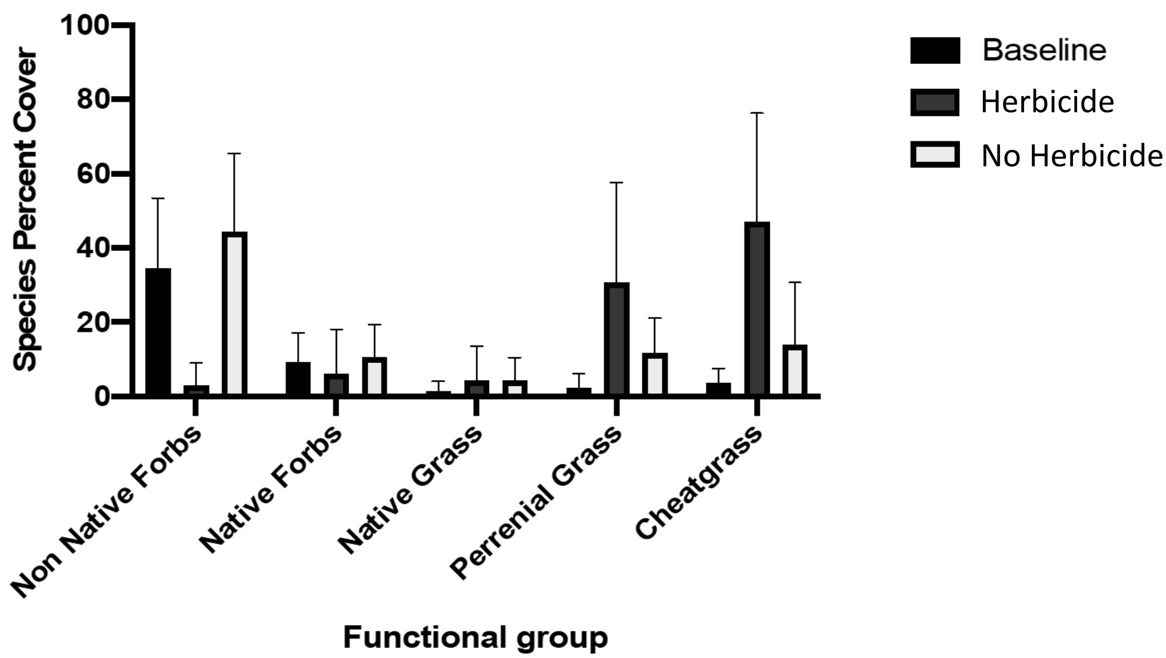

Figure 2.16 Clustered bar plot of the mean community composition and standard deviation by functional group for pre and two years post treatment.

2.7 Discussion

Ecological restoration in semi-arid rangelands is complicated due to nutrient and moisture limitations, uncontrolled disturbance and the nature of the desired keystone species (Aronson et al., 1993). In 1985 Schaffer pointed out that “ecologists will probably never be able to write down the complete governing equations for the order in chaos that is any natural system”. This experiment did offer a snapshot of the effects of integrating common invasive plant management techniques over a highly degraded rangeland in Western Canada.

Herbicide: Unfortunately, no treatment completely achieved the goal of a knapweed-free perennial grass system, resistant to invasion. The most successful method of removing or reducing knapweed cover was herbicide application, but it came with the trade-off of an annual grass invasion. Herbicide treated plots decreased from a mean knapweed cover of 29.4±18.2 to 1.4±3.9 after the first year of treatment which resulted in mean cheatgrass cover to increase by 55% (±26) the following year. Cheatgrass had a strong negative correlation to knapweed and a strong relationship was observed by these two invasive grassland weeds. The highest growth of cheatgrass was associated with the sprayed/low ash treatment in both years so it's likely the interaction effect of those treatments was of influence. Other studies (Ortega and Pearson, 2011) have observed secondary cheatgrass invasion when using picloram on knapweed, but not to this magnitude. This highlights the importance of planning herbicide applications according to the presence of invasive grasses as well as the value of conducting small field trials before large treatment as every site will have different ecological factors to consider.

Ash: Ash application did not have any noticeable ability to reduce knapweed cover. In both years after treatment there were no significant differences between ash treatments. However, ash did have other positive effects regarding desirable grass cover. Ash significantly increased Kentucky bluegrass and Junegrass cover in the sprayed blocks. It is important to note that the increase in Junegrass was not observed in all blocks. While we cannot conclude that ash decreased knapweed biomass, it should be investigated further. It is possible that additional ash applications over time, or higher rates may prevent increased establishment of knapweed, if not an actual reduction in knapweed cover or biomass but those may be accompanied by either negative effects such as increased pH and nutrient toxicity. Surprisingly, there were no noticeable differences in individual species cover over the years in the unsprayed block. The knapweed cover was too dominant to be effected by seeding treatments or ash application without the additional step of herbicide. It is evident in highly invaded rangelands, knapweed needs to be chemically or physically removed for any other treatment options to be viable.

Further studies could look at simply mowing the standing biomass to facilitate treatment contact with the soil and modify or eliminate the seed production and input to the system.

Seed: While no seed treatment established significantly in any of the plots, it should be noted that intermediate wheatgrass did have some establishment within the sprayed treatment of site 3 in 2020. This is two years after treatment, exhibiting that grass establishment may not occur immediately after seeding. Mangold et al. (2015) reported similar results where there was very little seed establishment until 4 years after treatment. There are several factors that could explain poor seed establishment. First, the uncharacteristic temperature in December after seeding. Between December 13 and 31, approximately 2 weeks after applying seed, the mean daily temperature reached 10°C on three occasions, which melted the protective layer of snow preventing germination. This could have led to some germination and then dying when temperatures dipped again or being eaten by birds. Increases in Kentucky bluegrass and Junegrass were also associated with the high ash treatment and were naturally occuring in the seed bank.

A second possible factor is timing between herbicide application and seeding. In general practise it has always been common to seed any time after spraying with a broadleaf selective herbicide but there is little research surrounding these practices. According to the Environmental Protection Agency's (EPA's) recommendation (EPA, 2019) picloram can be applied in the spring or early summer and grass be seeded in the fall. However, there is some speculation that residual herbicide can actually hurt young grass seedlings. In fact, shortly after the planning and set up of this research project, a paper by McManamen et al. (2018) was released which investigated the timing of seed after application of picloram. They looked at the germination of several different species generally used in prairie restoration at five time intervals after picloram application and determined that picloram treated plots had 76-96% fewer grass seedlings than the control plots and that these effects persisted up to 11 months. There were only four months between herbicide application and seeding in our study. This was the first study to look at herbicide impacts on seed past two months in a field setting.

Diversity: Herbicide treatments significantly lowered diversity, however two years after treatment it increased by 45%. It is important to note that both Shannon's diversity and species richness decreased in the unsprayed plots. Two years after herbicide application diversity began to increase while no treatment at all resulted in a slow decline of diversity. Many ecological studies have concluded that productive, high-diversity grasslands are resistant to invasion (Abernathy et al. 2016; Maron and Marler, 2007; Connoly et al., 2018). Results of NMDS show community composition of the main plots (herbicide vs no herbicide) to be widely different from one another. These two communities are now set on a different trajectory, and hopefully long-term monitoring will determine that the sprayed plots will have higher diversity than before treatment. When applying research results to invasive plant management plans additional seeding should be done to increase diversity in the sprayed plots to achieve a species rich community.

Conclusion

Herbicide treatment was the most effective way to remove spotted knapweed, but the herbicide treatment also resulted in a secondary invasion. Ash did not noticeably decrease knapweed in any plots but did augment Junegrass and Kentucky bluegrass growth, likely due to fertilization effects. Herbicide did increase Kentucky bluegrass on its own, but that was enhanced by the addition of the high ash treatment. This indicated a fertilizing impact from both the herbicide and ash treatment which was not observed in native bunchgrasses. There were few differences between what was seen between the first and second year after treatments. Most notable is the surge of grasses, June grass and Kentucky bluegrass which were not as prominent in the first year. The other important change is the diversity indices which began to trend in different directions depending on herbicide treatment. Future invasive plant management activities should be monitored for at least two years to ensure the site is progressing as desired. The below ground soil chemistry interactions will provide important insight to what is observed above ground.

2.8 References

Abernathy J, Graham, D., Sherrard, M. 2015. Productivity and Resistance to Weed Invasion in Four Prairie Biomass Feedstocks with Different Diversity. Global Change Biology Bioenergy 8(6), 1082–1092.

Aronson, J., Floret, C., Le Floc'h, E., Ovalle, C. and Pontanier, R. 1993. Restoration and Rehabilitation of Degraded Ecosystems in Arid and Semi-Arid Lands. I. A View from the South. Restoration Ecology. 1, 8-17. https://doi.org/10.1111/j.1526-100X.1993.tb00004

Blumenthal, D. M., Jordan, N. R., & Russelle, M. P. 2001. Soil Carbon Addition Controls Weeds and Facilitates Prairie Restoration. Ecological Applications. 13(3), 605–615.

Brouillet, L., Coursol, F., Favreau, M. and Anions, M. 2016. VASCAN, The Database Vascular Plant of Canada.

Connolly, J, Sebastià, M-T, Kirwan, L, et al. 2018. Weed suppression Greatly Increased by Plant Diversity in Intensively Managed Grasslands: A continental-scale experiment. Journal of Applied Ecology, 55(2), 852–862.

Dormaar, J.F, Johnston, A.M., and Smoliak, S. 1978. Long-term soil changes associated with seeded stands of crested wheatgrass in southeastern Alberta, Canada. Proceedings of the 1st International Rangeland Congress; 14-18 August 1978, CO. Society for Range Management. 623-625

Environmental Protection Agency. 2019. PICLORAM + 2,4-D RTU. https://www3.epa.gov/pesticides/chem_search/ppls/081927-00074-20190826.pdf

Groh, H. 1944. Canadian Weed Survey. 2nd Annual Report. Canada Department of Agriculture. 74.

Grzędzicka, E., & Reif, J. 2020. Impacts of an Invasive Plant on Bird Communities Differ Along a Habitat Gradient. Global Ecology and Conservation, 23, e01150. https://doi.org/https://doi.org/10.1016/j.gecco.2020.e01150

Gurevitch, J. and Padilla, D.K. 2004. Are Invasive Species a Major Cause of Extinctions? Trends in Ecology and Evolution, 19, 470–474

Jacobs, J. S., & Sheley, R. L. 2013. Life History of Spotted Knapweed Observation. Society for Range Management, 51(6), 665–673.

Langmaier, M., and Lapin, K. 2020. A Systematic Review of the Impact of Invasive Alien Plants on Forest Regeneration in European Temperate Forests. Frontiers in Plant Science. https://www.frontiersin.org/article/10.3389/fpls.2020.524969

Lesica, P. and DeLuca, T.H. 1996. Long-term harmful effects of crested wheatgrass on Great Plains grassland ecosystems. Journal of Soil and Water Conservation. 51, 408-409.

Leonardo Frid (ESSA Technologies Ltd.), Duncan Knowler (Simon Fraser University), Carol Murray (ESSA Technologies Ltd.), Judy Myers (The University of British Columbia) and Lisa Scott (Eco-Matters Consulting). 2008. Economic Impacts of Invasive Plants in British Columbia. BC Invasive Species Council.

Lloyd, D., Angove, G.D. Hope, and Thompson, C. 1990. A Guide to Site Identification and Interpretation for the Kamloops Forest Region. Ecosystems Land Management Handbook BC Government.

Lucchetti MA, Glauser G, Kilchenmann V, Dübecke A, Beckh G, Praz C, Kast C. 2016. Pyrrolizidine Alkaloids from Echium vulgare in Honey Originate Primarily from Floral Nectar. J Agric Food Chem. 64(25):5267-73. doi: 10.1021/acs.jafc.6b02320

Kansas State University. 2016. "Left uncontrolled, Weeds Would Cost Billions in Economic Losses Every Year." ScienceDaily. www.sciencedaily.com/releases/2016/05/160516130720.htm

Kedzie-Webb, S., Sheley, R., Borkowski, J., and Jacobs, J. 2001. Relationships between *Centaurea maculosa* and indigenous platn assemblages. Western North American Naturalist, 61(1), 43-49. Retrieved January 2, 2021, from http://www.jstor.org/stable/41717075

Kettenring, K. M., and Adams, C. R. 2011. Lessons learned from Invasive Plant Control Experiments: A Systematic Review and Meta-analysis. Journal of Applied Ecology, 48(4), 970–979. https://doi.org/10.1111/j.1365-2664.2011.01979.x

Majerus, M., L. Holzworth, D. Tilley, D. Ogle and M. Stannard. 2009. Plant guide for Sandberg bluegrass (Poa secunda J. Presl). USDA.Natural Resources Conservation Services.

Mangold, J., Orloff, N., Parkinson, H., and Halstvedt, M.2015. Integrating Herbicides and Re-Seeding to Restore Rangeland Infested by an Invasive Forb-Annual Grass Complex. Ecological Restoration, 33(1), 16–19. https://doi.org/10.3368/er.33.1.16

Maron, J., and Marler, M. 2007. Native Plant Diversity Resists Invasion at Both Low and High Resource Levels. Ecology, 88(10), 2651–2661. https://doi.org/10.1890/06-1993.1

Maxwell, J.F., Drinkwater, R., Clark, D.A.V.I.D. and Hall, J.W., 1992. Effect of grazing, spraying, and seeding on knapweed in British Columbia. Rangeland Ecology & Management/Journal of Range Management Archives, *45*(2), 180-182.

McManamen, C., Nelson, C.R., Wagner, V. 2018. Timing of seeding after herbicide application influences rates of germination and seedling biomass of native plants used for grassland restoration. Restoration Ecology. 26(6) 1137-1148.

Mitchell, R. M., and Bakker, J. D. 2011. Carbon Addition as a Technique for Controlling Exotic Species in Pacific Northwest Prairies. Northwest Science, 85(2), 247–254. https://doi.org/10.3955/046.085.0213

Murray, J.V., Lehnhoff, E.A., Neve, P., Poggio, S.L. and Webber, B.L. 2012. 'Raising the Bar': Improving the Standard and Utility of Weed and Invasive Plant Research. New Phytol, 196: 678-680. https://doi.org/10.1111/j.1469-8137.2012.04343.x

Natural Resources Conservation Service. 2006. Invasive Species Sheets – Spotted knapweed. https://www.nrcs.usda.gov/wps/portal/nrcs/detail/ct/technical/ecoscience/invasive/?cid=nrcs142p2_011131. Accessed on 10/04/2020.

Oksanen, Jari & Blanchet, F. Guillaume & Kindt, Roeland & Legendre, Pierre & O'Hara, Bob & Simpson, Gavin & Solymos, Peter & Stevens, Hank & Wagner, Helene. 2010. Vegan: Community Ecology Package.

Pimentel, D., L. Lach, R. Zuniga, and D. Morrison. 2005. Update on the Environmental and Economic Costs Associated with Alien-Invasive Species in the United States. Ecol. Econ. 52:273–288.

Shaffer, W.M. 1985. Order and Chaos in Ecological Systems. Ecology 66:93-106.

Sheley, R. L., Jacobs, J. S., and Velagala, R. P. 1999. Enhancing Intermediate Wheatgrass Establishment in Spotted Knapweed Infested Rangeland. Journal of Range Management, 52(1), 68–74. https://doi.org/10.2307/4003494

Sheley, R. L., Krueger-mangold, J., Krue, J. 2003. Principles for Restoring Invasive Plant-Infested Rangeland. Weed Science, 51(2), 260–265.

United Sates Environmental Protection Agency. 2019. Notification per PRN 98-10 – Add ABN "Tordon 22K" and updated trademark. https://www3.epa.gov/pesticides/chem_search/ppls/062719-00006-20190920.pdf

Watson, A. K. and A. J. Renny. 1974. The Biology of Canadian weeds: *Centaurea diffusa* and *Centaurea maculosa.* Canadian Journal of Plant Science. 54, 687-701.

Westbrooks, R. 1998. Invasive Plants, Changing the Landscape of America: Fact Book. Washington, DC: Federal Interagency Committee for the Management of Noxious and Exotic Weeds. 109.

Wickham H. 2016. ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag New York. ISBN 978-3-319-24277-4, https://ggplot2.tidyverse.org.

Winslow, S. 2013. Sanberg bluegrass – A Native Grass for Conservation Use in Montana and Wyomming. Natural Resource Conservation Services. Plant Technical Note.

3 Soil Chemical Changes after Herbicide and Wood Ash Application in an Invaded Rangeland

3.1 Introduction

Invasive plants are generally viewed as an above ground problem, but the severity of the plant invasion are also influenced by the timing and availability of soil resources. In semiarid grasslands, one of the goals of restoration activities is renewal of soil processes that favor native perennial species, rather than invasive species that are known to increase with rising levels of nitrogen (N) availability (Blumenthal, 2009; Perry et al., 2010). Spotted knapweed is a deeply tap-rooted perennial forb that was introduced to North America over 130 years ago and is known as one of the most ecologically harmful exotic plant invaders of semiarid grasslands (Foster et al., 2021; DiTomaso et al., 2013) Increased availability of soil resources such as soil phosphorus (P) (Fraser and Carlyle, 2011) and nitrate (NO_3^-) (Herron et al., 2001) are known to increase the dominance of spotted knapweed, especially when soil water is not limiting (Pearson et al., 2017).

One research topic in invasive plant management is introducing N immobilization as a means to increase native perennial grasses. This was observed by Blumenthal et al. (1991) and Mitchel and Baker (2011) using other forms of carbon (activated carbon and sugar) on other prairie weeds. Addition of wood ash has also been reported to enhance soil fertility through a number of mechanisms such as increasing soil carbon, adsorbing allelochemicals or increasing nutrient availability (DeLuca and Gao, 2019).

In British Columbia the use of wood ash as a soil amendment is governed by Code of Practise for Soil Amendments (CoPSA) which falls under the Public Health Act and Environmental Management Act (Hannam et al., 2016). Ash must be accompanied by an analysis that meets the criteria for trace elements and foreign matter and volumes of more than 5 m^3 must be accompanied by a land application plan (LAP) (Hannam et al., 2016). According to Hannam et al. (2018), the legislation for wood ash application rates in BC is very simple as it is only restricted by heavy metal loading. In Alberta, the maximum application rates of wood ash to agricultural soils is set at 15 Mg ha^1(Alberta Environment, 2002).

In addition to this, there is relatively little research involving wood ash application to grassland soils. A literature review only retrieved eight relevant papers, with two having a North American context. Current wood ash research focuses around forests, and specifically the boreal where acidic soils are common. High alkalinity may present a challenge as application of ash typically raises forest soils 0.5-3 pH units (Huotari et al., 2015). This pH increase can have profound increases or decreases on microbial composition and productivity, depending on the microbial communities (Cruz-Paredes et al., 2017).

The field study was undertaken at the same location and plots as described in Chapter 2. I combined the wood ash treatments within herbicide and seed treatments with a split-plot design in order to determine if there was a specific combination that was more successful. I hypothesized that the introduction of carbon in the form of wood ash would stimulate the size and activity of the microbial biomass and reduce or delay soil inorganic N supply, making conditions less favourable for spotted knapweed. There is little research on herbicide effects on soil nutrients and results depend on site specific conditions (Kanissery et al., 2018). However, the effect on soil nutrient supply following input of plant material is much more understood. Endress et al. (2012) found an increase in annual brome grasses persisted following herbicide application to a degraded bluebunch/Idaho grassland in Northern Oregon that was invaded by *Potentilla recta*. Annual bromes were quick to colonize following canopy removal with herbicide application utilizing available soil resources that become available with herbicide use.

3.2 Materials and Methods

Three composite soil samples were taken along the site transect at three equal sectors in mid-June to establish baseline conditions at 0-15 cm and 15-30 cm belowground. Soil samples were stored in sealed bags in the laboratory fridge until soil analysis could be done. Soils were analyzed for: pH, electrical conductivity (EC), ICP-OES major elements and total N and C. pH and electrical conductivity were sampled with a pH probe using a 1:1.25 (soil:H_2O) dilution.

ICP-OES were analyzed by the provincial laboratory in Victoria, BC and total N and C were analyzed with a Thermofisher elemental analyzer with two replicates being run for each soil sample.

Plant Root Simulator (PRS) probes were used to assess differences in available soil nutrients in sub-plots in 2019 and 2020. PRS probes are ion exchange resin membranes held in plastic supports that can be easily inserted into soil to measure ion supply in situ with minimal disturbance (Western Ag). In each year, two PRS probe burial periods of four weeks were deployed from April 20-May 17 (deployment 1) and June 3-July 4 (deployment 2) in 2019 and April 22-May 20 (deployment 3) and June 4-July 2 (deployment 4) in 2020. After approximately 30 days PRS probes were removed and washed free of soil with deionized water and scrubbed with a coarse brush to remove all remaining soil particles. These were sent back to Western Ag Innovations for analysis of nitrate (NO^{-3}), ammonia (NH_4^+), calcium (Ca^{2+}), magnesium (Mg^{2+}), potassium (K^+), phosphates ($H_2PO_4^-$and HPO_4^{-2}), iron (Fe), manganese (Mn), copper (Cu), zinc (Zn), boron (B), sulfate ($SO_4^{2})^-$, lead (Pb), aluminum (Al) and cadmium (Cd).

In spring 2019, 2020 and October 2020, each sub-plot was sampled at a depth of 0-15 cm during vegetation sampling and soil was collected and analyzed for pH and EC. Samples from 2020 were analyzed for C% and N%.

The metal analysis for the wood ash that was used was obtained from a private environmental consulting company who was using the same ash for reclamation projects. Analysis was done by ALS in Kamloops, BC (Table 3.1). The data for three samples from the larger batch of ash was provided which were believed to be representative of the ash used in this study; however, as we did not submit our own we are making generalizations about the actual concentration used in this study. Metal concentrations were below provincial guidelines for maximum allowable concentrations for soil amendments (Organic Matter Recycling Regulations, 2002). Inorganic carbon ranged from 4.25% to 4.69%, total organic carbon from 2.5% to 3.15 and total carbon from 6.75% to 7.8% (Table 3.1).

Table 3.1 Metal analysis of wood ash from Merritt Viola Energy Plant. [1]Ashnet – Ash Chemistry Database (http://www.nrcan..gc.ca/forests/research-centres/gifc/ashnet/20288) Common characteristics of fly ash produced across Canada. [2] Wood ash trace element concentration limits (Canadian Council of Ministers of the Environment 2005).

	Mean	St Dev	Ashnet[1]	CSR-IL Env Protection Limit[2]
pH	12.96	0.01	11.30	-
Inorganic Carbon (%)	4.49	0.18	2.6	-
Total Carbon (%)	7.26	0.43	-	-
Total Organic Carbon (%)	2.77	0.25	18.1	-
Aluminum (Al) (mg/kg)	23100.00	828.65	-	-
Arsenic (As) (mg/kg)	27.57	3.91	-	0.04
Boron (B) (mg/kg)	174.33	7.04	-	-
Cadmium (Cd) (mg/kg)	5.02	0.41	-	0.075
Calcium (Ca) (mg/kg)	137666.67	2054.80	153500	-
Chromium (Cr) (mg/kg)	43.13	2.33	-	0.25
Cobalt (Co) (mg/kg)	9.79	0.34	-	0.2
Copper (Cu) (mg/kg)	92.27	3.48	-	0.3
Iron (Fe) (mg/kg)	20233.33	910.43	-	-
Lead (Pb) (mg/kg)	14.93	2.02	-	1
Magnesium (Mg) (mg/kg)	17400.00	216.02	14600	-
Manganese (Mn) (mg/kg)	5690.00	127.54	-	2
Molybdenum (Mo) (mg/kg)	7.04	0.76	-	0.15
Nickel (Ni) (mg/kg)	28.23	0.53	-	0.25
Phosphorus (P) (mg/kg)	7580.00	88.32	6300	-
Potassium (K) (mg/kg)	42066.67	2332.86	33100	-
Selenium (Se) (mg/kg)	0.49	0.06	-	0.002
Sodium (Na) (mg/kg)	9443.33	151.07	-	1
Sulfur (S) (mg/kg)	7666.67	1126.45	11000	-
Tin (Sn) (mg/kg)	4.17	0.48	-	0.3
Zinc (Zn) (mg/kg)	1201.33	147.12	-	0.45

3.3 Statistical Analysis

PRS probe results were analyzed by a mixed model, repeated measures ANOVA was ran using the lmer model in R to analyze values for herbicide and ash block effects during all four deployments. Statistical analysis were done in R using the lme4 package (Bates et al., 2015) for fitting linear mixed-effect models. If data was non-normal by interpretation of Shapiro-Wilks test ($p<0.05$) it was transformed by a square root function. As is common in ecological data, outliers were present in most samples. Outliers were left in the reported analysis, however tests were also ran with the removal of extreme outliers (more than 3 times the interquartile range, as assessed by boxplots) to determine if the overall conclusion changed. If outliers had an effect on the outcome, that was discussed.

3.4 Results

pH and Electrical Conductivity

Baseline soils had a mean pH of 7.3±0.1 and electrical conductivity at 36.1 µS.cm^2 with little variability among the three sites. Changes in pH in the first and second years after treatment application were compared to the baseline soil values using a mixed-model ANOVA in R. Results of the Shapiro-Wilks test determined data was distributed normally. Sampling time and ash application were associated with changes in pH and EC in our study; herbicide had no significant effect on either. Eight months after ash application (2019) mean pH was significantly different ($p=0.002$) between treatments with 8.1±0.3 and 7.5±0.4 in the high and low treatments compared to 7.1±0.6 in the controls (Figure 3.1). One year later (2020), the high and low treatments decreased to 7.5±0.6 and 7.2±0.5, respectively, compared to the control with a pH of 6.9±0.5 with ash treatment still being significantly different ($p=0.04$). Four months later those values were 7.7±0.2, 7.0±0.3 and 6.9±0.5 for high, low and control treatments, respectively; however, these were still significantly different ($p=0.002$). Ash application significantly increased the pH of site proportionally to the level applied, however that effect diminished over time.

An ANOVA test was conducted for electrical conductivity (µS/cm) between the three sampling periods post treatment and determined that the only significant difference was between ash applications but only in the October 2020 sampling time ($p=0.001$), where it was higher than the control and low level applications (Figure 3.2). Values were higher across the board during the 2019 sampling period, which may be attributed to an incorrect calibration.

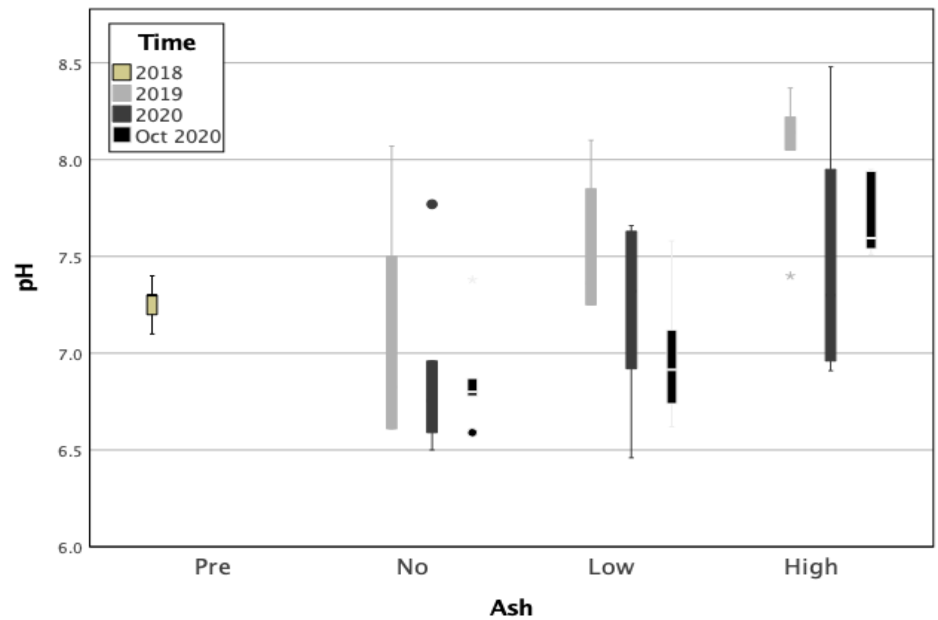

Figure 3.1 Clustered boxplot of pH values for all sampling periods. Stars represent extreme outliers (more than 3 times the IQR).

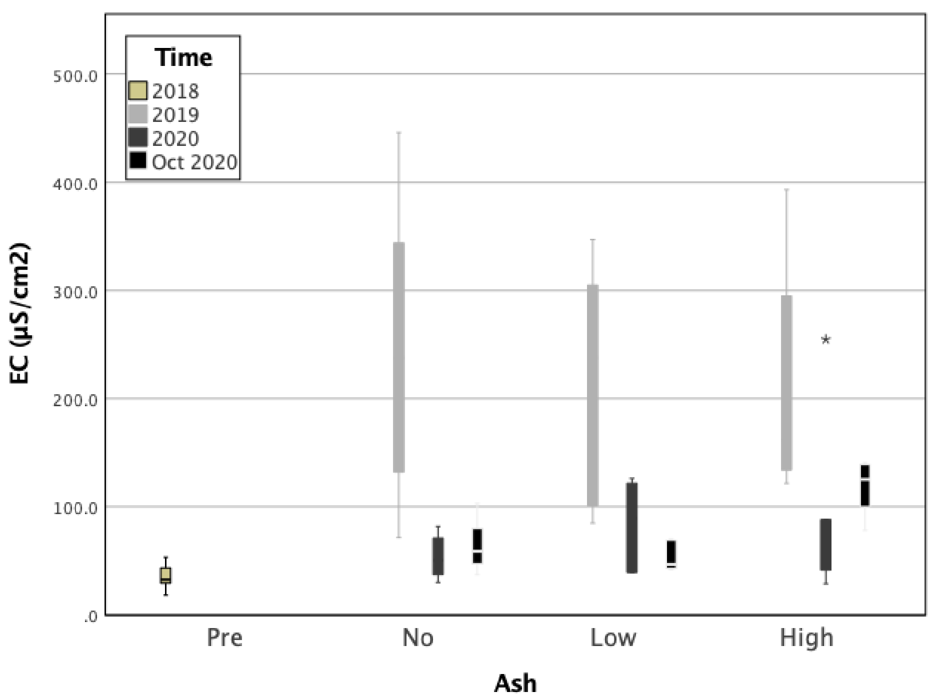

Figure 3.2 Clustered boxplots of Electrical Conductivity in microSiemens/cm for all sampling periods. Stars represent extreme outliers (more than 3 times the Interquartile Range).

Plant Root Simulator Results

Soil inorganic N is measured as nitrate (NO_3^-) and ammonium (NH_4^+), but nitrate was the dominant form and therefore, only considered in the analysis. The only significant difference for ammonium values was between blocks (p=0.01). Herbicide X ash interactions and time significantly affected nitrate values (p=0.003, <0.0001, respectively (Table 3.2). Herbicide application increased the nitrate values of all plots with a mean of 118.4 µg/cm^2/4 weeks which was 8 times higher than the unsprayed plots (13.92 µg/cm^2/4 weeks±17.3). Ash significantly affected nitrate levels, but post-hoc tests determined it was only in the herbicide treated plots. The herbicide with high and low ash treatments did have higher NO_3^- than the unsprayed plots however it was not significantly higher. Figure 3.3 depicts the difference in plant available nitrogen in sprayed vs. unsprayed plots, with the sprayed/no ash treatment having approximately 20 times more nitrate than any of the plots without herbicide. Within the herbicide treated plots, amount of nitrate decreased conversely with level of ash application.

Table 3.2 Analysis of Variance Table with Satterthwaite's method for nitrate values. Significance is indicated by p<0.05 or highlighted in grey.

NO_3^-	Sum Sq	Mean Sq	DF	F value	P
Site	2.35	1.18	2	2.42	0.29
Herbicide	15.52	15.52	1	31.97	0.03
Ash	6.73	3.36	2	6.93	0.002
Deployment	50.06	16.69	3	34.38	0.000
Herbicide x Ash	6.61	3.31	2	6.81	0.003
Herbicide x Deployment	2.18	0.73	3	1.50	0.23
Ash x Deployment	3.04	0.51	6	1.04	0.41

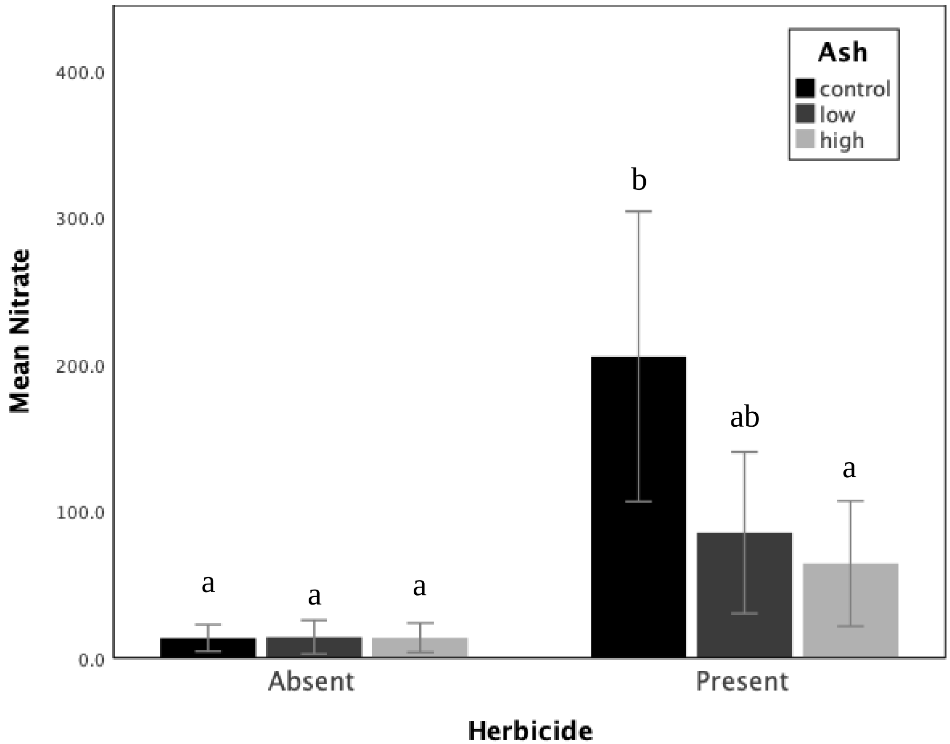

Figure 3.3 Mean values of NO_3^- for all sampling times at high, low and no ash levels and when herbicide is absent or present. Error bars are standard error (multiplier of 2). Bars sharing the same letters are not statistically different.

A mixed model, repeated measures ANOVA was used to analyze all the PRS probe results for herbicide and ash effects and differences within sampling times. The main treatment effects varied depending on the nutrient. There were no three-way interactions for any nutrient. Deployment time was a significant factor in differences for all nutrients except potassium and zinc.

Herbicide X ash and herbicide X time interactions significantly affected phosphorus (p=<0.0001 and 0.04). This was expressed as phosphorus being higher in herbicide treated plots as well as in the fourth deployment (Figure 3.4). When outliers were removed, the herbicide x deployment interaction was not significant, but all other observations had the same conclusion. Potassium values were four times higher in the high ash application during the first year of sampling compared to the no and low ash treatments which returned a significant p-value (< 0.0001), however block 3 had significantly elevated K. Sulphur values were significantly different for the ash X time interaction (p=0.01) however post-hoc tests determined those differences were only in the high ash treatment during deployments 1 and 2. Additionally, when sulphur was analyzed with outliers removed there were no differences between treatments. Magnesium differed between sampling times with the June deployments having higher plant available magnesium and herbicide X ash interaction (p=0.01). Sampling time significantly affected calcium availability in soil (p <0.001) with the June sampling period of both years having higher calcium. The two spikes correlated with times of high precipitation (Figure 3.9). Interaction between herbicide and ash returned a statistically significant difference (p=0.01).

Table 3.3 Analysis of Variance Table with Satterthwaite's method for PRS probe macronutrients. Significance is indicated by p<0.05 or highlighted in grey.

Phosphorus	**Sum Sq**	**Mean Sq**	**DF**	**F value**	**P**
Site	0.33	0.67	2	1.16	0.46
Herbicide	9.97	9.97	1	17.38	0.05
Ash	0.51	0.26	2	0.45	0.64
Deployment Time	22.02	7.34	3	12.80	<0.001
Herbicide x Ash	10.97	5.49	2	9.57	<0.001
Herbicide x Deployment	5.02	1.67	3	2.92	0.04
Ash x Deployment	7.42	1.24	6	2.16	0.07
Potassium	**Sum Sq**	**Mean Sq**	**DF**	**F value**	**P**
Site	342.33	171.16	2	5.41	0.008
Herbicide	65.28	65.28	1	2.06	0.16
Ash	2388.28	1194.14	2	37.73	<0.0001
Deployment Time	121.37	40.46	3	1.28	0.29
Herbicide x Ash	178.39	89.19	2	2.82	0.07
Herbicide x Deployment	10.63	3.54	3	0.11	0.95
Ash x Deployment	140.80	23.47	6	0.74	0.62
Sulphur	**Sum Sq**	**Mean Sq**	**DF**	**F value**	**P**
Site	0.63	0.32	2	2.65	0.27
Herbicide	0.10	0.10	1	0.86	0.45
Ash	2.44	1.22	2	10.22	0.0002
Deployment Time	2.55	0.85	3	7.11	0.001
Herbicide x Ash	0.40	0.20	2	1.68	0.20
Herbicide x Deployment	0.23	0.08	3	0.65	0.59
Ash x Deployment	2.40	0.40	6	3.35	0.01
Magnesium	**Sum Sq**	**Mean Sq**	**DF**	**F value**	**P**
Site	42438.90	21219.45	2	4.35	0.19
Herbicide	66.49	66.49	1	0.01	0.92
Ash	12907.69	6453.85	2	1.32	0.28
Deployment Time	205587.30	68529.10	3	14.06	0.0001
Herbicide x Ash	48886.86	24443.43	2	5.02	0.01
Herbicide x Deployment	20125.50	6708.50	3	1.38	0.26

Ash x Deployment	27493.97	4582.33	6	0.94	0.48
Calcium	**Sum Sq**	**Mean Sq**	**DF**	**F value**	**P**
Site	426.96	213.48	2	9.17	0.10
Herbicide	177.42	177.42	1	7.62	0.11
Ash	133.92	66.96	2	2.88	0.07
Deployment Time	2074.12	691.37	3	29.71	<0.001
Herbicide x Ash	217.84	108.92	2	4.68	0.01
Herbicide x Deployment	79.84	26.61	3	1.14	0.34
Ash x Deployment	113.38	18.90	6	0.81	0.57

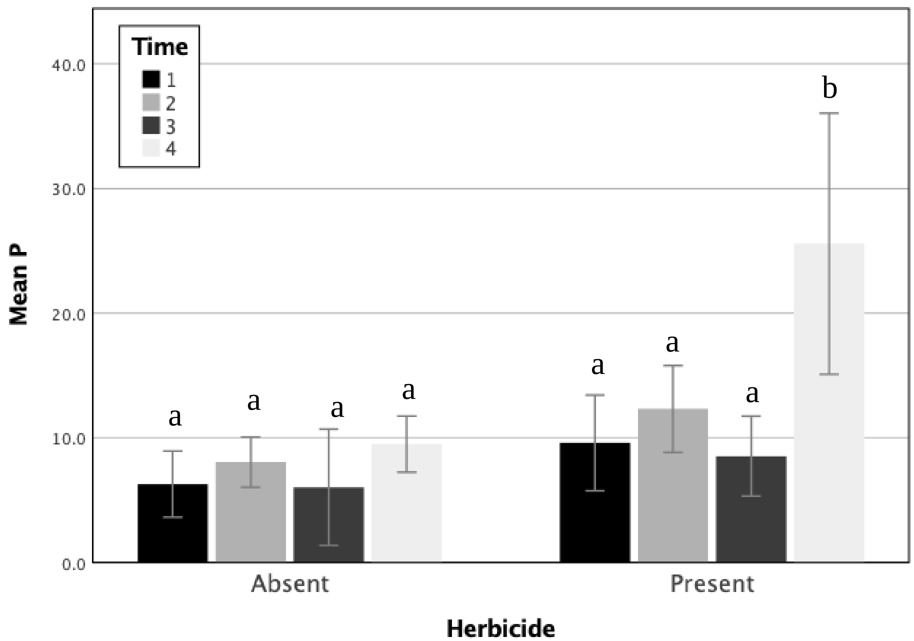

Figure 3.4 Mean phosphorus value by herbicide treatment over deployment period. P is measured in µg/cm2/4 weeks. Error bars show standard error (multiplier of 2). Bars sharing the same letters are not statistically different.

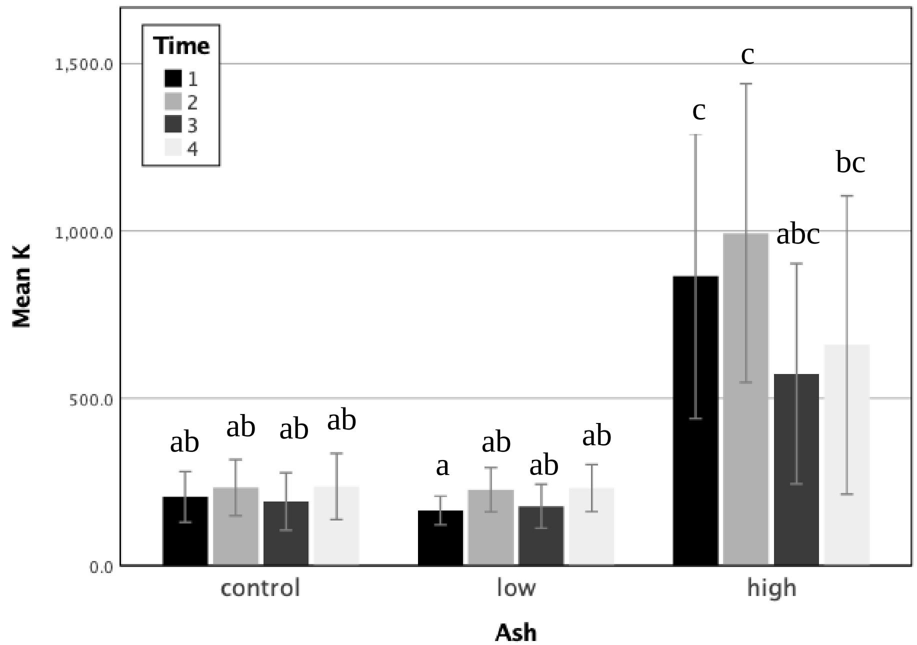

Figure 3.5 Mean potassium values by ash treatment over deployment time. K is measured in µg/cm^2/4 weeks. Error bars show standard error (multiplier of 2). Bars sharing the same letters are not statistically different.

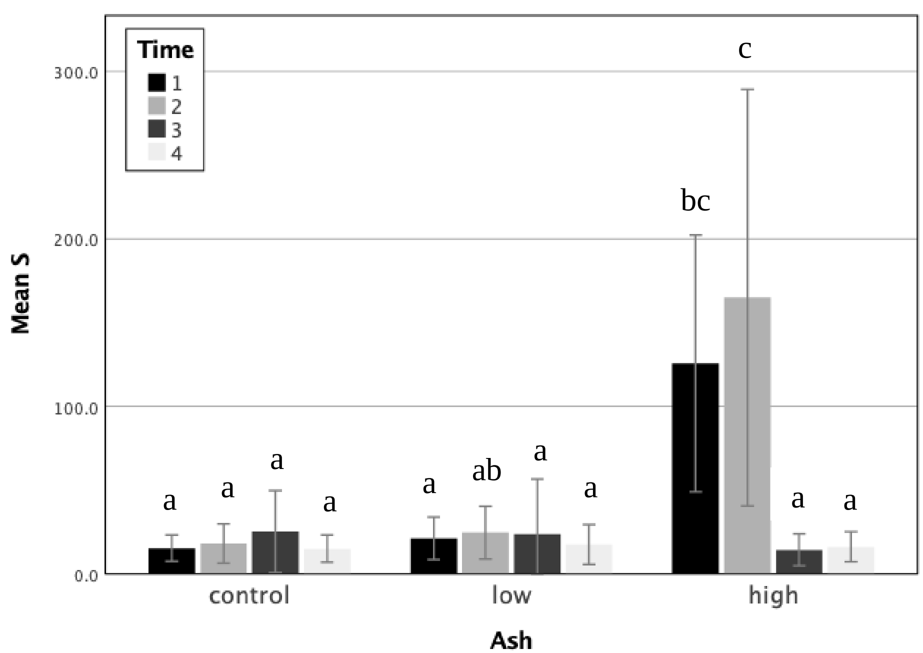

Figure 3.6 Mean sulphur values by ash treatment over deployment time. S is measured in µg/cm^2/4 weeks. Error bars show standard error (multiplier of 2). Bars sharing the same letters are not statistically different.

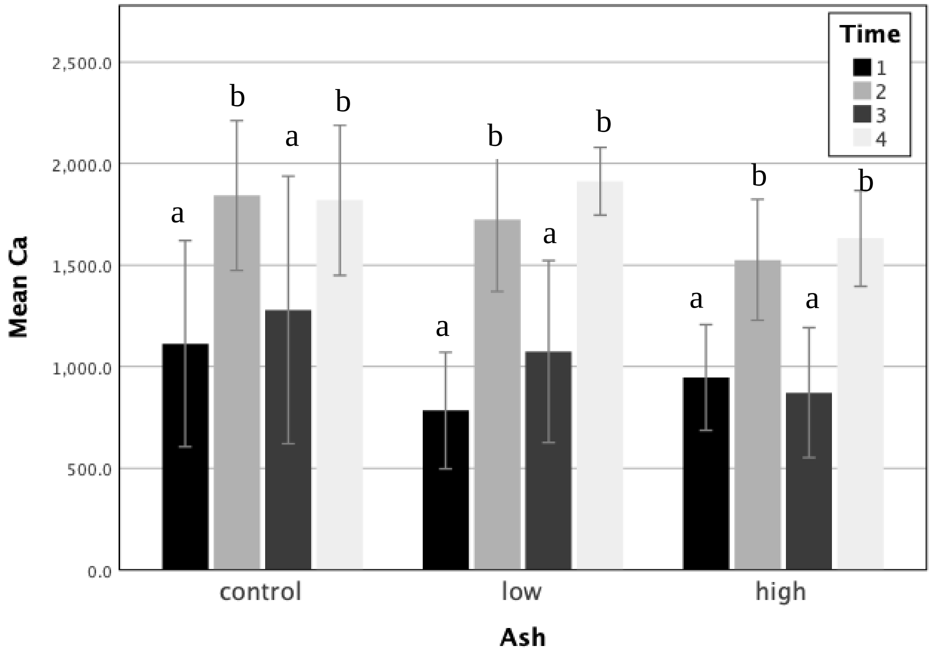

Figure 3.7 Mean calcium values by ash level at each deployment. Ca values are reported in µg/cm2/4 weeks. Error bars show standard error (multiplier of 2). Bars sharing the same letters are not statistically different.

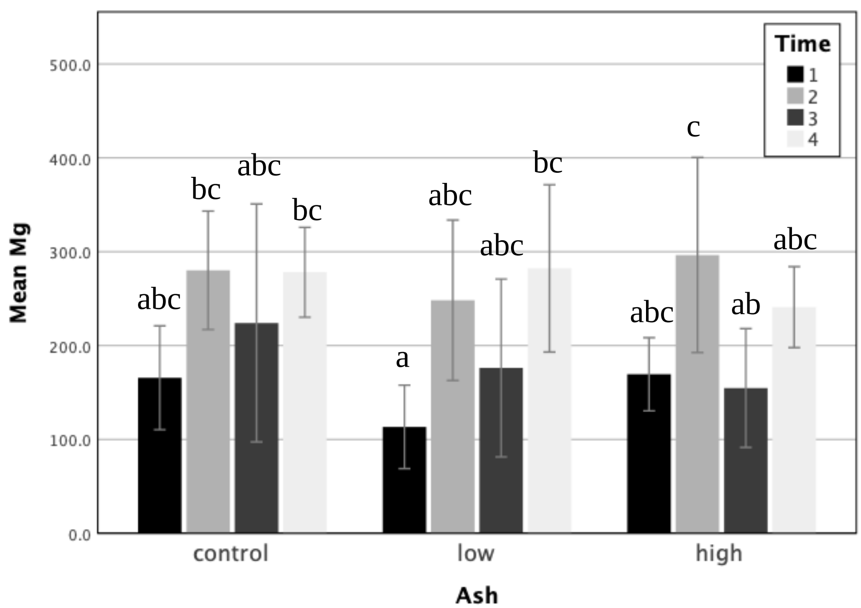

Figure 3.8 Mean magnesium values by ash treatment. Mg values are reported in µg/cm2/4 weeks. Error bars show standard error (multiplier of 2). Bars sharing the same letters are not statistically different.

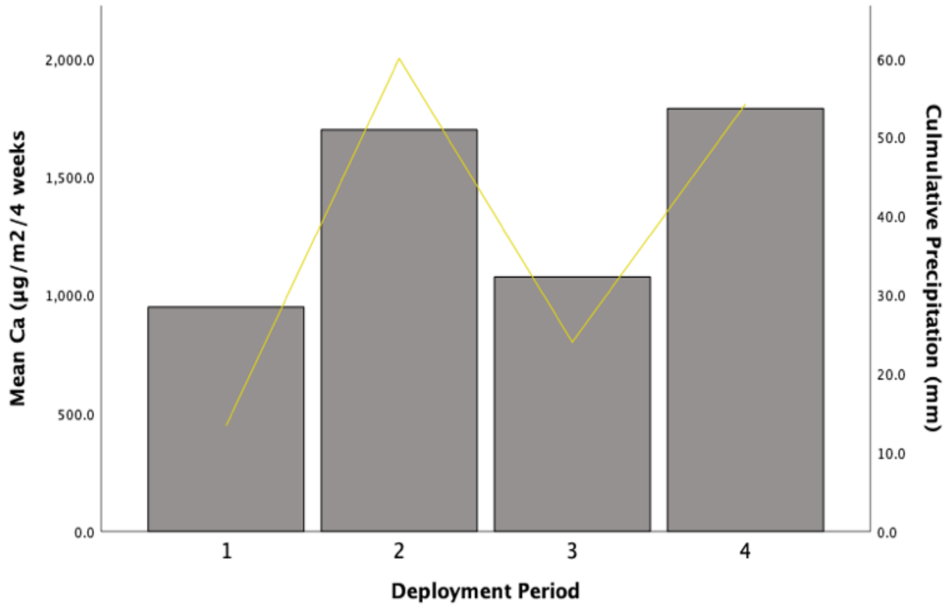

Figure 3.9 Mean Ca (grey bars) and cumulative precipitation (yellow line) by deployment period.

Micronutrients were classified as iron (Fe), boron (B), manganese (Mn), zinc (Zn), copper (Cu) and molybdenum (Mo). There were no significant differences for iron, manganese, copper or molybdenum ($P > 0.05$). B was significantly different between sampling times with the 4th deployment having the highest boron (Table 3.10) but there was no relationship between ash application and boron concentration. High ash application significantly increased zinc in all sampling periods except for the 3rd deployment. Lead and cadmium levels were below detectable levels in almost every sample and therefore no statistics were completed. Aluminum was present for each sample but the only significant difference was between sampling times where deployment 3 had significantly less aluminum available. Ash did not significantly increase the availability of any plant toxic elements.

Figure 3.10 Analysis of Variance Table with Satterthwaite's method for plant available B, Zn and Al. Significance is indicated by $p<0.05$ or highlighted in grey.

Boron	**Sum Sq**	**Mean Sq**	**DF**	**F value**	**P**
Site	0.42	0.21	2	2.59	0.09
Herbicide	0.03	0.03	1	0.40	0.53
Ash	0.39	0.19	2	2.37	0.11
Deployment Time	2.91	0.97	3	11.84	0.000
Herbicide x Ash	0.61	0.31	2	3.76	0.03

Herbicide x Deployment	0.48	0.16	3	1.95	0.14
Zinc	**Sum Sq**	**Mean Sq**	**DF**	**F value**	**P**
Site	2.43	1.22	2	0.59	0.63
Herbicide	0.65	0.65	1	0.32	0.63
Ash	19.47	9.74	2	4.71	0.01
Deployment Time	12.54	4.18	3	2.02	0.12
Herbicide x Ash	10.90	5.45	2	2.64	0.08
Herbicide x Deployment	10.68	3.56	3	1.72	0.18
Aluminum	Sum Sq	Mean Sq	DF	F value	P
Site	5.99	3.00	2	0.28	0.76
Herbicide	19.85	19.85	1	1.85	0.18
Ash	0.03	0.02	2	0.00	0.99
Deployment Time	134.77	44.92	3	4.18	0.01
Herbicide x Ash	59.54	29.77	2	2.77	0.07
Herbicide x Deployment	49.60	16.53	3	1.54	0.22

Soil Carbon and Nitrogen

It should be noted that an unknown error occurred part way during analysis in the thermofisher elemental analyzer. Soils were misplaced before they could be reanalyzed. Due to this, results from site 3 and half of site 2 were not accurate so they were removed from analysis and baseline soil conditions were averaged and extrapolated from site 1 and part of site 2. Site factors including pH and EC were similar, suggesting they were representative of 3. Carbon percentages were 1.63±0.3 (0-15 cm depth) and 1.43±0.6 (15-30 cm depth). Nitrogen percent for the site soils were 0.18±0.02 (0-15 cm depth) and 0.16±0.06 (15-30 cm depth). The C:N ratio of the site was 9. The wood ash sample was 0.05% nitrogen and 10.88% carbon

After treatment, mixed model ANOVA tests determined that nitrogen and carbon values in June 2020 had no significant differences regarding main effects but the interaction effect of herbicide and ash was (p=0.008 for carbon and p=0.01 for nitrogen). CN ratios were also analyzed in the same model, and ash treatment as well as herbicide x ash interactions returned significant differences (p=0.03 and 0.02, respectively) where the unsprayed/high ash treatment had a higher C:N ratio. Sites were resampled after the growing season and at that time there were no significant differences between treatments on nitrogen or carbon but C:N ratios were significantly higher due to the effects up ash in the sprayed/high ash treatment (p=0.0004) with a mean of 11.2±0.7 (Table 3.5).

Table 3.4 Summary of C:N Ratios at subplots during two sampling times.

June 2020				October 2020			
Herbicide	**Ash**	**C:N Ratio**	**St Dev.**	**Herbicide**	**Ash**	**C:N Ratio**	**St Dev.**
No	High	11.4	1.1	No	High	10.8	0.6
	Low	10.2	0.5		Low	10.2	0.4
	No	10.6	0.4		No	10.5	0.7
Yes	High	11.1	0.7	Yes	High	11.2	0.7
	Low	11.1	0.4		Low	10.3	0.6
	No	11.3	0.6		No	10.6	0.7

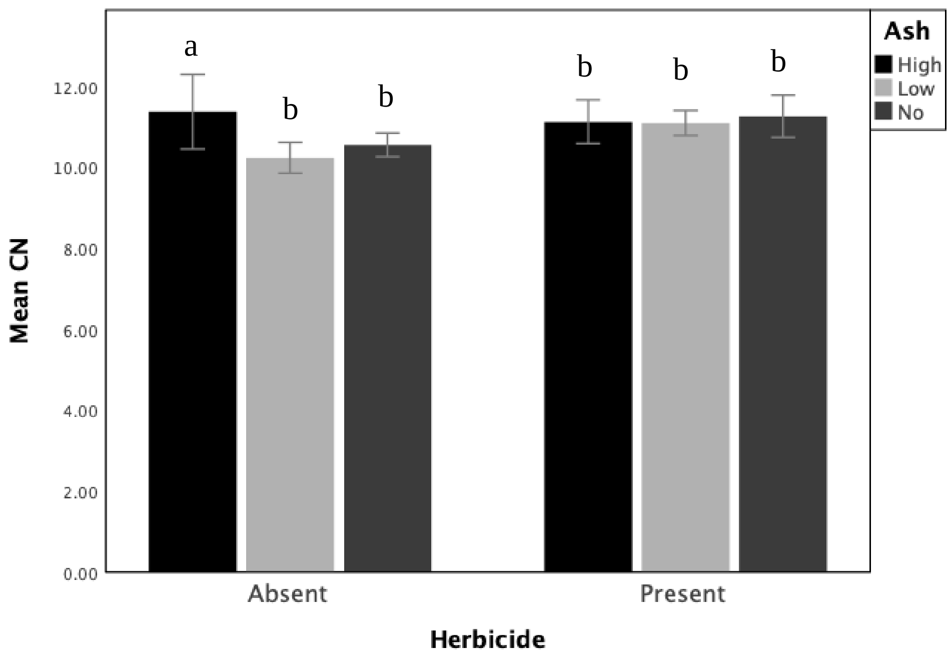

Figure 3.11 Mean CN ratio by treatment in June 2020. Error bars show standard error (multiplier of 2). Bars sharing the same letters are not statistically different.

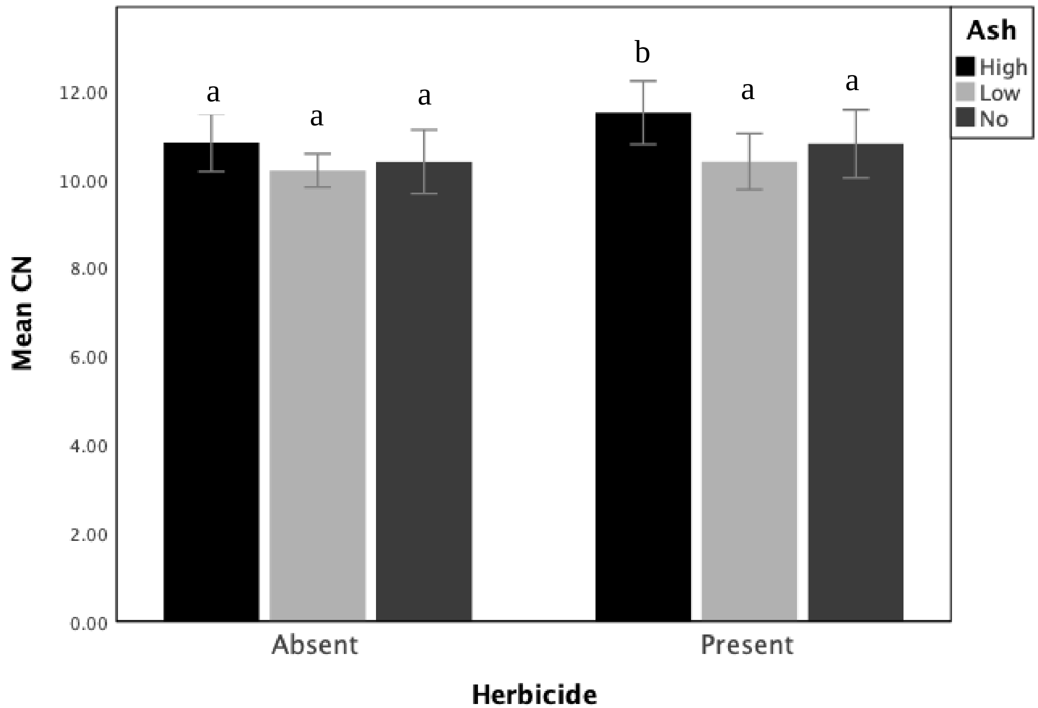

Figure 3.12 Mean CN ratio by treatment in October 2020. Error bars show standard error (multiplier of 2). Bars sharing the same letters are not statistically different.

3.5 Discussion

This study helped provide some unique understanding of the soil properties after broadcast herbicide treatment and wood ash amendments in a highly invaded grassland. Spotted knapweed has consistently been shown to alter soil properties, specifically soil C and P (Fraser and Carlyle, 2011; Hook et al., 2004). The experiment was replicated on 3 very large patches of spotted knapweed (approximately 15 ha at each site) and Fraser and Carlyle (2011) showed that the size of spotted knapweed patch is related to degree of soil alteration. My hypothesis was that the introduction of wood ash would helped modify soil properties to resemble natural conditions and we recorded and analyzed the effects on pH, EC, plant available nutrients and carbon and nitrogen values in the soil.

Effects of wood ash amendment on soil pH and EC

Wood ash amendment increased soil pH proportionally with the level applied. This increase was evident 8 months after amendment application but by the second year of sampling or 20 months after ash application the “low” concentration of ash amendment had decreased close to control conditions (7.2 compared to 6.9). By the last sampling period in October 2020, high ash application was still elevated but the low application was only 0.1 point higher than the control. In sum, the 100 g/m^2 ash level returned to baseline pH levels by 2 years after application, but the 1000 g/m^2 level was still noticeably elevated. A study by Bieser and Thomas (2019) looked at soil properties after applying two wood ash amendments (poplar biochar and high carbon wood ash) at a rate in between the two levels used in our study (500 g/m^2). Three years after application the high-carbon wood ash treatment still had elevated pH, but the poplar biochar treatment did not. The wood-ash in our study had a higher calcium content (14%) than either of the treatments in the Bieser and Thomas study (0.61±0.02 and 10.0±0.24), so the high treatment may maintain elevated pH for a while longer. It was expected that EC would have followed the same trend as pH but that proved to be untrue with only the high ash application in the final sampling period having significantly different EC. While we did not measure microbial properties, it is documented that wood ash induced pH can have positive or negative effects on certain groups of bacteria and that bacterial richness and diversity strongly decreases with increasing levels of ash (Bang-Andreasen et al., 2017).

Effects of Wood Ash on Plant Available Nutrients

Nitrate Availability

Plant available nitrate was higher in the herbicide treated plots when the repeated measures model examined all deployment times. It was only significantly higher in the herbicide and no ash plot; ash application (low and high) lowered nitrate enough that it was not significantly different from the control plots. This is likely due to the decaying knapweed which was treated releasing nitrogen back into the soil at high rates, also observed by Hooker and Stark (2008, 2012). All herbicide treated plots had elevated nitrate, with those levels being reduced over time. Hook and Stark (2012) noted that soils with vegetation treated by herbicide had N pools 3-5 times greater than the control and these samples were taken within the same season. This data was not taken until 10 months after treatment, so it is likely our nitrate values would have been even higher the previous year. One theory attributed to spotted knapweeds rapid dominance is its ability to exploit resources previously inaccessible to other species (Suding et al., 2004), likely due to its long tap root. The sheer volume of spotted knapweed detritus is the most obvious explanation for this result. Nitrogen is thought to be one of the limiting factors in a grassland

(Gleeson and Tillman, 1990), so an increase of NO_{3-} suddenly being available in a generally limited area can have important implications for management, such as secondary cheatgrass invasion. Ash had a significant effect on nitrate concentration in the herbicide treated plots, with nitrate levels being proportionally lower by level of ash applied. Other studies have found a decrease in nitrogen concentration in the upper soil layers after wood ash application with many attributing it to the increase of pH promoting immobilization of nitrogen (Mandre, 2006; Bamryd and Fransman, 1995). That is a logical conclusion in our study as nitrate availability followed the same pattern as pH, decreasing steadily after application.

Other Nutrients

Other than nitrate, ash treatment was the driving factor of potassium, sulphur and zinc increases only. This is surprising as other studies (Bieser and Thomas, 2019) found biochar or wood ash application to increase availability in copper, zinc, boron, sulphur and lead and Hansen et al. (2017) found their ash amendment to significantly increase calcium, potassium, magnesium, manganese and sodium. This highlights how variable results can be when applying wood ash amendments, as they will be dependent on the composition of the ash, climatic conditions, pH, baseline soil conditions (nutrients as well as texture) as well as application method. The wood ash used had high levels of calcium which drove the pH changes and although the differences of plant available calcium was not quite significant with the control group actually having higher calcium than the low and high ash applications (1514.16, 1374.76 and 1244.24) which is contradictory to what would be expected. The high ash treatment added a large spike of sulphur to the soil for the first year after application, which was then undistinguishable between the other ash treatments for the second year. Phosphorus also significantly differed during deployment times, but the 4th deployment had close to double available P than the 2nd deployment which was due to 6 outlier values that were above the mean. Zinc did not follow a clear pattern with respect to ash application, in general it was higher in the high ash treatment, but that was not true for sampling time 3. Zinc is an essential plant nutrient but at high levels can be toxic (Rout and Das, 2003) An important thing to note is the level of toxic plant elements (lead, cadmium and aluminum) were not elevated in the high ash treatment. Metal toxicity in plants can be a cause of concern when applying wood ash treatments and can negate any positive effects from the ash (Saarsalmi et al., 2004).

Soil moisture

All nutrients significantly differed between sampling time other than zinc and potassium. Soil moisture levels are known to increase plant nutrient uptake (Metwally and Pollard, 1959; Veresoglou and Fitter, 1984) and can explain some of the patterns we saw with regards to nutrient availability in this study. The spikes in calcium and magnesium availability in deployment 2 and 4 correspond very closely to the periods of high precipitation and by default, high soil moisture. Nitrate also followed the same pattern however the degree diminished over time most likely due to immobilization taking place.

Carbon and Nitrogen response to wood ash amendment

Wood ash did not significantly increase the value of available carbon in the soil in the two sampling times as expected. One possible reason for this is the carbon content of the ash amendment used. Comparing to other studies, Blumenthal et al. (2007) used carbon amendments that were 39% and 42% C while Mitchel and Baker (2011) used carbon amendments of 42% and 100% C. Our wood ash amendment was around 10% C, with a lesser amount (~3%) as organic carbon. A higher organic carbon content would have been more likely to increase soil carbon percent. The particular ash was used as it is a local waste product and was the most feasible at large scales. One of the successful studies where carbon addition controlled invasive plants found that very high levels of carbon needed to be added to illicit a response (greater than 394 C per square meter) and that this may not be a practical invasive plant management technique (Blumenthal et al., 2003). With our wood ash being 10% C and the high ash treatment would have been approximately 100 g of C/m^2 and would explain why we did not see an reduction in spotted knapweed. Carbon on its own did not significantly increase, we were also interested in the CN ratio which was significantly higher in some of the high ash plots, suggesting that N was very low. CN ratios in native grassland soils tend to range from 8 to 13 (Zhang et al., 2013 and Xu et al., 2019) while the global average is thought to be 13.3 (Xu et al., 2013). The soils in this study were within average values of grassland soils, however were on the lower end. The lower the CN ratio the more rapidly nitrogen will be released into the soil and it generally takes a CN ratio of greater than 35 for microbial immobilization to occur (Brust, 2019).

This chapter discussed some soil properties following two years after a herbicide and ash amendment grassland treatment. Ash positively increased some important plant nutrients and immobilized some of the nitrate from the knapweed detritus. Ash increased soil pH, but those effects diminished over time and did not appear to negatively affect plant growth. Ash did not have any effect on total soil carbon as hypothesized and therefore was not an effective way to reduce spotted knapweed. The next chapter visits how changes in soil properties may have contributed to above ground plant responses and recommendations for management.

3.6 References

Alberta Environment. 2002. Standards and Guidelines for the Use of Wood Ash as a Liming Material for Agricultural Soils. T/666. Public Health Act and Environmental Management Act.

Bang-Andreasen, T., Nielsen, J.T., Voriskova, J., Heise, J., Rønn, R., Kjøller, R., Hansen, H.C. and Jacobsen, C.S. 2017. Wood ash induced pH Changes Strongly Affect Soil Bacterial Numbers and Community Composition. Frontiers in Microbiology. 8, 1400.

Bieser, J., Thomas, S. 2019. Biochar and high-carbon wood ash effects on soil and vegetation in a boreal clearcut. Canadian Journal of Forest Research. 49. 10.1139/cjfr-2019-0039.

Blumenthal, D. M., Jordan, N. R., and Russelle, M. P. 2001. Soil Carbon Addition Controls Weeds and Facilitates Prairie Restoration. Ecological Applications, 13(3), 605–615.

Bramryd, T., and Fransman, B. 1995. Silvicultural Use of Wood Ashes – Effects on the Nutrient and Heavy Metal Balance in a Pine (*Pinus sylvestris L*.) Forest Soil. Water Air Soil Pollution., 85, 1039–1044.

Brust, G. 2019. Chapter 9 - Management Strategies for Organic Vegetable Fertility. Safety and Practice for Organic Food. 193-212. Biswas and Micallef. Academic Press.

Cruz-Paredes C, Wallander H, Kjøller R, Rousk J (2017) Using community trait-distributions to assign microbial responses to pH changes and Cd in forest soils treated with wood ash. Soil Biology & Biochemistry, 112, 153–164.

DeLuca, T., and Gao, S., 2020. Biochar alters nitrogen and phosphorus dynamics in a western rangeland ecosystem. Soil Biology and Biochemistry. 148, 107868.

DiTomaso J, GB Kyser, SR Oneto, RG Wilson, SB Orloff, LW Anderson, SD Wright, JA Roncoroni, TL Miller, TS Prather, C Ransom, KG Beck, C Duncan, KA Wilson, JJ Mann. 2013. Weed Control in Natural Areas in the Western United States. Weed Research and Information Center, Univ. of California, Davis. 544 pages

Foster, J. G., Fraser, L., Van Hamme, J., Higgins, R., Gosselin, L., Ford, A. 2019. Effects of the invasive plant spotted knapweed (Centaurea stoebe L.) on grassland arthropod communities: Use of genomic barcoding tools for ecosystems reclamation management. Retrieved from Thompson Rivers University

Fraser, L. H., and Carlyle, C. N. 2011. Is Spotted Knapweed (*Centaurea stoebe L.*) Patch Size Related to the Effect on Soil and Vegetation Properties? Plant Ecology, 212(6), 975-983. doi:10.1007/s11258-010-9878-7

Gleeson, S. K. and Tilman, D. 1990 Allocation and the transient dynamics of succession on poor soils. Ecology 71 1144–1155.

Hannam, K.D., Deschamps, C., Kwiaton, L., Venier, L., and Hazlett, P.W. 2016. Regulations and guidelines for the use of wood ash as a soil amendment in Canadian forests. Natural Resources Canada, Canadian Forest Service. Information report GLC-X-17.

Herron, G. J., R. L. Sheley, B. D. Maxwell, and J. S. Jacobsen. 2001. Influence of nutrient availability on the interaction between spotted knapweed and bluebunch wheatgrass. Restoration Ecology. 9:326-331.

Hook, P. B., Olson, B. E., and Wraith, J. M. 2004. Effects of the invasive forb Centaurea maculosa on grassland carbon and nitrogen pools in Montana, USA. Ecosystems, 7(6), 686–694

Hooker, T.D. and Stark, J.M. 2008 Soil C and N cycling in three semiarid vegetation types: response to an in situ pulse of plant detritus. Soil Biol Biochem 40:2678–2685

Hooker, T.D., and J.M. Stark. 2012. Carbon flow from plant detritus and soil organic matter to microbes—Linking carbon and nitrogen cycling in semiarid soils. Soil Sci. Soc. Am J. 76:903-914.

Huotari, N., Tillman-Sutela, E., Moilanen, M., Laiho, R., 2015. Recycling of ash – For the good of the environment?. Forest Ecology and Management. 348. 10.1016/j.foreco.2015.03.008.

Kanissery R., Gairhe B., Kadyampakeni D., and Kelly M. 2018. Herbicide - Nutrient Interactions in Soil: A Short Review. Agri Res & Tech: Open Access Journal. 15(2): 555951. doi: 10.19080/ARTOAJ.2018.15.555951.

Mandre, M., Pärn, H., Ots, K., 2006. Short-term effects of wood ash on the soil and the lignin concentration and growth of Pinus sylvestris L. Forest Ecology and Management, 223, 1–3. https://doi.org/10.1016/j.foreco.2005.11.017

Metwally, S. Y., and Pollard, A. G. 1959. Effects of Soil Moisture Conditions on the Uptake of Plant Nutrients by Barley and on the Nutrient Content of the Soil Solution. Journal of the Science of Food and Agriculture, 10(11), 632–636. https://doi.org/10.1002/jsfa.2740101109

Mitchell, R. M., & Bakker, J. D. (2011). Carbon Addition as a Technique for Controlling Exotic Species in Pacific Northwest Prairies. Northwest Science, 85(2), 247–254. https://doi.org/10.3955/046.085.0213

Organic Matter Recycling Regulation, BC Reg 18/2002, https://canlii.ca/t/53k28. Retrieved on 2021-03-08

Perry, L.G., D. M. Blumenthal, T. A. Monaco, M. W. Paschke, and E. F. Redente. 2010. Immobolizing nitrogen to control plant invasions. Oecologia 163: 13-24.

Rout, G. R., and Das, P. 2009. Effect of Metal Toxicity on Plant Growth and Metabolism: I. Zinc. Sustainable Agriculture, 873-884. doi:10.1007/978-90-481-2666-8_53

Suding, K. N., LeJeune, K. D., and Seastedt, T. R. 2004. Competitive impacts and responses of an invasive weed: Dependencies on nitrogen and phosphorus availability. Oecologia, 141(3), 526-535. doi:10.1007/s00442-004-1678-0

Xu, E., Hongqi, Z., and Yongmei, X. 2019. Effects of Large-Scale Cultivated Land Expansion on the Balance of Soil Carbo and Nitrogen in the Tarim Basin. Agronomy. 9(2), 86. https://doi.org/10.3390/agronomy9020086.

Xu, X., Thornton, P., and Post, W. 2013. A Global Analysis of Soil Microbial Biomass Carbon, Nitrogen and Phosphorus in Terrestial Ecosystems. Global Ecology and Biogeography. 22, 737-749.

Zhang, J., Wang, X., Wang, J., and Wang, W. 2014. Carbon and Nitrogen Contents in Typical Plants and Soil Profiles in Yanqi Basin of Northwest China. Jounal of Integrative Agriculture. 13(3), 648-656.

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4 Plant and Soil Interactions and Implications for Management

4.1 Introduction

The intention of ecologically based weed management is to use one or more invasive plant control methods that mitigate the above and the belowground effects of invasive plant invasions. It is not enough to just talk about the vegetation or the soil responses to the treatment, we must also discuss how these impacted each other, and the feedback mechanisms. There were several interesting but unexpected observations from this study which pave the way for more research. The most intriguing observations were the nitrogen cycle's influence on above ground plant responses such as secondary invasion and the boost in non-seeded grass growth associated with the high ash addition.

Secondary Invasion

One of the most concerning results is secondary invasion of *B. tectorum*. It was unexpected as, there were only small amounts noted in the baseline surveys, and seed treatments were expected to fill the open spots to prevent an aggressive increase of undesirable species. One part of the explanation could be the increase of soil nitrogen as a result of the herbicide treatments. Previous studies have suggested that invasive annuals dominate in disturbed environments with high soil nitrogen (Bidwell et al., 2006; Blumenthal, 2006). My research proposal discussed the relationship of spotted knapweed and nitrogen, but failed to investigate cheatgrass. Vasquez et al., (2008) looked at competition of cheatgrass and native grasses on a nitrogen gradient (0, 137 and 280 mg N/kg) and found that cheatgrass biomass increased with increasing N concentrations and thus greater soil N leads to an increased competitive ability of cheatgrass. A similar relationship was seen with the vegetation data from 2020 and the nitrate data from deployment 4 (Figure 4.1). This trend was there for other deployments but it was most obvious in the 4th. The combined effects of elevated nitrogen due to the herbicide treatment, the poor success of seedling establishment and the open space left by the removal of knapweed all compounded the incidence of secondary cheatgrass invasion. The highest growth of cheatgrass was associated with the sprayed/low ash treatment in both years, so it's possible that the high level of ash did cause some immobilization of nitrogen early on and prevent the cheatgrass from increasing as greatly, while the low ash treatment had high nitrogen, plus additional micronutrients from the ash to promote growth.

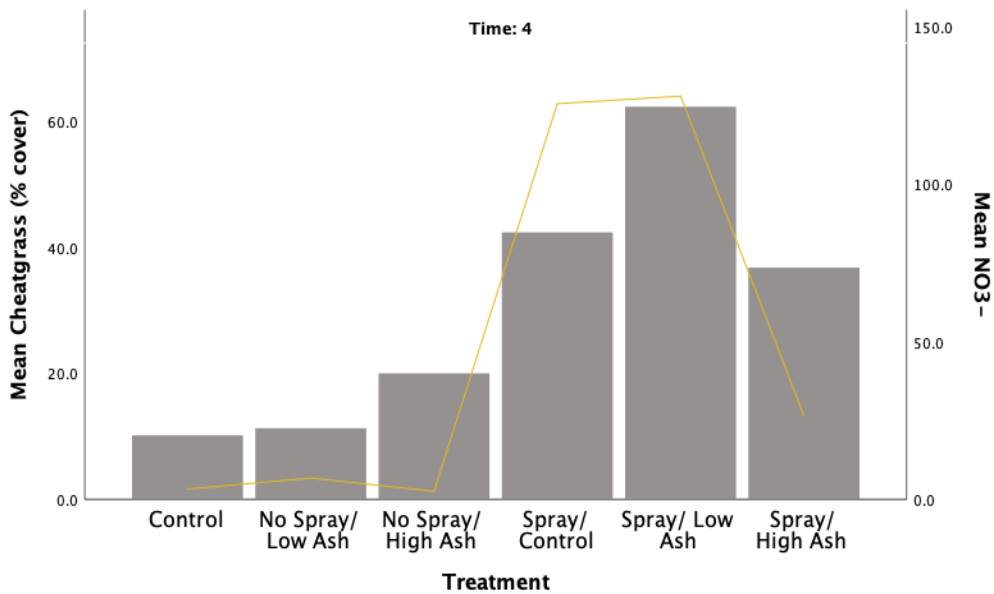

Figure 4.1 *Bromus tectorum* (cheatgrass) % cover (grey Bars) by treatment (deployment 4) and corresponding nitrate values (Yellow Line).

So what came first? The nitrogen or the weeds?

One hypothesis surrounding invasion ecology is that species poor communities may be unable to fully use available nutrients, which can increase nutrient availability and in turn promote dominance by exotic species (Heckman and Carr, 2016). Several experiments have found that nitrogen addition increases the competitive ability of spotted knapweed (Gao et al., 2015; Story et al., 1989) and suggests that spotted knapweed will invade ecosystems with high nitrogen availability, however; it is also known to invade nutrient poor systems. Thorpe and Callaway (2011) found that soil nitrate was 60% lower in communities invaded with spotted knapweed than in the control plot and suggested that it alters soil nitrogen cycling by decreasing the rate of nitrification. In this study there is only one data point to compare the invaded site to the uninvaded site from the October 2020 sampling period but it determined nitrogen was significantly lower than any treatment in the invaded research plots. This contradicts Thorpe and Callaway (2011), but is consistent with other studies (Sheley and Krueger-Mangold, 2006; Blumenthal et al., 2003) where high N availability was found to facilitate invasion by non-native and invasive plant species. It is still unclear if the invaded site became N rich, possibly from organic fertilizer of grazing animals which then facilitated invasion? Or did spotted knapweed change the N cycle by decreasing the rate of nitrification. Due to the contrasting research it would be worth further investigation.

Positive graminoid responses

Despite having poor success with seeding treatments, we did see a noticeable increase in *Poa pratensis* and *Koeleria macrantha*, two desirable species that were not seeded, and thus naturally occurring in the seedbank. This increase was significant in only the herbicide/high ash treatment which suggests that the suppression of the knapweed via herbicide was important for the high ash treatment to act as a fertilizer. *P. pratensis* tends to reach its maximum uptake of nutrients early in the season and is resistant to low temperatures (Veresoglou and Fitter, 1984) which could explain its increase in comparison to other graminoids. A relevant study (Gundale and DeLuca, 2007) found that wildfire created charcoal had a positive effect on the growth of *K. macrantha* with increasing soil charcoal concentration.

Carbon amendment as a control method

Many scientific studies have examined the use of various carbon amendments and their ability to suppress invasive plants via inducing immobilization of plant available nitrogen (Blumenthal et al., 2007; Reever Morghan and Seastedt, 1999). These results have ranged from very successful (Mitchel and Bakker, 2011; Kulmatiski and Beard, 2006) to no significant effect (Krueger-Mangold and Sheley, 2008), and this study did not prove it to be a viable alternative to chemical treatment. Ideally, we would discover the missing link needed to credit cheap carbon amendments as the leading alternative to chemical weed control, reversing invasion and fertilizing the grasslands but in 30 years of studies we still have not isolated the exact environmental conditions that make it successful. Still, many researchers have reiterated that controlling nutrient levels is a viable invasive plant management strategy (Seastedt et al., 1991). Instead of using soil amendment, Herron et al., (2001) used annual ryegrass to sequester nitrogen and saw that this tipped the competitive advantage from spotted knapweed to bluebunch wheatgrass.

4.2 Management Implications and Study Limitations:

While this study was important for adding to the growing body of literature about rangeland invasive plant management, and one of few studies applying wood ash to rangeland soils the goal of the project was to better understand how to manage the Laurie Guichon Memorial Grassland Interpretive Site. As most ecological restoration studies have concluded; it is important to determine site specific factors when applying a control prescription (Kettenrig and Adams, 2011); this study was a building block towards a restoration plan.

The most alarming result of our study was the influx of cheatgrass in herbicide treated plots. This suggests that herbicide should not be used in areas with high knapweed density and cheatgrass present or should be used sparingly. Herbicide was the only successful treatment that lowered spotted knapweed cover, therefore its use must not be dismissed, just improved. A limitation to our study was that only one type of herbicide was used. There are many herbicides on the market and some may have prevented secondary invasion. In fact, there are new herbicides available that target annual grasses which could eventually be used in conjunction with a broadleaf specific herbicides to treat knapweed and cheatgrass in one application. Another key part of rangeland invasive plant control is replacing the invasive species with your desired species which we failed to do in this experiment. In the future, seeding practices should be adaptive with multiple applications if needed. Also, as indicated by McManamen et al. (2018), picloram (used in this study) had a far greater negative effect on seedling germination than aminopyralid. They also found that fall herbicide and spring seeding had better success than the converse. Aminopyralid may be a better option in preventing cheatgrass invasion by improving perennial seed establishment.

There have been many conflicting studies regarding the efficacy of carbon amendments for weed control, and this study has not cleared up any confusion. While neither level of wood ash application significantly lower knapweed cover we can at least conclude there were no negative effects. Wood ash soil amendments can sometimes be a source of metals that are toxic to plants; however, no high levels of Cd, Pb or Al were observed in this study. The slight positive correlation between wood ash level and perennial grass growth may be enough to support wood ash application in conjunction with the improved seeding practices. There is also evidence that the high ash treatment did lower nitrogen in the soil and may have been the reason cheatgrass was lower in the high ash treatment than the low ash treatment.

A limitation to this study was a lack of soil sampling. As the project evolved it was more evident that there were many complex nitrogen interactions happening as a result of the wood ash treatments. There were only two soil samples taken after ash application as well as only one sample being taken from each sub-plot. Analysis of the C% and N% closer to the application of the ash and more times throughout the growing season to see how long the carbon persisted and what rate it was used is a key missing piece. There also should have been more uniform sampling throughout the plot to get a clearer picture of nutrient levels as well as PRS probe deployments in more locations. PRS deployment in an uninvaded site would have helped to further understand the soil chemical property changes associated with high invasion levels, and a gradient from high invasion, to low, to no invasion would answer questions about the changes to the site over the time of invasion. There are also many micro vertical changes associated with the pH and base cations after wood ash application as discussed by Hansen et al. (2017) which we did not capture. Hopefully this study encourages more research on wood ash in grasslands.

Another limitation was the composition of wood ash used. In order to have achieved the increased C:N ratio and therefore immobilize nitrogen, the wood ash would have needed to have a higher organic carbon (OC) content. The OC was much lower than the inorganic C so it was not as accessible to microbes as we hoped. The wood ash was chosen as it was a local waste product and it would not be economical or feasible to pay or ship large amounts of a more suitable ash, or manufactured biochar. It would be worth monitoring the supply of local wood ash to see compositional changes depending on the most recent materials used in its generation.

Conclusion

This study looked at above ground plant response to several invasive plant management techniques, the belowground soil responses include plant available nutrients, pH and electrical conductivity as well as C:N ratios. We have barely grazed the surface of soil properties associated with spotted knapweed invasions and wood ash while not even beginning to talk about the effects of ash on soil microbes. An expert in invasion ecology would need to have a graduate level of understanding in plant science, soil chemistry and soil bio-geochemistry at the bare minimum. I have previously discussed some future directions of research and suggest that future large scale invasive plant studies should be cross disciplinary and involve multiple experts as it is impossible for one or two people to understand everything. Land managers have noted that invasive plant research can be disconnected from actual efforts to conserve and restore ecosystems, (Kettenring and Adams, 2011) therefore it was important to use feasible techniques that were realistic for future management efforts. The improved herbicide practices discussed and use of wood ash as a fertilizer to aid revegetation will be useful tools in future restoration efforts in knapweed invaded rangelands.

4.3 References

Blumenthal, D.M. 2006. Interactions between resource availability and enemy release in plant invasion. Ecology Letters, 9: 887-895. https://doi.org/10.1111/j.1461-0248.2006.00934.x

Blumenthal, D. M., N. R. Jordan, and M. P. Russelle. 2003. Soil carbon addition controls weeds and facilitates prairie restoration. Ecological Applica- tions 13:605–615.
Booth,

Bidwell, S., Attiwill, P.M. and Adams, M.A. 2006. Nitrogen availability and weed invasion in a remnant native woodland in urban Melbourne. Austral Ecology, 31: 262-270. https://doi.org/10.1111/j.1442-9993.2006.01575.x

Cruz-Paredes C, Wallander H, Kjøller R, Rousk J. 2017. Using community trait-distributions to assign microbial responses to pH changes and Cd in forest soils treated with wood ash. Soil Biology & Biochemistry, 112, 153–164.

Endress, B., Parks, C., Naylor, B., Radosevich, S. 2008. Herbicide and Native Grass Seeding Effects on Sulfur Cinquefoil (Potentilla Recta)-Infested Grasslands. Invasive Plant Science and Management. 1 50-58. 10.1614/IPSM-07-005.1.

Gao, Y., Yu, H. W., and He, W. M. 2014. Soil space and nutrients differentially promote the growth and competitive advantages of two invasive plants. Journal of Plant Ecology, 7(4), 396–402. https://doi.org/10.1093/jpe/rtt050

Gundale, M.J., DeLuca, T.H. 2007. Charcoal effects on soil solution chemistry and growth of Koeleria macrantha in the ponderosa pine/Douglas-fir ecosystem. Biol Fertil Soils 43, 303–311. https://doi.org/10.1007/s00374-006-0106-5

Hansen, M., Bang-Andreasen, T., Sørensen, H., and Ingerslev, M. 2017. Micro vertical changes in soil pH and base cations over time after application of wood ash on forest soil. Forest Ecology and Management, 406, 274-280. doi:10.1016/j.foreco.2017.09.069

Heckman, R. W., and Carr, D. E. 2016. Effects of soil nitrogen availability and native grass diversity on exotic forb dominance. Oecologia, 182(3), 803–813. https://doi.org/10.1007/s00442-016-3692-4

Herron, G. J., Sheley, R. L., Maxwell, B. D., and Jacobsen, J. S. 2001. Influence of nutrient availability on the interaction between spotted knapweed and bluebunch wheatgrass. Restoration Ecology, 9(3), 326–331. https://doi.org/10.1046/j.1526-100X.2001.009003326.x

Kettenring, K. M., and Adams, C. R. 2011. Lessons learned from invasive plant control experiments: A systematic review and meta-analysis. Journal of Applied Ecology, 48(4), 970–979. https://doi.org/10.1111/j.1365-2664.2011.01979.x

Krueger-Mangold, J. M., Sheley, R. L., and Svejcar, T. J. 2006. Toward ecologically-based invasive plant management on rangeland. Weed Science, 54(3), 597–605. https://doi.org/10.1614/WS-05-049R3.1

Kulmatiski, A., & Beard, K. H. 2006. Activated carbon as a restoration tool: Potential for control of invasive plants in abandoned agricultural fields. Restoration Ecology, 14(2), 251–257. https://doi.org/10.1111/j.1526-100X.2006.00127.x

Mangold, J. M., & Sheley, R. L. 2008. Controlling Performance of Bluebunch Wheatgrass and Spotted Knapweed Using Nitrogen and Sucrose Amendments. Western North American Naturalist, 68(2), 129–137. https://doi.org/10.3398/1527-0904(2008)68[129:CPOBWA]2.0.CO;2

McManamen, C., Nelson, C. R., & Wagner, V. 2018. Timing of seeding after herbicide application influences rates of germination and seedling biomass of native plants used for grassland restoration. Restoration Ecology, 26(6), 1137–1148. https://doi.org/10.1111/rec.12679

Mitchell, R. M., & Bakker, J. D. 2011. Carbon Addition as a Technique for Controlling Exotic Species in Pacific Northwest Prairies. Northwest Science, 85(2), 247–254. https://doi.org/10.3955/046.085.0213

Reever Morghan, K. T., and Seastedt, K.T. 1999. Effects of soil nitrogen reduction on nonnative plants in restored grasslands. Restoration Ecology 7:51–55.

Seastadt, T. R., J. M. Briggs, and D. J. Gibson. 1991. Controls of nitrogen limitation in tall-grass prairie. Oecologia 87:72–79

Mangold, J. M., and Sheley, R. L. 2008. Controlling Performance of Bluebunch Wheatgrass and Spotted Knapweed Using Nitrogen and Sucrose Amendments. Western North American Naturalist, 68(2), 129–137. https://doi.org/10.3398/1527-0904

Story, J. M., K. W. Boggs, and D. R. Graham. 1989. Effects of nitrogen fertilization on spotted knapweed and competing vegetation in Western Montana. Journal of Range Management 42:222–225

Thorpe, A. S., & Callaway, R. M. (2011). Biogeographic differences in the effects of Centaurea stoebe on the soil nitrogen cycle: Novel weapons and soil microbes. Biological Invasions, 13(6), 1435–1445. https://doi.org/10.1007/s10530-010-9902-9

Vasquez, E., Sheley, R., and Svejcar, T. 2008. Nitrogen Enhances the Competitive Ability of Cheatgrass (Bromus tectorum) Relative to Native Grasses, 287–295. https://doi.org/10.1614/IPSM-08-062.1

Appendix I List of Species

Scientific name	Common name	Origin
Cenaurea stoebe	Spotted knapweed	Introduced
Lupinus sericea	Silky Lupine	Native
Lithersperma ruderal	Lemon weed	Native
Symphyotrichum ericoides	Hairy aster	Native
Eriogonum flavum	Buckwheat	Native
Alyssum allysoides	Desert alyssum	Introduced
Erigeron compositus	Cut-leaved daisy	Native
Kochia scoparia	Kochia	Introduced
Microsteris gracilis	Pink twink	Native
Tragopogon dubius	Yellow salsify/Goats beard	Introduced
Arabis holboellii	Holboell's Rockcress	Native
Epilobium ciliatum	Fringed willowherb	Native
Verbascum thapsus	Mullein	Introduced
Sisymbrium altissimum	Tall tumble mustard	Introduced
Solidago canadensis	Goldenron	Native
Lactuca serriola	Prickly lettuce	Introduced
Vicia americana	American vetch	Native
Achillea millefolium	Yarrow	Native
Antennaria parvifolia	Nuttal's pussytoes	Native
Taraxacum officinale	Dandelion	Introduced
Arenaria serpyllifolia	Thyme leaved sandwort	Native
Lepidium latifolium	Peppergrass	Introduced
Gallairdia aristata	Gallairdia	Native
Erysimum inconspicuum	Small wallflower	Native
Lomatium macrocarpum	Desert parsley	Native
Collomia tenella	Slender collomia	Native
Myosotis stricta	Blue forget-me-not	Native
Eriogonum heracleoides	Parsnip flowered buckwheat	Native
Bromus tectorum	Cheatgrass	Introduced
Pascopyrum smithii	Bluebunch wheatgrass	Native
Poa pratensis	Kentucky bluegrass	Introduced
Festuca idahoensis	Idaho fescue	Native
Agropyron cristatum	Crested wheatgrass	Introduced
Koeleria macrantha	June grass	Native
Poa secunda	Sandberg bluegrass	Native
Juncus balticus	Baltic rush	Native

Appendix II: Germination Experiment

Method: In September soil was collected from each plot to run a greenhouse seed bank analysis. In each plot a 1 m^2 square frame was placed in the bottom section of each plot and four 10 cm diameter soil bulk density corers were used to collect the top 10 cm of soil to equal 3% of the frame. This number was recommended by Plue and Hermy (2012) who presented a method of sampling to accurately estimate presence/absence of species and increase comparability of future seed bank community studies. Soil from each plot was placed into a large ziploc freezer bag and then placed in a freezer for 1 month to simulate winter dormancy. A 2 cm soil sieve was used to remove large coarse material. 11 x 11 inch trays were lined with sterilized sand to improve drainage and then filled with soil samples. Trays were kept at 21 ℃ and under a grow light. Trays were watered as needed to keep moist. When germination had stalled trays were stirred to mimic disturbance. Once a species was identified and counted it was removed from the tray. After 5 months Giberellic acid was applied to further enhance germination. 15 mL of 1 g 92% Giberellic acids to 1 L water was sprayed on the soil surface. The experiment continued until 1 week after germination had ceased.

Results: There were 12 species recorded. Only *Bromus tectorum* (cheatgrass) and *Centaurea stoebe* (spotted knapweed) were recorded in large amounts. Herbicide presence significantly increased the number of cheatgrass seeds and significantly decreased the number of spotted knapweed seeds per m^2. Overall, spotted knapweed seeds were quite low as Shirman (1981) noted natural seed production ranged from 5,000 to 40,000 seeds/m^2. The low seed production is most likely attributed to the presence of seed feeding biocontrol agents which were observed at the site.

Species	Herbicide	Mean	St Dev.	P value
	Absent	32.92	45.87	
Bromus tectorum	Present	134.81	90.61	0.0001
	Absent	9.42	7.67	
Centaurea stoebe	Present	5.53	6.71	0.02

Works Cited

Plue, J., & Hermy, M. (2012). Consistent seed bank spatial structure across semi-natural habitats determines plot sampling. *Journal of Vegetation Science*, *23*(3), 505–516. https://doi.org/10.1111/j.1654-1103.2011.01361.x

Shirman, R. 1981. Seed production and spring seedling establishment of dif- fuse and spotted knapweed. J. Range Manage. 34:45-47