THE LONG-TERM EFFECTS OF BIOSOLIDS ON RANGELAND SOIL QUALITY AND PLANT COMMUNITY IN THE CENTRAL INTERIOR OF BRITISH COLUMBIA

by

Emelia Avery

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The following individuals certify that they have read, and recommend to the Faculty of Graduate and Postdoctoral Studies for acceptance, a thesis/dissertation entitled:

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Examining Committee:

Dr. Maja Krzic
Supervisor

Dr. Brian Wallace
Supervisory Committee Member

Dr. Sean Smukler
Supervisory Committee Member

Dr. Chris Chanway
Additional Examiner

Additional Supervisory Committee Members:

Dr. Gary Bradfield

Abstract

Biosolids have been shown to improve forage production and soil quality on semi-arid rangelands in the short-term. The objective of this study was to assess the long-term effects of a single, surface biosolids application on rangeland soil quality, forage production and plant community composition. In 2002, the experiment was established on a ranch in the central Interior of British Columbia, where two treatments were evaluated: surface biosolids application at 20 Mg ha^{-1} and a control (no biosolids). Both treatments were replicated in four blocks, which were then excluded from grazing for 14 years. In 2016, soil samples were collected in April, June, August and October to assess various soil quality indicators, while forage quality indicators were assessed in June 2016. Fourteen years following the biosolids application, aboveground plant biomass was almost two times greater with biosolids application than on control. Exposed mineral soil was significantly decreased in biosolids plots. Despite differences in aboveground biomass there was no difference in total soil C, permanganate-oxidizable C, or aggregate-protected total C and polysaccharides contents between biosolids and control plots. However, biosolids amended soil did exhibit significantly greater aggregate stability, lower pH, increased spring soil water content, and increased availability of soil P, Fe, Zn and Cu. Forage grown on biosolids plots had lower protein concentrations than control plots, but greater total protein due to the greater biomass. The biosolids application resulted in higher cover of native bluebunch wheatgrass in the long-term, along with >25% cover of agronomic perennial, Kentucky bluegrass. These results offer a demonstration of the potential long-term improvement in forage production that can occur under biosolids application without grazing, which was accompanied by somewhat mixed effects on soil quality and plant species composition.

Lay Summary

In an effort to divert biosolids (treated municipal sewage solids) from landfilling, the majority of the 38,000 annual dry tonnes of biosolids produced in British Columbia (BC) are applied to land, including to rangeland in the central and southern Interior. Biosolids have been shown to improve forage production and soil quality on semi-arid rangeland, and have been suggested to be beneficial in the restoration of previously degraded grasslands. The objective of my study was to assess the long-term effects of a single biosolids application on the soil quality, forage production and plant community composition on a ranch in the central Interior of BC. Fourteen years after the biosolids application, forage production was almost two times greater on plots with biosolids application than on plots without biosolids, and the amount of bare soil was significantly lower. Improvements in soil structure and soil water content were found under biosolids application, along with greater availability of soil phosphorus. However, soil carbon sequestration was not evident under the biosolids application. Native perennial grasses performed better in the biosolids plots, as did a non-native agronomic grass which may have benefitted from the enrichment of soil water and nutrients. The findings of my study offer insight into the long-term effects of biosolids on forage production, plant community composition and soil quality, and may be useful to those involved in management of BC's rangeland.

Preface

This thesis represents unpublished work which I conducted with assistance from undergraduate students and advisors. I was the lead investigator in the studies included in Chapter 2, 3 and 4 and was responsible for major areas of research question formation, data collection, data analysis and thesis composition. The experimental site was established in 2001 by Dr. Reg Newman. Sample collection assistance was provided by Dr. Reg Newman, Dr. Brian Wallace, Dr. Maja Krzic, Roz Kempe and Ivan Nesic. The Sustainable Agriculture Lab (SAL) Coordinator, Katie Neufeld, provide guidance and support on several laboratory procedures included in this study. Laboratory assistance with processing samples was provided by several outstanding undergraduate students. Specifically, a study by Thea Rodgers provided the permanganate-oxidizable carbon data in Chapter 2, while Michael Oh and Karly Vanichuk assisted with the soil aggregate stability and polysaccharide analyses. Dr. Maja Krzic was the supervisory author on this project and was closely involved in all aspects of the studies included in this thesis. This project was also done in close collaboration with Dr. Brian Wallace and Dr. Reg Newman, who contributed to the experimental design, project development and interpretation and presentation of the manuscript. Dr. Gary Bradfield and Dr. Sean Smukler also contributed project guidance and advice on data analysis and presentation.

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List of Abbreviations

ADF – Acid detergent fiber

BC – British Columbia

CSR – Contaminated Sites Regulation

FC – Field capacity

IDFxm - Very Dry Mild Interior Douglas-fir biogeoclimatic zone

MV – Metro Vancouver

MPN - most probably number

MWD – Mean weight diameter (of water-stable aggregates)

OMRR – Organic Matter Recycling Regulation

POXC - Permanganate-oxidizable carbon

PRS - Plant Root Simulator

PWP – Permanent wilting point

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Chapter 1: General Introduction

1.1 Background on biosolids

At one time, it was common practice across North America to release untreated sewage directly into waterways. Today, as regulated by the Municipal Wastewater Regulation under the BC Environmental Management Act, most municipalities in British Columbia (BC) have wastewater facilities to protect public health and keep waterways clean. The main objective of these facilities is to remove organic materials from wastewater (sewage) through various physical, chemical, and biological processes. The by-products of this process are solid residuals, termed biosolids once they have been further treated. Biosolids are defined as the stabilized municipal sewage sludge resulting from a municipal wastewater treatment process which has been sufficiently treated to meet requirements for safe land application (OMRR 2002).

1.1.1 The production of biosolids

The combination of physical, chemical, and biological processes used to treat wastewater and produce biosolids varies by municipality. Generally, wastewater treatment involves a pre-treatment, followed by primary, secondary, and sometimes tertiary treatments to effectively separate the effluent (which is returned to waterways) from the sludge (which is further processed to create biosolids). Pre-treatment involves screening wastewater to remove debris and grit before it enters the treatment facility. Primary treatment usually involves gravity sedimentation of wastewater to remove suspended solids. Secondary treatment generally involves a biological process, in which microorganisms are added to the wastewater to further remove suspended solids. Tertiary treatment is used to produce a higher

quality effluent, generally via chemical precipitation or other processes designed to remove nitrogen (N) and phosphorus (P) from the effluent (U.S. Environmental Protection Agency 1999).

The solids separated during the wastewater treatment processes are further processed to become a nutrient-rich, stable organic matter product. The biosolids treatment processes vary by municipality and according to the desired end-use of the product. The two most widely used biosolids treatment processes are stabilization and dewatering (U.S. Environmental Protection Agency 1999). Stabilization reduces pathogen levels, odor, and creates a less volatile product. Methods of stabilization include anaerobic digestion, aerobic digestion, composting, heat drying, and alkali (lime) stabilization. Dewatering is the process of removing excess water from the biosolids, and is a necessary step for most biosolids that will be transported or processed further (i.e., heat dried, incinerated, or composted). Common methods of dewatering include air drying, centrifuges, and belt filter presses.

Metro Vancouver (MV), which processes 440 billion litres of sewage per year, employs primary (physical) and secondary (biological) treatments at three of five wastewater treatment facilities, while the remaining two facilities employ primary treatment only (Metro Vancouver 2017). At the MV wastewater treatment facilities, 50-60% of the total suspended solids are removed by primary treatment, while 95% are removed by secondary treatment (Metro Vancouver 2017). The sludge, which includes the solids that were removed during the primary and secondary treatment as well as the bacterial biomass used during secondary treatment, are further treated to create biosolids.

In MV, all biosolids are anaerobically digested at either thermophilic (55^{o}C) temperatures for 18 days or mesophilic (38^{o}C) temperatures for 23-29 days to produce a stabilized product. Thermophilic digestion produces Class A biosolids, which contain fecal coliform levels of less than 1,000 MPN (most probably number) per gram of total solids, while mesophilic temperatures produce Class B biosolids, with fecal coliform levels of less than 2,000,000 MPN per gram of total solids (OMRR 2002). Following digestion, biosolids are dewatered by centrifuge.

The type of wastewater and biosolids treatment processes used, as well as the initial composition of the wastewater, influence in turn the quantity and quality of the biosolids produced by a municipality. Typically, biosolids contain between 31-67% organic matter (Sommers 1977), while greases, oils and waxes make up 5-20% of total solids (Moffet et al. 2005). Biosolids that are applied to rangeland in western North America generally contain between 2-5% N concentrations, a similar range as composted livestock manure (Blumenthal et al. 2017).

1.1.2 End use of biosolids

In BC, 38,000 dry tonnes of biosolids are produced at wastewater treatment facilities across the province every year. The ultimate management challenge is finding disposal or recycling options for this significant amount of organic material. The most common end-uses of biosolids in North America are landfilling, incineration, and land application (Roy et al. 2011). In 2016, 94% of biosolids produced in BC were directed to land application and the remaining 6% went to a landfill or were otherwise disposed (i.e., dumped at sea) (British Columbia Ministry of Environmental Protection and Sustainability 2016). Landfilling of

biosolids has become a less attractive option over the past decade, due to environmental concerns regarding greenhouse gas emissions (methane, nitrous oxide, and carbon dioxide) from landfilled material and the impact of landfill leachate on groundwater and surface waters (Powlson et al. 2011; Kjeldsen et al. 2002). Further to this, landfilling materials is becoming more costly as a result of the increase in materials from growing populations and decreasing landfill space. Land application involves spreading of biosolids on the soil surface, or incorporating or injecting biosolids into the soil. Incineration of biosolids exposes them to high temperatures in a combustor, and reduces them to ash that is one-fifth of the original volume (U.S. Environmental Protection Agency 1999). The ash is then disposed of in landfills or used in certain industrial processes such as cement production. While there is ongoing research as to the optimal treatment and use of biosolids, this report will focus on land application.

1.2 Land application of biosolids

Biosolids contain high levels of organic matter and nutrients including N and P, making them a valuable fertilizer and soil amendment. The land application of biosolids has been shown to increase levels of soil organic matter and accelerate nutrient cycling in degraded soils (Ippolito et al. 2010; Haney et al. 2015; Sullivan et al. 2006). Since 1990, Metro Vancouver has delivered hundreds of thousands of tonnes of biosolids for projects in rangeland and forestry fertilization, landfill, gravel pit and mine site reclamation, and city park landscaping. MV aims to send 100% of biosolids produced to such beneficial re-use projects, as opposed to sending it to the landfill. However, contract availability and public perception can greatly affect their ability to achieve this goal. Generally in BC, a higher

proportion of biosolids is directed to mine site reclamation than rangeland fertilization, reflecting the scale of those land use types in the province (Larney and Angers, 2012). In 2014, about 11,000 tons of MV biosolids went to rangeland fertilization (Force 2015). Most of that rangeland fertilization occurs in the southern interior of the province, where the majority of BC's grasslands are found.

1.2.1 Rates and regulation of rangeland application of biosolids in BC

Biosolids in Canada are provincially regulated. In BC, the Organic Matter Recycling Regulation (OMRR) of B.C. governs the land application process of biosolids under the *Environmental Management Act*. The OMRR sets forth standards for pathogen reduction, vector attraction reduction, as well as limits on allowable concentrations of metals for both Class A and Class B biosolids. Wastewater treatment facilities are required to perform frequent sampling of biosolids quality to ensure they meet the OMRR criteria for safe land application. Additionally, the OMRR requires that a Land Application Plan is developed by a Professional Agrologist prior to land application of Class A biosolids in quantities greater than 5 cubic meters (m^3) or of any quantity of Class B biosolids.

The Land Application Plan includes the proposed rate of application, which is based on providing the target crop's N requirement for a single growing season (McDougall et al. 2008). In dry areas of the province, applications can be made to provide the crop's N requirement for two growing seasons. These rates are calculated based on the crop N requirement, the N levels in the native soil, and the N levels in the biosolids amendment. Rangeland applications rates, however, are not based on agronomic rates due to the unique characteristics of the grassland ecosystem (McDougall et al. 2008). Although native

bunchgrasses have low N requirements, fertilization with biosolids can increase forage yields above unfertilized levels, especially in rangeland that has previously been degraded. Once N levels are increased, however, often soil moisture limits yield. To reduce the environmental degradation (i.e., grass smothering, soil compaction) that would occur with annual fertilization, large applications that far exceed annual forage uptake of N are made infrequently. In 2008, the industry rate for rangeland fertilization was an application that occurred only once every five years, which provided a maximum of 200-250 kg ha^{-1} of available N in the first year following application (McDougall et al. 2008). This is around 10 times the amount of N that is removed via grazing from a healthy bunchgrass range in a year (25 kg N ha^{-1}). Applications of biosolids to rangeland are spread directly onto the soil surface instead of incorporated, to avoid disturbing native vegetation. Semi-arid ecosystems offer unique benefits and risks for biosolids land application. The low levels of precipitation slow mineralization and reduce the risk of nutrient leaching, while typically alkaline soils have high sorption capacities for metals and P (White et al. 1997). Risks of biosolids application to grasslands are surface runoff during winter snowmelt, ammonia volatilization and other greenhouse gas emissions, and plant community degradation through undesirable species establishment (McDougall et al. 2008). Following the application of Class B biosolids, rangeland sites cannot be grazed for 60 days. No such restriction exists for Class A biosolids, due to their extremely low levels of pathogens.

1.2.2 Considerations regarding biosolids application to rangeland

In addition to receiving household sewage, wastewater treatment plants receive wastewater from industrial and commercial areas, which often have higher levels of trace

elements and organic contaminants. Prescription drugs, household products, beauty products, and a myriad of other synthetic products that are part of modern life also enter the sewers. The presence of organic contaminants in biosolids has caused public concern over the safety of biosolids for land application within the last decade.

The OMRR (2002) lists restrictions for 11 metals that are considered potential pollutants in the land application of biosolids: arsenic, cadmium, chromium, cobalt, copper, lead, mercury, molybdenum, nickel, selenium, and zinc. Conservative limits are imposed for the concentration of each of these metals in biosolids material, to ensure that biosolids can be safely applied to land. Whether the metals will be available or fixed in the soil receiving biosolids application will depend on several characteristics of the biosolids and the soil, such as pH, organic matter type and content, clay type and content, and the presence of Fe and Al hydrous oxides. Under the OMRR application regulations, levels of metals in the native soil must be assessed prior to biosolids application. In a study conducted by the BC Ministry of Environment, (2016) metal levels in the soil at the Jesmond study site used in this research project were well below the OMRR and Contaminated Sites Regulation (CSR). Most metals were actually more concentrated in the control, for unclear reasons. Cu, Ag, S, Sn were significantly higher in the biosolids plots, but they were well below the regulation limits.

To date, there are no regulations in place for organic contaminants, or “emerging substances of concern”. In a review of the contaminant levels in biosolids produced from Metro Vancouver’s five wastewater treatment facilities, levels of several potential organic contaminants commonly found in biosolids were assessed (Bright and Healey 2003). The few organic contaminants that were found in the biosolids included p-cresol, phenol,

phenanthrene, pyrene, naphthalene, and heavy extractable petroleum hydrocarbons. However, it was concluded that only the petroleum hydrocarbons posed an environmental risk during the land application of those biosolids at rates based on the metal limits set forth by the OMRR. The recent study by the BC Ministry of Environment (2016) evaluated various emerging substances of concern such as polycyclic aromatic hydrocarbons, phenolics, polychlorinated biphenyls, and polychlorinated dioxins, and furans in the soil at the Jesmond study site. All substances were well below the CSR standards. However, certain phenolics (o-Cresol, m-Cresol, and p-Cresol) and polychlorinated dioxins and furan concentrations were significantly higher in the biosolids plots. A recent literature review of the risks of biosolids land application in the Nicola Valley of BC (Land Resource Consulting Services 2016) concluded that more research is needed to better understand the potential for land-applied biosolids to contaminate ground and surface-water and impact wildlife and natural aquatic ecosystems.

1.3 Effects of biosolids application on rangeland health

1.3.1 The importance of grasslands as rangeland in BC

Grasslands cover approximately 0.74 million hectares in BC, representing less than 1% of the province's land base (Wikeem and Wikeem 2004). The majority of grassland ecosystems are located in the Central and Southern interior, Cariboo Chilcotin, Rocky Mountain trench and Peace River regions. Most grasslands in BC are found in the rain shadow of the coastal or interior mountain ranges, in dry valley bottoms or plateaus where moisture is not sufficient to support tree growth. The plateaus and valleys where grasslands are found are the result of glacial activity. The soil is derived from till, glacio-lacustrine or

glacio-fluvial deposits left by receding glaciers, or covered by subsequent caps of loess (Gayton 2003).

Plant species composition of BC's grasslands vary by latitude, elevation, climate and soils (Wikeem and Wikeem 2004). The Cariboo Chilcotin grasslands occupy 230,000 ha of land, 90% of which is found in the Fraser River Basin, Cariboo Basin, and the Chilcotin Plateau. The Lower Grasslands found along the Fraser and Chilcotin River valleys below 650 m elevation are shrub-steppe plant communities dominated by bluebunch wheatgrass [*Pseudoroegneria spicata* (Pursh) A. Löve], and big sagebrush (*Artemesia tridentata* Nutt.). Between 650 and 850 m elevation, the shrub steppe is replaced by grasslands dominated by bluebunch wheatgrass, needle-and-thread grass [*Hesperostipa comata* (Trin. & Rupr.) Barkworth], and mixed forbs as the landscape transitions from steep river valley slopes to rolling plateaus. Grasslands occupying the plateau surfaces along the Fraser and Chilcotin rivers are part of the Interior Douglas-fir biogeoclimatic zone. A climax plant community of these grassland phases would support short-awned porcupinegrass [*Hesperostipa curtiseta* (Hitchc.) Barkworth], bluebunch wheatgrass, spreading needle grass [*Achnatherum richardsonii* (Link) Barkw.], and Rocky Mountain fescue (*Festuca saximontana* Rydb.) ; mostly grass species that benefit from the increased moisture of higher elevation. The majority of the grassland soils below 1000 m elevation vary from Brown to Dark Brown Chernozem depending on elevation. Around 1200 m, the grasslands are largely replaced by the Sub-Boreal Pine-Spruce zone, characterized by stands of lodgepole pine, white spruce and wetlands ringed by trembling aspens (Wikeem and Wikeem 2004).

Grasslands in BC provide many important ecosystem services (including carbon sequestration, nutrient and water cycling, soil preservation, biodiversity maintenance) as well as play an important role in the ranching industry. Ranching has been an integral part of BC's interior landscape since the influx of goldrush miners in the late 1850s (McLean 1982). Today, approximately 4,086 cattle ranches operate in BC, representing 5% of cattle in Canada (BC Cattlemen's Association 2018). There are over 10 million hectares of active rangeland in BC, 85% of which is tenured Crown land. Eight million ha of BC rangeland is forested, while 1.3 million ha are located on grasslands (Wikeem et al. 1993).

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When properly managed, grasslands can be productive forage systems while maintaining their ecological integrity. However, many grasslands in BC and across North America have been substantially altered following the introduction of livestock by European settlers in the mid-19th century, and some continue to be impacted today by urban development, crop production, recreational activities, and overgrazing. Once degraded, grasslands can take a long time to recover; large areas of grasslands in the interior of BC still show the effects of extensive overgrazing that occurred over a century ago (Wikeem and Wikeem 2004). There is interest, therefore, in the use of organic amendments such as

biosolids to increase forage production to levels that were previously supported by BC's grasslands, as well as questions around the role of biosolids in the restoration of degraded grasslands. Comprehensive assessments of range health and restoration include both soil and plant quality indicators (Newman et al. 2011), as outlined below.

1.3.2 Effects of biosolids on rangeland soil properties

1.3.2.1 Soil physical properties

Aggregate stability is an important indicator of soil structure, one which connects soil physical, chemical and biological processes. Stable aggregates contribute to a soil's ability to resist erosion, maintain rates of water infiltration, and sequester carbon (C) (Ryals et al. 2014; Blankinship et al. 2016). Generally, the literature on the effects of biosolids application to rangeland and forage crops has shown that biosolids have positive effects on aggregate stability (García-Orenes et al. 2005; Wallace et al. 2009; Wallace et al. 2016). Biosolids, like any other organic soil amendment, act as a food source for microbes and plants, which both play important roles in the biological formation of aggregates (Blankinship et al. 2016). Increases in microbial activity, plant root exudates, and above ground plant litter inputs are the necessary catalysts for increased aggregate creation and stability. On a semi-arid grassland site in the interior of BC, a single application[1] of anaerobically digested biosolids at 20 and 60 Mg ha^{-1} significantly increased the size and stability of soil aggregates eight years after the application (Wallace et al. 2016) as compared to the control and a N + P fertilizer treatment, suggesting that one biosolids application can have an ongoing positive effect on soil structure for many years. There was no significant difference between the aggregate

[1] All rates of biosolids application are presented in dry weight, unless otherwise specified.

stability of the two levels of biosolids treatment (20 and 60 Mg ha^{-1}); however, an identical trial established in the same year on a pasture of crested wheatgrass found that soils applied with biosolids at 60 Mg ha^{-1} had significantly greater aggregate stability eight years later, while the 20 Mg ha^{-1} rate appeared more similar to the control and fertilizer treatments. However, biosolids have not unequivocally shown to improve soil aggregation. A no-till perennial forage production system of coastal bermudagrass (*Cynodon dactylon* L.) in Travis County, Texas, received anaerobically digested Class B biosolids for seven years at 20, 40, and 60 Mg ha^{-1} y^{-1} (Jin et al. 2015). While soil organic carbon levels were positively correlated with biosolids application rate in the top 0-15 cm, along with increases in available water content, the quantity of soil water stable aggregates decreased linearly with application rate. The authors speculated that this was due to the chemical constituents of the biosolids, which can include dispersers such as humic substances, anionic and non-ionic surfactants, and high amounts of Na^+, K^+, and organic P.

Other studies have focused on the effects of biosolids on soil hydrological properties. Harris-Pierce et al. (1995) demonstrated that semiarid blue grama (*Bouteloua gracilis*) plots amended with biosolids had higher infiltration rates, but were not significantly different in runoff to precipitation ratios when compared to untreated plots during simulated rain events that occurred two week after application. Sediment content was, however, increased in runoff from biosolids plots. Moffet et al. (2005) showed that biosolids application to Chihuahuan Desert grassland increased infiltration, and decreased runoff and erosion. The greatest marginal reductions in erosion were achieved at the lower biosolids application rates (7 and 34 Mg ha^{-1}), while the difference between the higher rates (34 and 90 Mg ha^{-1}) was not significant. Sullivan et al. (2006) found that the effects of biosolids on soil water content

depended on the amount of soil water; after a rain event they found that the plots receiving at least 5 Mg biosolids ha^{-1} had significantly greater water content than nonamended control plots, but this difference was not seen at dryer times.

Soil bulk density has not generally been shown to change significantly as a result of biosolids application (Wallace 2007; Ryals et al. 2014). However, biosolids applied to degraded agricultural and salinized soils in the Southwest region of Spain resulted in a decrease in bulk density (García-Orenes et al. 2005). Conversely, another study (Haney et al. 2015) found increasing bulk density with increasing biosolids application rate, which was attributed to the greater machinery traffic required for higher rates of application. Bulk density did not increase with duration of applications (number of years for which applications were made), suggesting the soil compaction was not exacerbated over time.

1.3.2.2 Soil chemical properties

Biosolids contribute organic matter to soils, which in turn improves soil structure and fertility. Organic matter has many measurable benefits to soil quality, including improved soil water retention, soil aggregation, soil nutrient balance, and reduction of soil erosion (Tisdall and Oades 1982). Organic amendments, such as biosolids, have been of interest as a means of increasing C stocks in degraded rangeland soils, which would improve soil functioning and have the potential added benefit of sequestering global C. This increase in C stocks can occur directly through the incorporation of the C in the amendment into the soil system, and indirectly via increased primary production (Ryals et al. 2014). For C to be truly considered "sequestered", it must involve a net transfer of C from the atmosphere to soil (via photosynthesis), where it is incorporated into a fraction of organic matter resistant to

decomposition (Powlson et al. 2011). One mechanism of protecting organic matter from decomposition is the physical protection of C within aggregates, sometimes termed the physically "occluded" organic matter fraction. An amendment of organic green waste compost was applied at 70 Mg ha^{-1} to two degraded grasslands in California (Ryals et al. 2014). Both sites experienced increases in total soil C and N three years after the application. In that study, it was found that the organic amendment had been incorporated into the free light fraction (a labile pool of soil C) at both grassland sites, as well as the physically occluded organic matter fraction at the coastal grassland in just three years. The heavy fraction (mineral particle associated carbon) did not change at either site. These results demonstrate that organic matter directly applied to the soil surface can be incorporated into the labile and occluded organic fractions over a relatively short period of time, despite slow changes to the mineral associated C pool. Indirect increases in organic matter via increased plant production due to an organic amendment application are less well documented. Single biosolid applications at rates greater than 21 Mg ha^{-1} to semi-arid short grass steppe rangeland in Colorado resulted in concomitant increases in soil total C as observed 13 years after application (Ippolito et al. 2010). However, it was unclear whether this was attributable to direct additions of C from the biosolids, or indirect C additions from long-term increases in plant productivity.

Biosolids applications to rangeland have often been found to lower the pH of the native soils (Fresquez et al. 1990), an effect that also was noted 20 years after a single application of biosolids to semi-arid grassland in Colorado (Ippolito et al. 2010). One simple explanation for this is that the biosolids material is often more acidic than the native grassland soil, and their addition therefore lowers the pH. However, there are additional

reactions that occur over many years of microbial decomposition and mineralization of organic matter that have acidifying effects on the soil environment.

When applied to rangelands, biosolids contribute significant amounts of N and P, typically the first and second most limiting nutrient in these ecosystems. Dried, anaerobically digested biosolids were surface-applied to a degraded semi-arid grassland site in New Mexico, at rates of 0, 22.5, 45, and 90 Mg ha^{-1} (White et al. 1997). Nine years after the application, the soils with the highest amendment rates (45 and 90) had significantly greater N mineralization rates than the low rate and the control, indicating that the sites with higher biosolids application rates were benefitting from higher levels of fertility. Despite initial increases in concentration proportional to application rates within the first four years, eight years after the application most nutrients and water soluble elements showed no treatment effect, having either been taken up by plants or microorganisms, leached or washed away. Only P, B, and Mn levels did not decrease with time since application, and Al was not affected by either biosolids application rate or time since application. In another study at the coastal bermudagrass forage production fields in central Texas, Haney et al. (2015) examined the effects of repeated applications of biosolids on soil N, P, and K nutrient loading and depth distribution. Biosolids were applied at 0, 20, 40, and 60 Mg ha^{-1} y^{-1} for eight consecutive years. At the end of the eight years of applications, total soil extractable inorganic N and P increased linearly with application rate, while extractable K was not affected. They also examined the effect of application duration by using a chronosequence fields receiving 20 Mg ha^{-1} y^{-1} of biosolids for 0, 8, and 20 years, and found that only extractable P increased with duration of applications. Extractable N levels increased with increasing rates of biosolids application, but were not affected by duration of application,

likely due to the labile nature of inorganic N. Elevated concentrations of nitrate were found throughout the soil profile (0-110 cm) while increases of orthophosphate were found in surface soils (0-40 cm) applied with biosolids. Higher rates of application (40 and 60 Mg ha^{-1} y^{-1}) were also found to result in higher N mineralization rates. Similarly, a one-time application of biosolids to shortgrass steppe in Colorado was shown to enhance N cycling over 12 years following a single application (Sullivan et al. 2006).

1.3.2.3 Soil biological properties

The application of biosolids to the soil surface provides a fresh food source for microbial populations which can affect the composition of the microbial community. Sullivan et al. (2006) recorded increases in bacteria-to-fungal ratios in shortgrass steppe rangeland plots receiving 30 Mg ha^{-1} applications of biosolids on both long-term (>12 years previously) and short-term (<2 years previously) timeframes. Dennis and Fresquez (1989) found a positive relationship between microbial populations and biosolids application rates, as well as decreases in fungal diversity at the higher application rates. Barbarick et al. (2004) examined microbial responses in semi-arid grasslands applied with biosolids at 30 Mg ha^{-1} and found increased indicators of microbial activity (increased CO_2 evolution and actively metabolizing microbial biomass) six years after the application. Indications that microbial activity are increased in the long term due to a single biosolids application has positive implications for long term soil structure and nutrient cycling ability.

1.3.3 Effects of biosolids on rangeland forage production

The benefits of biosolids to forage production are well documented. The use of biosolids has been shown to increase forage quantity and quality (McFarland et al. 2007; Jurado-Guerra et al. 2013; Paschke et al. 2005) in semi-arid grasslands. Forage quality involves measures of protein (N) content, palatability, nutrient content, and trace elements. The Cu/Mo ratio is of particular importance due to the occurrence of molybdenosis in ruminants that consume forage with Cu/Mo ratios lower than 2, which causes Cu deficiency. Perennial grass biomass was increased by up to 300% under 25 Mg ha^{-1} biosolid application rates in a semi-arid shrubland dominated by mountain big sagebrush (*Artemisia tridentata)* and mixed grass species (Pierce et al. 1998). Additionally, the biosolids application increased the Cu/Mo ratio from below 1.2/1 to above 2/1 for western wheatgrass and bluebunch wheatgrass one year after application at low rates (10 or 15 Mg ha^{-1}) and two years after application at intermediate rates (25-40 Mg ha^{-1}). Perennial grass tissue N concentrations also increased by 60-70%, which enhanced the nutritional value of the forage. Sullivan et al. (2006) also found a doubling of plant tissue N concentration from the control to the highest application rate (30 Mg ha^{-1}), and a decrease in plant tissue C:N ratio with increasing biosolids application rates in both short-term and long-term plots. The ability of biosolids to improve forage quality and quantity has been demonstrated, but less is known as to how long these benefits last.

1.3.4 Effects of biosolids on rangeland plant community

Biosolids are often surface applied to degraded grasslands with the intention of restoring them to their previous productive state. The application of biosolids, which

provides a slow-release source of N and P and micronutrients, has been shown to stimulate plant growth and litter production (Ippolito et al. 2010; Wallace et al. 2009; Pierce et al. 1998). In the restoration process, not only the productivity but also the composition of the plant community is of importance. Native perennial grasses contribute high levels of C to the soil in the form of plant root exudates and root sloughing from their extensive root system. Forbs and annual grasses have less extensive root systems and contribute less C to the soil. Exotic grass and forb species can dramatically alter the ecosystem functioning of a grassland and reduce forage levels for wildlife and livestock (Blumenthal et al. 2017). Applications of inorganic N, the most limiting nutrient in grassland ecosystems, has repeatedly been shown to favour invasive plant species with high reproductive rates and an ability to capitalize on an excess of nutrients at the expense of more slow-growing native perennials, which are adapted to low nutrient levels. However, the effects of organic N additions, which are more slowly released, are not as well understood (Ryals et al. 2014).

Studies focused on the impact of biosolids on native plant communities produced mixed findings. Some studies have shown that biosolids surface applications to degraded rangeland benefit native plant communities (Newman et al. 2014; Sullivan et al. 2006; Paschke et al. 2005), while others have shown applications to provide competitive advantage to early seral or exotic species (Newman et al. 2014; Blumenthal et al. 2017; Wallace et al. 2016). Newman et al. (2014) originally established the study site used in my research project in Jesmond, BC as part of a larger study examining the effects of a 20 Mg ha^{-1} biosolids application on the plant community composition of three semi-arid rangeland sites. After four years, biosolids application had increased the abundance of late-seral target species bluebunch wheatgrass (*Pseudoroegneria spicata)* at the Jesmond site, while negative and

non-significant responses were found at the other two sites, which were drier and had higher levels of invasive species pre-application. This study highlighted the importance of the state of the pre-application plant community as a factor in the long-term effect of the application. At a native mixed-grass prairie site in Wyoming, Blumenthal et al. (2017) performed plant productivity measurements on plots that had received fresh cattle manure, composted cattle manure, and composted biosolids (at 22.4 Mg ha^{-1}) 22 years previously. Despite initial increases in range productivity recorded within the first four years after the application, the composted cattle manure application resulted in the long-term invasion of cheatgrass (*Bromus tectorum)* at the site. Biosolids, however, did not result in a significant increase in cheatgrass abundance. Blumenthal et al. (2017) suggested that a lack of long-term studies on the effects organic amendments on grassland plant species composition may result in overestimations of the benefits of such applications to the native plant community. The concept of a "lag phase" in the community structure of a degraded grassland that has received nutrient additions has been proposed, based on a fertilized sandy grassland that did not diverge along a distinct successional pathway from the control until five years after the fertilization (Faust et al. 2012). More long-term studies are needed to understand the effects of organic amendments such as biosolids on rangeland plant species composition.

1.4 Summary of general introduction

Biosolids represent both a management challenge and potential opportunity for beneficial re-use. As urban populations increase, municipalities are looking for alternative destinations for biosolids beyond landfills, such as the application of biosolids to rangeland. Biosolids destined for land application are regulated to limit the concentration of heavy

metals and pathogens to safe levels, and application rates are made based on N content. Rangeland in BC is well suited to biosolids application due to its dry climate and alkaline soils. Additionally, the inherent limitations of water and nutrients in semi-arid rangeland suggest that these agroecosystems could benefit greatly from biosolids application.

Rangeland forage production has been shown to increase under biosolids application, likely due in part to the N and P contents of the material. Biosolids differ from a synthetic fertilizer, however, in that they contain appreciable amounts of organic matter, which can increase total soil C, increase infiltration, decrease erosion, increase aggregate stability, and provide a slow-release source of nutrients. Since long-term studies on the effects of biosolids application on rangeland soil are relatively few, it is unclear how long these benefits to soil quality, and by extension, forage production will be sustained. Furthermore, few studies examine the long-term effects of biosolids application on rangeland plant species composition, which is an important aspect of rangeland health. It has been suggested recently that more long-term studies are needed to ensure that slow, unanticipated changes in plant species composition do not occur as a result of organic matter applications to rangeland (Blumenthal et al. 2017).

1.5 Thesis objectives

This study examines the effects of biosolids application on rangeland soil quality and plant community productivity and composition. It is guided by the following objectives and associated hypotheses.

Objective 1 – Determine the effects of a single surface biosolids application on soil properties [the stability of aggregates, bulk density, total C and N, permanganate-oxidizable

carbon (POXC), polysaccharides, pH, nutrient availability, and water holding capacity] 14 years following application to ungrazed rangeland in the central Interior of BC.

Hypothesis 1 – Fourteen years after biosolids application, there will be evidence of improvements in soil properties, including increased stability of soil aggregates, lower bulk density, increased total soil C and N, increased POXC, increased polysaccharides, increased availability of soil nutrients, and/or improved soil water holding capacity relative to control without biosolids application.

Study objective 1 will be addressed in the Chapter 2 of this thesis.

Objective 2 – Determine the effects of a single surface application of biosolids on forage quality and quantity 14 years after application to ungrazed rangeland in the central Interior of BC.

Hypothesis 2 – Fourteen years after biosolids application, there will be improved forage quality and quantity in the biosolids treatment relative to control treatment.

Study objective 2 will be addressed in the Chapter 3 of this thesis.

Objective 3 – – Determine the effects of a single surface application of biosolids on plant species composition 14 years after application to ungrazed rangeland in the central Interior of BC.

Hypothesis 3 – Fourteen years after a single application, biosolids will improve plant species composition in the long-term by increasing cover of native perennials and decreasing cover of forbs and non-natives relative to control treatment.

Study objective 3 will be addressed in the Chapter 3 of this thesis.

Objective 4 - Determine the effects of surface applied biosolids on soil aggregate stability and the distribution of total C, total N, and polysaccharides within soil aggregates 14 years after application to ungrazed rangeland in the central Interior of BC.

Hypothesis 4 - Fourteen years after a single application, biosolids will increase C and N and polysaccharide concentrations found within stable soil aggregates relative to control treatment.

Study objective 4 will be addressed in the Chapter 4 of this thesis.

Chapter 2: The Long-Term Effects of Biosolids on Soil Properties of Ungrazed Semiarid Rangelands[2]

2.1 Introduction

Biosolids are stabilized sewage sludge resulting from municipal wastewater treatment processes, which meet requirements for safe land application (OMRR 2002). They contain appreciable amounts of nitrogen (N), phosphorus (P), and organic matter, and are regulated to meet safe pathogen levels and metal contents. In British Columbia (BC), 38,000 dry tonnes of biosolids are produced at wastewater treatment facilities every year, the majority of which are destined for land applications such as mine reclamation and to a lesser extent rangeland fertilization (Larney and Angers 2012). The majority of rangeland fertilization in BC occurs on open grassland rangelands in the Central and Southern Interior, where the semi-arid climate reduces the risk of nutrient leaching. For example, in 2014, about 11,000 tons of Metro Vancouver biosolids went to rangeland fertilization (Force 2015) in the Interior of BC.

The land application of biosolids has been shown to increase soil organic matter and plant nutrients availability in rangeland soils (Ippolito et al. 2010; Haney et al. 2015; Sullivan et al. 2006). Long-term increases in N mineralization have been found after biosolids applications of 30 Mg ha^{-1} or greater (Sullivan et al. 2006, White et al. 1997), suggesting a long-term shift in microbial community. When applied to the soil surface, biosolids can reduce soil water evaporation and moderate soil temperatures by acting as a physical mulch

[2] NOTE: A version of this chapter will be submitted for publication in the Canadian Journal of Soil Science - E. Avery, M. Krzic, B. Wallace, R.F. Newman, S.M. Smukler, and G. Bradfield. The Long-Term Effects of Biosolids on Soil Properties of Ungrazed Semiarid Rangelands.

(Wester et al. 2011). Biosolids have also been shown to increase soil aggregation up to eight years after a single application (Wallace et al. 2016). While many studies have documented the effects of biosolids on rangeland soil properties, relatively fewer studies examine the long-term (i.e., greater than 10 year) impacts of a single application (Ippolito et al. 2010; Sullivan et al. 2006; Paschke et al. 2005). How long the effects of a one-time biosolids application on rangeland soil properties last, and which effects are still evident after 10^{+} years is of interest to land managers and regulatory bodies of organic matter land applications alike.

The objective of this study was to determine the effects of a single surface biosolids application on soil properties [the stability of aggregates, bulk density, total C and N, permanganate-oxidizable carbon (POXC) and polysaccharides, pH, nutrient availability, and water holding capacity] 14 years following application to ungrazed rangelands in the central Interior of BC.

2.2 Materials and Methods

2.2.1 Site Description

The study was conducted in 2016 on the long-term field experiment established in 2002 at a working ranch near Jesmond, in the South Cariboo region of BC (51° 25' N, 122° 09' W; elevation 1,100 m). The site is located on the Fraser Plateau, on the eastern side of the Fraser river valley. The soil is a loam Orthic Dark Brown Chernozem formed on glacial moraine, overlaid by a thick layer of aeolian deposits (Valentine et al. 1987; Wikeem and Wikeem 2004). It belongs to the Chimney association, which are the dominant grassland soils of the northeast Fraser Plateau. The topography is very gently (2-5%) to gently (6-9%)

sloping. The soil at the study site has a loam texture with 14% clay, 46% silt, and 40% sand, with negligible amounts of coarse fragments (see Appendix A for more detail on soil texture). No carbonates were found in the top 35 cm of soil at the study site.

The study site is in the grassland phase of the Very Dry Mild Interior Douglas-fir (IDFxm) biogeoclimatic subzone (Wikeem and Wikeem 2004). The dominant late-seral vegetation of this zone is bluebunch wheatgrass [*Pseudoroegneria spicata* (Pursh) A. Löve], spreading needlegrass [*Achnatherum richardsonii* (Link) Barkw.], and short-awned porcupinegrass [*Hesperostipa curtiseta* (Hitchc.) Barkworth], along with an understory of native forbs and cryptogams. At the time of the experiment establishment in 2002, 26% of the site area had exposed mineral soil and the vegetation was dominated by needle-and-thread grass [*Hesperostipa comata* (Trin. & Rupr.) Barkworth], with secondary species of Junegrass [*Koeleria macrantha* (Ledeb.) J.A. Schult. f.], low pussytoes [*Antennaria dimorpha (Nutt.) T. & G.]*, white pussytoes (*Antennaria microphylla* Rydb.), and prairie sagewort (*Artemisia frigida* Willd.); indicating an early- to mid-seral stage and site degradation (Newman et al. 2014).

This region is located the rain shadow of the Coastal mountains, receiving a total of 415 mm precipitation annually, of which 146 mm as snow. The highest rates of precipitation occur in late spring or early summer (May, June and July). In the summer, dry heated air rises from the Fraser river valley and contributes to dry conditions on the adjacent plateau, which are interrupted by intense precipitation events (Wikeem and Wikeem 2004). The mean annual temperature is 3.8ºC, while the mean January temperature is -7.8ºC and the mean July temperature is 14.8ºC. The region receives 101 frost-free days per year, and 1,174 growing

degree days (base 5ºC) (Climate BC 2017). In the year of data collection for this study (2016) Jesmond, BC had an unusually wet growing season, receiving 276.2 mm precipitation from May to September (Appendix B).

In the fall of 2002, establishment of the long-term field experiment at the Jesmond site began with the selection of four randomly located blocks with uniform plant species composition and cover. Each block (60 m × 70 m in size) was fenced with a 1.5 m-high barbed wire to prevent cattle grazing. The experiment was laid out in a randomized complete block design with two treatments: (i) no biosolids (control) and (ii) single surface application of biosolids at 20 dry Mg ha^{-1} replicated once in each of four blocks. The biosolids were surface applied using a pull-type manure spreader without subsequent incorporation. The biosolids were supplied by the Annacis Island wastewater treatment plant in Metro Vancouver, BC where they had been anaerobically digested and dewatered. They contained total N and P contents of 54.91 g kg^{-1} and 29.99 g kg^{-1} (dry weight), respectively. More detailed information on the biosolids used in this study can be found in Appendix C.

2.2.2 Sampling and Analysis

Soil aggregate stability

Soil samples were collected on 28 April (the beginning of the growing season and time of maximum precipitation in this region), 6 June (time of maximum growth of cool-season grasses in this region), 29 August (mid-late growing season, post-peak grass production time), and 6 October (end of growing season) of 2016 to assess soil aggregate stability. Three 50 m long transects (Appendix D) were established per treatment plot (i.e., biosolids and control plots within each block). One composite sample per transect was

obtained by combining six subsamples taken every 7 m along the transect. Subsamples were collected with a trowel from the 0 to 7.5 cm depth. Any loose organic residues (including biosolids), sod layer, or microbial crust present on the soil surface were removed before sampling.

Samples were transported to the laboratory in lidded plastic containers and refrigerated at 4ºC until analysis. The stability of soil aggregates was assessed using a variation of a wet sieving method (Nimmo and Perkins 2002). In the laboratory, field moist samples were sieved through a 6-mm sieve and aggregates were collected on a 2-mm sieve. Fifteen grams of soil aggregates (2-6 mm) were placed on top of a nest of sieves with openings of 2, 1, and 0.25 mm after being thoroughly moistened with a humidifier. This was done to minimize aggregate disruption during wet sieving due to air trapped inside of the aggregates. Wet sieving was performed for 10 min at a vertical stroke rate of 30 strokes per minute and an oscillating action through an angle of 30º. After removing the sieves from the water, the material retained on each sieve was oven dried at 105ºC for a minimum of 12 hours. Once dry, the material on each sieve was crushed in a mortar and pestle and passed back through its respective sieve. The mass of the mineral particles remaining on each sieve was subtracted from the dry soil mass that passed through the sieve, to avoid biased estimates of water stable aggregates. The results were expressed as mean weight diameter (MWD), which is equal to the sum of products of (1) the mean diameter of each aggregate size fraction and (2) the proportion of total weight occurring in the corresponding size fraction.

To adjust for the water content of the soil prior to wet sieving, 5 g of each soil sample was weighed and placed into an oven at 105ºC for 12 hours of drying, and weighed again to

determine gravimetric soil water content. This was then used to adjust the initial mass of the aggregates prior to wet sieving, so as to obtain accurate proportions of the total mass of aggregates within each size class.

Soil water retention

Intact soil cores (35.5 cm^3) were collected at 5-cm below the soil surface on 5 October 2016. A total of four cores were collected from each plot for a total of 32 cores for soil water retention determination using a pressure plate apparatus (Klute 1986). These data were then used to establish the soil water retention characteristics curve.

Soil volumetric water and temperature

Volumetric soil water content was monitored from late April 2016 to February 2017. Soil sensors that measure the dielectric permittivity of the soil using capacitance and frequency domain technology (Decagon Devices, Pullman, WA) were installed in one of the four blocks (in both control and biosolids plots) at three depths which allowed to monitor the following portions of the soil profile: soil surface (0-10 cm), main root zone (20-30 cm), and below the main root zone (40-50 cm). The soil sensor installed at the soil surface (0-10 cm depth) also measured soil temperature.

Soil bulk density

Bulk density was determined on undisturbed soil core samples taken from the 0- 7.5 cm depth on 5 October 2016. Soil samples were taken with a double-cylinder, drop-hammer sampler, with a 7.5-cm diameter by 7.5-cm deep core. A total of four cores were collected from each treatment plot for a total of 32 samples. The cores were dried for 24 h at 105^{o}C,

and soil bulk density was determined as the mass of oven dry soil per unit volume of field moist soil (Blake and Hartge 1986).

Soil carbon fractions

Total soil C and N, polysaccharide, and POXC were determined on soil samples collected on 29 August 2016, using the same sampling procedure as described for aggregate stability analysis. One composite sample per transect was obtained by combining six subsamples taken every 7 m along the transect. Subsamples were collected with a trowel from the 0 to 7.5 cm depth. In the laboratory, field moist samples were passed through a 6 mm opening sieve, air dried, crushed with a rolling pin, and sieved through a 2 mm sieve.

Total soil C and N were determined by dry combustion method (Nelson and Sommers 1996) using a LECO CNS-2000 automated analyzer (LECO Corp., St. Joseph, MI).

Soil polysaccharides were determined using the phenol-sulfuric acid method for labile polysaccharide analysis described by Lowe (1993). To release the polysaccharides by hydrolysis with sulfuric acid, 0.50 g of soil was placed in an Erlenmeyer flask with 100 mL 0.5 *M* H_2SO_4 and autoclaved for 1 h at 103 kPa. The samples were cooled and filtered into 250-mL volumetric flasks and made to volume using distilled water. Glucose standards of 20, 30, 40, 50 and 60 μg mL^{-1} were prepared fresh for each analysis by diluting a stock glucose solution (1000 μg mL^{-1}) with various amounts of deionized water. 1 mL of the polysaccharide solution was combined in a cuvette with 5 mL concentrated H_2SO_4 and 1 mL phenol solution. The same was done with 1 mL of each of the glucose standards, and 1 mL of deionized water to create a blank. To ensure proper mixing of the cuvette contents, the H_2SO_4 was added using a burette. Samples were heated at 30ºC for 25 min. Three 250 μL drops

from each sample (as well as the blank and the glucose standards) were added to a 96 well-plate, and the absorbance of the samples was read at 490 nm in a microplate spectrophotometer (TECAN Group Ltd., Zurich, Switzerland). Quantification of polysaccharides was assessed based on a standard curve made with glucose standards.

The analysis of POXC, also referred to as active C, was based on the Weil et al. (2003) method and the detailed procedure by Culman et al. (2012). A stock solution of 0.2 *M* $KMnO_4$ was prepared in a $CaCl_2$ solution at a pH 7.2. Standards of 0.005 *M*, 0.01 *M*, 0.015 *M* and 0.02 *M* were produced by diluting the $KMnO_4$ stock solution with various amounts of deionized water. To prepare the samples, 5 g of soil were placed into 50-mL screw-top centrifuge tubes. 2 mL of $KMnO_4$ and 18 mL of deionized water was added to each centrifuge tube. The tubes were sealed with a lid and shaken for exactly 2 minutes at 240 oscillations per minute on an oscillating shaker. Samples were placed in a dark room and left to settle for exactly 10 minutes. Following this step, 0.5 mL of the supernatant were added to new centrifuge tubes containing 49.5 mL of H_2O. The tubes were sealed and inverted to mix the solution, and three 250 µL drops from each sample were added to a 96-well plate. A blank of deionized water and four standard stock solutions (0.005 *M*, 0.01 *M*, 0.015 *M* and 0.02 *M* $KMnO_4$) were added to the same well-plate. Plates were run on a microplate spectrophotometer (TECAN Group Ltd., Zurich, Switzerland) at 550 nm to determine absorbance. Deionized water blanks were then subtracted from all values and a standard curve was produced. The following equation was used to determine POXC (AC):

$$AC\ (mg\ Kg^{-1}\ soil) = [0.02\ mol/L - (B_0 + B_1 \times Abs)] \times (9000\ mg\ C/mol) \times (0.02L\ solution/Wt)$$

Where:

0.02 mol/L = initial solution concentration
B_0 = intercept of the standard curve
B_1 = slope of the standard curve
Abs = absorbance of the sample
9000 = milligrams of C oxidized by 1 mole of MnO_4 changing from Mn^{7+} to $Mn4^{+}$
0.02 L = volume of stock solution reacted
Wt = weight of air-dried soil sample in kg

Soil pH

Soil pH was determined on soil samples taken on 29 August 2016, using the same sampling method as described for soil carbon fractions. Soil samples were air dried, crushed with a rolling pin and sieved through a 2 mm opening sieve. Soil pH was determined using a 1:2 (v/v) soil to 0.01 *M* $CaCl_2$ slurry (Hendershot and Lalande 1993).

Plant available nutrients

Plant Root Simulator (PRS®) probes were used to determine plant available nutrients during four sampling periods throughout the 2016 growing season. The PRS® probes (Western Ag Innovations, Saskatoon, SK) allow *in situ* evaluation of the net rate of ion adsorption in a soil during the burial period (i.e., ion availability; μg ion 10 cm^{-2} 2 wk^{-1}). The four burial periods in this study were: 27 April – 12 May, 26 May – 6 June, 6 June – 20 June, and 20 July – 3 August 2016. Four root exclusion cylinders (25 x 20 cm) per plot were first inserted into the soil to eliminate the competition for ions from adjacent plants. In each cylinder, two probes (a single anion and a single cation probe) were inserted perpendicular to the soil surface to a depth of 10 cm using a soil knife of similar dimension to the probe. This ensured complete contact between the resin membrane on the probe and soil. The probes

remained in the soil for 12 to 16 days for each burial period. Before installation and after removal from the soil, probes were stored in plastic bags and kept cool with ice packs. Probes were quickly rinsed with deionized water in the field to remove any residual soil until a thorough cleaning with a brush and more deionized water could be done in the laboratory. The probes were sent to laboratory of the Western Ag Innovations, Saskatoon, SK for determination of the following ions: NO_3^-, NH_4^+, $H_2PO_4^-$, K^+, SO_4^{2-}, Ca^{2+}, Mg^{2+}, Fe^{3+}, Cu^{2+}, Zn^{2+}, B^{3+}, Mn^{2+}, Al^{3+}, Pb^{2+}, and Cd^{2+}.

2.2.3 Statistical Analysis

Soil data were analyzed as a randomized, complete block design with four replications. Sub-samples collected on treatment plots were averaged by treatment plot prior to analysis. All statistical analyses were preformed using R statistical software version 3.4.0 (R Core Team 2017). Mixed effect models were constructed using the *nlme* package (Pinheiro et al. 2017). For soil properties that were sampled only once (bulk density, pH, total C and N, polysaccharides, POXC, water retention characteristics), treatment (biosolids or no biosolids) was the only fixed effect in the model. Blocks were included as random effects. For soil properties that were assessed multiple (3-4) times throughout the 2016 growing season (aggregate stability, aggregate water content and plant available nutrients), treatment, sampling date and a treatment × sampling date interaction were the fixed effects. Random effects included blocks and the nested experimental units (treatment plots) within blocks. I also included a continuous autoregressive correlation structure to account for temporal correlation for repeated measures of a sample in time, where necessary. Following a significant F-test generated by ANOVA, post-hoc tests (Bonferonni method) were done to

identify differences between fixed effect levels. An example ANOVA for both single measure type data and repeated measures type data is included in Appendix E. For all models, assumptions were assessed for each model and log transformations were made where necessary to meet assumptions. A significance level of 0.05 was used for all tests.

2.3 Results and Discussion

Soil aggregate stability

Averaged across sampling dates (Figure 2-1), the biosolids treated plots had a MWD of 1.6 mm (±0.06 SEM), which was significantly higher ($p=0.04$) than on the control plots with MWD of 1.4 mm (±0.06 SEM). These findings are similar to another study carried out on a semiarid grassland in BC with a single surface biosolids application (Wallace et al. 2016) in which biosolids plots eight years following the application still had greater MWD (1.8 mm) Generally, it has been reported that soil applied with sewage sludge reached maximum aggregate stability anywhere from one week to four years after application (Abiven et al. 2009). The more recalcitrant nature of composted biosolids leads to more gradual, but longer lasting changes in aggregate stability as compared to more labile organic matter additions such as raw sewage sludge and green manures. It is possible that the increase in MWD noted in my study, 14 years after the application, could be a result of a positive feedback loop between increased plant growth and plant-induced changes in soil structure. Perennial grass cover increased from 41% to 99% in the first 4 years after biosolids application, as the plant community responded to an influx of available nutrients (Newman et al. 2014), and has remained at high levels since then as will be shown in Chapter 3. Plant roots secrete polysaccharides and other organic compounds that can act both as a glue to bind

mineral particles (thus starting the aggregation process) and as a food source soil microbes (Tisdale and Oades 1982; Angers and Caron 1998). Further to this, fine roots physically enmesh aggregates, along with their mycorrhizal fungi counterparts (Angers and Caron 1998).

Aggregate MWD was also significantly affected by sampling date ($p < 0.01$), being at least 34% lower in April than any other sampling date (Figure 2-2). There was no interaction ($p = 0.14$) between treatment and sampling date. The effect of sampling date on MWD was likely due in part to soil water content, which has been shown to be negatively correlated to MWD (Krzic et al. 2000; Wallace et al. 2009). Soil particle contact points and capillary forces decrease with increasing soil water content, which leads to overall decreases in soil strength (Angers and Caron 1998). Although aggregate water content in April was not statistically different than August or October (Appendix F), it is likely that the recent snow melt and typical higher spring precipitation that had maintained high moisture levels for several weeks leading up to the April sampling.

The largest size class (aggregates of 2 – 6 mm in diameter) had a similar trend to the MWD (Figure 2-3) which is not surprising because this size class has the greatest mass and therefore the greatest influence in the calculation of the MWD. However, the treatment effect on the proportion of stable aggregates 2 – 6 mm was not significant ($p = 0.06$). The 1 - 2 mm aggregate size class was significantly more abundant in the biosolids treated plots ($p = 0.03$), while no treatment effect was found for the smallest (0.25 – 1 mm) aggregate size class ($p = 0.3$). The proportion of soil in the size class < 0.25 mm (which includes soil microaggregates and non-aggregated mineral particles) was significantly greater in the control treatment than

the biosolids treatment ($p = 0.01$), and followed an inverse trend to the largest aggregate size class (2 – 6 mm). This size class represented the greatest proportion of soil of all aggregate size classes in both treatments, representing 55% and 47% of total soil aggregates in the control and biosolids treatments, respectively.

All aggregate size classes were significantly affected by sampling date (Figure 2-4) suggesting that this soil property is highly temporal in nature. While the largest aggregates (2-6 mm) followed a trend similar to the MWD over time, likely affected by water content and duration of wet periods, the other fractions followed different trends over time, suggesting that there are other mechanisms affecting these size classes.

Soil water retention characteristics

Despite a trend of increased volumetric water in the biosolids plots, no significant increases were found between the biosolids and control plots at any of the seven soil water tension values assessed (Appendix G). Soil texture, soil structure, and soil organic matter content determine a soil's water retention characteristics, affecting total pores space and pore size distribution (Hillel 1982). Increases in volumetric water content at saturation (Point 1) indicate an increase in total pore space, whereas increases in volumetric water content at high levels of soil matric potential indicate an increase in micropores, which hold soil water more tightly than macropores. Small increases in both the FC and PWP in the biosolids plots resulted in the same plant available water content as the control plots (15.1% and 14.9%, respectively).

Soil volumetric water

The growing season of 2016 was uncharacteristically wet (Appendix B), which was

reflected in the soil water content (Figure 2-5). In the early season (May and June), the biosolids plot exhibited slower draw down of volumetric water content during dry periods in the soil surface (0-10 cm) and the rooting zone (20-30 cm). The average values determined by the Soil water characteristics curve (Appendix G) for FC (25.8%) and PWP (10.9%) at the Jesmond site (plotted as horizontal dotted lines in the 0-10 cm depth of Figure 2-5) indicate that there was sufficient plant available water for most of the growing season with the exception of a drought period in June which coincides with a period of very low precipitation in 2016. In the biosolids plot, this soil volumetric water content was below the PWP from June 20th to July 5th, whereas in the control plot the drought conditions lasted from June 8th to July 5th. Although the volumetric water probe data was not replicated, the dynamics of the soil surface water content (0-10 cm) was statistically confirmed by replicated gravimetric water content assessments taken for the aggregate stability measurements from the 0-7.5 cm depth. These measurements showed a significant interaction between sampling date and treatment. Biosolids plots had significantly greater gravimetric soil water content on 28 April and 6 June, but no differences were found on 29 August and 6 October (Figure 2-6). Below the rooting zone, the 40-50 cm depth showed little fluctuation in volumetric water content, and the biosolids water content was slightly above the control water content the entire growing season (Figure 2-5).

The higher soil water content and shorter drought conditions observed in the biosolids plot in the early season could have significant impacts on plant productivity in moisture limited ecosystems such as the Jesmond site. However, the reasons for the higher soil water content in the biosolids plots in May and June 2016 at 0-10 cm and 20-30 cm depths are not entirely clear. One possibility is that soil evaporation was the controlling mechanism for soil

water content early in the season, while plant transpiration became more important later in the season after peak above ground biomass was reached (late June). Higher soil temperature (as seen in bottom graph, Figure 2-5) and greater exposure of mineral soil measured in the control would result in faster draw down of soil water in the control than in the biosolids plots throughout the growing season. Later in the growing season, soil water evaporation will decrease in both plots due to greater soil cover provided by aboveground biomass, a mechanism which may affect the control plots more drastically due to the greater magnitude of change of soil cover in those plots. Meanwhile, the aboveground biomass on the biosolids plots, being more than double that on the control plots, would increase transpiration losses of soil water. The combination of increased soil water transpiration from above ground biomass on the biosolids plots, and reduced soil water evaporation from the control could result in the synchronization of soil water dynamics between the biosolids and control treatments that we recorded later in the growing season (July-October 2016). This explanation is supported by the fact that the lowest depth (40-50 cm), where the biosolids plots retain marginally higher soil water content throughout the growing season, is below the main rooting depth and therefore would not be affected by greater plant transpiration.

It is also possible that the biosolids plots, having greater aboveground biomass, retained a greater snowpack over the 2016 winter, which in turn resulted in increased soil water content in the spring. Vegetation height, density and cover greatly influence the depth of snow retained on site, especially in open grassland ecosystems (Pomeroy and Brun 2001). It is estimated that 77% of the total snow received in fallow fields in the southern Canadian prairies is eroded by wind (Pomeroy and Brun 2001), and is subsequently either sublimated or redistributed to sites with higher aerodynamic roughness (i.e., sites with greater amounts

of vegetation) (Pomeroy and Brun 2001). It is possible that the biosolids plots, having greater vegetation cover and height, accumulate and retain a deeper snowpack over the winter which results in greater soil water contents in the early spring.

Soil bulk density

Soil bulk density was not affected by the biosolids treatment 14 years after application (Table 2-1). Similarly, Wallace et al. (2009) found no effects of biosolids on soil bulk density, four and five years after the surface application of biosolids to a crested wheatgrass rangeland in the southern interior of BC. Most studies that have reported significant effects of biosolids on soil bulk density (Epstein et al. 1976; García-Orenes et al. 2005; Kladivko and Nelson 1979; Hemmat et al. 2010) involve the incorporation (via tillage or mixing) of high rates of biosolids into the soil profile, as opposed to the surface application of relatively lower rates of biosolids as done in my study. A review of the effects of biosolids on soil properties by Clapp et al. (1986) reported a general trend of decreasing bulk density with increasing biosolids application and found that the reduction of bulk density was most affected by the rate of biosolids application. The effects of biosolids on bulk density in this review were only noted up to 48 months after application. The literature suggests that given the application method, rate and the duration of time since application at the Jesmond site, it is not surprising to find no effects of the biosolids on soil bulk density. This being the case, any changes in bulk density occurring 14 years after a 20 Mg ha^{-1} biosolids surface application would be expected to be a result of long-term increased levels of above ground biomass contributing to improved soil structure on the site. This was not yet evident, however, at the Jesmond site.

Total soil C

Fourteen years following biosolids application, there was no difference in total soil C stock (kg m^{-2}) in plots with and without application (Table 2-1). Several studies have found long term increases in soil C contents with increasing rates of biosolids application (Tian et al. 2009; Ippolito et al. 2010). Generally, changes in soil C are seen after cumulative loading rates that are much larger than the rates used in this study (Tian et al. 2009; Jin et al. 2015)). Likely the closest in application rate to my study was the study by Ippolito et al. (2010), where a single application of biosolids at 30 Mg ha^{-1} in semi-arid grasslands in Colorado increased soil C by 6 to 10 g kg^{-1} in the 0-8 cm depth relative to the control 14 years after application. Most short-term studies that have reported changes in soil C level after biosolids application have found the differences in the top 0-8 cm, which is expected when biosolids are surface applied to forage without tillage (Cogger et al. 2013, Ippolito et al. 2010). However, eight years of continuous applications did result in linear increases of soil C with application rate in the 0-15 cm depth. Furthermore, long-term increases in aboveground perennial production caused by biosolids application should be matched by increases in below-ground root production. At my study site, the root zone (0-30 cm) may therefore have had accumulations of soil C on the biosolids plots below the depth that was studied here (0-7.5 cm).

Organic matter amendments can increase soil C levels directly from the C inputs in the amendment, and indirectly from increased plant production. Organic matter additions of biosolids at 20 Mg ha^{-1} contribute relatively small amounts of C to the soil, especially given a mineralization rate of around 30-50% in the first year (Wallace 2007; McDougall et al.

2008). In fact, Cogger et al. (2006) calculated that within the year of application an average biosolids application of 20 Mg ha^{-1} with a moderate first season decomposition rate of 30% would directly increase the soil C content of a soil with an initial organic C content of 2.50% (much like in my study) to 2.56% (calculated to the 30 cm depth). This was considered unlikely to result in any substantial changes in soil C or other soil properties such as bulk density or aggregate stability. Additionally, organic amendments applied to the soil surface likely contribute less C to the soil than when tilled or otherwise incorporated due to limited contact between the amendment material and the soil. This in turn leads in organic matter decomposition with reduced opportunities for physical protection (or stabilization) of organic matter by mineral soil particles (Powlson et al. 2011; Hopkins et al. 2009). It is likely that, 14 years after the single application, most direct C additions to the soil from the biosolids application in Jesmond, BC have been already mineralized and the fraction of potentially mineralizable organic matter that remains is negligible.

It was hypothesized that biosolids application would result in indirect increases in soil C due to increased plant growth in the biosolids plots over the 14 years of the experiment's duration. Management practices that increase plant productivity, such as N-fertilization of grasslands, are often accompanied by increases in soil C storage (Derner et al. 2007; Fornara and Tilman 2012). However, not all management practices that increase plant productivity have been found to increase soil C in the long-term. The level of C stored in a soil at any given time is based on an equilibrium between losses of C to decomposition and the inputs of C from net primary production. For example, Condron et al. (2014) found that grazed pastures that were irrigated for 62 years contained 34% less soil organic C than dry-land pastures, despite supporting 74% higher pasture production. Consistently greater respiration

rates of soil organisms in irrigated plots led to greater organic matter mineralization, which likely outweighed the C additions from the increased plant production.

Carbon sequestration occurs when C generated by net primary productivity is added to the soil, through root exudates, sloughing of root material, or litter decomposition. To be sequestered, C must be "stabilized" or protected from microbial decomposition by physico-chemical mechanisms (Six et al. 2002; Gregorich et al. 1994). It has been hypothesized that all soils have a C saturation point, or a maximum C storage potential based on ecosystem characteristics, climate, geomorphology and soil mineralogy (Six et al. 2002; Masiello et al. 2004). In accordance with the C saturation theory put forth by Six et al. (2002), soils with the lowest organic C content (e.g., degraded soils) have been shown to have a greater rate of C stabilization than those already close to saturation (Powlson et al. 2012; Larney and Angers 2012).

Given the relatively high C levels in the control of the study sites, it is possible that this site is near to its maximum C storage capacity, and therefore accumulates C more slowly. In the study at Meadow Springs Ranch in Colorado (Ippolito et al. 2010), the C concentration (0-8 cm) they found at the highest biosolids application rate (30 Mg ha^{-1}) 14 years after the application was 2.51%, whereas the C concentration in control in my study (0-7.5 cm) was 2.54%. The soils at the Meadow Springs Ranch received 330-380 mm of mean annual precipitation, had a loam texture and 1.59 % C in the control, suggesting greater levels of C degradation than those seen at the Jesmond site. Presence of early seral plan species in 2002 at the time of study establishment at the Jesmond study site (Newman et al. 2014) is an indication that site was in degraded state, but since soil C levels on plots with and without

biosolids application were the same (Table 2-1) 14 years later suggests that level of degradation was not extensive.

Finally, total soil C is a large, slow changing pool, and any significant changes via plant inputs can take many decades to occur, especially in dry grassland ecosystems (Burke et al. 1995). It is possible that, given more time, the increased plant productivity in the biosolids plots of my study would result in changes to the C pool.

The C:N ratio of biosolids is typically within 7-10 (Cogger et al. 2006). In my study, the C:N ratio of the 0 – 7.5 cm soil layer on both treatments with and without biosolids application was 11 (Table 2-1). The lack of effect of biosolids application on C:N ratio suggests that these soils have reached an equilibrium level of C and N mineralization that is no longer affected by the biosolids application. Sullivan et al. (2006) found an increase in C mineralization rates in a semi-arid shortgrass steppe one year after biosolids application, whereas 12 years after application there was no difference in C mineralization rates between plots with and without biosolids. They also found that biosolids application increased soil bacterial biovolumes (i.e., visual counts of bacteria and fungi present) in the short term (1 year), but no differences were found in the long term (i.e., 12 years after application). Hence, it is possible that any effects that biosolids had on microbial activity immediately after application in Jesmond are no longer occurring.

Labile C pools

POXC and polysaccharides represent fractions of organic C that are labile, and more responsive to management changes than total organic C. While aggregate stability has been shown to correlate with total organic C, there is evidence that it is more strongly correlated

with C fractions that are more labile (Degens 1997; Liu et al. 2005). POXC, also known as 'active C', includes the C most readily decomposable by microorganisms, as well as C loosely bound to soil mineral particles. POXC has been considered more useful than total C in monitoring short-term changes in organic matter, due to its more rapid turn-over rate of two to five years (Lucas and Weil, 2012). Labile polysaccharides (also referred to as dilute acid extractable, or hydrolysable, polysaccharides) are microbial and plant root exudates that have been shown to correlate significantly with aggregate stability and are thought to be involved in aggregation (Martin 1971; Liu et al. 2005).

Despite moderate increases in MWD in the biosolids amended soil relative to the control, no differences were found in POXC ($p=0.24$) (Table 2-1). There was, however, a significantly greater polysaccharide content in the control than on biosolids treatment ($p=0.02$) (Table 2-1). Given the lack of treatment effect on the aggregate polysaccharide concentration, polysaccharides are not likely the main mechanism leading to the increased MWD in the biosolids treated soil. It is possible that there are other dynamics at play in aggregation processes in these semi-arid grasslands. Evans et al. (2012) found a significant negative correlation with polysaccharides and soil aggregate MWD, in their study on the effects of grazing time and rate on soil quality indicators. Their study was conducted on similar soils to those in this study: Dark Brown and Brown Chernozem soils of loam texture, at a Bluebunch wheatgrass grassland in Southern Interior of BC. They found the most significant positive correlations with MWD to be root biomass, bulk density and pH. In another study, the stability of macroaggregates under grassland was directly related to the length of external vesicular arbuscular mycorrhizae (VAM) and to the length of fine roots (0.2 – 1 mm in diameter) (Miller and Jastrow, 1990).

The greater polysaccharide content in the soil of the control plots was particularly unexpected, as polysaccharides have been correlated with aggregation, which was greater in the biosolids treated plots (Figure 2-1), and increased plant inputs (presumed higher in the biosolids treated plots due to higher above ground biomass). Polysaccharides include a larger class of compounds that range in their degradability and effectiveness in soil binding (Martin et al. 1971). The greater polysaccharides in the control could signify that either the plant root exudates and/or soil microbial community differ between the control and biosolids plots, which in turn may affect the levels of soil polysaccharides. It is notable that the C:N ratio of the forage grown in the control plots is significantly lower than forage grown in the biosolids plots, as will be discussed in Chapter 3. Differences in the C:N ratio of plant inputs (via roots and surface residue) affects microbial activity in the soil and could result in different production rates of polysaccharides.

Soil pH

Fourteen years after application, soil pH was significantly lower in the biosolids treated plots (Table 2-1). Biosolids plots had a mean pH of 5.8, while the control plots had a mean pH of 6.2. Similarly, Ippolito et al. (2010) found that soil pH was significantly lower on biosolids amended plots 13 years after a single application of biosolids to semi-arid grassland in Colorado. However, the pH of the biosolids in the study by Ippolito et al. (2010) was 5.0. The pH of the biosolids used in Jesmond was around 8.0, so it was not the material that lowered the pH directly. Rather, several soil processes triggered by the addition of organic matter can contribute to the acidification of soil over time. Soluble cations coming from biosolids can displace H^+ ions from soil clay and organic matter binding sites, while the

conversion of organic N and sulfur to nitrates and sulfates produces acidity (Sullivan et al. 2015). Therefore, 14 years of organic matter decomposition along with enhanced root activity has likely had an acidifying effect on the soil on my study site.

Total soil N

Total soil N content (kg m^{-2}) was hypothesized to be greater in the biosolids treatment, but exhibited no treatment effect in 2016 at the Jesmond site (Table 2-1). The application of 20 Mg ha^{-1} at the Jesmond site contributed 1,000 kg total N ha^{-1} to the biosolids amended plots in 2002. Based on the Biosolids Land Application Guide (McDougall et al. 2008), biosolids residuals contain around 20% mineral N (almost entirely in the form of NH_4^+), which is immediately available in the year of application, along with a further 10-20% of organic N that will mineralize in the first year. A further 10 and 5% of the organic N is predicted to mineralize in year two and three. Given that N is highly mobile in soil, it is not surprising that there is no longer any difference in total soil N content in biosolids and control plots.

Biosolids studies that do report increase in total soil N either had much higher application rates (Martínez et al. 2003) or were done on soils that were very low in N at the time of application. For example, in a study by Ippolito et al. (2010) on semi-arid rangeland in Colorado where 30 Mg ha^{-1} of biosolids were applied resulted in 0.19% total soil N relative to 0.11% total soil N on the control, 14 years after application.

Plant available nutrients

The availability of most plant nutrients and metals measured by the PRS probes varied significantly throughout the growing season, but only the availability of P ($H_2PO_4^-$

ion) and Fe^{3+} exhibited a significant treatment effect (Figure 2-7 and Figure 2-8). Both were higher in the biosolids plots across all sampling dates. The availability of Ca^{2+}, Mg^{2+}, K^{+}, B^{3+}, SO_4^{2-}, Mn^{2+}, Al^{3+}, NH_4^{+} and NO_3^{-} was the same in the biosolids and control treatments. Cu^{2+} and Zn^{2+} exhibited a significant interaction between treatment and sampling date, whereby Cu^{2+} was higher in the biosolids plots during the 3rd and 4th burial periods (June 6-June 20 and July 20-August 3), while Zn^{2+} was significantly higher in the biosolids plots during the 3rd period only. The only elements unaffected by either biosolids treatment or by sampling date were Al and B.

It should be noted that these results represent the availability of various elements (macronutrients, micronutrients, and Al^{3+}) to plants as opposed to their total content in the soil. The relationship between total nutrient content in the soil and the nutrient ion availability in the soil solution is not necessarily linear, as it depends on environmental conditions such as pH, soil water content, and concentrations of competing ions. The plant availability of several soil micronutrients (i.e. zinc, manganese, and iron) decreases with increasing pH, while the availability of molybdenum tends to increase with increasing pH (Sillanpaa 1982). It is generally believed that slightly acidic soils with a pH between 6 and 7 have optimal plant availability of soil micronutrients, as well as soil P (Brady and Weil 2007). Given the difference in pH between the biosolids and control treatments was a matter of 0.4 pH units (6.2 in the control plots and 5.8 in the biosolids plots) it seems unlikely that the relatively small difference in pH between the biosolids and control plots is responsible for the significant increase in availability of P or select micronutrients in the biosolids plots (Sims 1986; Sillanpaa 1982; Hinsinger 2001).

The biosolids application added a total of 600 kg ha^{-1} of P to the plots 14 years ago. Unlike the more mobile cations, losses of inorganic P from the soil are generally very small. Given that no plant removal has occurred since the application of biosolids, and the immobile nature of P in soils, it is likely that there is a greater total P pool due to the biosolids application that continues to be available to plants.

All nutrients that were found to be either significantly higher in the biosolids plots across all dates or significantly higher at certain dates due to an interaction between treatment and sampling dates (P, Fe, Zn and Cu) are relatively immobile in soils. All nutrients that showed no effect of biosolids treatment are somewhat to very mobile in soils (except for Mg, which is considered immobile). It is likely that the excess mobile nutrients which were not taken up into above ground biomass and litter within the first few years after application have been lost from the soil. The lack of treatment effect on both NH_4 and NO_3 ions agree with the earlier finding that the amount of total N is not different between biosolids and control plots (Table 2-1).

The regulation of biosolids that are to be land applied includes restrictions on metal contents. Of the elements measured with the PRS probes, only Zn and Cu are included in the list of heavy metals whose concentration in biosolids is regulated, due to their ability to adversely affect human and ecosystem health when present in high concentrations (OMRR 2002). Both Zn and Cu were well below the allowable concentrations in the original biosolids material (Appendix C) so there is little reason to believe that the increased levels of available Zn and Cu measured in the soil in 2016 are averse to ecosystem or human health. However, the long-term effect of biosolids application on the availability of these metals is worth

noting. Recent studies on the distribution of Cu and Zn in biosolids have found Cu in biosolids to be mostly bound by organic matter, whereas Fe-oxides are more important for binding Zn (Donner et al. 2012; Tella et al. 2016). Thus, the availability of Zn is governed by desorption rates from Fe oxides, while Cu availability is largely governed by the mineralization of organic C. The fact that Cu was significantly higher in the last two sampling periods of 2016 season suggests that either mineralization of the original biosolids material is continuing to occur later in the season, or that Cu that was released via mineralization of biosolids was subsequently bound by soil organic matter and continues to be released under conditions favorable to mineralization.

The availability of most nutrient ions (Ca^{2+}, Mg^{2+}, Fe^{3+}, K^{+}, $H_2PO_4^{-}$, SO_4^{2-}, Mn^{2+}, NH_4^{+} and NO_3^{-}) was directly affected by sampling date (Figure 2-7 and Figure 2-8). Most of these nutrients (except NH_4^{+}) were significantly higher in the last sampling dates than the other dates. This was likely due to a large precipitation event (27.3 mm) that occurred on August 2nd, 2016, the day before PRS probes were collected (Figure 2-5). Soil water content has a large effect on the diffusion rate, solubility, and mineralization or immobilization of soil ions, which all in turn affect plant uptake. In semi-arid grasslands, "pulse" precipitation events such as the event on August 2, 2016 are typical and increase nutrient availability by creating conditions conducive to increased mineralization, solubility of inorganic compounds and ion diffusion (Austin et al. 2004). As we saw with Cu and Zn, soil conditions can alter the treatment effects of biosolids.

2.4 Conclusions

Fourteen years after a single surface biosolids application at 20 Mg ha^{-1} to rangeland in Jesmond, BC, greater soil aggregate stability, increased early season soil water content, lower pH and greater availability of certain soil nutrients (P and Fe at all sampling dates, and Cu and Zn at select sampling dates) were observed relative to the control. Significant differences in soil water retention, total soil N, and availability of mobile soil nutrients were not found between biosolids and control treatments. Additionally, despite 14 years of increased plant productivity in the biosolids treatment, no significant differences were found in bulk density, total soil C content or the labile soil POXC fraction, while significantly greater contents of soil polysaccharides were found in the control relative to the biosolids treatment.

The biosolids application improved aspects of soil structure and soil water regime in the long-term exclosures, likely in part due to increased plant production and resulting accumulation of litter in the biosolids treatment over 14 years. The long-term increased availability of P and of less mobile trace elements (Fe, Cu and Zn) in the biosolids treatment highlights the importance of testing pre-application soil levels of these nutrients, especially on sites that have received biosolids previously. The lack of difference in soil C between biosolids and control plots suggests that either the site soil C levels are not as degraded as previously thought, or the plant C inputs are offset by rapid mineralization, perhaps made possible by greater soil water content and improved structure. Overall, land managers may see long-term improvements in certain soil properties resulting from rangeland biosolids application in the absence of grazing. Soil C restoration from biosolids application will

depend on the state of the site, as well as other factors determining the equilibrium between carbon inputs and respiration.

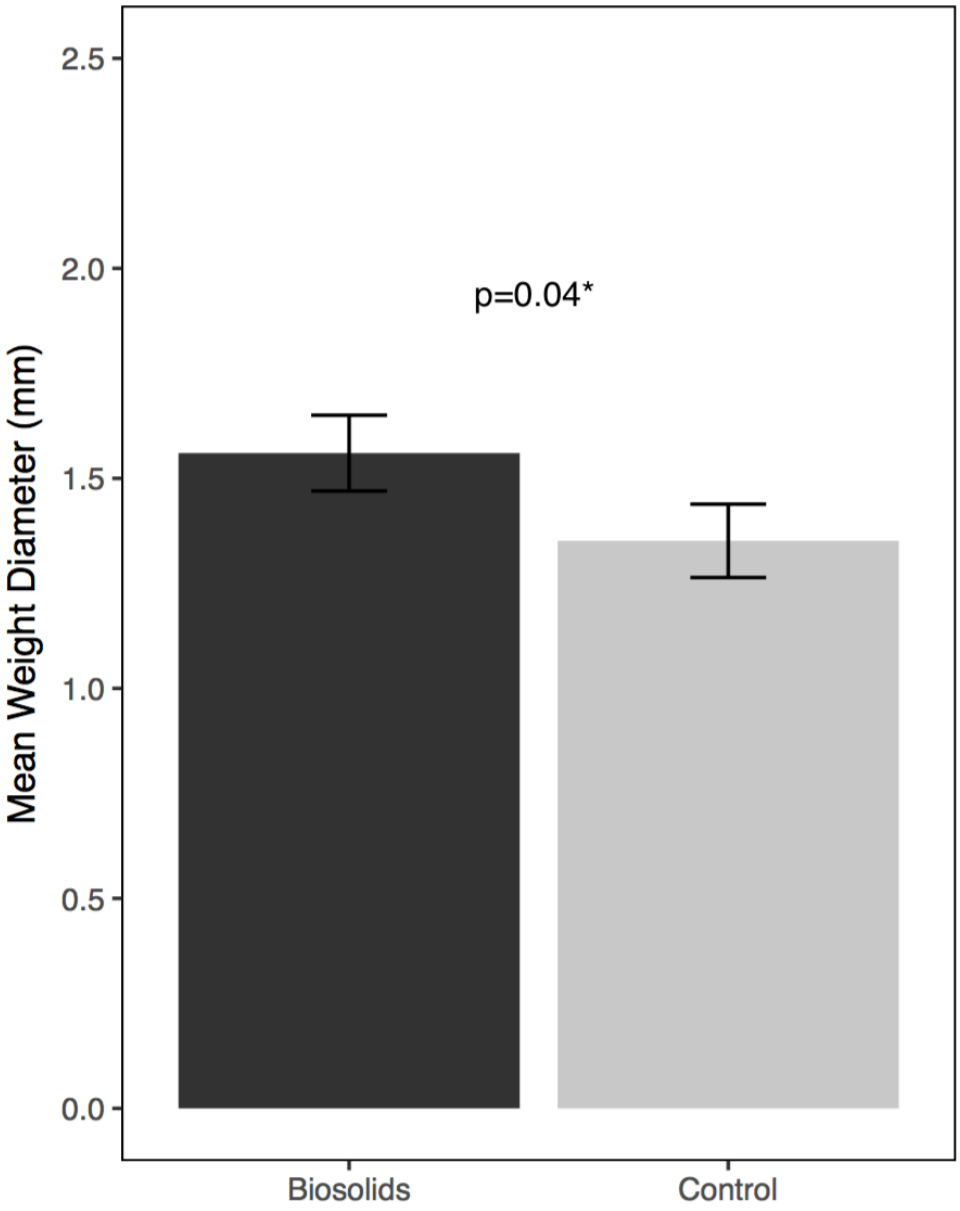

Figure 2-1 Mean weight diameter (MWD) of water stable aggregates biosolids and control treatment, 14 years after surface biosolids application on rangeland in the central Interior of British Columbia (BC). Error bars represent the standard error of the mean (n=4); * indicates significant difference at $p < 0.05$

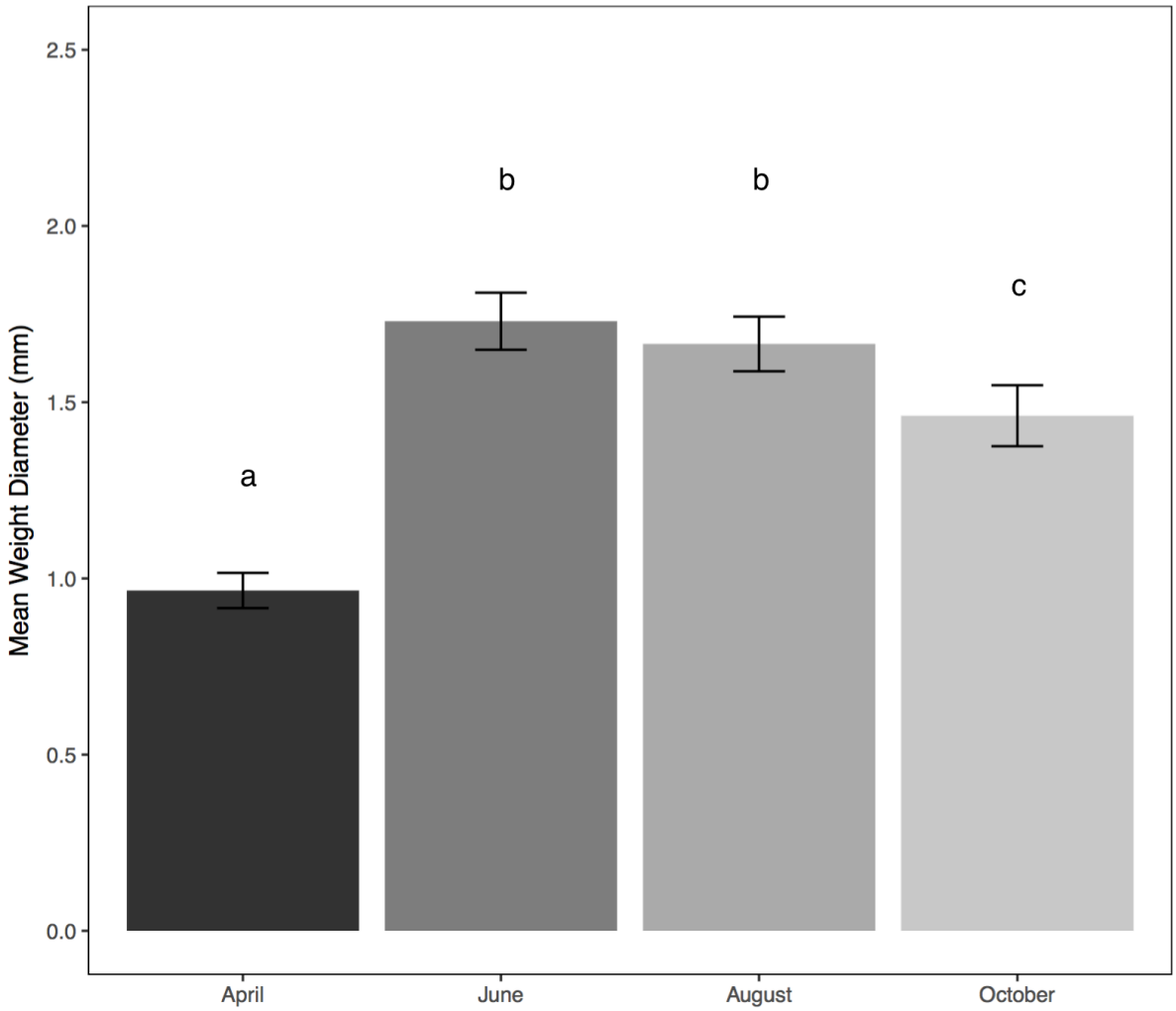

Figure 2-2 Mean weight diameter (MWD) of water stable aggregates by sampling date as determined 14 years after surface biosolids application on rangeland in the central Interior of British Columbia (BC). Error bars represent the standard error of the mean (n=4). Bars with different letters are considered significantly different at $p \leq 0.05$.

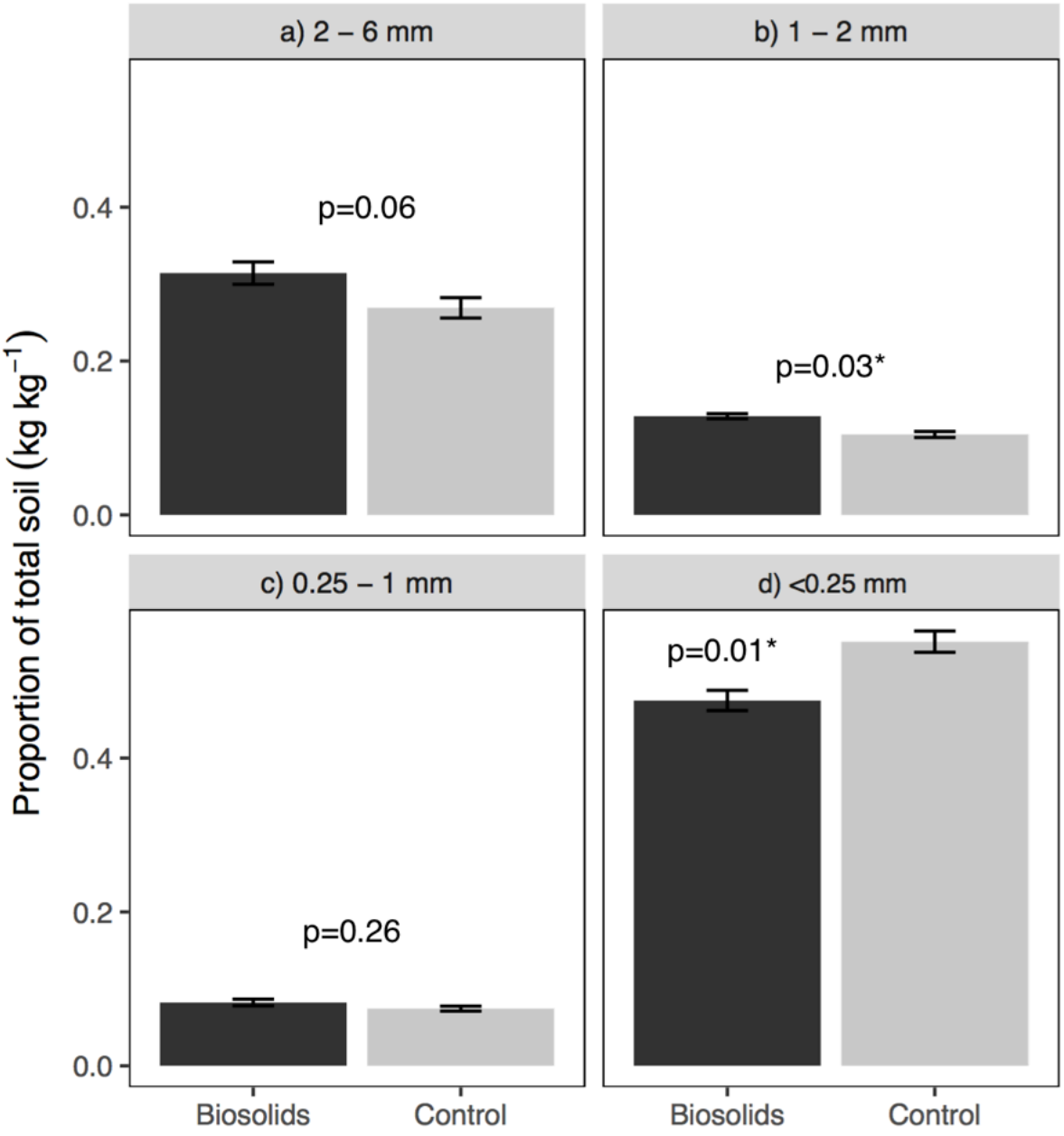

Figure 2-3 The proportion of total aggregates in (a) the 2 – 6 mm water-stable size class, (b) the 1 – 2 mm water-stable size class, (c) the 0.25 – 1 mm water-stable size class, and (d) the <0.25 mm size class between biosolids and control treatments 14 years after surface biosolids application on rangeland in the central Interior of British Columbia (BC). Error bars represent the standard error of the mean (n=4). * indicate significant difference at $p < 0.05$

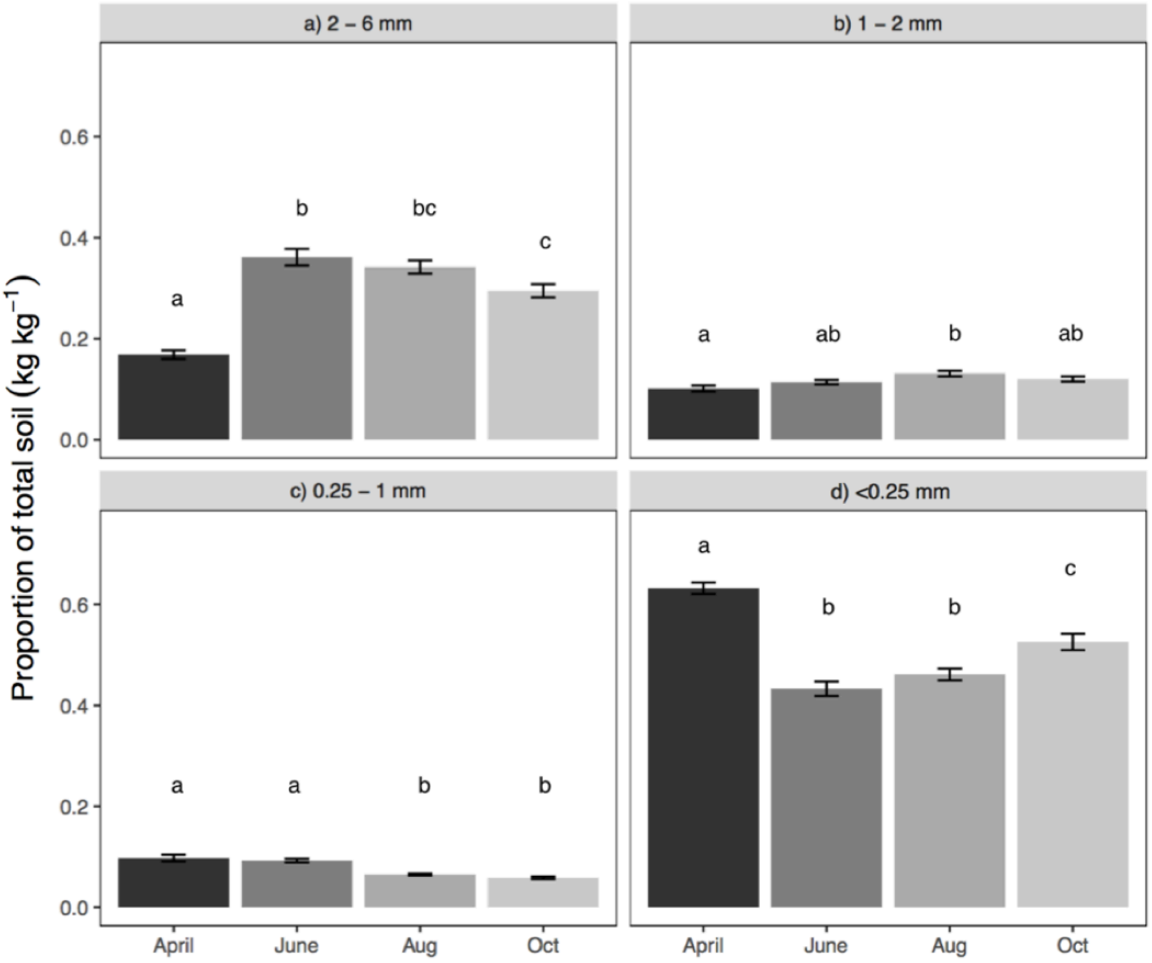

Figure 2-4 The proportion of total aggregates in (a) the 2 – 6 mm water-stable size class, (b) the 1 – 2 mm water-stable size class, (c) the 0.25 – 1 water-stable mm size class, and (d) the <0.25 mm size class among four sampling dates 14 years after surface biosolids application on rangeland in the central Interior of British Columbia (BC). Error bars represent the standard error of the mean (n=4). Bars with different letters are considered significantly different at $p \leq 0.05$.

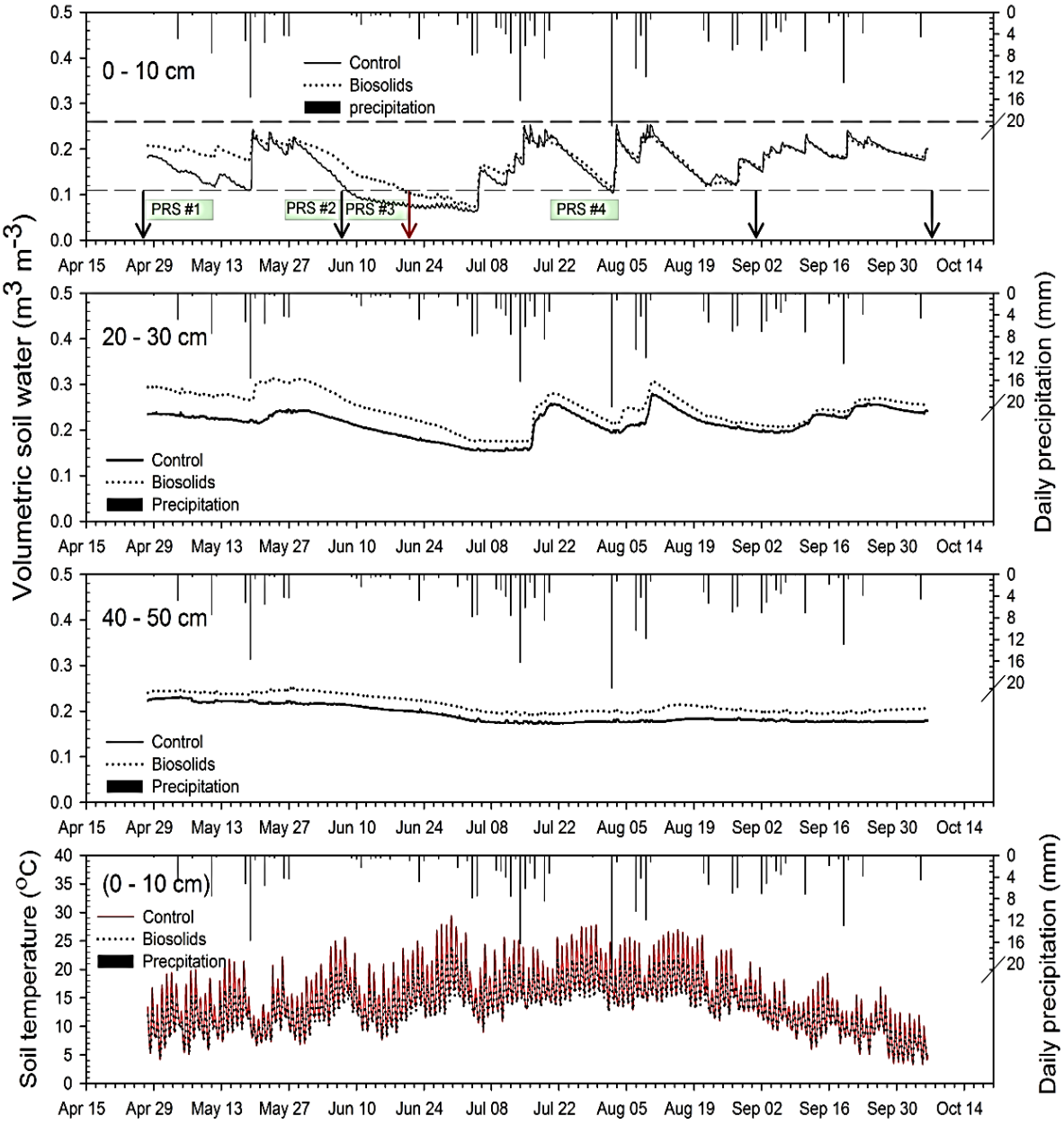

Figure 2-5 Soil volumetric water content and soil temperature (y-axis left) at three depths and daily precipitation (y-axis right) from April-October 2016 on rangeland in the central Interior of British Columbia (BC). The broken parallel lines in the 0-10 cm panel indicate the range of easily available soil water for plant uptake. The labels PRS#1-4 refer to the *in situ* incubation periods and the black arrows refer to sampling dates for aggregate stability and water content, and the red arrow refers to the sampling date for vegetation assessments.

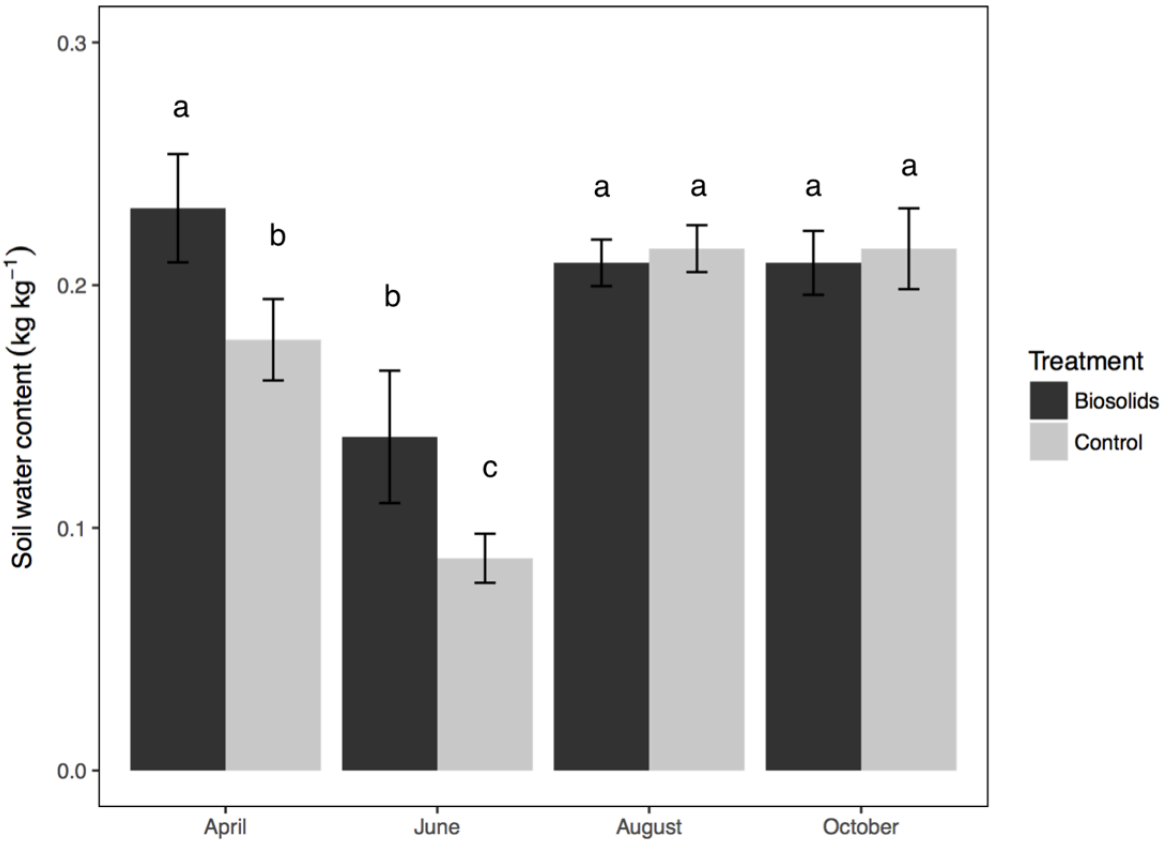

Figure 2-6 Gravimetric water content of soil aggregates collected at four sampling dates in 14 years after surface biosolids application on rangeland in the central Interior of British Columbia (BC). Different letters indicate significantly different levels in soil water content in biosolids and control treatments.

Table 2-1 Soil properties at 0-7.5 cm depth, percentages of ground cover and aboveground biomass of forbs and grasses as determined 14 years after surface biosolids application and control. Values in brackets are standard errors of the mean (*n*=4).

Property	Treatment		
	Biosolids	Control	*p-value*
Bulk density (Mg m^{-3})	0.95 (0.02)	0.96 (0.03)	0.79
Total soil C (kg m^{-2})	1.82 (0.02)	1.82 (0.12)	0.99
Total soil N (kg m^{-2})	0.17 (0.00)	0.17 (0.01)	1.00
C:N	11.0 (0.00)	11.0 (0.01)	0.81
Polysaccharides (kg m^{-2})	0.74 (0.01)	0.86 (0.03)	0.02
POXC (kg m^{-2})	0.046 (0.001)	0.048 (0.003)	0.24
Soil pH	5.76 (0.09)	6.20 (0.06)	<0.01
Litter (%)	88.76 (2.57)	34.58 (4.04)	<0.01
Microbiotic crust (%)	0.44 (0.26)	31.29 (3.49)	<0.01
Thatch (%)	25.86 (6.92)	0.13 (0.07)	<0.01
Bare soil (%)	1.01 (0.35)	5.96 (1.58)	0.04
Grass biomass (kg ha^{-1})	1267 (158.2)	556 (123.6)	0.01
Forbs biomass (kg ha^{-1})	274 (146.5)	235 (65.1)	0.59
Total biomass (kg ha^{-1})	1541 (132.6)	791 (63.5)	< 0.01

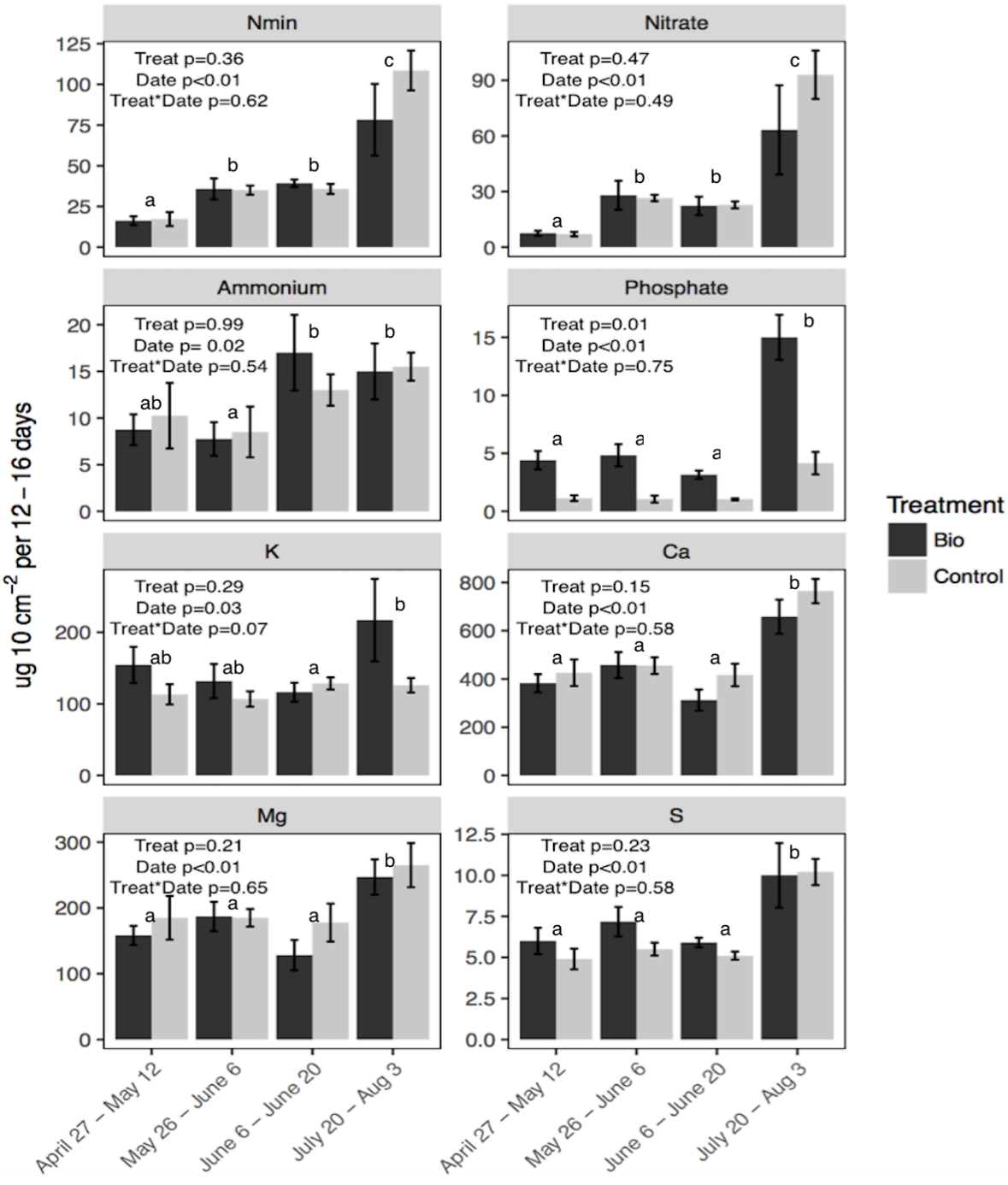

Figure 2-7 Concentration of available soil macronutrients 14 years after surface biosolids application on rangeland in the central Interior of British Columbia (BC). Error bars represent the standard error of the mean (n=4). Results of repeated measures ANOVA for treatment effect ("Treat"), sampling date ("Date") and treatment by sampling date interaction ("Treat*Date") included for each element. Results are considered significantly different at $p<0.05$. Different letters indicate significant differences in nutrient concentrations found on different sampling dates. Note the change in scale of the y-axis on each graph panel.

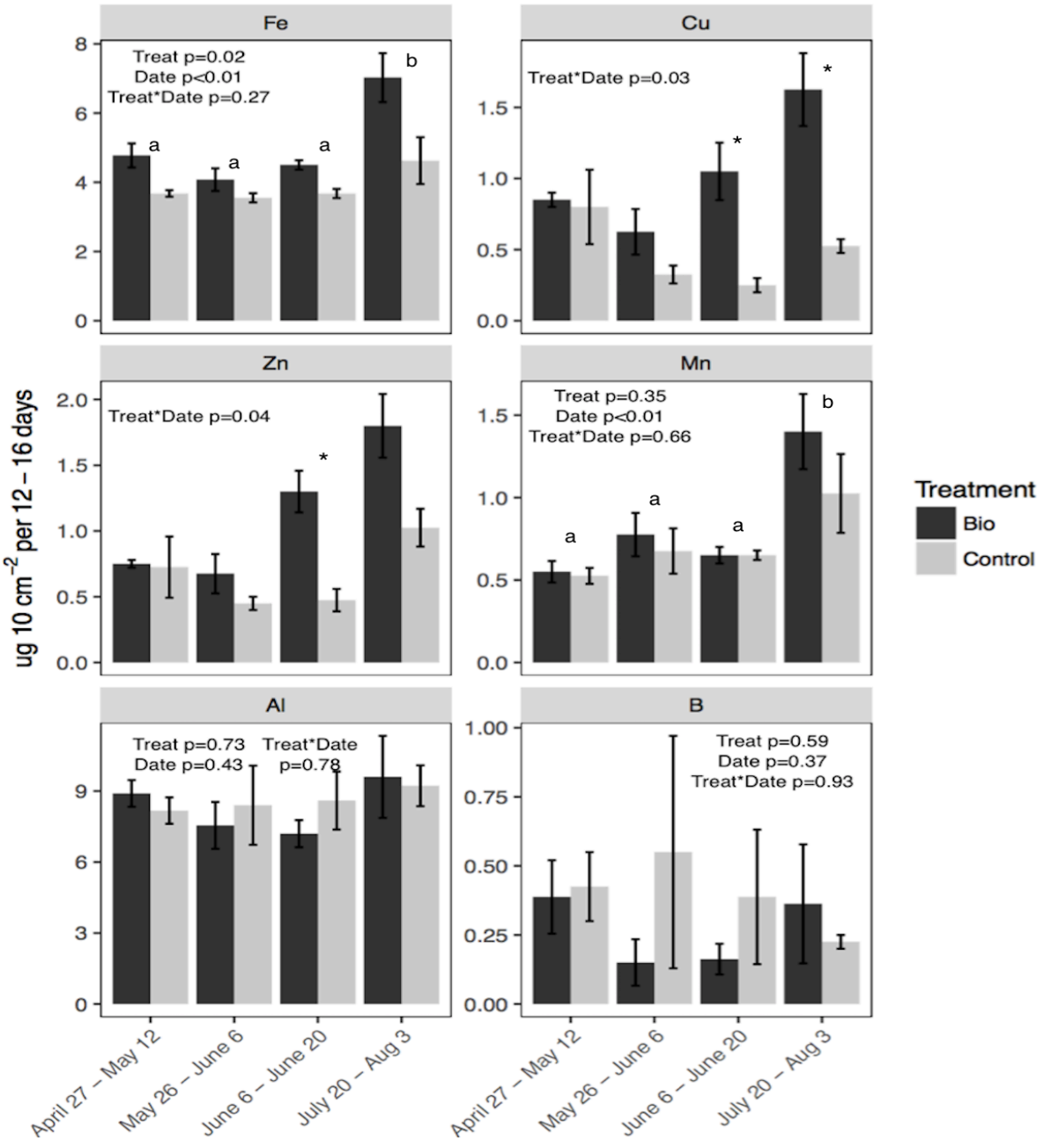

Figure 2-8 Concentration of available soil micronutrients and metals 14 years after surface biosolids application on rangeland in the central Interior of British Columbia (BC). Error bars represent the standard error of the mean (n=4). Results of repeated measures anova for treatment effect ("Treat"), sampling date ("Date") and treatment by sampling date interaction ("Treat*Date") included for each element. Results are considered significantly different at $p<0.05$. Different letters indicate significant differences in nutrient concentrations found on different sampling dates. In the case of a treatment by date interaction, * indicates the sampling dates at which micronutrient concentrations differed significantly between biosolids and control treatments. Note the change in scale of the y-axis on each graph panel.

Chapter 3: Forage Production and Plant Species Composition on Ungrazed Rangeland 14 Years after Biosolids Application [3]

3.1 Introduction

Biosolids are the solid by-product of municipal wastewater facilities, which are further processed to meet requirements for safe land application (OMRR 2002). The most common end-uses of biosolids in North America are landfilling, incineration, and land application (Roy et al. 2011). In 2016 in British Columbia (BC), 94% of biosolids were directed to land application (e.g., mine reclamation, fertilization of forest plantations and rangelands) and the remaining 6% went to a landfill or were otherwise disposed (British Columbia Ministry of Environmental Protection and Sustainability 2016).

Many grasslands in BC have been substantially altered following the introduction of livestock by European settlers in the mid-19th century, and no longer support the forage levels that they once did. Once degraded, grasslands are slow to recover; large areas of grasslands in the interior of BC still show the effects of extensive overgrazing that occurred over a century ago (Wikeem and Wikeem 2004). Biosolids, containing high levels of organic matter and nutrients including N and P, offer valuable resources for these nutrient and water limited ecosystems. There is interest in the use of organic amendments such as biosolids to improve rangeland health and productivity.

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It has been shown that three to five years after application, biosolids result in higher forage yields due to increased supply of nutrients and improved water holding capacity (McDougall et al. 2008; Jurado-Guerra et al. 2013; Jurado and Wester 2001; Martínez et al. 2003). Some studies have documented much longer-lasting increases in forage production after biosolids application (Ippolito et al. 2010; Wester et al. 2011; Sullivan et al. 2006), but it is not as clear how long the effect on forage production can last. Similarly, while biosolids generally improve forage quality and protein content in the short-term, the long-term relationship between biosolids application and forage quality is not clear.

Plant species composition is an important factor to consider both in terms of forage quality, as well as ecological integrity. For example, Wallace et al. (2016) found increased plant production eight years after a single biosolids application, but the increases came from annual and perennial forbs at the expense of perennial grasses, lowering the quality of the forage. A recent study on the long-term impact of organic matter applications to degraded rangeland (Blumenthal et al. 2017) has demonstrated that deleterious shifts in plant community composition can occur more than 10 years after the application, reinforcing the importance on long-term studies that consider plant species composition.

The objective of this study was to assess the effects of a single, surface application of biosolids on forage quality and quantity and plant species composition 14 years after the application on a rangeland that was protected from grazing in the central Interior of BC.

3.2 Materials and Methods

3.2.1 Site Description

The study was conducted in 2016 on the long-term field experiment established in 2002 at a working ranch near Jesmond, in the South Cariboo region of BC (51° 25' N, 122° 09' W; elevation 1,100 m). The site is located on the Fraser Plateau, on the eastern side of the Fraser river valley. The soil is a loam Orthic Dark Brown Chernozem formed on glacial moraine, overlaid by a thick layer of aeolian deposits (Valentine et al. 1987; Wikeem and Wikeem 2004). It belongs to the Chimney association, which are the dominant grassland soils of the northeast Fraser Plateau. The topography is very gently (2-5%) to gently (6-9%) sloping. The soil at the study site has a loam texture with 14% clay, 46% silt, and 40% sand, with negligible amounts of coarse fragments (see Appendix A for more detail on soil texture). No carbonates were found in the top 35 cm of soil at the study site.

The study site is in the grassland phase of the Very Dry Mild Interior Douglas-fir (IDFxm) biogeoclimatic subzone (Wikeem and Wikeem 2004). The dominant late-seral vegetation of this zone is bluebunch wheatgrass [*Pseudoroegneria spicata* (Pursh) A. Löve], spreading needlegrass [*Achnatherum richardsonii* (Link) Barkw.], and short-awned porcupinegrass [*Hesperostipa curtiseta* (Hitchc.) Barkworth], along with an understory of native forbs and cryptogams. At the time of the experiment establishment in 2002, 26% of the site area had exposed mineral soil and the vegetation was dominated by needle-and-thread grass (*Hesperostipa comata* [Trin. & Rupr.] Barkworth), with secondary species of Junegrass [*Koeleria macrantha* (Ledeb.) J.A. Schult. f.], low pussytoes [*Antennaria dimorpha (Nutt.) T. & G.]*, white pussytoes (*Antennaria microphylla* Rydb.), and prairie

sagewort (*Artemisia frigida* Willd.); indicating an early- to mid-seral stage and site degradation (Newman et al. 2014).

This region is located in the rain shadow of the Coastal mountains, receiving a total of 415 mm precipitation annually, of which 146 mm as snow. The highest rates of precipitation occur in late spring or early summer (May, June and July). In the summer, dry heated air rises from the Fraser river valley and contributes to dry conditions on the adjacent plateau, which are interrupted by intense precipitation events (Wikeem and Wikeem 2004). The mean annual temperature is 3.8ºC, while the mean January temperature is -7.8ºC and the mean July temperature is 14.8ºC. The region receives 101 frost-free days per year, and 1,174 growing degree days (base 5ºC) (Climate BC 2017). In the year of data collection for this study (2016) Jesmond, BC had an unusually wet growing season, receiving 276.2 mm precipitation from May to September (Appendix B).

In the fall of 2002, establishment of the long-term field experiment at the Jesmond site began with the selection of four randomly located blocks with uniform plant species composition and cover. Each block (60 m × 70 m in size) was fenced with a 1.5 m-high barbed wire to prevent cattle grazing. The experiment was laid out in a randomized complete block design with two treatments: (i) no biosolids (control) and (ii) single surface application of biosolids at 20 dry Mg ha^{-1} replicated once in each of four blocks. The biosolids were surface applied using a pull-type manure spreader without subsequent incorporation. The biosolids were supplied by the Annacis Island wastewater treatment plant in Metro Vancouver, BC where they had been anaerobically digested and dewatered. They contained

total N and P contents of 54.91 g kg^{-1} and 29.99 g kg^{-1} (dry weight), respectively. More detailed information on the biosolids used in this study can be found in Appendix C.

3.2.2 Sampling and Analysis

Aboveground plant biomass and nutrients

On 20-21 June 2016, green standing biomass was clipped as close to the soil surface as possible within a 1-m^2 frame, and separated into functional groups of grasses and forbs. Five frames were clipped per plot. The plant material was dried in an oven at 60°C for 24 hours, and weighed by functional group to assess aboveground biomass (kg ha^{-1}).

Tissue nutrient analysis was done on total aboveground biomass. The forb and grass functional group components from each frame were combined and ground together using a 2mm mesh screen Wiley Mill. The macronutrients N, C and S were determined by dry combustion (Nelson and Sommers 1996) using LECO analyser (LECO Corp., St. Joseph, MI), while the remaining macro- and micronutrients (B, Ca, Cu, Fe, K, Mg, Mn, Mo, Na, P and Zn), and Al were determined by microwave digestion followed by inductively coupled plasma optical emission spectrometry (Kalra and Maynard 1991).

Plant tissue macro and micronutrients are presented both as tissue concentrations (kg kg^{-1} or mg kg^{-1}) and total nutrient uptake (kg ha^{-1}). Total nutrient uptake was calculated as the product of nutrient concentration in plant tissue and dry matter yield (Jarrell and Beverly 1981).

Forage quality

The total aboveground biomass, sampled and ground for nutrient analysis as described above, was also analyzed for forage quality. Acid Detergent Fibre (ADF) analysis was conducted to determine amounts of fibre, lignin (ash free), and cellulose in the forage (Goering and Van Soest 1970). Protein content was analyzed by acid hydrolysis followed by colorimetric analysis (Marks et al. 1985).

Plant Species Composition and Ground Cover

Plant species composition was assessed on June 20-21, 2016 using canopy cover sampling (Daubenmire 1959). Percent canopy cover for individual vascular plants and ground cover types was estimated within fifty 0.2×0.5 m plots located randomly along five 50-m long transects. Transects were systematically arranged 5-m apart to provide balanced coverage within each treatment unit (Appendix D). Plant species composition was previously determined in June/July 2002 (i.e., before biosolids were applied) and annually in the same months thereafter for four years by Dr. Reg Newman (Newman et al. 2014). Plant nomenclature followed Douglas et al. (1998).

Thatch analysis

Thatch sampling was performed along the 5 plant transects per plot, at 5 random locations per transect (Appendix D). At each location, depth (cm) of thatch was recorded, or 0 was recorded if thatch was not present. At each location that thatch was present, a 10 cm × 10 cm (up to 10 cm depth) sample was collected with a flat shovel and placed into a sealed plastic bag. In the lab, thatch samples were cut to a known volume (using a sharp-edged cylinder), weighed, dried in the oven at 60^{o}C for 24h days, and weighed again to determine oven-dry thatch density. Because all thatch originated from Kentucky Bluegrass, the cover of

Kentucky Bluegrass by block was multiplied by the average depth of thatch by block to obtain the volume of thatch (m^3 ha^{-1}) by block. The volume of thatch was then multiplied by the density of the thatch to obtain the biomass of that by block (kg ha^{-1}).

To prepare thatch samples for C analysis, root and soil components were physically separated using a 2 mm sieve. Soil and roots were weighed separately to determine the portion (by mass) of the thatch that each represented. Roots were washed with dionized water. Carbon concentration of soil and roots was determined by dry combustion (Nelson and Sommers 1996) using LECO analyser (LECO Corp., St. Joseph, MI).

3.2.3 Statistical Analysis

Forage data were analyzed as a randomized, complete block design with four replications. Sub-samples collected within treatment plots were averaged by treatment plot prior to analysis. All statistical analysis was preformed using R statistical software version 3.4.0 (R Core Team 2017). Mixed effect models were constructed using the *nlme* package (Pinheiro et al. 2017). Treatment (with and without biosolids) was the only fixed effect in the model. Blocks were included as random effects. For all models, assumptions were assessed for each model and log transformations were made where necessary to meet assumptions. A significance level of 0.05 was used for all tests. An example ANOVA for a similar mixed effect model is included in Appendix E (b).

3.3 Results and Discussion

Aboveground biomass

Fourteen years after surface biosolids application, standing aboveground biomass in the biosolids plots was nearly two times greater than in control plots (1540.8 kg ha^{-1} in

biosolids and 791.1 kg ha^{-1} in control; Table 3-1). An examination of biomass by functional group shows that forbs were equally abundant (p=0.59) in the biosolids and control treatments, while the grass biomass was more than double in the biosolids plots.

It has been estimated that the bunchgrass rangeland in southern BC in excellent condition yields 580 to 1,300 kg per ha of forage dry matter per season (McDougall et al. 2008). By that measure, the control plots in my study produced an average dry biomass, while the biosolids plots produced an exceptionally high biomass. While biosolids have been widely shown to increase forage production in the short term (Wallace et al. 2009; Jurado and Wester 2001), there are a few studies that have found similarly long lasting increases in forage productivity as a result of a single biosolids application. Sullivan et al. (2006) found long-term increases in aboveground biomass with increasing application rates (2.5, 5, 10, 21, and 30 Mg ha^{-1}) on a semi-arid shortgrass steppe rangeland dominated by native perennials (western wheatgrass and needle-and-thread grass). The 21 Mg ha^{-1} rate, which is closest to the rate in my study, achieved a 60% increase in aboveground biomass 12 years after the application. The differences were noted in the first year of the study when the site received 50% greater rainfall than regional averages, while no differences in biomass were found the subsequent year, which received 50% less rainfall than regional averages.

Forage nutrients

Forage uptake of all macronutrients (N, S, Ca, K, Mg, and P) and uptake of most micronutrients (Al, B, Cu, Mn, Mo, and Zn) were significantly higher in plants from the biosolids amended plots (Figure 3-2 and Figure 3-4). On the other hand, the uptake of Fe and Na was not affected by biosolids application.

In contrast to the nutrient uptake values, only the concentrations of P and Cu were significantly greater in the plants from the biosolids plots, while N and Mg concentrations were greater in plants from the control plots (Figure 3-1 and Figure 3-3). No other element showed a difference in tissue concentration between biosolids and control plots. Concentrations of Al, Fe, and Na in plants on control plots, however, represented a 61, 83, and 126%, respective increase over the concentrations in plants grown in biosolids plots. The plant biomass C:N ratio of 39.5 in biosolids treatment was significantly higher than in the control (33.8) (Table 3-5). The difference was due to the higher N concentration in the plants in the control plots.

The reason for the difference between the plant uptake and tissue concentrations of macronutrients is referred to as the "dilution effect", a well-known effect reviewed by Jarrell and Beverly (1981), wherein yield increases due to fertilization, irrigation, and other environmental conditions, result in the decrease in concentration of nutrients in the plant tissue. In my study, there was more than double the plant biomass in the biosolids plots, despite similar availability of most nutrients (as shown by the PRS soil probe data, Figure 2-7 and Figure 2-8). Consequently, the higher concentrations of N and Mg in forage on the control plots relative to the plots that received biosolids were most likely explained by lower forage biomass on the control.

Another possible explanation the higher N concentrations in the plants in the control plots, besides the dilution effect, could be the increased presence of forbs in the control plots. Forbs represented 18% of the biomass from the biosolids plots and 30% from the control plots (Table 3-1). Forbs have been shown in other grassland forage quality assessments to

have higher N and lower C:N ratios than grasses (Wilsey and Wayne Polley 2006). It has also been shown that within plant functional groups, different species on the same site often have different N concentrations due to different uptake mechanisms or differences in rooting depth (Koerselman and Meuleman 1996). These niche characteristics allow for coexistence of a diversity of species, especially on resource limited sites (Harpole and Tilman, 2007). The increase in forbs could also account for the high variability for certain elements (Al, Fe and Na) in the control plots.

At the Jesmond study site, P was the only macronutrient that was significantly more available in biosolids amended soil, and was also the only macronutrient that had significantly greater concentration in the plants growing in biosolids plots. The two most limiting nutrients across most ecosystems are N and P. The fact that P is more concentrated in plants that received biosolids 14 years ago may suggest that the plants continue to benefit from elevated levels of soil P. Copper, which was more available in the biosolids amended soil at two out of four sampling dates (as discussed in Chapter 2), was also the only other nutrient more concentrated in plant tissues from biosolids plots. However, Fe, the only micronutrient with a clear increase in availability in the biosolids amended soil at all sampling dates, was 83% more concentrated in the control plant tissues (though non-significant). This suggests the lack of direct connection between plant available nutrients and plant tissue concentrations and highlights the complexity of soil-plant interactions. As such, Jarrell and Beverly (1981) recommended the consideration of total nutrient uptake be coupled with consideration of concentrations wherever possible.

Forage quality

Protein and fiber content of forage are often considered together to assess the overall forage quality as feed (McFarland et al. 2007). The higher protein concentration in the forage in the control plots than in the biosolids plots (Table 3-2) is in accordance with the finding of higher N concentration discussed above (Figure 3-1), and can be explained in the same way as the higher N concentration: likely due to a dilution effect of the 2-fold increase in aboveground biomass in the biosolids plots. The bunchgrass forage in southern and central BC on average has 12% of protein (McDougall et al. 2008), and total protein content ranges from 70 to 156 kg ha^{-1}, which suggests that both control and biosolids plots are below regional average protein concentrations, but are within the average total contents of protein per hectare. Despite a 34% increase in total protein per ha (concentration × yield) in the biosolids plots, there was no significant difference in total protein content per hectare in biosolids and control plots.

The portion of a forage that is insoluble in acid detergent (i.e., ADF), contains the poorly digestible components of the plant cell wall (cellulose and lignin). Higher values of ADF suggest lower-quality feed, due to decreased digestibility and increased chewing time for the livestock. Grass and legume forages with ADF values under 35% are considered good quality. The ADF levels found in the forage at the Jesmond site are therefore marginally higher than ideal (Table 3-2), and no long-term treatment effect of biosolids application was found. Similarly, no differences were found in cellulose or lignin (the components of ADF) content between biosolids and control treatments. While other studies (McFarland et al. 2007) have shown that biosolids can improve forage quality by increasing protein content and digestibility in the short term, the effect was not evident in my study conducted 14 years after biosolids application.

An important aspect of forage quality is nutrient ratios, such as the Cu:Mo ratio, which can result in Cu deficiency in ruminants when below 2. Biosolids application at the Jesmond study site did not have a long-term effect on the forage Cu:Mo ratio, which was 1.2 in the biosolids plots and 1.0 in the control 14 years after the application. Biosolids application to a semi-arid shrubland increased the Cu:Mo ratio from below 1.2/1 to above 2/1 for western wheatgrass and bluebunch wheatgrass one year after application at low rates (10 or 15 Mg ha^{-1}) and two years after application at intermediate rates (25-40 Mg ha^{-1}) (Pierce et al. 1998). It is possible that any positive effects of biosolids application on forage Cu:Mo ratio are relatively short term.

Plant species composition

The cover of late-seral, native bluebunch wheatgrass, was almost 16 times greater in the biosolids plots 14 years after a single biosolids application (Table 3-3). The most abundant grass at the site was early-seral needle-and-thread grass, covering 33.3% and 31.0% of biosolids and control plots, respectively. Agronomic Kentucky bluegrass (*Poa pratensis* L.*)* was significantly greater in the biosolids treatment, covering over 25% of biosolids plots and less than 1% of control plots. Cover of mid-seral bunch grass Nevada bluegrass (*Poa secunda ssp juncifolia* Scribn.*)* and late-seral Junegrass were not significantly affected by biosolids. Early-seral Sandberg's bluegrass (*Poa secunda ssp secunda* Presl*)* cover was low and similar in both biosolids and control plots.

Pussytoes (*Antennaria spp*) were significantly more abundant in the control plots. Prairie sagewort, field milk-vetch (*Astragalus agrestis* Dougl. ex G. Don*)*, and nodding onion (*Allium cernuum* Roth*)* were not significantly affected by biosolids. Common dandelion

(*Taraxacum officinale* G.H. Weber ex Wiggers*)* was over 54 times more abundant in the biosolids plots (p=0.06).

Overall, the long-term perennial grass response to the biosolids application has been positive, resulting in an 82% increase in cover compared to the control plots. This positive effect was noted in earlier sampling in 2006 reported by Newman et al. (2014), who attributed the perennial response to adequate precipitation and a lack of cheatgrass (*Bromus tectorum* L.*)* on site at the time of biosolids application. These two features distinguished the Jesmond site from two drier sites included in the study by Newman et al. (2014), where biosolids led to an increase of exotic forbs at the expense of perennial grasses. Total cover of native forbs is 11% greater in the control plots than in the biosolids plots, at 24.3% and 13.2%, respectively. Total cover of exotic forbs, composed mainly of common dandelion, was 5.4% greater in the biosolids plots in 2016, at 5.8% in the biosolids plots and 0.4% in the control plots. See Appendix H for complete list of species sampled from 2002-2006 and 2016 at the Jesmond study site.

At the 2006 sampling, cover of low-growing, early seral pussytoes was lower in the biosolids plots, which was attributed to the smothering effect of biosolids (Newman et al. 2014). Pussytoes are often considered indicators of highly degraded grasslands, and are associated with the early-seral stage of grassland recovery. The continued low levels of pussytoes in the biosolids in 2016 suggests that the smothering effect of biosolids on pussytoes can have a long-lasting effect when biosolids cover is subsequently replaced by plant litter (Figure 3-6). The cover of common dandelion in the biosolids plots in 2016 had

decreased slightly in the 10 years since the 2006 sampling date, suggesting that this forb is not threatening to invade, but persisting at low levels in the biosolids plots (Figure 3-6).

Needle-and-thread grass, the most dominant plant species in both the biosolids and control plots, increased sharply in the biosolids in the first three years after application, while at the same time decreasing in the control (Figure 3-5). However, 14 years after the application, this early-seral perennial grass is at similar levels in both treatment plots. It appears to have reached its peak capacity in the biosolids plots in 2005, when it was at just over 50% cover. This grass is known to replace bluebunch wheatgrass in more southern grasslands in BC where heavy grazing has occurred (Wikeem and Wikeem, 2004). Its persistence at this site could point to the history of extensive use.

In 2016, 76% of the increased perennial cover in the biosolids plots as compared to the control was attributed to Kentucky bluegrass, which was at less than 1% cover in 2006 (Figure 3-5). Kentucky bluegrass is a cool-season, C3 perennial that spreads via rhizomatous mats close to the soil surface. While it has good forage value, it has garnered a lot of attention recently due to its prolific expansion in the Northern Great Plains of the United States and the southern Canadian Prairies over the past 2-3 decades (Bork et al. 2017; White et al. 2012; Toledo et al. 2014; DeKeyser et al. 2015; Otfinowski et al. 2017; Sanderson et al. 2017, among others). Several authors have investigated mechanisms of Kentucky bluegrass encroachment (White et al. 2012; Bork et al. 2017), as well as documented negative effects of Kentucky bluegrass invasion on native plant cover (White et al. 2012; Sanderson et al. 2017), species richness and diversity (Dekeyser et al. 2015). The impact of Kentucky bluegrass on grassland communities of the Norther Great Plains and southern Canadian

Prairies has been attributed to its ability to transform the structure and function of the ecosystem via continuous mats of roots and litter (thatch) (White et al. 2012),

The extent of Kentucky bluegrass dominance in BC grasslands is not well documented, but the species has been present in the province likely since European settlement in the mid to late 1800s and is now common in many grasslands (Wikeem and Wikeem 2004). It is widely used as a forage grass and in landscaping. In the central Interior of BC, Kentucky bluegrass has come to dominate sites in the IDFxm biogeoclimatic subzone that are typically wetter than my study site, such as toe slopes, shallow depressions and swales (Coupé and Iverson 2014). A combination of overgrazing and the aggressive spreading capability of Kentucky bluegrass has resulted in few remaining late seral plant communities at these sub-hygric sites, and an alternate seral association has been created that accounts for Kentucky bluegrass dominance (Coupé and Iverson 2014).

The presence of Kentucky bluegrass (species often associated with cooler and wetter sites), on the biosolids plots in my study 14 years after biosolids application suggests that the biosolids application has altered the moisture regime of the site. Indeed, increased soil water was measured in May and June 2016 in the biosolids plots as discussed in Chapter 2 (Figure 2-5 and Figure 2-6). Accumulation of litter and reduced bare soil exposure may be the mechanism of this change. Kentucky bluegrass has been shown to increase in long-term ungrazed, lightly and moderately grazed pastures that accumulate heavy loads of litter (DeKeyser et al. 2009; Sanderson et al. 2017). It has also been shown to increase under heavy grazing, suggesting that availability of resources such as light, moisture and nutrients control its invasion, and grazing can hasten or delay its spread depending on the environmental

conditions at that site (Bork et al. 2017). It seems likely that the lack of grazing and long-term accumulation of litter at my sub-mesic to mesic study site encouraged the spread of Kentucky bluegrass. It is also possible that Kentucky bluegrass benefited from the additional available P in the biosolids plots, though no sources have been found that link Kentucky bluegrass specifically to soil P levels. Overall, a long-term enrichment in the soil water and nutrient resources in plots that received biosolids has resulted in the spread of Kentucky bluegrass to >25% cover.

Mature plant communities of mesic to submesic grassland sites in the IDFxm, such as my study site, are expected to be dominated (>25% cover) by bluebunch wheatgrass, accompanied by a diverse grass, forb and cryptogamic community (Coupé and Iverson 2014). While the bluebunch wheatgrass in the biosolids plots is not dominating the plant community to its full potential, increased bluebunch wheatgrass cover in the biosolids plots has been evident since two years after the application of biosolids (Figure 3-5). Cover of bluebunch wheatgrass in both the biosolids and control plots was greatest in 2006, and has declined in both treatments since then. However, the bluebunch wheatgrass plants growing in the biosolids plots in 2016 were greater in circumference, height, and nearly five times greater in standing biomass than bluebunch wheatgrass plants in the control plots (Table 3-4). The number of flower stalks of bluebunch wheatgrass plants (an indicator of plant reproductive vigor) in biosolids plots was more than double that that of plants in control plots; however, high variability resulted in no significant difference ($p= 0.23$). Flower stalks ranged in count from 0 - 117 per plant in the biosolids plots, and from 0 - 54 per plant in the control plots. Among the yield indicators used in assessing plant vigor, the number of flower stalks (in conjunction with maximum flower stalk length) has been shown to be the most

sensitive indicators of bluebunch wheatgrass vigor recovery (Mueggler 1975). Similarly erratic results in bluebunch wheatgrass flower production have been noted elsewhere, especially on sites in long-term exclosures (Anderson 1991). As such, a large sample size (80-100 plants from each treatment) is suggested to improve the accuracy of bluebunch wheatgrass flower stalks as an indicator of vigor recovery (Anderson 1991).

Individual bluebunch wheatgrass plants can take 8-10 years to recover to full forage production and reproductive capacity after intense grazing, and can take longer if additive stresses such as drought, insect herbivory and competition are occurring (Anderson 1991; Mueggler 1975). At a population level, bluebunch wheatgrass recovery is limited by its poor competitive ability, erratic seed production, and poor seedling establishment and/or survival. Low vigor plants will stop producing seed almost entirely. As such, very long time periods (over a decade) and the right environmental conditions are likely required for bluebunch wheatgrass recovery (Anderson,1991). The competition at the site with needle-and-thread grass in both the biosolids and control plots, and Kentucky bluegrass in the biosolids plots is likely limiting bluebunch wheatgrass recovery.

Soil cover

Exposed mineral soil shrunk quickly from 2002-2006 in both the biosolids and control before reaching what appears to be a steady state amount in 2006 (Figure 3-7). This rapid decline was likely due to the protection from grazing for both treatments initiated in 2002. The biosolids treatment reached a steady state level of significantly less exposed soil than the control in 2016 (Table 3-3).

In 2016, the control treatment had a significantly greater microbiotic crust than the biosolids treatment, while litter cover was 54% greater in the biosolids treatment than the control (Table 3-3). Microbiotic crust cover dropped drastically in the biosolids plots in 2003, obscured by the biosolids mulch. In following years, biosolids were replaced with litter cover, and the microbiotic crust remained at very low levels in the biosolids plots. Microbiotic crust plays an important role in this ecosystem by protecting the soil in the interspaces between bunchgrasses from erosion and heavy rainfalls, and reducing evaporation from the interspaces. In the biosolids plots, however, the roles of protection against erosion and evaporation have been replaced by a nearly continuous cover of plant litter (88.8%) and increased perennial grass production.

In 2016, we also measured an additional ground cover component - the layer of thatch found beneath Kentucky bluegrass. The thatch layer was composed of thickly matted Kentucky bluegrass roots, dead plant material and enmeshed organic matter and mineral soil particles that accumulated between the aboveground vegetation and the mineral soil surface. While the percent cover of the thatch was not measured directly, it was assumed to have the same percent cover as the Kentucky bluegrass. Accordingly, the thatch represented a layer of enmeshed soil and fine roots that covered around 25% of the biosolids plots to an average depth of 3.8 cm, and was composed of 0.6% to 17% roots by volume (6% on average). The impacts of thatch on bunchgrass ecosystems may include greater soil insulation, reduced seed germination due to restricted access of seeds to light, and changes in soil hydrological cycles. The full impacts of thatch on the ecosystem functioning are not yet well understood and need further study (Toledo et al. 2014; DeKeyser et al. 2015).

Aboveground C Stock

While biosolids application did not affect the soil C stock 14 years after application, the aboveground C stock was nearly doubled in size (kg ha^{-1}) in the biosolids plots as compared with the control plots (Table 3-5). Additionally, the thatch covering 25% of the biosolids plots represents a substantial C pool in the plant-soil interface. The matted rhizomes that form the thatch contribute an additional 775 kg of C per ha, which is even greater than the C stored in the aboveground biomass on biosolids plots (688 kg ha^{-1}). This was particularly surprising because the thatch only covered 25% of the biosolids plots, and yet represented a greater C stock than the above ground standing biomass on those plots. This can be attributed dense concentration of matted rhizomes in the thatch. Zhang et al. (2018) reported root C stocks (from the top 15 cm soil depth) that were 3 times greater than the standing biomass C stock in a grassland vegetated by foothills rough fescue (*Festuca campestris* Rydb), with co-dominant Parry's oat grass (*Danthonia parryi* Scribn) and Kentucky bluegrass. Thatch is the buildup of plant organic matter (living and dead) that occurs when aboveground production outpaces belowground decomposition of organic material (Murray and Juska 1970). Kentucky bluegrass commonly creates a thatch layer in grassland ecosystems, likely due to the combination of its vigorous aboveground production and the relatively slow belowground decomposition that occurs in moisture-limited grasslands. Furthermore, the absence of grazing on the site for 14 years is believed to have contributed to the accumulation of thatch at the soil surface. While the biosolids application did not result in any changes in soil C stocks, there was certainly an accumulation of aboveground C on biosolids plots.

Additionally, the soil enmeshed within the thatch had a higher C concentration (9.2%) as compared to the soil below the thatch (2.6%), which was likely due to the high concentration of roots and partially decomposed organic matter in close contact with mineral soil particles in the thatch. This thatch soil represents another important pool of C that was missed in the original evaluation of the belowground soil C stock contained in the mineral soil.

3.4 Conclusions

Fourteen years after a single, surface biosolids application at 20 Mg ha^{-1} to rangeland in southern Interior of BC, more than two times the aboveground biomass of grasses and plant litter cover was found in the biosolids treatment relative to the control, along with reductions in bare soil and microbiotic crust cover. Significantly greater plant uptake of all macronutrients, and Cu, Mn and Zn occurred in the biosolids treatment. The only two nutrients to be more concentrated in the biosolids treated forage relative to the control forage were P and Cu, while N, Mg, and protein were more concentrated in the control forage. No significant differences in uptake of most micronutrients and forage quality were found between biosolids and control treatments. Bluebunch wheatgrass, the late-seral native grass species, had significantly increased cover and aboveground biomass in the biosolids treatment relative to the control; however, between 2006 and 2016, non-native Kentucky bluegrass had reached greater than 25% cover in the biosolids plots, perhaps restricting the full recovery of bluebunch wheatgrass.

This study suggests that biosolids application combined with exclusion of grazing can increase forage production in the long-term and reduce bare soil, which is a crucial aspect of

rangeland improvement. It is likely that the accumulation of plant litter perpetuated increased plant productivity by acting as a mulch to conserve soil water. Increases in water and nutrients may have benefitted Kentucky bluegrass in the long-term. While biosolids application on this study site that was protected from grazing for 14 years did result in a long-term shift away from the late-seral plant community trajectory, the effects of this shift on rangeland health and productivity should be further monitored before being ruled negative or positive.

Table 3-1 Above ground biomass (kg ha^{-1}) of forb and grass functional groups 14 years after biosolids application on the study site in the central Interior of British Columbia (BC). Standard error of the mean is shown in brackets (n=4).

Treatment	Aboveground biomass (kg ha^{-1})		
	Forbs	Grasses	Total
Biosolids	274 (146.5)	1267 (158.2)	1541 (132.6)
Control	235 (65.1)	556 (123.6)	791 (63.5)
p-value	0.59	0.01	< 0.01

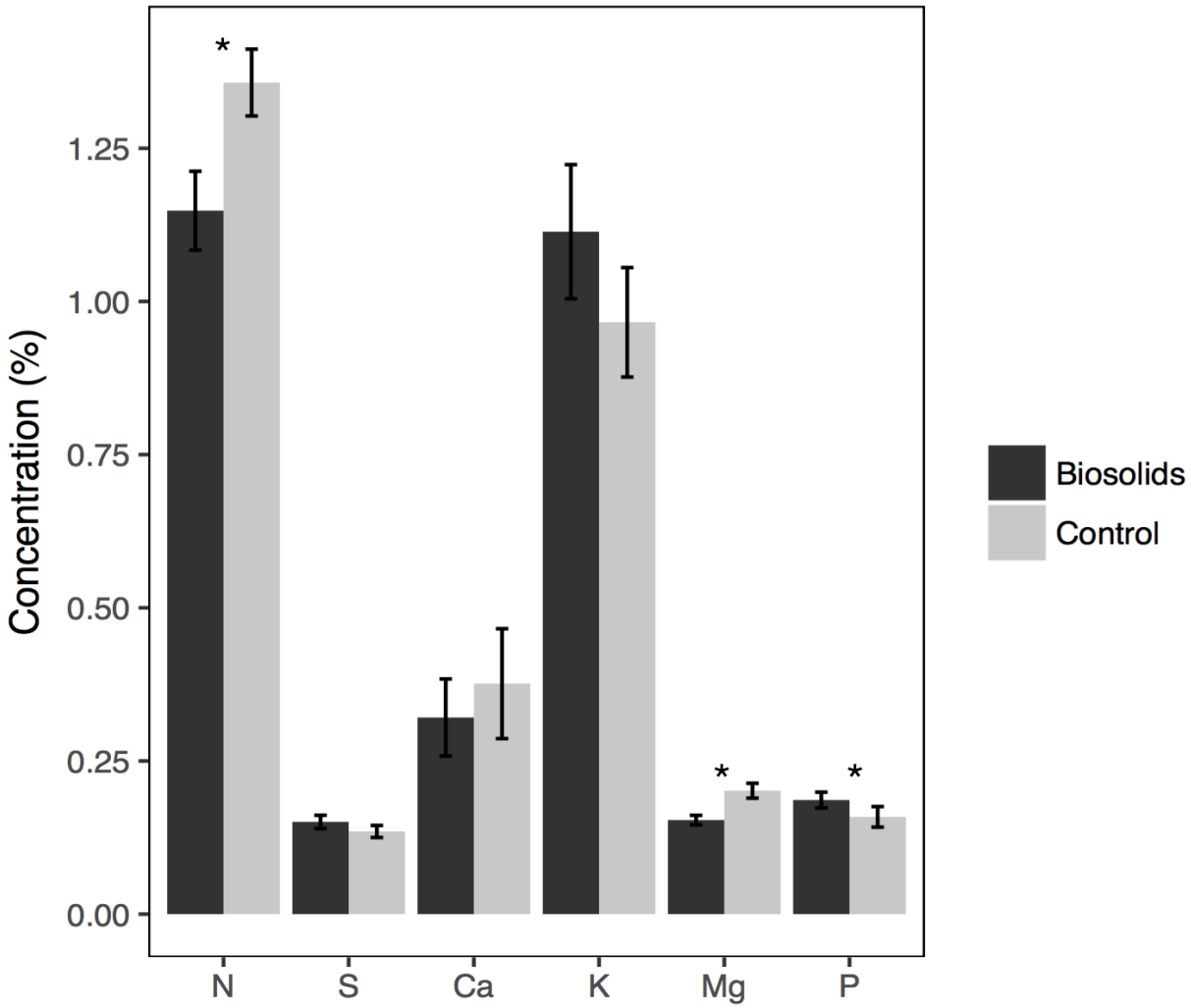

Figure 3-1 Concentration of plant macronutrients (N, S, Ca, Mg, K, and P) in treatments with and without biosolids application 14 years after biosolids application on the study site in the central Interior of British Columbia (BC). Error bars represent the standard error of the mean (n = 4). * indicates a significant treatment effect at $p<0.05$.

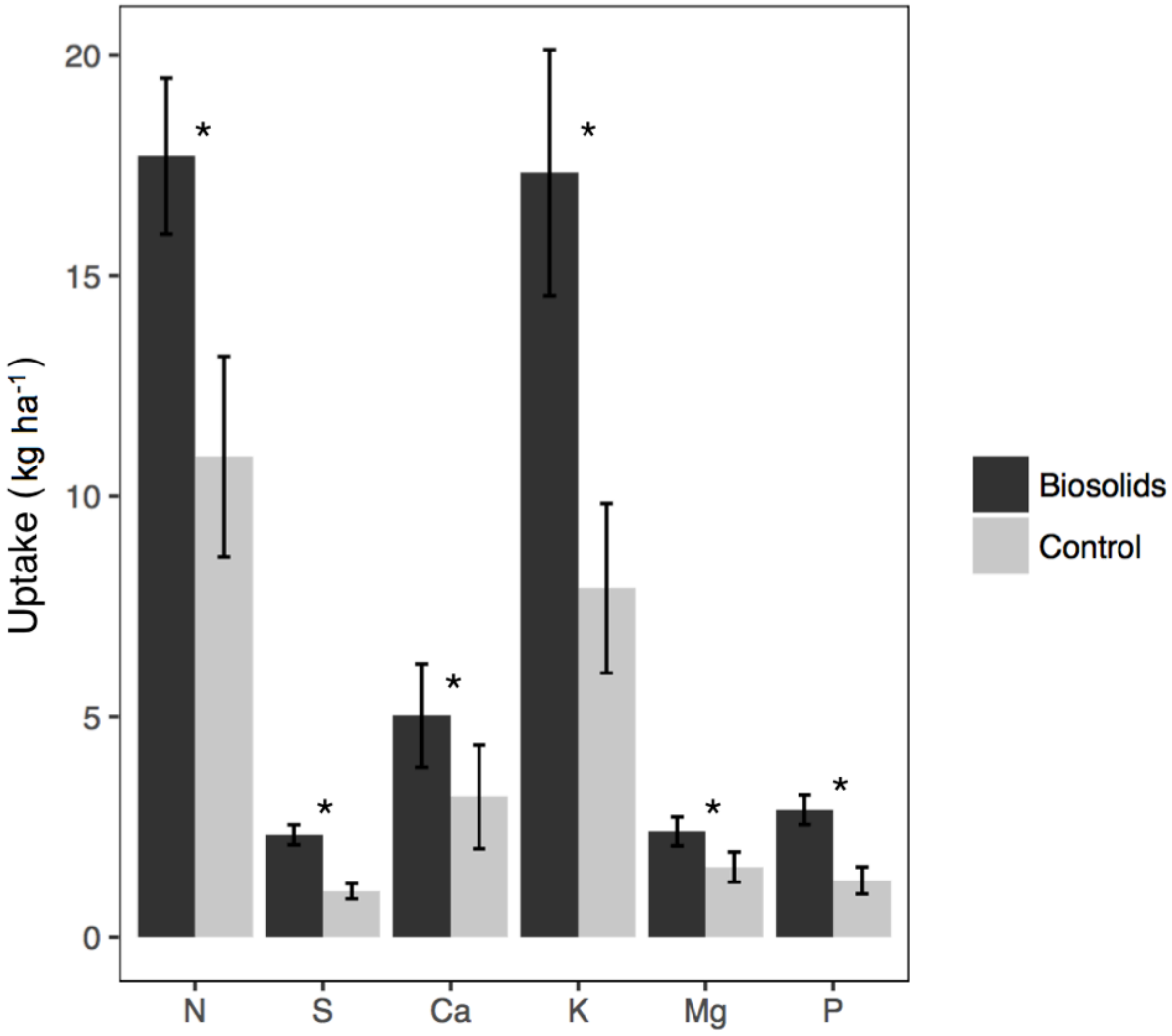

Figure 3-2 Uptake (kg ha^{-1}) of plant macronutrients (N, S, Ca, Mg, K, and P) in treatments with and without biosolids application 14 years after biosolids application on the study site in the central Interior of British Columbia (BC). Error bars represent the standard error of the mean (n = 4). * indicates a significant treatment effect at $p<0.05$.

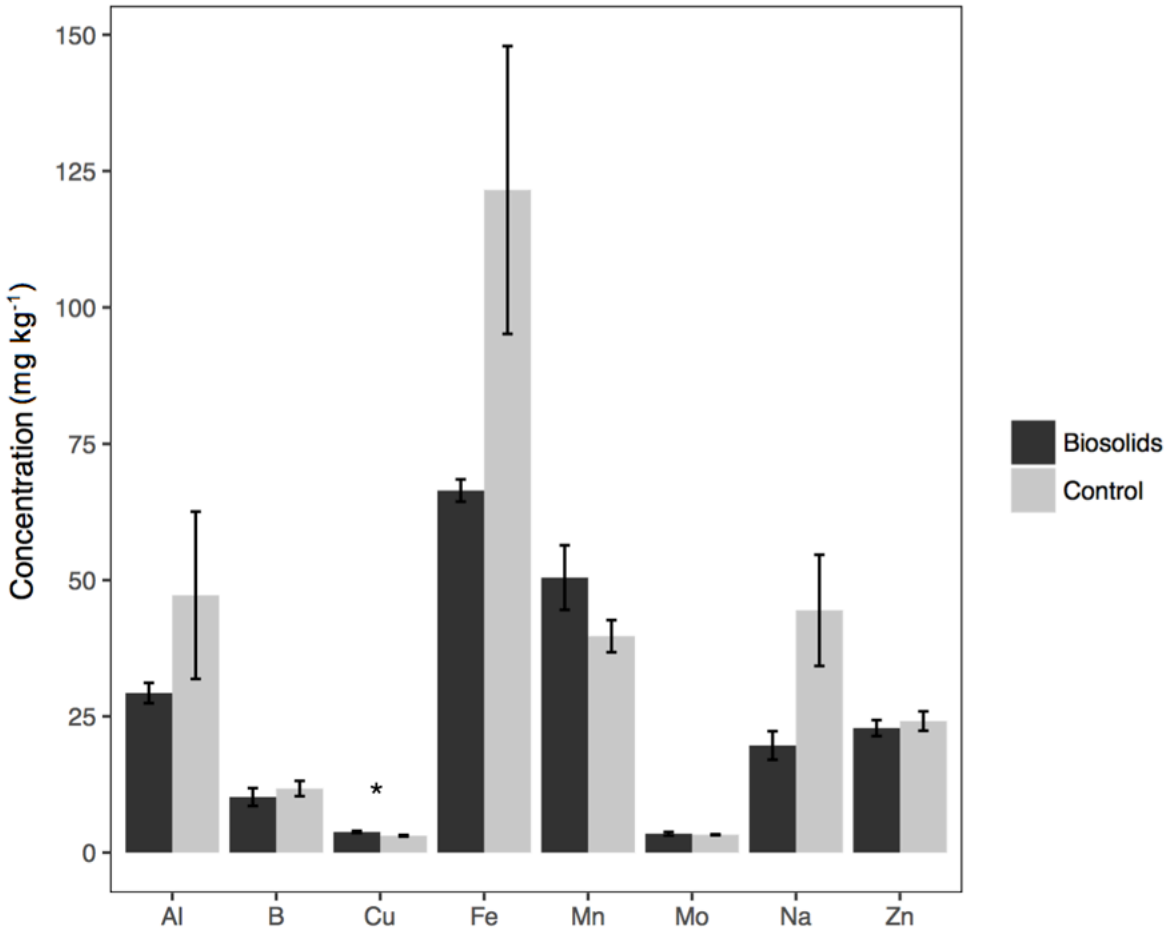

Figure 3-3 Concentrations (a) and uptake (b) of plant micronutrients (B, Cu, Fe, Mn, Mo, Na, Zn) and Al in treatments with and without biosolids application 14 years after biosolids application on the study site in the central Interior of British Columbia (BC). Error bars represent the standard error of the mean (n = 4). * indicates a significant treatment effect at $p<0.05$.

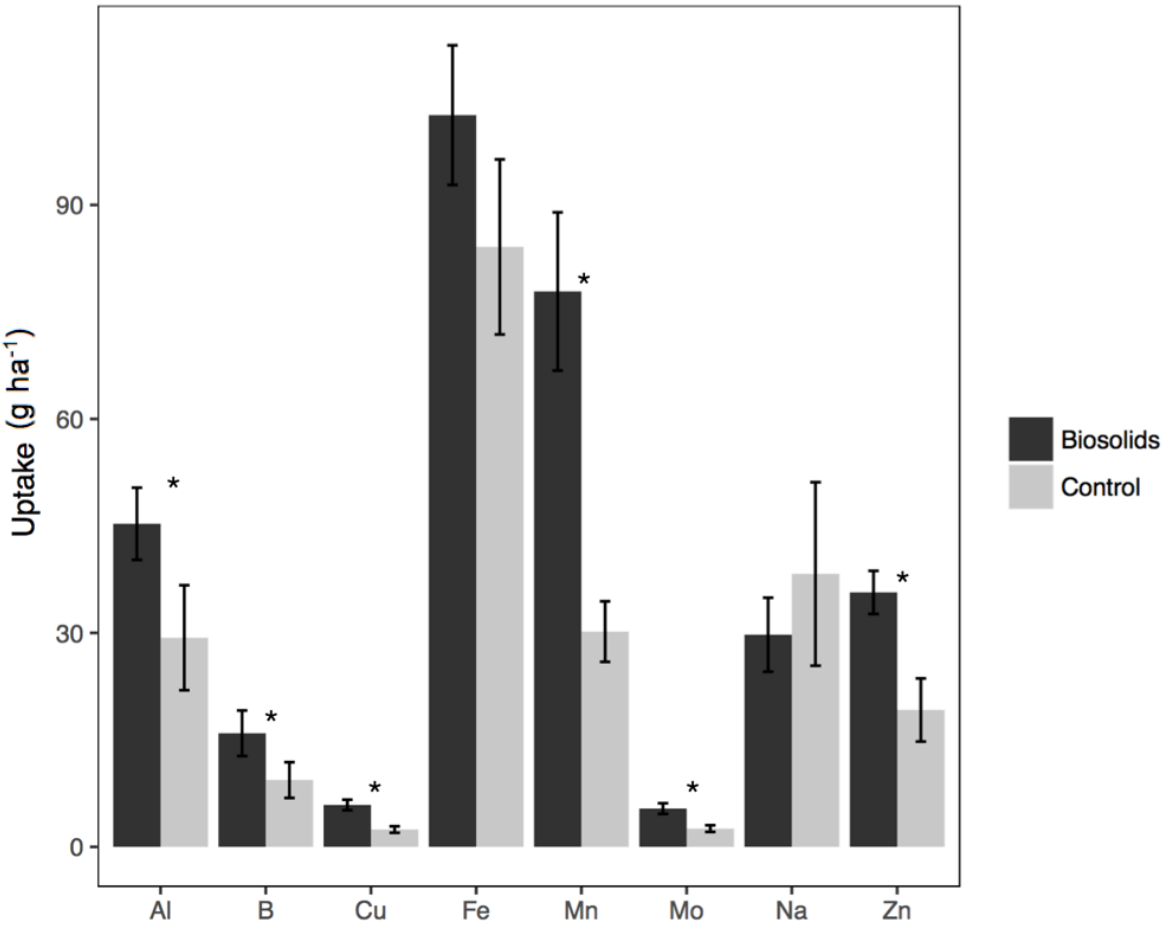

Figure 3-4 Uptake (g ha^{-1}) of plant micronutrients (B, Cu, Fe, Mn, Mo, Na, Zn) and Al in treatments with and without biosolids application 14 years after biosolids application on the study site in the central Interior of British Columbia (BC). Error bars represent the standard error of the mean (n = 4). * indicates a significant treatment effect at $p<0.05$.

Table 3-2 Forage quality in biosolids and control plots 14 years after biosolids application on the study site in the central Interior of British Columbia (BC). Standard error of the mean is shown in brackets (n=4).

Forage quality indicator	Biosolids	Control	*p-value*
Protein (%)	7.0 (0.47)	10.2 (0.52)	0.01
Protein (kg ha^{-1})	109.1 (11.35)	81.3 (17.46)	0.05
Fiber (ADF[a]) (%)	38.9 (1.13)	38.7 (1.82)	0.86
Cellulose (%)	34.7 (1.22)	33.9 (1.68)	0.29
Lignin (%)	4.2 (0.23)	4.8 (0.15)	0.13
Cu:Mo	1.2 (0.11)	1.0 (0.06)	0.23

[a]ADF = Acid detergent fiber

Table 3-3 Percent cover for individual plant species and ground cover 14 years after biosolids application on the study site in the central Interior of British Columbia (BC). Standard error of the mean are shown in brackets (n=4).

Category	Species†	Origin	Late seral‡	Treatment		
				Biosolids	Control	p-value
Perennial grasses	Needle-and-thread grass (*Hesperostipa comata)*	native	<1.0	33.26 (5.27)	31.03 (4.27)	0.32
	Junegrass (*Koeleria macrantha)*	native	3.0-9.9	1.93 (1.01)	6.97 (1.45)	0.07
	Nevada bluegrass (*Poa secunda ssp juncifolia)*	native	-	7.35 (5.90)	0.30 (0.15)	0.16
	Kentucky bluegrass (*Poa pratensis)*	agronomic	<1%	25.86 (6.92)	0.13 (0.07)	<0.01
	Sandberg's bluegrass (*Poa secunda ssp secunda)*	native	-	0.83 (0.79)	2.64 (0.99)	0.20
	Bluebunch wheatgrass (*Pseudoroegneria spicata)*	native	>25.0	6.06 (3.24)	0.38 (0.21)	0.04
Perennial forbs	Nodding onion (*Allium cernuum)*	native	-	4.19 (3.30)	5.60 (4.74)	0.41
	Field milk-vetch (*Astragalus agrestis)*	native	-	2.73 (1.03)	4.32 (1.43)	0.43
	Pussytoes (*Antennaria spp)*	native	3.0-9.9	0.43 (0.32)	8.05 (1.96)	0.02
	Prairie sagewort (*Artemisia frigida)*	native	3.0-9.9	1.26 (0.45)	3.05 (0.89)	0.16
	Common dandelion (*Taraxacum officinale)*	exotic	-	4.44 (2.39)	0.08 (0.03)	0.06
Ground cover	Mineral soil	-	-	1.01 (0.35)	5.96 (1.58)	0.04
	Microbiotic crust	-	-	0.44 (0.26)	31.29 (3.49)	<0.01
	Litter	-	-	88.76 (2.57)	34.58 (4.04)	<0.01

† Species with >2.5% cover in either biosolids or control plots are included in the table.
‡ Coupé and Iverson (2014).

Table 3-4 Vigour indicators of bluebunch wheatgrass plants growing in biosolids and control treatments 14 years after biosolids application on the study site in the central Interior of British Columbia (BC). Standard error of the mean are shown in brackets (n=4).

Indicator of plant vigor	Biosolids	Control	*p-value*
Circumference (cm)	37.7 (9.55)	22.1 (6.39)	0.03
Height (cm)	45.9 (4.12)	35.6 (3.06)	0.03
# of flower stalks per plant	17.6 (6.45)	7.3 (3.20)	0.23
Standing biomass (g per plant)	39.9 (15.26)	8.0 (3.54)	0.08

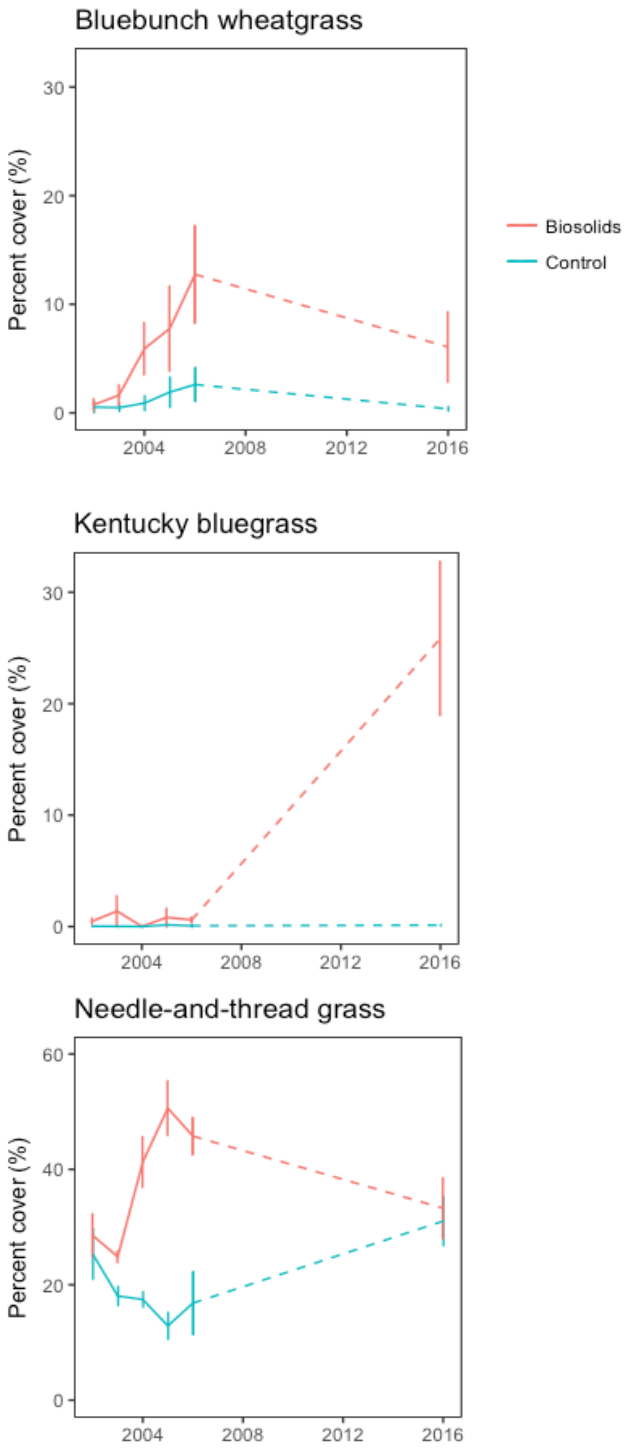

Figure 3-5 Cover of three most dominant perennial grasses (bluebunch wheatgrass, Kentucky bluegrass, and needle-and-thread) from 2002 – 2016 in biosolids and control plots on the study site in the central Interior of British Columbia (BC). Dotted line indicates the estimated trend during years (2006-2015) without data collection. Note that the y-axis scales differ between graphs.

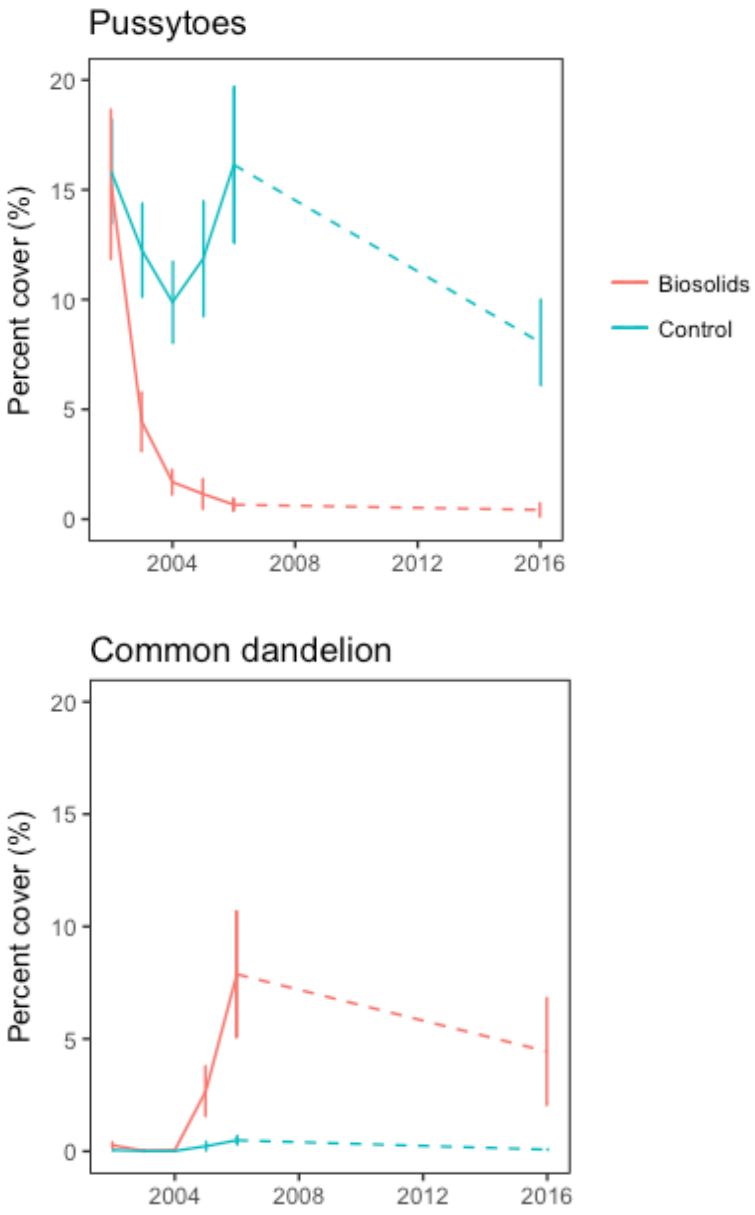

Figure 3-6 Cover of two forbs from 2002 – 2016 in biosolids and control plots on the study site in the central Interior of British Columbia (BC). Dotted line indicates the estimated trend during years (2006-2015) without data collection.

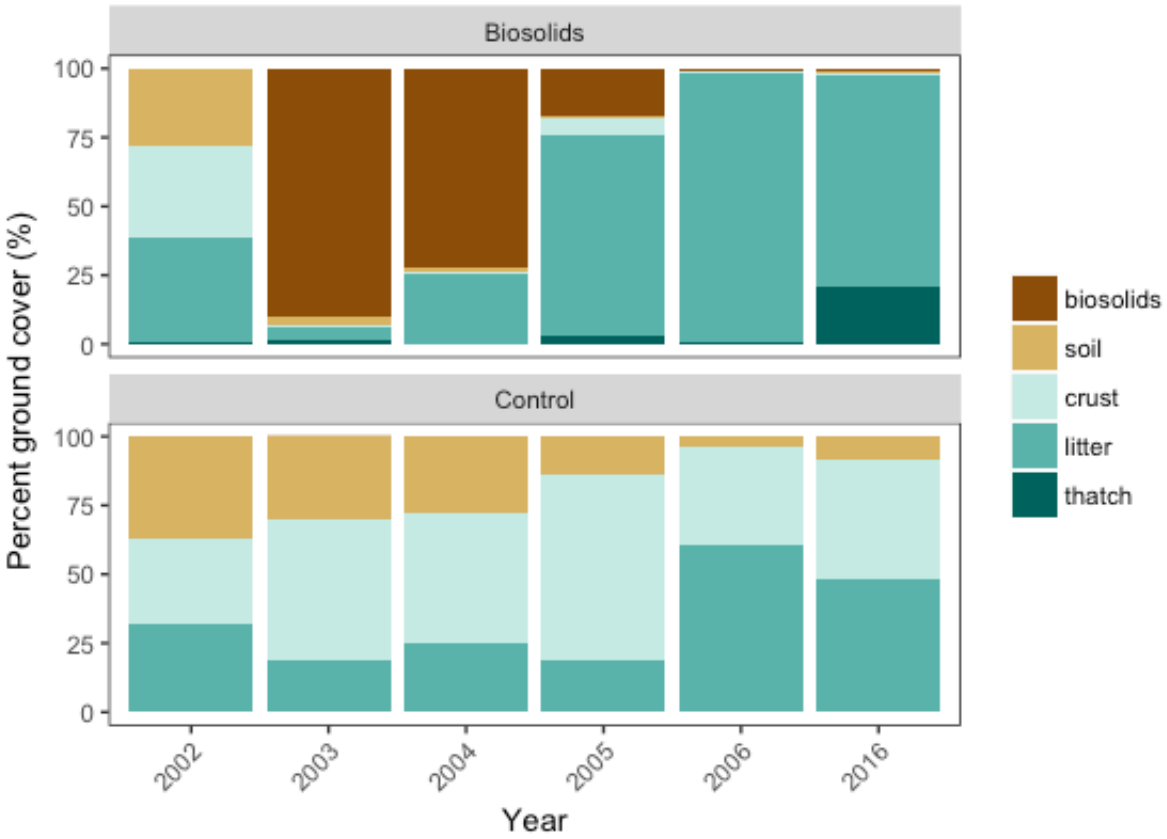

Figure 3-7 Percent of ground occupied by biosolids, bare soil, microbiotic crust and litter in biosolids and control plots from 2002-2006 and 2016 14 years after biosolids application on the study site in the central Interior of British Columbia (BC).

Table 3-5 The size, concentration and C:N ratio of soil, vegetation and thatch C pools 14 years after biosolids application in biosolids and control treatments on the study site in the central Interior of British Columbia (BC). Standard error of the mean is shown in brackets (n=4).

C Stock Property	Biosolids	Control	*p-value*
---------- C (kg ha^{-1}) ----------			
Soil (kg $ha^{-1}$to 7.5cm depth)	18220 (90)	18235 (120)	0.99
Vegetation (kg ha^{-1})	687.8 (51.35)	358 (64.38)	<0.01
Thatch soil (kg ha^{-1})	5624.9 (1391.44)	0	NA
Thatch roots (kg ha^{-1})	774.6 (229.36)	0	NA
---------- C (%) ----------			
Soil C %	2.55	2.54	0.95
Vegetation C %	44.66 (0.19)	45.28 (0.08)	0.02
Thatch soil C%	9.16 (0.34)	-	NA
Thatch roots C%	41.88 (0.79)	-	NA
---------- C:N ratio ----------			
Soil C:N	11.05 (0.00)	11.01 (0.01)	0.81
Vegetation C:N	39.47 (2.02)	33.76 (1.3)	<0.01
Thatch soil C:N	11.67 (0.13)	-	NA
Thatch roots C:N	39.44 (4.93)	-	NA

Chapter 4: Biosolids Increase Soil Aggregation but Not Aggregate Protected Carbon in Ungrazed Rangeland Soils 14 Years after Application[4]

4.1 Introduction

In the face of widespread rangeland degradation across North America due largely to historical overgrazing, there is interest in the use of organic matter amendments such as biosolids to increase soil C in rangelands with depleted organic matter stocks (Ryal et al. 2014). In British Columbia (BC), rangeland fertilization with biosolids is a common practice to increase forage production and improve soil quality. Every year, 38,000 dry tonnes of biosolids are produced at wastewater treatment facilities across the province, a portion of which are destined for rangeland application in the southern and central Interior. In 2014, about 11,000 tons of Metro Vancouver biosolids went to rangeland fertilization (Force 2015) in the interior of BC. Many studies have documented positive effects of biosolids on forage production in semi-arid rangeland (Jurado-Guerra et al. 2013; Sullivan et al. 2006; Martínez et al. 2003); however, information regarding the long-term effect of biosolids on soil C sequestration in rangelands is limited.

A single, surface application of biosolids to rangeland has been shown to increase soil aggregate stability up to eight years after the application (Wallace et al. 2016). Soil aggregates promote C sequestration by providing physical protection to soil organic matter

[4] A version of this chapter will be submitted for publication in the Journal of Environmental Quality - E. Avery, B. Wallace, M. Krzic, R.F. Newman, S.M. Smukler, and G. Bradfield. Biosolids increase soil aggregation and soil carbon on rangelands 14 years after application.

from microbial decomposers (Tisdall and Oades 1982; Elliot 1986). According to the Tisdall and Oades (1982) model of hierarchical aggregate organization, aggregates of different sizes are stabilized by distinct binding agents, which results in varying stability and turnover rate of aggregate-protected C. Macroaggregates (>0.25 mm) are typically bound by temporary binding agents such as roots and fungal hyphae, while microaggregates (<0.25 mm) are bound by aromatic organic compounds and chemical bonds which make them more resistant to decomposition. Microaggregates, therefore, have slower turnover rates than macroaggregates. It is believed that fresh organic matter additions to soil are first incorporated into macroaggregates, where they are further decomposed and in time become associated with mineral particles, forming microaggregates within the macroaggregates (Six et al. 2000). As such, microaggregates are thought to be responsible for long-term C sequestration, but macroaggregates contribute to the stabilization process of fresh organic matter into microaggregate-protected C (Balabane and Plante 2004). The microaggregate-within-macroaggregate fraction has been shown to detect early changes in soil C resulting from changes in management (Six 2014).

Labile polysaccharides (also termed dilute acid extractable, or hydrolysable, polysaccharides) are a fraction of chemically labile soil C that originates from soil micro-organisms, plant root exudates, and the easily decomposable parts of plant residues (Cheshire et al. 1973). Polysaccharides have been shown to correlate strongly with aggregate stability, and are thought to be an important transient binding agent that binds microaggregates together within macroaggregates (Tisdall and Oades 1982). Although polysaccharides were theorized to be of greater importance in microaggregation, several studies have found them to be at greater concentrations in macroaggregates than in microaggregates (Liu et al. 2005;

Haynes and Swift 1990). Others have not found good correlations between polysaccharide content and aggregate stability, suggesting that other factors such as root enmeshment, earthworm activity and fungal hyphae play more important roles in some soils (Carter et al. 1994; Degens et al. 1994).

Numerous studies have focused on the effects of cultivation on aggregate stability and C sequestration in aggregates (Six et al. 2000; Tan et al. 2006); while there are relatively few studies examining the long-term effects of organic amendments on aggregate stability and C sequestration in rangelands. The main objective of this study was to assess the effects of surface applied biosolids on soil aggregate stability, and distribution of total C and N and polysaccharides within soil aggregates 14 years after application to an ungrazed rangeland in the central Interior of BC.

4.2 Materials and Methods

4.2.1 Site Description

The study was conducted in 2016 on the long-term field experiment established in 2002 at a working ranch near Jesmond, in the South Cariboo region of BC (51° 25' N, 122° 09' W; elevation 1,100 m). The site is located on the Fraser Plateau, on the eastern side of the Fraser river valley. The soil is a loam Orthic Dark Brown Chernozem formed on glacial moraine, overlaid by a thick layer of aeolian deposits (Valentine et al. 1987; Wikeem and Wikeem 2004). It belongs to the Chimney association, which are the dominant grassland soils of the northeast Fraser Plateau. The topography is very gently (2-5%) to gently (6-9%) sloping. The soil at the study site has a loam texture with 14% clay, 46% silt, and 40% sand,

with negligible amounts of coarse fragments (see Appendix A for more detail on soil texture). No carbonates were found in the top 35 cm of soil at the study site.

The study site is in the grassland phase of the Very Dry Mild Interior Douglas-fir (IDFxm) biogeoclimatic subzone (Wikeem and Wikeem 2004). The dominant late-seral vegetation of this zone is bluebunch wheatgrass [*Pseudoroegneria spicata* (Pursh) A. Löve], spreading needlegrass [*Achnatherum richardsonii* (Link) Barkw.], and short-awned porcupinegrass [*Hesperostipa curtiseta* (Hitchc.) Barkworth], along with an understory of native forbs and cryptogams. At the time of the experiment establishment in 2002, 26% of the site area had exposed mineral soil and the vegetation was dominated by needle-and-thread grass (*Hesperostipa comata* [Trin. & Rupr.] Barkworth), with secondary species of Junegrass [*Koeleria macrantha* (Ledeb.) J.A. Schult. f.], low pussytoes [*Antennaria dimorpha (Nutt.) T. & G.]*, white pussytoes (*Antennaria microphylla* Rydb.), and prairie sagewort (*Artemisia frigida* Willd.); indicating an early- to mid-seral stage and site degradation (Newman et al. 2014).

This region is located the rain shadow of the Coastal mountains, receiving a total of 415 mm precipitation annually, out of which 146 mm as snow. The highest rates of precipitation occur in late spring or early summer (May, June and July). Almost half of total precipitation (194 mm) occurs from May to September. In the summer, dry heated air rises from the Fraser river valley and contributes to dry conditions on the adjacent plateau, which are interrupted by intense precipitation events (Wikeem and Wikeem 2004). The mean annual temperature is 3.8ºC, while the mean January temperature is -7.8ºC and the mean July temperature is 14.8ºC. The region receives 101 frost-free days per year, and 1,174 growing

degree days (base 5ºC) (Climate BC 2017). In the year of data collection for this study (2016) Jesmond, BC had an unusually wet growing season, receiving 276.2 mm precipitation from May to September (Appendix B).

In the fall of 2002, establishment of the long-term field experiment at the Jesmond site began with the selection of four randomly located blocks with uniform plant species composition and cover. Each block (60 m × 70 m in size) was fenced with a 1.5 m-high barbed wire to prevent cattle grazing. The experiment was laid out in a randomized complete block design with two treatments: (i) no biosolids (control) and (ii) single surface application of biosolids at 20 dry Mg ha^{-1} replicated once in each of four blocks. The biosolids were surface applied using a pull-type manure spreader without subsequent incorporation. The biosolids were supplied by the Annacis Island wastewater treatment plant in Metro Vancouver, BC where they had been anaerobically digested and dewatered. They contained total N and P contents of 54.91 g kg^{-1} and 29.99 g kg^{-1} (dry weight), respectively. More detailed information on the biosolids used in this study can be found in Appendix C.

4.2.2 Sampling and Analysis

Soil samples were collected on 28 April (the beginning of the growing season and time of maximum precipitation in this region), 6 June (time of maximum growth of cool-season grasses in this region), 29 August (mid-late growing season, post-peak grass production time), and 6 October (end of growing season) in 2016 to assess aggregate stability. Total C and N and polysaccharides in aggregates were assessed on 28 April, 29 August, and 6 October sampling dates.

Three 50-m long transects were established per treatment plot. The transects (Appendix D) were laid out 5 m apart running parallel to the length of the treatment plots (i.e., biosolids and control plots within each block). One composite sample per transect was obtained by combining six subsamples taken every 7 m along the transect. Subsamples were collected with a trowel from the 0 to 7.5 cm depth. Any loose organic residues (including biosolids), sod layer, or microbial crust present on the soil surface was removed before sampling.

Samples were transported to the laboratory in lidded plastic containers and refrigerated at 4^{o}C until analysis. In the laboratory, field moist samples were sieved through a 6-mm sieve and aggregates were collected on a 2-mm sieve. Two fifteen gram subsamples of sieved soil aggregates (first subsample was for aggregate stability analysis, and second subsample was for aggregate C, N and polysaccharide analysis) were placed on top of a nest of sieves with openings of 2, 1, and 0.25 mm after being thoroughly moistened with a humidifier. This was done to minimize aggregate disruption during wet sieving due to air trapped inside of the aggregates. Wet sieving was performed for 10 min at a vertical stroke rate of 30 strokes per minute and an oscillating action through an angle of 30^{o}. After removing the sieves from the water, the material retained on each sieve of first subsample was oven dried at 105^{o}C for 12 hours to complete the aggregate stability analysis. The material retained on each sieve of the second subsample was oven dried at 60^{o}C for 24 hours in preparation for aggregate C, N and polysaccharide analysis. Once dry, the material on each sieve was crushed in a mortar and pestle and passed back through its respective sieve. For aggregate stability samples, the mass of the mineral particles remaining on each sieve was subtracted from the dry soil mass that passed through the sieve, in order to avoid biased

estimates of water stable aggregates. The results were expressed as mean weight diameter (MWD), which is equal to the sum of products of (1) the mean diameter of each aggregate size fraction and (2) the proportion of total weight occurring in the corresponding size fraction.

Total soil C and N, and polysaccharides were determined on soil that passed through the sieves mentioned above, representing stable aggregates of three size fractions (2-6 mm, 1-2 mm, and 0.25-1 mm). C and N content were determined by dry combustion (Nelson and Sommers 1996) using a LECO CNS-2000 automated analyzer (LECO Corp., St. Joseph, MI). Soil polysaccharides were determined using the phenol-sulfuric acid method for labile polysaccharide analysis described by Lowe (1993). To release the polysaccharides by hydrolysis with sulfuric acid, 0.50 g of soil was placed in an Erlenmeyer flask with 100 mL 0.5 *M* H_2SO_4 and autoclaved for 1 h at 103 kPa. The samples were cooled and filtered into 250-mL volumetric flasks and made to volume using distilled water. 1 mL of the polysaccharide solution was combined with 5 mL concentrated H_2SO_4 and 1 mL phenol solution. Samples were heated at 30ºC for 25 min. Three 250 µL drops from each sample was added to a 96 well plate and the absorbance of the samples was then at 490 nm in a microplate spectrophotometer (TECAN Group Ltd., Zurich, Switzerland). Quantification of polysaccharides was assessed based on a standard curve made with a stock solution of glucose.

4.2.3 Statistical methods

Soil aggregate stability and total aggregate C and N and polysaccharide data was analyzed as a randomized, complete block design with four replications. Sub-samples collected within

treatment plots were averaged by treatment plot prior to analysis. All statistical analysis was preformed using R statistical software version 3.4.0 (R Core Team 2017). Mixed effect models were constructed using the nlme package (Pinheiro et al. 2017). Treatment, sampling date and treatment by sampling date interaction were fixed effects. Random effects included blocks and the nested experimental units (treatment plots) within blocks. Following a significant F-test generated by ANOVA, post-hoc tests (Bonferonni method) were done to identify differences between fixed effect levels. An example ANOVA for repeated measures soil data is included in Appendix E (a).

4.3 Results and Discussion

Aggregate stability

Biosolids application significantly increased aggregate stability at the Jesmond study site 14 years after a single, surface application, indicated by increasing MWD relative to the control (Table 4-1). MWD was also affected by sampling date, being lowest in April and highest in late June. There was no interaction between sampling date and treatment. The largest macroaggregate size class (2-6 mm) followed a very similar pattern as MWD (being the heaviest and most well represented size class it has significant influence on the MWD), but was not found to be significantly greater on biosolids plots relative to the control ($p=0.06$). Only the 1-2 mm aggregate size class was significantly greater under biosolids application then on the control, while no effect of biosolids was found in the 0.25-1 mm size class.

The sampling dates were arranged throughout the growing season to include both wetter and drier months. However, June, generally one of the wettest months of the year, was one of the driest months in 2016, while August received nearly double the average

precipitation (Appendix B). All aggregate size classes were affected by sampling date. Again, the 2-6 mm aggregate size class followed a nearly identical pattern over time to MWD, whereas changes in the smaller two aggregate size classes over time were less notable. The proportion of aggregates in the1-2 mm size class was most abundant in August, whereas the proportion of aggregates in the 0.25-1 mm size class more abundant in April and June than in August and October. Overall, biosolids application increased aggregate stability, a trend which was consistent across four sampling dates.

The two-fold increase in aboveground plant biomass in the biosolids plots at the Jesmond study site likely contributed to the greater MWD on biosolids plots relative to the control. Blankinship et al. (2016) found that removing plants from a seasonally dry grassland for two years resulted in a 22-33% decrease in MWD, due to an overall shift from large macroaggregates (2-9 mm) to small macroaggregates (0.25-2 mm). Plants affect macroaggregation directly via physical enmeshment of soil into aggregates by roots, and indirectly by providing C substrates (plant litter, root exudates, and root dieback) that feed microbes and stimulate aggregation.

Total C and N of soil aggregates

We hypothesized that 14 years after application, biosolids would increase total C and N concentrations of water stable aggregates, but found no evidence of this (Table 4-2). Biosolids application did not affect the C:N ratio of the aggregates either, with the aggregates from biosolids treatment having a ratio of 11.01 and the control aggregates at 11.09 (Table 4-2). Eight years after a single biosolids applications at 60 Mg ha^{-1} or 20 Mg ha^{-1} to semi-arid rangeland, Wallace et al. (2016) found that biosolids application significantly lowered the

C:N ratio in aggregates in the 2 – 6 mm and 1 – 2 mm size class relative to aggregates in the non-amended soil. Similar results were reported four and five years after the same application rates in a nearby crested wheatgrass pasture (Wallace et al. 2009), but only for the higher application rate (60 Mg ha^{-1}), whereas the 20 Mg ha^{-1} application showed no effect. Additionally, Wallace et al. (2016) found more than two-fold increase in spring C and N concentrations in water-stable aggregates in biosolids treated plots as compared to the control. The increased C and N contents were only noted in the spring sampling date, while there were no significant differences in the summer or fall sampling date, suggesting a large flux of nutrients in the early season in the biosolids treated plots.

The lack of long-term biosolids effect on total C and N content in the stable aggregates in my study suggests that whatever biosolids material that was originally incorporated into aggregates has since been mineralized. Hence, the greater MWD in the biosolids plots is not attributable to residual organic matter from the application still being physically protected. It also suggests that the greater plant growth, indicated by more than double aboveground plant biomass (Table 3-1 in Chapter 3) on the biosolids plots is not contributing to greater concentrations of aggregate-protected C.

Despite being unaffected in the long-term by biosolids treatment, stable aggregate C and N contents did change significantly with sampling date (Table 4-2). All aggregate size classes had significantly greater C concentrations in April than in August or October, and the same was true for N concentrations in all aggregates except the smallest size class (0.25-1 mm), which did not change significantly over time (Table 4-2). There was no interaction between biosolids treatment and sampling date effect on aggregate C or N. Aggregate C:N

ratio was constant across sampling dates in the 2-6 mm and 1-2 mm aggregates, as C and N concentrations decreased at similar rates. In the smallest (0.25-1 mm) aggregates, however, the C:N ratio was lower in August and October than in April due to the lack of change in N concentration over the growing season.

This sampling date effect on aggregate C and N content is similar to that found by Wallace et al. (2016) in similar grassland soils, except that it was found in both biosolids and control plots as opposed to just biosolids plots. This spring influx of C and N in aggregates is likely a reflection of soil conditions (i.e., moisture and temperature) that are conducive to microbial activity and in turn, aggregation. Aggregates sampled later in the growing season were likely formed during the previous spring dry-down period (Blankinship et al. 2016). The transition of weaker, C-rich macroaggregates early in the season to stronger aggregates with less C later in the growing season supports the theory that fresh C inputs are first bound in macroaggregates, where decomposition continues to slowly occur (Six et al. 2000).

Polysaccharides of soil aggregates

Biosolids application did not have a long-term effect on polysaccharide concentration in water-stable aggregates (Table 4-2). Across all aggregate size fractions, the polysaccharide concentration ranged from 1.0-1.6% (by mass) and no aggregate size class exhibited a temporal trend in polysaccharide concentration across the three sampling dates (April, August, and October). There was no interaction between treatment and sampling date. Polysaccharide content of stable aggregates was hypothesized to be greater in aggregates from biosolids plots due to the expectation that the greater plant productivity would result in plant C inputs (some of which as labile polysaccharides) which would provide a food source

for microbial communities and stimulate aggregation via the production of microbial gums (also polysaccharides). While we do see greater proportion of 2-6 mm and 1-2 mm aggregates in the biosolids plots relative to the control, this cannot be attributed to greater concentration of polysaccharides. It has been suggested that soil carbohydrates (including polysaccharides) are of limited importance in macroaggregate development in soil with relatively high organic C as often found in grasslands (Carter et al. 1994; Miller and Jastrow 1990). While dilute-acid extractable polysaccharides may be useful indices of improvements in soil structure in cropped soils (Liu et al. 2005), they may be less so in rangelands. Other factors such as plant roots and mycorrhizal fungi activity may be of more importance in regulating improvements in soil structure in high C soils (Carter et al. 1994). This suggestion was confirmed in the study by Blankinship et al. (2016), where the positive effect of plant cover on macroaggregation was attributed mostly to root activity, as follow-up laboratory soil incubations demonstrated that in seasonally dry grassland ecosystems, the microbe population did not depend on fresh plant C inputs to produce microbial gums. Instead, it appeared that the microbes sourced mineral-associated C during dry periods to produce polysaccharides and engineer aggregates.

4.4 Conclusions

Although biosolids application resulted in long-term moderate increases in aggregate stability, there was no indication that greater C sequestration was occurring in stable aggregates amended with biosolids. Any biosolids material that was directly occluded in macroaggregates has since been decomposed. Temporal trends in aggregate C and N did appear to be inversely related to trends in aggregate stability: in April, when MWD was

lowest and 2-6 mm aggregates were least abundant of any sampling date, aggregate C was at its highest concentration. Aggregate polysaccharide concentrations were not affected by biosolids application or by sampling date, staying relatively constant throughout the season. As such, it seems that labile polysaccharides are not the mechanism causing the greater aggregate stability in the biosolids plots in these relatively C rich grassland soils, a finding that agrees with other studies on grassland aggregate dynamics. Furthermore, with no evidence of cultivation in the last 50 years, along with grazing exclusion for last 14 years, it is possible that the aggregate-protected C pool in these soils is near saturation, and as such the increased plant C inputs from the greater aboveground biomass on the biosolids plots is not readily stabilized. However, improvements to soil structure can still occur as a result of long-term increased forage production following biosolids application, via other mechanisms such as increases in root and fungal hyphae growth. Land managers hoping to increase C sequestration through organic amendments application to rangelands should consider the management history and present soil C levels to determine whether sequestration is likely to occur with additional C inputs.

Table 4-1. Mean weight diameter (MWD) and proportion by weight (%) of aggregates in each aggregate size class over four sampling dates in 2016 at the study site in the central Interior British Columbia (BC). Standard errors of the mean are shown in the brackets (n=4). ns, nonsignificant difference among treatments.

Aggregate size class (mm)	Treatment	Sampling date in 2016				*p-value*		
		April	June	August	October	Treatment	Date	Treatment × Date
	----------MWD (mm)----------							
N/A	Biosolids	1.05 (0.042)	1.74 (0.117)	1.78 (0.064)	1.67 (0.042)	0.04	<0.01	ns
	Control	0.88 (0.043)	1.72 (0.05)	1.55 (0.055)	1.26 (0.049)			
	---------- Proportion by weight (%)----------							
2-6	Biosolids	0.18 (0.012)	0.36 (0.03)	0.37 (0.017)	0.34 (0.01)	ns (0.06)	<0.01	ns
	Control	0.15 (0.011)	0.36 (0.014)	0.32 (0.017)	0.25 (0.013)			
1-2	Biosolids	0.12 (0.006)	0.12 (0.006)	0.14 (0.006)	0.13 (0.006)	0.03	<0.01	ns
	Control	0.08 (0.007)	0.11 (0.007)	0.12 (0.008)	0.11 (007)			
0.25-1	Biosolids	0.11 (0.01)	0.10 (0.005)	0.06 (0.002)	0.06 (004)	ns	<0.01	ns
	Control	0.09 (0.008)	0.09 (0.004)	0.07 (0.004)	0.06 (0.003)			

Table 4-2. Total soil C (%), total N (%), C:N ratio, and soil polysaccharide (%) in water stable aggregate size classes over three sampling dates in 2016 at the study site in the central Interior British Columbia (BC). Standard errors of the mean are shown in the brackets (n=4). ns, nonsignificant difference among treatments.

Aggregate size class (mm)	Treatment	Sampling date in 2016			*p-value*		
		April	August	October	Treatment	Date	Treatment × Date
----------C (%)----------							
2-6	Biosolids	3.31 (0.17)	3.15 (0.17)	2.84 (0.18)	ns	0.01	ns
	Control	3.46 (0.23)	2.73 (0.12)	2.79 (0.21)			
1-2	Biosolids	2.92 (0.05)	2.74 (0.07)	2.57 (0.08)	ns	<0.01	ns
	Control	3.09 (0.19)	2.54 (0.07)	2.59 (0.11)			
0.25-1	Biosolids	3.15 (0.13)	2.60 (0.10)	2.80 (0.11)	ns	0.01	ns
	Control	3.20 (0.29)	2.76 (0.17)	2.78 (0.10)			
----------N (%)----------							
2-6	Biosolids	0.30 (0.02)	0.28 (0.02)	0.26 (0.02)	ns	0.02	ns
	Control	0.31 (0.02)	0.25 (0.02)	0.25 (0.01)			
1-2	Biosolids	0.27 (0.01)	0.26 (0.01)	0.24 (0.01)	ns	<0.01	ns
	Control	0.28 (0.2)	0.24 (0.01)	0.24 (0.01)			
0.25-1	Biosolids	0.28 (0.01)	0.24 (0.01)	0.26 (0.01)	ns	ns (0.05)	ns
	Control	0.28 (0.02)	0.25 (0.02)	0.24 (0.01)			
----------Polysaccharides (%)----------							
2-6	Biosolids	1.45 (0.14)	1.56 (0.02)	1.33 (0.14)	ns	ns	ns
	Control	1.56 (0.19)	1.57 (0.11)	1.39 (0.04)			
1-2	Biosolids	1.22 (0.03)	1.14 (0.12)	1.21 (0.10)	ns	ns	ns
	Control	1.18 (0.06)	1.31 (0.07)	1.23 (0.15)			
0.25-1	Biosolids	1.15 (0.06)	1.28 (0.07)	1.35 (0.04)	ns	ns	ns
	Control	1.26 (0.08)	1.28 (0.04)	1.3 (0.04)			
----------C:N----------							
2-6	Biosolids	11.16 (0.1)	11.17 (0.23)	11.13 (0.09)	ns	ns	ns
	Control	11.19 (0.12)	11.1 (0.19)	11.22 (0.28)			
1-2	Biosolids	10.98 (0.12)	10.74 (0.16)	10.72 (0.11)	ns	ns	ns
	Control	10.97(0.16)	10.76 (0.11)	10.76 (0.13)			
0.25-1	Biosolids	11.42 (0.07)	10.9 (0.09)	10.85 (0.15)	ns	<0.01	ns
	Control	11.41 (0.11)	11.04 (0.08)	11.38 (0.19)			

Chapter 5: General Conclusions and Recommendations for Future Studies

5.1 General Conclusions

Fourteen years after a single biosolids application at 20 Mg ha^{-1} to rangeland in Jesmond, BC, more than two times the above ground grass biomass and plant litter cover was found in the biosolids treatment relative to the control, along with reductions in bare soil and microbiotic crust cover. The long-term increase in plant productivity was accompanied by increased aggregate stability, lower pH, increased spring soil water content, and increased availability of soil P, Fe, Zn and Cu. The long-term increased availability of P (along with other immobile soil nutrients Fe, Zn and Cu) is a residual effect of the biosolids application 14 years after the application. On the other hand, improved aspects of soil structure and soil water regime are likely in part due to a feedback mechanism from the increased plant production and resulting accumulation of litter in the biosolids treatment over 14 years.

The biosolids application benefitted native grass production at the expense of forbs, and increased target restoration species bluegunch wheatgrass cover. The long-term enrichment of soil resources (P and water availability) resulting from the biosolids application also resulted in the >25% of Kentucky bluegrass cover, an agronomic perennial grass that dominates typically wetter sites in this region. Kentucky bluegrass is valued as a forage species, but the effect of its dominance on bunchgrass ecosystem functioning remain largely unknown. Its success in the ungrazed biosolids plots suggests that non-native species may benefit in the long-term from resource-enriched soil environment brought about by

organic matter amendments to rangeland. This study makes an important contribution to the uncertainty around the mechanisms of Kentucky bluegrass invasion in grassland ecosystems seen in the Great Plains and Canadian Prairies.

Despite 14 years of greater plant production on the biosolids plots, no differences were found in soil C levels. Total C, POXC, and aggregate-protected total C and polysaccharides contents were the same in the biosolids and control treatments. Only bulk polysaccharides (found in total soil <6 mm, not just aggregates) differed between the treatments, being greater in the control plots. It is possible that this site is near C saturation levels, suggested by high levels of total soil C in the control and a land-use history of minimal to no cultivation. However, it is also possible that there are differences in the soil C stored at depths below 7.5 cm, which were missed by the sampling regime. The greater cover of deep-rooted perennials on the biosolids plots which would contribute C-rich plant root exudates lower in the soil profile suggests that this may be the case. Another explanation of the lack of differences in C levels could be that microbial respiration rates are greater on the biosolids plots. Despite no documented differences in belowground C, aboveground C was significantly increased by biosolids application in the long-term, located in the aboveground plant biomass and the thatch layer.

5.2 Management Implications and Recommendations for Future Research

A single biosolids application has been shown to increase forage production by more than 2-fold after 14 years without grazing at my study site. Evidently, this is an unrealistic length of time for most ranchers to leave land ungrazed, and as such, the results should be treated as an example of the potential long-term restoration of forage production that can occur under

biosolids application without grazing. From a rangeland restoration perspective, the observed shift away from native plant community cover represented by the invasion of Kentucky bluegrass should be balanced with consideration of general improvements in site health (lower exposed mineral soil, greater cover of perennial grasses, improved soil structure).

To better understand the long-term impacts of biosolids on actively managed rangeland, future research should be undertaken to explore the interactions between grazing and biosolids applications in terms of their long-term effects on the rangeland soil and plant properties evaluated in this study.

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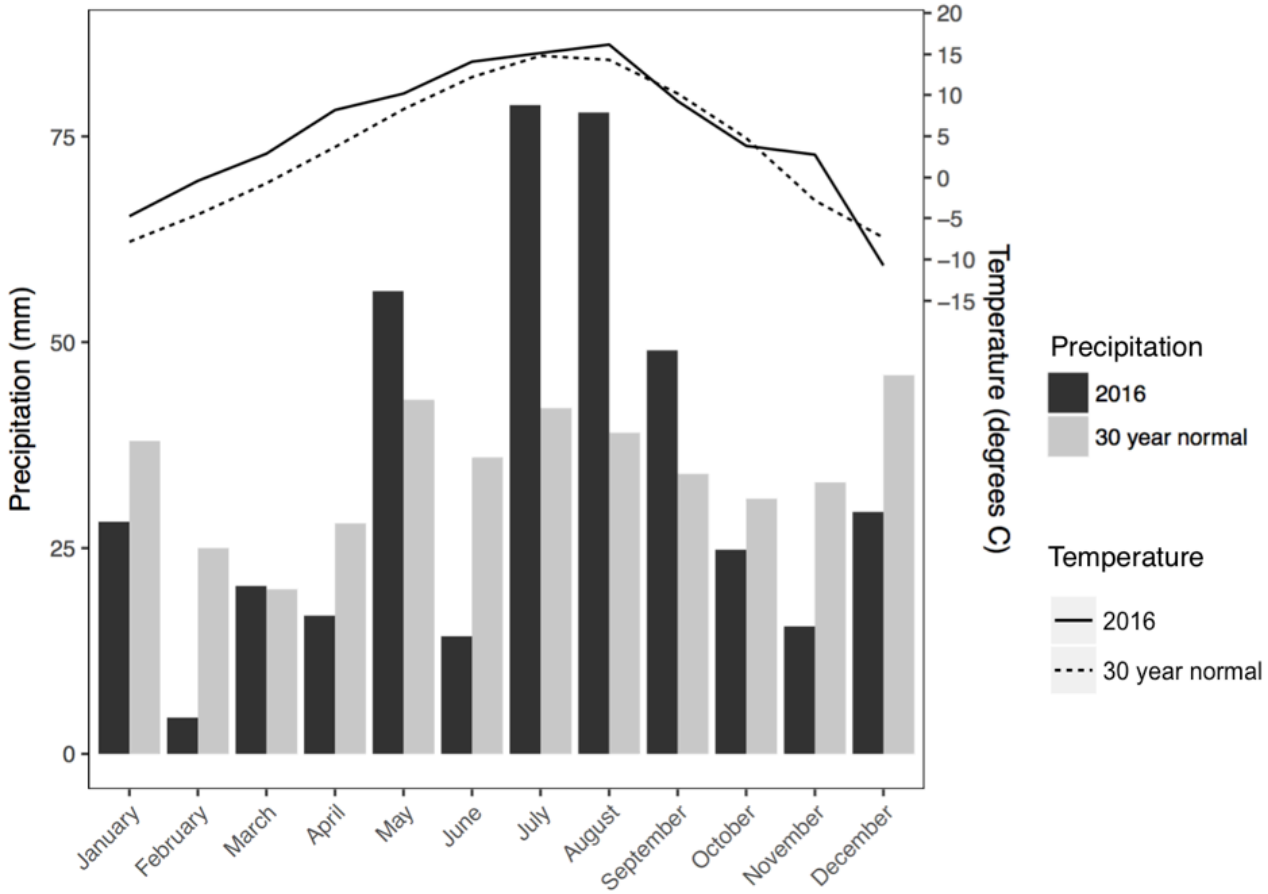
Appendices

Appendix A- Particle size analysis by block at the Jesmond study site in 2016. Four replicates were run from each block and then averaged for the analysis. Standard errors of the mean are shown in brackets.

Block	Sand (%)	Silt (%)	Clay (%)	Texture class
1	39.1 (1.16)	47.4 (1.36)	13.6 (0.2)	Loam
2	39.4 (0.28)	46.4 (0.11)	14.2 (0.39)	Loam
3	41.1 (0.31)	45.6 (0.31)	13.3 (0.00)	Loam
4	41.03 (1.5)	45.2 (1.31)	13.8 (0.19)	Loam
p-value	0.1	0.1	0.8	-

Appendix B - Monthly air temperature and precipitation in rangeland in the central Interior of British Columbia (BC) in the 2016 growing season (April-Oct) and 30-year historical average (1961-1990) (Climate BC 2017).

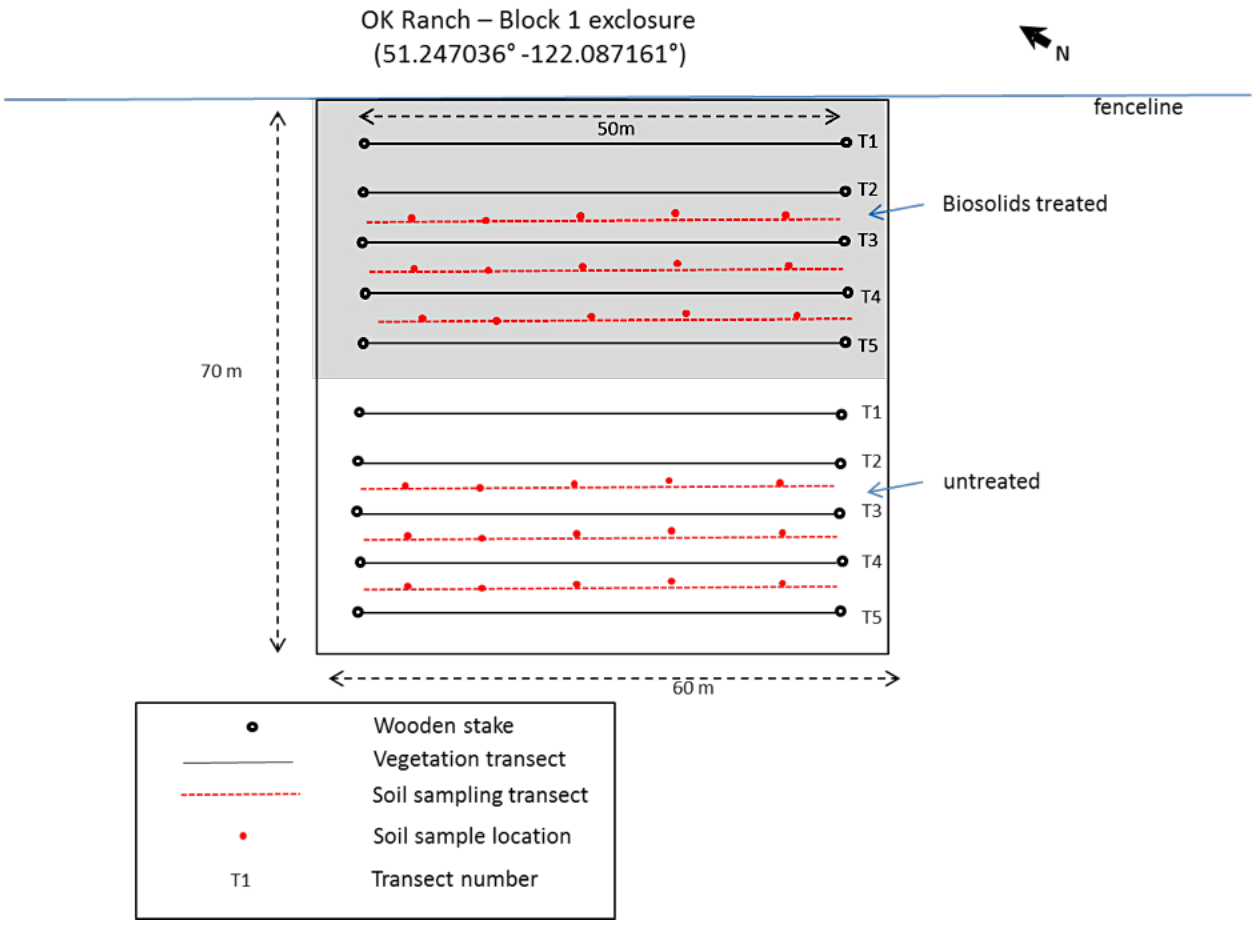


Appendix C – Properties of biosolids used in this study and OMRR criteria for Class A and B Biosolids.

Variables	Biosolids used in this study	Regulatory limits – Class B	Regulatory limits – Class A
Total solids (g kg^{-1})	301	-	
Volatile solids (g kg^{-1})	200	-	
Electrical conductivity (dS m^{-1})	4.1	-	
pH	8.5	-	
Macronutrients (g kg^{-1} dw)			
Total N	54.91	-	
Total P	29.99	-	
Total K	1.47	-	
Total Ca	25.94	-	
Total Mg	4.63	-	
Trace elements	(mg kg^{-1} dw)	(mg kg^{-1} dw)	(kg ha^{-1} in 45 yrs)[†]
Cd	3.3	20	4
Cu	1352	2,200	150
Cr	70	1,060	210
Pb	84	500	100
Hg	3.2	5	1
Ni	19	180	36
Zn	902	1850	370

[†] Class A Biosolids regulatory limits are determined as the "Maximum Acceptable Cumulative Metal Additions to Soil over 45 Years (kg metal/ha)" by the Trade Memorandum T-4-93 Standards for Metals in Fertilizers and Supplements

Appendix D - Sampling design for soil and plant properties in one of four blocks at the study site in the central Interior of British Columbia (BC).



Appendix E – ANOVA output examples of repeated (a) and single (b) measure type data.

a) ANOVA output example for the repeated measures mixed effect model (MWD ~ Treatment*Time, random effects = Block/Treatment plot) and the post-hoc test results.

Source	numDF	denDf	F-value	p-value
Intercept	1	18	823.1112	<0.0001
Treatment	1	3	12.2956	0.0393
Date	3	18	77.1324	<0.0001
Treatment X Date	3	18	2.0349	0.1450

Bonferonni method post-hoc test for difference amongst sampling dates

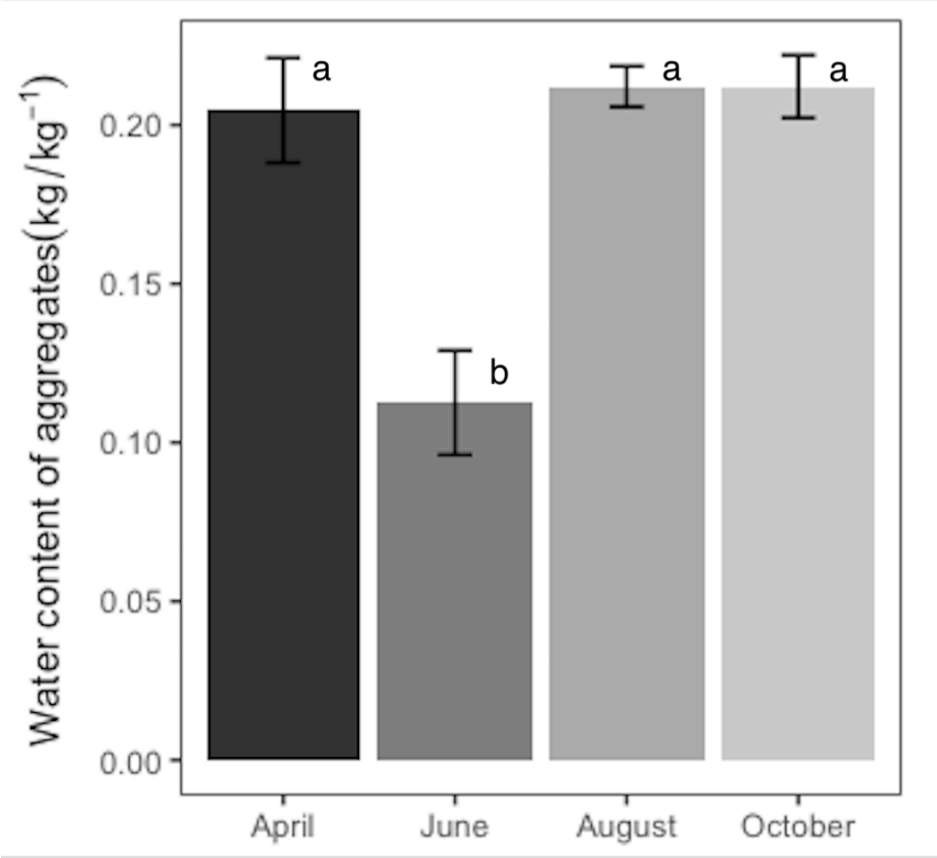
contrast	df	t-ratio	p-value
April - June	18	-9.71	<0.0001
April - Aug	18	-8.89	<0.0001
April - Oct	18	-6.30	<0.0001
June - Aug	18	0.82	1.000
June - Oct	18	3.41	0.019
Aug - Oct	18	2.59	0.111

Results are averaged over the levels of: Treatment
P value adjustment: Bonferroni method for 6 tests

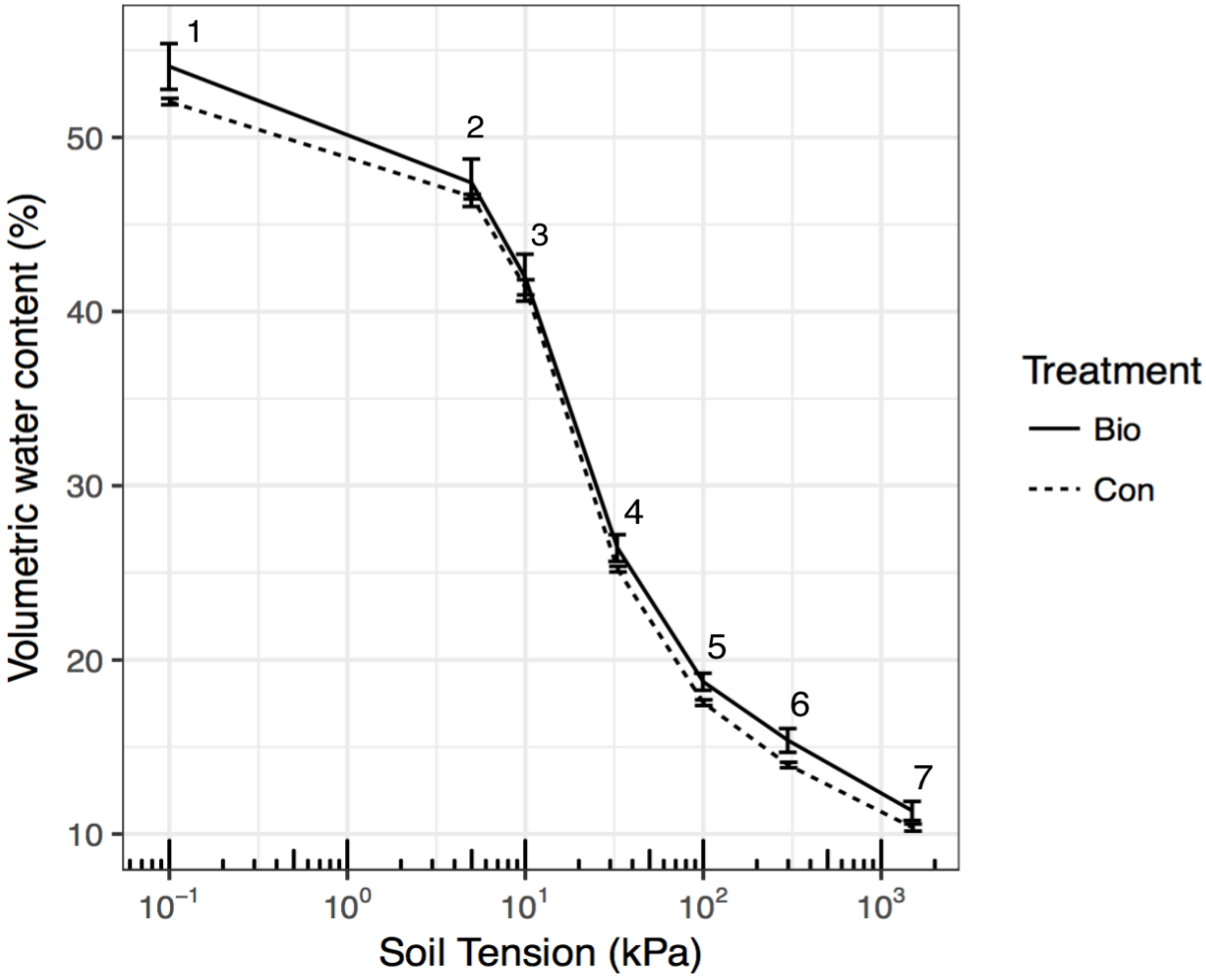
b) ANOVA output example for the mixed effect model (Bulk density ~ Treatment, random effect = Block).

Source	numDF	denDf	F-value	p-value
Intercept	1	3	3757.97	<0.0001
Treatment	1	3	0.089	0.7849

Appendix F- Gravimetric water content of soil aggregates by sampling date in Jesmond, BC 2016, averaged over biosolids and control treatments.



Appendix G- Soil water characteristics curve obtained 14 years after surface biosolids application on rangeland in the central Interior of British Columbia (BC). Point 1= saturation (0kPa), point 4 = field capacity (33kPa), and point 7 = permanent wilting point (1,500 kPa). Significant differences in volumetric water content between the control and biosolids treatments are not indicated on the figure as none were found at any of the 7 points of increasing water tension. Error bars represent the standard error of the mean (n=4). The x-axis is presented in the $\log_{(10)}$ scale, while the y-axis is linear.



Appendix H - Species list of all species recorded at the study site in Jesmond, BC during 2002-2006 and 2016 plant cover sampling.

Scientific name	Authority	Common name	Family	Life form	Origin
Achillea millefolium	L.	yarrow	Asteraceae	Forbs	Native
Achnatherum richardsonii	(Link) Barkw.	spreading needlegrass	Poaceae	Grass and grasslike	Native
Allium cernuum	Roth	nodding onion	Liliaceae	Forbs	Native
Antennaria dimorpha	(Nutt.) T. & G.	low pussytoes	Asteraceae	Forbs	Native
Antennaria microphylla	Rydb.	white pussytoes	Asteraceae	Forbs	Native
Artemisia campestris	L.	northern wormwood	Asteraceae	Forbs	Native
Artemisia frigida	Willd.	prairie sagewort	Asteraceae	Forbs	Native
Arabis holboellii	Hornem.	Holboell's rockcress	Brassicaceae	Forbs	Native
Astragalus agrestis	Dougl. ex G. Don	field milk-vetch	Fabaceae	Forbs	Native
Astragalus miser	Dougl. ex Hook.	timber milk-vetch	Fabaceae	Forbs	Native
Bromus tectorum	L.	cheatgrass	Poaceae	Grass and grasslike	Exotic
Carex filifolia	Nutt.	thread-leaved sedge	Cyperaceae	Grass and grasslike	Native
Camelina microcarpa	Andrz. ex DC.	littlepod flax	Brassicaceae	Forbs	Exotic
Carex obtusata	Lilj.	blunt sedge	Cyperaceae	Grass and grasslike	Native
Carex petasata	Dewey	pasture sedge	Cyperaceae	Grass and grasslike	Native
Carex sp.		sedge	Cyperaceae	Grass and grasslike	Native
Cerastium arvense	L.	field chickweed	Caryophyllaceae	Forbs	Native
Comandra umbellata	(L.) Nutt.	bastard toadflax	Santalaceae	Forbs	Native
Descurainia pinnata	(Walt.) Britt.	western tansy mustard	Brassicaceae	Forbs	Native
Elymus trachycaulus	(Link) Gould ex Shinners	slender wheatgrass	Poaceae	Grass and grasslike	Native
Erigeron compositus	Pursh	cut-leaved daisy	Asteraceae	Forbs	Native
Festuca occidentalis	Hook.	western fescue	Poaceae	Grass and grasslike	Native
Gaillardia aristata	Pursh	brown-eyed Susan	Asteraceae	Forbs	Native
Galium boreale	L.	northern bedstraw	Rubiaceae	Forbs	Native
Geum triflorum	Pursh	old man's whiskers	Rosaceae	Forbs	Native
Hesperostipa comata	(Trin. & Rupr.) Barkw.	needle-and-thread grass	Poaceae	Grass and grasslike	Native
Juncus balticus	Willd.	Baltic rush	Juncaceae	Grass and grasslike	Native
Koeleria macrantha	(Ledeb.) J.A.	junegrass	Poaceae	Grass and	Native

	Schult. f.			grasslike	
Linum lewisii	Pursh	western blue flax	Linaceae	Forbs	Unknown
Lomatium macrocarpum	(Nutt. ex T. & G.) Coult. & Rose	large-fruited desert-parsley	Apiaceae	Forbs	Native
Orthocarpus luteus	Nutt.	yellow owl-clover	Scrophulariaceae	Forbs	Native
Oxytropis campestris	(L.) DC.	field locoweed	Fabaceae	Forbs	Native
Penstemon procerus	Dougl. ex Graham	small-flowered penstemon	Scrophulariaceae	Forbs	Native
Potentilla hippiana	Lehm.	woolly cinquefoil	Rosaceae	Forbs	Native
Poa secunda ssp juncifolia	(Scribn.) Soreng	Nevada bluegrass	Poaceae	Grass and grasslike	Native
Poa pratensis	L.	Kentucky bluegrass	Poaceae	Grass and grasslike	Native
Poa secunda ssp secunda	J. Presl	Sandberg's bluegrass	Poaceae	Grass and grasslike	Native
Pseudoroegneria spicata	(Pursh) A. Löve	bluebunch wheatgrass	Poaceae	Grass and grasslike	Native
Rosa acicularis	Lindl.	prickly rose	Rosaceae	Trees and shrubs	Native
Senecio canus	Hook.	woolly groundsel	Asteraceae	Forbs	Native
Sisymbrium altissimum	L.	tall tumble-mustard	Brassicaceae	Forbs	Exotic
Taraxacum officinale	G.H. Weber ex Wiggers	common dandelion	Asteraceae	Forbs	Exotic
Tragopogon dubius	Scop.	yellow salsify	Asteraceae	Forbs	Exotic
Tragopogon pratensis	L.	meadow salsify	Asteraceae	Forbs	Exotic
Zigadenus venenosus	S. Wats.	meadow death-camas	Liliaceae	Forbs	Native
Silene douglasii	Hook.	Douglas' campion	Caryophyllaceae	Forbs	Native
Lepidium densiflorum	Schrad.	prairie pepper-grass	Brassicaceae	Forbs	Exotic
Rhinanthus minor	L.	yellow rattle	Scrophulariaceae	Forbs	Native

Appendix I- Polysaccharides (%), total C (%) and total N (%) in water stable aggregate size fractions and bulk soil, averaged over biosolids and control treatments. Soil fractions within a given soil property with different letters are significantly different at $p<0.05$

Soil property	Bulk soil†	2-6 mm	1-2 mm	0.25-1 mm
Polysaccharides (%)	1.12 (0.04)a	1.48 (0.05)c	1.22 (0.04)b	1.27 (0.02)b
C (%)	2.55 (0.10)a	3.04 (0.09)c	2.74 (0.06)b	2.88 (0.07)b
Total C as polysaccharide (%)	0.44 (0.02)a	0.49 (0.02)b	0.45 (0.01)ab	0.45 (0.01)ab
N (%)	0.23 (0.01)a	0.27 (0.01)c	0.25 (0.01)b	0.26 (0.01)b
C:N	11.03 (0.11)ab	11.16 (0.07)a	10.82 (0.05)b	11.17 (0.07)a