USING BIOSOLIDS TO MITIGATE DROUGHT EFFECTS IN A SEMI-ARID GRASSLAND WITH AND WITHOUT GRAZING BY CATTLE

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Abstract

Climate extremes are becoming more common throughout semi-arid rangelands of British Columbia; therefore, finding climate mitigation techniques is paramount. Biosolids are a soil amendment made from treated municipal wastewater that can be surface applied to grasslands to aid in reclamation and increase primary productivity. The purpose of this work was to examine the ability of a single application of biosolids to mitigate drought effects in a semi-arid, grazed and ungrazed, grassland 19 years after application. In 2002, biosolids were applied at a rate of 20 Mg ha^{-1} in two study sites, then one of these sites was fenced to create a grazing exclosure. In 2020 rainout shelters were constructed to simulate a growing season drought for approximately two years, where the experimental drought plots had a median 55% reduction in soil moisture. Before and after sampling included above and belowground biomass as well as plant community composition. Further, soil bulk density, pH, organic matter, total carbon and nitrogen, and soil water content were analysed. Rainout shelters reduced root biomass but the biosolids treatment mitigated this loss. Additionally, biosolids significantly increased aboveground biomass and litter. Biosolids lowered diversity, whereas open grazing increased diversity. Biosolids also increased total carbon, total nitrogen, and soil organic matter. The only soil metric altered by the experimental drought treatment was soil water content, which was lowered. Overall, a single application of biosolids had positive lasting effects on the ecosystem nearly two decades later. Although some undesirable outcomes were observed, grazing may be used as a management tool, in combination with biosolids, to meet reclamation targets in a semi-arid rangeland. Further research is needed to understand the relationship between drought, biosolids, and grazing in semi-arid grasslands in British Columbia.

Lay summary

Droughts can cause economic and ecological stress in the agricultural industry. Biosolids are a fertilizer-like product that are made from treated municipal wastewater, which is rich in organic matter, and due to this, may be useful as a soil amendment in preventing drought stress. The goal of this work was to test the effects of drought on plants and soils 19 years after a single surface application of biosolids compared to a control plot. I also tested the effects of grazing by allowing one site, in both the biosolids amended plots and control plots, to be grazed by cattle and the other site fenced from grazing. Drought stress was created using experimental drought shelters and then plant and soil samples were collected for analyses. I found that biosolids generally increased plant growth and soil health, while grazing helped to prevent unwanted effects, like lower plant diversity, due to the biosolids. More research is needed to determine if biosolids can mitigate drought, as there were few observed drought effects.

Preface

The initial idea for this thesis was created by Dr. Lauchlan Fraser and those at the Fraser Lab. Further, the Fraser Lab (Gwen Freeze, Keenan Baker, Matthew Coghill, Jared Frasca, Gillain Spencer, and Solenn Vogel) were responsible for the construction of the research site in 2020 and assisted in field work throughout this project. Further, Solenn Vogel and Gwen Freeze collected the 2020 vegetation cover data referenced within this work. Additionally, Dane Goh provided some laboratory assistance. Data analysis was completed by myself. My supervisory committee, Dr. Lauchlan Fraser, Dr. Melanie Jones, Dr. John Klironomos, Dr. Darcy Henderson, and Dr. Miranda Hart, provided feedback throughout the various stages of this project.

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Dedication

To the cows.

1 Introduction

Climate models predict changing precipitation across the globe (IPCC, 2014a). In the next fifty years, North America is predicted to have increased growing seasons with less than average baseline amounts of precipitation (IPCC, 2014b). Changes to precipitation regimes can be expected to put pressure on established agricultural practices. Grasslands experiencing drought may become less productive resulting in economic hardship or food scarcity and thus, are less able to support robust ranching and agricultural activities (Fraser, 2019; Gibson and Newman, 2019). For example, in the Great Plains of the USA, farm income is predicted to be reduced by 33% under severe, long-term drought (Osei et al., 2015).

Each subset within the agricultural industry has unique challenges that could impact the drought response of the ecosystem. Ranching operations, for example, must consider the direct and indirect effects of grazing on semi-natural areas in conjunction with any climate or drought challenges that may impact the plant community or soil health. Any negative impacts on the plant community and soils can have downstream effects on forage availability causing logistical problems for ranching operations. Therefore, finding mitigation techniques that allow for the continuation of these agricultural practices in the face of drought is vital.

One technique is the application of biosolids, a product of municipal waste that can be treated until to become a suitable soil amendment (Ministry of Environment and Climate Change Strategy, 2016; Ministry of Environment of British Columbia, 2016a). Municipalities are searching for sustainable uses for biosolids and applying them to land allows for nutrients and organic matter to be reused. This practice is already occurring, with 9% of all biosolids produced in British Columbia being used for agricultural purposes (Ministry of Environment and Climate Change Strategy, 2016; Ministry of Environment of British Columbia, 2016a). However, there is little research examining the separate and combined effects of drought, grazing, and biosolids on grasslands. This research is much needed as ranchers and land managers are actively applying biosolids to grasslands in British Columbia. Thus, the aim of this research is to identify the effects of drought, grazing, and biosolids on the plant community and soil metrics in a semi-arid, C3-dominated grassland in British Columbia.

1.1 Drought

Droughts within grasslands are well documented and have the potential to act as an ecosystem disturbance if conditions are severe enough (Tilman and Haddi, 1992; Weaver and Albertson, 1939). Characteristically, droughts have less than typical amounts of precipitation, higher evapotranspiration, and lower soil moisture which can increase stress (Weaver and Albertson, 1940). Additionally, the length, timing, and severity of drought are all variables that contribute to ecosystem response (Carroll et al., 2021). Species that utilize C3 photosynthetic pathways undergo much of their primary productivity when temperatures are mild, in spring and early summer (Hahn et al., 2021; Pearcy and Ehleringer, 1984). Large influxes of soil moisture, like snowmelt, lead to earlier flowering times as well (Munson and Long, 2017). Thus, in C3 dominated grasslands, such as my study system, drought that occurs later in the season, well after the initial snowmelt, may not have the same effect as drought that decreases winter or early spring precipitation. Due to this, calculating the outcome or ecological impact of drought can be difficult.

Although, wide scale modeling can help predict future ecological problems relating to drought, large-scale climate models may be too coarse to inform local management decisions. *In situ* experiments, while less broad, can help inform local decision makers about specific systems of interest and mitigation strategies. A common way to study drought *in situ* is by using rainout shelters that prevent precipitation from falling on the ground below (Fahey et al., 2018; Kundel et al., 2018; Stampfli et al., 2018; Yahdjian and Sala, 2002). These shelters typically consist of four support legs, a roof, and gutter (Yahdjian and Sala, 2002). Depending on the objective of the experiment, the roofs can be adjusted to allow for some precipitation to fall to the ground below. Further, as the sides of the shelters are open, precipitation will be able to enter this way but this prevents the creation of a microclimate (Kundel et al., 2018). Additionally, rainout shelters can be used in combination with other treatments to test multiple factors, like drought and grazing, drought and temperature, or drought and biosolids (Carlyle et al., 2014).

1.1.1 Grassland vegetation response to drought

As previously stated, drought can be a dynamic type of ecosystem disturbance that can greatly impact vegetation. Within a system of mixed cool and warm season grasses, site dryness has been shown to account for 70% of variation in plant diversity among treatments (Souther et al., 2020). Further, drought typically contributes much more of an effect on plant community composition than land management techniques, like grazing (Deléglise et al., 2015; Liang et al., 2018). In grasslands, however, there is little consensus on how plant community composition will be altered under drought (Ploughe et al., 2019). Grasses had higher biomass during and after experimental drought compared to other functional groups (Carlsson et al., 2017). Sometimes, drought-driven shifts are seen as changes to species dominance, where dominant species decrease in biomass and subordinate species increase in biomass (Mariotte et al., 2013). The mechanisms and interactions that drive these changes are still under investigation (Ploughe et al., 2019).

Broadly, grasslands under experimental drought created by rainout shelters have showed decreased growing season aboveground biomass and annual net primary production (Hartmann and Niklaus, 2012; Stampfli et al., 2018). Species richness of native and exotic species has been shown repeatedly to decrease after both natural and experimental drought (Fahey et al., 2018;

Souther et al., 2020; Tilman and Haddi, 1992). Additionally, grass belowground biomass has been shown to decrease after short term or experimental drought (Carroll et al., 2021; de Vries et al., 2016). Commonly, these changes are not detectable in future growing seasons or after typical precipitation regimes resume (Deléglise et al., 2015; Hartmann and Niklaus, 2012; Stampfli et al., 2018). In years after growing season experimental drought, grassland roots showed no changes in biomass (Koerner and Collins, 2014; Li et al., 2021).

Diversity is expected to be highest at moderate levels of disturbance as less stress tolerant species may decline in favour of more tolerant ones (Grime, 1972). Thus, it would be expected that under severe enough drought, grassland diversity will decrease. Tilman and Haddi (1992) discussed that, although there was a reduction in species richness during drought years, there may not be a genuine or long-term loss of species. These species may still be present in the seed bank or on a larger scale, and as recovery occurs, they will establish again (Tilman and Haddi, 1992).

Aside from the direct plant community response to drought, the composition of a grassland impacts the ecosystem level response to drought. Communities with higher species richness were less susceptible to drought and exhibited more resilience than those with lower species richness before drought (Klaus et al., 2016; Tilman and Downing, 1994). Although there is evidence that higher biodiversity increases the stability of a grassland, the temporal scale examined may influence whether an effect is seen (Ploughe et al., 2019; Tilman et al., 2006). For example, after short term drought, changes to species richness, a component of diversity, could not predict a pattern in drought resistance (Wang et al., 2007). Similarly, increased functional diversity did not result in increased drought resistance (Carlsson et al., 2017). Tilman et al. (2006) suggested that an increase in ecosystem stability in relation to diversity would only be detected if the system was examined over longer term, such as over the course of ten years; high inter-interannual variability in the data might over-shadow diversity effects in shorter-term investigations. Although there is mixed evidence that plant community composition is related to drought tolerance, drought effects should be considered in any land management program to preserve ecosystem services.

1.1.2 Soil physical properties response to drought

Drought can also influence soil health, mostly related to soil carbon. Bulk density decreased under experimental drought, most likely due to changes in aggregate stability (Yang et al., 2019). Under drought, plants lower photosynthetic rates while respiration continues, leading to lower soil carbon (Kuzyakov and Domanski, 2000; Lawlor and Cornic, 2002; Mooney et al., 1966). Another study found a significant decrease in soil organic carbon above 10 cm after drought, but not below this depth (Zhang et al., 2019). This, in conjunction with lower microbial biomass after drought, indicates lower carbon within soils under drought (Su et al., 2020). Therefore, drought can be expected to negatively impact soil carbon. In a semi-arid grassland under experimental summer drought, mineralization and nitrification rates were higher than controls (Yang et al., 2020). Lower plant biomass and CO_2 flux under drought contributed to soils more concentrated with nitrogen, as decomposition processes were uncoupled (Evans and Burke, 2013; Yang et al., 2020). Further, soil nitrogen is susceptible to leaching depending on moisture regimes and fertilization, thus in drought systems more nitrogen is present in the surface soils compared to those with heavy precipitation (Ledgard et al., 2011)(Ledgard et al., 2011).

Soil organic matter has been shown to predict a systems drought response. Under drought, study sites with high soil organic matter were more productive than sites with low soil organic matter (Buttler et al., 2019). Further, sites with lower soil organic matter had ongoing effects from drought, whereas drought effects were undetectable at sites with high soil organic matter (Buttler et al., 2019). Variation in site-specific traits, like aspect or soil texture, could have a conflating effect on the measured drought response, yet it may be beneficial to add organic matter to a system, when at low levels, to mitigate the lower productivity associated with drought.

1.2 Cattle grazing in grasslands

Grasslands are a particular system of interest due to the commercial and agricultural value of them as rangelands. Cattle ranching is a significant part of the agriculture industry within Canada. As of 2020, there were 11.2 million cattle found on various farm types within the country (Statistics Canada, n.d.). Although grazing is a common ecosystem process in British Columbia, over-grazing from commercial activities has the potential to disrupt typical succession or function.(Wikeem and Wikeem, 2004). To minimize ecological damage, legislation has set out requirements for conserving ecosystem components like biodiversity and soil health within these systems (*Forest and Range Practices Act: Range Planning and Practices Regulation*, 2004). Therefore, it is important to fully understand the impacts of cattle grazing on grasslands independently and with respect to other management practices.

1.2.1 Plant community response to grazing

Previously, site productivity was thought to dictate the way grazing impacts the plant community (Bakker et al., 2006; Olff and Ritchie, 1998). In a 7-year field experiment that looked at small and large herbivore grazing, it was found that at more productive sites, grazing increased plant species richness. However, this effect was only significant when large and small herbivore grazing was included, opposed to non-significant effects with only small herbivore grazing (Bakker et al., 2006). Despite this, a recent global study that covered a broad range of productivity levels, found that herbivory did not decrease richness at less productive sites (Koerner et al., 2018). Moreover, the researchers found that changes to diversity were the result of changes in species dominance due to grazing, and not linked to site productivity (Koerner et al., 2018).

It is likely that herbivory alters the plant community by reducing the most dominant plants, through dietary choices, and therefore reducing the competitive exclusion of other, less dominant plants (Bradfield et al., 2020; Grime, 1972; Olff and Ritchie, 1998; Rook et al., 2004). Therefore, I expect to see increased species richness in grazed plots, as growth of the dominant plant, Kentucky bluegrass, would be controlled by grazing, allowing for other species to establish. After 6 years of sheep grazing exclusion, a semiarid grassland plant community had lower species richness and Shannon diversity than plots that were continuously grazed (Ren et al., 2018). Similar trends were reported in a large review, where herbivory resulted in neutral or positive increases to species richness (Koerner et al., 2018). In a long-term grazing exclusion experiment, species richness was found to be lower in grazed semi-arid grasslands in China (Cheng et al., 2016). Globally, grazing can have mixed results but within a semiarid grassland, grazing most commonly results in an increase in richness.

In both long- and medium-term studies, grazed plots had lower aboveground and belowground biomass than grazing exclusion plots (Cheng et al., 2016; Ren et al., 2018). Additionally, the composition of the aboveground biomass has been shown to change with grazing (Norman, 1957; Weaver and Darland, 1948). For example, biomass of some perennial grasses and forbs was significantly higher in grazed plots, while shrub biomass decreased (Cheng et al., 2016). Litter also increased in grazing exclusion plots (Cheng et al., 2016). However, under extreme grazing and lower precipitation, forbs and shrubs both became more abundant, reflective of the general weakening of the grassland (Weaver and Darland, 1948).

As noted, grazing alone can alter the plant community, but the interaction of grazing and drought is also of interest. Although, drought or precipitation have a much larger effect on plant community traits than grazing or competition, there is also a significant interaction between drought and grazing on plant species richness, diversity, and aboveground biomass (Liang et al., 2018; Wilson, 2007). Wilson (2007) found that several grass species were not found in drought years yet would inconsistently recover as abiotic factors became desirable, rather than possible successional changes. Moderate levels of grazing, which were defined as leaving 50% of aboveground biomass, resulted in the quickest recovery of plant species richness after a year of drought compared to high levels of grazing or grazing exclusion (Souther et al., 2020). Further, moderate grazing levels resulted in, and maintained, the lowest exotic species richness for 5 years after drought (Souther et al., 2020).

1.2.2 Soil responses to grazing

The effect of grazing on soil carbon is variable across systems, with grazing either increasing, decreasing, or not significantly altering soil carbon. Models used to predict the direction of soil carbon are complex and based on a variety of factors, like community composition, nitrogen availability, annual net primary production, and soil respiration (Pineiro et al., 2010). Additionally, the magnitude and directionality of the effect of grazing on soil carbon is influenced by soil type and annual precipitation. For example, in a large review, sandy soils with low precipitation yielded a smaller negative effect size, where clay soils at the same precipitation level had a larger positive effect size (Mcsherry and Ritchie, 2013). Poor management, like overgrazing, can lead to a reduction of carbon storage especially outside of temperate grasslands (Eze et al., 2018). In these situations, grazing exclusion leads to increased soil carbon storage (Cheng et al., 2016; Eze et al., 2018; He et al., 2012; Ren et al., 2018). However, Canadian grasslands have the potential to sequester carbon in the soil under correct management practices, like no till agriculture and sustainable stocking rates, to a finite amount (Wang et al., 2014). A study looking at grazing effects in the interior of British Columbia on

Black Chernozems soil, found no changes to total carbon or nitrogen with long term grazing (25 – 75 years) (Krzic et al., 2014).

Both medium- and long-term grazing exclusion has been shown to decrease bulk density in the top 10 cm of soil, whereas grazing increased bulk density (He et al., 2012; Krzic et al., 2014; Pineiro et al., 2010; Ren et al., 2018). Total nitrogen was also a soil component that declined with grazing in semiarid grasslands (Cheng et al., 2016; Ren et al., 2018). Authors suggested that this is due to a combination of mechanisms, like changes in energy transfer between vegetation and cattle or lower organic matter deposition, which reduce total nitrogen in rangelands (Ren et al., 2018).

1.3 Biosolids

Many municipalities, through wastewater treatment plants, process sewage residuals into an end-product called biosolids. These treatment plants include a three-step process: large materials are removed; then waste solids are broken down; and lastly, metals, nutrients, and pathogens are removed (Lu et al., 2012; Sullivan et al., 2022). After the initial wastewater processing, the treated wastewater, or sludge, is further processed via thickening and dewatering to create concentrated solids. Stabilization, through aerobic or anaerobic digestion, is the final industrial process and inactivates remaining pathogens in the solids (Lu et al., 2012; Wijesekara et al., 2016). Several options exist for biosolids that are left at the end of these processes: landfilling, incineration, or land application. Land application of biosolids allows for nutrient recycling and energy recovery, whereas landfilling and incineration are primarily disposal methods with disadvantages, like cost and environmental impact (Wijesekara et al., 2016).

After processing, biosolids are comprised of organic matter and nutrients, and are somewhat comparable to traditional manure fertilizer which makes them suitable for land for various reasons (Ministry of Environment of British Columbia, 2016b). In British Columbia, biosolids are typically used as compost, in land reclamation, or for agricultural purposes (Ministry of Environment of British Columbia, 2016a). However, reclamation and agriculture do not necessarily value the same outcomes. Ranchers primarily see value in increased forage biomass and quality, while reclamation practitioners value establishing green plant cover and lowering soil erosion to meet regulatory targets (Ayambire et al., 2021; Drescher and Warriner, 2022) . Additionally, biosolids have been found in the soil at rates of 32% of the original application almost 20 years later, indicating a slow decomposition rate in arid and semi-arid environments (Walton et al., 2001). Therefore, understanding the impacts of biosolids under future climate change and land management is necessary to determine if biosolids are a sustainable choice for stakeholders.

There is significant public hesitation around land applications of biosolids and, while perceptions vary, in general, increased research on the topic is desired by affected communities (Robinson et al., 2012; Whitehouse et al., 2022). In British Columbia, biosolids undergo testing to ensure that they meet regulatory guidelines before they are applied to land (Ministry of Environment of British Columbia, 2016b; *Organic Matter Recycling Regulation*, 2022). Composition of biosolids vary greatly with the individual source of the wastewater (Feizi et al., 2019). Therefore, using high quality biosolids that meet regulatory quality standards and applying best management practices for their use ensures any potential human health risks are minimized. However, negative environmental impacts are harder to predict due to the complexity of ecosystems. This is especially apparent in semi-natural areas, like rangelands, where many ecosystem services are being carried out. Continuing to investigate the broader impacts enables regulators and managers to adapt best management practices for land application of biosolids.

1.3.1 Plant community impacts of biosolids

Although application rate is sometimes substantial, in general, a biosolids application will increase primary production for years after an application (Ploughe et al., 2021). At an application rate of 30 Mg ha^{-1} yr^{-1}, biosolids resulted in a significantly increased cotton yield in a three-year experiment (Tsadilas et al., 2005). Alternatively, an increase in aboveground biomass in a semi-arid grassland was significant 12 years after an application rate as low as 5 Mg ha^{-1} (Sullivan et al., 2006a). Along with significant increases to biomass in general, grass biomass in semi-arid grasslands can more than double that of the control 14 years after a single application (Avery et al., 2019). A large meta-analysis looking at degraded prairies and rangelands, showed no impact on plant diversity or species richness after a biosolids treatment and suggested that plant community impacts may be dependent on the level of site degradation prior to treatment (Ploughe et al., 2021). However, Ploughe et al. (2021) stated a need for further research on the influence of biosolids on exotic plant species, as well as functional group diversity.

The plant community composition is an important metric to examine as species cover can be used to assess species level changes due to biosolids treatments. In a semi-arid grassland in British Columbia, bluebunch wheatgrass (*Pseudoroegneria spicata*) increased in treatment plots; this was described as a positive outcome as it is a late seral species in this ecosystem (Avery et al., 2019). Additionally, the late seral forb pussytoes (*Antennaria* spp.) was extirpated from the system (Avery et al., 2019). In grasslands, lower abundances of pussytoes have been correlated with lower levels, or historical, but not current, disturbance (Delesalle et al., 2009; McLean and Tisdale, 1972). Thus, they are sometimes used as an indicator of degradation in the monitoring of British Columbian grasslands (Delesalle et al., 2009) . In a semi-arid grassland, bluebunch wheatgrass increased after a biosolids application, but in another similar grassland with an annual precipitation less than <400 mm, bluebunch wheatgrass did not increase (Newman et al., 2014). Another study in a semi-arid grassland ecosystem, found that perennial grass cover increased in biosolids plots while forbs and annual grass cover decreased with increasing rates of application (Ippolito et al., 2010). Further, control plots had very similar plant communities whereas plots with any rate of biosolids application had unique and varied communities (Sullivan et al., 2006a). Thus, biosolids can be expected to increase variation in plant community composition, while sometimes favouring late serial species, like bluebunch wheatgrass.

Application of biosolids may also increase the productivity of certain plants over others, an outcome that may not be desirable (Newman et al., 2014). *Poa pratensis*, or Kentucky bluegrass, is an exotic cool season grass, native to Europe and Asia, but that has become well established in many native grasslands across Canada and the United States (Dekeyser et al., 2013). From a ranching perspective, Kentucky bluegrass is an acceptable forage during the cool seasons, but during the summer heat there is the risk of decreasing forage availability in Kentucky bluegrass-dominated pastures because it is less tolerant of extreme temperatures (Toledo et al., 2014). Although there has been limited evidence of negative downstream effects on cattle production during Kentucky bluegrass invasion (Toledo et al., 2014) , invasion can result in landscape level changes and changes to the composition of native plant communities (Dekeyser et al., 2013; Palit et al., 2021; Toledo et al., 2014). Kentucky bluegrass invasion is facilitated by anthropogenically disturbances; management decisions, like grazing, fertilization, or prescribed burning; and genetic advantages attained through turfgrass cultivation (DeKeyser et al., 2015; Gasch et al., 2020; Palit et al., 2021);therefore, it is expected that application of biosolids will increase its abundance (Avery et al., 2019; DeKeyser et al., 2015; Gasch et al., 2020).

Further, Bluebunch wheatgrass is often considered a poor competitor compared to introduced grasses. In the spring, Bluebunch wheatgrass will start producing new growth at temperatures above 7 °C, where Kentucky bluegrass can start growing at temperatures as low as 2 °C, providing better access to soil moisture and nutrients (Evans, 1949; Miller et al., 1986) . Additionally, as an adaptation to water limited systems, mature Bluebunch wheatgrass produces deep roots that increase tolerance to water stress. As a trade off, bluebunch wheatgrass has inconsistent seed production and does not produce rhizomes (Miller et al., 1986). Kentucky bluegrass, although with a shallower root network, aggressively produces a thick layer of biomass through rhizomes that limit other species ability to establish by limiting space and access to light (Borer et al., 2014; DeKeyser et al., 2015; Evans, 1949).

Life histories play an important role in predicting the outcome of biosolids treatments. In mixed C_3-C_4 grasslands, grasses had higher first year survivorship and lived longer compared to forbs, which were both factors that predicted species dominance (Lauenroth and Adler, 2008). Further, under fertilization, grasses produced more seeds per shoot than forbs, indicating a larger reproductive advantage (Scotton and Rossetti, 2021). Once grasses have established, forbs would continue to be at a competitive disadvantage, as grasses like Kentucky bluegrass, will shade out low growing species and have a harder time accessing ground water (Borer et al., 2014; DeKeyser et al., 2015). Therefore, an application of biosolids would be expected to significantly lower forb biomass as grasses are to benefit most from the nutrient influx.

1.3.2 Soil properties affected by biosolids

It is well documented that biosolids applications alter soil properties and that the effects can be in the short (< 5 years) and medium term (5-20 years) after application. Many biosolids studies only examine the soil <15 cm deep, as there is less evidence of significant chemical and physical alterations deeper than 15 cm or that surface applied biosolids persist at lower depths in rangelands (Ippolito et al., 2010; Sullivan et al., 2006b; Walton et al., 2001). Chemical properties, like total carbon and total nitrogen, increased with a single biosolids application and were detectable more than 12 years later in a semi-arid rangeland located in Colorado, USA (Ippolito et al., 2010; Sullivan et al., 2006a, 2006b). However, in a study previously done at OK Ranch at the application rate of 20 Mg ha^{-1}, total carbon and total nitrogen were not statistically different from the control treatments in the top 7.5 cm of soil (Avery et al., 2018). Further, phosphorus and iron were the only plant nutrients found to be elevated in biosolids plots 14 years after application (Avery et al., 2018). Notably, biosolids increased soil organic carbon more than other amendments, like chemical fertilizer and animal manure (Hemmat et al., 2010). This difference may be attributed to large component of organic matter that makes up biosolids.

Repeated biosolids applications at rates of 10, 30, and 50 ton ha^{-1} yr^{-1} have been shown to decrease bulk density of soils compared to unamended soils, but increased application rate did not result in a reciprocal decrease in bulk density. In another study on calcareous soils, the reapplication of biosolids only caused significant decreases in bulk density at the comparatively high application rate of 100 Mg ha^{-1} (Hemmat et al., 2010). The differences between the findings could be due to varying biosolids compositions, as the biosolids used in Tsadilas *et al.* (2005) and the Hemmat *et al.* (2010) were different in pH, soil carbon, and organic matter. Additionally, at lower application rate and single application, there was no difference in the bulk density of soils in a semi-arid grassland, indicating single versus repeated applications may influence outcomes (Avery et al., 2018; Wallace et al., 2009). This stresses the importance of site-specific monitoring, as these studies otherwise took place over similar timelines and overlapping application rates.

Soil moisture was irregularly impacted by a single biosolids application. Biosolids application generally resulted in higher soil water content but not always significantly or consistent with application rate or season (Avery et al., 2018; Wallace et al., 2009). An application of biosolids at a rate of at least 20 Mg ha $^{-1}$ increased the mean weight diameter of stable aggregates 5, 9 and 14 years after a single application (Avery et al., 2018; Wallace et al., 2016a, 2009). In larger stable aggregates, the C:N ratio was significantly lower than control treatments years after biosolids application (Wallace et al., 2016b). Further, there was no statistical difference in aggregate mean weight diameter between a biosolids application rate of 60 Mg ha $^{-1}$ and 20 Mg ha $^{-1}$ (Wallace et al., 2016a, 2009). In conclusion, changes to soil physical properties are detectable in the decade after biosolids application that vary in frequency and application rate (Tsadilas et al., 2005; Wallace et al., 2016a).

1.4 Biosolids for drought mitigation in rangelands

The use of biosolids for drought mitigation is not well understood, but it has been suggested that biosolids may increase drought tolerance in grasslands (Boudjabi et al., 2019; Chang et al., 2014; Zhang et al., 2009). Although, the application of biosolids yields different results than the application of traditional fertilizer, it is worth noting that grasslands that were fertilized with nitrogen had higher drought resistance, in the way of higher vegetation productivity, than unfertilized grassland plots (Carlsson et al., 2017; Klaus et al., 2016). On this note, and like the increased drought tolerance seen with nitrogen fertilization, biosolids application increased drought tolerance as measured by reduced leaf wilting and increased proline concentration in potted *Poa pratensis* (Chang et al., 2014). In a greenhouse study, grasses grown in soils with added biosolids showed greater drought tolerance (e.g., lower percentage leaf wilting) than grasses grown with chemical fertilizer control (Zhang et al., 2009). Further, root growth under drought was increased with a biosolids application compared to regular watering regimes and without a biosolids treatment (Chang et al., 2014). Similarly, wheat grown in a soil-biosolids mixture was morphologically and physiologically more adapted for drought than wheat grown in a soil-chemical fertilizer mixture (Boudjabi et al., 2019), suggesting that, although chemical fertilizers may increase drought tolerance, the composition of biosolids, aside from the increased nutrients, increases drought tolerance even more.

It is important to examine the interaction of biosolids and other factors in field experiments. As an example, if biosolids result in increased drought tolerance in *P. pratensis*, land managers may have a harder time meeting reclamation targets. Grazing may provide a way to manage the dominance of *P. pratensis* so that species richness and community composition can be preserved after a biosolids application during drought (Gasch et al., 2020; Otfinowski et al., 2017); however, in the semi-arid grasslands of southern British Columbia, bluebunch wheatgrass is more susceptible to grazing than Kentucky bluegrass (Jones and Nielson, 1997)(Jones and Nielson, 1997). There are likely additional ways in which drought, grazing, and application of biosolids will interact to affect these plant communities.

Species are classified as r- or K-selected species by their life histories (Hamilton, 1968; MacArthur and Wilson, 1967); r-selected species reach maturity quickly and allocate most resources towards reproduction, whereas K-selected species mature slower, allocating fewer resources to prompt reproduction (Hamilton, 1968; Parry, 1981). Species can also be categorized based on the C-S-R model, which distinguishes species as "competitors", "stress tolerators", or "ruderals"(Grime, 1988). Competitors are most like K-selected species and ruderals are like r-selected species; stress tolerators are characterised by their ability to access resources in high stress environments, like water-limited systems (Grime, 1988; Wilson and Lee, 2000). When considering r/K selected species, Kentucky bluegrass has traits most like an r-selected species, as it grows quickly and reproduces earlier than other grass species (Evans, 1949; Palit et al., 2021). Yet, under the more complex model of C-S-R, Kentucky bluegrass seems to be more of a competitor and often dominates a community (DeKeyser et al., 2015). Contrastingly, Bluebunch wheatgrass is most like a K-selected species and a stress tolerator, where it established deep root networks that allow productivity to be maintained in times of low precipitation (Miller et al., 1986).

The fast growing and early reproduction of Kentucky bluegrass is likely to be favoured under biosolids. Kentucky bluegrass can aggressively spread and is likely to quickly utilize any influxes of nutrients (Evans, 1949). As a result of this, a large amount of biomass accumulates as thatch, which in turn shades out seedlings and disrupts typical moisture dynamics, allowing Kentucky bluegrass to become the dominant plant species (Gasch et al., 2020). This dynamic is likely to exist until a disturbance occurs that allows other species to establish, and effectively slows down the feedback loop (Connell and Slatyer, 1977). As previously discussed, grazing, a type of disturbance, had been shown to reduce competitive exclusion of strong competitors, like Kentucky bluegrass, which may allow for other species, like less aggressive native grasses, to establish again(Otfinowski et al., 2017; Toledo et al., 2014). Further, under drought, Bluebunch wheatgrass is likely to have an advantage being a K-selected species and stress tolerator, as it spends more resources establishing deep root systems (Miller et al., 1986).

1.5 Objectives and Hypotheses

The aim of this research was to assess the interaction of drought, grazing, and biosolids on ecosystem health 19 years after a single application of biosolids in a semi-arid grassland. At the time, Ok Ranch had experienced historical heavy grazing which degraded the ecosystem and biosolids were applied as a restoration technique to reverse this damage (Sylvis, 2016). This study focused on grassland plant community structure, species composition, and soil health as these are commonly used indicators of grassland status in British Columbia (Grasslands Conservation Council of British Columbia, 2017). The goal of this research was to make inferences about the effects of drought, grazing, and biosolids on rangelands that can be used to inform land management decisions. Further, this work can be used to generate hypotheses regarding the sustainability of biosolids and ranching that would help guide future biosolids research in British Columbia. Six hypotheses were made:

1) A biosolids application will increase overall plant biomass, while altering the plant community through a reduction in forb biomass.
2) A biosolids application will increase soil organic matter but result in lower soil bulk density.
3) one year of drought will reduce plant biomass and diversity, soil water content, total soil carbon, and bulk density.
4) A biosolids application will mitigate some drought effects, like decreased plant biomass and total soil carbon (Figure 1A), and exacerbate other drought effects, like reduced diversity (Figure 1B).
5) Grazing will increase plant richness but will lower above and belowground plant biomass. Under a biosolids application, grazing will mitigate changes to diversity in biosolids applied plots (Figure 1C).
6) Grazing will increase soil bulk density.

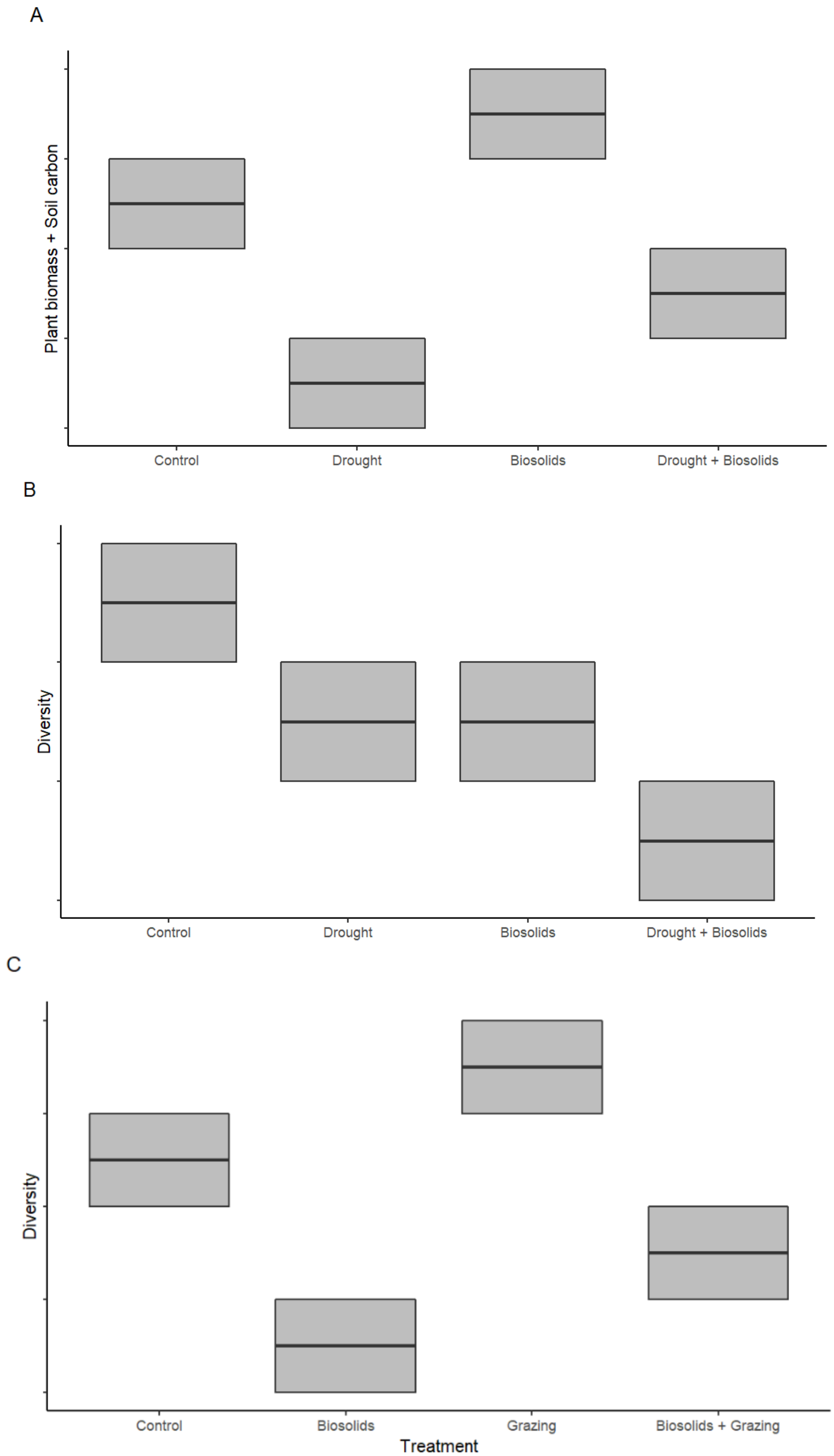

Figure 1 Qualitative visualization of effect trends for hypotheses 3-5 at OK Ranch based on drought (rainout shelters), grazing, or biosolids treatments.

2 Methods

2.1 Site description and experimental design

This work was done on a working cattle ranch, OK Ranch, near Jesmond B.C. The site is 1200 m above sea level, located in the Very Dry Mild Interior Douglas-fir biogeoclimatic zone (Wikeem and Wikeem, 2004). The mean annual precipitation is 401 mm, with about half of this falling in the spring and summer season, and the mean temperature is 3.0 °C (Newman et al., 2014). The soil type is Dark Brown Chernozem and the soil texture is loamy (Avery et al., 2018; Newman et al., 2014).

Late stages of the plant community include *Pseudoroegneria spicata*, bluebunch wheatgrass and *Achnatherum richardsonii*, Richardson's needlegrass or spreading needlegrass (Wikeem and Wikeem, 2004). *Koeleria macrantha,* junegrass and naturalized *Poa pratensis*, Kentucky bluegrass, are commonly found in grasslands in this area due to degradation from historical heavy grazing (Wikeem and Wikeem, 2004). *Hesperostia comata*, needle-and-thread grass, is also commonly found in middle seral stages after degradation (Wikeem and Wikeem, 2004). Common forbs include *Antennaria spp.*, pussytoes; *Tragopogon dubius*, yellow salsify; and Artemisia *frigida*, pasture sage depending on level of ecosystem disturbance (Wikeem and Wikeem, 2004). There are no established trees or shrubs greater than 1 m within the study sites. All plants surveyed were C3 plants. From this point on, the above species will be referred to by the common names provided.

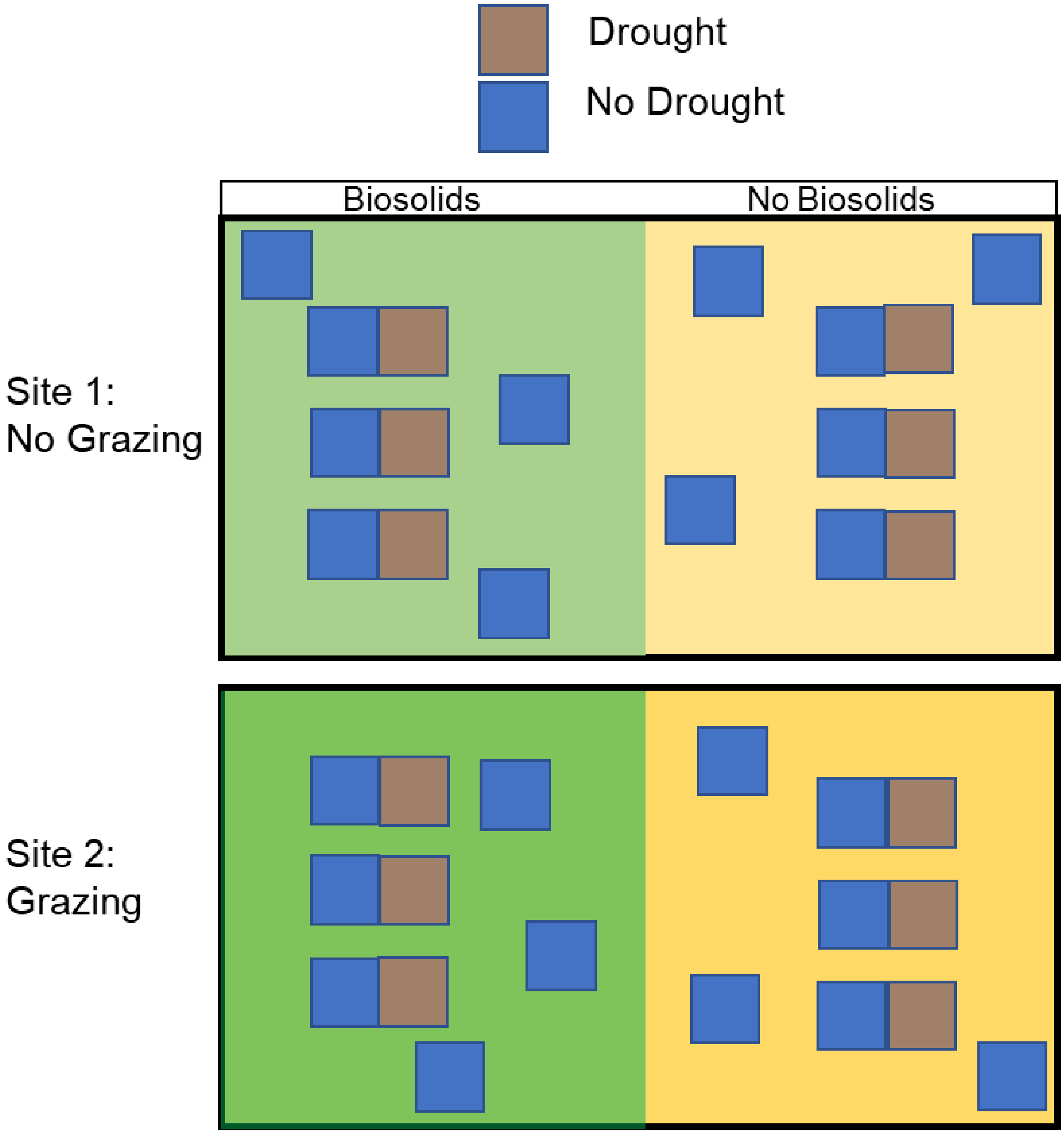

Figure 2 Treatment layout at OK Ranch. The two research blocks are outlined in black and labeled as "Site 1" and "Site 2". The green background represents the 2002 biosolids application within Site 1 and Site 2. The brown squares represent rainout shelters drought plots and blue squares represent ambient control plots.

In 2002, two 70 m x 60 m research blocks were created with barbwire fencing (Figure 2; see Newman et al., 2014 for site description). Then, in a split-block design, each block had biosolids surface spread on one half of the block at a rate of 20 Mg ha^{-1}. As the biosolids were applied via surface spreading using large machinery, it limited the ability to replicate and size these plots. Ever since construction in 2002, Site 1 was used as an exclosure with cattle excluded through a closed gate; Site 2 had an open gate and was periodically grazed based on regular ranching activities. This resulted in an "Exclosure" and "Open" treatments for the grazed factor. The fencing and gate still allowed for any small herbivores and ungulates to access the sites.

In June of 2020, twelve 3 m x 3 m rainout shelters were constructed across the two blocks, along with twelve paired ambient condition plots without rainout shelters. The total of 24 rainout shelters was the maximum considered feasible based on material and time restrictions. In August 2021, an additional 12 randomized ambient condition plots were sampled for a total of 36 plots (Figure 2). The rainout shelters were constructed using a 2.5 m x 2.5 m wood frame with a roof of flexible polyethylene (Figure 3). The roofs of the rainout shelters were removed in October 2020 for the winter and were replaced in May 2021to prevent any snow damage to the structures or plastic. Since the construction of the rainout shelters, no cattle grazing has occurred in Site 2 to protect the shelters and equipment.

Figure 3 Rainout shelters that were constructed in June 2020. The horizontal roof was the only piece deconstructed during the winter months. The gutter and hose directed runoff water away from the plot area.

From July 2020 to August 2021, soil moisture and temperature data were collected every 30 minutes in one rainout shelter and one control plot in each subsite, using a HOBO USB Micro Station Data Logger (Onset Computer Corporation, Bourne, Massachusetts). These sensors were placed at 5 cm below the soil surface, away from the edges of the plots. This allowed for the monitoring of the experimental drought to detect any difference in soil moisture between treatments. Further, monitoring the temperature of the plots helped to detect any microclimates created by the rainout shelters (Kundel et al., 2018). Unfortunately, several of the sensors became corrupted over the course of the experiment but two units had enough salvageable data to show trends from August 2020-August 2021.

2.2 Vegetation sampling

Vegetation sampling took place in June 2020, prior to installation of rainout shelters, and in August 2021, approximately one year after the rainout shelter construction. Sampling occurred randomly in 24 plots in 2020 and again, randomly within those same plots in 2021. In 2021, an additional 12 plots were sampled throughout Site 1 and Site 2 to increase the sample size and area sampled (Figure 2). Plant species and community composition, and diversity were determined. Firstly, percent species cover of vegetation, to be used for examining the coverage of specific species of interest, was recorded using a 0.5 m x 0.5 m quadrat within each plot. Additionally, but only in 2021, within the quadrats, all biomass was clipped to the soil surface and then taken to the laboratory to be dried and weighed. Prior to this, all standing green biomass separated into functional groups (forbs and grasses) before being processed. Litter was collected separately by hand raking to the cryptogram layer. Cryptograms were present and accounted for in the percent cover estimates but not collected for biomass estimation due to the inability to separate them from the soil. Biomass samples, sorted by forbs, grasses, and litter, were dried at 60 °C for 24 hours before being weighed.

2.3 Soil sampling

Soil sampling occurred at the same time as the vegetation sampling in August of 2021. Soils were not collected in 2020. Samples were not taken within 0.5 m from the edge of all plots to avoid any edge effects from the rainout shelters. Detached litter and any vegetation crowns were removed from measurement areas and were not included in any of the soil samples. There were no biosolids that were visually identified at the sampling locations. For the belowground plant biomass estimates, a 10 cm diameter soil core was used to a depth of 20 cm for soil collection once per plot. The samples were sieved using a 2 mm sieve and then the remaining roots were dried for 24 hours at 60 °C and weighed.

Other soil tests were conducted using sieved soil from the top 10 cm of soil, due to the lack of evidence for biosolids application effects at greater depths (see Chapter 1: Biosolids). Approximately 30 g of soil was needed from each plot for the total carbon/nitrogen (TCN) and soil organic matter (SOM) analysis. This was taken from three places within a plot and pooled to account for soil heterogeneity. The TCN samples were sent to the Analytical Chemistry Services Laboratory (NRL) through the British Columbia Ministry of Environment and Climate Change Strategy in Victoria, BC, for elemental analysis. For the SOM analysis, the loss on ignition method was used. Firstly, 10 g of soil was dried at 65 °C for 48 hours and weighed. After, the samples were placed in a muffle furnace at 550 °C for 5 hours to remove the organic matter and then weighed again to estimate the SOM content (Nelson and Sommers, 1996)(Nelson and Sommers, 1996).

Soil pH, bulk density, and water content measurements were also taken once per plot. The pH was measured using a pH probe and a 10:2 volume to mass, water to soil dilution. Bulk density within the top 10 cm was taken using a 10 cm deep and 1cm diameter core. Soil water content was determined gravimetrically. For the aforementioned analyses, samples were dried at 65 °C for 48 hours.

2.4 Data analysis

Non-metric multidimensional scaling (NMDS), via the METAMDS function, was used to visualize plant cover data for the sampling years 2020 and 2021. A permutational multivariate analysis of variance (PERMANOVA) was done using the ADONIS function. Both functions were from the VEGAN package in R 4.0.4 (Oksanen et al., 2020; R Core Team, 2022). Due to difference in sampling time, there was no direct statistical comparison between 2020 and 2021 plant community data (See Chapter 5). Shannon and Simpson diversity indices were also calculated using the DIVERSITY function from the VEGAN package (Oksanen et al., 2020). As there are several ways of quantifying diversity, using more than one diversity indices, like one that emphasizes rare species and one that emphasises dominance, is best practice and provides the most robust analysis (Morris et al., 2014).

A three-way analysis of variance (ANOVA) was then used to examine the variation between the drought, grazing, and biosolids treatments for all variables except for percent cover and 2020 measurements. A two-way analysis of variance (ANOVA) was used for 2020 data as these data were taken prior to construction of the rainout shelters. Drought, grazing and biosolids were all considered fixed factors, each with two levels; drought= rainout/ambient control; grazing= exclosure/open; biosolids = biosolid/no biosolid. The most complete model was used for analysis (drought * grazing * biosolids) that included all three-way interactions. When a significant interaction was identified, then a Tukey Honest Significant Difference test was performed using the TUKEYHSD function. This was done using the AOV function from the STATS package in R 4.0.4 (R Core Team, 2022). Residuals were checked for normality using Q-Q plots.

Due to the desire to examine legacy effects of biosolids at this specific site, the grazing and biosolids lacked true replication or independence. The lack of replicates of the grazing treatment limits the conclusions that can be drawn from this treatment, as variation may be attributed to site effects. Secondly, the biosolids treatment was pseudoreplicated, as several measurements were taken from the same geographic application. Despite this, observations from this research can be used to inform future hypotheses of complementary research (Davies and Gray, 2015).

3 Results

3.1 Environmental data

Based on environmental data, there were several precipitation events when the rainout shelters were in place (Figure 4). Temperature sensors recorded a variation of less than one degree across the entire experiment; however, when the rainout shelters were in place, the drought plots recorded a maximum of 2 °C warmer (Figure 5). Across the sampling dates, the volumetric water content in rainout shelter plots was 58% lower in biosolids plots and 50% lower in no biosolids plots from June 2020 to October 2021 (Figure 5).

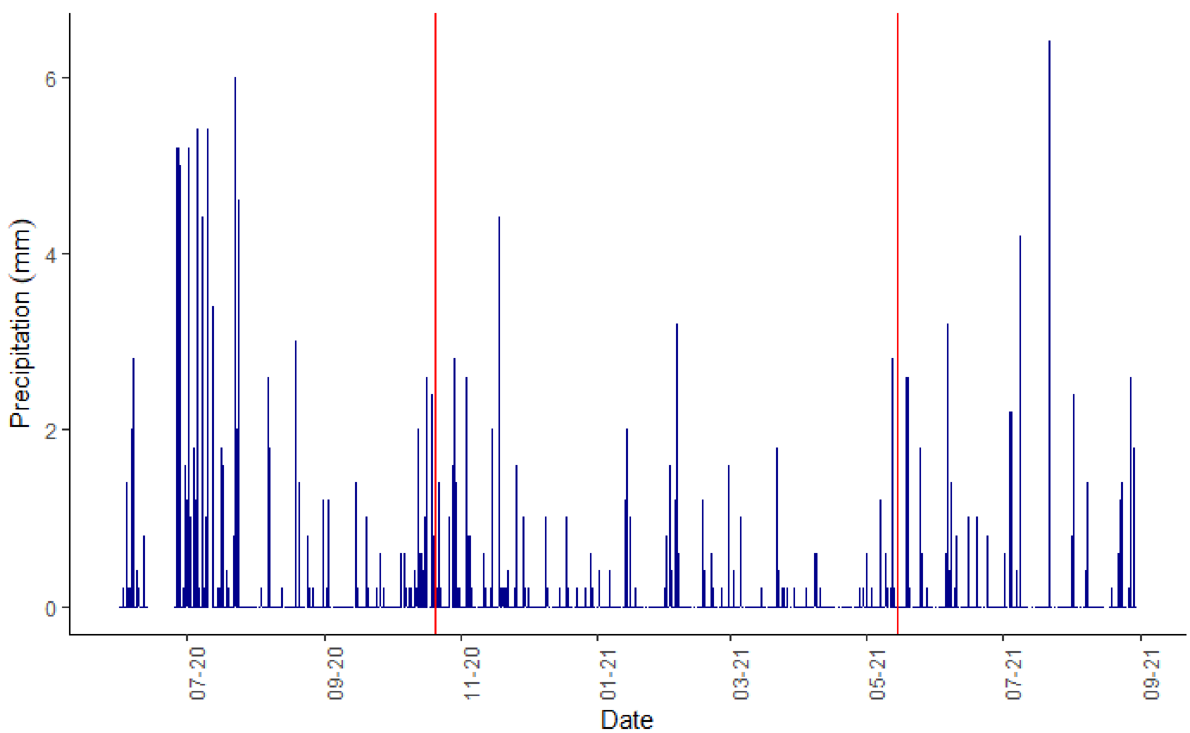

Figure 4 Precipitation (mm) recordings from the FLRNO Meadow Lake weather station, 29 km northeast of OK Ranch from June 2020 to August 2021. The redlines indicate the removal (October 2020) and subsequent replacement (May 2021) of the rainout shelters for the winter season.

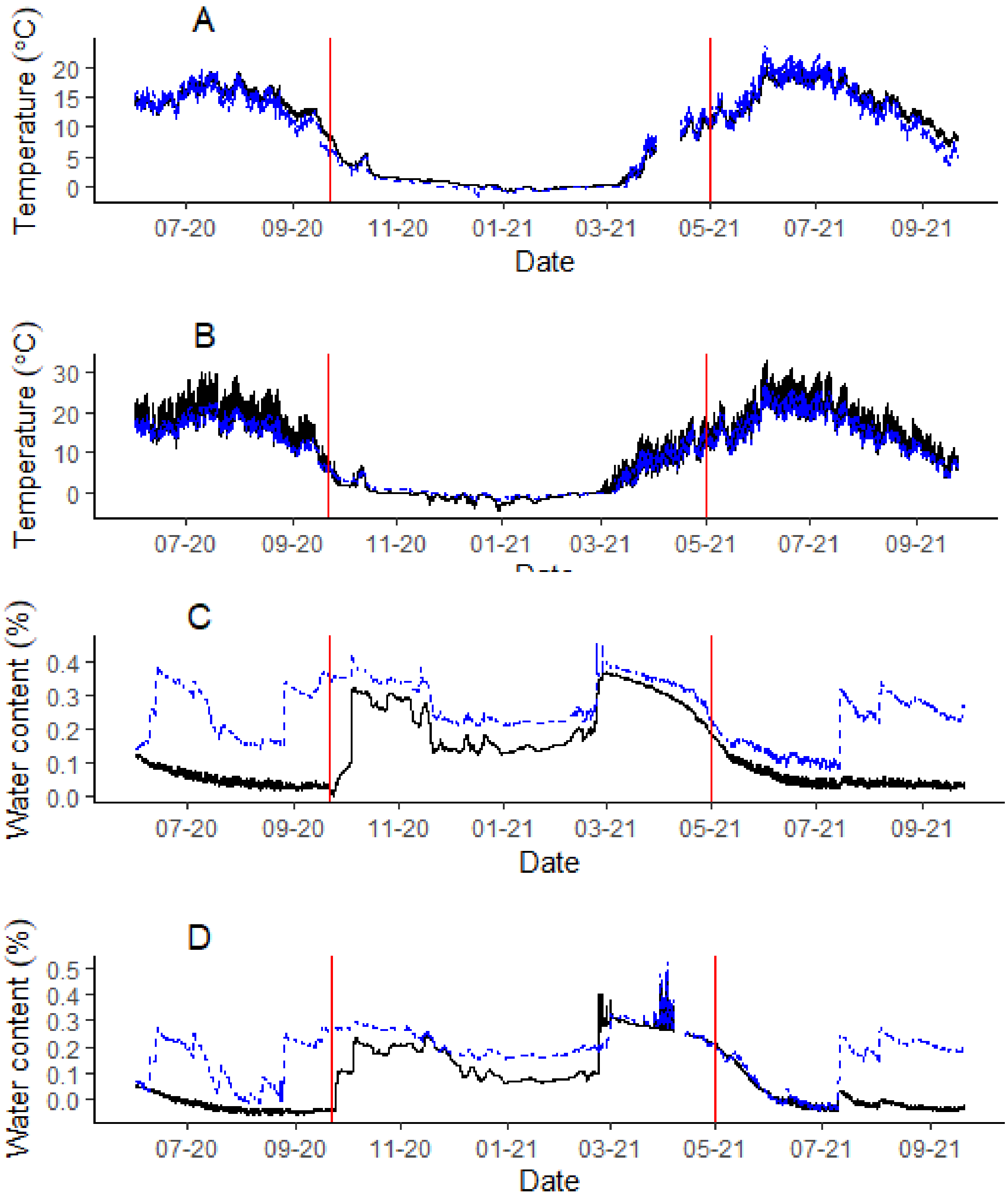

Figure 5 Soil conditions recorded by four Onset HOBO data loggers from June 2020 to October 2021 every 30 minutes. Plots (A) and (C) show soil temperature and water content with biosolids. Plots (B) and (D) show soil and water content without biosolids. Dashed, blue lines indicate ambient conditions, where solid, black lines are experimental drought conditions. Between the red lines indicate when the rainout shelters were removed for the winter.

3.2 Plant community responses

3.2.1 Vegetation biomass

Biomass was measured only in 2021. At that time, the mean aboveground biomass was approximately 3.5x higher with a biosolids application, compared to plots without biosolids (Table 1, Figure 6). The grazing exclosure site had a higher mean aboveground biomass than the open site (Table 1, Figure 6). There was a significant interaction between grazing and biosolids, where in plots with biosolids, no grazing resulted in a significantly higher aboveground biomass than in open grazing areas (Figure 6). By contrast, without biosolids, there was no difference between the means of in plots that were under grazing exclosure or open grazing (Figure 6). Rainout shelters had no significant effects on aboveground biomass.

In biosolids plots, the mean belowground biomass was higher than in the control plots (Table 1, Figure 7). Rainout shelters lowered mean belowground biomass to less than half of ambient conditions plots (Table 1, Figure 7). Grazing and interactions were not significant for belowground biomass.

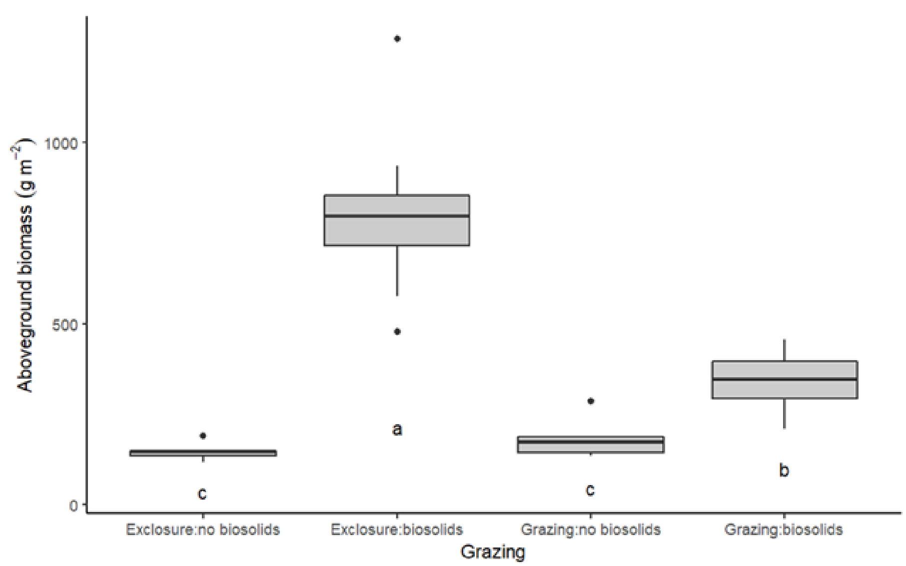

Figure 6 Aboveground biomass (g m^{-2}) showing the significant interaction between the grazing and biosolids treatments taken during 2021 (n=9). Lower case letters indicate significantly different means according to Tukey's Honest Significant Difference test. The shaded area of each box represents the interquartile range while the line within each box represents the medium of

sample. The lines extending from each box indicate values less than 1.5 times the interquartile range but greater than the interquartile range itself, and any outliers represent values less than 3 times the interquartile range.

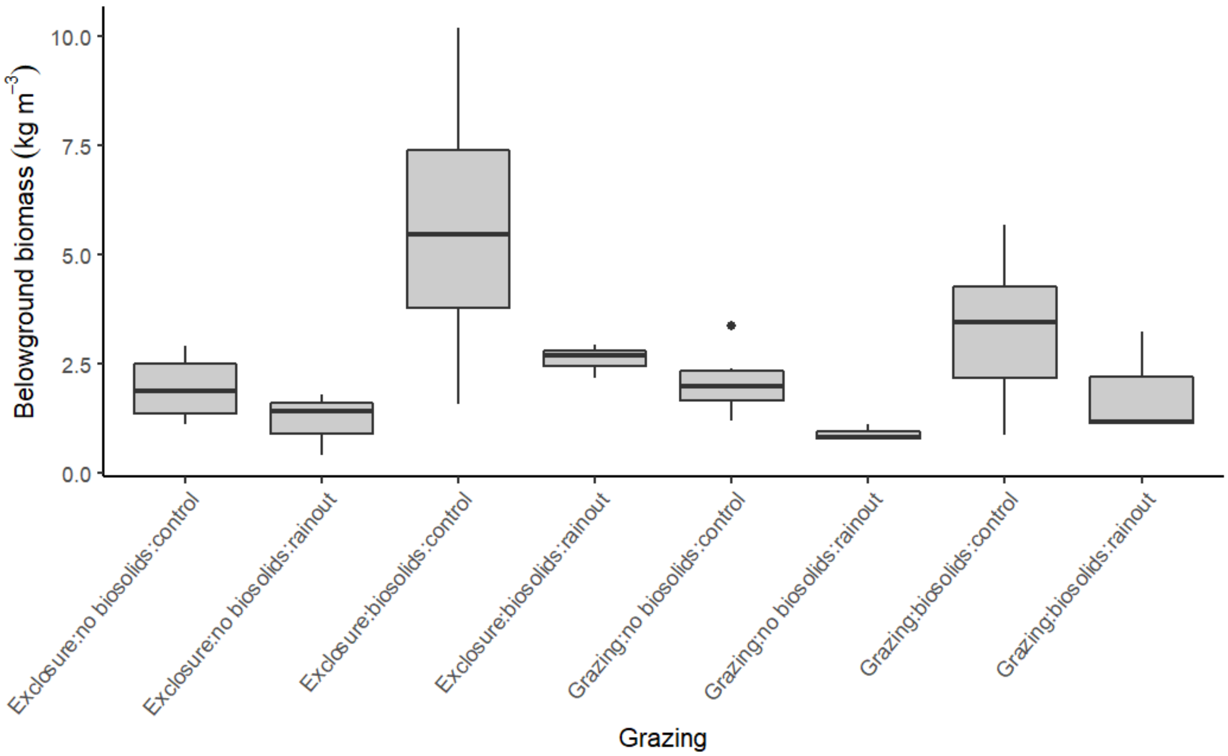

Figure 7 Belowground biomass (kg m^{-3}) for grazing, biosolids, and rainout treatments taken during 2021 (n=3). Biosolids had a significant effect of $p<0.001$ and rainout shelters had significant effect of $p<0.009$.

Table 1 Three-way ANOVA results for aboveground and belowground biomass, sampled in August 2021, by factor, grazing, biosolid, and rainout. (n = 9). Values with significant p-values are denoted with asterisks (*p<0.05; **p<0.01, ***p<0.00

	Aboveground Biomass					Belowground biomass				
Factor	**df**	**Sum Sq**	**Mean Sq**	**F value**	**Pr(>F)**	**df**	**Sum Sq**	**Mean Sq**	**F value**	**Pr(>F)**
grazing	1	0.179	0.179	15.348	<0.001***	1	18.619	18.619	2.886	0.100
biosolid	1	2.270	2.270	194.671	<0.001***	1	91.043	91.043	14.111	<0.001***
rainout	1	7.85E-05	0.000	0.007	0.935	1	50.921	50.921	7.893	0.009**
grazing:biosolid	1	0.456	0.456	39.090	<0.001***	1	18.447	18.447	2.859	0.102
grazing:rainout	1	0. 9.11E-05	9.11E-05	0.008	0.930	1	1.665	1.665	0.258	0.615
biosolid:rainout	1	0. 4.15E-06	4.15E-06	0.0003	0.985	1	8.127	8.127	1.260	0.271
grazing:biosolid:rainout	1	0.001	0.001	0.114	0.738	1	5.265	5.265	0.816	0.374
Residuals	28	0.326	0.012	NA	NA	28	180.649	6.452	NA	NA

3.2.2 Litter biomass and functional groups

Aboveground litter, grass, and forb biomass was only sampled in 2021. Aboveground litter biomass was higher in plots with biosolids than without (Table 2, Figure 8). Further, the open grazing treatment had lower mean litter biomass than the grazing exclosure treatment (Table 2, Figure 8). Like aboveground biomass, the pattern of effect was similar for grazing and biosolids on litter biomass. The interaction between grazing and biosolids was significant, where in plots with biosolids, grazing exclosure resulted in significantly higher litter biomass than in open grazed plots (Figure 8). In plots without biosolids, there was no significant difference between the mean litter biomass of plots that were under grazing exclosure or open grazing (Figure 8). Again, with biosolids, there was no difference between in means between the two levels of the grazing treatment (Table 2, Figure 8). The 2021 sampling had 227% ($p = 0.00004$) more litter coverage than the 2020 year.

Aboveground grass biomass was decreased by rainout shelters, while increased by biosolids (Table 2, Figure 9). Additionally, forbs were significantly decreased in plots with biosolids (Table 2, Figure 10). No other factors were significant for grass or forb biomass. Several of the biosolids plots had no forb biomass.

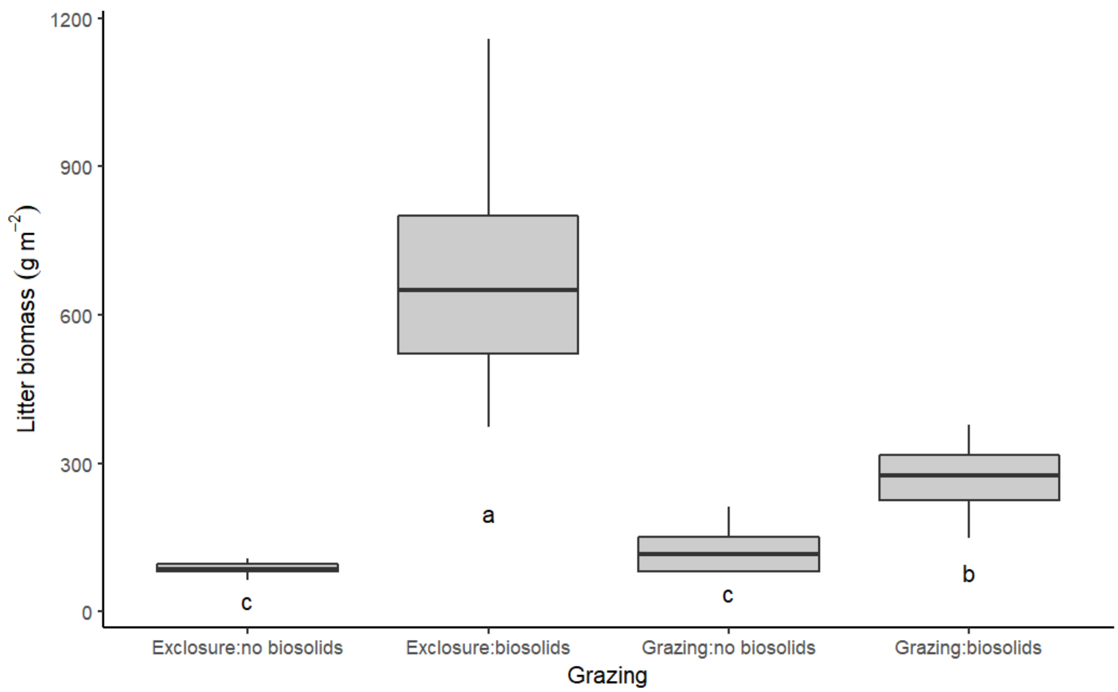

Figure 8 Litter biomass (g m^{-2}) showing the significant interaction between the grazing and biosolids treatments taken during 2021 (n=9). Values with different lowercase letters indicate significantly different means according to Tukey's Honest Significant Difference test.

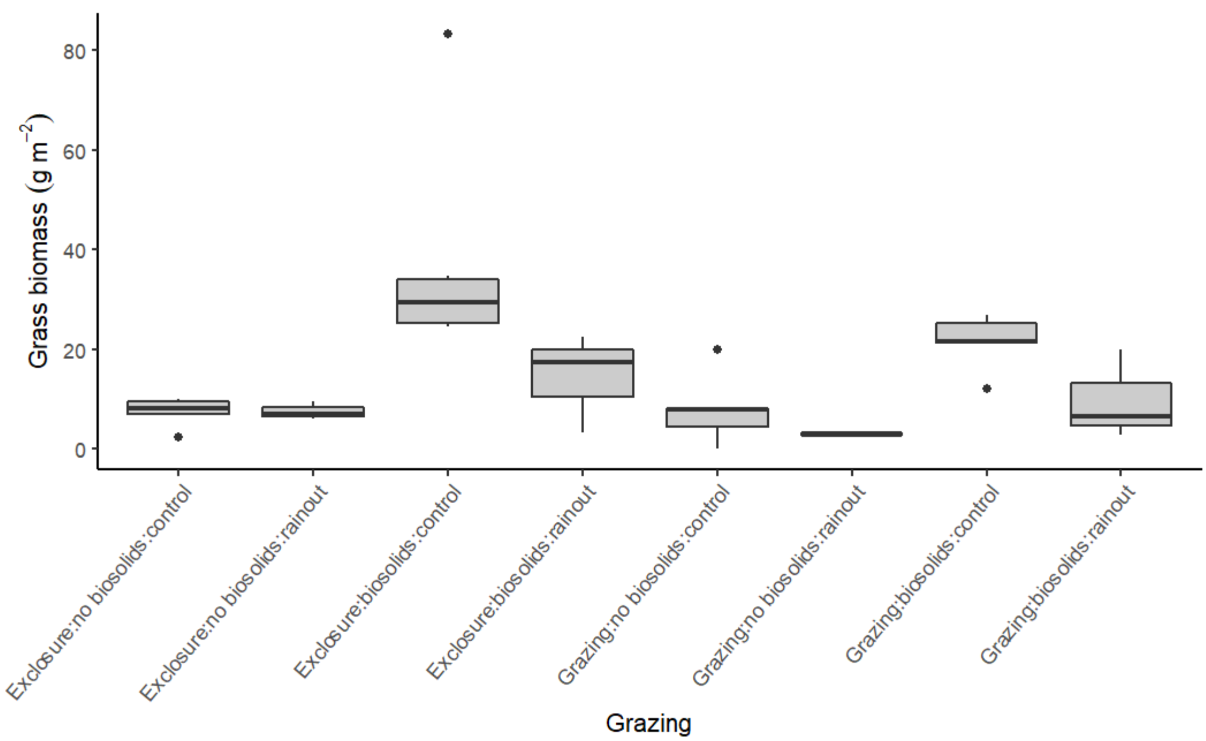

Figure 9 Grass aboveground biomass (g m^{-2}) for grazing, biosolids, and rainout shelter treatments from 2021 sampling (n = 3). Grazing had a significant effect at $p<0.011$ and the biosolid treatment had significant effect at $p<0.001$.

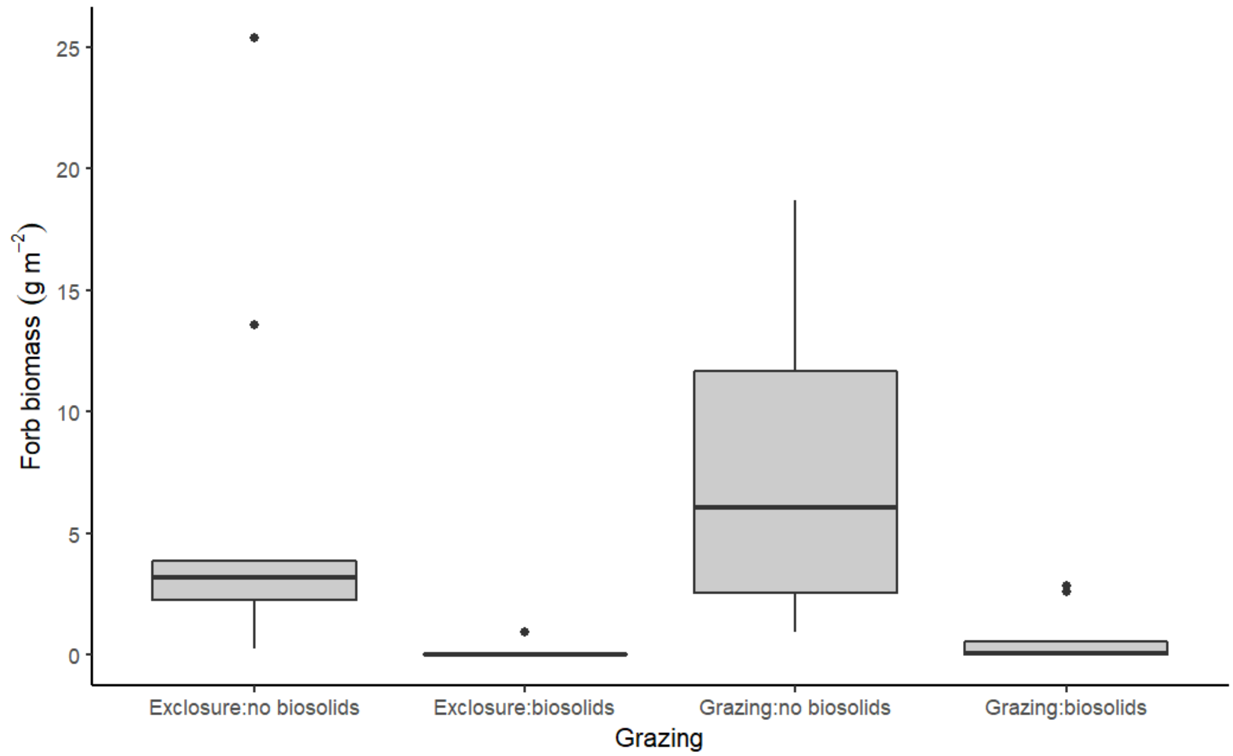

Figure 10 Forb above-ground biomass (g m^{-2}) for grazing and biosolids treatments from 2021 sampling (n = 9). Biosolids had a significant main effect at $p<0.001$.

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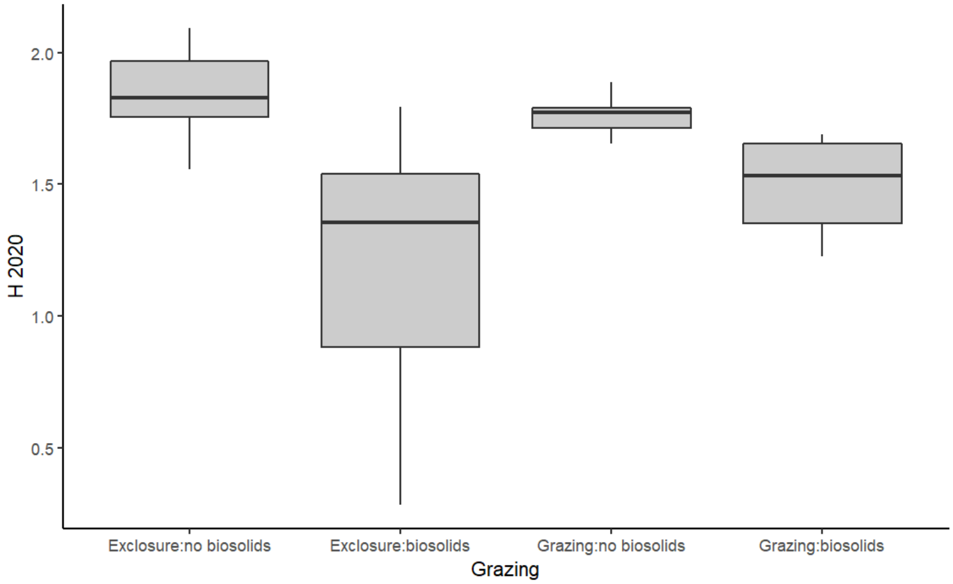
Table 2 Three-way ANOVA results for litter, grass, and forb biomass (g m^{-2}) for grazing, biosolids, and rainout shelter treatments taken during 2021 (n=9). Values with significant p-values are denoted with asterisks (*p<0.05; **p<0.01, ***p<0.001).

	Litter biomass					Grass biomass					Forb biomass				
Factor	**Df**	**Sum Sq**	**Mean Sq**	**F value**	**Pr(>F)**	**Df**	**Sum Sq**	**Mean Sq**	**F value**	**Pr(>F)**	**Df**	**Sum Sq**	**Mean Sq**	**F value**	**Pr(>F)**
grazing	1	0.180	0.180	11.295	0.002**	1	0.2916	0.2916	3.9783	0.056	1	0.203	0.203	2.133	0.155
biosolid	1	3.195	3.195	200.571	<0.001***	1	2.0329	2.0329	27.7323	<0.001***	1	3.602	3.602	37.847	<0.001***
rainout	1	0.019	0.019	1.223	0.278	1	0.5416	0.5416	7.3889	0.011*	1	0.073	0.073	0.770	0.388
grazing:biosolid	1	0.598	0.598	37.538	<0.001***	1	0.0005	0.0005	0.0064	0.937	1	0.003	0.003	0.031	0.861
grazing:rainout	1	0.029	0.029	1.832	0.187	1	0.0169	0.0169	0.2303	0.635	1	0.106	0.106	1.119	0.299
biosolid:rainout	1	0.005	0.005	0.331	0.570	1	0.2348	0.2348	3.2036	0.084	1	0.000	0.000	0.005	0.944
grazing:biosolid:rainout	1	0.010	0.010	0.598	0.446	1	0.0330	0.0330	0.4499	0.508	1	0.025	0.025	0.262	0.612
Residuals	28	0.446	0.016	NA	NA	28	2.0525	0.0733	NA	NA	28	2.665	0.095	NA	NA

3.2.3 Diversity

Based on percent cover, plots with a biosolids application had lower Shannon and Simpsons diversity than plots without biosolids for both 2020 and 2021 sampling dates (Tables 3 and 4, Figure 11 and 12). In the 2021 sampling year, but not in 2020, grazing was also a significant factor, with grazing exclusion plots having lower Shannon diversity and inverse Simpson diversity compared to the open grazing treatment (Table 4, Figure 12). There were no significant interactions found between factors or with the rainout treatment.



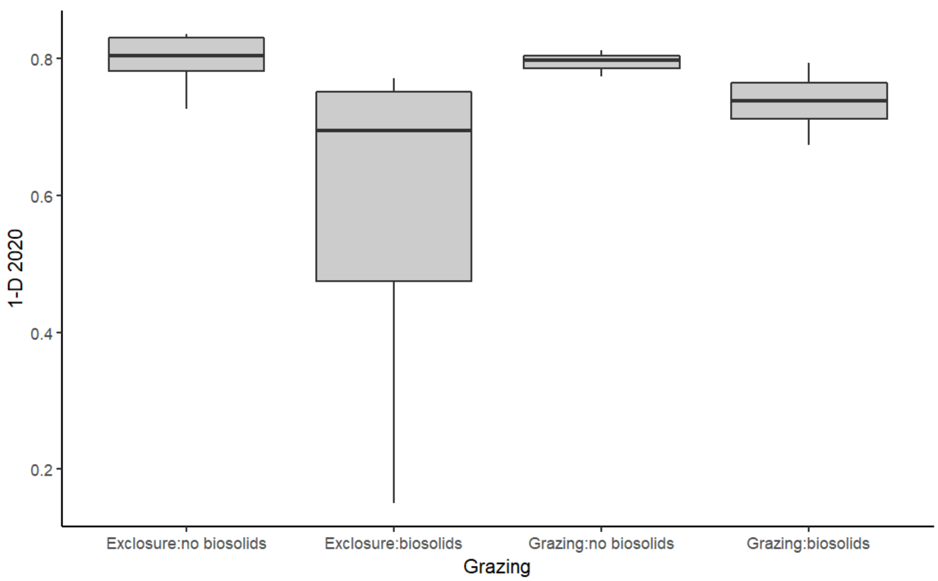


Figure 11 Shannon Diversity (H') and Simpson Diversity (D) indices for the biosolids treatment for both 2020 sampling of cover data (n=9). Biosolids had a significant effect on both Shannon ($p<0.002$) and Simpson diversity ($p<0.017$) .

Table 3 Two-way ANOVA results for Shannon Diversity (H') and Simpson Diversity (D for grazing and biosolids treatments taken during 2020 (n=9). Values with significant p-values are denoted with asterisks (*p<0.05; **p<0.01, ***p<0.001).

	Shannon diversity (H') 2020					Simpson diversity (D) 2020				
Factor	**Df**	**Sum Sq**	**Mean Sq**	**F value**	**Pr(>F)**	**Df**	**Sum Sq**	**Mean Sq**	**F value**	**Pr(>F)**
grazing	1	0.080	0.080	0.796	0.383	1	0.036	0.036	2.123	0.161
biosolid	1	1.282	1.282	12.698	0.002**	1	0.113	0.113	6.784	0.017*
grazing:biosolid	1	0.225	0.225	2.233	0.151	1	0.038	0.038	2.252	0.149
Residuals	20	2.019	0.101	NA	NA	20	0.335	0.017	NA	NA

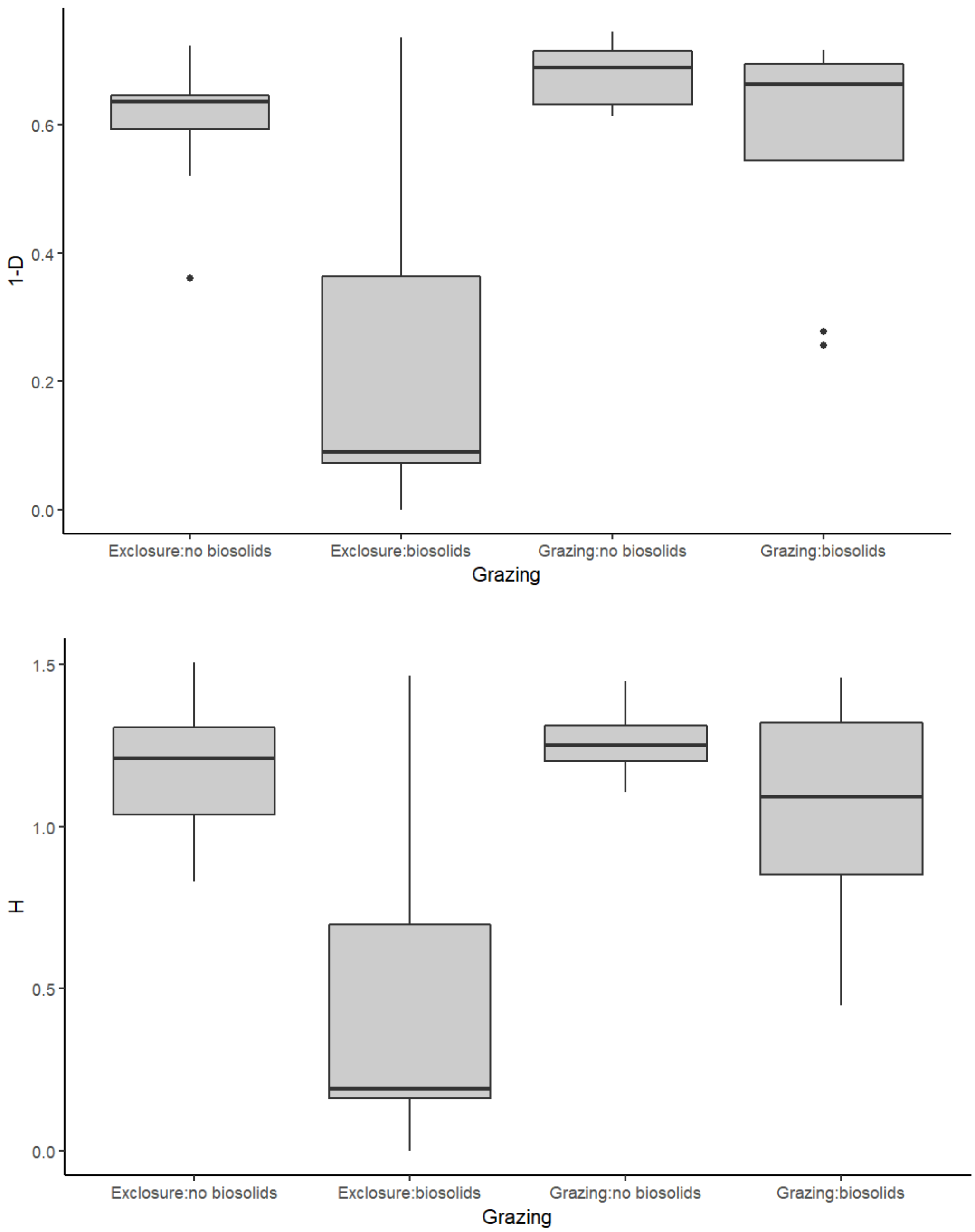


Figure 12 Shannon Diversity (H') and Simpson Diversity (D) indices for the biosolids treatment for both 2021 sampling of cover data (n=9). Grazing and biosolids had significant main effects on Shannon ($p<0.021$; $p<0.001$) and Simpson diversity ($p<0.005$; $p<0.001$).

Table 4 Three-way ANOVA results for Shannon Diversity (H') and Simpson Diversity (D for grazing, biosolids, and rainout shelter treatments taken during 2021 (n=9). Values with significant p-values are denoted with asterisks (*p<0.05; **p<0.01, ***p<0.001).

	H diversity					D Diversity				
Factor	**Df**	**Sum Sq**	**Mean Sq**	**F value**	**Pr(>F)**	**Df**	**Sum Sq**	**Mean Sq**	**F value**	**Pr(>F)**
grazing	1	0.820	0.820	6.022	0.021*	1	0.323	0.323	9.196	0.005***
biosolid	1	1.883	1.883	13.823	<0.001***	1	0.470	0.470	13.371	<0.001***
rainout	1	0.040	0.040	0.293	0.593	1	0.009	0.009	0.269	0.608
grazing:biosolid	1	0.468	0.468	3.434	0.074	1	0.122	0.122	3.459	0.073
grazing:rainout	1	0.085	0.085	0.628	0.435	1	0.041	0.041	1.172	0.288
biosolid:rainout	1	0.045	0.045	0.330	0.570	1	0.000	0.000	0.008	0.928
grazing:biosolid:rainout	1	0.075	0.075	0.552	0.464	1	0.026	0.026	0.740	0.397
Residuals	28	3.813	0.136	NA	NA	28	0.984	0.035	NA	NA

The application of biosolids resulted in significantly different plant communities in 2020, but not 2021, based on species cover measurements versus those without biosolids (Tables 5 and 6, Figure 13). Further, grazing was a significant factor in both sampling years. In 2021 only, there was a significant interaction between the grazing and rainout treatments, where rainout shelters within grazing exclosures had distinct plant communities compared to other treatment combinations (Figure 14).

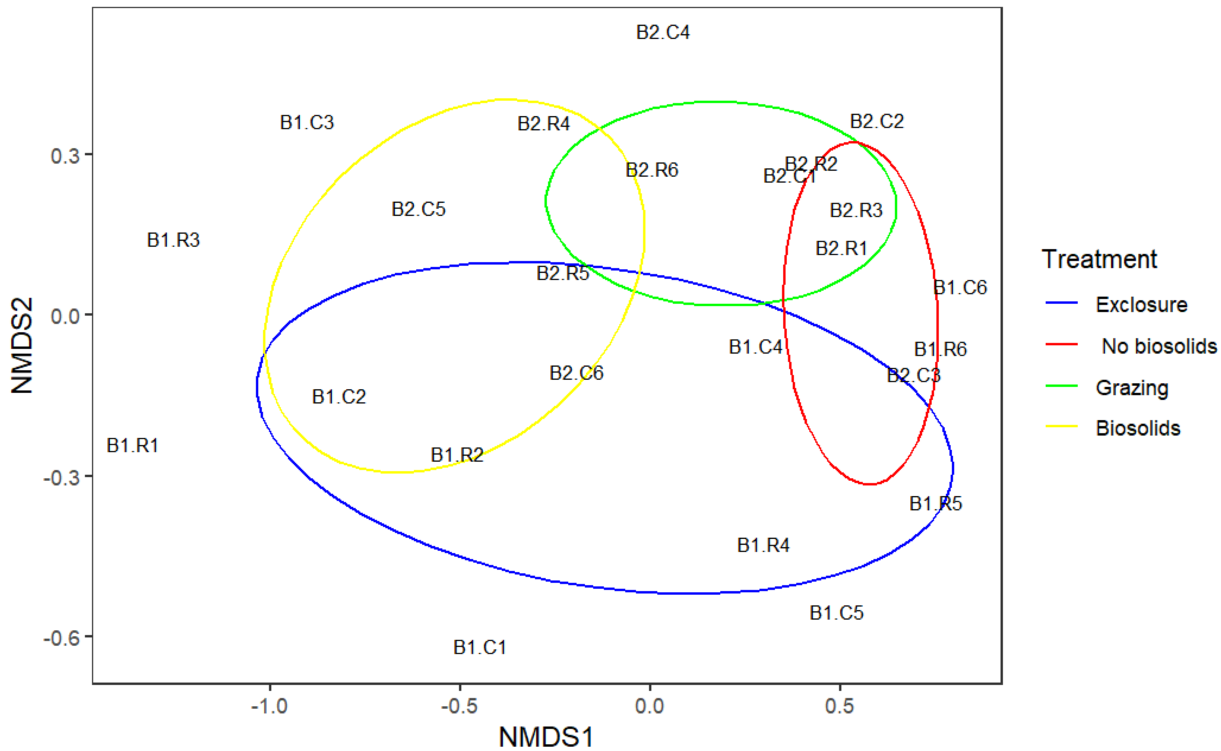


Figure 13 Ordination of 2020 plant percent cover data by plot ID with grazing and biosolid treatments indicated (n = 9). Ellipses indicate 95% confidence limits of treatments as shown.

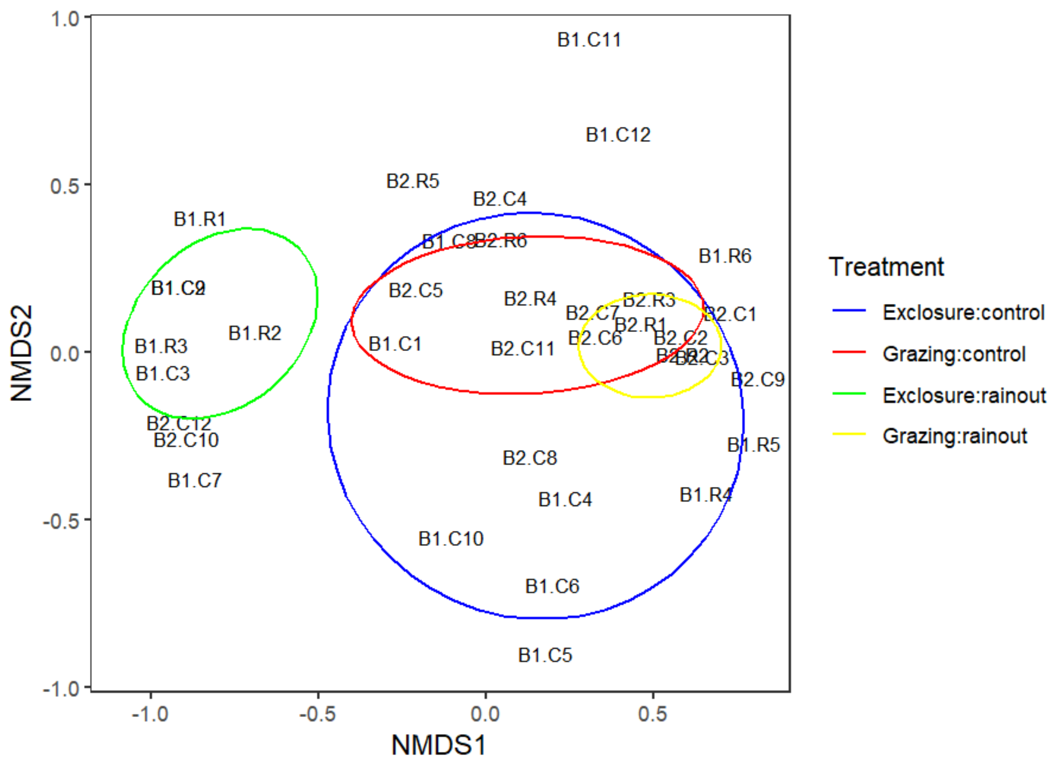


Figure 14 Ordination of 2021 plant percent cover data by plot ID with grazing and rainout treatments indicated (n = 3). Ellipses indicate 95% confidence limits of treatments as shown.

Table 5 P PERMANOVA results from plant percent cover data for grazing and biosolids treatments taken during 2020 (n=9). Values with significant p-values are denoted with asterisks (*p<0.05; **p<0.01, ***p<0.001).

PERMANOVA 2020						
Factor	**Df**	**Sum Sq**	**Mean Sq**	**F value**	**R2**	**Pr(>F)**
grazing	1	0.392	0.392	4.858	0.088	0.013*
biosolid	1	2.338	2.338	28.967	0.524	<0.001***
grazing:biosolid	1	0.114	0.114	1.407	0.025	0.2
Residuals	28	1.614	0.081	NA	0.362	NA
Total	35	4.458	NA	NA	1	NA

Table 6 PERMANOVA results from plant percent cover data for grazing, biosolids, and rainout shelter treatments taken during 2021 (n=9). Values with significant p-values are denoted with asterisks (*p<0.05; **p<0.01, ***p<0.001).

PERMANOVA 2021						
Factor	**Df**	**Sum Sq**	**Mean Sq**	**F value**	**R2**	**Pr(>F)**
grazing	1	0.437	0.437	4.834	0.107	0.013*
biosolid	1	0.136	0.136	1.505	0.033	0.203
rainout	1	0.029	0.029	0.320	0.007	0.742
grazing:biosolid	1	0.027	0.027	0.304	0.007	0.74
grazing:rainout	1	0.848	0.848	9.387	0.207	<0.001***
biosolid:rainout	1	0.044	0.044	0.482	0.011	0.624
grazing:biosolid:rainout	1	0.050	0.050	0.555	0.012	0.611
Residuals	28	2.531	0.090	NA	0.617	NA
Total	35	4.103	NA	NA	1	NA

3.2.4 Species of interest

In both years, the most common forbs were pussytoes, pasture sage, or wild onion. Of the forb species present, pussytoes were identified as of interest due to the use of them as ecosystem indicators and the absence of them found in the area in 2016 (Avery et al., 2019; McLean and Tisdale, 1972)(Avery et al., 2019; McLean and Tisdale, 1972). Means of species cover data from 2020 and 2021 can be found in Appendix A. Pussytoes were significantly less abundant in biosolids plots compared to plots without in both sampling years (Tables 7 and 8, Figures 15 and 16). None of the other factors had significant effects on the cover of pussytoes.

In 2020, the most abundant grasses were needle- and-thread grass and Kentucky bluegrass. The total cover of native grasses (the sum of bluebunch wheatgrass, June grass, and needle-and-thread grass), was significantly lower with grazing exclosure, than in open grazing plots (Table 9, Figure 17). No factors had significant effects on the abundance of bluebunch wheatgrass, when evaluated individually. Kentucky bluegrass was less abundant in open grazing plots, than in grazing exclosure plots (Table 9, Figure 17). Kentucky bluegrass was approximately 40x more abundant in the biosolids plots than in non-biosolids plots. No interactions were found to be significant.

In 2021, bluebunch wheatgrass and Kentucky bluegrass were the most abundant grasses. The mean cover of native grasses (the sum of bluebunch wheatgrass, June grass, and needle-and-thread grass) and bluebunch wheatgrass varied only slightly between each other, indicating that much of this grouping consists of bluebunch wheatgrass (Table 10, Figure 18 and Figure 19). Open grazing increased cover of native grasses in 2020 and 2021. Only in 2021, did open grazing result in significantly higher bluebunch wheatgrass (Table 10, Figure 18). Native grasses and bluebunch wheatgrass were not significantly affected by the biosolids or rainout shelter treatments. Kentucky bluegrass was less abundant in plots with open grazing compared to grazing exclosure and more abundant in plots with biosolids compared to non-biosolids plots (Table 10, Figure 19). Rainout shelters also resulted in less Kentucky bluegrass compared to ambient conditions plots but did not affect cover of native grasses.

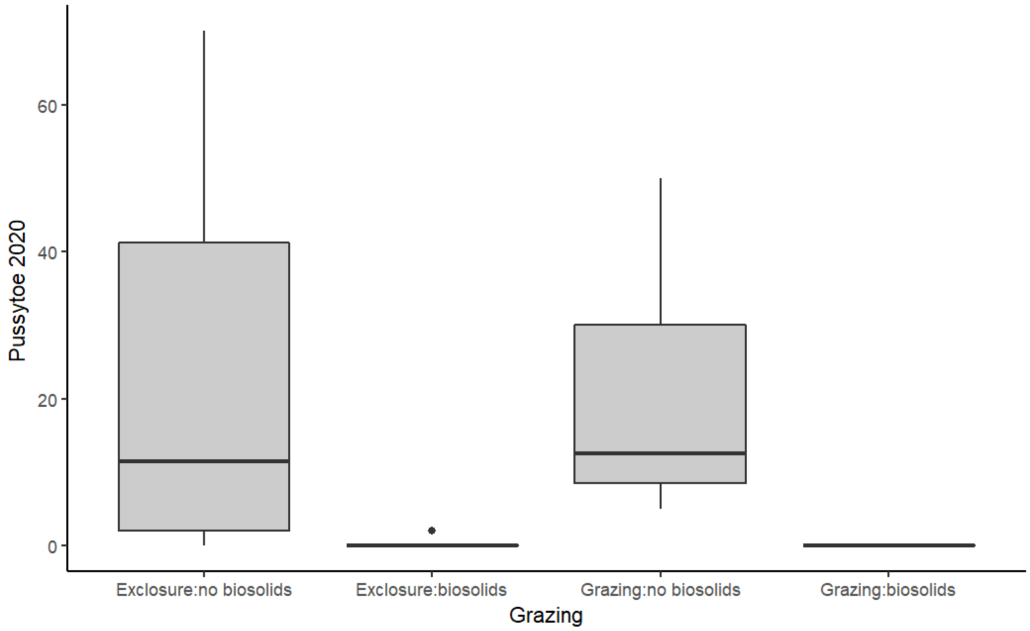


Figure 15 Percent cover data of pussytoes from 2020 (n=9). The biosolid treatment had significant ($p<0.005$) main effect on pussytoe cover.

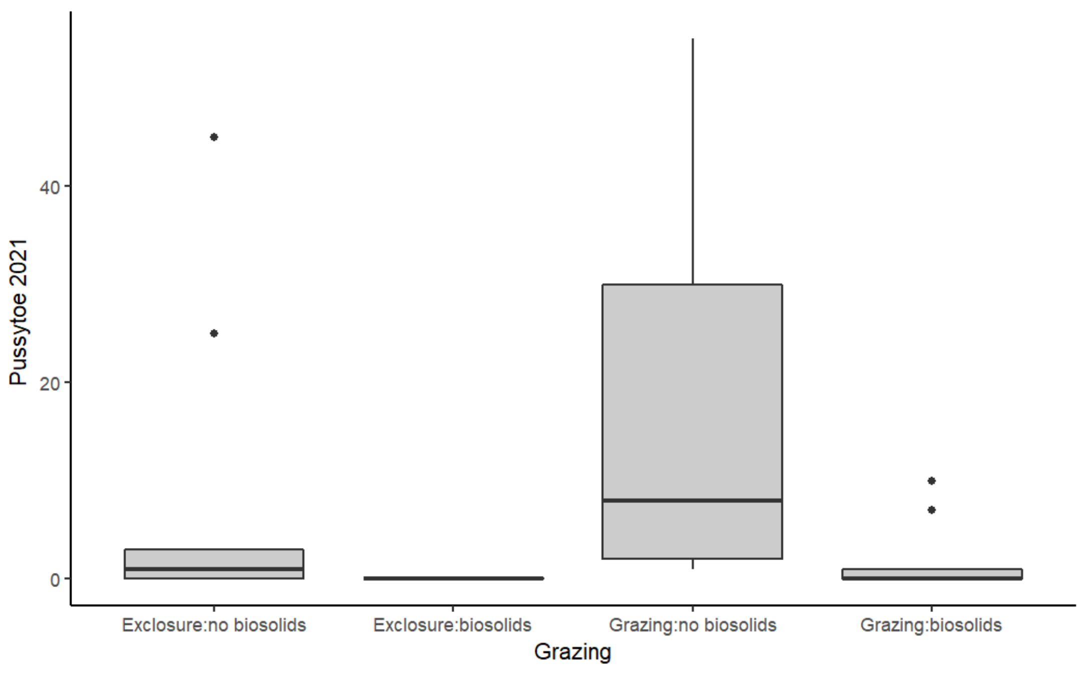


Figure 16 Percent cover data of pussytoes from 2021 (n=9). The biosolid treatment had significant ($p<0.009$) main effect on pussytoe cover.

Table 7 Two-way ANOVA results for percent cover of pussytoes for grazing, biosolids, and rainout shelter treatments taken during 2020 (n=9). Values with significant p-values are denoted with asterisks (*p<0.05; **p<0.01, ***p<0.001).

	Pussytoes 2020				
Factor	**Df**	**Sum Sq**	**Mean Sq**	**F value**	**Pr(>F)**
grazing	1	20.167	20.167	0.068	0.796
biosolid	1	2904.000	2904.000	9.841	0.005**
grazing:biosolid	1	13.500	13.500	0.046	0.833
Residuals	20	5901.667	295.083	NA	NA

Table 8 Three-way ANOVA results for percent cover of pussytoes for grazing, biosolids, and rainout shelter treatments taken during 2021 (n=9). Values with significant p-values are denoted with asterisks (*p<0.05; **p<0.01, ***p<0.001).

	Pussytoes 2021				
Factor	**Df**	**Sum Sq**	**Mean Sq**	**F value**	**Pr(>F)**
Grazing	1	245.444	245.444	1.540	0.225
Biosolid	1	1248.444	1248.444	7.832	0.009**
rainout	1	159.014	159.014	0.998	0.326
grazing:biosolid	1	93.444	93.444	0.586	0.450
grazing:rainout	1	203.347	203.347	1.276	0.268
biosolid:rainout	1	70.014	70.014	0.439	0.513
grazing:biosolid:rainout	1	100.347	100.347	0.629	0.434
Residuals	28	4463.5	159.411	NA	NA

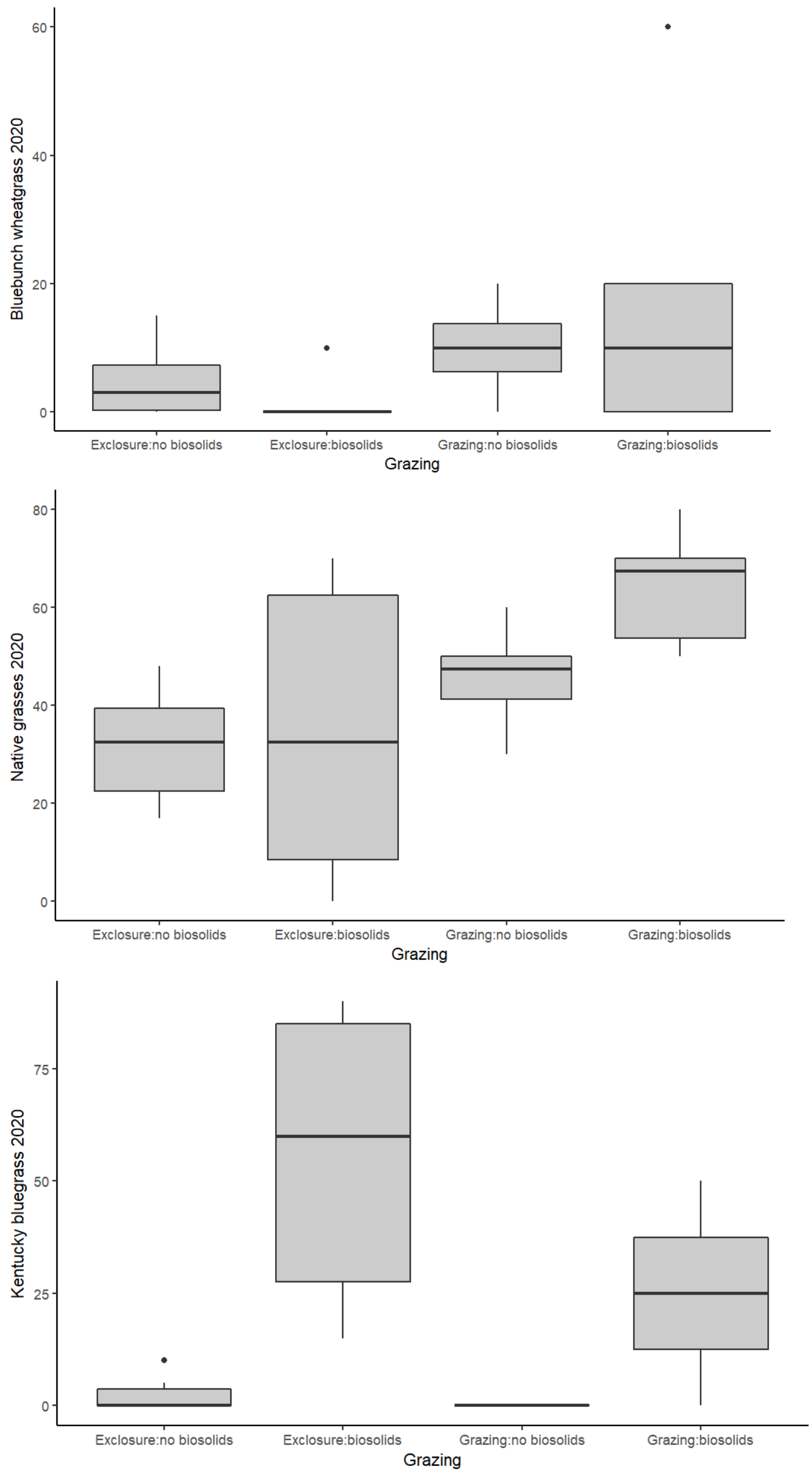


Figure 17 Percent cover data of Bluebunch wheatgrass (top), native grasses, including Bluebunch wheatgrass (middle), and Kentucky bluegrass (bottom) from 2020 (n=9). Grazing had a significant effect on native grasses ($p<0.009$) and Kentucky bluegrass ($p<0.046$), while the biosolid treatment had significant ($p<0.001$) main effect on Kentucky bluegrass. No treatments had significant effects on Bluebunch wheatgrass.

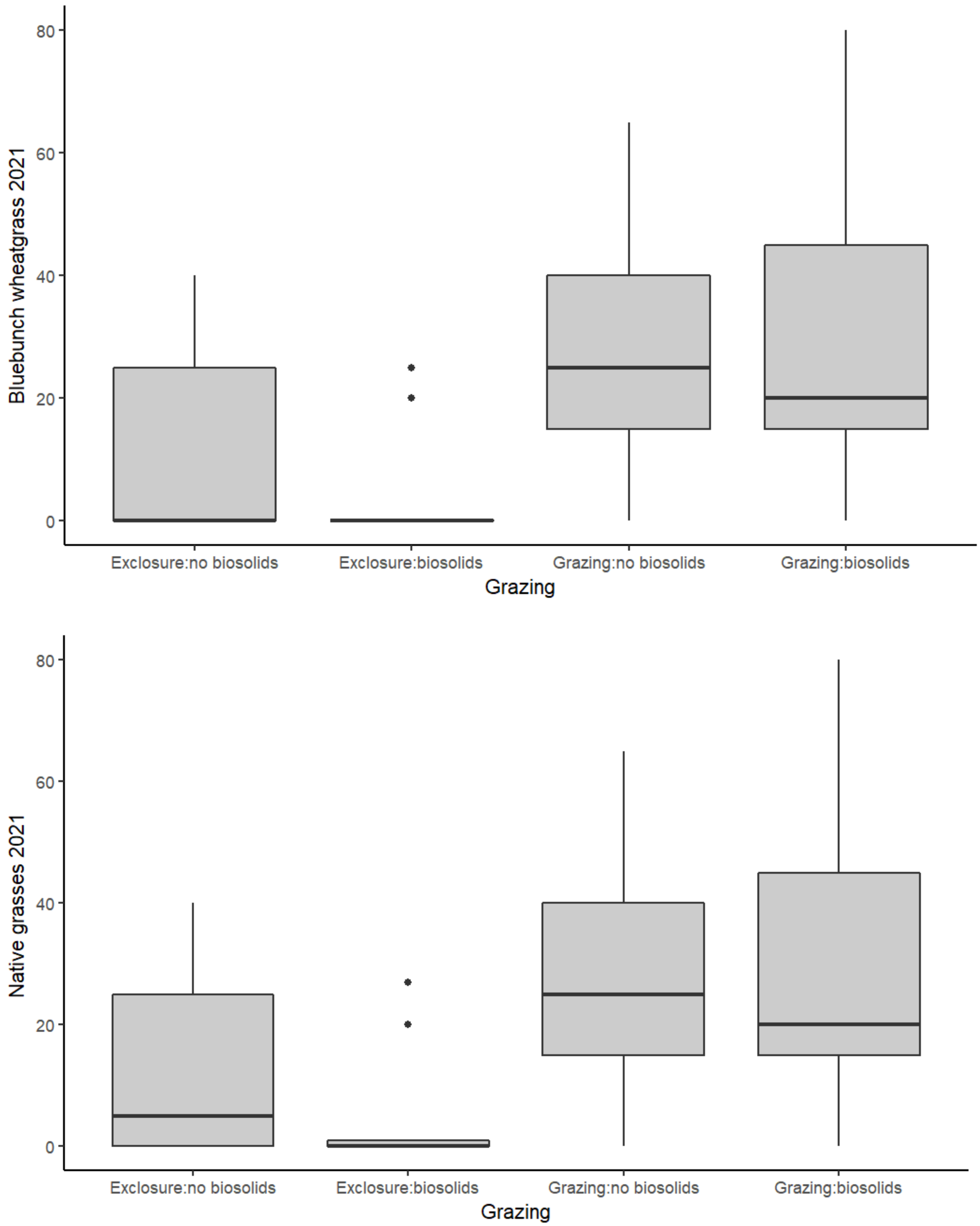


Figure 18 Percent cover data of Bluebunch wheatgrass (top) and Native grasses, including Bluebunch wheatgrass (bottom) from 2021 (n=9). Grazing had significant main effects on Bluebunch wheatgrass ($p<0.009$) and native grasses ($p<0.011$).

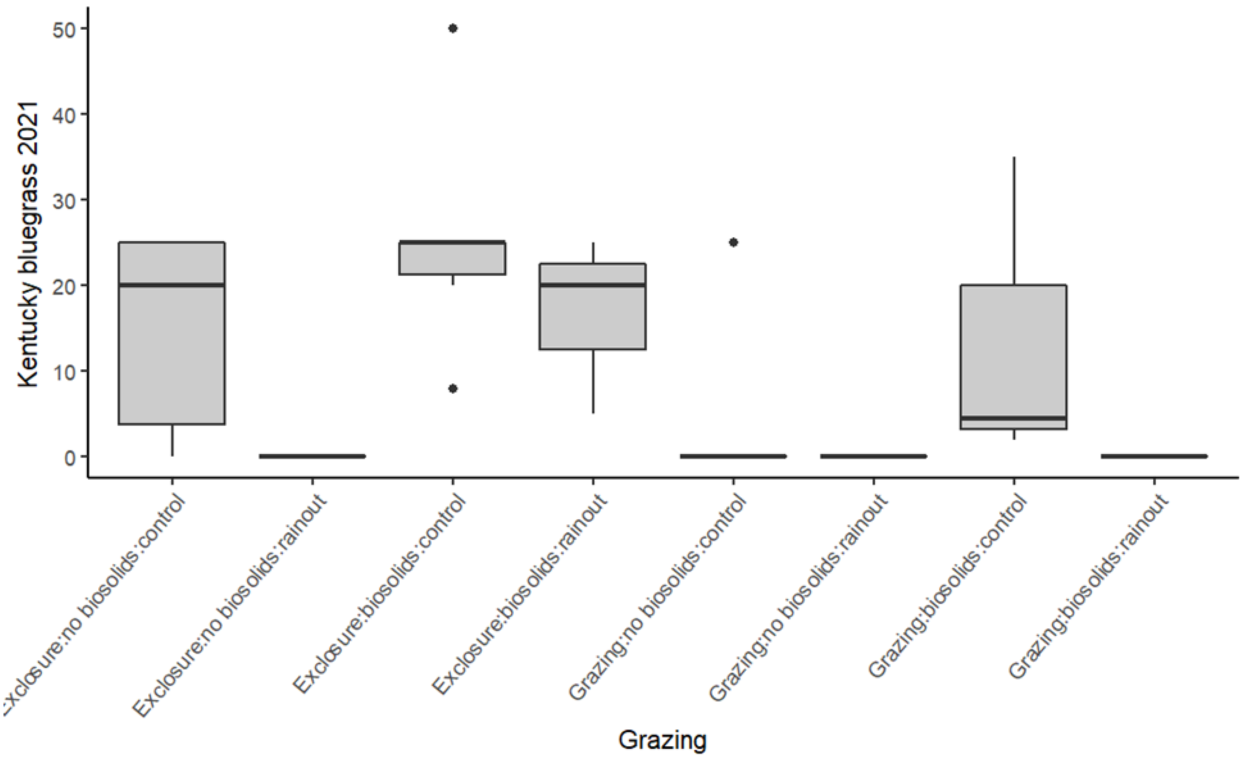


Figure 19 Percent cover from 2021 of Kentucky bluegrass (n=3). Grazing, biosolid, and rainout shelter treatments all had significant main effects on Kentucky bluegrass (p<0.007; p<0.021; p<0.015).

Table 9 Two-way ANOVA results for percent cover of Kentucky bluegrass, bluebunch wheatgrass, and native grasses (bluebunch wheatgrass, June grass, and needle-and-thread grass) for grazing, biosolids, and rainout shelter treatments taken during 2020 (n=9). Values with significant p-values are denoted with asterisks (*p<0.05; **p<0.01, ***p<0.001).

	Kentucky bluegrass 2020					Bluebunch wheatgrass 2020					Native grasses 2020				
Factor	**Df**	**Sum Sq**	**Mean Sq**	**F value**	**Pr(>F)**	**Df**	**Sum Sq**	**Mean Sq**	**F value**	**Pr(>F)**	**Df**	**Sum Sq**	**Mean Sq**	**F value**	**Pr(>F)**
grazing	1	1666.667	1666.667	4.530	0.046*	1	610.042	610.042	3.764	0.067	1	2838.37	2838.375	8.368	0.009**
biosolid	1	9204.167	9204.16	25.017	<0.001***	1	18.375	18.375	0.113	0.740	1	672.042	672.042	1.981	0.175
grazing:biosolid	1	1204.167	1204.167	3.273	0.085	1	145.042	145.04	0.895	0.355	1	360.375	360.375	1.062	0.315
Residuals	20	7358.333	367.91	NA	NA	20	3241.500	162.075	NA	NA	20	6783.833	339.192	NA	NA

Table 10 Three-way ANOVA results for percent cover of Kentucky bluegrass, bluebunch wheatgrass, and native grasses (bluebunch wheatgrass, June grass, and needle-and-thread grass) for grazing, biosolids, and rainout shelter treatments taken during 2021 (n=9). Values with significant p-values are denoted with asterisks (*p<0.05; **p<0.01, ***p<0.001).

	Kentucky bluegrass 2021					Bluebunch wheatgrass					Native grasses				
Factor	**Df**	**Sum Sq**	**Mean Sq**	**F value**	**Pr(>F)**	**Df**	**Sum Sq**	**Mean Sq**	**F value**	**Pr(>F)**	**Df**	**Sum Sq**	**Mean Sq**	**F value**	**Pr(>F)**
grazing	1	1045.444	1045.444	8.568	0.007**	1	3211.111	3211.111	7.847	0.009**	1	3043.361	3043.361	7.490	0.011*
biosolid	1	729.000	729.000	5.975	0.021*	1	69.444	69.444	0.170	0.684	1	78.028	78.028	0.192	0.665
rainout	1	813.389	813.389	6.666	0.015*	1	401.389	401.389	0.981	0.330	1	445.014	445.014	1.095	0.304
grazing:biosolid	1	113.778	113.778	0.933	0.342	1	336.111	336.111	0.821	0.373	1	354.694	354.694	0.873	0.358
grazing:rainout	1	26.889	26.889	0.220	0.642	1	138.889	138.889	0.339	0.565	1	115.014	115.014	0.283	0.599
biosolid:rainout	1	2.000	2.000	0.016	0.899	1	234.722	234.722	0.574	0.455	1	224.014	224.014	0.551	0.464
grazing:biosolid:rainout	1	102.722	102.722	0.842	0.367	1	5.556	5.556	0.014	0.908	1	7.347	7.347	0.018	0.894
Residuals	28	3416.333	122.012	NA	NA	28	11458.333	409.226	NA	NA	28	11376.833	406.315	NA	NA

3.3 Soil Metrics

Soil was sampled in 2021 only. Soil metrics mostly responded to treatments as expected. Open grazing increased bulk density compared to grazing exclosure. Biosolids lowered the mean bulk density compared to plots without biosolids (Table 11, Figure 20). Rainout shelters or any interactive effects had no effects on bulk density. Soil water content was higher in ambient condition plots and was lower in rainout shelter plots (Table 11, Figure 21). Further, a biosolids application increased the soil water content compared to plots without biosolids in ambient conditions plots. Grazing exclosure also increased soil water content. The soil pH was lower in plots with biosolids compared to plots without (Table 11, Figure 20). There were no other effects on pH detected.

Total carbon and total nitrogen were both higher in plots with biosolids, compared to plots without (Table 12, Figure 22). Open grazing resulted in lower total carbon and total nitrogen. Lastly, soil organic matter was higher in plots with biosolids and lower in plots that had open grazing (Table 12, Figure 22). There were no between-factor interactions nor any effects from the rainout shelters.

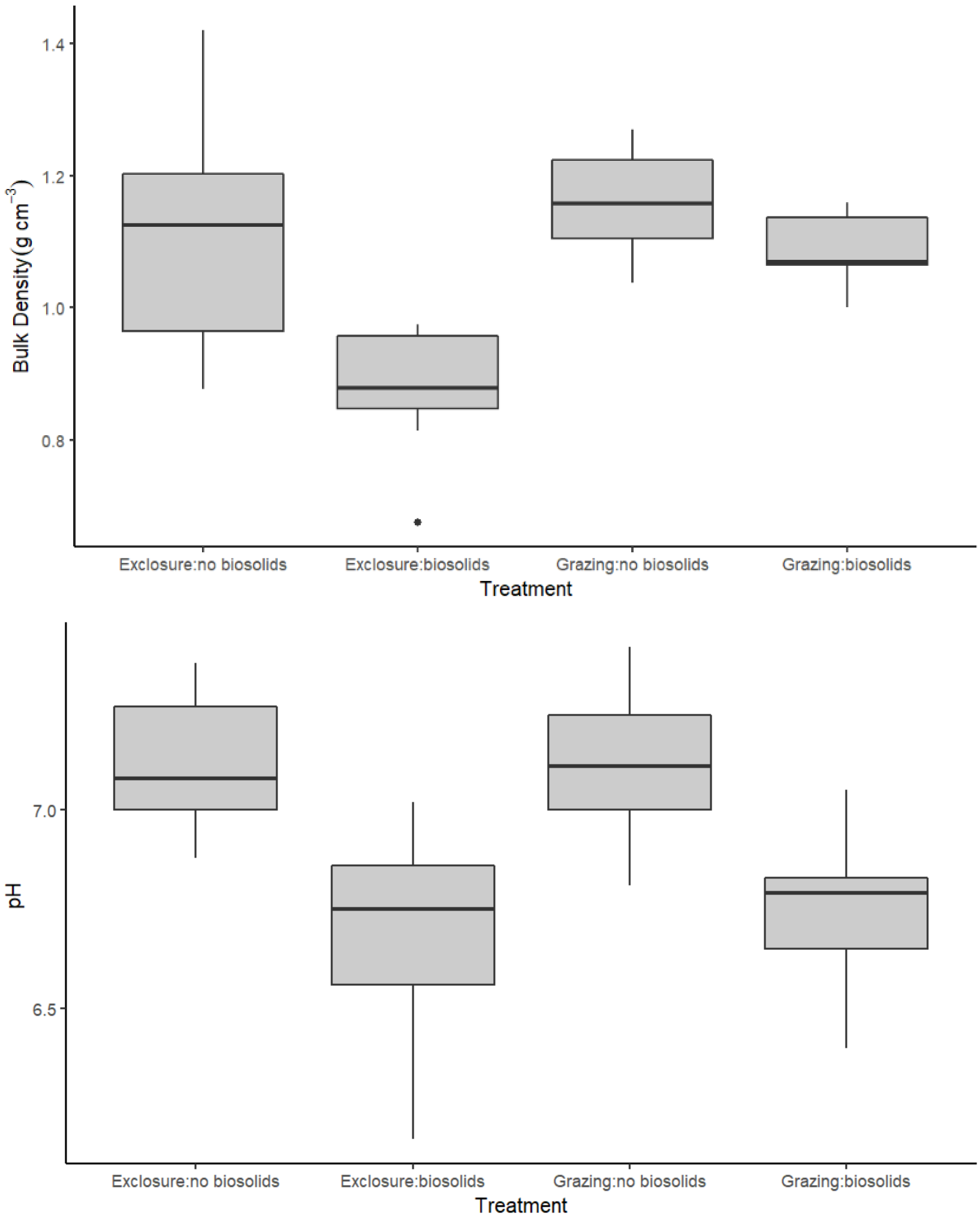


Figure 20 Bulk density (g/cm^3) and pH measurements of the soil for grazing and biosolids treatments in 2021 (n = 9). Grazing had a significant effect ($p<0.002$) on bulk density and the biosolid treatment had significant ($p<0.001$) main effects on both bulk density and pH.

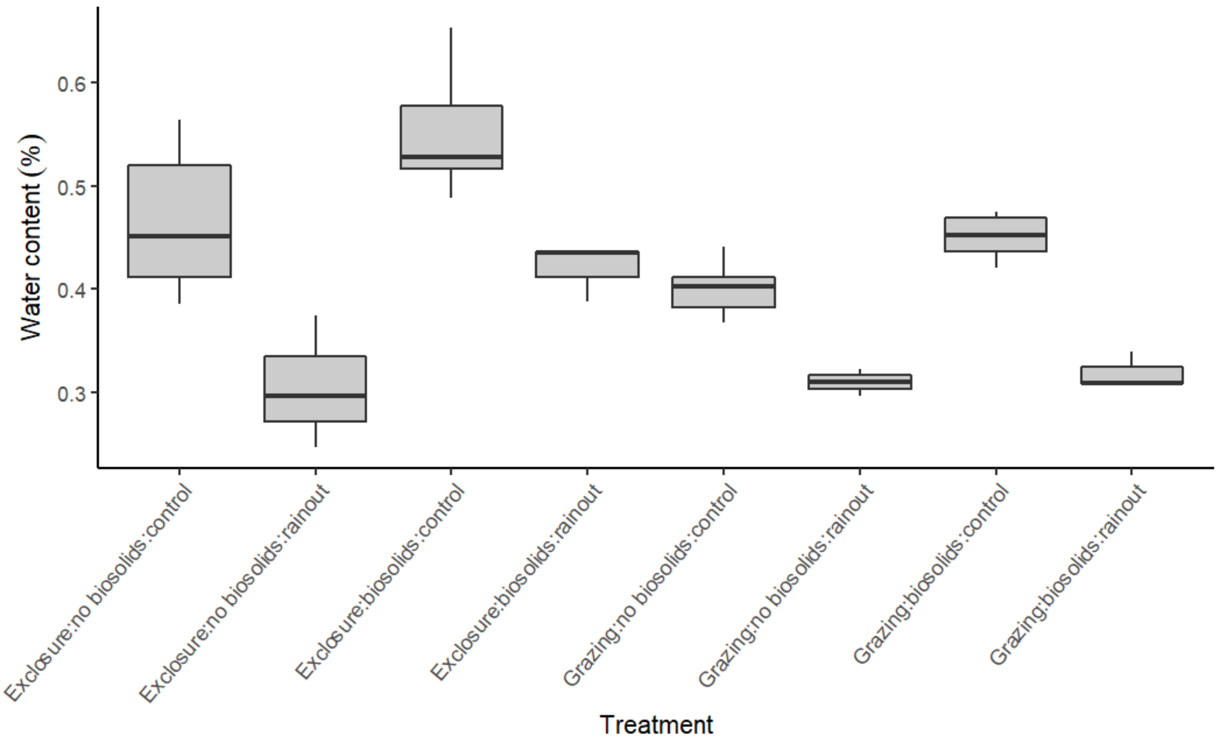


Figure 21 Water content (%) measurements of the soil for grazing, biosolids, and rainout shelters treatments in 2021 (n = 9). Grazing, biosolid, and rainout shelter treatments all had significant main effects with a p<0.001.

Table 11 Three-way ANOVA results for bulk density (g/m [3]), water content (%), and pH of soil for grazing, biosolids, and rainout shelter treatments taken during 2021 (n=9). Values with significant p-values are denoted with asterisks (*p<0.05; **p<0.01, ***p<0.001).

	Bulk density					Water content					pH				
Factor	**Df**	**Sum Sq**	**Mean Sq**	**F value**	**Pr(>F)**	**Df**	**Sum Sq**	**Mean Sq**	**F value**	**Pr(>F)**	**Df**	**Sum Sq**	**Mean Sq**	**F value**	**Pr(>F)**
grazing	1	0.152	0.152	12.139	0.002**	1	0.045	0.045	20.549	<0.001***	1	0.011	0.011	0.245	0.624
biosolid	1	0.220	0.220	17.526	<0.001***	1	0.039	0.039	17.505	<0.001***	1	1.381	1.381	30.692	<0.001***
rainout	1	0.009	0.009	0.696	0.411	1	0.131	0.131	59.657	<0.001***	1	0.08	0.08	1.778	0.193
grazing:biosolid	1	0.051	0.051	4.041	0.054	1	0.008	0.008	3.445	0.074	1	0.005	0.005	0.104	0.750
grazing:rainout	1	0.004	0.004	0.354	0.557	1	0.002	0.002	1.038	0.317	1	0.0002	0.0002	0.004	0.947
biosolid:rainout	1	0.003	0.003	0.270	0.607	1	0.000	0.000	0.033	0.858	1	0.106	0.106	2.352	0.136
grazing:biosolid:rainout	1	0.018	0.018	1.469	0.236	1	0.002	0.002	1.120	0.299	1	0.002	0.002	0.049	0.826
Residuals	28	0.351	0.013	NA	NA	28	0.062	0.002	NA	NA	28	1.260	0.045	NA	NA

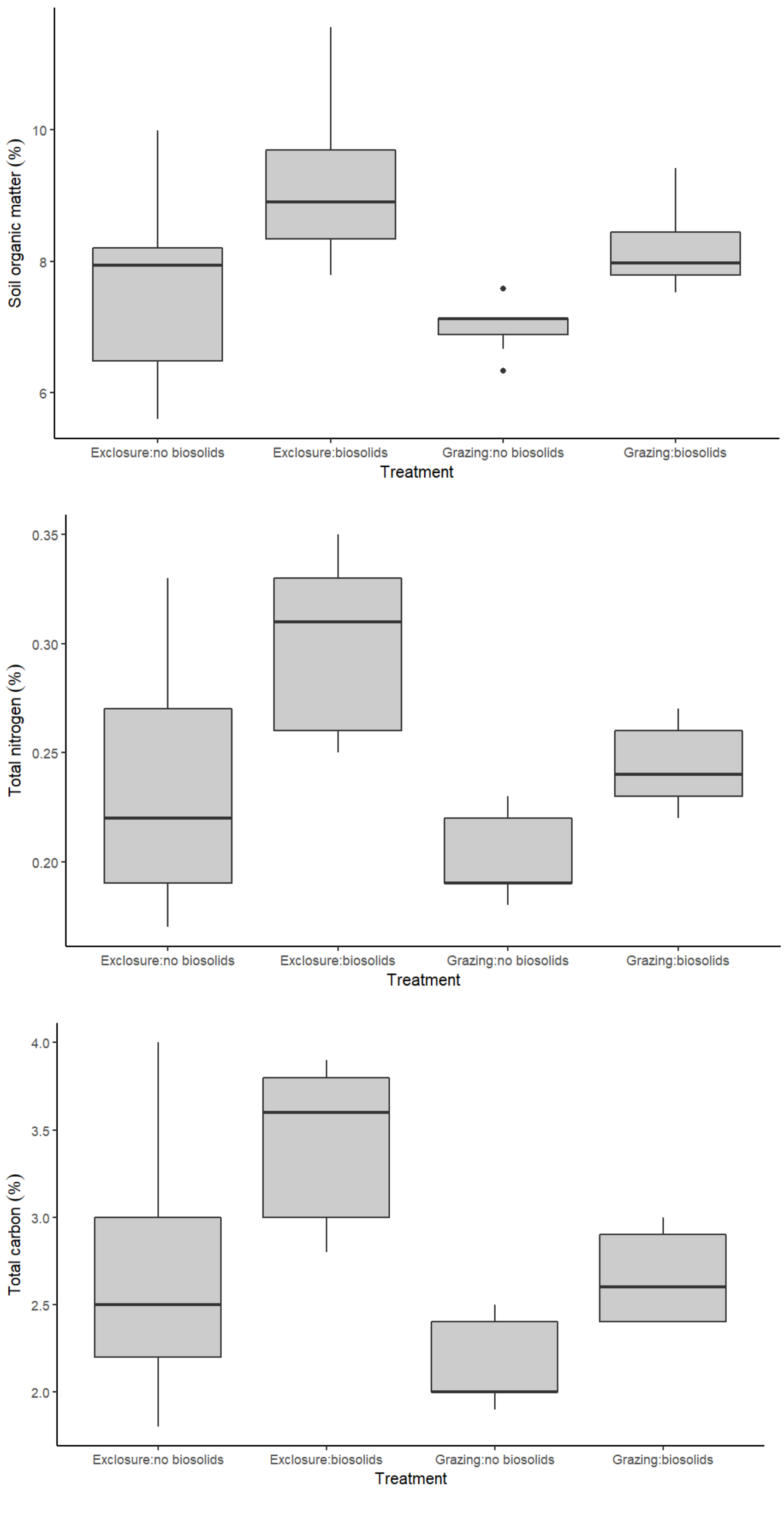


Figure 22 Total carbon (%), total nitrogen (%), and soil organic matter (%) measurements of the soil for grazing and biosolids treatments in 2021 ($n = 9$). Grazing and biosolids treatments both had significant main effects on total carbon ($p<0.001$;$p<0.001$), total nitrogen ($p<0.01$;$p<0.001$), and soil organic matter ($p<0.025$;$p<0.01$),.

Table 12 Three-way ANOVA results for Total carbon (%), total nitrogen (%), and soil organic matter (%) of soil for grazing, biosolids, and rainout shelter treatments taken during 2021 (n=9). Values with significant p-values are denoted with asterisks (*p<0.05; **p<0.01, ***p<0.001).

	Total carbon					Total nitrogen					Soil organic matter				
Factor	**Df**	**Sum Sq**	**Mean Sq**	**F value**	**Pr(>F)**	**Df**	**Sum Sq**	**Mean Sq**	**F value**	**Pr(>F)**	**Df**	**Sum Sq**	**Mean Sq**	**F value**	**Pr(>F)**
grazing	1	3.180	3.180	15.595	<0.001***	1	0.0169	0.0169	13.255	0.01**	1	6.664	6.664	5.582	0.025*
biosolid	1	3.674	3.674	18.014	<0.001***	1	0.0278	0.0278	21.786	<0.001***	1	16.059	16.059	13.452	0.01**
rainout	1	0.067	0.067	0.330	0.570	1	1.20E-33	1.20E-33	9.44E-31	1.000	1	0.015	0.015	0.013	0.912
grazing:biosolid	1	0.234	0.234	1.146	0.294	1	0.002	0.002	1.473	0.235	1	0.141	0.141	0.118	0.734
grazing:rainout	1	0.094	0.094	0.460	0.503	1	0.001	0.001	0.627	0.435	1	0.370	0.370	0.310	0.582
biosolid:rainout	1	0.436	0.436	2.136	0.155	1	0.001	0.001	0.736	0.398	1	1.305	1.305	1.093	0.305
grazing:biosolid:rainout	1	0.036	0.036	0.174	0.679	1	0.002	0.002	1.259	0.271	1	0.047	0.047	0.040	0.843
Residuals	28	5.71	0.204	NA	NA	28	0.0357	0.0013	NA	NA	28	33.426	1.194	NA	NA

4 Discussion

4.1 Plant community

Contrary to my hypotheses, experimental drought had few reported effects on the plant community. Although the rainout shelters were successful in reducing soil moisture (Figure 5), a C_3 grassland undergoes most primary production in early spring, likely before the installation of the rainout shelters in May (Hahn et al., 2021; Pearcy and Ehleringer, 1984). I found that rainout shelters did not influence aboveground biomass, although belowground biomass was lower in the rainout treatment (Table 1). Carroll et al. (2021) found comparable results in C_3 grasslands where experimental drought lowered belowground productivity but had no effect on aboveground productivity. Frank (2007) explained that while aboveground biomass was more dependent on snowmelt in species that had early bursts in productivity, root growth was more reliant on growing season precipitation, which may explain the results seen in this study. A reduction of winter precipitation may have yielded different results in this C_3 dominated grassland as that is when most growth occurs (Fay et al., 2000). The minimal response to the rainout shelters is probably due to the timing of the drought, calling into question the effectiveness of the rainout shelter treatment on the plant community.

However, my research provided evidence that a single application of biosolids had legacy effects on the plant community in a semi-arid grassland up to two decades after application. Biosolids caused an increase in aboveground and belowground biomass, where open grazing caused a decrease in aboveground biomass and had no effect on belowground biomass. The effects of grazing were able to mitigate some species and community levels effects of the biosolids application.

Belowground, biosolids increased root biomass almost 20 years after a single application (Table 1, Figure 7). It is unclear what property caused the increase in root biomass as biosolids change several soil variables in ways that could contribute to increased belowground plant biomass. Increased nutrients, like nitrogen, are a possible mechanism, as the applications of biosolids in comparison to a control treatment and a urea treatment increased the dry root weight of durum wheat in a greenhouse experiment (Boudjabi et al., 2019) . However, the urea treatment did not receive similar additions of soil organic matter or other biosolids components, like micronutrients or hormones, suggesting the effect may not only be from increased nutrients (Boudjabi et al., 2019. Another greenhouse study comparing grass root growth in untreated biosolids with indole-3-acetic acid (IAA) and IAA-treated biosolids, found that the untreated biosolids did not increase tall fescue root productivity, whereas the IAA treatment and the IAA-treated biosolids did (Zhang et al., 2009). Thus, the increase of belowground biomass in my study may have been due to inherent biosolids properties, like biologically active substances, IAA, higher phosphorus content or organic matter, and would not be seen with traditional nitrogen fertilizer applications (Chang et al., 2014; Zhang et al., 2009).

Previously, biologically active properties had been especially beneficial for grasses facing drought stress, yet there was no interactive effect between biosolids and the rainout shelter treatment on belowground biomass (Boudjabi et al., 2019; Chang et al., 2014; Table 1). Rainout shelters did result in lower mean belowground biomass compared to control plots, but the increase in root biomass due to the application of biosolids was large enough to offset this (Table 1, Figure 7). The lack of interaction may be explained by the scale and duration of the previous studies, as those were short term (< 1 year), greenhouse studies on a single species. Regardless of mechanism, carbon from belowground biomass had a longer residence time in grasslands compared to aboveground biomass and thus slowed down carbon outflux; increasing belowground biomass growth is a beneficial reclamation outcome as carbon cycles are maintained and greenhouse gas emissions are slowed (Soussana and Lemaire, 2014).

As expected, aboveground biomass was much greater with the biosolids application than in plots without biosolids (Table 1, Figure 6). I found a larger increase in aboveground biomass, nearly 3.5x more, than a study using the same biosolids and same application regime in a nearby location that only reported 2.5x more biomass due to biosolids (Avery et al., 2019). Plots with biosolids resulted in increased aboveground and litter biomass under grazing exclosure, than in plots with open grazing and biosolids. Without biosolids, there was no difference in means between open grazing and grazing exclosure (Table 1 and 2, Figure 6 and Figure 8). Herbivores, when given free choice, spent more time in biosolids-treated areas than in untreated area, perhaps due to increased forage availability (Wester et al., 2011). Thus, as cattle spend more time in biosolid treated areas they reduce the amount of biomass through increased grazing, explaining a possible cause of the interaction between grazing and biosolids on aboveground biomass and litter. Although increasing biomass is beneficial as it increases forage, large amounts of litter contribute to larger fuel loads which can open these systems up to severe fire and subsequent invasions (Chambers et al., 2014).

A biosolids application increased aboveground grass biomass, while rainout shelters decreased grass biomass (Table 1, Figure 9). Thus, biosolids may be useful for ranchers to ensure bulk forage under the possibility of drought. However, Avery et al., (2019) found forage grown in biosolids had lower concentrations of N and Mg, possibly due to nutrient dilution, which may result in lower quality forage. Although, high-quality grass pasture or grass hay can be produced with biosolids. With abundant N and S (key nutrients in protein), biosolids effectively increase forage protein (Sullivan et al., 2022). Biosolids also supply plant-essential micronutrients such as Cu, Zn and Mn (Sullivan et al., 2022). Therefore, a single biosolids application can be an efficient way to increase aboveground grass biomass, but forage quality may need to be monitored.

Additionally, biosolids significantly reduced forb biomass (Table 1, Figure 10). Specifically, there was a reduction in pussytoe cover (Tables 7 and 8, Figures 15 and 16). Pussytoes have consistently been found to be at, or near 0 % cover with biosolids applications, whereas non-treated plots had a percent cover of between 5-8 % in 2016 (Avery et al., 2019; Newman et al., 2014). In 2020 and 2021, pussytoe coverage ranged from 12-22 % in plots without biosolids. The difference between pussytoe's coverage could indicate the continued degradation of the non-treated plots because pussytoes have been used as a grassland health indicator as they consistently decreased with restoration efforts, like stopping overgrazing (McLean and Tisdale, 1972; Newman et al., 2014). Alternatively, as pussytoes are prostrate growers they may be shaded out as light becomes limited at the bottom of the canopy, which is observed with any soil amendment, rather than an indication of continued degradation of the plots without biosolids (Borer et al., 2014).

In both sampling years, grazing and biosolids had independent effects on Kentucky bluegrass, where open grazing decreased cover and biosolids increased cover (Tables 9 and 10, Figures 17 and 19) (see Chapter 5.1 Limitations). Several studies looking at the ability of grazing to reduce Kentucky bluegrass in unamended soils found nonsignificant effects (Hendrickson et al., 2020; Otfinowski et al., 2017). However, these studies were relatively short in duration, around 5 years, whereas OK Ranch had been grazed for at least 18 years. Biosolids have been found to increase Kentucky bluegrass cover under grazing exclusion (Avery et al., 2019; Newman et al., 2014). Under grazing exclosure, higher amounts of litter accumulation increased soil moisture and facilitated Kentucky bluegrass growth, where open grazing plots had lower amounts of Kentucky bluegrass cover in the same semi-arid grassland (Avery et al., 2019).

Further, the rainout shelters also had lower Kentucky bluegrass cover compared to ambient conditions plots (Table 9 and Figure 19). Kentucky bluegrass has a shallow root system, preventing the plant from accessing deeper water resources: thus, it would be sensitive to conditions that lower soil water content, like rainout shelters and open grazing (Canadell et al., 1996; Weaver, 1958). This causes concern for ranching operations as forage needs to be maintained at sustainable levels, however, there has been limited evidence to show negative downstream effects on cattle production during Kentucky bluegrass invasion (Toledo et al., 2014).

Biosolids did not have significant effects on Bluebunch wheatgrass cover or native grasses cover (June grass, bluebunch wheatgrass, and spreading needle grass) in either sampling year. In 2020, open grazing resulted in higher cover of native grasses while in 2021, open grazing resulted in higher cover of both Bluebunch wheatgrass and native grasses (Table 9 and 10, Figure 17 and 18). In a similar grassland, a grazing regime increased native grass cover when the ecosystem had previously been dominated by Kentucky bluegrass (Hendrickson et al., 2020). Kentucky bluegrass exhibited traits most like a competitor with large amounts of biomass and high abundance, while Bluebunch wheatgrass showed characteristics of stress tolerance, as the positive response to grazing and no response to drought (DeKeyser et al., 2015; Grime, 1988; Table 9 and 10). Further, as Kentucky bluegrass had lower cover and native grasses were higher in cover under grazing, grazing was likely acting as a disturbance that allowed the establishment of more stress tolerant species (Connell and Slatyer, 1977). In other words, cattle reduced competitive exclusion by grazing the dominant plant species, Kentucky bluegrass, which increased resource access for other species (Borer et al., 2014; Grime, 1972; Olff and Ritchie, 1998; Rook et al., 2004). Therefore, grazing may be able to preserve some native grass cover regardless of if biosolids are present.

Application of biosolids resulted in distinct plant communities in 2020 (Table 5, Figure 13). Also, plant diversity was lower in plots with a biosolids treatment in both years (Tables 3 and 4, Figures 11 and 12). A meta-analysis showed biosolids applications have inconsistent or non-significant effects on Shannon diversity in degraded, but uninvaded grasslands (Ploughe et al., 2021)(Ploughe et al., 2021). The lower diversity seen at OK Ranch could be explained by the increased abundance of Kentucky bluegrass grazing exclosure resulted in lower plant diversity compared to open grazing (Table 3 and 4, Figures 11 and 12). Although there was no significant interaction reported, Shannon diversity had a mean of 1.84 under grazing exclosure without biosolids, 0.50 under grazing exclosure and biosolids, and 1.03 under open grazing and biosolids. This suggests that at OK Ranch, grazing has the to potential dampen the loss of diversity seen with the biosolids application. This is in line with previous ideas for Kentucky bluegrass management, as sustainable levels of grazing can be used to slow invasions yet includes another layer of complexity when considering biosolids treatment (Gasch et al., 2020).

Further, the multivariate analysis found plots with grazing exclosure and rainout shelters to be dissimilar in plant cover from the other treatment combinations (Table 6, Figure 13). As this was the only metric that detected an effect between grazing and rainout shelters, there is the potential for this result to be an artifact of experimental design. Although, the combination of grazing and rainout shelters could have lowered soil moisture cumulatively enough that it resulted in changes in plant community composition typically seen in less-than-ideal abiotic conditions (Wilson, 2007).

4.2 Soil properties

A single biosolids application had lasting effects on the soil and my second hypothesis was supported, as a biosolids treatment increased soil organic matter and decreased bulk density (Tables 11 and 12, Figure 20 and 22). Biosolids also increased soil water content, total carbon, and total nitrogen. My third hypothesis was mostly rejected as rainout shelters had no effect on bulk density or total carbon but was somewhat supported as rainout shelters lowered soil water content. My sixth hypothesis was supported as open grazing increased bulk density, along with significantly decreasing total carbon, total nitrogen, and soil organic matter (Tables 11 and 12, Figure 20 and 22).

Total nitrogen was lower in grazed plots and higher in biosolids applied plots. Rainout shelters had no effect on total nitrogen (Table 12, Figure 22). Villa and Ryals (2021) indicated that persistent nitrogen after biosolids applications was either due to management, like grazing, as nitrogen would be replenished from manure, or from lack of irrigation regimes that caused leaching. There was no interaction between biosolids, grazing, or rainout shelters at OK Ranch. Open grazing resulted in lower total nitrogen (Table 12, Figure 22), and may have been due to increased rates of nitrogen leaching through cattle urine deposition, as seen in other semi-arid grasslands (Ledgard et al., 2011; Somda et al., 1997). There was no evidence at OK Ranch to suggest that under a biosolids treatment, experimental drought plots leached nitrogen less than ambient condition plots, as experimental drought was not a significant factor (Table 12, Figure 22).

The biosolids treatment increased total carbon and soil organic matter in the top 10 cm at OK Ranch (Table 12, Figure 22). A large component, around 20%, of biosolids is organic matter and thus biosolids increase soil carbon, especially in comparison to other soil amendments with lower or more liable organic carbon (Hemmat et al., 2010). Ippolito et al. (2010)Ippolito et al. (2010) also suggested that biosolids, due to the large proportions of humic and fulvic acids, are less susceptible to degradation and therefore increase carbon in arid soils. The pH was also found to be lower in biosolids-treated plots (Table 11, Figure 20). Avery et al. (2019) suggested that the lower pH was a result of organic matter decomposition and increased biological activity in the years after treatment.

Application of biosolids resulted in lower bulk density (Table 11, Figure 20). Hemmet et al. (2010) found that increasing levels of organic carbon in biosolids, and related soil amendments, consistently lowered soil bulk density. Lower bulk density can increase water infiltration, making water more accessible at lower depths and reducing surface runoff; this, combined with increased organic matter, may explain the positive effect of biosolids on soil water content (Döbert et al., 2021; Noorvy Khaerudin et al., 2017)Table 11, Figure 20) Under similar application rates, biosolids have been shown to increase field capacity while also increasing organic matter and lowering bulk density (Tsadilas et al., 2005). Increased root growth in biosolids treatments may be attributed, not only to increased nutrients, but to lower soil resistance and increased soil water content (Chapter 5.1).

Globally, grazing often lowers soil carbon with increasing detrimental effects at higher grazing intensities (Eze et al., 2018). Here, grazing reduced both soil organic matter and total carbon (Table 12, Figure 22). This is likely due to lower rates of carbon assimilation by the plant biomass after grazing, leading to lower amounts of humification and less rhizodeposition (Kuzyakov and Domanski, 2000; Schmitt et al., 2013). Total soil carbon with open grazing and without biosolids was 2.2 % and 2.6% with open grazing and with biosolids. Practically, as the positive effect of biosolids on soil carbon was larger than the negative one from grazing, biosolids may be able to mitigate some of this loss. Additionally, lower soil organic matter, and compaction from trampling, lead to higher bulk densities in open grazing plots (Alderfer and Robinson, 1947; van Haveren, 1983). Although, bulk densities observed under open grazing were still low enough to not impede root growth and showed no evidence of causing lower root biomass in the grazed plots (Zimmerman and Kardos, 1960; Chapter 5.1). Water content was also lower in open grazing plots, which is consistent as these plots had less litter to retain soil moisture (Sharafatmandrad et al., 2010). Further, the higher bulk densities would lower infiltration and lower soil organic matter reducing water retention (Franzluebbers, 2002; Knoll and Hopkins, 1959; Rawls et al., 2003).

Although, the rainout shelters were removed from October 2020 to May 2021, there were several rainfall events when the shelters were functional (Figure 3). Rainout shelters lowered soil water content significantly compared to plots without the shelters (Table 11, Figure 21). Rainout shelters also resulted in lower volumetric soil water content throughout the study as measured with the Onset HOBO devices (Figure 4). Despite this, many of the expected drought effects, on both the plant community and soil properties, were not seen and thus my ability to reach conclusions about biosolids mitigating drought is limited. As discussed in the previous section, the lack of effect could be due to the magnitude by which the rainout shelters reduced precipitation or due to timing of the reduction.

5 Conclusions and land management implications

Grasslands make up a small portion of land area within British Columbia and are heavily relied on for various ecosystem services. Historically, some of these areas have been over-grazed, which resulted in the degradation of the plant community and soil properties. Biosolids are used as a reclamation technique yet are not completely understood. Currently, biosolids are being applied to rangelands despite limitations in the literature, further signaling the need for this research. The purpose of this research was to identify any possible interactions between biosolids, grazing, and experimental drought on a semi-arid grassland, as to my knowledge, this had not been done before. It was hypothesized that biosolids were to mitigate some drought effects like lower biomass and soil carbon, while diversity would be lowered. Further, grazing would be able to mitigate unwanted outcomes, like lower forb biomass, that occur with a biosolids treatment, while biosolids were to minimize experimental drought effects on the plant community and soil properties.

The study produced limited evidence regarding interactive effects of biosolids and experimental drought, or rainout shelters. Although water content was lower in experimental drought plots, there was little effect on other soil metrics or on the plant community. When experimental drought did cause effects, like lowering root biomass or water content, biosolids had a larger, mitigating effect. Further, no negative effects were observed that suggested that biosolids treatments should not be used as a reclamation tool under the threat of drought or drying climate. Thus, there is some evidence that biosolids can mitigate drought effects, but much more robust research is needed. More research examining droughts at differing the severities and lengths would be beneficial as well.

Biosolids can be used to increase belowground biomass and aboveground biomass, mostly in the form of grasses, but will lower forb biomass. Open grazing lowered aboveground and litter biomass but had no significant effects on forb and grass biomass. Kentucky bluegrass cover was lower in grazed plots than exclosure plots, while in biosolids treated plots Kentucky bluegrass was higher. This is indicative that grazing may be able to mitigate the growth of Kentucky bluegrass after a biosolids treatment. Biosolids improved soil health, like increased water content and lower bulk density. Soil carbon and nitrogen were also increased in the biosolids treated plots. As open grazing depleted total carbon in the soil, the use of biosolids on rangelands may be a useful technique to lessen the effect of cattle. Although outside the scope of this research, a relevant follow up would be to investigate the microbial communities at OK Ranch to better understand underlying mechanisms.

Biosolids can be a useful soil amendment in semi-arid rangelands with a mix of native and naturalised agronomics, like Kentucky bluegrass. Further, the addition of grazing may help to reach reclamation targets, rather than the use of biosolids alone. Additionally, there was no evidence that moderate decreases in growing season precipitation lower the reclamation benefits of biosolids. However, caution should be used when applying biosolids, even at modest rates, as the lasting effects of a single application are detectable almost two decades later.

5.1 Study limitations and strengths

This study faced several challenges that should be considered when examining the results. Chapter 2: Data analysis discusses the statistical constraints caused by experimental design for this research. Additionally, due to the 2021 drought and subsequent wildfires, vegetation identification was completed in late summer. This limited my confidence in grass species identification as much of the plant material was senescent. To address this, I included analysis for a pooled native grasses variable to increase accuracy of grass cover analysis. Regardless, many of these effects were quite strong and should still be considered.

There were notable deficiencies in research looking at the impact of grazing and drought on the effects of biosolids. So, although imperfectly replicated and constrained by logistical challenges, this study provided an opportunity to study the long-term effects of biosolids *in situ* in combination with grazing and drought. Additionally, as this area has previously been used for other biosolids research, there was an ability to compare my results with those previous works (Avery et al., 2019, 2018; Newman et al., 2014; Wallace et al., 2016b, 2009). Further, this study provided a realistic reflection of biosolids as a reclamation technique on a working ranch, as the conditions in the research blocks were reflective of actual management strategies. This work started to create more robust research regarding interactions between biosolids and other factors within British Columbian rangelands.

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Appendices

Appendix A: Species cover data

Table 13 Species cover means from baseline sampling in 2020 by treatment.

Species ID	Exclosure	Grazing	No biosolids	Biosolids
Moss	1	1	0	2
Yarrow	5	5	0	9
Pussytoe	12	10	22	0
Yellow salsify	1	0	0	1
Dandelion	0	0	0	0
Yellow owl clover	1	0	1	0
Pasture sage	1	8	5	3
Vetch (unknown)	3	0	2	1
Field milk vetch	3	1	0	4
Wild onion	12	2	11	3
Unknown long leafy	1	0	0	1
Mustard	0	1	1	0
Death camas	1	0	1	0
Cut leaf fleabane	1	0	1	0
Geranium (unknown geranium)	1	0	1	0
Unknown grass	0	0	0	0
Needle and thread grass	25	27	16	35
Kentucky bluegrass	29	13	1	40
June grass	5	15	15	5
Bluebunch wheatgrass	3	13	7	9

Table 14 Means of species cover from 2021 sampling by treatment.

Species ID	Exclosure	Grazing	No biosolids	Biosolids	Control	Rainout
Moss	0	1	0	1	1	0
Yarrow	0	0	0	0	0	0
Pussytoe	4	10	13	1	8	4
Yellow salsify	1	0	0	0	0	0
Dandelion	0	0	0	0	0	0
Pasture sage	0	2	2	0	1	2
Vetch (unknown)	0	0	0	0	0	0
Field milk vetch	0	0	0	0	0	0
Pale comadra	1	0	1	0	0	1
Small flowered penstemon	1	0	1	0	1	1
Cut leaf fleabane	0	0	0	0	0	0
Sticky geranium	0	0	0	0	0	0
Arnica (unknown)	0	0	0	0	0	0
Arugula (unknown)	0	0	0	0	0	0
Yellow owl clover	0	0	0	0	0	0
Kentucky bluegrass	16	6	6	15	14	4
Bluebunch wheatgrass	9	28	20	18	21	14
Needle and thread grass	0	0	0	0	0	0
June grass	0	0	0	0	0	0
Native grass	10	28	21	18	22	14

Appendix B: Main effects graphs

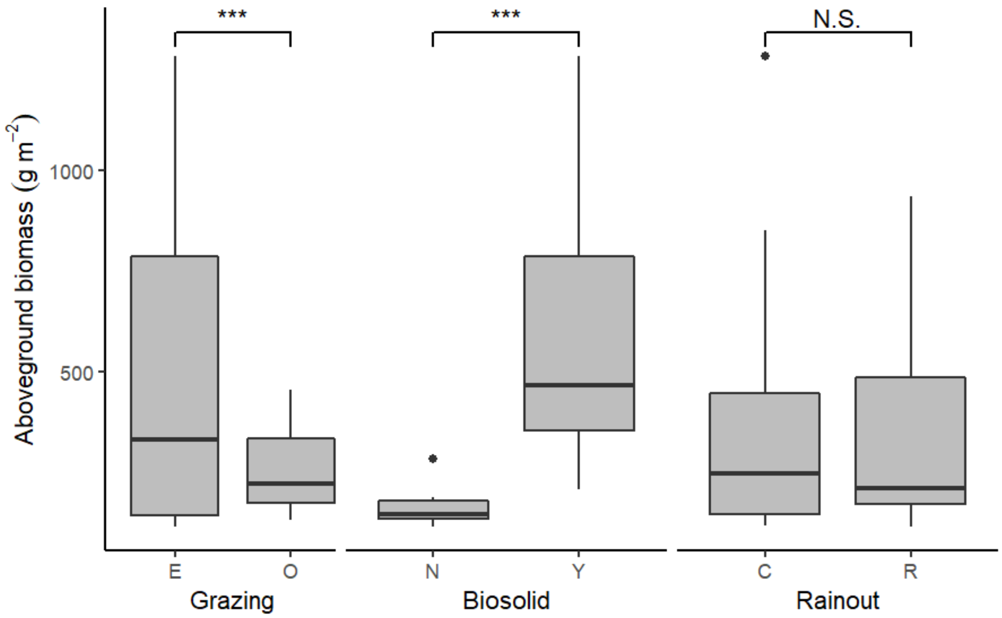

Figure 23 Aboveground biomass (g m^{-2}) for grazing, biosolids, and rainout shelter treatments taken during 2021 (n=12). Values with significant p-values are denoted with asterisks (*p<0.05; **p<0.01, ***p<0.001). E = exclosure, O = open; N = no biosolids, Y= biosolids; C= ambient conditions, R= rainout shelters.

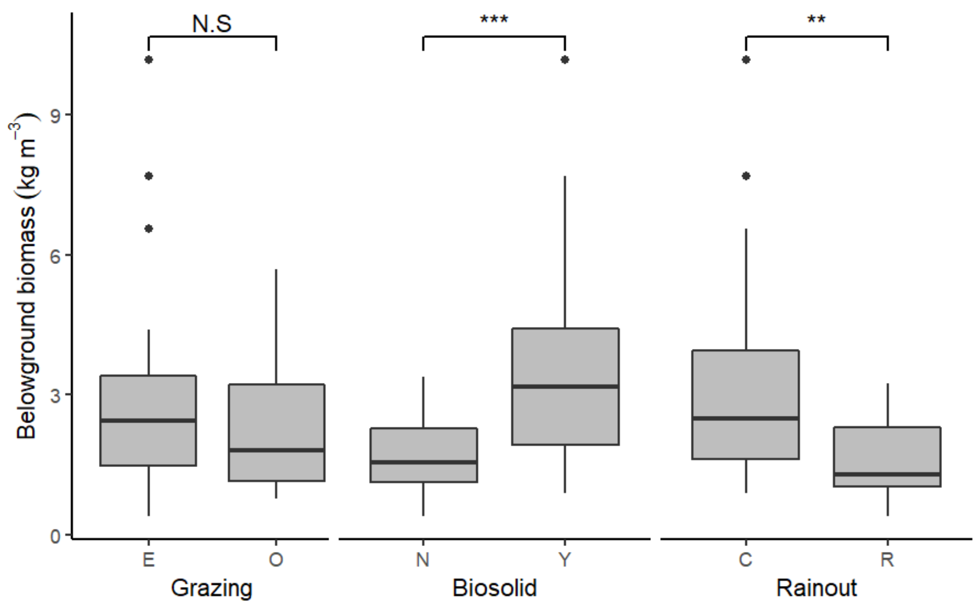

Figure 24 Figure 8 Belowground biomass (kg m^{-3}) for grazing, biosolids, and rainout shelter treatments taken during 2021 (n=12). Values with significant p-values are denoted with asterisks (*p<0.05; **p<0.01, ***p<0.001). E = exclosure, O = open; N = no biosolids, Y= biosolids; C= ambient conditions, R= rainout shelters.

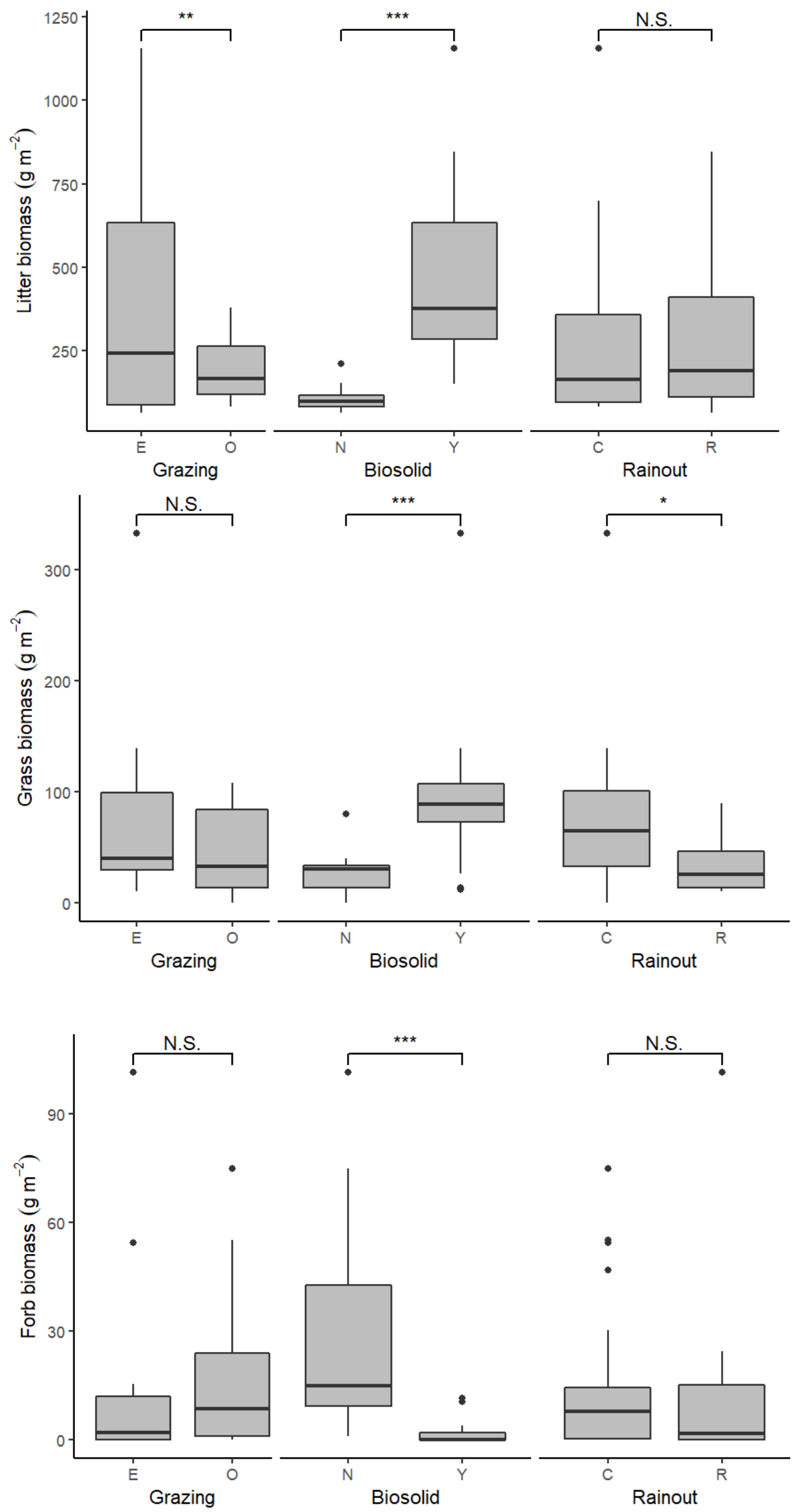

Figure 25 Functional group (top = litter biomass, middle = grass biomass, bottom = forb biomass) above-ground biomass (kg ha^{-1}) for each factor from 2021 sampling (n = 12). Values with significant p-values are denoted with asterisks (*p<0.05; **p<0.01, ***p<0.001). E = exclosure, O = open; N = no biosolids, Y= biosolids; C= ambient conditions, R= rainout shelters.

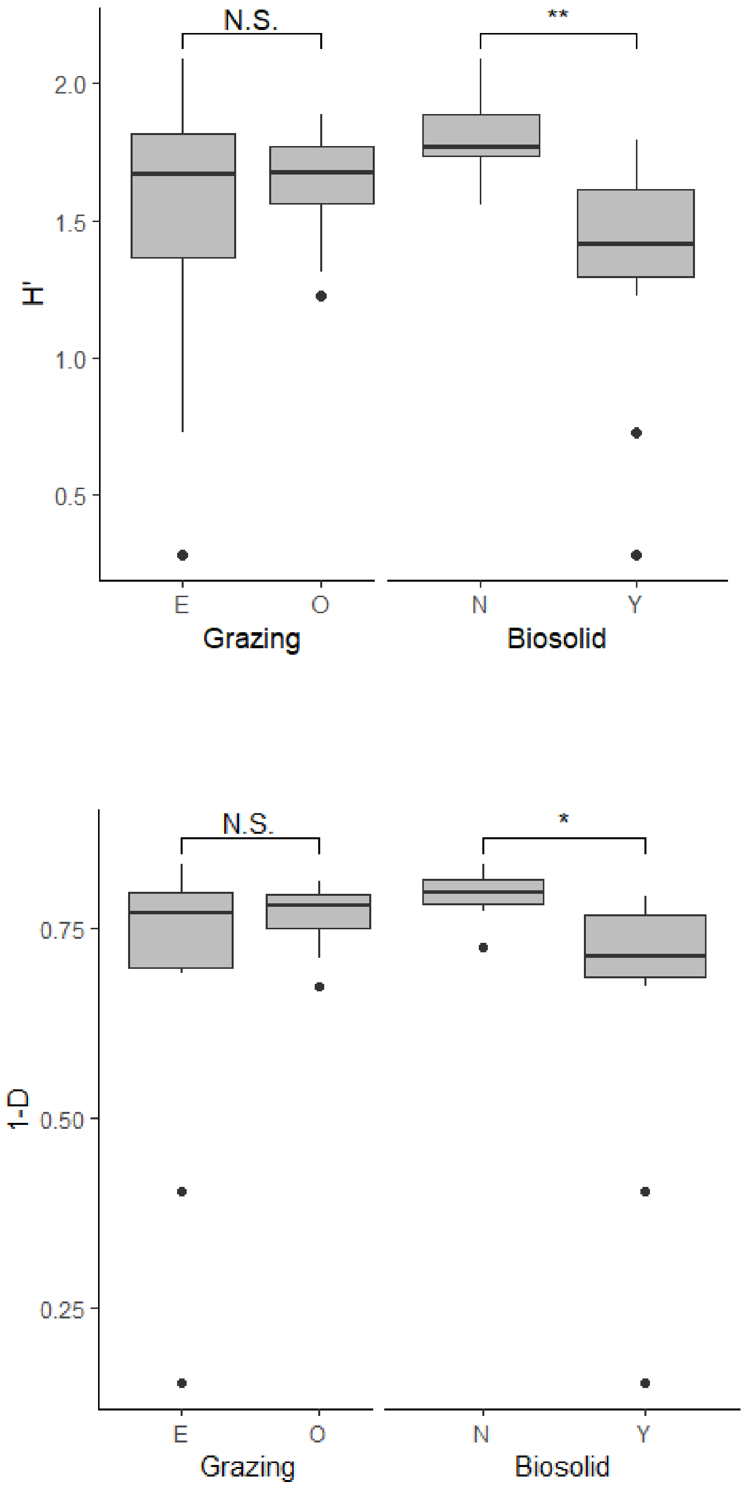

Figure 26 Shannon Diversity (H’) and Simpson Diversity (D) indices for the biosolids treatment for both 2020 sampling of cover data (n=12). Values with significant p-values are denoted with asterisks (*p<0.05; **p<0.01, ***p<0.001). E = exclosure, O = open; N = no biosolids, Y= biosolids; C= ambient conditions, R= rainout shelters.

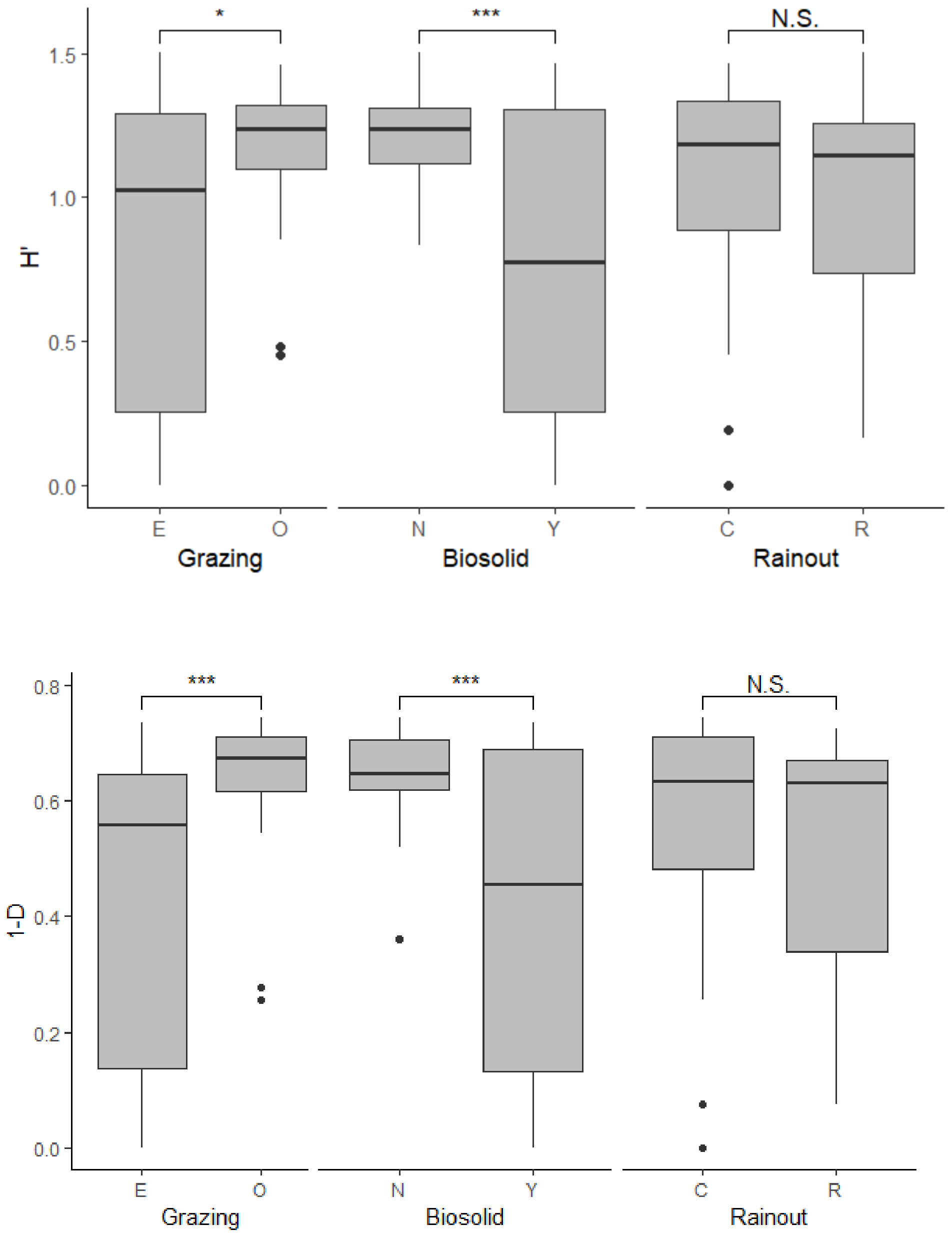

Figure 27 Shannon Diversity (H') and Simpson Diversity (D) indices for the biosolids treatment for both 2021 sampling of cover data (n=12). Values with significant p-values are denoted with asterisks (*p<0.05; **p<0.01, ***p<0.001). E = exclosure, O = open; N = no biosolids, Y= biosolids; C= ambient conditions, R= rainout shelters.

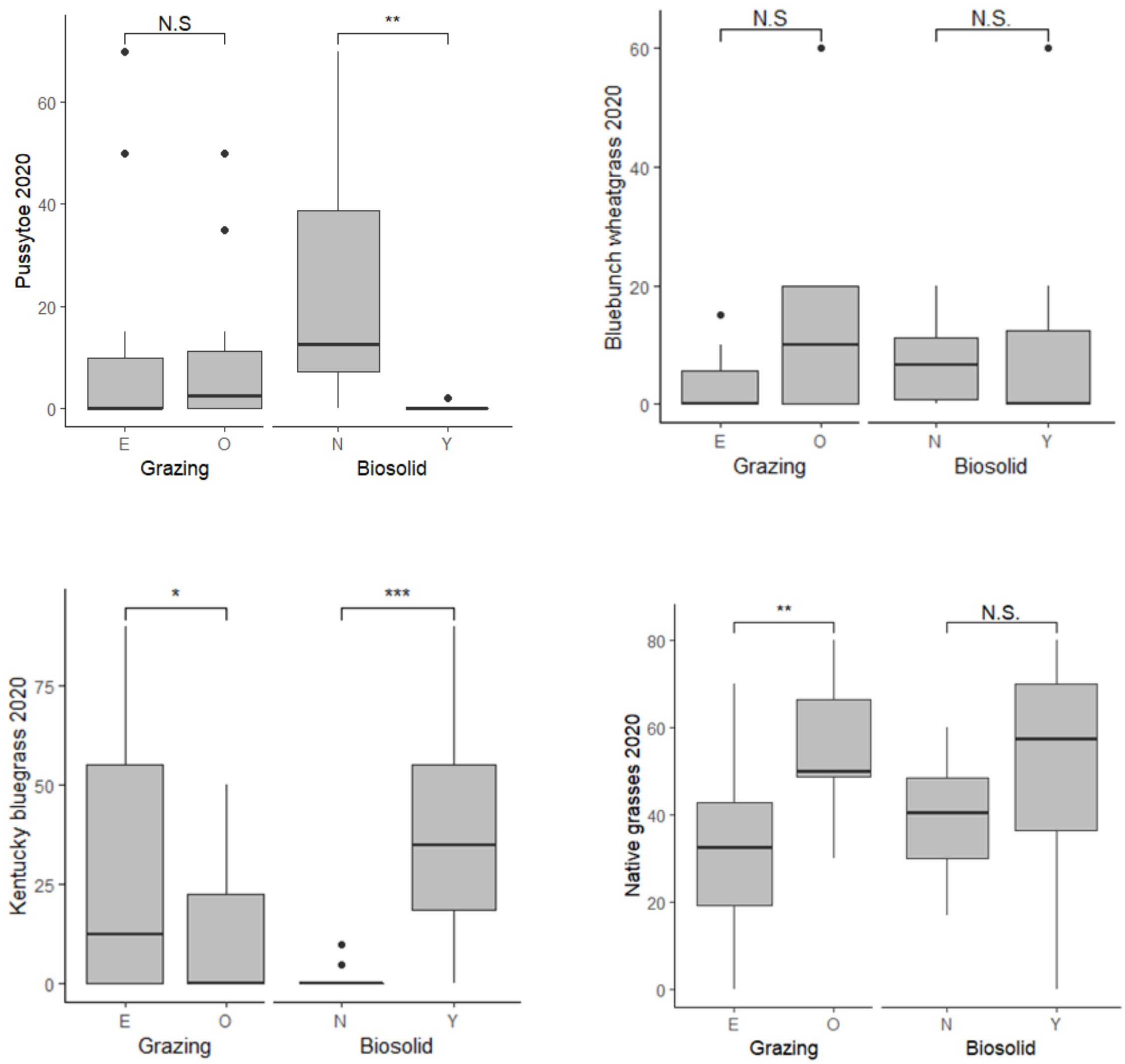

Figure 28 Cover of species of interest by factor from 2020 sampling (n= 12). Values with significant p-values are denoted with asterisks (*p<0.05; **p<0.01, ***p<0.001). E = exclosure, O = open; N = no biosolids, Y= biosolids; C= ambient conditions, R= rainout shelters.

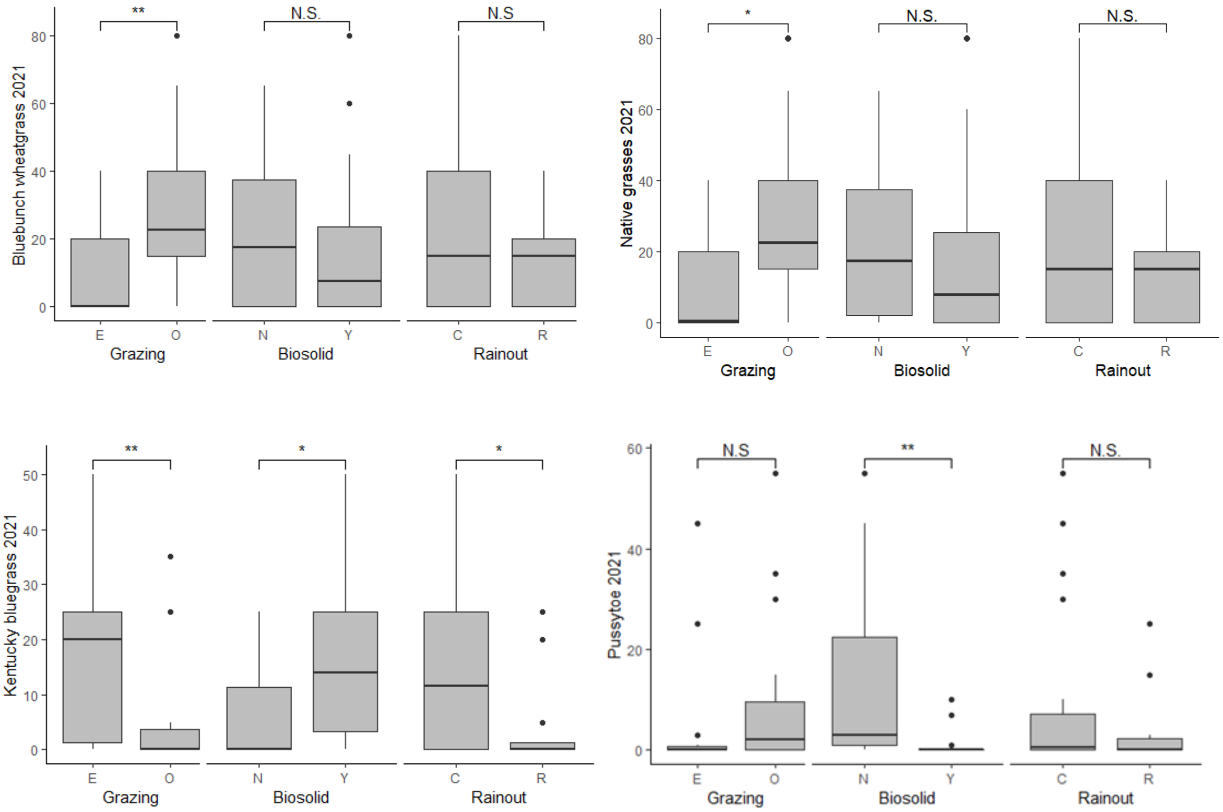

Figure 29 Cover of species of interest by factor from 2021 sampling (n= 12). Values with significant p-values are denoted with asterisks (*p<0.05; **p<0.01, ***p<0.001). E = exclosure, O = open; N = no biosolids, Y= biosolids; C= ambient conditions, R= rainout shelters.

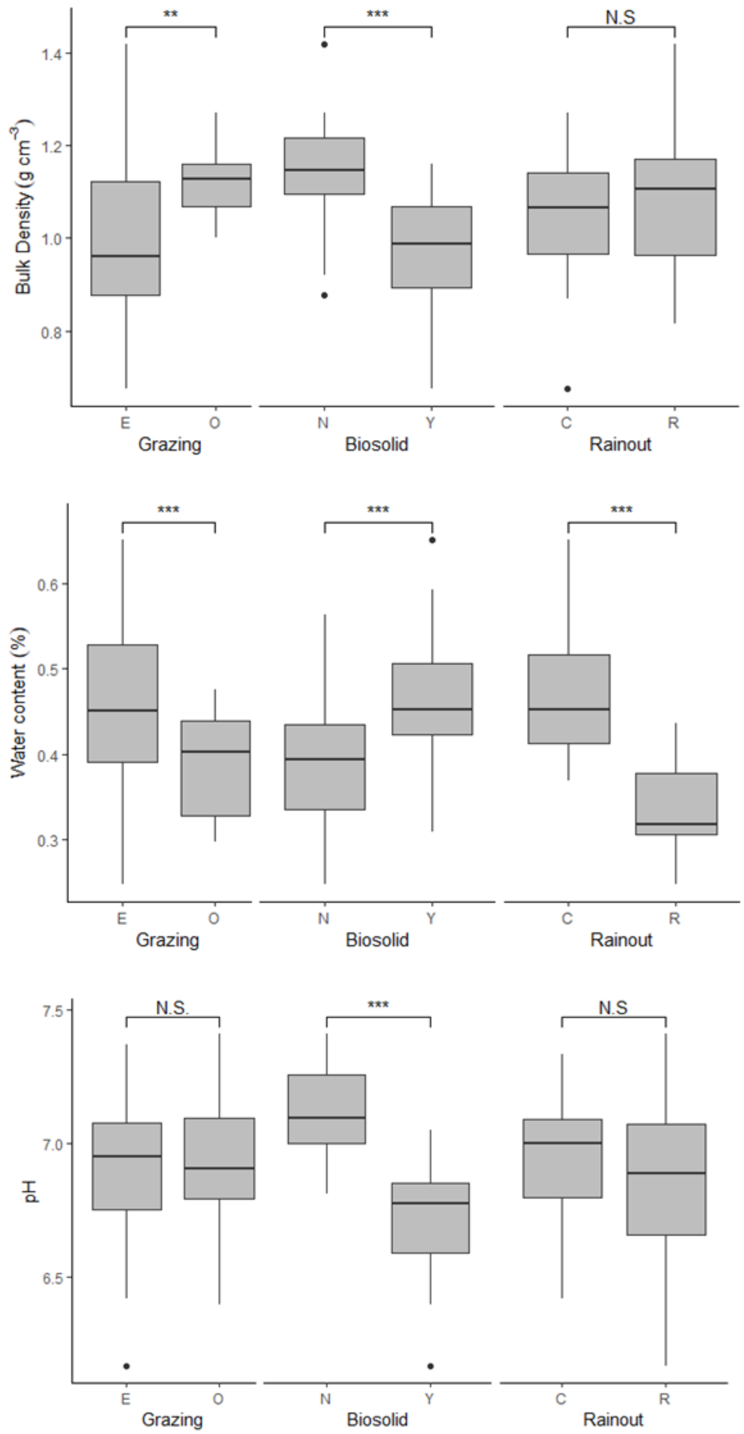

Figure 30 Bulk density (g/cm^3), water content (%), and pH measurements of the soil by factor (n= 12). Values with significant p-values are denoted with asterisks (*p<0.05; **p<0.01, ***p<0.001). E = exclosure, O = open; N = no biosolids, Y= biosolids; C= ambient conditions, R= rainout shelters.

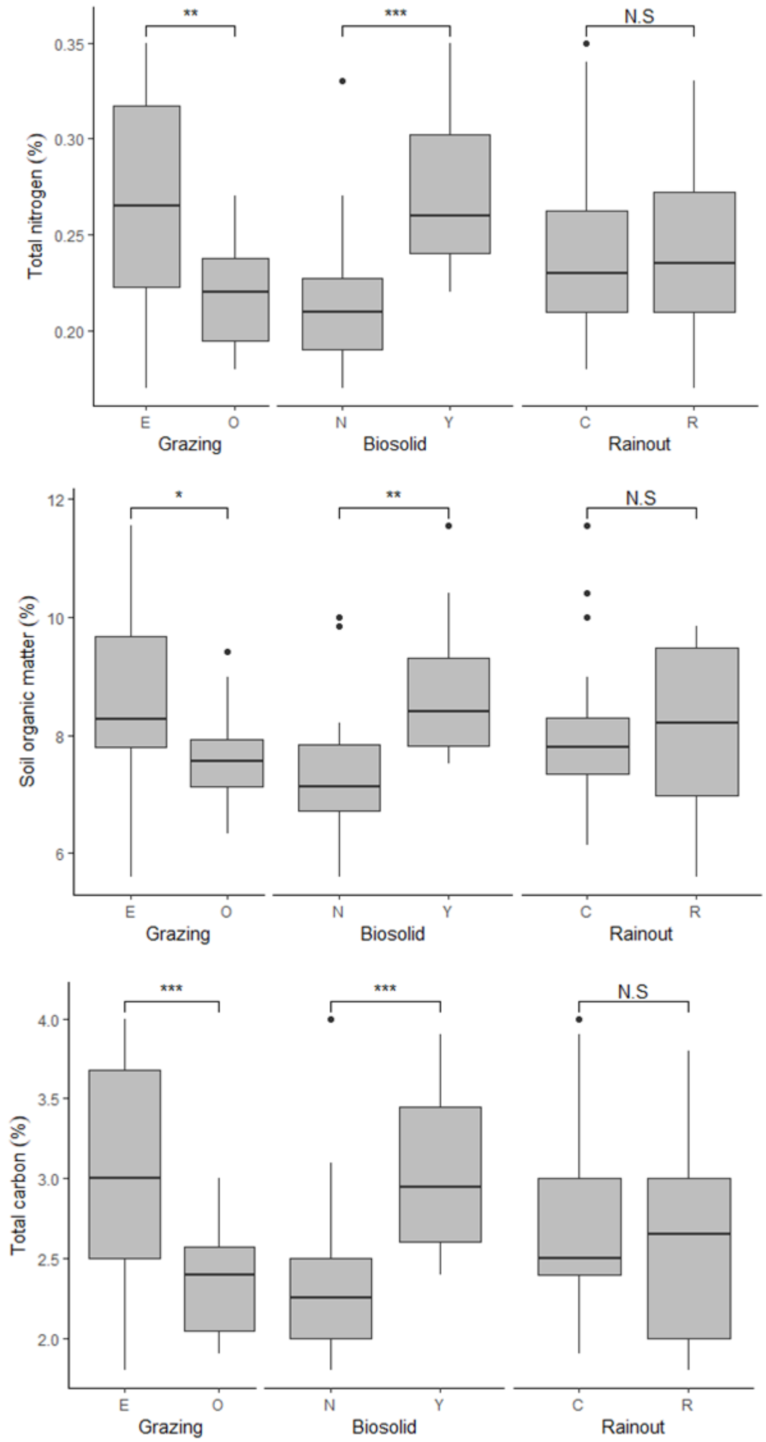

Figure 31 Total carbon (%), total nitrogen (%), and soil organic matter (%) measurements of the soil by factor (n= 12). Values with significant p-values are denoted with asterisks (*p<0.05; **p<0.01, ***p<0.001). E = exclosure, O = open; N = no biosolids, Y= biosolids; C= ambient conditions, R= rainout shelters.