REDUCING CRESTED WHEATGRASS (*AGROPYRON CRISTATUM* (L.) GAERTN) AND IMPROVING SOIL HEALTH TO FACILITATE NATIVE PLANT RESTORATION

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The Supervisory Committee certifies that this ***disquisition*** complies with North Dakota State University's regulations and meets the accepted standards for the degree of

MASTER OF SCIENCE

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ABSTRACT

Land managers seek to restore diversity of native plant species to areas dominated by introduced crested wheatgrass (*Agropyron cristatum* (L.) Gaertn.; CWG). This study was initiated by the Bureau of Land Management (BLM) to find the most effective treatment or treatment combination in reducing CWG cover and enhancing soil for native species establishment. Our treatments used alone and in combination were tillage, herbicide, cover crops, and a soil amendment.

We found that CWG reduction was dependent on tillage or herbicide, with the highest reduction in their combination. The combination of these included the addition of cover crops and amendment but still led to the lowest total microbial abundances, along with the lowest fungal-to-bacterial ratio. Cover crop was highest in microbial abundances in the greenhouse and amendment was highest in the field. Herbicide led to increased soil nitrate and low labile carbon, but this was offset by cover crop addition.

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DEDICATION

I want to dedicate this work to my sister Stephanie for inspiring my love of the natural world. You taught me to respect all life and appreciate what nature has to offer us, shaping who I am today. This is also dedicated to those who seek to understand nature's complexity.

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LIST OF ABBREVIATIONS

A	Amendment.
AM	Arbuscular Mycorrhizal.
AH	Amendment and Herbicide.
BLM	Bureau of Land Management.
C	Cover Crop (Chapter 1).
C	Carbon (Chapter 2).
CA	Cover crop and Amendment.
CAT	Cover crop, Amendment, and Tillage.
CH	Cover crop and Herbicide.
CT	Cover crop and Tillage.
CWG	Crested Wheatgrass.
EC	Electrical conductivity.
H	Herbicide.
KS	Kitchen Sink (Cover crop, amendment, tillage, herbicide).
MBC	Microbial biomass carbon.
POXC	Permanganate oxidizable carbon.

LIST OF SYMBOLS

™..Trademark symbol

GENERAL INTRODUCTION

According to Johannes Le Roux’s book “The Evolutionary Ecology of Invasive Species,” (Le Roux 2022) invasive species “live life in the fast lane” by rapidly adapting, reproducing, and expanding throughout new ecosystems. These abilities allow them to outcompete native species for valuable resources above and belowground, leading to reductions in biodiversity and ecosystem services around the world. Additionally, anthropogenic activities and climate change have shifted survival and distribution of species, favoring the adaptive potential of invasive species over native species (da Silva et al. 2021). Plant-soil feedbacks are the processes that plants use to change soil properties as they grow, which in turn alters the habitat for the plants (Reinhart 2012). These feedbacks can be considered positive or negative depending on the altered soil properties effects on soil biota and plant community structure. These properties include biotic factors like the soil biological community and abiotic factors like moisture, bulk density, or nutrient availability. Although most plant species tend to perform worse in soils they have repeatedly grown in, research has shown that invasive species are either unaffected or benefit from the feedback created by conspecific growth (Perkins and Nowak 2012).

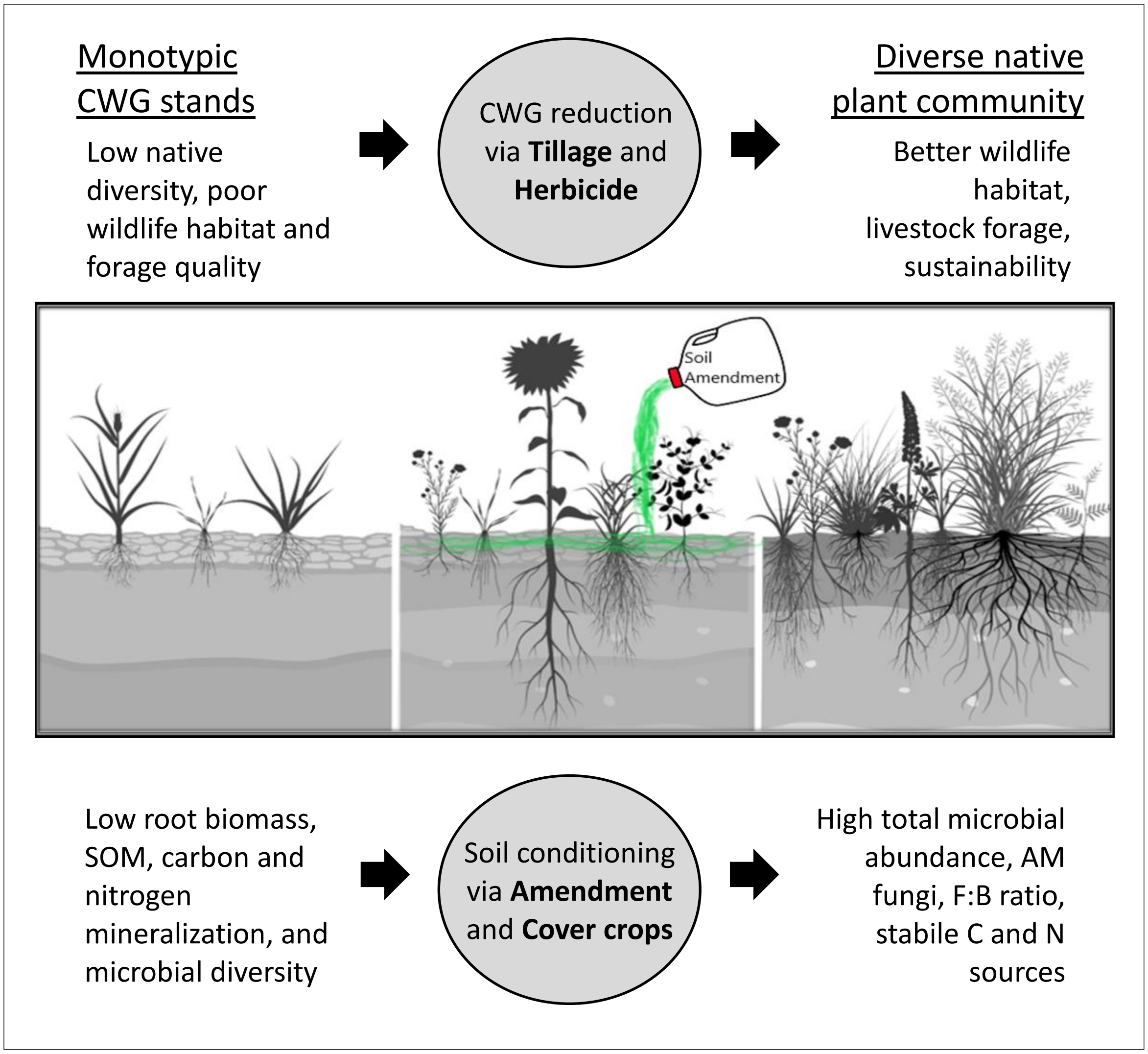
The mixed-grass prairies of the Northern Great Plains are especially susceptible to invasions due to their highly variable climate conditions and increased fragmentation caused by agricultural land conversion (DeKeyser et al. 2013). Prairies, also known as grasslands, are semi-arid ecosystems with wide variability in abiotic factors. The variability in these factors often translates to variability of success in certain land management decisions (Bakker et al. 2003), including those involving invasive species. Invasive grasses are especially difficult to manage due to their morphological and physiological similarities to native grasses, which means control methods often negatively impact native species along with the targeted invasive ones (Gaskin et al. 2021). Perennial grasses have meristems and large seed banks to regrow from when defoliated, making management approaches like herbicide or grazing only temporarily effective (Bakker et al. 2003, Wilson et al. 2010; NRCS 2021).

The semi-arid climate of the United States is characterized by low precipitation, and climate models of this region project more intense, but less frequent precipitation events (IPCC 2007). This shift in precipitation leaves more opportunity for invasive grasses with higher drought tolerance like crested wheatgrass (*Agropyron cristatum*, CWG) to invade these ecosystems (Bansal et al. 2014). CWG is the most common exotic introduced grass in the western US because of its drought and cold tolerance, plus its availability for livestock grazing during early spring (Krzic et al. 2000). However, CWG decreases in forage quality during summer, having lower protein concentration and digestibility (Holt 1996, Jefferson and Coulman 2008). This leads to livestock seeking other warm season vegetation during the summer, decreasing the native plant cover. Native vegetation cover and density are higher in grazed versus ungrazed CWG stands (Nafus et al. 2016). Research has found unintended consequences of its introduction, including decreased native plant species diversity and decline in soil health properties like root biomass, soil organic matter, aggregate stability, and more (Lesica and DeLuca 1996; Dormaar et al. 1978, Dormaar et al. 1995; Smoliak et al. 1967).

We hypothesize that CWG's alteration of the soil environment for its own benefit contributes to its dominance over native species. Chapter 1 of the research project is dedicated to investigating tillage and herbicide's effectiveness at reducing CWG. In chapter 2, we inspected the soil health implications of these management methods and evaluated if the addition of cover crops and a biological soil amendment can create more stable, biologically diverse soils. We believe using soil conditioning methods with traditional invasion reduction methods can improve future native establishment (0.1). This research hopes to aid land managers looking to reduce CWG and improve soil conditions before restoring native species. Monotypic CWG stands contain low aboveground and belowground diversity as well as low SOM, carbon and nitrogen mineralization. Successful reduction of CWG occurs with tillage and herbicide, but these often degrade soil quality further.

This project proposes to add soil conditioning methods to these aboveground control methods to increase soil health parameters (0.1). The soil conditioning methods and CWG control methods we will be testing in various combinations are tillage, herbicide, cover crops, and a biological soil amendment. A field study was conducted near Glasgow, MT from 2022-2023 to reach our objectives. In chapter 1, we focus on aboveground impacts of the treatments, measuring CWG reduction, percent cover crop, vegetation biomass, percent bare soil, and native plant diversity, and treatment costs. We hypothesize that CWG reduction and cover crop growth will be greatest in the herbicide and tillage treatments, with the greatest in their combination. We also believe the tillage treatments will have the greatest amount of bare soil and Native diversity will be low in the tillage and herbicide treatments.

Chapter 2 focuses on the soil conditioning methods and their abilities to offset the impacts of tillage, herbicide, and the CWG soil legacy. A field and greenhouse study were conducted. We measured each treatment’s total organic carbon, permanganate oxidizable carbon (POXC), total nitrogen, nitrate, ammonium, microbial abundances, the greenhouse vegetation biomass, and greenhouse plant functional groups. We hypothesized that these treatments, especially when used together, will have the highest microbial abundances (with focus on arbuscular mycorrhizal fungi) and increased carbon and nitrogen levels.



0.1. Conceptual diagram of the starting crested wheatgrass ecosystem, the secondary conditioning stage, and the goal native plant community.
The above and belowground conditioning stage (center) represents our project’s approach for crested wheatgrass suppression and soil health improvement to facilitate the future restoration of native species to a crested wheatgrass dominant ecosystem.
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CHAPTER 1: EVALUATING SOIL AND VEGETATION TREATMENTS FOR CRESTED WHEATGRASS REDUCTION

Abstract

Land managers throughout the Northern Great Plains have recognized the degradation of native mixed-grass prairies due to the introduction of the non-native crested wheatgrass (CWG). The Northern Great Plains is 1 of the 4 last intact temperate grasslands in the world and spans 180 million acres, crossing five U.S. states and two Canadian provinces. Millions of these hectares have been altered to monotypic CWG stands. There has been limited success in reducing CWG in North American grasslands, and most management methods have focused on aboveground management strategies. Our goal was to combine aboveground vegetation control with soil manipulations to reduce CWG. We conducted a two-year field study in northeast Montana to evaluate four different management methods (tillage, herbicide, soil amendment, and cover crop) and their combinations. In vegetation surveys, we found the highest CWG reduction in the combination of all four treatments, (Cover Crop + Amendment + Tillage + Herbicide), followed by Cover Crop + Herbicide. The treatments with the highest CWG cover were those without tillage and/or herbicide. The use of tillage led to the highest rates of bare soil, which increased significantly between 2022 and 2023 in those treatments. This study demonstrates that soil manipulations, in combination with strategies targeting the aboveground vegetation, are effective at reducing CWG within a two-year period.

Introduction

Crested wheatgrass (CWG) [*Agropyron cristatum* (L.) Gaertn.] is one of the most widespread invasive species in the Great Plains and western North American rangelands; being planted somewhere within 6-10 million hectares (Wilson 2003; Ambrose & Wilson 2004), with hundreds of thousands of these hectares being in the public lands of Eastern Montana (Lesica & Cooper 2019). Its first introduction into the United States was in 1898 but it wasn't seriously considered for planting until the year of 1915 (Lesica & Cooper 2019; Rogler & Lorenz 1983). It is native to the steppes of central Asia and has thrived and spread throughout the arid sagebrush steppe and mixed grass prairie biomes in the United States (NRCS 2021). The sagebrush steppe biome is also located throughout the state of Montana and CWG's introduction there began at the agricultural experiment station near the town of Havre, MT (Reitz et al. 1936). Researchers at this station concluded that, when compared to brome grass (*Bromus spp.)* and slender wheatgrass (*Elymus trachycaulus*), CWG was best adapted to the dry, cold, and unirrigated landscape of Montana.

The semi-arid mixed grass prairie of eastern Montana covers about two-thirds of the state and is characterized by low precipitation that leads to a dominance of grass and shrub species with few trees (Luna and Vance 2017). Silver sagebrush (*Artemesia cana*) and diverse native grassland plants are essential species for forage, soil protection, and the hydrological cycle in this region (Hickman et al. 2013, Baker et al. 1988). Many wildlife species are adapted to the heat and drought of this region and rely on sagebrush and diverse grasses for wildlife habitat.

Bird species in the mixed-grass prairie include the golden eagle (*Aquila chrysaetos*), sagebrush sparrow (*Artemisiospiza nevadensis*), burrowing owl (*Athene cunicularia*), Greater sage-grouse (*Centrocercus urophasianus*), and many more (Vance et al. 2017). Many of these animals are species of concern, with populations declining due to habitat loss. (Hemstrom et al. 2002, Luna and Vance 2017) The Greater sage-grouse is one of the species of conservation concern, with a decline in population primarily attributed to loss of sagebrush habitat (Timmer et al. 2019). In northeast Montana, Lloyd and Martin (2005) found that nestlings of the chestnutcollard longspur (*Calcarius ornatus*) had slower growth and 17% less chance of survival in CWG stands versus native grasslands. Other songbird communities have also shown reduced diversity in areas of CWG (Sutter and Brigham 1998).

CWG is a cool-season perennial bunchgrass and its primary introduction to the United States was during the drought of the 1930s for livestock forage production, reducing soil erosion, and preventing establishment of weedy species (DiAllesandro et al. 2013). It's palatable to all classes of wildlife and livestock in the spring and fall if a resurgence occurs from adequate moisture (Ogle et al. 2023). It's unpalatable during summer and animals require protein supplements in winter for proper nutrition. Its lack of summer forage means landowners often seek to restrict its range and/or spread (Kral-O'Brien 2020). It establishes easily due to its tolerance of various soil types, ability to sequester moisture at lower temperatures as a seedling, weed resistance, ability to take up phosphorous more quickly than native species, and its drought and frost tolerance (Lesica and Cooper 2019; Lesica and DeLuca 1996).

CWG can be considered desirable due to its hardy attributes, but further research has shown the negative implications of its introduction, like a reduction in native plant species diversity and soil quality (Christian & Wilson 1999). Restoring native species to near monoculture stands of CWG will increase the ecological services provided by the plant community (Morris et al. 2019). Desirable ecological services of native plant species include providing habitat and food for wildlife, creating resilience to potential disturbances, and maintaining healthy soil properties. These all have been altered by invasions of introduced species (Le Roux 2022). Recovery of invaded native ecosystems through restoration is not focused on duplicating past conditions, but recovering the dynamic ecological services of that system using past conditions as a reference point to what is possible (Howell et al. 2012).

Many aboveground management techniques have been used for controlling CWG with limited success. One such method is herbicide application. In a 5-year study from McAdoo et al. (2017) glyphosate application initially suppressed the growth of CWG, but the grass eventually reemerged and exotic weed species were introduced, outcompeting the seeded native species. This response is partly attributed to CWG's persistent seedbank which allows seeds to remain viable for at least 2-4 years (Morris et al. 2019). The high growth rate of CWG also allows its reemergence (Gerry & Wilson 1995). Ambrose and Wilson (2003) showed this by looking at herbicide effects on CWG over 4 years and found that emergence from the seed bank did not decrease, possibly because seed production increases to compensate for decreased plant abundance.

Defoliation methods like grazing or clipping have been used to suppress CWG with varied success. Wilson and Pärtel (2003) found clipping reduced CWG cover by 90% and doubled the cover of native species. Other research found that long term grazing of CWG has no significant impact on the forage yield or seedbank of the grass, with stands persisting after 20 years of annual heavy grazing (Hubbard 1949, Hull and Klomp 1966). CWG's persistent seedbank is a large factor in its success, so reductions to the seedbank via tillage and herbicide will require repeated applications to primary and secondary growth of the grass, since the physical soil disturbance can cause secondary invasions (Hulet et al. 2010). Marlette and Anderson (1986) showed CWG's stands aren't invaded by native species in the seedbank even when native species are surrounding the grass. However, the chances improve at least 30-50 years later when the CWG stands have aged. Little is known about the seed dispersal or seedling recruitment in CWG, but Marlette and Anderson (1986) found a strong relationship between the standing CWG cover and density of emerging seedlings in each of their study sites. They also found that propagules of CWG are numerous and readily reseed themselves, as well as surrounding native stands.

Various land managers have sought ways to restore CWG dominated pastures to a more diverse native landscape, but there has been varied success with singular management methods like herbicide and soil disturbance (Davies et al. 2013, Hulet et al. 2010, McAdoo et al. 2017). Various studies have emphasized the need for more research on combined and repeated applications (Morris et al. 2019, Fansler and Mangold 2011). We hypothesize that using combined reduction methods of herbicide and disk tillage over 2 years will lead to greater CWG reduction. We also believe the limited success in native establishment is caused by the limited effort to address soil impacts of CWG control methods. Examining soil properties under CWG management methods and restoring diversity underground may aid in the management of this invasive grass species.

Soil microbial diversity is one of the main soil health indicators in both natural and managed ecosystems (Shu et al. 2022). Biologically diverse soils create stable soil structure, decompose organic matter, cycle nutrients, and increase plant productivity (Bender et al. 2016). Jordan et al. (2017) found that CWG alters soil biota to its own benefit and prevented native forb growth. Forbs rely on arbuscular mycorrhizal (AM) fungal relationships to receive soil nutrients, and CWG has lower AM fungal richness and colonization than native plant communities (Mummey and Ramsey 2017; Jorden et al. 2012). CWG’s low root inputs into the soil have shown lower amounts of organic matter, mineral and total nitrogen, and less total carbon than native perennial grasses (Dormaar et al. 1995; Christian and Wilson 1999; Gasch et al. 2015).

Soil microorganisms rely on living roots and plant detritus for important nutrients like carbon and nitrogen (Glessman 2020). Cover crops are a popular method of building soil biology, protecting the soil surface, sequestering nutrients like carbon and nitrogen, and improving moisture capture/availability. Organic amendments are another method popular in regenerative agriculture and include manure, composts, and biosolids (Gravuer et al. 2019). They have proven to be more successful than chemical fertilizers in improving microbial diversity since they increase crop productivity, soil fertility, and microbial biomass (Shu et al. 2022; Gravuer et al. 2019). Studies have shown that using certain cover crops in conjunction with organic amendments can lead to higher crop yield and biomass and suppress root pathogens (Wang et al. 2007). However, this effect is not universal, since there are many forms of amendments, with their components varying in plant available nutrients and mineralization rates (Li 1998). Exploring the soil impacts of various combinations of amendments and cover crops can add valuable insight to soil restoration methods.

This chapter of the research evaluates the effectiveness of both aboveground and belowground approaches for reducing CWG, including tillage, herbicide, cover crops, biological amendments, and their combinations. The main objectives of cover crops and amendment were to create a diverse soil environment in which native species can establish and sustain themselves. Soil impacts of all methods will be explored in chapter 2. The expectation for this chapter is that tillage and herbicide treatments will reduce CWG, and the combination of these will have the greatest reduction.

Methods

The study site (near 48.315, -106.663) is located about 16km (just under 10 miles) northwest of the town of Glasgow, MT. The field is approximately 805 meters long (0.5 miles) and 610 meters wide (0.38 miles). Most of the field’s soil is listed as a Thoeny-Phillips complex with 1-5 percent slope, and a portion consists of a Phillips loam with 0-4 percent slope (USDA NRCS WSS 2023).

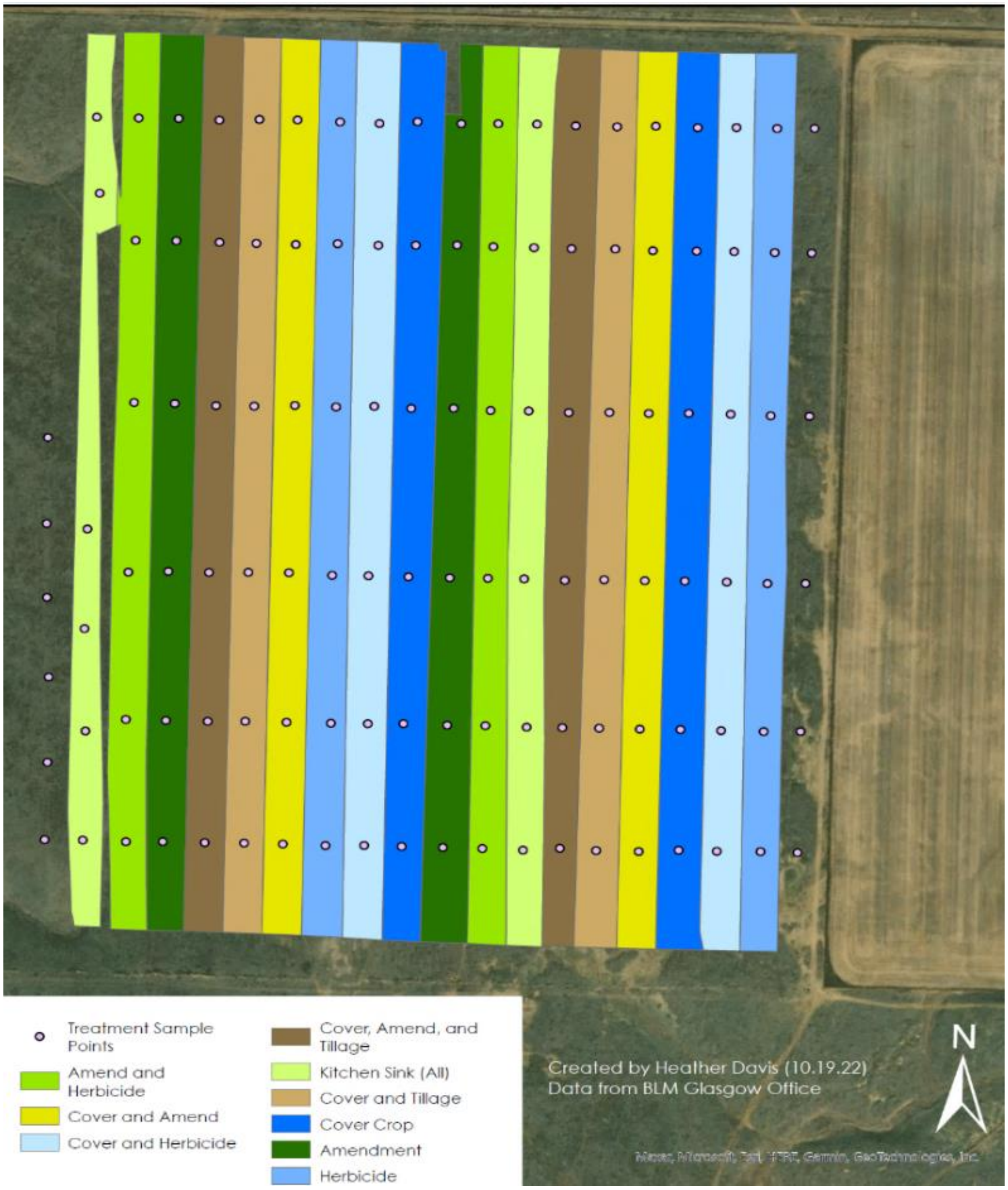
This mixed grass prairie has dominant vegetation of needle and thread grass (*Hesperostipa comata*) and rhizomatous wheatgrasses; primarily western (*Pascopyrum smithii*) and/or thickspike (*Elymus lanceolatus*). Other grasses include prairie junegrass (*Koeleria macrantha*), blue grama (*Bouteloua gracilis*), Sandberg bluegrass (*Poa secunda*) and plains reedgrass (*Calamagrostis montanensis*). Silver sagebrush (*Artemisia cana*) is the most common shrub (USDA NRCS ESD 2023). The primary native vegetation found at our field site includes silver sagebrush, blue grama, prairie junegrass, and western wheatgrass. Some of the forbs found are yarrow (*Achillea millefolium*), scarlet globemallow (*Sphaeralcea coccinea*), fringed sagewort (*Artemisia frigida*), plains pricklypear (*Opuntia polyacantha*), and brittle pricklypear (*Opuntia fragilis*; Luna and Vance 2017).

According to the National Weather Service, the 2022 annual mean minimum temperature for the Glasgow, MT area occurred in December at -18.39 degrees Celsius (-1.1 degrees Fahrenheit), and the maximum occurred in August at 13.67 degrees Celsius (92.3 degrees Fahrenheit). The average annual precipitation in 2022 was 27.54 cm (10.84 inches) and in 2023 was 30.99 cm (12.2 inches, excludes November and December). In 2022, the monthly precipitation from May to September in cm was 5.36, 3.66, 4.93, 0.74, and 0.33. In 2023, it was 9.88, 6.12, 1.63, 1.35, and 1.80 cm. The average growing season for the state of Montana is fairly short, with an estimate of less than 122 frost-free days and fewer than 34 cm (14 inches) of precipitation. (Montana State University 2023)

Our project's field site has a plant species dominance of CWG. The field is nicknamed Mooney Coulee and its exact year of acquisition by the Bureau of Land Management is unknown but was likely in the early 1930's (Borgreen, Bureau of Land Management, personal communication). Mooney Coulee was private cropland prior to its acquisition, with some rusted metal remnants of old farming equipment still found throughout the plot. The Dust Bowl's extreme drought conditions in the 1930's caused many abandoned crop fields to be reseeded to CWG after federal acquisition in hopes of stabilizing the soil (Rogler and Lorenz 1983).The plot of land is also part of the Bankhead-Jones Farm Tenant Act, which was signed by President Roosevelt on July 22, 1937 (Maddox 1937). This law created both long term loans for farm tenants and sharecroppers, plus short-term loans for farmers in need of supplies, equipment, and livestock.

Mooney Coulee was first acquired by the Federal agency titled the US Grazing Service, which is the precursor of the current BLM (Borgreen, Bureau of Land Management, personal communication). The US Grazing Service was founded in 1939 to enforce the Taylor Grazing Act, which allowed federal agencies to lease public lands for grazing. The Grazing Service was later combined with the General Land Office to create the Bureau of Land Management, which now manages the plot. Mooney Coulee has been used as a livestock grazing allotment ever since the acquisition (M. Borgreen, Bureau of Land Management, personal communication).

We established ten treatments (9 treatments and control) across the Mooney Coulee site to investigate the effectiveness of the treatments in reducing CWG and monitoring cover crop germination and growth. Each treatment strip runs north to south and is approximately 31 m by 0.8 km (100 ft by 0.5 mile). We duplicated each of the 10 treatments, creating 20 treatment strips in total (1.1). The treatments are indicated in the legend of 1.1 (the control strips located on each end have no color) and are listed in Table 1.1 with their abbreviations used in the s throughout the chapter.



1.1. CWG Field Study Treatment Map.
Mooney Coulee research plot, which includes the 10 CWG reduction treatments, each replicated twice. The individual strips include Control (no treatment), Amendment, Herbicide, Cover crop, Tillage, and combinations of each (20 total). The pink dots within the strips represent sampling points for soil and vegetation. The control strips have no color and are located on each end of the treatment strips.

1.2. Native Prairie Study Site Map.
This site is located 4.83 km (3 miles) NE of treatment plot, which is shown on the smaller map on the left. The larger map on the right shows the blue dots, which serve as sample points for vegetation and soil data.

Table 1.1. Treatments and their abbreviations.
The amendment is a mixture of Rejuvenate™ and SeaShield™ from Advancing Eco Agriculture. The cover crops are a mixture of 15 species that have success in the region. The tillage applied was from a Degelman Pro-Till high-speed disk with a 15 cm (six-inch) disturbance depth.

Abbreviation	Treatment
Native	Native prairie
Control	control (no treatment)
KS	amendment + herbicide + cover crops, + tillage (kitchen sink)
AH	amendment + herbicide
A	amendment
CAT	cover crop + amendment + tillage
CT	cover + tillage
CA	cover crop + amendment
H	herbicide
CH	cover crop + herbicide
C	cover crop

We located 6 points within each of the strips that serve as sample points for the vegetation surveys and soil sampling (total of 12 sample points per treatment). The location of most of these points were determined by evenly spacing 6 points within the length of the strip, keeping them in the center of the strip to avoid any treatment edge effects. During treatment and sample point installation in 2022, the 2 strips to the west were adjusted due to treatment application error (visible in 1.1).

In addition to the Mooney Coulee plot, we also took soil samples and monitored vegetation from a native prairie site located 4.28 km (3 miles) northeast of Mooney Coulee in order to gain insight on soil properties of a closeby native prairie ecosystem that is not dominated by CWG (1.2). We sampled from this location because it was the closest BLM land with intact native prairie. This reference area has been grazed by cattle, but has no history of tillage. There are some limitations in comparing data collected from this site to Mooney Coulee due to the native sampling site having no history of agriculture like Mooney Coulee. It also has different soil texture and topography than the Mooney Coulee treatment plot. However, its purpose is to serve as a reference for native prarie with no CWG invasion in this study.

Treatment applications at Mooney Coulee began in spring of 2022. On May 23, 2022, the following herbicide formulation was applied: Glystar 5 EPA Reg No 42750-61 (68.14 L), with Brimstone (activator; 9.5 L), Crosshair (drift control 1.89 L), and Bronc Max (water treatment; 3.79 L) to herbicide strips by a private contractor, at a Glystar application rate of 3.26 kg/ha. On May 25, 2022, the tillage treatment was conducted by BLM staff with a Degelman Pro-Till high-speed disk, which fractures the soil surface and partially inverts the soil. The disturbance depth in our treatments was approximately 15 cm.

On May 27th, 2022, the commercial amendments Rejuvenate™ and SeaShield™ (Advancing Eco Agriculture, Middlefield, OH) were applied at a rate of 130.96 L/ha (45.42 L water plus 3.79 L of each amendment) and cover crops were seeded at about 5.3 kg/ha at a 2.54 cm depth with 19.1 cm spacing (28.7 lbs/ac, 1-inch depth, 7.5 inch spacing). These were seeded with a John Deere 1890 disk drill with over the press wheel stainless liquid tubes. The cover crop species and individual seeding rates are shown below in Table 1.2. Both were applied by a private contractor. The cover crop mix was chosen due to the success of these species in the region and for their abilities to add soil organic matter to increase microbial activity, lower soil-borne pathogens, and create plant available nitrogen (Paudel et al. 2021; Siczek and Lipiec 2016).

The Rejuvenate™ product is a liquid amendment containing complex carbohydrates, humates, and magnesium, intended to stimulate soil microbial activity and residue decomposition. The SeaShield™ product is a cold pressed liquid crab, fish, and shrimp concentrate, intended to deliver nutrients and stimulate soil biological activity. All costs for installing treatments (materials, custom rates, equipment, etc.) were calculated for each year and are reported in the results.

The same treatment methods were re-applied in the spring 2023 on the same strips. Herbicide was applied on May 18th. Tillage was applied on June 6th and the cover crops and amendment were both applied on June 7th.

Table 1.2. Cover crop seed mix and seeding rates.
The cover crop seed mix used in rehabilitation treatments of rangeland dominated by CWG. The total seeding rate was 5.3 kg/ha (28.7 lbs/ac). Cover crop species are listed from smallest to largest percentage of the seed mix.

Species common name	Scientific name	Origin	% Mix	Seeding Rate (lbs/ac)
Lacy phacelia	*Phacelia tanacetifolia*	OR	1.0	0.3
White wonder foxtail millet	*Setaria italica*	NE	2.1	0.6
Forage Collards (variety not stated)	*Brassica oleracea*	ID	2.4	0.7
Pg584 ethiopian cabbage	*Brassica carinata*	OR	2.6	0.7
Dixie crimson clover	*Trifolium incarnatum*	OR	3.5	1
Peredovik Sunflower (variety not stated)	*Helianthus annuus*	SD	3.5	1
Koto buckwheat	*Fagopyrum esculentum*	SD	3.7	1
Horizon white proso millet	*Panicum miliaceum*	SD	6.9	2
Turbo brand bmr brachytic hybrid sudangrass	*Sorghum bicolor spp. drummondii*	TX	6.9	2
Common Vetch (variety not stated)	*Vicia sativa*	OR	6.9	2
Certified neela flax	*Linum usitatissimum*	SD	7.8	2
White wonder foxtail millet	*Setaria italica*	SD	8.3	2.4
M59000 sorghum c sudangrass hybrid (conventional)	*Sorghum bicolor spp. drummondii*	TX	10.3	3
Faba Bean (variety not stated)	*Vicia faba*	CAN	17.0	5
Morton oats	*Avena sativa*	SD	17.0	5

To evaluate the response of CWG to each set of treatments, we conducted annual vegetation surveys (beginning of July in 2022 and 2023). At each of the 126 points, we followed a modified version of the Bureau of Land Management's protocol for line-point intersect to collect data on soil surface and vegetation cover within the plot. This protocol is found within the terrestrial version of the Assessment, Inventory, and Monitoring Strategy manual of the Bureau of Land Management (Herrick et al. 2021).

At each sample point we created 12.5 m long vegetation survey transects, at a bearing of 8 degrees (to avoid treatment strip edge effects and to avoid transects falling in line with the 0 degrees tillage or seeding lines). The original protocol calls for 3 transects that are 25 m long at each sample point, but due to our size limitations in our plots, we altered this to one 12.5 m long transect at each of the 6 points within each strip. Along these transects, we collected data every 5 m for a total of 25 points. We identified vegetation species, if identifiable, and cover crops. All cover crop species were categorized as either forb or grass. We also collected data on ground cover, which included litter, clubmoss (Lycopodiopsida), or bare ground. Clubmoss is a low growing vascular plant that provides extensive ground cover in the Northern Mixed Grass Prairie of North America (Romo 2011). The soil surface at our site consisted of either clubmoss or soil. Vegetation surveys and biomass collections for the Native reference site were only conducted in 2023.

Starting on September 11th of both 2022 and 2023, near the end of each growing season, we collected aboveground herbaceous biomass at each of the 126 points to gain insight on vegetation growth within each treatment. This is done by placing a 0.5 square meter frame 5 meters from each sample point at a bearing of 188 degrees. Then we clipped all vegetation, including CWG, at the ground surface, dried the combined plant samples to a constant weight, then weighed them in grams. We included CWG to see the general impact of treatments on all vegetation.

We used R (R Core Team 2018) along with the 'agricolae' (de Mendiburu & Yassen 2020), 'dunn.test' (Dunn 1964), 'tidyverse' (Wickham et al. 2019), 'ggplot2' (Wickham 2016), 'FSA' (Ogle 2023), and 'dplyr' (Wickham et al. 2023) packages for data organization, analysis, and visualization.

To analyze the vegetation survey and biomass clipping data, we compared the mean of the response variables across the field treatments (n=12 and n=6 in the Native site). The response variables presented here include percent cover of CWG, cover crop, bare soil; native species Shannon Diversity index (Shannon 1948); and herbaceous biomass. We calculated summary statistics, then compared the mean of the response variables across treatments and years. To compare within each year and across treatments, we used the Kruskal-Wallis Test, which is a rank-based nonparametric test that determines if there are significant differences between groups (Kruskal and Wallis 1952). We then performed the post hoc Dunn-Bonferroni, which compares the means of groups and tells you which specific groups are significantly different (Dunn 1961). We also performed a Mann-Whitney U test, also known as Wilcoxon rank sum test, to see if there were significant differences between the 2022 to 2023 means within the same treatment. (Mann & Whitney 1947; Wilcoxon 1945).

Results

We assembled Table 1.3 to give an idea of costs for individual and combinations of these treatments for our project, based on the costs of implementing the treatments during the study. Not treating CWG is free in the short term, but will be more cost intensive as CWG dominates further. Addition of more treatments increased the costs per acre, with amendment + herbicide +

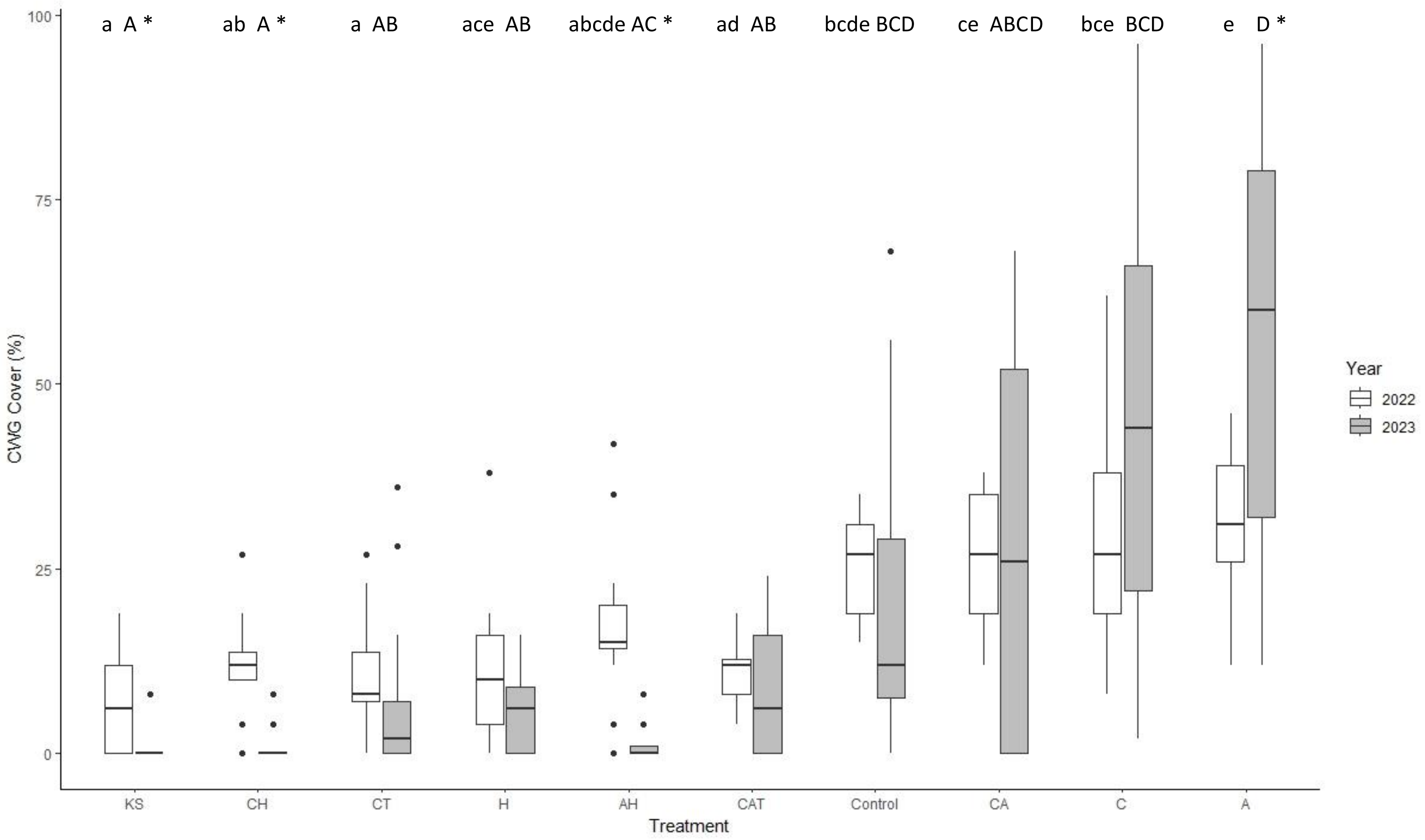
cover crops + tillage being the most expensive. Prices of treatments increased from 2022 to 2023.

Table 1.3. Costs of CWG reconstruction treatments and their combinations.
Costs for each treatment calculated from installation costs (materials, custom rates, and equipment) in each year. The third column is the average cost of the treatment based on both years. Treatments are listed from least to most expensive in US dollars.

Treatment	2022 Cost/acre	2023 Cost/acre	Average Cost/acre
Control (no treatment)	$0	$0	$0
Herbicide	$30.33	$32.64	$31.49
Tillage	$56.62	$66.23	$61.43
Cover crop	$68.60	$72.32	$70.46
Amendment	$75.51	$89.79	$82.65
Cover crop + herbicide	$98.93	$104.96	$101.95
Amendment + herbicide	$105.84	$122.43	$114.14
Cover crop + tillage	$125.22	$138.55	$131.89
Cover crop + amendment	$144.11	$162.11	$153.11
Cover crop + amendment + tillage	$200.73	$228.34	$214.54
Amendment +herbicide + cover crops + tillage (Kitchen Sink)	$231.06	$260.98	$246.02

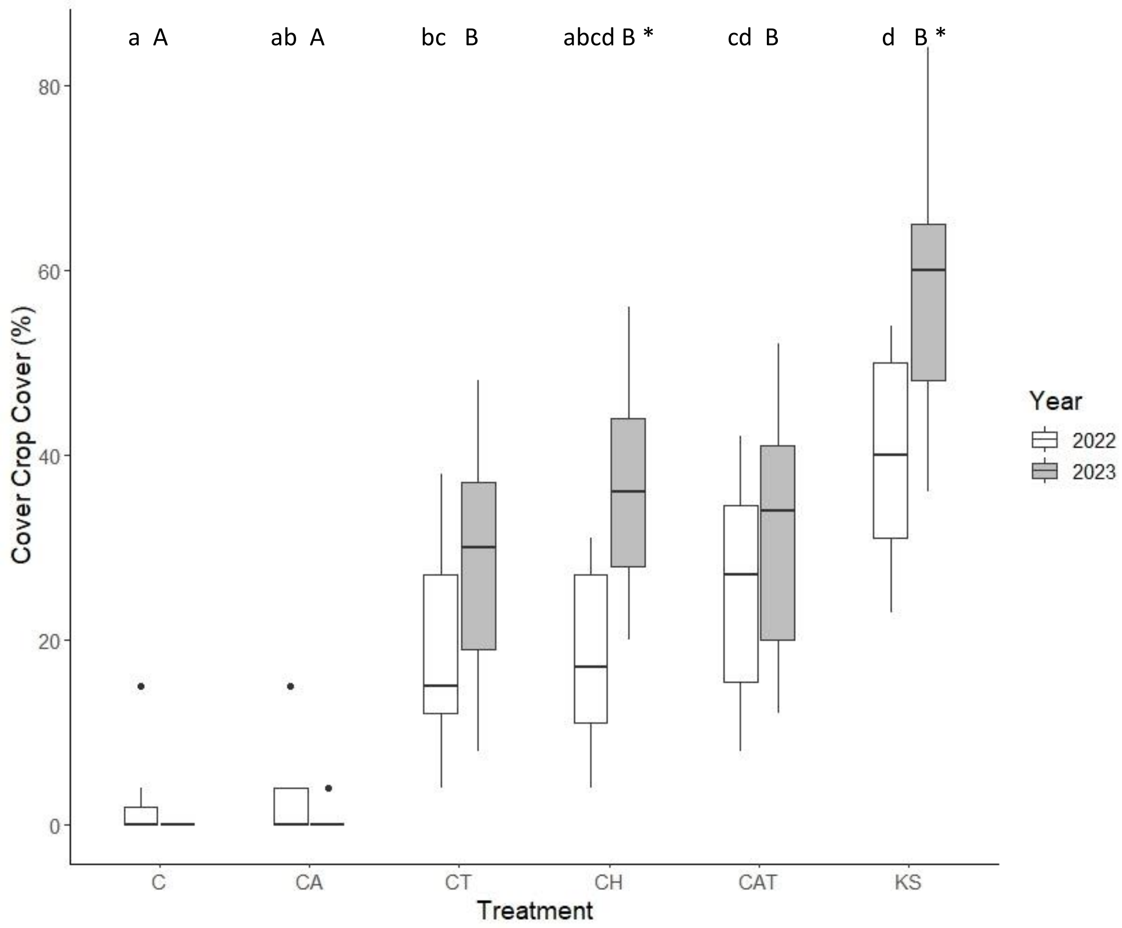
The cover of CWG varied by treatment and year. In 2022, the treatment with all methods combined (KS) had the lowest CWG cover and was significantly lower than the Amendment (A), Cover crop (C), Cover crop + Amendment (CA), and Control treatments, which are treatments without herbicide and/or tillage (Fig. 1.3). In 2023, this intensive treatment was still the lowest, but was followed closely by Cover crop + Herbicide (CH). The Amendment (A) treatment had the highest CWG cover in both years, and was similar to Control, Cover + Amendment (CA), and Cover (C) treatments.

When comparing the CWG cover of each treatment between the two years, Amendment + Herbicide (AH), Cover crop + Herbicide (CH), and the treatment with all methods (KS) had significant decreases in CWG, with mean CWG cover ranging from 0-2 percent in 2023. Most of the other treatments had no statistically significant differences between years besides Amendment, which significantly increased between years, from 31.1 to 58.3 mean percent cover. Overall, there was a general decrease in CWG cover between years and there was greater reduction in treatments including tillage and/or herbicide.



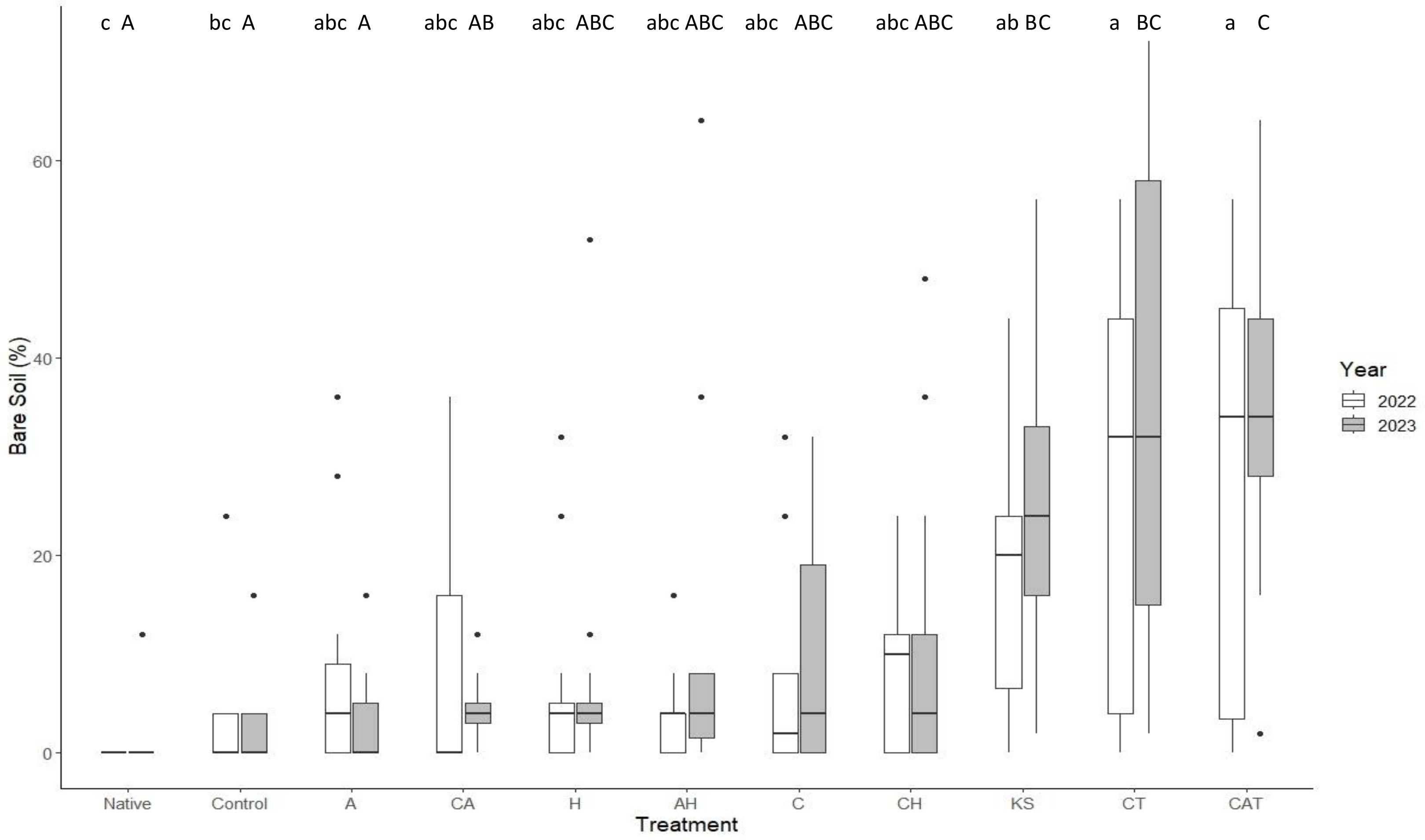
1.3. CWG cover of each treatment in 2022 and 2023.
The distribution of average crested wheatgrass cover (calculated by average hits of crested wheatgrass in each transect (n=12 per treatment, n=6 in the Native site) in each treatment and each year. The letters on the x-axis are abbreviations of the full treatment name. A stands for amendment, C stands for cover crop, H stands for herbicide, T stands for tillage, and KS stands for kitchen sink (all 4 treatments). Treatments are ordered from lowest (left) to highest, (right). Significant differences ($p < 0.05$) between treatment means within a year are indicated by different letters (lowercase for 2022 and upper case for 2023). Significant differences ($p < 0.05$) between years within a treatment are indicated by an asterisk (*).

Six of the 10 treatments included cover crops (seed mix is listed in Table 1.2) and had variable emergence across the treatments. 1.4 shows the distribution of cover crop cover between these 6 treatments and in 2022 and 2023. In both years, Cover crop (C) and Cover crop + Amendment (CA) had the lowest mean cover in both years. The most intensive treatment (KS) had the highest mean cover crop cover in both years. When comparing each treatment between years, the Cover Crop + Herbicide (CH) and treatment with all methods (KS) had statistically significant increases from 2022 to 2023.



1.4. Cover crop percentage of each treatment in 2022 and 2023.
The distribution of average cover crop percent cover (calculated by average hits of cover crop in each transect (n=12 per treatment) in each treatment and each year. The letters on the x-axis are abbreviations of the full treatment name. A stands for amendment, C stands for cover crop, H stands for herbicide, T stands for tillage, and KS stands for kitchen sink (all 4 treatments). Treatments are ordered from lowest (left) to highest (right). Significant differences ($p < 0.05$) between treatment means within a year are indicated by different letters (lowercase for 2022 and upper case for 2023). Significant differences ($p < 0.05$) between years within a treatment are indicated by an asterisk (*).

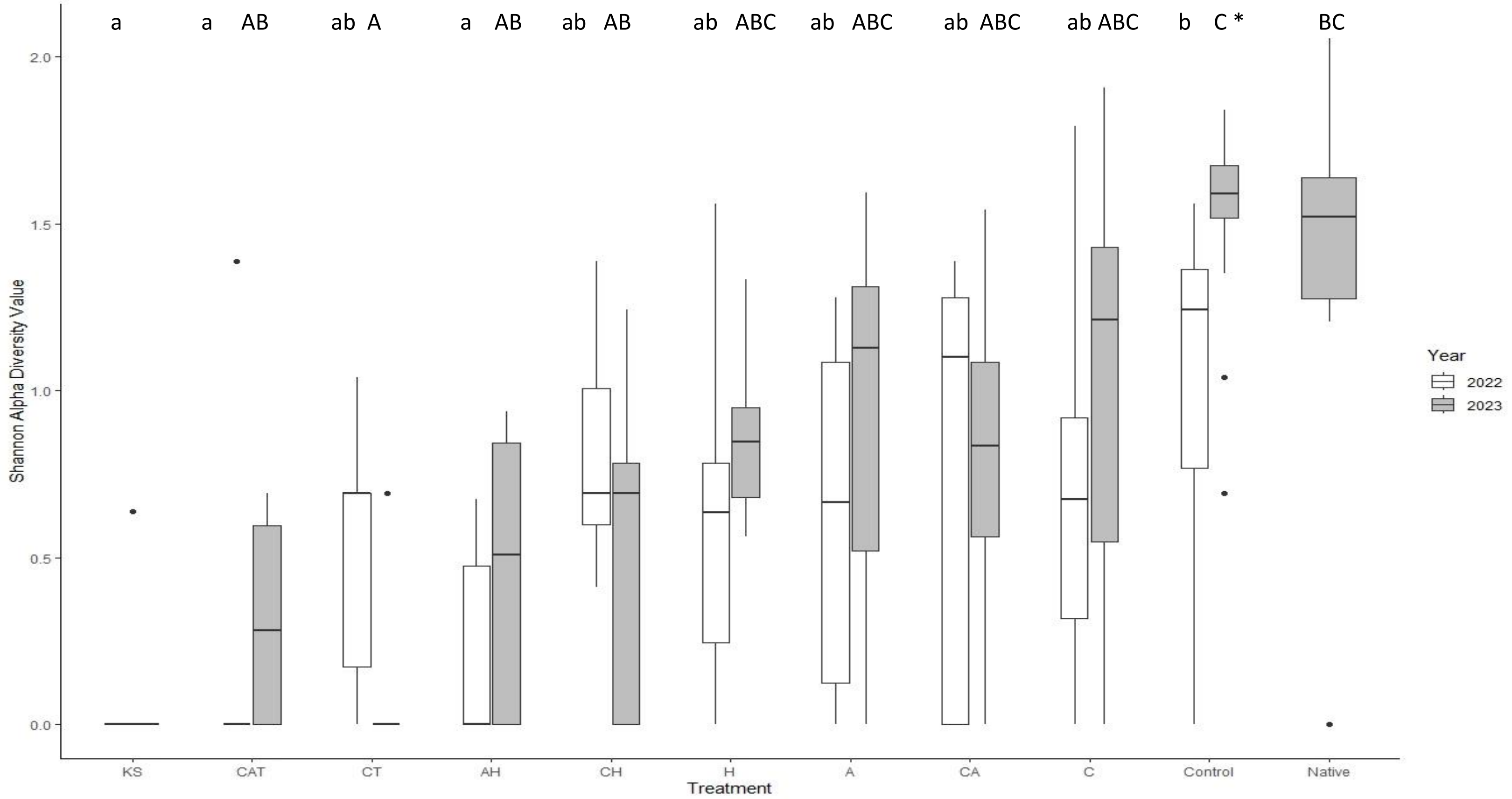
The percent cover of bare soil was variable within and across treatments. In 2022, we found that the native plot and control treatment had significantly less bare ground than Cover crop + Tillage (CT) and Cover crop + Amendment + Tillage (CAT). In 2023, native, control, and Amendment (A) had significantly less bare soil than all treatments (KS), CT, and CAT. Between the years, there were no significant increases or decreases in bare soil in any of the treatments. Overall, the tillage treatments had the highest percent of bare soil in both years.



1.5. Bare soil percentage of each treatment in 2022 and 2023.
The distribution of average bare ground percent cover (calculated by average hits of bare ground in each transect (n=12 per treatment, n=6 in the Native treatment)) in each treatment and each year. The letters on the x-axis are abbreviations of the full treatment name. A stands for amendment, C stands for cover crop, H stands for herbicide, T stands for tillage, and KS stands for kitchen sink (all 4 treatments). Treatments are ordered from lowest (left) to highest (right). Significant differences ($p < 0.05$) between treatment means within a year are indicated by different letters (lowercase for 2022 and upper case for 2023).

We calculated the mean Shannon Diversity Index from vegetation transect data within each treatment and each year to compare native species richness and evenness in each treatment. In 2022, Amendment + Herbicide (AH), Cover crop + Amendment + Tillage (CAT), and the treatment with all methods (KS) had significantly lower mean diversity indices than the Control treatment. The other treatments did not have statistically significant differences in 2022. In 2023, KS and Cover crop + Tillage (CT) had significantly lower Shannon diversity than the control and native plot.

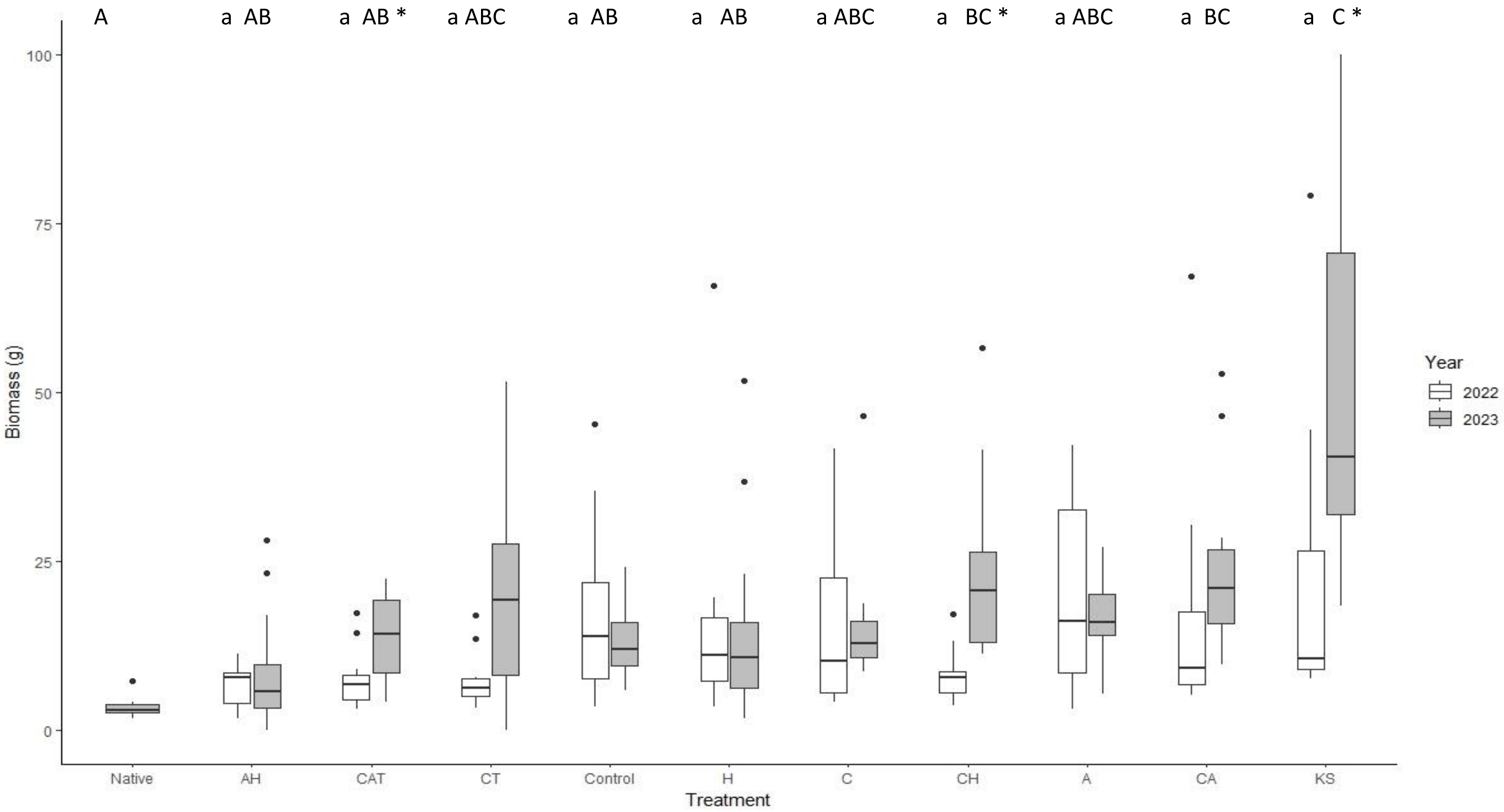
When comparing each treatment's diversity index between years, the only statistically significant change between years was in the Control plot, which had an increase in the mean diversity value.



1.6. Native Shannon Diversity Index Values of each treatment, 2022 and 2023.
The distribution of mean Shannon Diversity Index values (calculated by average hits of native species in each transect (n=12 per treatment, n=6 in the Native site)) in each treatment and each year. The letters on the x-axis are abbreviations of the full treatment name. A stands for amendment, C stands for cover crop, H stands for herbicide, T stands for tillage, and KS stands for kitchen sink (all 4 treatments). Treatments are ordered from lowest (left) to highest (right). Significant differences ($p < 0.05$) between treatment means within a year are indicated by different letters (lowercase for 2022 and upper case for 2023). Significant differences ($p < 0.05$) between years within a treatment are indicated by an asterisk (*).

Mean native vegetation herbaceous biomass in each treatment and each year is shown in 1.7. In 2022, we found no significant difference between the mean vegetation biomass values across treatments. In 2023, the most intensive treatment (KS) had significantly higher mean biomass than Amendment + Herbicide (AH), Cover crop + Amendment + Tillage (CAT), Control, Herbicide (H), and the Native (N) prairie. Also in 2023, the native site also had significantly lower mean biomass than Cover crop + Amendment (CA) and Cover crop + Herbicide (CH).

From 2022-2023, the Cover crop + Amendment + Tillage (CAT), Cover crop + Herbicide (CH), and the treatment with all methods (KS) all had significant increases in their mean biomass. Overall, the most intensive treatment (KS) had the highest mean vegetation biomass in 2023.



1.7. Vegetation biomass (grams (g) / 0.5 m^2) in each treatment, 2022 and 2023.
The distribution of average vegetation biomass in each treatment and each year. The letters on the x-axis are abbreviations of the full treatment name. A stands for amendment, C stands for cover crop, H stands for herbicide, T stands for tillage, and KS stands for kitchen sink (all 4 treatments). Treatments (n=12 per treatment, n=6 in the Native treatment) are ordered from lowest (left) to highest (right). Significant differences ($p < 0.05$) between treatment means within a year are indicated by different letters (lowercase for 2022 and upper case for 2023). Significant differences ($p < 0.05$) between years within a treatment are indicated by an asterisk (*).

Discussion

Our objective was to see which treatments or treatment combination of cover crop, tillage, herbicide, and soil amendment was most successful in reducing CWG in 2022 and 2023. Our results matched our expectation that the use of all treatments would be the most effective. We also expected tillage treatments to cause the highest bare ground percentage and cover crop emergence; but lowest species diversity, which was observed in our results.

Our results coincide with other studies that show how successful reduction of CWG is dependent on soil disturbance to reduce the seedbank and herbicide to remove aboveground vegetation for reseeding. (McAdoo et al. 2017; Morris et al. 2019; Vaness et al. 2008). Our significant differences in CWG cover were between treatments with tillage and/or herbicide and those without. In both years, there were no significant differences between the treatments that included herbicide or tillage, but the Kitchen Sink, Cover crop + Herbicide, and Amendment + Herbicide all had a significant decrease in CWG cover the 2nd year, indicating the importance of repeated applications in reducing CWG and preventing its recovery (Davies et al. 2020; Morris et al. 2019).

Using the biological soil amendment alone appeared to provide a benefit to CWG growth, with a significant increase between years in CWG cover in treatments with amendment. This is likely because of CWG's rapid nutrient acquisition and growth compared to native species (Gunnel et al. 2010). When compared to native grasslands, total soil nitrogen and carbon is lower in CWG fields, which may be because of CWG's higher allocation to aboveground biomass than belowground (Vaness and Wilson 2007; Dormaar et al. 1995; Christian and Wilson 1999). It was likely that the CWG plants outcompeted any native plants for the supplemented sources of carbon, nitrogen, and other nutrients.

For the percent cover of cover crops, our results followed expectations that tillage and herbicide would increase percentage cover, with the 2 treatments without these methods having the lowest cover crop averages. This is likely because tillage increases the seed-soil contact (Blunk et al. 2021) and herbicide eliminates weedy species and other competition (Tharp and Kells 2000). Especially at our semi-arid field site in northeast Montana, a large majority of the ground surface is dominated by clubmoss and a biological soil crust. These protect against erosion, but also make seed to soil contact difficult for cover crops which likely led to low establishment. Kitchen Sink (all treatments) had the highest average cover crop emergence, suggesting that using herbicide with tillage was more successful than using them by themselves. This indicates that cover crop emergence could be contingent on elimination of competing plants.

Tillage treatments are known for causing higher amounts of bare soil, which has implications for erosion and future invasive weed establishment. But higher rates of bare soil also removed the soil crust and clubmoss to allow higher seed to soil contact (Blunk et al. 2021). We had a wide range of bare soil values within tillage treatments the first year, but year 2 saw a significant increase in the percentage of bare ground in Cover crop + Amendment + Tillage and Kitchen Sink treatments. These two are the most intensive treatments, but there are also patches of bare soil throughout the study site, so environmental variation could also be impacting results. We assume this since the Cover crop + Tillage treatment is identical to Cover crop + Amendment + Tillage besides the addition of the amendment, which is unlikely to have influenced the amount of bare soil within the treatment.

Native plant diversity within the treatments, as measured by the Shannon Diversity Index, followed the expectations of having the highest average diversity values in the Native and

Control treatments. This is because herbicide and tillage have been shown to reduce native plant species diversity, richness, cover, and community composition (Smith et al. 2023; Lamm et al. 2022). Although the Control treatment was significantly higher than 5 of the treatments in the 2nd year, it's surprising there was no significant difference between Control and Cover crop + Herbicide or Cover crop + Amendment + Tillage. This is unexpected due to the Control plot still containing some native vegetation unimpacted by herbicide or tillage. It could indicate that these two treatments have not reduced the native plant community diversity well below the level of diversity the Control plot contains.

Vegetation biomass between treatments had no significant differences in the first year's data. However, 2023 had significantly higher values for the Kitchen Sink treatment than Amendment + Herbicide, Cover crop + Amendment + Tillage, Herbicide, and Control, indicating that combination of all the treatments led to the most ideal vegetation and soil environment for the cover crops and vegetation to grow in. The native plot's vegetation was significantly lower in vegetation biomass, which was expected since CWG produces much higher aboveground biomass than native species (Hamerlynck et al. 2016). It could also be because of the native prairie site being grazed by cattle very shortly before our vegetation surveys. Future surveys should make sure to exclude grazing where biomass samples are being collected.

Another possibility for the low biomass in the native plot is that the vegetation there was more diverse and included earlier blooming species that reached their peak biomass earlier in the season. Timing and amount of precipitation also could have varied between the two sites. Or it could be extended growing seasons of the native species that grow alongside CWG since they must compete for more resources above and belowground. Finally, a large amount of biomass clippings taken in the treatment strips included cover crops and CWG, which are both heavier than the other species present. CWG is much heavier since it has been established on the research plot for many years; reaching knee height in many cases.

Overall, we found that reducing CWG was most successful in our Kitchen Sink treatment, followed closely by Cover crop + Herbicide and Amendment + Herbicide in 2023. Although Kitchen Sink was most successful in reduction, it led to low plant diversity, which may be undesirable. Reducing CWG below our control plot levels within two years was dependent on tillage or herbicide. Cover crop is also dependent on use of these. Use of cover crop and amendment without tillage or herbicide led to increases in CWG.

For land managers, budget and logistic restraints may interfere with the ability to use all four treatment methods. So, reasonable alternatives of Kitchen Sink for similar results in this timeframe are Cover crop + Herbicide and Amendment + Herbicide since these had the lowest CWG cover in 2023. However, it is important to keep in mind that herbicide has been shown to only temporarily control CWG, since it rapidly regenerates from its seed bank and its seeds remain viable in the seedbank for at least 2-4 years (Gunnel 2009; Hulet et al. 2010; Pyke 1990; Wilson and Partel 2003). For higher control of CWG, we suggest at least 2 years of herbicide application, if used. Future research could also investigate if more than 2 years of herbicide application further reduces CWG cover. However, changes in soil properties under herbicide are an important consideration and are explored in chapter 2.

Other research suggests prevention of CWG resurgence and better native seeding success could be found through combining repeated applications of herbicide with another defoliation treatment like tillage, grazing, or mowing, which helps remove the seed before it enters the seedbank (Morris et al. 2019, Hansen and Wilson 2006). This is supported by our results, in which the only treatment with herbicide and tillage (Kitchen Sink) had the lowest CWG cover. However, tillage and herbicide risk the destruction of non-target species and secondary invasions of exotic annual weed species (Rinella et al. 2009; Courchamp et al. 2011; Davies et al. 2000; Pearson et al. 2016). We suggest adding cover crops to herbicide and tillage use to reduce risk of CWG resurgence and exotic plant invasions.

For future recommendations, cover crop + herbicide + tillage is a treatment we did not test and is another viable combination to test for CWG control. Future research can also look at repeated treatment applications over a longer period of time, which may reduce the amount of CWG regrowth. Our site had high a very high dominance of CWG over native species, so soil disturbance and herbicide were more viable control methods of CWG. However, future studies could be done to investigate control methods that reduce impact on the native plant community like spot spraying or timed spring grazing.

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CHAPTER 2: IMPACTS OF CRESTED WHEATGRASS CONTROL STRATEGIES ON SOIL HEALTH

Abstract

Increased interest in restoring native plant diversity to monotypic crested wheatgrass (*Agropyron cristatum*; CWG) stands has led to investigations behind CWG's competitive strategies. Since successful management strategies for CWG have been limited to herbicide and tillage applications, we wanted to investigate if addition of liquid soil amendment mixture and cover crops to these methods could help restore soil health indicators under these management strategies. We also wanted to explore the impacts of long established CWG stands on soil properties. Through a 2-year field study completed near Glasgow, Montana, and a greenhouse study, we compared the response of soil properties to treatments used alone and with each other. Throughout our field site, CWG is the dominant plant species and has been grazed by cattle since it was planted with CWG in the 1930's. We measured physical, chemical, and biological soil characteristics including water content, bulk density, phospholipid fatty acid analysis, and pH, electrical conductivity (EC), and carbon and nitrogen pools.

Soil results were variable between properties. Nitrate was higher in treatments including tillage and herbicide, but especially herbicide. Biologically, we saw the highest microbial abundances in the Amendment treatment. The field study's most intensive field treatment Kitchen Sink (KS) (Cover crop + Amendment + Tillage + Herbicide) had the lowest rates of total and arbuscular mycorrhizal (AM) fungal abundances out of the treatments, having lower rates than control. Our most intensive greenhouse treatment combination of Cover crop + Amendment + Herbicide (CAH), had the highest rates of total microbial and AM fungal abundance out of the treatments during the 2nd stage of the experiment and had highest native

species emergence. Combining cover crops and the amendment with tillage and herbicide had mixed results regarding restoration of soil biology. Previous studies have demonstrated how herbicide leads to higher nitrate levels, which risks future nutrient leaching and benefits exotic species over natives. Future research should increase the soil conditioning application and project time period to further distinguish the effects of the individual treatments and their combinations.

Introduction

Many invasive species alter soil properties and make an inhospitable environment for native species to establish and persist (Perkins and Hatfield 2014).The exact mechanisms are not well understood, but research has hypothesized it is due to the soil "legacy" effects they create. This is similar to land-use legacies, the concept that land uses have lasting impacts on ecosystems (Morris et al. 2011). Invasive species leave soil legacies by altering the aboveground litter accumulation, soil moisture, carbon and nitrogen cycles, and microbial community, which interferes with future attempts to restore native plant diversity (Jurand et al. 2013; Tanner and Gange 2013). When comparing the performance of two native tallgrass prairie species versus two invasives, Shivega and Aldrich-Wolfe (2017) found that a native soil microbial community either had no effect or was detrimental for the exotic species. They also found that elevated nitrogen compared to native prairie levels only benefitted the exotic species.

Crested wheatgrass (*Agropyron cristatum*; CWG) is a non-native perennial grass species known to alter the soil's physical, chemical, and biological components (Jordan et al 2012; Perkins and Nowak 2013a, b). Perkins and Nowak (2012) found that CWG's advantages over native species bottlebrush squirreltail and bluebunch wheatgrass (*Elymus elymoides* and *Pseudoroegneria spicata)* are seen especially during seedling stages, where competition for water, nutrients, and light is high. CWG was also more competitive in soils previously hosted by CWG, supporting the need to understand CWG’s ability to condition the soil (Nafus et al. 2020; Hooker and Stark 2008). Aboveground techniques of restoring native species to CWG stands have had limited success (Lesica and Cooper 2019; Hulet et al. 2010; Davies et al. 2013), perhaps because they have not addressed the less understood belowground factors to CWG’s success.

Research about CWG’s impact on soil is limited, but there is research supporting how CWG decreases overall soil health. Wilson & Pärtel (2003) looked at multiple soil impacts, finding that previously cultivated land planted with CWG in the late 1940s had lower plant diversity, soil carbon (C), and nitrogen (N) than similarly age fields dominated by native grasses. CWG also alters soil physical structure, creating weak soil aggregates and low soil respiration rates compared to native rangeland (Broersma et al. 2000; Norton et al. 2012). CWG’s lack of strong aggregates indicates the lack of microorganisms in the soil and leads to higher rates of compaction (Wilpiszeski et al. 2019). Wallace et al. (2009) found that using a biosolid treatment on CWG dominated rangelands successfully increased the soil’s proportion of water stable aggregates, C, and N. Biosolids and other organic matter applications methods to improve soil nutrient availability, soil C sequestration, and soil water availability, which are limited under CWG and most soils of semi-arid grasslands. (Chen and Stark 2000; Hook et al. 2019; Schlesinger et al. 1990; Vinton and Burke 1995)

CWG takes up phosphorus faster than other species, especially when it has been supplemented (Diallesandro et al. 2013). CWG provides soil with relatively high amounts of carbohydrates, but little organic N. These soil conditions result in immobilization of soil N since high concentrations of carbohydrates in soils with limited N results in net demand on soil N by microbes. When comparing the availability of plant available N between the invasive winter annual grass cheatgrass (*Bromus tectorum*) and CWG, CWG released 30% less total soil N and organic C (Morris et al. 2016). This was attributed to CWG having lower rates of root exudation and release of organic matter with labile N. CWG may provide little to soil organic matter, having a coarse root system with less root biomass production than native species (Christian & Wilson 1999).

As for the biological effects on soil, soil dominated by CWG has been shown to have a much lower microbial abundance when compared to soils of native species following reclamation (Gasch et al. 2016). Soil microbiota are essential in helping plants acquire nutrients and cope with environmental stress (Clarholm 1985; Ingham 1985). Soil rhizosphere microbiota are largely dependent on nutrients released by plant root exudates (Klein et al. 1988). Klein et al. (1988) compared root exudates from CWG to western wheatgrass (*Argropyron smithii*) and blue grama (*Bouteloua gracilis*) in a simulated growing season and found significantly lower total C and N released from CWG root exudates than the other species. However, CWG's pattern of low-quality root seems to change under stressful environmental conditions, giving it the advantage over native species. Henry et al. (2007) found that root exudates of CWG are influenced significantly by water and nutrient stress, with large increases of total organic carbon (TOC) with drought and potassium limited soil treatments.

All plants rely on the soil microbial communities for their rapid responses to changes in the environment (Hawkins & Crawford 2018). Arbuscular mycorrhizal (AM) fungi are some of the most important of these microbes, forming mutualistic associations with 80-90 percent of vascular plants (Anderson et al. 1984). These AM fungal associations improve plants' uptake of water and inorganic nutrients, especially P (Otgonsuren & Lee 2010). AM fungi are also essential to soil stabilization, with their hyphae binding soil particles together to increase aggregation (Duchicela et al. 2012; Rillig & Steinberg 2002). CWG has been shown to either lack or have fewer AM fungal molecular root signatures and taxa compared to native grass and shrub species surveyed (Reinhart & Rinella 2021). Jordan et al. (2012) found that soils conditioned with CWG had significantly lower AM fungal richness when compared to native species and reduced future growth of native species. Grassland forbs grown in soil inoculated by AM fungi have been shown to have greater competitive responses with grasses (Neuenkamp et al. 2019).

CWG has been used to stabilize rangelands throughout the western US, establishing easily in cold and drought tolerant environments. It has provided soil cover after the drought of the 1920’s-30’s but has created monotypic stands that many land managers seek to convert to native species for wildlife habitat, ecological diversity, and soil health (Miller et al. 2021; Misar et al. 2016). Since CWG dominates both aboveground vegetation and the seedbank for multiple decades, successful reduction of CWG has been tied to repeated herbicide or soil disturbance applications (Morris and Monaco 2019). These methods have been successful in suppressing CWG seed production and removing the existing plants with repeated applications, but these management strategies have negative implications for soil health Henderson and Naeth 2005; Trognitz et al. 2016).

The application of Glyphosate-based herbicide has been shown to increase soil nitrate and phosphate levels (Gaupp-Berghausen et al. 2015), risking nutrient leaching into groundwater aquifers, streams, and lakes. Increases in soil N have been found to favor invasive plant growth over native ones (Shivega and Aldrich-Wolfe 2017, Mattingly and Reynolds 2014, Daehler 2003). As for underground impacts, increased N can lead to declines in bacterial and fungal biomass as well as the abundance and diversity of AM fungi (Farrer et al. 2013, Treseder 2004; Leff et al. 2015). Reduced tillage practices have also been shown to increase bacterial and AM fungal biomass, fungal-to-bacterial (F:B) ratios, and soil moisture content (van Groenigen et al. 2010, Frey et al. 1999, Hendrix et al. 1986).

Increasing soil microbial diversity has been shown to promote plant diversity and suppress soil-borne pathogens (Vukicevich et al. 2016). Cover crops' root exudates have C-rich compounds like amino acids, sugars, and proteins that influence both beneficial and pathogenic microorganisms (Badri and Vivanco 2009, Akiyama et al. 2005, Nicol et al. 2003). The composition of the root exudates depends on the host plant identity, which indicates higher diversity of cover crop species leads to higher diversity of the microbes attracted to the exudates (Broeckling et al. 2008, Bardgett and van der Putten 2014). Some land managers have also applied amendments to the soil to increase organic soil C and reverse the soil legacy effects of invaders and decrease their growth (Kulmatiski 2011). Previously disturbed soils of agricultural lands, pastures, or other restoration areas have low C pools and could have greater proportional increases of soil C from amendment applications (Gravuer et al. 2019, Lal 2018).

This project's goal was to evaluate the use of cover crops and a biological soil amendment mixture for improving soil physical, chemical, and biological properties that may be degraded by CWG and its management methods. The treatments we evaluated alone and in combination with each other are cover crops, amendment, herbicide, and disk tillage. Our objectives were to evaluate the response of soil water content, bulk density, soil microbial community abundances, pH, EC, particle size, C pools, and N pools to these treatments. We met these objectives through a 2-year long field study in NE Montana and a greenhouse study. We expected to see higher microbial abundances, soil moisture, bulk density, C, and N pools in the treatments containing cover crop and amendment due to more organic matter and nutrient inputs. We also expected higher plant counts and forb to grass ratio in our greenhouse treatments with cover crop and amendment.

Methods

The study site (near 48.315, -106.663) is located about 16km (10 miles) northwest of the town of Glasgow, MT. The field is approximately 805 meters long (0.5 miles) and 610 meters wide (0.38 miles). According to the web soil survey, a little over half of the site's soil is a Thoeny-Phillips complex with 1-5 percent slope (USDA NRCUS WSS 2023). The other portion of the study site consists of a Phillips-Elloam complex with 0-4 percent slope. Textures vary from loam, clay loam, to clay, and are found on till plains. They are very deep and drain easily. These soils' clay horizons can begin in depths of 15.24-25.4 cm (6-10 in.) or 7.62-15.24 cm(3-6 in.), respectively (USDA NRCS WSS 2023).

The silt deposited by glacial meltwater created the fertile soils of the Great Plains. The soils of the Brown Glaciated Plains consist of about 58,679 square kilometers (14.5 million acres) across North-central Montana and are suited to dryland farming, consisting of soils in the Mollisol and Vertisol orders. This area was glaciated during the Late Wisconsin glaciation, which was at its full expanse around 20,000. This is the driest and westernmost part of the Northern Great Plains' prairie pothole region.

According to the National Weather Service, the 2022 annual mean minimum temperature for the Glasgow, MT area occurred in December at -18.39 degrees C (-1.1 degrees F), and the maximum occurred in August at 13.67 degrees C (92.3 degrees F). The average annual precipitation in 2022 was 27.54 cm (10.84 inches) and in 2023 was 30.99 cm (12.2 inches, excludes November and December). The average growing season for the state of Montana is fairly short, with an estimate of less than 122 frost-free days and fewer than 34 cm (14 inches) of precipitation. (Montana State University 2023)

CWG is the most abundant plant species throughout our entire field site. The field is nicknamed Mooney Coulee and its exact year of acquisition by the Bureau of Land Management is unknown but was likely in the early 1930's (Borgreen, personal communication). The Dust Bowl's extreme drought conditions in the 1930's caused many abandoned crop fields to be reseeded to CWG after acquisition in hopes of stabilizing the eroded soil. The Bureau of Land Management owns the plot, which is nicknamed after a nearby coulee, Mooney Coulee. Mooney Coulee has been used as a livestock grazing allotment for landowners ever since the original acquisition (Borgreen, personal communication).

To address our project objectives, we established 10 treatments across the Mooney Coulee site in 2022 and re-applied them in 2023. Each treatment strip runs north to south and is approximately 30.48m by 0.8km (100ft by 0.5 mi). We duplicated each of the 10 treatments, making 20 treatment strips in total. The treatments and their abbreviations are listed in Table 1.1 of chapter 1. The treatments are shown in 1.1 (the control strips located on each end have no color) and the native site map is shown in 1.2 of chapter 1.

The cover crop species name, scientific name, origin, percentage of the mix, and their individual seeding rates are listed below (Table 2.1). These species were chosen because of their success in the semi-arid climate of northeastern Montana. Cover crops improve soil health by increasing microbial biomass and activity, with many species promoting beneficial soil microbes like arbuscular mycorrhizal fungi, which are utilized by plants to receive essential nutrients in the soil (Finney et al. 2017).

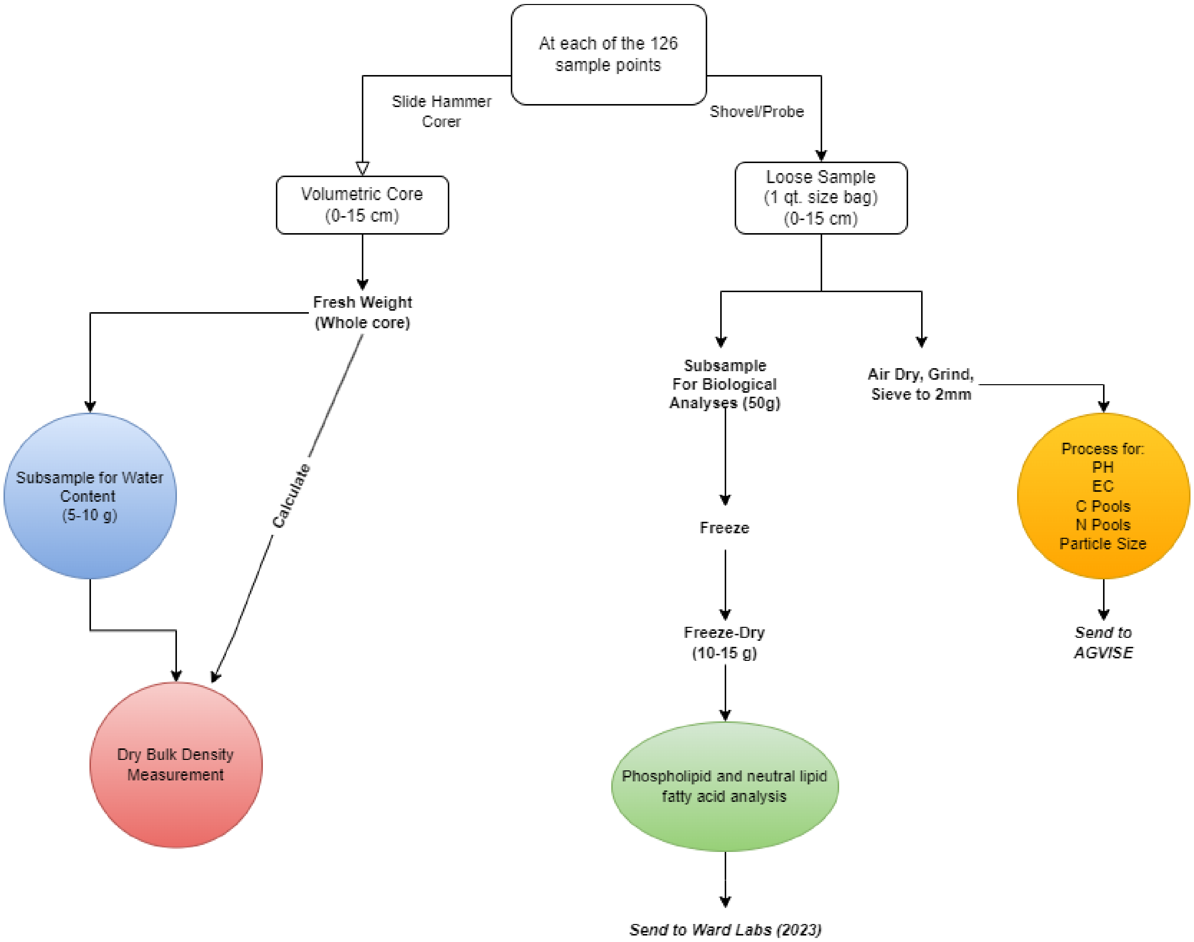
Table 2.1. Cover crop seed mix and seeding rates.
The cover crop seed mix used in rehabilitation treatments of rangeland dominated by CWG. The total seeding rate was 5.3 kg/ha (28.7 lbs/ac). Cover crop species are listed from smallest to largest percentage of the seed mix.

Species common name	Scientific name	Origin	% Mix	Seeding Rate (lbs/ac)
Lacy phacelia	*Phacelia tanacetifolia*	OR	1.0	0.3
White wonder foxtail millet	*Setaria italica*	NE	2.1	0.6
Forage Collards (variety not stated)	*Brassica oleracea*	ID	2.4	0.7
Pg584 ethiopian cabbage	*Brassica carinata*	OR	2.6	0.7
Dixie crimson clover	*Trifolium incarnatum*	OR	3.5	1
Peredovik Sunflower (variety not stated)	*Helianthus annuus*	SD	3.5	1
Koto buckwheat	*Fagopyrum esculentum*	SD	3.7	1
Horizon white proso millet	*Panicum miliaceum*	SD	6.9	2
Turbo brand bmr brachytic hybrid sudangrass	*Sorghum bicolor spp. drummondii*	TX	6.9	2
Common Vetch (variety not stated)	*Vicia sativa*	OR	6.9	2
Certified neela flax	*Linum usitatissimum*	SD	7.8	2
White wonder foxtail millet	*Setaria italica*	SD	8.3	2.4
M59000 sorghum c sudangrass hybrid (conventional)	*Sorghum bicolor spp. drummondii*	TX	10.3	3
Faba Bean (variety not stated)	*Vicia faba*	CAN	17.0	5
Morton oats	*Avena sativa*	SD	17.0	5

We located 6 points within each of the strips to serve as sample points for soil and biomass sampling. The location of most of these points were determined by evenly spacing 6 points within the length of the strip, keeping them in the center of the strip to help avoid any treatment edge effects. During treatment and sample point installation, the 2 strips to the west were adjusted due to operation error (visible in 1.1).

In addition to the Mooney Coulee plot, we also took soil samples and monitored vegetation from a native prairie site located 4.28 km (3 miles) northeast of Mooney Coulee in order to gain insight on soil properties of a closeby native prairie ecosystem that is not dominated by CWG (1.2). We sampled from this location because it was the closest BLM land with intact native prairie. This reference area has been grazed by cattle, but has no history of tillage. There are some limitations in comparing data collected from this site to Mooney Coulee due to the native sampling site having no history of agriculture like Mooney Coulee. It also has different soil texture and topography than the Mooney Coulee treatment plot. However, its purpose is to serve as a reference for native prarie with no CWG invasion this study.

At the end of the growing season in mid-September of 2022 and 2023, we took 2 different soil samples at each of the 126 sample points. The two samples were taken 5 meters from each sample point at a 188-degree bearing. The 1st soil sample (0-15 cm depth) was taken using a shovel. The 2nd was a volumetric core (0-15 cm depth) using a hammer-driven corer (5-cm diameter). We kept all samples in coolers then transferred them to the fridge located in the Glasgow BLM office. After returning them to NDSU, we separated samples in the lab for further processing. 2.1 displays how we separated the soil samples for each process, and Table 2.2 lists the properties and protocols used for the analyses.



2.1. Flowchart representing the steps for how the field study soil samples were taken, prepared, and analyzed.
At each of the 126 points, we took a volumetric core sample and a loose sample. From the volumetric cores, we measured water content and bulk density. From the loose soil samples, we measured microbial community composition (phospholipid fatty acid analysis), microbial biomass carbon, pH, electrical conductivity (EC), carbon (C) pools, nitrogen (N) pools, and particle size (only in 2022).

Table 2.2. Soil Processing and analyses for field treatment plots.

Property	**Analysis**
Particle size distribution and texture (only analyzed in 2022)	Hydrometer method (Gee and Or 2002)
Water content	Gravimetric method (Gardner 1986)
pH	1:1 slurry method (Thomas 1996)
Electrical conductivity (EC)	1:1 slurry method (Rhoades 1996)
Carbon (C) pools (organic, inorganic, labile, microbial)	Dry combustion (Nelson and Sommers 1996), permanganate oxidizable carbon (Weil et al. 2003), and chloroform fumigation (Vance et al. 1987)
Nitrogen (N) pools (total, NO_3^- and NH_4^+)	Dry combustion and potassium chloride extracts (Mulvaney 1996)
Microbial community structure (total microbial, bacteria, fungi, arbuscular mycorrhizal fungi abundance) (only analyzed in 2023)	Phospholipid fatty acid analysis (Buyer and Sasser 2012) (Sharma and Buyer 2015)

Ward Labs performed the phospholipid fatty acid analyses and AGVISE conducted soil chemical analyses, while other analyses were completed in NDSU labs (Fig. 2.1).

The PLFA analysis included a ratio of the proportions of fungi to bacteria to understand relative shifts of these groups across treatments. Research has linked higher fungi to bacteria ratios to more sustainable agro-ecosystems (Wang et al. 2019), since they can store more soil C. Agricultural areas with tillage have been shown to have lower fungal activity and stored carbon than neighboring restored prairies (Bailey et al. 2002).

To understand general characteristics of each treatment's soil and how it changed throughout the project, we measured the pH, electrical conductivity (EC, salts), water content, and bulk density. We also measured the percentages of sand, silt, and clay to determine the mean soil textural classification within each treatment. The results of these tests are found in Table 2.3, and they serve as background site information, so they were not statistically analyzed. Soil texture class and its percentages were only measured in 2022 since this was not expected to be affected by our treatments. Dominant soil textures of our field site were loam and sandy loam. Values for all other properties were similar across treatments and years.

One of the soil health indicators we chose was permanganate-oxidizable carbon (POXC). POXC is a form of labile or biologically active carbon, which signifies the more rapid decomposing organic matter within soil. This carbon cycles through within a few days to a few years, unlike the intermediate pools that take a few years to decades, and stable pools that can take decades to centuries to turn over (Hurisso et al. 2016). Since POXC represents the active soil C, it has been shown to be more reactive to changes in management practices and a valuable metric of stabilization of soil organic matter. Some studies have reported a positive correlation between POXC and crop productivity. (Culman et al. 2021)

Table 2.3. Summary statistics for general field study soil characteristics.
The letters on the x-axis are abbreviations of the full treatment name. A stands for amendment, C stands for cover crop, H stands for herbicide, T stands for tillage, and KS stands for kitchen sink (all 4 treatments).

Treatment	**Texture Class**	**Sand**	**Silt**	**Clay**	**pH**	**Electrical conductivity**	**Water content**	**Bulk density**
		(%)	**(%)**	**(%)**		**(dS / m)**	**(g water / g soil)**	**(g / cm^3)**
					<u>2022</u>			
A	Loam	50	32	19	6.71 (0.56)	0.13 (0.08)	0.05 (0.02)	1.33 (0.07)
AH	Loam	48	33	19	6.47 (0.87)	0.18 (0.11)	0.06 (0.02)	1.32 (0.08)
C	Loam	48	33	19	6.82 (0.48)	0.15 (0.07)	0.05 (0.02)	1.33 (0.14)
CA	Loam	50	33	17	6.80 (0.46)	0.12 (0.08)	0.05 (0.02)	1.38 (0.11)
CAT	Loam	47	36	18	6.54 (0.36)	0.10 (0.05)	0.04 (0.02)	1.33 (0.11)
CH	Loam	49	33	18	6.70 (0.51)	0.14 (0.06)	0.05 (0.01)	1.41 (0.28)
CT	Loam	50	30	20	6.66 (0.44)	0.18 (0.17)	0.05 (0.02)	1.35 (0.13)
H	Sandy loam	55	29	17	6.33 (0.27)	0.12 (0.03)	0.05 (0.03)	1.23 (0.15)
KS	Sandy loam	54	28	18	6.38 (0.37)	0.12 (0.05)	0.04 (0.01)	1.30 (0.10)
Control	Sandy loam	56	27	17	6.70 (0.51)	0.13 (0.09)	0.04 (0.01)	1.32 (0.11)
Native	Loam	52	30	18	6.43 (0.48)	0.14 (0.07)	0.06 (0.02)	1.35 (0.13)
					<u>2023</u>			
A	---	---	---	---	6.68 (0.38)	0.10 (0.04)	0.18 (0.40)	1.23 (0.23)
AH	---	---	---	---	6.63 (0.74)	0.20 (0.10)	0.11 (0.03)	1.31 (0.12)
C	---	---	---	---	6.94 (0.44)	0.13 (0.07)	0.08 (0.04)	1.38 (0.08)
CA	---	---	---	---	6.85 (0.25)	0.13 (0.08)	0.06 (0.02)	1.35 (0.16)
CAT	---	---	---	---	6.68 (0.51)	0.15 (0.08)	0.06 (0.02)	1.34 (0.12)
CH	---	---	---	---	6.78 (0.42)	0.15 (0.09)	0.10 (0.04)	1.28 (0.08)
CT	---	---	---	---	6.82 (0.42)	0.15 (0.10)	0.07 (0.02)	1.37 (0.11)
H	---	---	---	---	6.55 (0.41)	0.19 (0.07)	0.12 (0.04)	1.33 (0.10)
KS	---	---	---	---	6.33 (0.46)	0.15 (0.09)	0.06 (0.02)	1.32 (0.13)
Control	---	---	---	---	6.98 (0.45)	0.12 (0.05)	0.06 (0.02)	1.31 (0.06)
Native	---	---	---	---	6.95 (0.51)	0.16 (0.13)	0.04 (0.01)	1.22 (0.15)

Greenhouse Experiment

Our greenhouse experiment involves 3 soil sampling stages to capture soil property changes over time under the same treatments as the field study (Table 2.4). In May 2022, soil for the greenhouse experiment was collected from the field site (west of the northwest corner of the Mooney Coulee field). Soil was collected using a shovel to 15 cm depth by excavating bunches of CWG and shaking soil from the root zone into a bucket. We also collected bulk soil from the vicinity of the grass bunches. Soil was transported back to the lab and spread out on a tarp in a greenhouse to air dry. Large rocks and chunks of vegetation were removed, and the soil was placed into storage buckets until use. These buckets were placed in a room temperature greenhouse. Cover crop seed was sub-sampled from the mix used to seed cover crop treatment strips in the field in May 2022 and transported to the lab for use in the greenhouse study. The liquid biological soil amendment used in the field treatments was also sub-sampled in May 2022 and frozen until use in the greenhouse study.

In January of 2023, we initiated the greenhouse project by filling 40 pots with 1250g of air-dried soil from the field site. The pots had a diameter of 15.24 cm (6-inch) with a capacity of 1.33L (81 cubic inches/1.41 quarts). Next, we added water to the pots to reach a desired water content of 0.22 g water/g of soil (approximate field capacity, based on soil texture and mass). We recorded the weight of each pot before watering to maintain the water content throughout the duration of the experiment. We adjusted the amount of water required to maintain field capacity each time we took soil samples from the pot. We then divided the pots into 8 treatments, each with 5 pots (replicates) per treatment:

1. Control
2. Amendment
3. Cover Crop
4. Herbicide

5. Herbicide + Cover Crop
6. Amendment + Herbicide
7. Amendment + Cover Crop
8. Amendment + Herbicide + Cover Crop

On January 13th, we began applying the treatments to the pots. The amendment and herbicide rates were determined by converting the field rates (L/ha) to a pot rate based on the pot size (milliliters per pot). We used a weed science pot sprayer to apply both the herbicide and amendment treatments. The field and converted pot rates of application are shown below in Table 2.4. The cover crop treatment conversion from field to pot was less than 0.0041 grams of seed, which is too little to be able to determine their effects on the soil. To provide the opportunity for each cover crop species to be represented in the greenhouse study, we applied 2 seeds of each of the cover crop species to each pot.

Table 2.4. Field application rates of amendment, herbicide, and cover crop treatments plus their converted rates for the greenhouse.
Pots that were assigned combinations of treatments received the additive rates of each individual application.

Treatment	Field Application Rate	Greenhouse Application Rate
Amendment	130 L/ha (14 gal/ac) Seashield = 9.4 L/ha (1 gal/ac) Rejuvenate = 9.4 L/ha (1 gal/ac) Water = 112.3 L/ha (12 gal/ac)	0.165 ml/ pot (premixed)
Herbicide	3.4 kg/ha (48 oz/ac) Glystar = 1.3 kg/ha (18 oz/ac) Brimstone= 0.5 kg/ha (6.4 oz/ac) Bronc Max= 0.2 kg/ha (2.56 oz/ac) Crosshair= 0.1 kg/ha (1.25 oz/ac)	3.72 ml/100ml tank Glystar= 3.19 ml Brimstone= 0.33 ml Bronc Max= 0.13 ml Crosshair= 0.07 ml
Cover crop	32.5 kg/hc (29 lb/ac)	2 seeds per cover crop species

Table 2.5. Soil sampling dates and sampling markers for the 2023 greenhouse experiment.
We took one soil sample per pot at each sampling stage, making 120 soil samples at the end of the experiment.

Sampling stage	Date of sampling	Sampling marker
Initial	January 15th	Amendment, Cover crop, Herbicide applied
Intermediate	March 9th	Cover crop maturity
Final	May 5th	Native species maturity

We applied the cover crop seeds by lightly pushing them down into the soil then covering them with a small amount of field soil. After the application of the treatments, we used a small plastic soil corer with about 3.54 cm (1 in) diameter to a depth of about 3.81 cm (1.5 in) to collect about 15g of soil from the pot to serve as our first soil sample for lab processing. The soil sample was frozen until analysis. Then, we randomized the pots and placed them in the growing chamber, located at the Jack Dalrymple Agricultural Research Complex on the NDSU campus. The growing chamber maintained a 12-hour light period from 6:00AM to 6:00PM, with the daytime temperature set to 25 °C and nighttime temperature set to 20°C. The relative day humidity was set to 60%, an estimated value of the early summer humidity averages in Glasgow, Montana. The average relative humidity in May and June are 52 and 51% (Weather Atlas staff, n.d,). Pots were checked every 3-5 days, watered to maintain approximate field capacity, and the location was randomized once per week. We maintained this routine until March 9th, when most cover crops flowered and began to set seed.

To end the cover crop soil conditioning stage on March 9th, we clipped the leaf biomass of the pots with cover crops at the soil surface. Then we used the same small corer to remove the second soil sample from each pot. The soil sample was frozen until analysis. To begin the last phase, we seeded native plant seeds directly into the soil in every pot. The following species were seeded at 2 seeds per pot: western wheatgrass (*Pascopyrum smithii*), green needle grass (*Nasella viridula*), blue grama (*Bouteloua gracilis*), purple prairie clover (*Dalea purpurea*), lewis flax (*Linum lewisii*), and annual sunflower (*Helianthus annuus*).

We chose these grass species to achieve a mixture of rhizamatous (western wheatgrass) and cespitose (green needle grass) cool season grasses. The forb species were chosen for their nitrogen fixation (purple prairie clover), fungal promoting roots (Lewis flax), and warm seasonality (annual sunflower). We brought the pots to field capacity again, randomized their location, and placed them back in the growing space. Then, we checked pots every 3-5 days and maintained field capacity by weighing the pots and adding deionized water as necessary. We re-randomized the pots once per week. We maintained this routine until May 5th to let the native plants establish and possibly flower. We removed the 3rd and final soil sample from each pot using the soil corer and stored them in the freezer. We clipped vegetative biomass at the soil surface of each pot and separated clippings into 2 groups: grass and forb. Finally, we dried the biomass in labeled bags or envelopes at 60-65 deg. C and weighed them. CWG was present in the pots (due to field soil containing seeds) but was unidentifiable without a seedhead due to its similarity in appearance to the native green needlegrass planted. Waiting for the seedhead to form would have likely resulted in the forb species to die since they were beginning to dry out towards the end of the experiment.

At the end of the experiment, there were 120 soil samples in frozen storage (40 pots with 3 sample events). The experiment had 3 soil sampling stages with 40 pots: initial treatment application (January 2023), an intermediate stage where cover crops grew until flowering (March 2023) and a final stage where native species grew until flowering (May 2023)(Table 2.5). These were processed and analyzed for soil microbial community structure using the phospholipid fatty acid analysis, performed by Ward Labs.

We performed the PLFA analysis on our greenhouse soil samples on a subset of the treatments used in the field study. We tested for significant differences in mean total microbial abundance between different treatments in the same conditioning stage and the same treatment in different conditioning stages (Fig. 2.9).

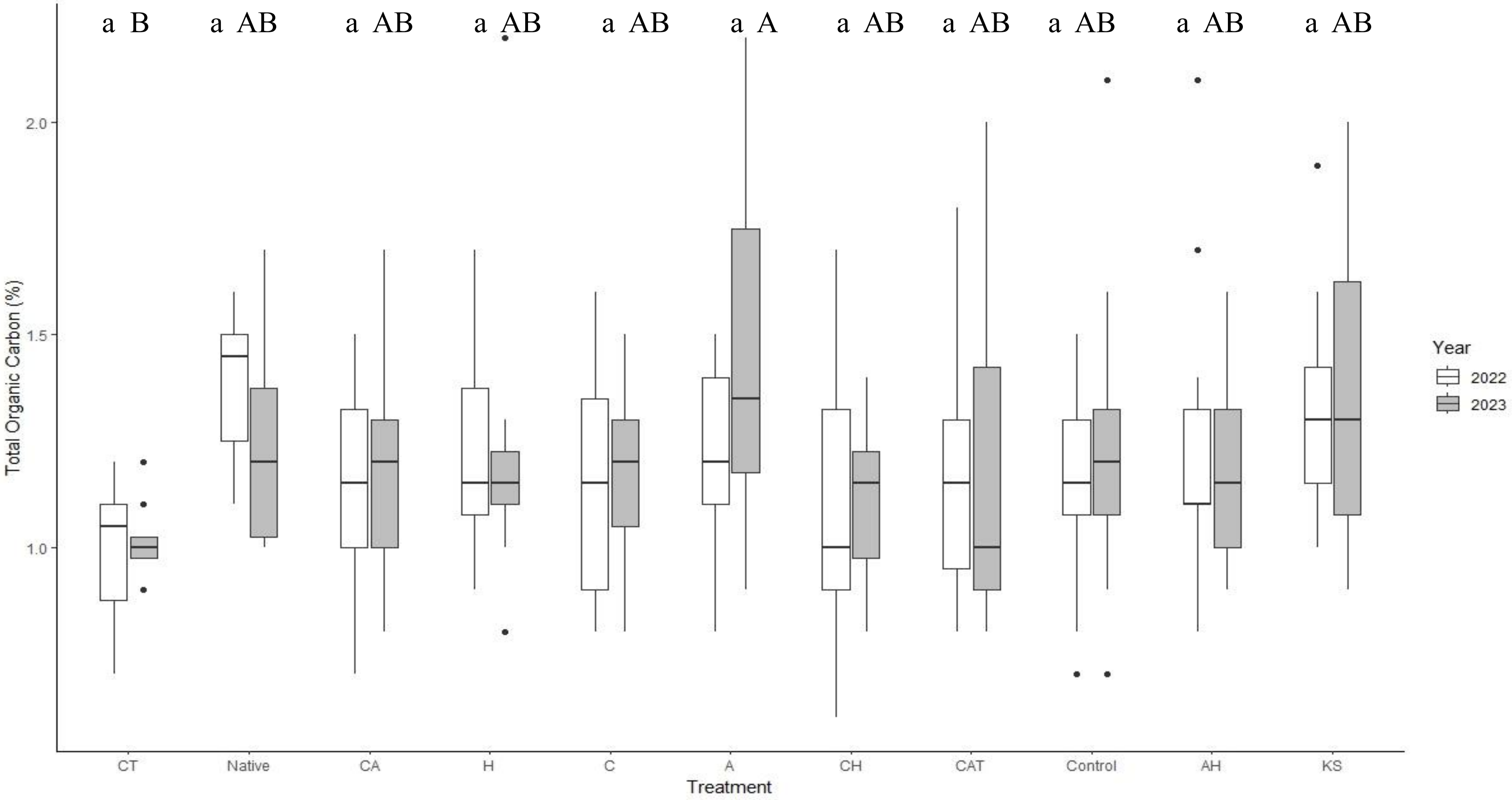
We used R (R Core Team 2018) along with the ‘agricolae’ (de Mendiburu & Yassen 2020), ‘dunn.test’ (Dunn 1964), ‘tidyverse’ (Wickham et al. 2019), ‘ggplot2’ (Wickham 2016), ‘FSA’ (Ogle 2023), ‘dplyr’ (Wickham et al. 2023), and ‘tidyr’ (Wickham et al. 2023) packages for data organization, analysis, and visualization.

To analyze the field-collected soil properties, we compared the mean of the response variables (n=12, n=6 in the Native site) across each of the 10 field treatments, using non-parametric means comparison tests (Kruskal-Wallis) and post hoc tests (Dunn’s test). For data that was collected in both years, we also compared the means within each treatment between years using the non-parametric Mann Whitney U test (or Wilcoxon rank-sum test).

To analyze the greenhouse data, we compared the mean of the response variables across each of the pot treatments (n=5). The greenhouse experiment included 3 soil sampling stages and a PLFA was performed on these samples to see microbial community structure. The soil response variables we measured were total microbial abundance, AM fungal abundance, and the fungal:bacterial ratio. We compared these between different treatments in the same conditioning stage and the same treatment in different conditioning stages. To further examine the plant communities present in each greenhouse treatment (n=5) during the final soil conditioning stage, we collected grass and forb counts in each of our pots. We used non-parametric means comparison tests (Kruskal-Wallis) and post hoc tests (Dunn’s test) to compare means between treatments and to compare means within treatments and across experiment stages.

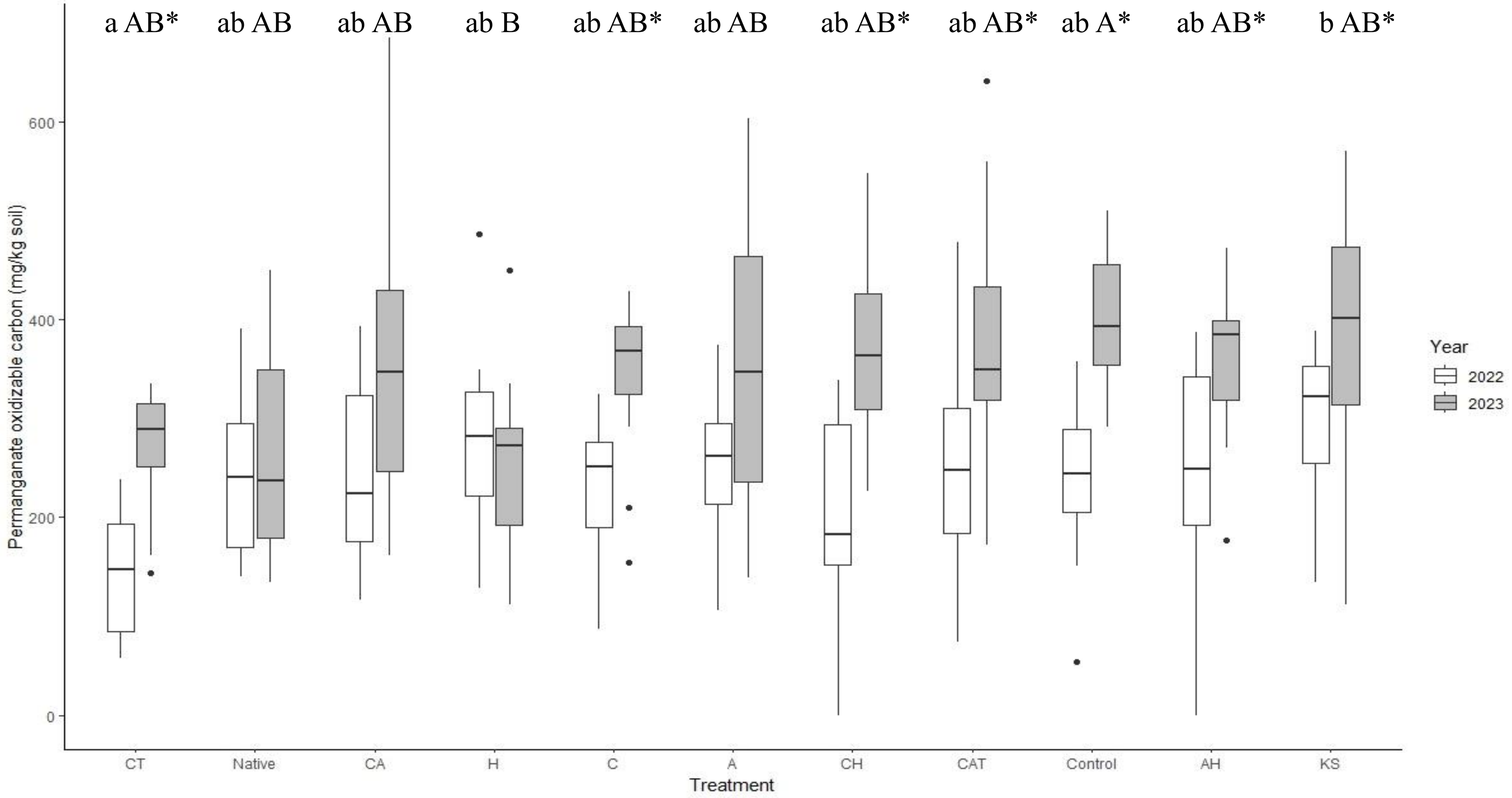
Results

There were few differences in the total soil organic carbon between treatments and years. We found no significant differences in the percentage of total soil organic carbon across treatments in 2022 (Fig. 2.4). In 2023, the organic carbon in the Amendment (A) treatment was significantly higher than in the Cover crop + Tillage (CT) treatment. When comparing each treatment’s values between the years, there were no significant differences.



2.2. Total organic carbon in each field study treatments, 2022 and 2023.
Boxplot representing how soil total organic carbon (y-axis) responded to the treatments (x-axis)(n=12)(n=6 for native site) and differences between years. The letters on the x-axis are abbreviations of the full treatment name. A stands for amendment, C stands for cover crop, H stands for herbicide, T stands for tillage, and KS stands for kitchen sink (all 4 treatments). Significant differences ($p < 0.05$) between treatment means within a year are indicated by different letters (lowercase for 2022 and upper case for 2023).

POXC, a form of labile C that is sensitive to management changes, did not have large variations between the treatments. Most treatments had similar rates of POXC in both years, with only one statistically different pairing for each year (Fig. 2.5.). In 2022, we found that the treatment with all methods (KS) had significantly higher POXC than Cover crop + Tillage (CT). In 2023, Control was significantly higher than Herbicide (H). Most treatments had significant increases in POXC from 2022 to 2023 besides the Native site (N), Cover crop + Amendment (CA), Herbicide (H), and Amendment (A).

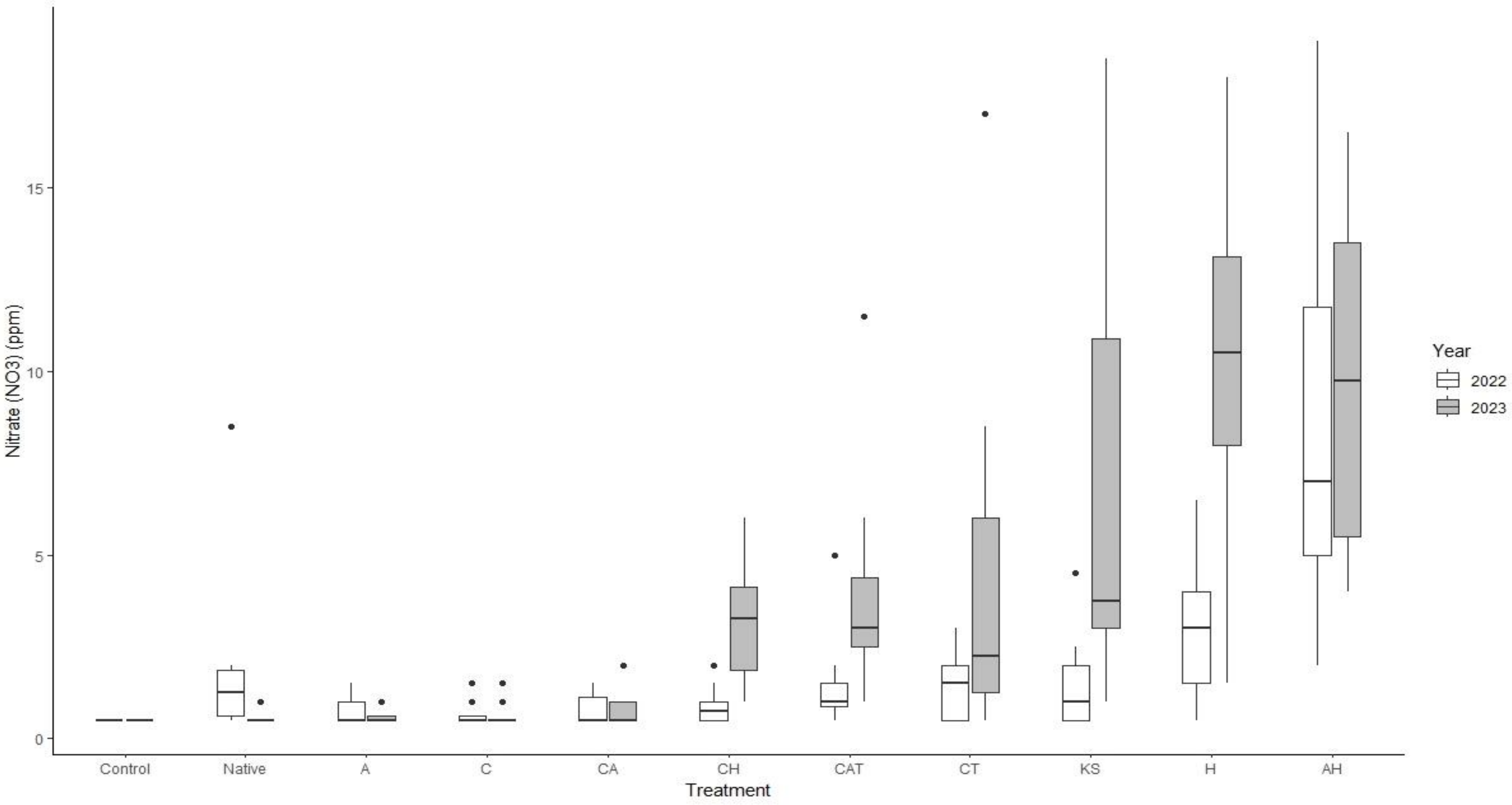


2.3. Permanganate oxidizable carbon (POXC) found in each field study treatment, 2022 and 2023. Boxplot representing how soil permanganate oxidizable carbon (y-axis) responded to the various treatments (x-axis)(n=12) and differences between years. The letters on the x-axis are abbreviations of the full treatment name. A stands for amendment, C stands for cover crop, H stands for herbicide, T stands for tillage, and KS stands for kitchen sink (all 4 treatments). Significant differences ($p < 0.05$) between treatment means within a year are indicated by different letters (lowercase for 2022 and upper case for 2023). Significant differences ($p < 0.05$) between years within a treatment are indicated by an asterisk (*). The native site had fewer sample points (n=6).

We found no statistically significant differences between any treatments in total soil nitrogen in 2022 or 2023. There were also no statistically significant changes in treatments from 2022 to 2023.

Nitrate varied between treatments and had some differences between years (Fig. 2.6). The control plot had very little nitrate, with all 12 observations measuring 0.5 ppm. The plots with significantly higher nitrate than control in both years were Amendment + Herbicide (AH) and Herbicide (H). In 2023, the 3 treatments that were significantly higher than the Native site were Amendment + Herbicide (AH), Herbicide (H), and the treatment with all methods combined (KS).

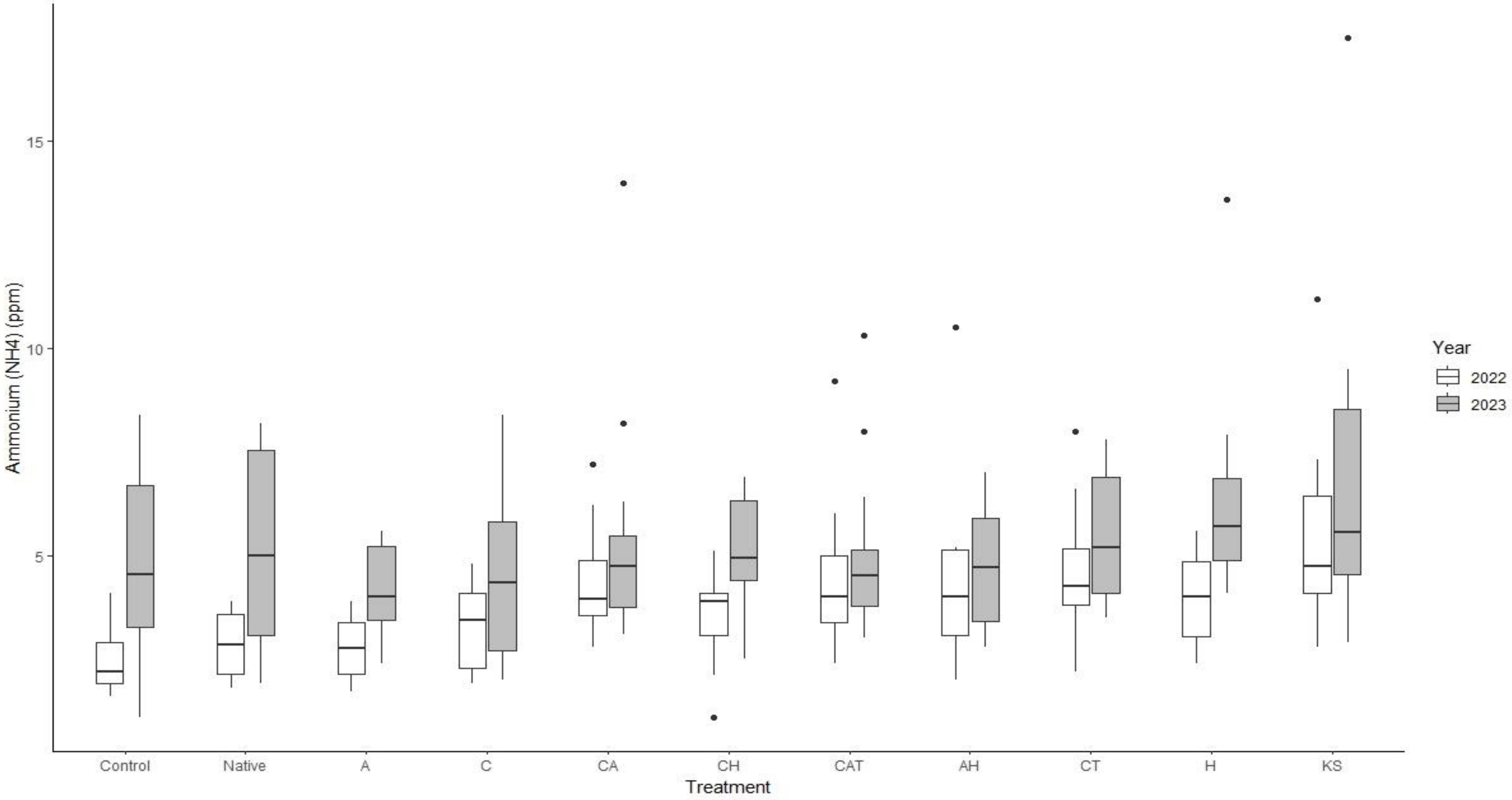
In both years, Amendment + Herbicide (AH) was also significantly higher in nitrate than Amendment (A), Cover crop (C), and Cover crop + Amendment (CA). In 2023, Herbicide (H) was also significantly higher than Amendment (A), Cover crop (C), CA, and the Native site (N).



2.4. Nitrate found in each field study treatment, 2022 and 2023.

Boxplot representing how soil nitrate (y-axis) responded to the various treatments (x-axis)(n=12) and differences between years. They are arranged from lowest to highest. The letters on the x-axis are abbreviations of the full treatment name. A stands for amendment, C stands for cover crop, H stands for herbicide, T stands for tillage, and KS stands for kitchen sink (all 4 treatments). Significant differences ($p < 0.05$) between treatment means within a year are indicated by different letters (lowercase for 2022 and upper case for 2023). Significant differences ($p < 0.05$) between years within a treatment are indicated by an asterisk (*). The native site had fewer sample points (n=6).

Some of the treatment’s mean ammonium levels were statistically different in 2022, but none were in 2023 (Fig. 2.8). More specifically, in 2022, Control was significantly lower than Cover crop + Amendment (CA), Cover crop + Tillage (CT), and the treatment with all methods combined (KS). Amendment (A) was also significantly lower than the most intensive treatment (KS). The 4 treatments that increased significantly between the years were Control, Amendment (A), Cover crop + Herbicide(CH), and Herbicide (H).



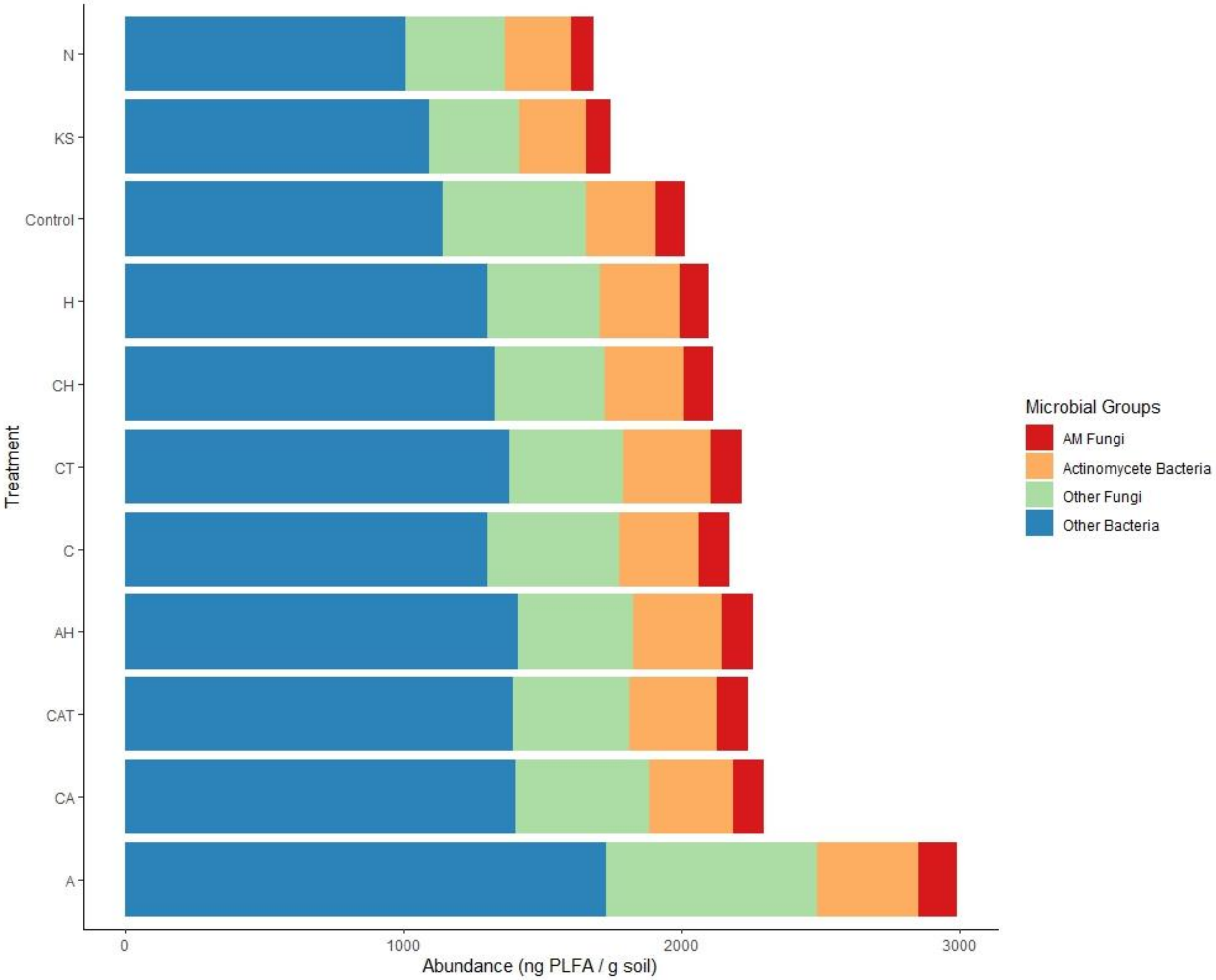
2.5. Ammonium found in each field treatment, 2022 and 2023.
Boxplot representing how soil ammonium (y-axis) responded to the various treatments (x-axis)(n=12) and differences between years. The letters on the x-axis are abbreviations of the full treatment name. A stands for amendment, C stands for cover crop, H stands for herbicide, T stands for tillage, and KS stands for kitchen sink (all 4 treatments). Significant differences ($p < 0.05$) between treatment means within a year are indicated by different letters (lowercase for 2022 and upper case for 2023). Significant differences ($p < 0.05$) between years within a treatment are indicated by an asterisk (*). The native site had fewer sample points (n=6).

We used a PLFA analysis to estimate microbial group abundances within each treatment (Table 2.6). The mean of the total microbial abundance was highest in the Amendment treatment, followed by Cover + Amendment (CA), then Cover + Amendment + Tillage (CAT)(Fig. 2.9). The lowest mean total abundance was in the Native site, which was similar to the control and most intensive treatments (KS)

Amendment (A) was significantly higher than treatment with all methods (KS) in all microbial groups except eukaryotes, which were generally low. The Native site (N) shared statistically similar values as intensive treatment (KS) in all the measurements, which were often the lowest. For the fungal-to-bacterial ratio (F:B ratio), treatments that were significantly lower than Control were Kitchen Sink, Amendment + Herbicide, CAT, CH, and CT.

Table 2.6. Field data – means (all units: ng PLFA / g soil) and standard deviations of phospholipid fatty acid (PLFA) analysis of microbial groups collected from CWG reconstruction treatments (n=12, n=6 for Native treatment). The treatments listed are abbreviations of their full names. A stands for amendment, C stands for cover crop, H stands for herbicide, T stands for tillage, and KS stands for kitchen sink (all 4 treatments). The smaller letters indicate significant differences (p = 0.05) in abundance of microbial groups across treatments. These comparisons are read down each column. The sum of microbial groups represents the total microbial abundance in each treatment.

Treatment	Total abundance	Bacteria	Fungi	Actinomycete Bacteria	AM Fungi	Other Eukaryotes	F:B ratio
A	3853 (1083)^{a}	1729 (562)^{a}	758 (253)^{a}	366 (116)^{a}	138 (39)^{a}	13 (8)^{a}	0.53 (0.13)^{ab}
AH	2990 (543)^{ab}	1413 (296)^{ab}	413 (147)^{ab}	323 (78)^{ab}	107 (30)^{ab}	5 (7)^{ab}	0.37 (0.10)^{c}
C	2987 (871)^{ab}	1303 (410)^{ab}	477 (168)^{ab}	283 (79)^{ab}	110 (33)^{ab}	9 (7)^{ab}	0.46 (0.10)^{abc}
CA	3105 (814)^{ab}	1406 (417)^{ab}	477 (178)^{ab}	302 (71)^{ab}	113 (33)^{ab}	7 (6)^{ab}	0.42 (0.04)^{abc}
CAT	3041 (593)^{ab}	1397 (303)^{ab}	418 (147)^{ab}	316 (53)^{ab}	110 (25)^{ab}	4 (6)^{ab}	0.37 (0.07)^{ac}
CH	2869 (494)^{ab}	1331 (255)^{ab}	395 (163)^{b}	284 (50)^{ab}	106 (25)^{ab}	2 (5)^{ab}	0.37 (0.09)^{ac}
CT	2971 (712)^{ab}	1385 (423)^{ab}	405 (179)^{b}	315 (99)^{ab}	111 (24)^{ab}	6 (10)^{ab}	0.37 (0.07)^{ac}
H	2852 (738)^{ab}	1304 (388)^{ab}	403 (177)^{b}	291 (69)^{ab}	99 (33)^{ab}	2 (4)^{b}	0.38 (0.08)^{abc}
KS	2448 (336)^{b}	1096 (163)^{b}	323 (108)^{b}	240 (40)^{b}	89 (11)^{b}	4 (5)^{ab}	0.38 (0.10)^{c}
Native	2431 (338)^{ab}	1008 (157)^{b}	358 (136)^{ab}	238 (40)^{ab}	82 (15)^{b}	5 (6)^{ab}	0.44 (0.12)^{abc}
Control	2790 (413)^{ab}	1141 (213)^{ab}	519 (120)^{ab}	249 (53)^{ab}	105 (20)^{ab}	5 (7)^{ab}	0.56 (0.13)^{b}



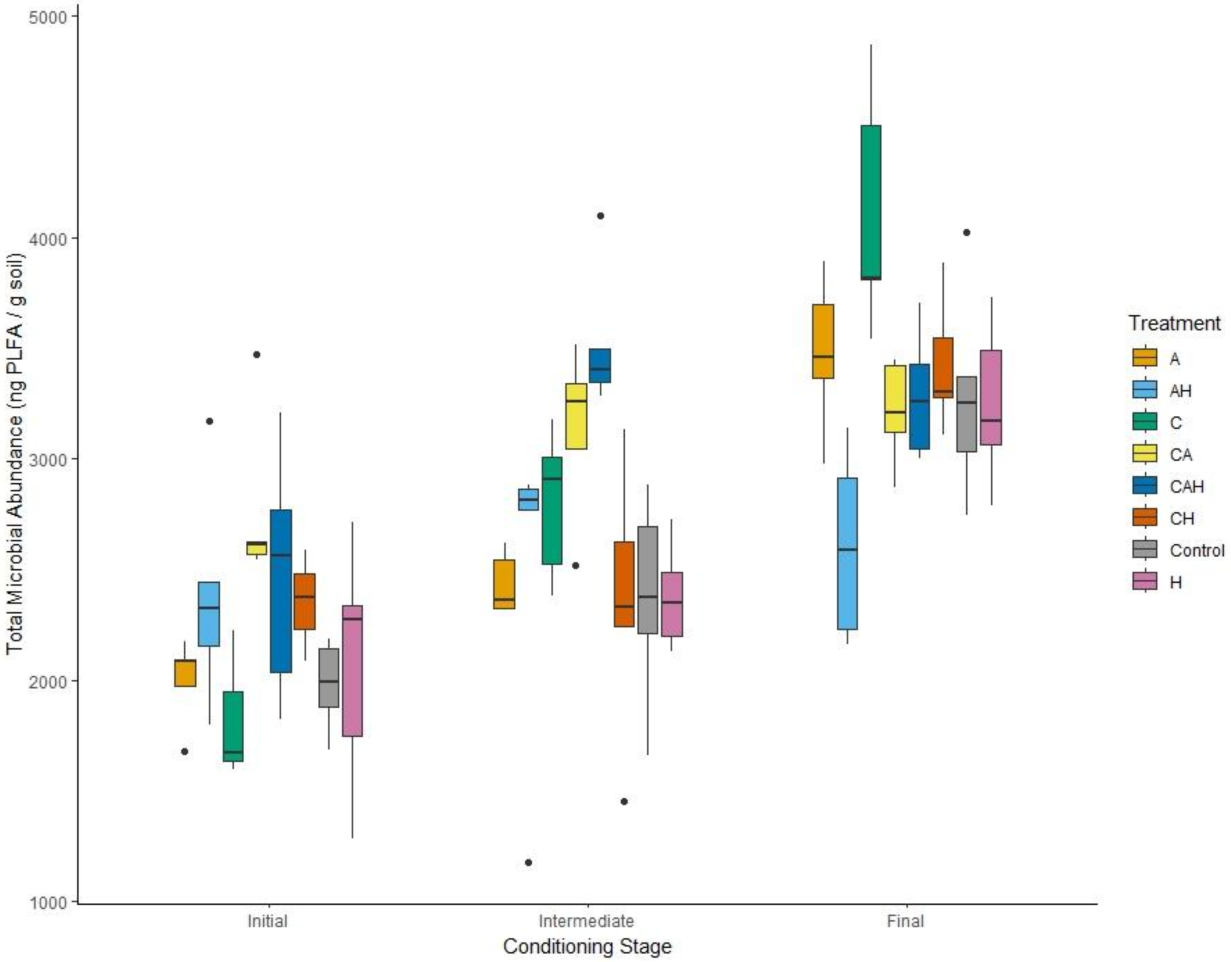
2.6. Field study – Treatments and their microbial group abundances.
Stacked bar chart representing mean abundances of microbial groups in various colors. The treatments (n=12) on the y-axis are abbreviations of their full names. A stands for amendment, C stands for cover crop, H stands for herbicide, T stands for tillage, and KS stands for kitchen sink (all 4 treatments). Each of the response variables are measured in nanograms of phospholipid fatty acid/gram of soil. The native site had fewer sample points (n=6).

We also tested for differences in the soil microbial communities in the greenhouse and found significant differences between treatments (Table 2.7)(2.10). In the initial conditioning stage at the start of the experiment, there were no significant differences between the treatments. In the intermediate stage with full cover crop growth, about 2 months later, we found that the microbial abundance in the Cover crop + Amendment + Herbicide treatment was significantly higher than Amendment. In the final native plant conditioning stage, there was a statistically significant difference in microbial abundance between Amendment + Herbicide and Cover crop. The Cover crop treatment had the highest total abundance and Amendment + Herbicide had the lowest abundance.

Within treatments, microbial abundance in most treatments significantly increased with the increasing duration of the study. Amendment, Cover crop, Cover crop + Herbicide, Control, and Herbicide all had significant increases from their initial stage abundance to their final stage abundance. Cover crop had the highest significant increase from the initial to final stage. Cover crop + Amendment + Herbicide increased significantly between the initial and intermediate stage. The treatments that had no statistical differences between stages were Amendment + Herbicide and Cover + Amendment.

Table 2.7. Greenhouse data - means and standard deviations of total phospholipid fatty acid (PLFA) abundance collected from soil conditioning treatments in the greenhouse (n=5).
Samples were collected at three stages of conditioning (initial, intermediate, final). The treatments listed are abbreviations of their full names. A stands for amendment, C stands for cover crop, H stands for herbicide, T stands for tillage, and KS stands for kitchen sink (all 4 treatments). Different lowercase letters indicate significant differences (p = 0.05) in abundance of microbial groups across treatments and within a conditioning stage. Different uppercase letters indicate significant differences in abundance of microbial groups within treatments and across conditioning stages.

	Total abundance (ng PLFA / g soil)		
Treatment	**Initial**	**Intermediate**	**Final**
A	2000 (194)^{aB}	2433 (140)^{aAB}	3478 (347)^{abA}
AH	2379 (504)^{aA}	2502 (742)^{abA}	2606 (426)^{aA}
C	1829 (342)^{aB}	2802 (336)^{abAB}	4109 (555)^{bA}
CA	2765 (396)^{aA}	3135 (384)^{abA}	3213 (235)^{abA}
CAH	2479 (559)^{aA}	3527 (329)^{bB}	3287 (290)^{abAB}
CH	2350 (253)^{aB}	2358 (614)^{aB}	3424 (301)^{abA}
Control	1978 (203)^{aB}	2364 (473)^{aAB}	3285 (476)^{abA}
H	2070 (558)^{aB}	2378 (240)^{aB}	3249 (366)^{abA}



2.7. Total microbial abundances of each greenhouse treatment in 3 stages. Soil samples were collected from the same pots at three stages of soil conditioning (initial, intermediate, final), which are listed on the x-axis. The treatments listed are abbreviations of their full names. A stands for amendment, C stands for cover crop, H stands for herbicide, T stands for tillage, and KS stands for kitchen sink (all 4 treatments). Total microbial abundance is measured in nanograms of phospholipid fatty acid / gram of soil.

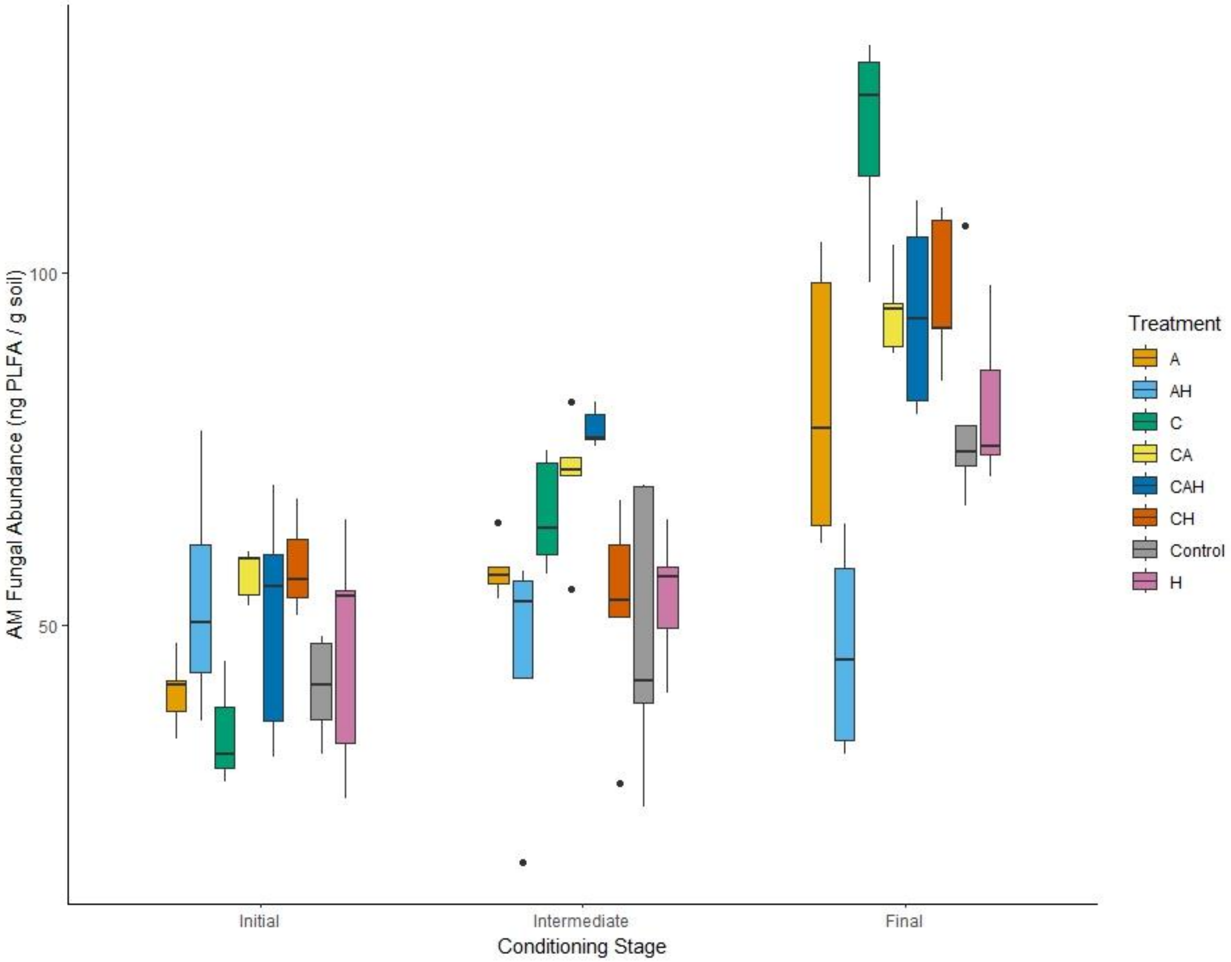
The PLFA method provides abundance of major microbial groups. For brevity, we only present results for AM fungi and the F:B ratio because we were especially interested if the treatments increased the absolute and relative abundances of soil fungi (Table 2.8).

For AM fungi (2.11), there were no significant differences between treatments in the initial stage. In the intermediate stage, Cover crop + Amendment + Herbicide was significantly higher than Amendment + Herbicide and Control. In the final stage, there was significantly more fungi in the Cover crop treatment than the Amendment +Herbicide treatment.

When looking at each treatment’s difference in AM fungi between stages, nearly all of them doubled in abundance from the initial to final stage (except Amendment + Herbicide) and the AM fungi in the cover crop treatment tripled from the initial to the final stages. Herbicide significantly increased from the intermediate to the final stage.

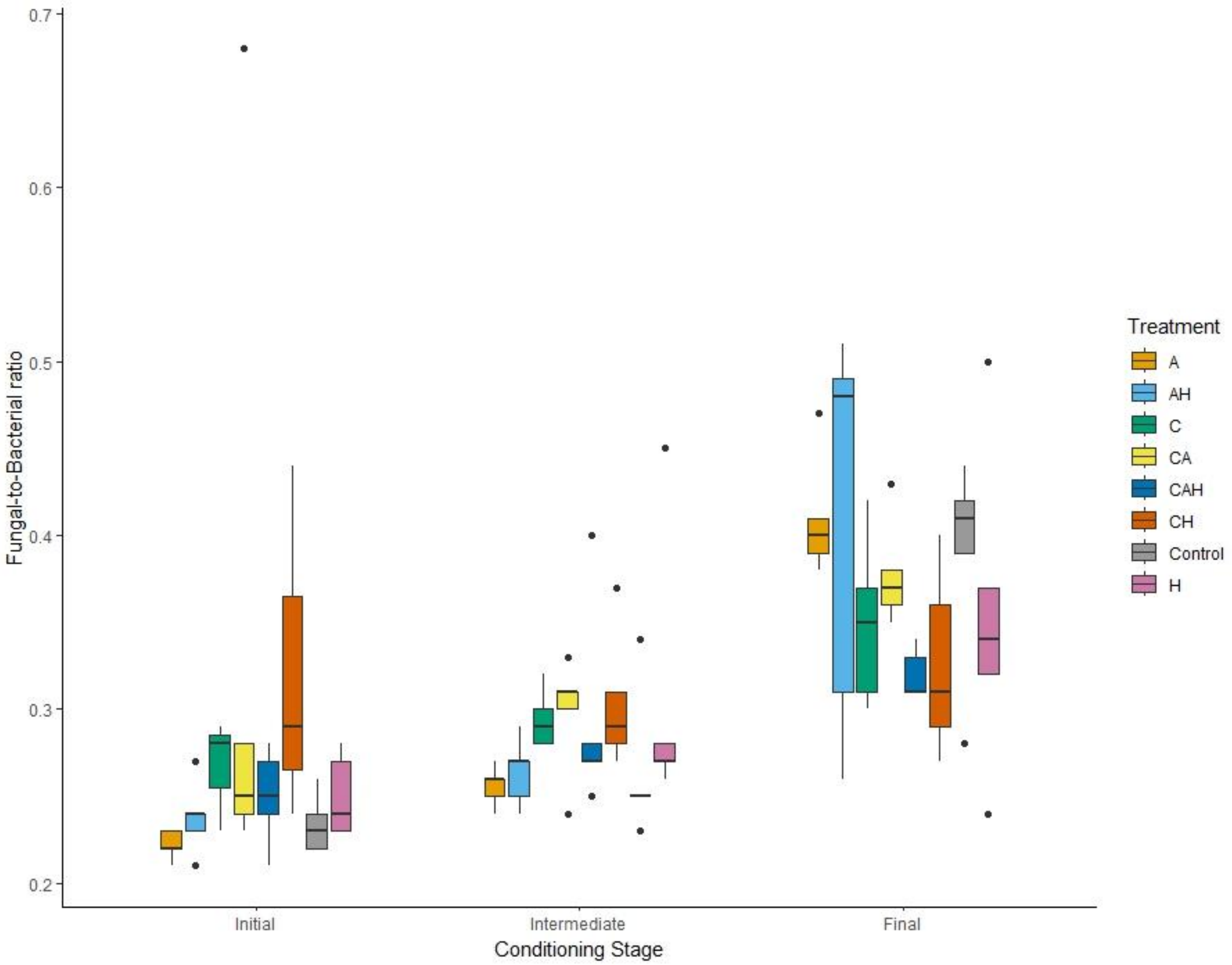
Table 2.8. Greenhouse Data - means and standard deviations of total arbuscular mycorrhizal fungi and Fungal: Bacteria ratios collected from soil conditioning treatments in the greenhouse (n=5).
The treatments listed are abbreviations of their full names. A stands for amendment, C stands for cover crop, H stands for herbicide, T stands for tillage, and KS stands for kitchen sink (all 4 treatments). Samples were collected at three stages of conditioning (initial, intermediate, final). Different lowercase letters indicate significant differences (p = 0.05) in abundance of microbial groups across treatments and within a conditioning stage. Different uppercase letters indicate significant differences in abundance of microbial groups within treatments and across conditioning stages.

Treatment	**Arbuscular Mycorrhizal Fungi (ng PLFA / g soil)**			**F:B ratio**		
	Initial	Intermediate	Final	Initial	Intermediate	Final
A	41 (5)^{aB}	58 (4)^{abAB}	81 (19)^{abA}	0.22 (0.08)^{aB}	0.26 (0.01)^{aAB}	0.41 (0.04)^{aA}
AH	54 (16)^{aA}	45 (17)^{aA}	47 (14)^{aA}	0.24 (0.02)^{aB}	0.26 (0.02)^{aAB}	0.41 (0.12)^{aA}
C	35 (9)^{aB}	66 (8)^{abAB}	120 (14)^{bA}	0.27 (0.03)^{aB}	29 (0.02)^{aAB}	0.35 (0.05)^{aA}
CA	57 (3)^{aB}	71 (10)^{abAB}	94 (6)^{abA}	0.34 (0.19)^{aA}	0.30 (0.03)^{aA}	0.38 (0.03)^{aA}
CAH	51 (16)^{aB}	78 (3)^{bAB}	94 (14)^{abA}	0.25 (0.03)^{aB}	0.29 (0.06)^{aAB}	0.32 (0.01)^{aA}
CH	59 (8)^{aB}	52 (15)^{abAB}	97 (11)^{abA}	0.32 (0.10)^{aA}	0.30 (0.04)^{aA}	0.33 (0.05)^{aA}
Control	41 (7)^{aB}	49 (20)^{aAB}	80 (16)^{abA}	0.23 (0.02)^{aB}	0.26 (0.04)^{aAB}	0.39 (0.06)^{aA}
H	47 (16)^{aB}	54 (9)^{abB}	81 (11)^{abA}	0.25 (0.02)^{aA}	0.31 (0.08)^{aA}	0.35 (0.09)^{aA}



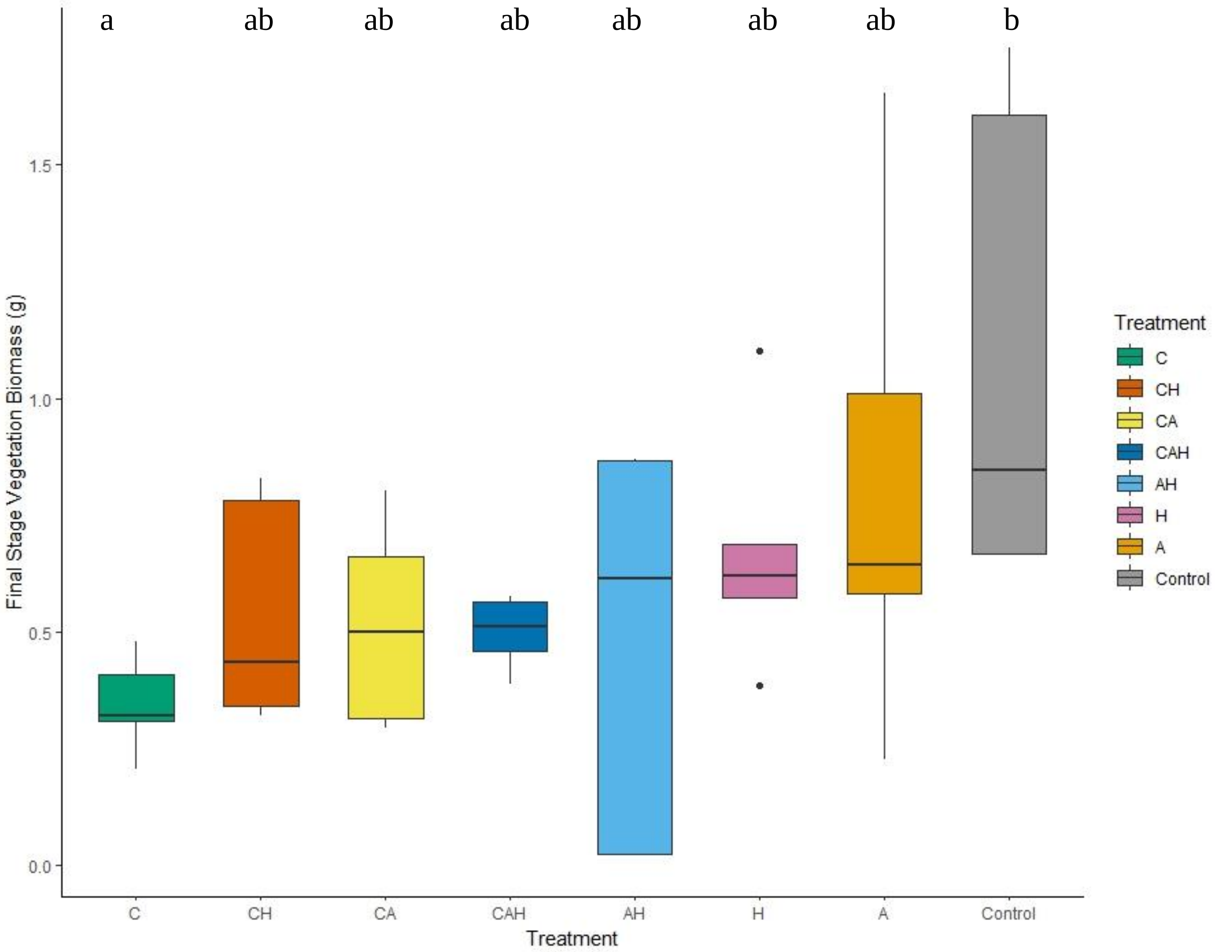
2.8. AM fungal abundances of each greenhouse treatment in 3 stages.
AM fungal abundance (y-axis) is measured in nanograms of phospholipid fatty acid / gram of soil. Soil samples were collected from the same pots at three stages of soil conditioning (initial, intermediate, final), which are listed on the x-axis. The treatments listed are abbreviations of their full names. A stands for amendment, C stands for cover crop, H stands for herbicide, T stands for tillage, and KS stands for kitchen sink (all 4 treatments).

The PLFA analysis included a ratio of the proportions of fungi to bacteria (F:B) to understand relative shifts of these groups across treatments (Fig. 2.12). Across treatments, there were no significant differences in F:B. Most treatments increased significantly from the initial to final stage besides Cover crop + Amendment, Cover crop + Herbicide, and Herbicide.



2.9. Fungal-to-bacterial ratios of each greenhouse treatment in 3 soil conditioning stages. Soil samples were collected from the same pots at three stages of conditioning (initial, intermediate, final), which are listed on the x-axis. The treatments (n=5) listed are abbreviations of their full names. A stands for amendment, C stands for cover crop, H stands for herbicide, T stands for tillage, and KS stands for kitchen sink (all 4 treatments).

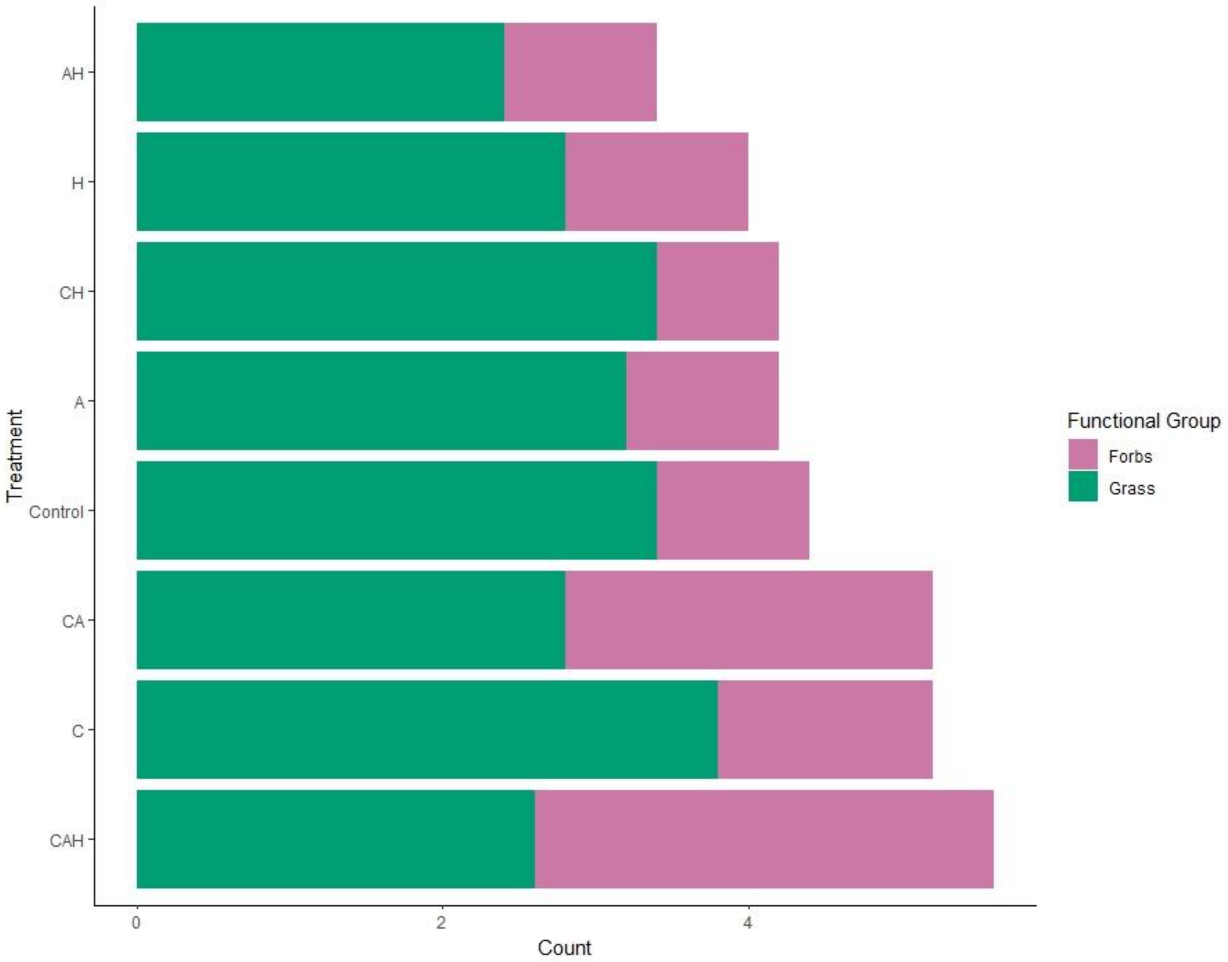
There were few statistically significant differences found in vegetation biomass across the pot treatments (Fig. 2.13). Vegetation biomass included all aboveground vegetation, including CWG. The native species were planted at the beginning of the final conditioning stage. Control had the greatest vegetation biomass, being significantly higher than cover crop (biomass included CWG). The treatments containing cover crops had the smallest biomass values, which could indicate lack of soil resources for native species when planted directly after cover crop growth.



2.10. 2023 Greenhouse Project - Vegetation biomass (grams) in the final stage of the treatments.
Boxplot showing the distribution of vegetation biomass weight in each treatment (n=5). The letters on the x-axis are abbreviations of the full treatment name. A stands for amendment, C stands for cover crop, H stands for herbicide, T stands for tillage, and KS stands for kitchen sink (all 4 treatments). Treatments are ordered from lowest (left) to highest (right).

We found no significant differences between the total number of plants and the number of plants within the grass and forb functional groups in each treatment (2.14).

For total plant counts, there were no statistically significant differences between treatments. Counts of forbs and grasses also had no significant differences. Cover crop + Amendment + Herbicide was highest with a mean and standard deviation of 5.6 plants (±1.52) plants. It also had the highest forb average, at 3.0 (±1.52) plants. Amendment + Herbicide had the lowest mean total count at 4.2 (±0.84) plants. The Control treatment was near the middle at a total count mean of 4.4 (±1.82). Control had a low forb count with a mean of 1.0 (±1.82), but the treatment with the lowest forb mean was Cover crop + Herbicide at 0.8 (±1.92).



2.11. 2023 Greenhouse Project – Count of various plant functional groups in the final stage of the treatments.

Stacked bar chart showing the count of grasses versus forbs in each treatment (n=5) after the final native species soil conditioning stage. The letters on the y-axis are abbreviations of the full treatment name. A stands for amendment, C stands for cover crop, H stands for herbicide, T stands for tillage, and KS stands for kitchen sink (all 4 treatments). Treatments are ordered from lowest (top) to highest (bottom) total functional group count, but total mean plant number and mean plant number within each functional group were not statistically significantly different.

Discussion

Our objectives were to evaluate the impacts of invasive plant management strategies on soil physical, chemical, and biological properties in a CWG dominated field. We met our objectives through a 2-year field study with annual treatment applications and a 4-month long greenhouse study with 3 soil conditioning phases.

There were no meaningful differences in the general soil properties (pH, EC, and bulk density) of our field study treatments. This indicates 2 years of amendment, herbicide, tillage, and cover crop did not meaningfully alter these soil properties at our field site. Tillage impacts soil structure by breaking down soil macroaggregates into microaggregates, which often leads to lower soil bulk density and water infiltration due to increased porosity. (Topa et al. 2021; Pagliai et al. 2004). We expected to see lower bulk density in our tillage treatments, but tillage did not appear to modify bulk density beyond observed variability across the site.

We anticipated soil TOC percentages and POXC to be higher in the Amendment treatments and Cover crop treatments, since they provide essential nutrients and organic matter for microbial mineralization (Hu et al. 2022). Cover crop also prevents soil erosion, which helps retain soil organic carbon (Adetunji et al. 2020). We only found a significant difference in TOC in 2023, when Amendment was higher than the Cover crop + Tillage treatment. This could indicate that cover crops used alone could not offset the negative impact of tillage on total organic carbon. POXC increased significantly in many of the treatments from 2022 to 2023, including Control. POXC response was similar in most treatments, with only one significantly different pairing in both years. In 2022, Cover crop + Amendment + Herbicide + Tillage had significantly higher POXC than Cover crop + Tillage. In 2023, there was significantly higher POXC in Control than Herbicide, possibly indicating a negative impact of herbicide on carbon mineralization. Chavez-Ortiz et al. (2022) found similar results, showing that glyphosate applied to soil with no history of glyphosate use negatively impacts soil carbon mineralization. Hosseini Bai et al. (2014) found that another labile C pool, microbial biomass carbon, also had a negative correlation with herbicide application, remaining lower than control levels. More research should be done with responses of POXC and other forms of soil C to herbicide to confirm if a correlation exists and the possible mechanisms. Our results also found a clear correlation between herbicide use and increase in soil nitrate, which is mobile in the soil profile and risks of leaching into water sources (Gaupp-Berghausen et al. 2015). Seven treatments increased in POXC significantly from 2022-2023, with 5 of them containing cover crops. One of these significant increases between years was also in control, which could indicate the change was due to something other than treatment applications, like environmental variations.

The limited changes in TOC and POXC could be attributed to the extensive time it takes soil carbon and nitrogen pools to noticeably change. Changes in soil organic carbon vary in soil type and land use history but have been shown to take 6-10 years to be detectable, requiring a very large number of samples if increases in carbon inputs are below 15% (Smith 2004). POXC is a form of labile carbon that has been found to respond strongly to tillage and addition of organic matter. It also has a strong relationship with TOC, making it a good indicator of soil carbon sequestration (Bongiorno et al. 2019; Hurisso et al. 2016).

Total nitrogen did not vary between treatments, but there were significant differences in nitrate (NO_3^-) and ammonium (NH_4^+) nitrogen. We observed greater soil nitrate availability in Herbicide and Herbicide+Amendment treatments than in the control, suggesting herbicide application increases soil nitrate (Gaupp-Berghausen et al. 2015). We also saw a correlation between high soil nitrate and high vegetation biomass in the use of all treatments (KS), indicating cover crop growth benefitted from high nitrate levels. Ammonium increased between years in some treatments, but did not follow a distinguishable pattern, suggesting that treatments may not have been responsible for the changes.

For our field study's microbial responses, we conducted a PLFA analysis. We expected Amendment treatments to bolster microbial communities through organic matter and nutrient inputs. Amendment was successful in improving the total microbial abundance, having some of the highest abundances in the field and greenhouse study. Treatments including amendment but not herbicide had the highest means, suggesting herbicide treatments had negative impacts on total microbial abundance in the field study. However, total microbial abundances did not follow this trend in our greenhouse study, with an increase occurring between each stage. This could be because our Herbicide's interaction in the greenhouse is much different than in the field. Herbicide in the field study interacted with vegetation, while herbicide was applied to bare soil in the greenhouse. In a field setting, glyphosate can be exuded by roots of the plants that were sprayed, plus be released from dead plants (Soares et al. 2019), which could have greater effects on soil microorganisms due to the longer persistence in the soil profile. Application of herbicide to bare soil in the greenhouse may have degraded the herbicide quickly or passed through the soil in the pots much quicker than in the field soil because of the consistent watering compared to infrequent precipitation in the field.

In our field study, Kitchen Sink had the lowest total microbial and AM fungal abundances, along with the lowest F:B ratio. This may indicate how addition of the amendment and cover crop was not enough to improve the microbial diversity depleted by herbicide and tillage applications. The greenhouse study treatment equivalent to our Kitchen Sink field treatment was Cover crop + Amendment + Herbicide (CAH). During the intermediate stage, after cover crop treatments had reached maturity, this treatment had the highest total abundance and AM fungi. It did not maintain the highest microbial abundances in the final stage, after native species reached maturity, but had the highest number of plants (and forbs) present. This could indicate that the plant species that grew, especially the forbs, need soils with higher total microbial biomass, but produced less soil organic matter in the final stage than the cover crops did during the intermediate stage. CAH treatment did not have one of the highest vegetation biomasses, but this is probably due to the high domination of forb species. Most treatments contained a higher grass to forb ratio. The grasses, including CWG, grew taller and faster growth than the native forbs.

The lack of significant differences in greenhouse plant counts may be attributed to our small treatment replication size, which was chosen due to constraints in the amount of field soil available. It could also be because the same number of plants were planted in each pot (2 per species), leaving little room for significant variation. It is important to note that the grass functional group in 2.13 included CWG due to trouble differentiating with the native green needlegrass without a seedhead.

TOC was significantly lower in the Cover crop + Tillage than the Amendment treatment, indicating cover crops were not as successful as the amendment in increasing carbon. In our field study, use of all treatments (KS) did not follow our biological expectations, having the lowest total microbial abundance, bacteria, and AM fungi of the treatments. This may indicate the use of herbicide and tillage had a significant negative impact on the soil microbial community, and the addition of cover crop and amendment were not successful in restoring them to a level higher than Control. There is limited literature exploring the interaction of herbicide and tillage and the impacts on the soil microbial community. Rosner et al. (2019) found conservation tillage coupled with herbicide application favored AM fungal root colonization and benefitted plant growth. This could indicate that certain mixtures of less intensive tillage techniques and herbicides could have lesser impact on soil biology than our techniques.

Our greenhouse project had different results than the field study for soil biology responses. Cover crop and soil amendments used together have been shown to increase soil organic C and N more effectively than used alone (Messiga et al. 2015). The use of the most treatments (CAH) had the highest total microbial abundance and AM fungi in the intermediate stage of the experiment, plus the highest emergence of native plant species and forbs. Although there was less total microbial and AM fungi than the cover crop treatment in the final stage of the experiment in CAH, the interaction of these treatments may have created a diverse soil community preferable for the native plant growth. Some of the variation in the soil microbial abundances may have been because of how field soil samples were handled and stored versus the greenhouse. The field soil samples were placed in a fridge for storage instead of a freezer since there were 126 samples that were much larger than the greenhouse ones. The field soil samples also went through a 9-hour drive in a cooler while being transported from the field.

The lack of significant changes in total nitrogen pools and total organic carbon pools can be attributed to the study’s short time frame, and future research could evaluate if there are more distinguishable results in more than 2 years. Some of our overlapping soil results could indicate the need for a longer project time period or another experimental design to identify clear differences between treatments. Other research could also test the effects of other soil conditioning treatments, tillage methods, and herbicides. We discovered herbicide use may correlate with high soil nitrate and low POXC. These effects were negated when cover crops were added to herbicide use. Cover crops also led to the highest total microbial and AM fungal abundances in the greenhouse, further showing their beneficial soil impacts. The addition of cover crop and amendment were not successful in negating the negative soil impacts of herbicide and tillage used together.

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MANAGEMENT IMPLICATIONS

Various recommendations can be made based on each land manager’s goals and budgets. The prices of the individual and combined treatments are found in Table 1.3. If land managers are simply looking for CWG reduction alone, both herbicide and tillage are effective. Herbicide is cheaper than tillage application. If budgets allow, we found that using herbicide and tillage together can lead to higher CWG reduction than when used alone. Using both also led to the highest cover crop averages. It’s important to note the negative soil implications of using the methods together, which led to high nitrate levels and the lowest total microbial and AM fungi abundances and fungi-to-bacteria ratios in our field study. Using both also risks the secondary invasion of noxious or invasive plant species like cheatgrass or Japanese brome (*Bromus tectorum L.* and *B. japonicus* Thunb. Ex Murray). We recommend native or cover crop seeding to suppress the establishment of these.

Addition of cover crops and soil amendment were not enough to offset the impact of using tillage and herbicide together. Herbicide is not recommended to be used without a soil conditioning method because it leads to low POXC and high nitrate levels. Cover crops were cheaper and slightly better than the amendment at offsetting herbicide’s nutrient impacts. Both the addition of cover crops and amendment to herbicide had higher POXC than herbicide alone in the second year of application. Amendment was not effective in lowering nitrate levels with herbicide. Both cover crop and the amendment showed beneficial soil biological impacts in the greenhouse and the field. Overall, we recommend cover crop + herbicide as a cost-effective method of suppressing CWG, limiting impact on bare soil, and achieving stable soil nutrient and microbial ranges for future native establishment.

GENERAL CONCLUSION

The introduction of crested wheatgrass (*Agropyron cristatum*, CWG) to the northern Great Plains region of the United States has led to lower native species diversity due to its competitive characteristics above and belowground (Vaness and Wilson 2007). Land managers have had limited success in both reducing CWG and establishing native plants within these stands (McAdoo et al. 2017). Herbicide and tillage have been shown to result in temporary suppression of CWG, often regenerating rapidly (Davies et al. 2013, Hulet et al. 2010, McAdoo 2017). Research has indicated that CWG reduction is contingent on combining these treatments, but this likely leads to degradation of soil properties (Gasch et al. 2016). This degradation may have negative implications for native seeding and establishment, plus cause exotic weed invasions (Morris et al. 2019).

Our research objectives were to evaluate the effectiveness of traditional management methods of herbicide and tillage in reducing CWG. We also evaluated whether the addition of a biological soil amendment and/or cover crops could restore soil physical, chemical, and biological properties under CWG dominated prairies. We conducted a 2-year long field study in NE Montana and a 4-month greenhouse study at North Dakota State University to reach our objectives. We collected vegetation data to measure CWG response to treatments and soil samples to observe soil physical, chemical, and biological responses to our treatments. Overall, we found that CWG reduction was dependent on the use of herbicide and tillage, and the combination of both led to the highest reduction in CWG. In our field study, we found that our most intensive treatment, the use of tillage and herbicide with the addition of cover crops and the biological soil amendment, did not increase soil microbial abundance and AM fungi as we hoped, with this treatment having the lowest abundances. Our most intensive greenhouse treatment, the combination of cover crop, amendment, and herbicide contrasted our field study findings, with the highest total microbial and AM fungal abundance in the intermediate stage of the experiment. It also had the highest number of native species emergence. Herbicide treatments had highest nitrate levels.

Native species restoration into CWG stands is complex due to separate management needs above and belowground. We found that herbicide and soil disturbance methods were necessary to suppress CWG growth, but they also degrade soil biological properties beneficial for native species establishment. In our field study, herbicide and tillage treatments led to high nitrate levels in the soil, which risks possible nutrient leaching and exotic weed invasions. Land managers should consider using soil conditioning treatments with control approaches to restore the soil life that is lost and host a more diverse soil community preferrable for native species.

Cost limitations may not allow the use of both tillage and herbicide, and our field results do not show a clear choice for which is better for soil microbiota, so it will need to be studied further to identify clear trends. We found that both led to decreases in microbial abundances and herbicide led to an increase in nitrate. As for choice between the amendment and cover crop, our results were mixed, with better soil microbial response to cover crops in the greenhouse and better response to Amendment in the field. The final native biomass abundance in the greenhouse experiment was higher in Amendment treatment than Cover crop. Cover crop was more successful in offsetting the soil nutrient impacts of herbicide on POXC and nitrate. A follow up with success of native seeding may shed more insight on which soil conditioning treatment was preferrable for future native restoration projects.

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