Vegetation and Soil Biodiversity Across Perennial Grassland-Annual Cropland Edges

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# ABSTRACT

The agroecosystem is composed of a mosaic of land uses and management types. As such, edges are prevalent and can have biological and physical effects on the surrounding area. For a deeper understanding of edge effects, both aboveground and belowground processes must be considered. To address edge effects in the agroecosystem, we investigated both aboveground and belowground properties across perennial grassland-annual cropland edges in central Saskatchewan, Canada. Specifically, we examined aboveground vegetation, belowground soil properties, belowground vegetation, and soil microbial community composition across edges of managed perennial grasslands and croplands. An *a priori* structural equation model (SEM) was created to analyze relationships between aboveground and belowground changes across the edge, specifically looking at drivers of the soil microbial community. Overall, our SEMs demonstrated that soil total nitrogen positively influenced bacterial richness and bacterial richness negatively influenced fungal richness. Belowground plant richness, rather than aboveground plant richness, had a positive relationship with fungal richness. Aboveground living biomass was a positive driver of soil total carbon and total nitrogen. At the community level, soil bacteria and fungi appear to be directly influenced by soil properties and microbial interactions, rather than plants directly. However, further investigation into the fungal community revealed specific fungal genera abundance was influenced by plant richness, while some were not; and may be due to specific plant associations. Understanding edge effects in the agroecosystem may aid in developing better management practices, bringing benefits to both the producer and agroecosystem health and resilience.

## LIST OF ABBREVIATIONS

| | |
|---|---|
| Al | Aluminum |
| AMF | Arbuscular Mycorrhizal Fungi |
| ASV | Amplicon Sequence Variant |
| C | Carbon |
| Ca | Calcium |
| CLC | Conservation Learning Centre |
| DNA | Deoxyribonucleic Acid |
| ITS | Internal Transcribed Spacer Region |
| K | Potassium |
| KCl | Potassium Chloride |
| LMM | Linear Mixed Model |
| Mg | Magnesium |
| N | Nitrogen |
| $NH_4$ | Ammonium |
| NMDS | Non-Metric Multidimensional Scaling |
| $NO_3$ | Nitrate |
| OTU | Operational Taxonomic Unit |
| P | Phosphorus |
| PCA | Principal Component Analysis |
| PCR | Polymerase Chain Reaction |
| PLFA | Phospholipid Fatty Acid |
| RNA | Ribonucleic Acid |
| SD | Standard Deviation |
| SDNWA | St. Denis National Wildlife Area |
| SEM | Structural Equation Model |
| SOM | Soil Organic Matter |

## 1.0 GENERAL INTRODUCTION

Agriculture covers approximately 40% of terrestrial land (Foley, 2005) and is expected to increase with the growing human population (Lambin and Meyfroidt, 2011). Expansion and intensification of agriculture can cause landscape simplification. Landscape simplification is an overall decrease in landscape structural diversity, which occurs in agricultural settings by increasing cropland patch size and connectivity (Meehan et al., 2011). One major consequence of agricultural expansion and intensification is loss of biodiversity (Gámez-Virués et al., 2015). Increasingly, farms are becoming larger as smaller farms are combined, creating landscapes with less non-cropped areas such as ponds and edges (Benton et al., 2003; Šálek et al., 2018). Edges in these landscapes, such as field boundaries, support biodiversity in agroecosystems and provide important ecosystem services (Wratten et al., 2012). Consequences of edges and their effects in the agroecosystem have not been widely considered before, particularly in North America.

Edges within the landscape contribute to landscape complexity and edges often harbour greater biodiversity than adjacent areas (Ries et al., 2004). Understanding edge dynamics in the agroecosystem is critical, as further insight can aid in developing management practices that benefit the larger ecosystem. Edges in the agroecosystem are largely anthropogenic, such as field borders and roads that can affect the adjacent areas. Edge effects can be abiotic, including changes in soil properties and microclimate, and also biotic, such as changes in species distribution and composition near the edge (Fischer and Lindenmayer, 2007). Commonly, edges in the agroecosystem are inhabited by weeds or other invasive species (Wilkerson, 2013). Invasive species are a concern for agroecosystem biodiversity and also to producers, where invasive plant species may reduce yield. However, edges can also provide habitat for species that provide ecosystem services, such as pollinators and natural enemies of pests (Tscharntke et al., 2012). Edge effects in the agroecosystem are not well understood, as studies on edge effects are focused on forest edges (Harper et al., 2015; Ohara and Ushimaru, 2015), wildlife populations (Alverson et al., 1988; Baker et al., 2002; Fortin et al., 2013), and in general, limited to aboveground effects (Marshall and Moonen, 2002; Ries et al., 2004; Fahrig, 2017).

Aboveground edge effects most often include changes in plant community composition and distribution. Edge habitat in the agroecosystem frequently attract weed species. Cropland

edges are favourable to weed species as there is less competition from crop and the available nutrients from spillover (Petit et al., 2011; Burel et al., 2013); resulting in plant communities largely composed of generalist species (Devictor et al., 2008). Outside of North America, the practice of planted field edges, known as planted field margins, are more common, especially in Europe where hedgerows often border cropland (Marshall and Moonen, 2002). Planted field margins too have edge effects on the adjacent areas (Musters et al., 2009) and changes in plant community composition at the edge may also affect invertebrate and other insect species (Musters et al., 2009; Wimp et al., 2011).

Edge effects may extend belowground, altering the plant community, soil properties, and the soil microbial community. Aboveground plant community changes occurring due to edge effects will likely alter belowground plant root community and distribution (Jackson et al., 2000). Root community changes can influence soil properties. For example, shrubland and grassland vegetation typically allocate more carbon (C) to roots than annual cropland plants, resulting in greater soil C and soil organic matter (Jackson et al., 2017). Plant species and their roots can also influence the soil microbial community through changes in physical soil properties (Gould et al., 2016) and through root exudates (Eisenhauer et al., 2017). Cropland edges may have spillover effects from various amendments applied to the cropland affecting adjacent areas (Fried et al., 2018). Raised nutrient availability levels can shift composition of the microbial community. Commonly, nitrogen (N) containing fertilizers promote certain microbial groups, such as ammonia oxidizing bacteria, effectively changing bacterial community structure, increasing ammonia oxidation, and altering N cycling (Enwall et al., 2007). Changes to the soil microbial community could alter ecosystem function, promoting certain groups of microbes or altering plant productivity that can influence nutrient cycling (Rout and Southworth, 2013).

To further understand edge effects in the agroecosystem, the edges between perennial grasslands and annual croplands were examined at two sites, the Conservation Learning Centre (CLC) and the St. Denis National Wildlife Area (SDNWA). Both sites are located in central Saskatchewan and in the greater prairie pothole region. The two main research objectives were:

1. To characterize aboveground plant community, belowground plant community, soil properties, and soil microbial communities across perennial grassland-annual cropland edges in central Saskatchewan.

2.    Determine key biotic (aboveground and belowground plant richness) and abiotic factors (total N and total C) influencing the soil microbial community across the perennial grassland-annual cropland edges in Saskatchewan.

It was hypothesized that the aboveground and belowground plant community would change in composition and richness across the perennial grassland-annual cropland edges, which in turn would influence soil properties and the soil microbial community across these edges.

This thesis is written in the manuscript format with four chapters, a general introduction, a literature review, one research chapter covering both objectives, and a synthesis and conclusions chapter to summarize findings. The following is an outline of the chapters.

Chapter 2 is a literature review with a brief overview of edge effects across ecosystems, focusing on agriculture. Edge effects on plants is the primary focus. Plant and soil property influences on the soil microbial community are also briefly discussed.

Chapter 3 provides a characterization of perennial grassland-annual cropland edges. Aboveground vegetation, belowground vegetation, soil properties, and the soil microbial communities are examined across the edges. Aboveground and belowground properties are linked by using structural equation modelling (SEM). Further exploration of fungi at the genus level show different relationships between plant and soil properties.

Chapter 4 summarizes key results, discusses implications, and provides suggestions for future research. Potential management strategies are also discussed for edges in the agroecosystem that promote biodiversity and potentially enhance ecosystem services in landscapes.

## 2.0 LITERATURE REVIEW

### 2.1 Ecotones, Edges, and Edge effects

The influence of spatial patterns on ecological processes are studied at a variety of spatial scales in landscape ecology. At the landscape level, elements can be placed in three different categories: patches, corridors, and matrices, collectively known as the patch-corridor-matrix model. A patch is defined as an area that is relatively similar but differs from its surroundings, a corridor as a narrow strip that differs from land on either side, and the matrix as the dominant ecosystem type that has high connectivity within a landscape (Forman, 1995). However, the concept of the matrix is changing to accommodate spatial and temporal variation, as well as, challenging the idea that the matrix is considered non-habitat (Driscoll et al., 2013). A newer continuum type model has also been developed, this model generally focuses on one species and considers resources and habitat suitability for that one species rather than patch-corridor-matrix. Hybrid models combining the patch-matrix-corridor and the continuum model are also starting to be implemented (Brudvig et al., 2017). Landscapes are typically heterogeneous and therefore are characterized by a mosaic of patches with transitional gradients between different patch types using the patch-corridor-matrix concept. Mechanisms behind this heterogenic spatial arrangement and patchiness of the landscape include topography, geology, climatic and edaphic gradients, vegetation change, herbivory, and natural disturbance (Wiens, 1976; Forman, 1995). Human activities and disturbance also have a large influence on landscape composition (Pielke et al., 2011). Disturbance can create patches by leaving a mosaic of affected and unaffected areas. Anthropogenic disturbance is a primary cause for creating patches, linear features, and edges within most landscapes (Godron, 1981). Urban development, resource extraction, agriculture, and other land uses increase patchiness (Forman, 1995).

Patch size, distribution, and connectivity are essential for how energy flows and how species disperse across the landscape. In general, smaller patches tend to support fewer native plant species (Fischer and Lindenmayer, 2007) and smaller patches have a greater edge-to-area ratio than larger patches. An ideal patch size does not exist and in a managed landscape setting, patch size will vary depending on the species or process of interest; hence, the optimum patch size will depend on the management goals for a particular landscape (Forman, 1995).

Where two patches of habitat or vegetation type meet, exists a unique space whose properties differ from the adjacent patches (Ries et al., 2004). On a large scale, this area may be an ecotone; originally a term that describes a gradual change in vegetation (Yarrow and Marín, 2007), or in more recent times, ecotones can mark a transition zone between two ecosystems (Forman, 1995). Ecotones usually have environmental gradients that influence species distribution, or gradient conditions that directly affect ecosystem processes (Gosz, 1991). Precipitation gradients can explain transitions from prairie to forest, or shrubland to desert (Myster, 2011). Ecotones are also often associated with edaphic gradients. A soil depth gradient was identified as the main cause of a shrub-forest ecotone in New Zealand (Lloyd et al., 2000), while a prairie-forest ecotone in Saskatchewan had an underlying soil pH gradient (Purton et al., 2015).

A sharper transition between patches or other boundaries in the landscape, are termed edges (Forman 1995). An edge can be described as an abrupt change in ecological and microclimatic conditions; the term itself was introduced more recently than ecotone (Yarrow and Mandrin 2007). In general, edge is often used to denote the physical location where two habitat types meet and edges can exist within an ecotone. Edges occur naturally and can be created by anthropogenic disturbance (Luck et al., 1999). Changes in vegetation frequently mark an edge, such as grassland to forest (Risser, 1995). Human induced edges can be created by various activities including timber harvesting (Euskirchen et al., 2001), roads (Gieselman et al., 2013), agricultural fields (Dutoit et al., 2007; Rostami et al., 2016), and urban development (Barnard et al., 2007; Vallet et al., 2010). Patches and their edges have a constant exchange of material (Levin and Paine, 1974). Edges can affect the surrounding area, which can be due to both physical and biological changes at the edge (Fischer and Lindenmayer, 2007). Improving our understanding of edge effects could help to predict energy and resource flow within the landscape (Ries et al., 2004).

Edges can intensify, constrict, or block material flow from adjacent patches; permeability of edges can be largely determined from plant structure (Ries et al., 2004). Plant communities with species that form dense canopies reduce light at the ground level and can alter microclimate. These microclimatic changes could be unfavourable for some plant species, preventing movement of those plant species (Ries et al., 2004). In this way edges can act as a filter, affecting certain species or processes more than others (Forman and Moore, 1992). Edges can also act as

type of barrier to biotic invasions, by providing habitat for beneficial predators (Magura et al., 2017). Various plant and animal species may also respond differently to edges. Species response can be positive, negative, or neutral (Ries et al., 2004). Negative responses to edges and habitat fragmentation include large mammal populations, specifically large carnivores (Woodroffe, 1998), cougars (*Puma concolor*) (Dickson et al., 2005), and desert bighorn sheep (*Ovis canadensis*) (Epps et al., 2005). However, edges can promote certain species, such as deer mice (*Peromyscus maniculatus*) and white-tailed deer (*Odocoileus virginianus*) by allowing access to resources in adjacent patches (Alverson et al., 1988; Bayne and Hobson, 1998). Insects can also be influenced by edges, where greater nest densities have been found at edges of agricultural fields (Dauber and Wolters, 2004).

Edge effects on vegetation composition and distribution have been examined across a variety of natural and anthropogenic edges. A natural forest edge had greater stem densities than the interior of a Eucalyptus forest in Australia (Wright et al., 2010). Along with different densities of plants found at the edge, different plant species can be found at the edge compared with the interior. Plant species commonly found at edges are often introduced or non-native plants (Gieselman et al., 2013). Microclimatic gradients at edges can influences plants, especially at forest edges, where shifts in vegetation composition have been attributed to considerable gradients in microclimate (Matlack, 1993; Baker et al., 2016). In one case, relative humidity at mixed mesophytic forest edges had the largest influence on vegetation composition (Gehlhausen et al., 2000). Shade from the vegetation itself can also drive temperature and humidity conditions (Baker et al., 2016). Changes in the aboveground vegetation community will also translate to belowground vegetation differences. Plant roots are extremely important as they influence soil structure, water dynamics, and soil nutrient status (Bronick and Lal, 2005). Yet, examining belowground plant community change across edges has not been specifically explored.

Gradients in soil properties can also be a large determinant of vegetation communities. Coniferous and mixed forest edges were found to have soil N and pH gradients that extended from the edge to the interior (Bergès et al., 2013). Soil pH was found to be highest at a forest edge and became more acidic towards the interior, the same pattern was also found for Ca, K, Mg, and Al (Wuyts et al., 2013). Temperature differences across a tropical forest edge were also found in the soil, these soil temperature differences were greater than aboveground temperature differences across the edge (Ewers and Banks-Leite, 2013).

Measuring and describing edge effects is difficult due to complexity and variability of edges, with many influencing and interacting abiotic and biotic factors (Coffin, 2007). Several metrics have been developed to describe edge effects including edge influence, magnitude of edge influence, distance of edge influence, and area of influence (Harper et al., 2005). Ewers and Didham, (2006) propose that the strength of edge effects should be analyzed in two parts: magnitude and extent. Magnitude of an effect would be determined based on the amount of difference in a given characteristic between the interior and the edge. Extent of an edge effect would be the distance the effect can be detected compared with the interior of the nearby patch.

## 2.2 Edges in Agricultural Landscapes

Agricultural expansion and intensification will continue as the human population grows and this has various consequences on ecosystems. Expansion of agriculture will likely result in more habitat fragmentation, increase anthropogenic edges with expansion, and reduce anthropogenic edges in intensification (Magura et al., 2017). Moreover, loss of natural habitat to agricultural expansion may be detrimental for overall biodiversity due to homogenization of the landscape (Gámez-Virués et al., 2015). The alternative solution, intensification, is to increase productivity of existing agricultural land (Lambin et al., 2001), which can also decrease biodiversity in agroecosystems (Ma et al., 2013). Different organisms respond differently to land use intensification, but plant community diversity has been severely reduced (Gossner et al., 2016). Both expansion and intensification of agriculture can modify edge effects that occur in these managed landscapes and intensification could potentially magnify edge effects (Didham et al., 2015).

Edges in agriculture are readily manipulated through alterations in farming practices or any other associated land management practices (Ma et al., 2013). Different types of farming can have different edge effects; for example, organic farms had greater plant species richness at field edges than conventional farms due to less disturbance of the seed bank (Gabriel et al., 2006). Amendments applied on croplands can spillover into non-target areas; fertilizer runoff may promote higher plant species richness at the edge (Rostami et al., 2016; Gabriel et al., 2006). Higher phosphate and nitrate ($NO_3$) were found at a cropland-heathland edge compared with a forest-heathland edge; likely due to fertilizer applications on croplands (Piessens et al., 2006). The edge and areas adjacent to croplands are also subject to herbicides applied to croplands

(Pogue and Schnell, 2001; Postma-Blaauw et al., 2010). Herbicide drift can occur and can negatively affect non-target plants and arthropods at edges (Egan et al., 2014). The extent of edge effects in agriculture are largely dependent on management practices in the cropland and the condition of the adjacent area. In Quebec, a one metre edge effect was observed when analyzing the plant community at cropland edges (Boutin and Jobin, 1998). Edges in the agroecosystem are often characterized by higher plant diversity that in turn can act as a food source for birds and insects (Fried et al., 2009).

Field edges are prime habitat for plants that can tolerate higher levels of disturbance and capitalize on open space and resources. Edges are a place where these plants establish and spread, a starting point of invasion (Cilliers et al., 2008). Because these types of plants, also known as ruderals, are adapted to disturbance, they will exploit resources rapidly (Grime, 2006). Disturbances under agriculture are frequent from tillage to harvest (Booth and Swanton, 2002) and act as a filter on plants that are not disturbance-tolerant. Often, ruderals are generally more adaptable, which allows them to survive in areas subject to frequent disturbance (Navas, 2012). Composition of the weed community is highly dependent on farming practices (Pogue and Schnell, 2001; Liira et al., 2008; Rotchés-Ribalta et al., 2015). Edges can function in three general ways when concerning invasive plants species; edges can act as a barrier preventing invasive species from expanding into the adjacent area, as a conduit that facilitates movement of invasive species, and finally as a source of invasive species (Wilkerson, 2013). An overall decrease of biodiversity in agroecosystems may render these systems more susceptible to invasive species; as plant diversity is lower, fewer niches are filled and these niches can be exploited by invasive plants (Richardson and Pyšek, 2006).

Edge effects are primarily measured aboveground, but belowground effects have not yet been thoroughly explored. A large proportion of biodiversity in agroecosystems resides in the soil (Brussaard et al., 2007). The soil microbial community and its relationship with plants are a large contributing factor of overall plant community structure and ecosystem function (Bardgett et al., 2005). Thus, understanding how soil microbial communities and soil-plant relationships change across edges is essential for fully understanding dynamics in the agroecosystem.

## 2.3 Soil Microbial Communities in Agriculture

Land use and agricultural practices can have a lasting effect on soil microbial communities. Microbes are sensitive to certain environmental conditions, and have their own optimal ranges (Rousk et al., 2010). These factors are directly related to both land use, as well as, landscape location (Singh et al., 2010). A long-term study found that even after 50 years post cropping, abandoned cropland plots still had a similar microbial community as the current cropland plots (Buckley and Schmidt, 2001). Generally, high-input agriculture with conventional tillage decreases diversity and abundance of the soil microbial community (Brussaard et al., 2007). Physical disturbance from conventional tillage is known to decrease fungal populations, specifically mycorrhizae (Paul, 2015). Desiccation and compaction from tillage can have a major influence on the microbial community (Lupwayi et al., 1998), in addition to different crops and crop rotations (Postma-Blaauw et al., 2010). A study compared three types of tillage found that no-till under maize had higher root colonization by mycorrhizae than conventional till or chisel plow (Mozafar et al., 2000). Additionally, abundances of fungi and bacteria were greater in a minimum tillage practice compared to more intensive conservation plough (Kaurin et al., 2018).

Amendments used in agriculture can affect the soil microbial community. Increasing soil nutrient availability, such as C and N, was found to increase overall microbial richness (George et al., 2019) and increase microbial biomass (Yao et al., 2000). N containing fertilizers are consistently observed to influence microbial community composition and function (Ramirez et al., 2012). One study found N fertilizers increased ß-glucosidase (Cong and Eriksen, 2018) and N mineralization (Wingeyer et al., 2015; Cong and Eriksen, 2018). Another study found N fertilizer, a combination of urea, $NH_4$, and $NO_3$, decreased dehydrogenase and alkaline phosphatase activity (Nivelle et al., 2018). Different forms of fertilizers can also affect the soil microbial community. A long-term fertilizer study found manure, mineral fertilizer (NPK) and the combination of the two, had an increased abundance of enzymes compared to the control. However, the same study also found the greatest bacterial richness in the manure treatment (Francioli et al., 2016). One study reported no effect of inorganic fertilizer on the microbial community, but found organic amendments did affect microbial community composition (Li et al., 2017). Another study also found the organic amendment, sewage sludge, to increase soil respiration more than inorganic fertilizer treatments ($CaNO_3$ and $(NH_4)_2SO_4$) and the control

(Enwall et al., 2007). Other nutrients, like phosphorus (P) can also influence the soil microbial community and function. Both saprophytic and pathogenic fungi increased with increasing soil P (Schmidt et al., 2019). Additionally, after a one-time application of inorganic P fertilizer, both the bacterial and fungal communities were altered, including a significant change in arbuscular mycorrhizae fungi (AMF) communities (Ikoyi et al., 2018). AMF was also found to be negatively correlated with $NO_3$ (Schmidt et al., 2019). When comparing bacterial and fungal community responses to soil properties, the bacterial community was more strongly related to soil type than the fungal community (Wakelin et al., 2008), suggesting that bacteria are more influenced by soil properties than fungi.

Soil pH is reported to consistently affect soil bacteria (Rousk et al., 2010; Erlandson et al., 2018; Bahram et al., 2018). N fertilizers can affect the soil microbial community through alterations of soil pH (Geisseler and Scow, 2014). At lower pHs, aluminum becomes more soluble and is toxic to microbes (Wasof et al., 2019). In one case, it was found that pH better explained the mycorrhizal community than vegetation (Dumbrell et al., 2010).

Fungicides, herbicides, and pesticides can also affect the soil microbial community. Studies examining fungicide effects on the soil microbial community find that fungi are more affected than bacteria (Smith et al., 2000; Adetutu et al., 2008). Long-term application of a fungicide on a tall grass prairie showed an increase of the bacteria to fungi ratio and the fungicide also showed to have negative effects on AMF (Smith et al., 2000). Slight shifts in the fungal community were found after application of three fungicides including azoxystrobin, tebuconazole, and chlorothalonil, but no effects were found on bacterial community composition (Bending et al., 2007). Inconsistent effects on bacterial community composition were observed with fungicide application on canola fields in Canada, some bacterial communities composition shifted, but the majority did not (Lupwayi et al., 2009). Puglisi et al., (2012) found that ammonia oxidizing bacteria and archaeal communities were not altered by fungicides. Herbicides, specifically glyphosate, has mixed effects on the soil microbial community. Minor shifts in bacterial composition were found with application of glyphosate, increasing abundance of the phylum, Bacteroides (Guijarro et al., 2018). Only a marginal decrease in fungal richness was found when glyphosate was applied at twice the recommended rate on a wheat field (Schlatter et al., 2018). Some studies find no effect at all; soil bacterial and archaeal richness, evenness, composition, and enzyme activity was not altered by the application of four herbicides at the

recommended rate (Dennis et al., 2018). However, long-term studies may find larger effects on soil microbial community composition as one long-term study found negative effects of glyphosate on AMF and *Rhizobium* spp. (Van Bruggen et al., 2018). Changes of AMF and other symbionts like *Rhizobium* spp. can have significant effects on the larger ecosystem, as plant production and composition may be affected by loss of these symbiotic microbes. Pesticides can also potentially affect the soil microbial community. Soil bacterial community structure was significantly altered by pesticide application in a winter wheat field (Girvan et al., 2004). Some studies find no effects of pesticides on bacterial community composition (Omar and Abdel-Sater, 2001; Lupwayi et al., 2009), others have observed negative effects on soil fungi and decreased acid phosphatase enzyme activity (Omar and Abdel-Sater, 2001).

### 2.4 Soil Microbial-Plant Relationships

Plant growth can be strongly influenced by soil microbial communities and plants can also influence soil microbial communities (Postma-Blaauw et al., 2010). Understanding the relationship between the microbial community, plant growth, and ecosystem function is crucial, especially under changing landscapes. Factors affecting the soil microbial community as previously discussed include edaphic factors, various agricultural chemicals, but also plant communities (Berg and Smalla, 2009; Singh et al., 2010)

Soil microbes can have direct and indirect influences on plants through associations with plant roots and as free-living soil microbes (Strickland et al., 2009). Many microbes associate with plant roots, which help plants acquire nutrients and improve overall health (Berg and Smalla, 2009). Microbes in return often receive C substrates from root exudates (Berg and Smalla, 2009). Plants can influence microbial communities through root exudates, such as hormones that may select for or against certain microbes (Lareen et al., 2016). Root biomass and quantity of root exudates was found to be positively correlated with fungal biomass (Eisenhauer et al., 2017). Mycorrhizae, a fungal root symbiont, helps facilitate nutrients to the host plant. Plants are often reliant on mycorrhizae to obtain P, a relatively immobile nutrient in soil (Leake et al., 2004). Bacteria can also have symbiotic relationships with plants. Lentil seeds that were inoculated with *Rhizobium* in Saskatchewan increased seed production by 45% (Gan et al., 2005). Bacteria can also promote plant growth through bacterial production of plant-growth

hormones, rendering nutrients available, and enhancing other symbiosis with the host plant (Vessey, 2003).

Plant biomass and plant species identity may also help shape soil microbial communities. A study comparing strawberry and canola found different bacterial community compositions in the rhizospheres (Costa et al., 2006). Differences of microbial community composition were even found among different canola varieties (Dunfield and Germida, 2001). Plant species can influence the microbial community belowground, but also aboveground through plant biomass. Strickland et al., (2009) found that by taking plant litter from different communities; grass, pine, and rhododendron; and placing the litter with a soil from a different plant community, resulted in reduced C mineralization. Native vegetation together with native soil had the highest rates of C mineralization. Based on this finding, the researchers suggest that the soil microbial communities were not functionally the same and previous vegetation likely has a large influence on soil microbes.

The influence of exotic plant species on soil microbial communities highlight the importance of plant-soil microbial interactions and may provide insight into plant-soil dynamics of common weeds. Frequently, a positive feedback cycle occurs when exotic plant species are introduced into a new environment (Klironomos, 2002). Microbes that once regulated or suppressed the plant in its native range, are absent in the new habitat (Callaway et al., 2004; Strickland et al., 2009). Pathogenic microbes and mycorrhizae are thought to be responsible for a negative and positive feedback cycle, respectively (Klironomos, 2002). In a study on smooth brome invasion of a native fescue grassland, the invaded grasslands compared to non-invaded grasslands had a higher total N content and N mineralization rates (Piper et al., 2015). Soil microbial populations were different between the invaded and native grasslands, with higher ammonia-oxidizing bacteria and archaea present in the invaded grassland. This suggests that smooth brome affects the soil microbial population altering nutrient cycling (Piper et al., 2015). Invasive plants, which are frequently found at edges, have the potential to alter microbial communities that ultimately affect nutrient cycles.


## 2.5 Ecosystem Function and Services at the Edge

Agricultural disturbance can negatively influence ecosystem functions and services (Lalibert et al., 2010). Expansion and intensification of agriculture leads to landscape

simplification that results in loss of biodiversity (Landis, 2017). Biodiversity and landscape heterogeneity has also been linked with natural enemies of crop pests and pollinator diversity (Kazemi et al., 2018). Plant and microbial diversity have also been linked to ecosystem functions, such as C and N cycling (Zak et al., 2003; Strickland et al., 2009). Alterations to the N cycle are concerning, as N is one of the most globally limiting macronutrients for plant growth (Havlin, 2013). Net N mineralization has been associated with the relative abundance of fungi and bacteria (Waring et al., 2013). Therefore, agricultural practice influencing the soil microbial community will subsequently alter nutrient cycling.

Plant and microbial diversity have been related to the provision of ecosystem services. Research conducted on plant species richness in an old-field site in Minnesota, found increased biomass production with greater plant species richness and greater soil microbial diversity. Higher N mineralization rates were also found in plots that had higher plant species richness (Zak et al., 2003). Edge effects can simultaneously reduce native plant diversity while increasing overall species richness leading to a more biodiverse environment at the edges (Cousins and Aggemyr, 2008; Gieselman et al., 2013). However, invasive plants and weeds frequently dominate agricultural edges (Gabriel et al., 2006) and may account for this increase in plant species richness. Considering the soil microbial community's functional diversity rather than taxonomic biodiversity may be important when assessing ecosystem function; a decrease in taxonomic diversity does not always reduce function (Chaparro et al., 2012). Soil N dynamics could be an indicator of microbial functional status (Tiemann and Billings, 2011) and examining how changes in soil microbial community composition are linked to these dynamics are important for understanding agricultural edges and their effects.

## 3.0 EDGE EFFECTS ACROSS PERENNIAL GRASSLANDS AND ANNUAL CROPLANDS

### 3.1 INTRODUCTION

The agricultural landscape, an agroecosystem, is a complex ecosystem heavily influenced and manipulated by anthropogenic activities. Humans alter inputs into the system through the addition of crop seeds, soil amendments, fertilizers, herbicides, and pesticides. Outputs are also altered, such as crop harvesting, mowing, or grazing. As a consequence, ecosystem processes, such as nutrient cycling, biological pest control, and pollination are modified, usually having a negative influence on the processes (Tscharntke et al., 2005). An agroecosystem is composed of various land uses and where two land uses meet, edges exist. Plant and soil characteristics at the edge are unique, as it is a product of two land uses, ecosystems, or habitats meeting. Edges between land uses can influence the surrounding area and changes can be both abiotic, including temperature, moisture and soil properties, and biotic, such as plant community or animal community composition and distribution (Fischer and Lindenmayer, 2007). Expansion and intensification of agriculture has already influenced and induced change in nearby habitats, observed for both plant communities and soil properties (Culman et al., 2010; Didham et al., 2015; Buhk et al., 2017). Edges and the surrounding areas experience increased stress from agricultural intensification that can magnify edge effects (Didham et al., 2015) resulting in reduced vegetation and soil biodiversity (Lambin et al., 2001). Intensive, frequent, and consistent management practices occur in agroecosystems from tillage to harvest (Booth and Swanton, 2002), which define edge habitats and influence edge effects. Edges and the adjacent land of agricultural fields are subject to runoff containing various chemicals and amendments applied to croplands (Pogue and Schnell 2001; Postma-Blaauw et al., 2010). However, the extent and characteristics of edges and their effects in agroecosystems remain poorly understood.

Vegetation at the edge is susceptible to adjacent land uses. In an agroecosystem compared to a natural ecosystem, overall vegetation richness is in the agroecosystem is lower (Cousins and Aggemyr, 2008). However, edges themselves frequently have greater vegetation diversity as observed at edges between grasslands and forests (Ohara and Ushimaru, 2015). Edge effects can simultaneously reduce native plant diversity while increasing the overall species richness leading to a more biodiverse environment at the edge (Cousins and Aggemyr, 2008; Gieselman et al., 2013). Weed population densities are highest near, or at, an edge (Cardina et

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al., 1997) because these plants are disturbance tolerant (Watling and Orrock 2002). Plant communities at the edge may be of concern to farmers, where weeds can compete with crops (Oerke and Dehne, 2004). While aboveground vegetation changes at the edge are evident, belowground changes may also occur, but belowground edge effects in agroecosystems remain obscure.

Underlying gradients of soil properties have been found at edges, such as pH, nitrogen (N), and carbon (C) (Pocewicz et al., 2007; Bergès et al., 2013). Different soil properties can support different plant species. For example, one of the most globally limiting macronutrients for plants is N and elevated soil N can increase overall plant productivity but may reduce plant species richness, as increased litter can prevent seedling establishment in a grassland (Foster and Gross, 1998). This demonstrates the importance of how aboveground and belowground processes are linked. Soil physicochemical properties and vegetation changes across the edge may also influence the soil microbial community.

Soil microorganisms are critical in maintaining ecosystem functions, especially in nutrient cycling, such as mineralization (Zak et al., 2003; van der Heijden et al., 2008). Soil microbes are sensitive to certain environmental conditions and have their own optimal ranges (Rousk et al., 2010). Factors affecting the soil microbial community can be abiotic and biotic, which include edaphic factors such as pH, soil texture, soil moisture, nutrient availability (Lauber et al., 2008), agricultural chemicals and practices (Schimel et al., 2007), and plant community composition (Berg and Smalla, 2009; Singh et al., 2010). However, the magnitude to which these factors influence the soil microbial community are complex, with various studies reporting different effects on the bacterial and fungal communities (Boer et al., 2005). For example; some studies conclude bacteria are more sensitive to soil properties than fungi (Sayer et al., 2017; Sun et al., 2018) and that fungi are also more resistant to climatic changes, such as drought (de Vries et al., 2018). Microbial interaction can also influence community structure and function; for example, mycorrhizae can potentially limit C resources for nearby free-living soil microbes (Moore et al., 2015). Plant community composition is another factor that influences microbial community composition; certain plant species may have specific associations with specific microbes, such as mycorrhizal associations with plant roots (Berg and Smalla, 2009). Additionally, invasive plant species can alter the soil microbial community by changing the quality and quantity of litter inputs (Callaway et al., 2004). How the soil microbial community

responds to edge effects is crucial, as the soil microbial community is essential for ecosystem function (van der Heijden et al., 2008).

To investigate edge effects in agroecosystems, we examined aboveground and belowground attributes across perennial grassland-annual cropland edges in central Saskatchewan. The central question considered in this study was: If aboveground edge effects alter plant community composition and distribution in agroecosystems at perennial grassland-annual cropland edges, then will it drive change in soil properties and the soil microbial community? To address this question, we investigated vegetation composition and structure, physiochemical soil properties, and the soil microbial community composition across the edge. Two objectives were developed, the first objective was to characterize the aboveground plant community, belowground plant community, soil properties, and the soil microbial community. The second objective was to understand the link between aboveground and belowground changes to identify the factors that are driving the soil microbial community.

### 3.2 Methods and Materials

#### *3.2.1 Site Description*

We examined perennial grassland-annual cropland edges at two sites, St. Denis National Wildlife Area (SDNWA) (52°12'59.2"N 106°05'32.7"W) and the Conservation Learning Centre (CLC) (53°01'57.1"N 105°46'37.4"W). SDNWA is located in the Moist Mixed Grassland ecoregion and CLC is in the Boreal Transition ecoregion (Shorthouse, 2010). Soils at SDNWA are largely composed of Dark Brown Chernozemic and CLC are predominantly Black Chernozemic soils (Pennock et al., 2011).

We sampled at SDNWA from June 25-28, 2017 and sampling at CLC took place June 29 – July 6, 2017. Both sites consisted of cropland interspersed with perennial grassland fields. Grassland fields are cut once a year for hay at both sites. Flax (*Linum usitatissimum* var. CDC Sorrel) was planted for the 2017 growing season at SDNWA. Prior to seeding in May 2017, glyphosate was applied to croplands. Granular fertilizer (80 N - 32 P - 15 S lbs/acre) was used at the time of seeding and herbicides (Buctril M-broadleaf and Centurion-grasses) were applied to fields in July 2017. Perennial grasslands at SDNWA were largely composed of smooth brome

(*Bromus inermis* Leyss.), Kentucky bluegrass (*Poa pratensis* L.), quackgrass (*Elymus repens* L. Gould), and alfalfa (*Medicago stavia* L.). Prominent weeds included Canada thistle (*Cirsium arvense* L. Scop.), lamb's quarters (*Chenopodium album* L.), flixweed (*Descuriana sophia* L.), and perennial sow thistle (*Sonchus arvensis* L.).

Canola (*Brassica napus* L., Nexera RR112 Roundup Ready) was planted in May of 2017 at CLC. At the time of seeding, anhydrous fertilizer was applied (100 N - 25 P - 25 S lbs/acre). Glyphosate was also applied at the time of seeding, then Topnotch/Eclipse (fungicides) in June and Lance (fungicide) in July 2017. Perennial fields at CLC were composed of *B. inermis* L., meadow brome (*Bromus biebersteinii* Roem. & Schult.), *M. stavia*, and yellow clover (*Melilotus officinalis* L.). Prominent weeds at include *S. arvensis* and *C. arvense*.

Mean temperature in June and July 2017 at SDNWA was 15.6°C and 19.2°C, respectively. The 2017 annual precipitation at SDNWA was 337 mm; 14.4 mm fell in June and 19.6 mm in July (Bam et al., 2018). Mean temperature of June and July 2017 at CLC was 15.3°C and 18.4°C, respectively (Wittrock, 2019). The 2017 annual precipitation at CLC was 264 mm; 44.9 mm fell in June and 17.6 mm in July. The 2017 precipitation at CLC was the lowest of the past six years of recorded data from the CLC meteorological station (Wittrock, 2019).

### 3.2.2 Study Design

We sampled edges at two locations within each site. At each sampling location, three transects were laid perpendicular to the perennial grassland-annual cropland edges and spaced 3 metres apart (Fig. 3.1). Along each of the three transects, samples were taken at the edge (0 m), 25 cm, 50 cm, 1 m, 2 m, 6 m, 8 m, 16 m and 33 m into each of the two land use types, the perennial grassland and annual cropland. To add randomness to the design, each sampling point along the transect was randomly assigned a position of either directly on the transect, or 1 m to the left or right of the transect.
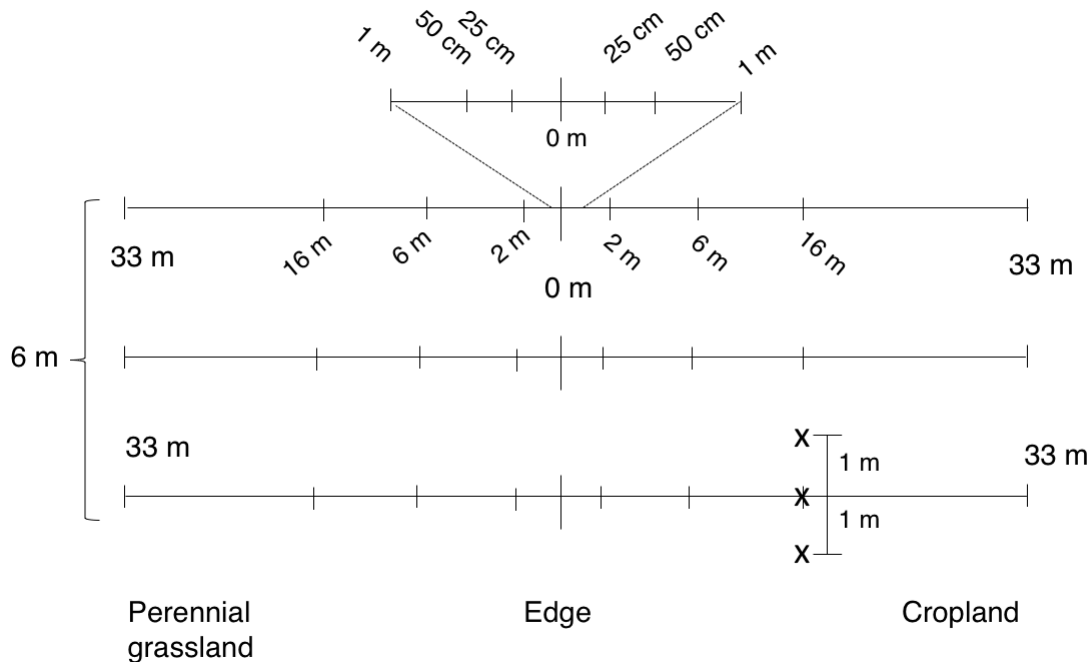
**Fig. 3.1**: Transect sampling design at perennial grassland-annual cropland edges. At each transect location, two per site, three transects (33m from edge into each land use) were spaced 3m apart. Each transect had 15 sampling point locations relative to the edge (25cm-33m). Each sampling point along the transect was randomly assigned to one of three positions (x): 1m left, 1m right or on the transect. At each site, there was a total of 90 sample points (2 sample locations * 3 nested transects * 15 sampling points per transect).

### *3.2.3 Aboveground Vegetation Sampling*

At each sampling point along a transect, we assessed percent cover for all plant species within a 1 $m^2$ quadrat (Fig. 3.2). Plant species presence/absence within a 1 m radius of the centre point were also recorded. Aboveground biomass was collected in a 20 cm x 50 cm quadrat that was placed in the centre of the 1 $m^2$ quadrat. Biomass was clipped at the surface and separated into three categories: grass, forbs, and plant litter. Biomass samples were then placed into a drying room at 40°C for four days and weighed to determine dry biomass. A voucher specimen collection for all plant species at the two sites was compiled at the WP Fraser herbarium, University of Saskatchewan. Plant specimens were collected during both summers of 2017 and 2018.

### 3.2.4 Belowground Soil Sampling

To characterize soil properties, we took a soil core (5 cm diameter x 10 cm depth) from the A horizon near the centre of the cover quadrat using a sledge core (AMS Soil Core Sampler, American Falls, ID) (Fig. 3.2). A composite sample of three smaller cores (2 cm diameter x 15 cm depth each) was also sampled near the centre of the cover quadrat (Fig. 3.2). The composite sample was used for molecular analysis of the soil microbial community (i.e. bacteria and fungi) and plant roots. Immediately following sampling, all soil samples were placed in a cooler, transported to the University of Saskatchewan, and subsequently stored at -20°C.
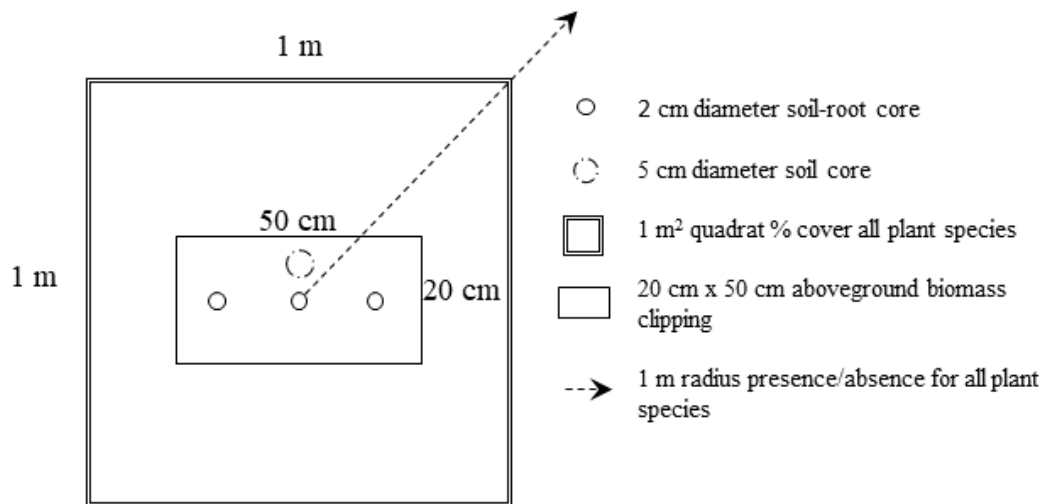


**Fig. 3.2**: Schematic of an individual sampling point along a transect. Percent vegetation cover was assessed first with the largest quadrat, 1m2. The dashed arrow represents a 1m radius of recorded plant species. The smaller nested quadrat (20cm x 50cm) was used to harvest aboveground biomass. Following biomass collection, a 5cm diameter soil core was taken near the centre of the sampling point as well as a composite sample of three 2cm diameter soil push cores.

### 3.2.5 Characterization of Soil Properties

Soil from the 5 cm diameter x 10 cm depth core was air-dried and passed through a 5 mm sieve to remove any large debris and rocks. Soil nitrate ($NO_3$) and ammonium ($NH_4$) extractions were performed using 50 mL of 2.0 M KCl from 5 g of soil and filtered using Whatman No. 42 filter papers (Carter and Gregorich, 2007). The filtered sample extracts were analyzed on an AutoAnalyzer 3 (SEAL, UK). Soil pH was measured with a pH probe (Mettler Toledo, USA) using a 1:2 soil to 0.1 M $CaCl_2$ solution (Thomas, 1996).

Total N and total C were determined by dry combustion. Air-dried, sieved soil was further homogenized using a ball-grinder (Retsch MM-400, Germany), 0.25 g of soil was used for analysis. Total C was combusted at 1100°C with a LECO C632 analyzer (LECO, USA). Total N was combusted at 1250°C with the TruMac CNS analyzer (LECO, USA).

### 3.2.6 Soil Microbial and Plant Community Sequencing

The composite soil samples were sub-sampled (5 g) and ground using a Retsch MM-400 (Retsch, Germany). Composite soil samples contained plant roots and these mixed root-soil sample were used for analysis of both microbial communities and plant communities. A pilot study conducted on different preparation methods to sequence plants, showed that there was no significant difference of plant operational taxonomic unit (OTU) richness between separating roots from soil and keeping root and soil together (mixed root-soil samples) (Appendix B). The capsule and grinding balls were thoroughly cleaned with bleach in between samples. We extracted DNA from 1 g of ground root-soil using the PowerPlant Pro Kit (Qiagen, Germany). The kit protocol was followed and DNA was eluted in 100 μL of EB solution. The DNA extract was quantified using the Qubit 2.0 Fluorometer (Invitrogen, Massachusetts, USA) with the Qubit HS assay kit (Invitrogen, Massachusetts, USA). All samples were standardized to 1 ng/μL of DNA for downstream amplification of the soil microbial community and the plant root community.

### 3.2.7 16S Amplicon Preparation

The 16S rRNA V4 region was amplified using the primers 515F/806R (Caporaso et al., 2011) to target the soil bacterial community. Reactions were performed at a final volume of 25

μL; 2 μL of template DNA, 12.5 μL of Platinum Green (2X) Master Mix (Thermo Fisher, Massachusetts, USA), 1.5 μL forward primer (10 μM), 1.5 μL reverse primer (10 μM), and 7.5 μL of PCR grade water. The PCR conditions were 3 mins at 94°C, 30 cycles: 94°C 45 s, 50°C 60 s, 72°C 90 s, and a final extension at 72°C for 10 mins. The PCR products were visualized on a 1.5% agarose gel to confirm amplification of the target region. Products were purified using the NucleoMag NGS Clean-up and Size Select magnetic beads (Macherey-Nagel, Germany) following the manufacture's protocol for single size selection with the exception of reduced drying time after the second ethanol wash (2 minutes).

### 3.2.8 ITS Amplicon Preparation

The internal transcribed spacer (ITS) region was amplified using the primer pair ITS1-F (Gardes and Bruns, 1993) and ITS2-R (White et al., 1990) to target the soil fungal community. The ITS region was selected based on its discriminatory power at lower taxonomic levels due to the high variability of the region and access to robust sequence reference databases (Lindahl et al., 2013). Reactions were performed at a final volume of 25 μL; 2 μL of template DNA, 12.5 μL of Platinum Green (2X) Master Mix (Thermo Fisher, Massachusetts, USA), 1 μL forward primer (10 μM), 1 μL reverse primer (10 μM), and 8.5 μL of PCR grade water. The PCR conditions were 3 mins 94°C, 35 cycles: 94°C 30 s, 52°C 30 s, 72°C 45 s, and 72°C for 7 minutes. The PCR products were visualized on a 1.5% agarose gel to confirm amplification of target region. Products were purified using the NucleoMag NGS Clean-up and Size Select magnetic beads (Macherey-Nagel, Germany) following the manufacture's protocol for double size selection with the exception of using a 2:5 initial ratio of beads to PCR product and reduced drying time after the second ethanol wash (2 minutes). The purpose of the double size selection procedure was to ensure that fragments larger than the target region were removed.

### 3.2.9 trnL Amplicon Preparation

Plant root DNA was amplified in the *trn*L intron region, using the *trn*L c-1 forward primer and *trn*L h-1 reverse primer set modified by Lamb et al. (2016). Reactions were performed at a final volume of 25 μL; 2 μL of template DNA, 12.5 μL of Platinum Green (2X) Master Mix (Thermo Fisher, Thermo Fisher, Massachusetts, USA), 1.5 μL forward primer

(10μM), 1.5 μL reverse primer (10 μM), and 7.5 μL of PCR grade water and cycling conditions of 5 mins at 95°C, 35 cycles: 95°C 30 s, 55°C 45 s, 72°C 60 s, and a final extension time at 72°C for 10 mins. The PCR products were visualized on a 1.5% agarose gel to confirm amplification of target region. Products were purified using the NucleoMag NGS Clean-up and Size Select magnetic beads (Macherey-Nagel, Germany) following the manufacture's protocol for single size with the exception of reduced drying time after the second ethanol wash (2 minutes).

### 3.2.10 Amplicon Library Preparation and Sequencing

Library preparation for Illumina MiSeq followed the Illumina Library Preparation Guide (#15044223 Rev. A), using Nextera XT Index Kit v2 Adapters (Illumina, San Diego, USA). The final concentration for the *trn*L library was 8 pM, 16S and ITS libraries were 10 pM, each library also had a 25% spike of PhiX (Illumina, San Diego, USA). A 300-cycle MiSeq v2 kit (Illumina, San Diego, USA) was used for 16S and *trn*L libraries, while ITS used a 500-cycle kit MiSeq v2 kit (Illumina, San Diego, USA). Sequencing was performed at the Toxicology Centre at the University of Saskatchewan. Reactions for all libraries were performed at a final volume of 25 μL; 2 μL of template DNA, 12.5 μL of Platinum Green (2X) Master Mix (Thermo Fisher, Thermo Fisher, Massachusetts, USA), 1.5 μL forward primer (10μM), 1.5 μL reverse primer (10 μM), and 7.5 μL of PCR grade water and cycling conditions of 5 mins at 95°C, 35 cycles: 95°C 30 s, 55°C 45 s, 72°C 60 s, and a final extension time at 72°C for 10 mins. Products were purified using the NucleoMag NGS Clean-up and Size Select magnetic beads (Macherey-Nagel, Germany) following the manufacture's protocol for single size with the exception of reduced drying time after the second ethanol wash (2 minutes).

### 3.2.11 Sanger Sequencing trnL

DNA was extracted from 0.05 g of plant material from voucher specimens collected in the field using the PowerPlant Pro Kit (Qiagen, Germany). The kit protocol was followed and DNA was eluted in 100 μL of EB solution. The DNA extract was quantified using the Qubit 2.0 Fluorometer (Invitrogen, Massachusetts, USA) with the Qubit HS assay kit (Invitrogen, Massachusetts, USA). PCR conditions and purification followed the same process as *trn*L for

Illumina sequencing, but stopping after the first purification. Purified PCR product was sent to the NRC facility at the University of Saskatchewan for Sanger sequencing.

### 3.2.12 Bioinformatics

Soil microbial sequences were processed through QIIME2 2018.11 (Caporaso et al., 2010) using the DADA2 pipeline (Callahan et al., 2016). Amplicon sequence variants (ASVs) are created instead of traditional operational taxonomic units (OTUs) with DADA2. For 16S sequences, only forward reads were used to ensure consistency due to poor overlap of sequences. Sequences were input into DADA2 that performed quality filtering and removal of chimeric variants. Reference sequences and taxonomy assignments were obtained from the Greengenes database (DeSantis et al., 2006). Archaeal, mitochondrial, and chloroplast sequences were removed from the dataset for downstream analysis on the bacterial community.

ITS sequences were also processed through the DADA2 pipeline that included quality filtering, removing chimeric variants, and merging forward and reverse reads. No trimming was performed on the reads due to the high variability of the ITS region (Halwachs et al., 2017). Reference sequences and taxonomy assignments were obtained from the UNITE database (Nilsson et al., 2019).

The *trn*L sequence bioinformatics process followed the pipeline developed by Lamb et al., (2016) and was conducted in mothur v.1.40. (Schloss et al., 2009). Briefly, forward and reverse reads were merged and trimmed to the primer region. Sequences were then aligned to a template created by Lamb et al. (2016), quality filtered, and chimeras were removed. The sequences were clustered into 99% similar OTUs and classified using a custom local database. The local database included plants recorded during percent cover and presence/absence measurements at CLC and SDNWA. The database was created from a combination of Sanger sequences from Lamb et al. (2016) and Sanger sequences obtained from the voucher specimens in this study. The Sanger sequences were combined into a single fasta file and a corresponding taxonomy file was created. The taxonomy file consisted of family, genus, and species for each plant in the fasta file. In mothur, the *list.seqs* command was used with the input as the taxonomy file, the resulting output was used in the *get.seqs* command along with the fasta file. The output file from the *get.seqs* command was used as the reference database.

### *3.2.13 Statistical Analyses*

All statistical analyses were conducted in R 3.5.2 (R Core Team, 2018) and code can be found in Appendix C. A non-metric multidimensional scaling (NMDS) was conducted for vegetation cover at each site using the *vegan* package (Oksanen et al., 2018). Plant cover data were Hellinger transformed before the NMDS to account for many zeros in the data set (Legendre and Legendre, 2012). Soil property vectors were created using the 'envfit' function in *vegan* and fit over the NMDS. From the NMDS, three groups based on sampling point location were apparent. Thus, we split sampling points into three edge locations: perennial grassland, edge, and cropland (n=30 for each edge location per site, n=5 for each sub-transect). Perennial grassland and cropland included sampling points from 1 m – 33 m on either side of the edge. The edge included samplings points at 0 m, 0.25 m, and 0.5 m into both perennial grassland and cropland.

To examine soil properties and vegetation biomass across the edge, we used linear mixed models (LMM). Fixed effects in the vegetation models included edge location, site, and their interaction, with biomass as the response variable. Random effects included sub-transect (n=3) nested within transect location (n=2). Models were fit with the *lme4* package (Bates et al., 2015) using restricted maximum likelihood (REML) estimation. Model fit was assessed by inspecting residuals to ensure homoscedasticity, AIC values, and adjusted $R_2$ values. We used the *lmerTest* package (Kuznetsova et al., 2017) to obtain degrees of freedom and *p*-values. Post-hoc testing was performed using Tukey's HSD to determine significant differences among edge location using the *emmeans* package (Lenth, 2019). Grass, forb, and litter biomass data were log transformed to meet the assumptions of normality. We also examined soil property relationships among edge location with the same LMM as described for biomass. $NO_3$ and $NH_4$ were log transformed to meet assumptions of normality.

Indicator plant species for each edge location were determined with the *indicspecies* package (Cáceres and Legendre, 2009). Plant species richness, evenness (Pielou's *J'*), and diversity (Shannon *H'*) were calculated with the *vegan* package (Appendix D). For the purpose of comparing the aboveground and belowground plant community, a Pearson's correlation matrix was created using the plant metrics: diversity, evenness, and richness. The best correlation was between aboveground and belowground plant species richness. Thus, we ran a linear model

with belowground plant richness as the response variable and aboveground richness as the explanatory variable.

A NMDS and a principal component analysis (PCA) were conducted for the soil microbial community. Both analyses were performed based on recent contention on how data obtained from high-throughput sequencing should be treated. Recent papers argue that this type of data needs to be treated as compositional, due to the capacities and limitations of next generation sequencing platforms (Gloor et al., 2017; Quinn et al., 2018). Compositional datasets have two distinct characteristics. First, is that the size of the library is an artifact and second, the difference between values are treated as proportions (Quinn et al., 2018). Sequences that are generated are not absolute and therefore do not reflect the true composition of the soil microbial community. Thus, we followed the process of analyzing microbial community composition using compositional data approaches from Gloor et al., 2017. Briefly, zero and NA values in the ASV tables were replaced with an estimate (Count Zero Multiplicative) using the *zCompositions* package (Palarea-Albaladejo and Martín-Fernández, 2015). The centered log-ratio transformation was calculated with the *CoDaSeq* package (Gloor and Reid, 2016) and PCA biplots were created with the 'prcomp' function. In addition to the PCA, an NMDS was used to examine the microbial community. Similar to the aboveground plant community, we used the Hellinger transformation as it places less weight on rare species (Legendre and Gallagher, 2001) and has previously performed well on microbial data (Mitra et al., 2010). Due to a horseshoe distortion of the bacterial PCA, the PCAs were placed in an appendix (Appendix E) and the NMDS is presented below in section 3.3.3. The horseshoe effect occurs when there is a linear gradient in the data and appears as a curve when plotting the first two dimensions, this could obscure interpretations of the communities (Morton et al., 2017).

Microbial diversity (*H'*), evenness (*J'*), and richness were also calculated from the ASV tables with the *vegan* package (Appendix F). Diversity, richness, and evenness were then used as response variables in LMMs to determine differences across the edge; however, only edge location was used as a fixed effect to fit the model. The same random effects were included.

To further investigate the fungal community, significant fungal genera were identified across the edge. Fungi were explored rather than bacteria because fungal community composition was different across the edge at both sites, based on the previous NMDS. To identify significant fungal genera, the ASV table was filtered at 20% prevalence across samples

to remove rare species. After filtering, the ASV table was centered log-ratio transformed and aggregated by genera using the 'tax_glom' function in the *phyloseq* package (McMurdie and Holmes, 2013). Welch's *t*-test was performed on each genus abundance across the edge using the previously established edge location: cropland, edge, and perennial grassland. The *p*-values were adjusted using the Bonferroni correction method and the resulting significant genera were used in further analyses. This process was completed for both sites together, and each site separately to acknowledge site differences.

Structural equation modelling (SEM) was used to investigate the relationship of the microbial community, plant community, and soil properties across the edge. An advantage of using SEM is the ability to include multiple complex relationships, with multiple variables. The first step in the SEM process is to develop a theoretical model or an *a priori* model (Grace, 2006). Observed variables used in the model were selected based on distinct trends across the edge from LMMs and how well the variables represented both sites. To capture the soil microbial community in the model, bacterial richness, fungal richness, and fungal genera abundance, as described in the previous paragraph, were used. Soil properties used in the model were soil total N and total C. Plant species richness for aboveground and belowground and living biomass was also included in the model. The *a priori* model included direct paths of C and N to fungi and bacteria, a direct path of plants to fungi, a direct path of bacteria to fungi, and a direct path of total biomass to total C and total N (Fig. 3.3).

The first SEM used total fungal richness and aboveground/belowground plant richness for both sites. The second set of SEMs used significant fungal genera in place of fungal richness and the last set of SEMs used site specific fungal genera. The variances of bacterial richness, fungal richness, and living biomass were large; thus z-scores were calculated using the 'scale' function in R. Models were fit and calculated using the *lavaan* package (Rosseel, 2012). Model fit was assessed by the chi squared value, associated *p*-value (*p*-value $> 0.05$ indicates good fit), Comparative Fit Index (CFI $> 0.95$ indicates good fit), Root Mean Square Error of Approximation (RMSEA $< 0.05$ indicates good fit) (Feinian Chen et al., 2008), and the Standardized Root Mean Square Residuals (SRMSR $< 0.09$ indicates good fit)(Iacobucci, 2010).
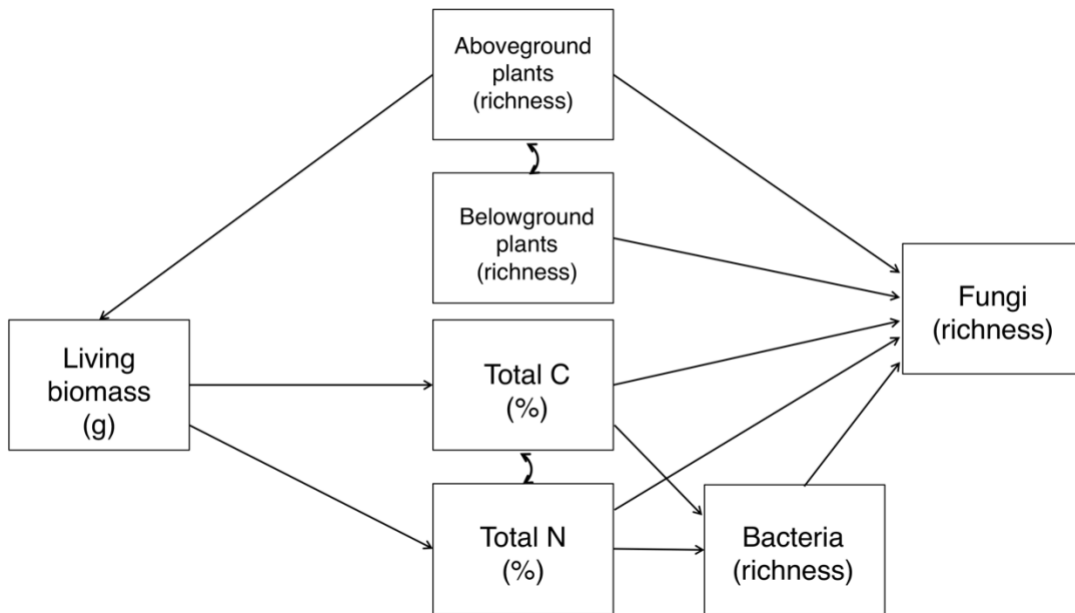
**Fig. 3.3**: A conceptual model demonstrating the hypothetical relationships (straight arrows) that occur aboveground and belowground across perennial grassland-annual cropland edges. Curved arrows represent covariates.

## 3.3 RESULTS

### 3.3.1 Vegetation Community and Biomass

Differences in plant community composition were strongly related to edge location (Fig. 3.4). Three distinct clusters were identified, the edge (0.5 m-0.5 m), the cropland (33 m-1 m), and the grassland (1 m-33 m) at both CLC and SDNWA. These plant communities across the edge appear to correlate with soil properties (Fig. 3.4). Increasing total C and total N was observed with perennial grasslands. Conversely, $NO_3$ appears elevated in the croplands (Fig. 3.7c).
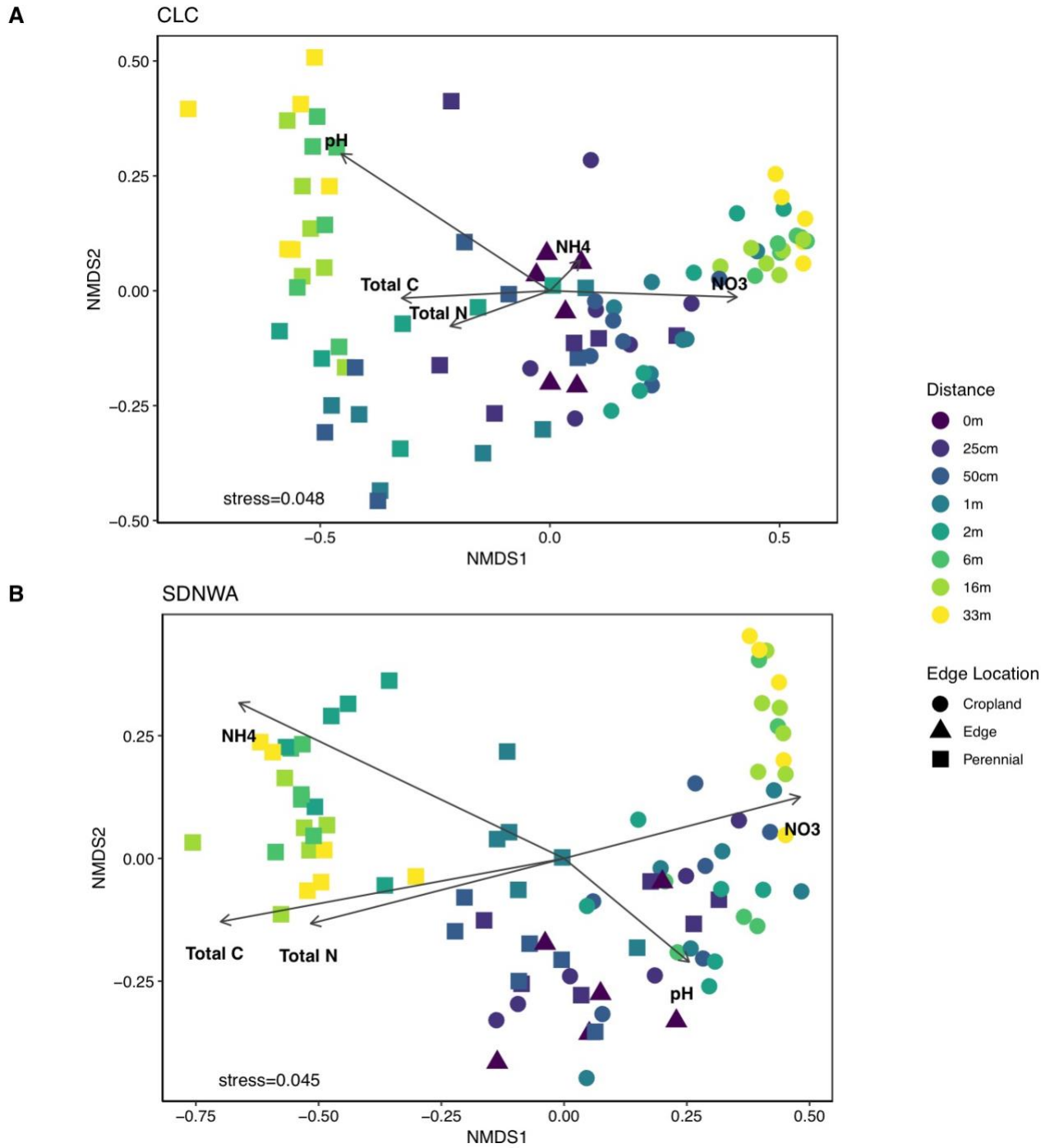
**Fig. 3.4:** A non-metric multidimensional scaling analysis of vegetation cover at (a) the Conservation Learning Centre and (b) the St. Denis National Wildlife Area. The colour gradient represents sampling points from 0 m to 33m (into either cropland or perennial grassland), with 0 m being the edge. Shapes represent the edge locations; triangles are edge points, squares represent perennial grassland and circles represent the cropland. Soil property vectors were fit to the plots.

Plant richness patterns also changed across the edge. Plant richness generally decreased with distance from edge (Fig. 3.5). Plant species evenness and diversity can be found in Appendix E. Plant species contributing to greater richness at the edge consisted of undesirable or weed species as determined by the *indicspecies* analysis (Table 3.1). Edge indicator species included hemp nettle (*Galeopsis tetrahit* L.) and cleaver's (*Galium aparine* L.) at both sites. Weed species at the edge were estimated to comprise 77% ± 8.9% (mean ± standard deviation) of plants recorded at CLC and 85% ± 7.4% at SDNWA. Grassland plant richness was generally lower than the edge, with *B. inermis* as the strongest indicator species at SDNWA, while at CLC, both *B. inermis* and *B. bieberstenii* were strong indicator species for the perennial grassland. Other indicator species for perennial grassland common to both sites included *M. satvia* and dandelion (*Taraxacum officinale* L.). Indicator species for cropland were the crops planted in 2017, *B. napus* and *L. usitatissimum* for CLC and SDNWA, respectively. A complete plant list with average cover can be found in Appendix G.
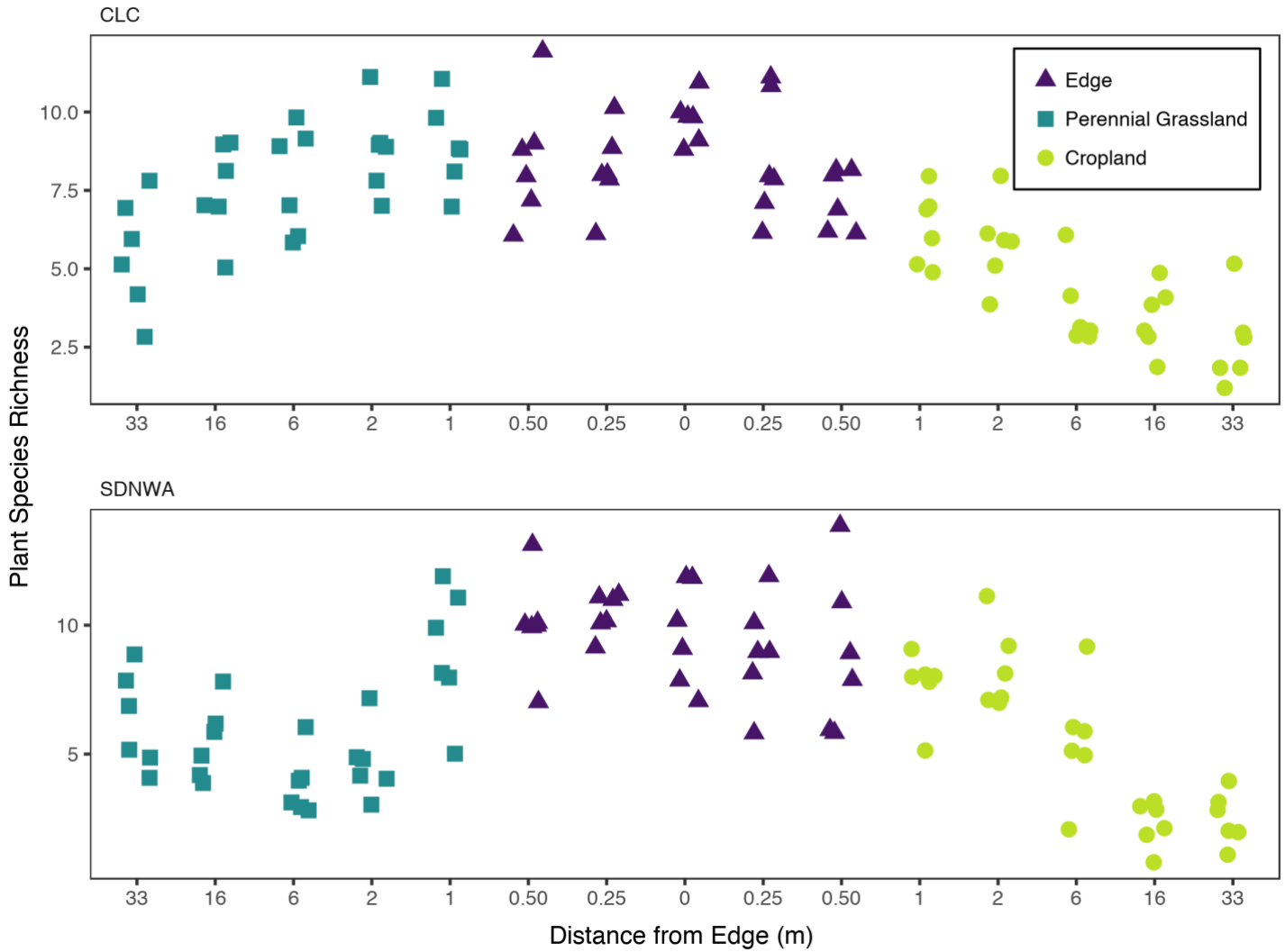
**Fig. 3.5**: Plant species richness for vegetation at sampling points across edge locations (perennial grassland, edge, and cropland) at the (a) Conservation Learning Centre (CLC) and the (b) St. Denis National Wildlife Area (SDNWA). The x-axis is distance from the edge of the perennial grassland and cropland and the y-axis is plant species richness. Colour represents perennial grassland, edge, and cropland, and shapes represent the location; triangles represent the perennial grassland, squares represent the edge, and circles represent cropland. Points are jittered.

**Table 3.1:** Indicator plant species for each edge location (perennial grassland, edge, and cropland) at the Conservation Learning Centre (CLC) and the St. Denis National Wildlife Area (SDNWA). Indicator species were determined by the *indicspecies* R package. Indicator species are also listed with edge + grassland and edge + cropland.

| Edge Location | Plant Species (CLC) | Plant Species (SDNWA) |
|---|---|---|
| Perennial Grassland | Meadow brome (*Bromus bieberstenii* Roem. & Schult.)<br>Alfalfa (*Medicago sativa* L.)<br>Smooth brome (*Bromus inermis* Lyess)<br>Kentucky bluegrass (*Poa pratensis* L.)<br>American vetch (*Vicia americana* Muhl. ex Willd.)<br>Rocky mountain fescue (*Festuca saximontana* Rydb.)<br>Showy aster (*Eurybia conspicua* (Lindl.) G.L.Nesom) | Smooth brome (*Bromus inermis* Lyess)<br>Alfalfa (*Medicago sativa* L.)<br>Dandelion (*Taraxacum officinale* F.H. Wigg.)<br>Kentucky bluegrass (*Poa pratensis* L.)<br>Slender wheatgrass (*Elymus trachycaulus* (Link) Gould ex Shinners) |
| Edge | Cleaver's (*Galium aparine* L.)<br>Hemp nettle (*Galeopsis tetrahit* L.)<br>unknown grass | Campion (*Silene latifolia* Poir.)<br>Hemp nettle (*Galeopsis tetrahit* L.)<br>Hawk's beard (*Crepis tectorum* L.)<br>Cleaver's (*Galium aparine* L.)<br>Rush skeleton weed (*Chondrilla juncea* L.) |
| Cropland | Canola (*Brassica napus* L.) | Flax (*Linum usitatissimum* L.)<br>Hoary cress (*Lepidium draba* L.) |
| Edge + Grassland | Dandelion (*Taraxacum officinale* F.H. Wigg.)<br>Canada thistle (*Cirsium arvense* (L.) Scop.)<br>Perennial sow thistle (*Sonchus arvensis* L.)<br>Canada goldenrod (*Solidago canadensis* L.) | Canada thistle (*Cirsium arvense* (L.) Scop.)<br>Perennial sow thistle (*Sonchus arvensis* L.)<br>Quackgrass (*Elymus repens* (L.) Gould) |
| Edge + Cropland | Canola (*Brassica napus* L.)<br>unknown grass<br>Bindweed (*Polygonum convolvulus* L.) | Flax (*Linum usitatissimum* L.)<br>Flixweed (*Descurainia sophia* (L.) Webb ex Prantl)<br>Wormseed mustard (*Erysimum cheiranthoides* L.) |

Living biomass was significantly different across the edge at SDNWA (Table 3.2). Living biomass was not only greater at SDNWA compared with CLC, but it was significantly different at all locations across the edge (Fig. 3.6a). Living biomass at CLC was only significantly higher in the perennial grassland than the edge (Fig. 3.6a). Forb biomass patterns across the edge were different at each site; the greatest forb biomass at CLC was in cropland, while greatest forb biomass at SDWNA was at the edge and cropland (Fig. 3.6b). At the edge, forbs consisted of 74% ± 31% and 88% ± 23% (mean ± standard deviation) of living biomass at CLC and SDNWA respectively. Elevated forb biomass in cropland compared to other edge locations at CLC was due to the crop, canola. Not surprisingly, the majority of grass biomass was in perennial grasslands. Grass biomass was different across all edge locations at SDNWA, whereas at CLC, grass biomass was higher in the perennial grassland compared to edge or cropland, but edge and cropland were not significantly different (Fig. 3.6c). Litter biomass was not significantly different across the edge at either site. Mixed model estimates and standard errors can be found in Appendix H; and means for the properties in Appendix I.

**Table 3.2:** F-values (*p*-values) from linear mixed models for biomass (g/m$_2$) across edge (perennial grassland, edge, and cropland), site (Conservation Learning Centre and St. Denis National Wildlife Area), and the interaction between edge and site. Significant *p*-values ($p < 0.05$) are denoted by *. Log transformed data are denoted by †.

| Factor | | Biomass Type | | | |
|---|---|---|---|---|---|
| | df | Living Biomass† | Grass† | Forbs† | Litter† |
| Edge Location | 2 | 28 (< 0.001*) | 200 (< 0.001*) | 19 (< 0.001*) | 4.1 (0.02*) |
| Site | 1 | 3.03 (0.11) | 0.50 (0.56) | 0.28 (0.61) | 0.04 (0.80) |
| Edge Location:Site | 2 | 8.7 (< 0.001*) | 12 (< 0.001*) | 11 (< 0.001*) | 3.7 (0.02*) |
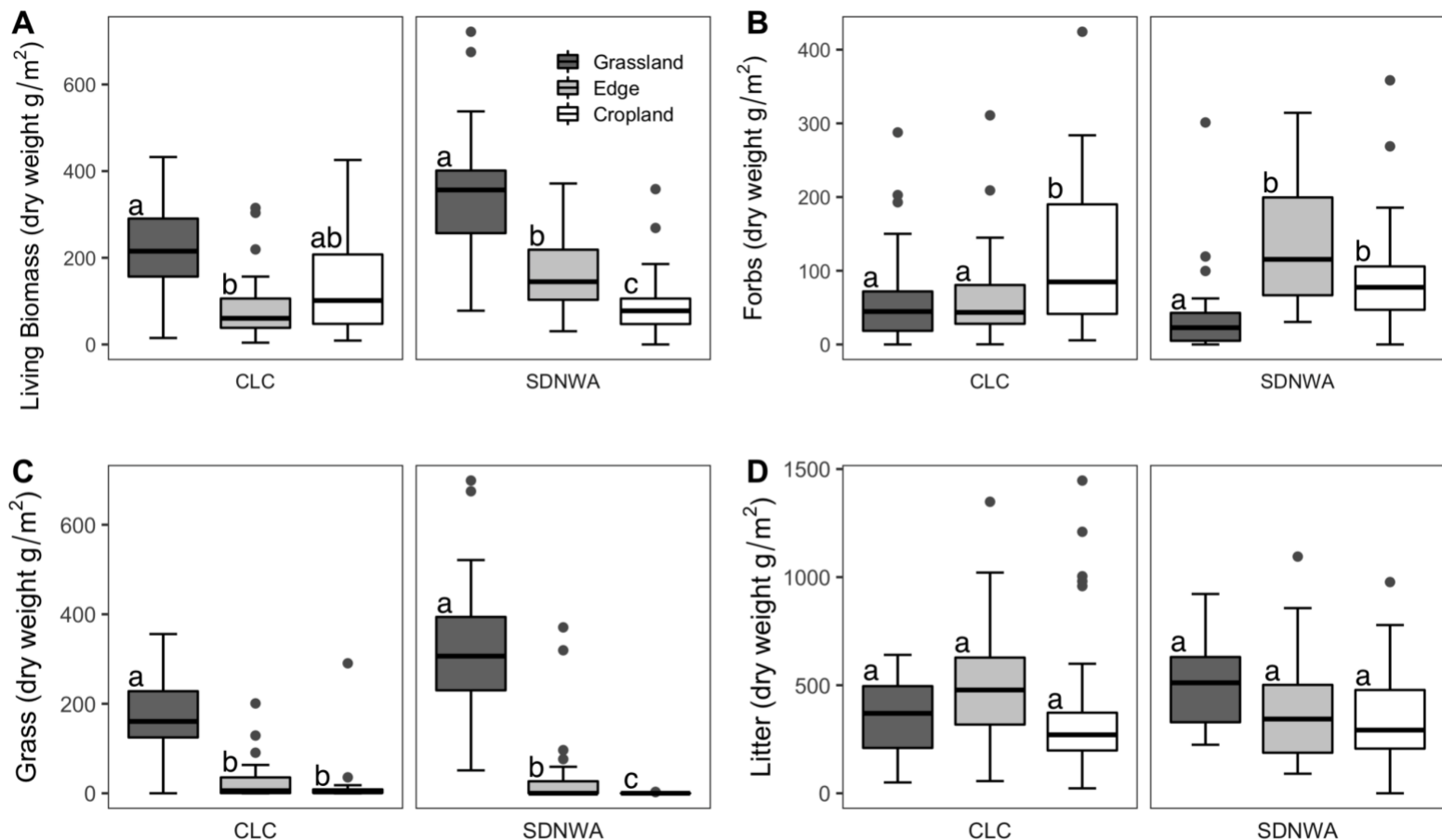
**Fig. 3.6:** Vegetation biomass across edge locations (perennial grassland, edge, and cropland) at the Conservation Learning Centre (CLC) and the St. Denis National Wildlife Area (SDNWA) sampled during June-July 2017; values are dry biomass (g/1 m2). Boxes encompass 25-75% quantiles of the data, while whiskers encompass 5-95%. The median is indicated by the black horizontal line, and outliers are shown as dots. Different letters indicate a significant difference ($p$-value $< 0.05$) between edge locations determined by Tukey-HSD post-hoc tests on the linear mixed models. Colour of the boxplots represent edge location, dark gray = grassland, light gray = edge, and white = cropland. (a) Living biomass (forbs and grass) (b) Forb biomass (c) Grass biomass and (d) Litter biomass.

### 3.3.2 Soil Properties

Soil properties were different across the edge at both sites (Table 3.3). Total C and total N were highest in the perennial grassland and decreased across the edge to the lowest observed values in the cropland at SDNWA (Fig. 3.7a-3.7b). Total C was only significantly greater in the perennial grassland compared to the edge and cropland, and only total N was significantly greater in perennial grassland compared to cropland at CLC (Fig. 3.7b). $NO_3$ had the opposite trend as total C and total N, with the lowest values in the perennial grassland and highest in the cropland (Fig. 3.7c). $NH_4$ did not share the same trend at both sites across edge. SDNWA had significantly higher $NH_4$ in perennial grassland compared with edge or cropland (Fig. 3.7d). There was no significant difference in $NH_4$ across the edge at CLC. Soil pH was significantly higher in the perennial grassland at CLC compared to edge and cropland, with pH values ranging across the edge from 4.8 - 6.9. At SDNWA, pH was not significantly different across the edge, with values that ranged from 6.5 - 7.5 (Fig. 3.7e). Overall, soil properties at each edge location were more variable at CLC than at SDNWA, but SDNWA had more significant differences in total N and total C across the edge. Mixed model estimates and standard errors can be found in Appendix H; and means for the properties in Appendix I.

**Table 3.3:** Linear mixed model F-value (*p*-values) results for soil properties across edge (perennial grassland, edge, and cropland), site (Conservation Learning Centre and St. Denis National Wildlife Area), and the interaction between edge and site. Significant p-values ($p < 0.05$) are denoted by *. Log transformed data are denoted by †.

| Factor | | Soil Properties | | | | |
|---|---|---|---|---|---|---|
| | df | Total C | Total N | $NH_4$† | $NO_3$† | pH |
| Edge location | 2 | 46 (< 0.001*) | 26 (< 0.001*) | 25 (< 0.001*) | 36 (< 0.001*) | 11 (< 0.001*) |
| Site | 1 | 0.95 (0.24) | 1.0 (0.23) | 11 (0.03*) | 5.9 (0.14) | 300 (< 0.001*) |
| Edge location:Site | 2 | 5.8 (0.003*) | 2.4 (0.09) | 17 (< 0.001*) | 0.36 (0.70) | 31 (< 0.001*) |
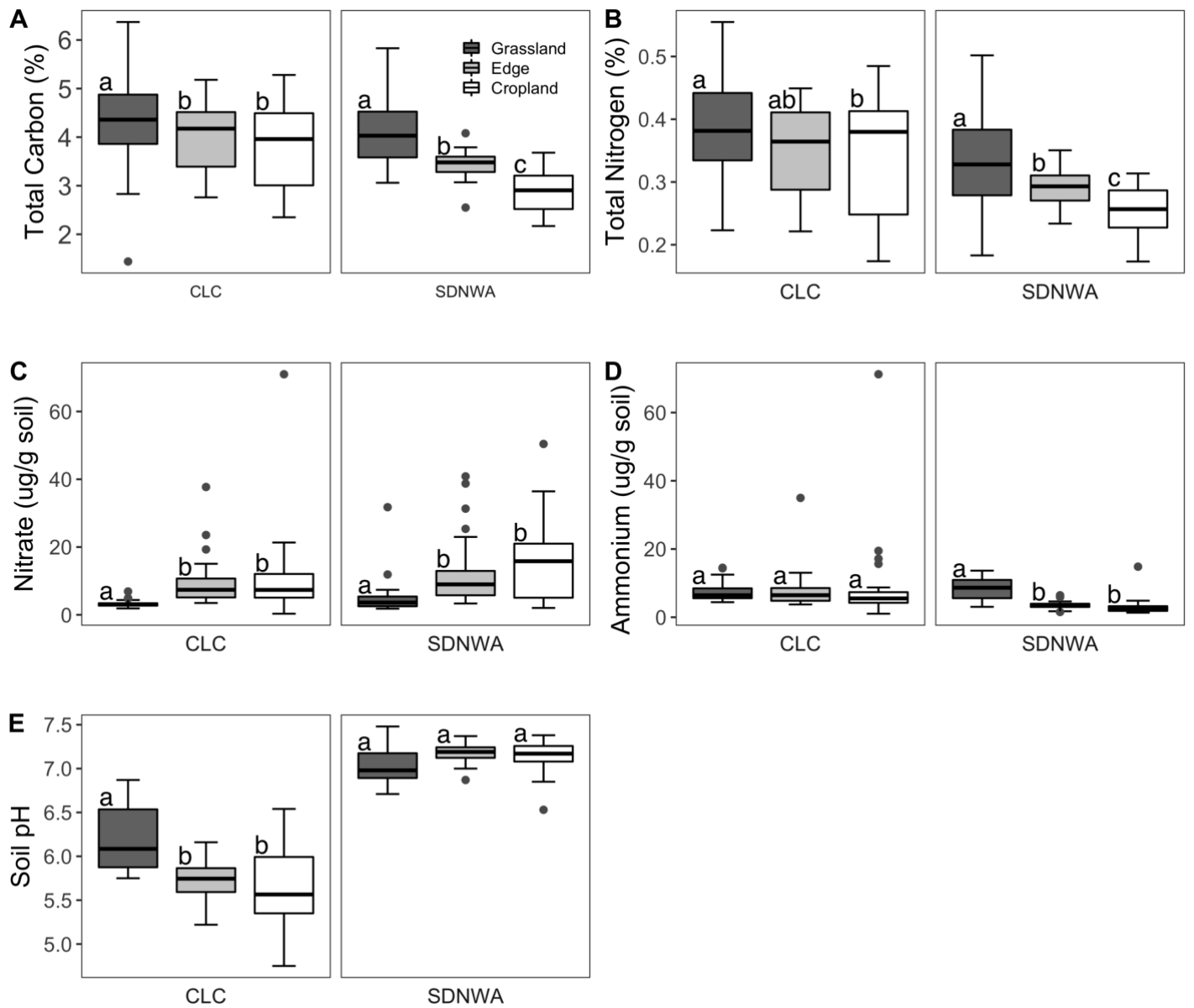
**Fig. 3.7**: Soil properties across the edge in each edge location (perennial grassland, edge, and cropland) at the Conservation Learning Centre (CLC) and St. Denis National Wildlife Area (SDNWA) sampled during June-July 2017. Boxes encompass 25-75% quantiles of the data, while whiskers encompass 5-95%. The median is indicated by the black horizontal line, and outliers are shown as dots. Different letters indicate a significant difference ($p$-value < 0.05) between edge locations determined by Tukey-HSD post-hoc tests on the linear mixed models. Colour of the boxplots represent edge location, dark gray = grassland, light gray = edge and white = cropland. (a) Total carbon (%) (b) Total nitrogen (%) (c) $NO_3$ (µg/g soil) (d) $NH_4$ (µg/g soil) (e) Soil pH.

### 3.3.3 Soil Microbial Community

Of the bacterial community, approximately 79% of taxa occurred in the top ten most abundant phyla (Fig. 3.8a-3.8b). The most abundant phyla were the Proteobacteria, Actinobacteria, and Acidobacteria. The fungal community had two dominant phyla, the Ascomycota and the Basidiomycota, followed by the Mortierellomycota (Fig. 3.8c-3.8d).

Fungal and bacterial diversity, richness, and evenness were not significantly different across the edge at either site from the LMMs ($p > 0.05$). The bacterial community did not have a clear pattern across the edge, however, SDNWA bacterial community composition appears to change more with respect to edge location than at CLC (Fig. 3.9a-3.9b). Fungal communities at both sites appeared to have a distinct perennial grassland community compared with the fungal community at the edge or in the cropland (Fig. 3.9c-3.9d).
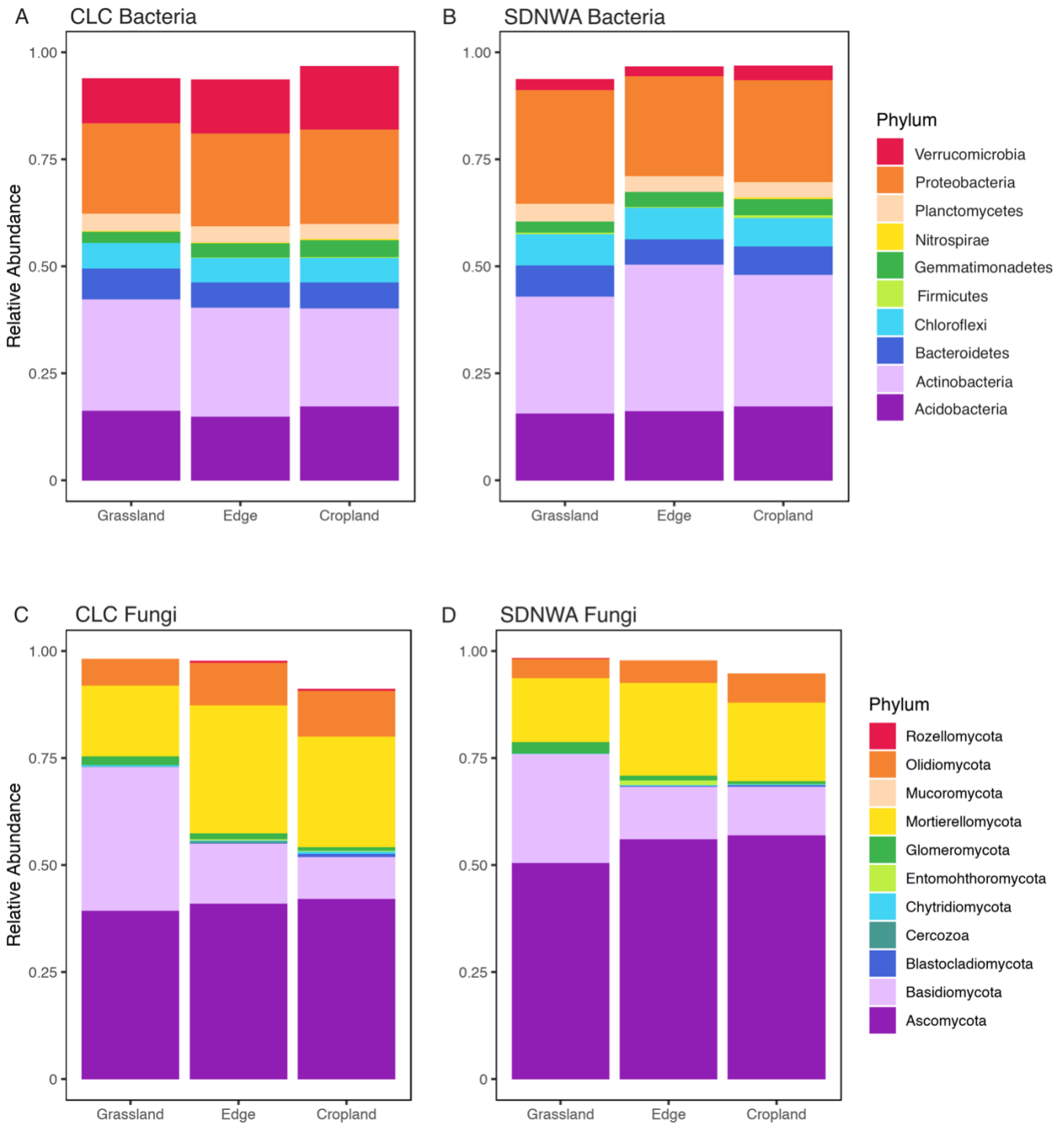
**Fig. 3.8:** Bacterial community composition across the edge at the Conservation Learning Centre (CLC) (a) and at the St. Denis National Wildlife Area (SDNWA) (b). Fungal community composition at CLC (c) and at SDNWA (d). The x-axis is relative abundance and the y-axis represents location relative to the edge.
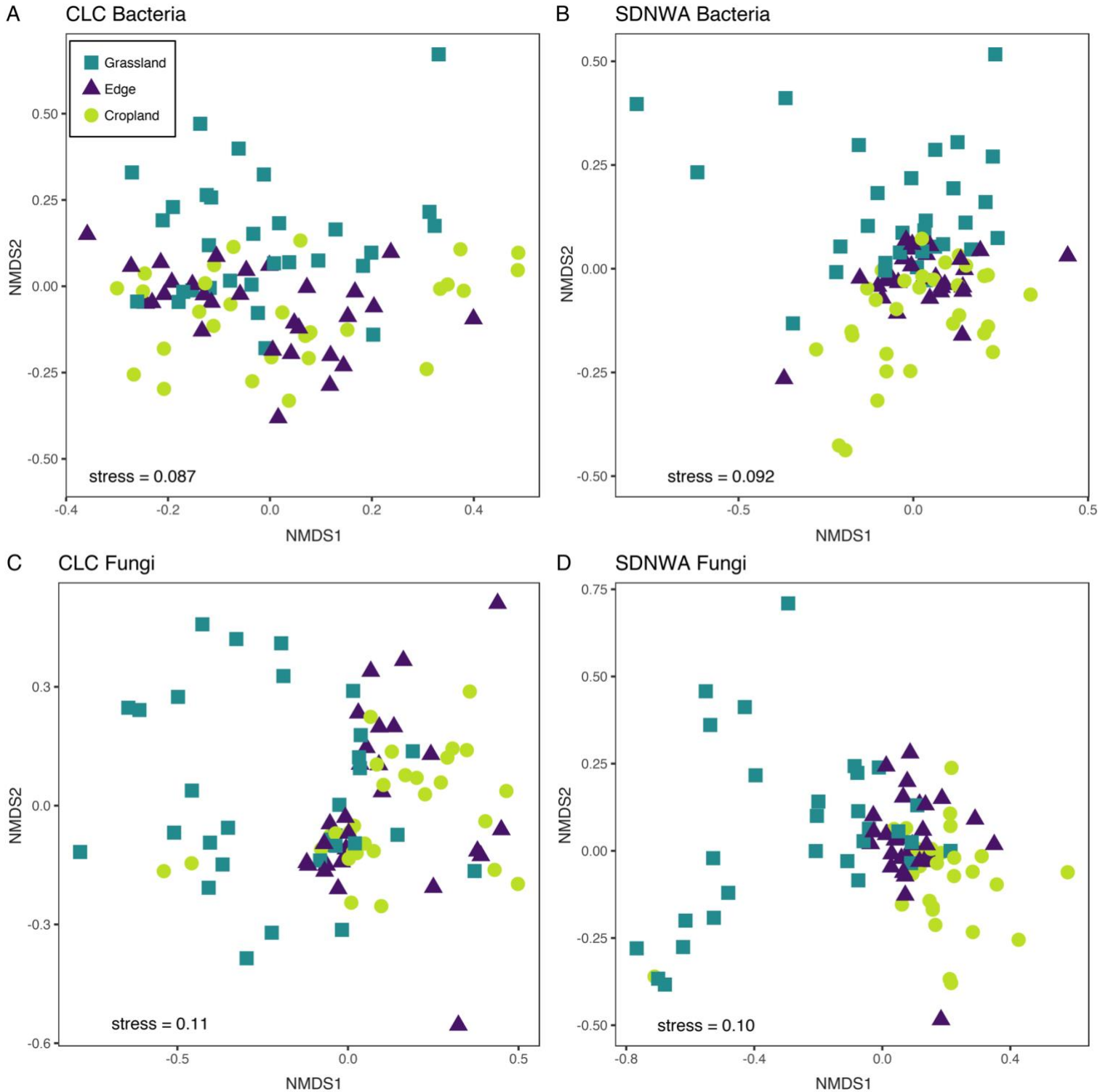
**Fig. 3.9:** Non-metric multidimensional scaling analysis for the (a, b) bacterial community and the (c, d) fungal community for both sites Conservation Learning Centre (CLC) and the St. Denis National Wildlife Area (SDNWA). Shape and colour of the points represent edge location across the edge of a perennial grassland and cropland. The data were Hellinger transformed before analysis.

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### 3.3.4 Aboveground and Belowground Plant Community

Comparing aboveground and belowground plant species richness, evenness, and diversity; plant species richness had the strongest Pearson correlation (0.18). Aboveground and belowground plant richness, determined by aboveground percent cover plots and sequencing of root-soil samples respectively, had a significant positive relationship, as determined by a linear model ($F_{1,175} = 6.4$ and $p = 0.01$), however the adjusted $R^2$ was 0.03 (Figure 3.10).
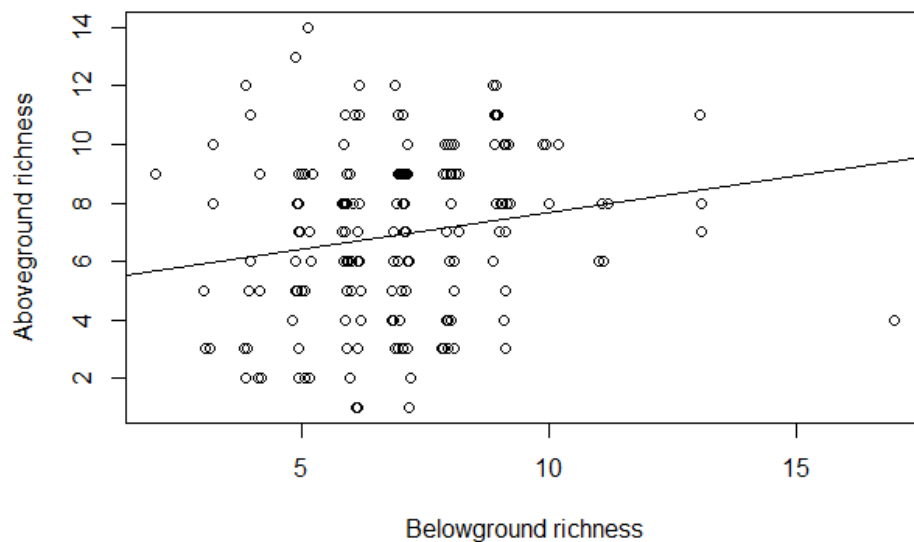


**Fig. 3.10**: Relationship between aboveground and belowground plant species richness at two sites. Aboveground richness was determined by a 1 $m^2$ quadrat and belowground richness was determined by a composite sample of three 2 cm by 10 cm soil cores. $R^2 = 0.03$.

### 3.3.5 Structural Equation Models

Belowground plant richness rather than aboveground plant richness had a significant positive relationship with total fungal richness, revealed by the first model (Fig. 3.11). Model coefficients and *p*-values can be found in Appendix J. Aboveground plant richness also had a positive relationship with fungal richness, but the relationship was not significant. Total N had a significant positive relationship with bacterial richness; and bacterial richness had a negative relationship with fungal richness. Living biomass had strong positive relationships with both total C and total N; and thus, was an indirect influence on bacterial and fungal richness.
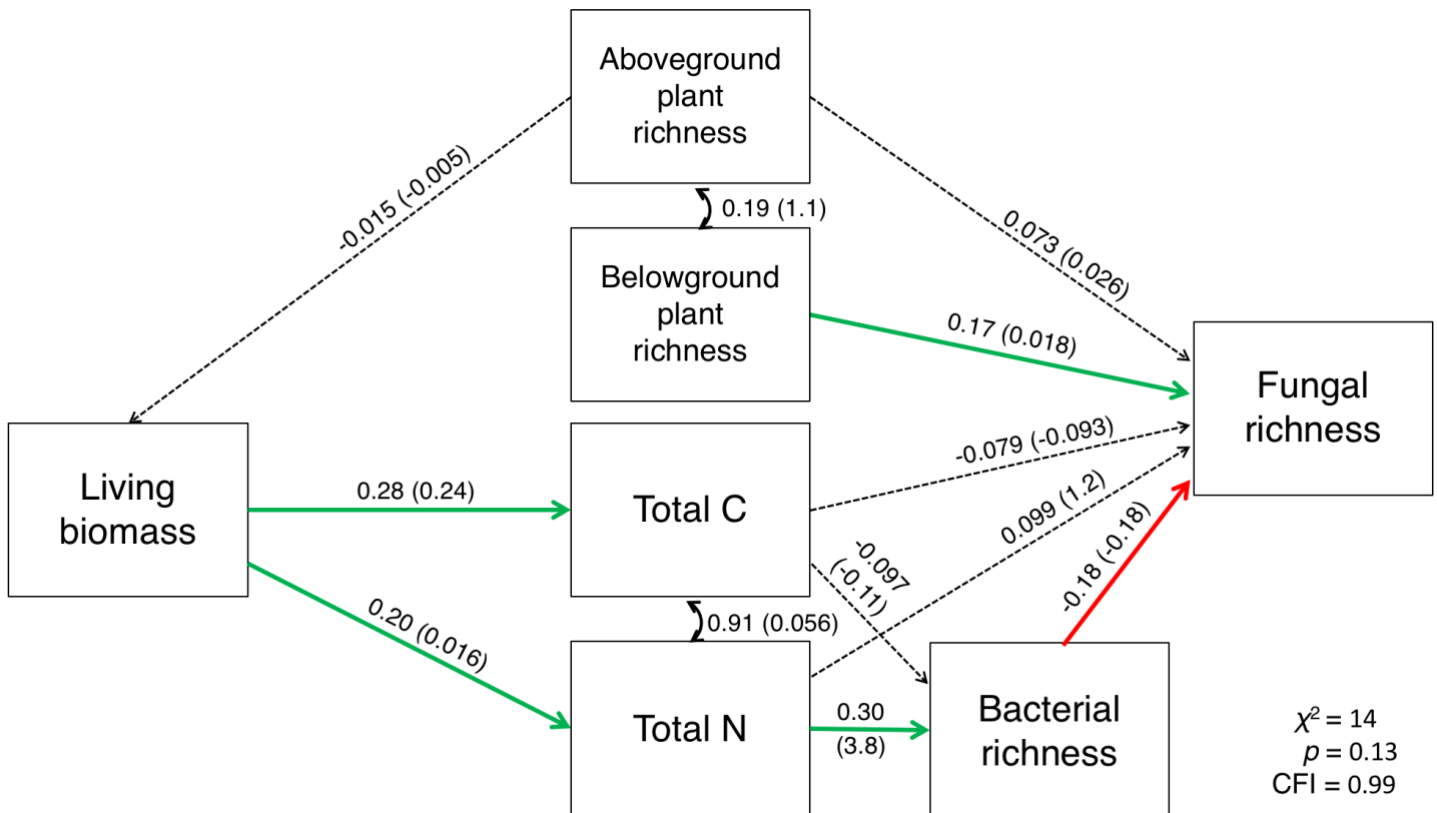


**Fig. 3.11**: Structural equation model with fungal richness and bacterial richness representing the soil microbial community. Other observed variables aboveground plant richness, belowground plant richness, living biomass, soil total N, and soil total C. Significant pathways (*p* < 0.1) are denoted by solid arrows and non-significant pathways denoted by dashed arrows. Green arrows represent significant positive pathways while red arrows represent significant negative pathways. Curved arrows represent covariant relationships. The standardized partial path coefficients and unstandardized partial coefficients are in parentheses next to the pathway arrows.

The second set of SEMs focused on fungal genera that were significant across the edge at both sites. After filtering the data set, 50 genera remained (from 392) and six genera were found to be significantly different across the edge (Table 3.4). Three genera had SEMs with poor fit ($p < 0.05$) and are not included in the model diagrams. Aboveground plant species richness had a positive relationship with *Paraphoma*, and a negative relationship with *Sarolcadium* (Fig. 3.12). This negative relationship may reflect that *Sarocladium* was most abundant in the cropland, while *Paraphoma* was most abundant at the edge. Belowground plant richness had no significant relationships to the fungal genera. *Parastagonospora* was the only genus to have a significant relationship with bacterial richnes and bacterial richness had a negative relationship to *Parastagonospora*. *Parastagonospora* was most abundant in the cropland. Total C had negative relationships with *Parastagonospora* and *Sarocladium*, total N had a significant positive relationship to *Sarocladium*.

**Table 3.4:** Fungal genera abundance that were significant ($p < 0.05$) across the edge of a cropland and perennial grassland at two sites, determined by Welch's *t*-test using abundance values after centered log-ratio transformation to obtain compositional abundance. * = cropland-grassland, † = edge-grassland, ‡ = edge-cropland

| Phylum | Subphyla | Class | Order | Family | Genus (*p*-value) |
|---|---|---|---|---|---|
| Ascomycota | Pezizomycotina | Dothideomycetes | Pleosporales | Phaeosphaeriaceae | *Chalastospora* (0.001*) |
| Ascomycota | Pezizomycotina | Dothideomycetes | Pleosporales | Phaeosphaeriaceae | *Clonostachys* (0.024*, <0.001†) |
| Ascomycota | Pezizomycotina | Sordariomycetes | Hypocreales | Nectriaceae | *Gibberella* (<0.001*, <0.001†) |
| Ascomycota | Pezizomycotina | Dothideomycetes | Pleosporales | Phaeosphaeriaceae | *Paraphoma* (0.002‡) |
| Ascomycota | Pezizomycotina | Dothideomycetes | Pleosporales | Phaeosphaeriaceae | *Parastagonospora* (0.001*) |
| Ascomycota | Pezizomycotina | Sordariomycetes | Hypocreales | Nectriaceae | *Sarocladium* (0.005*, 0.018†) |
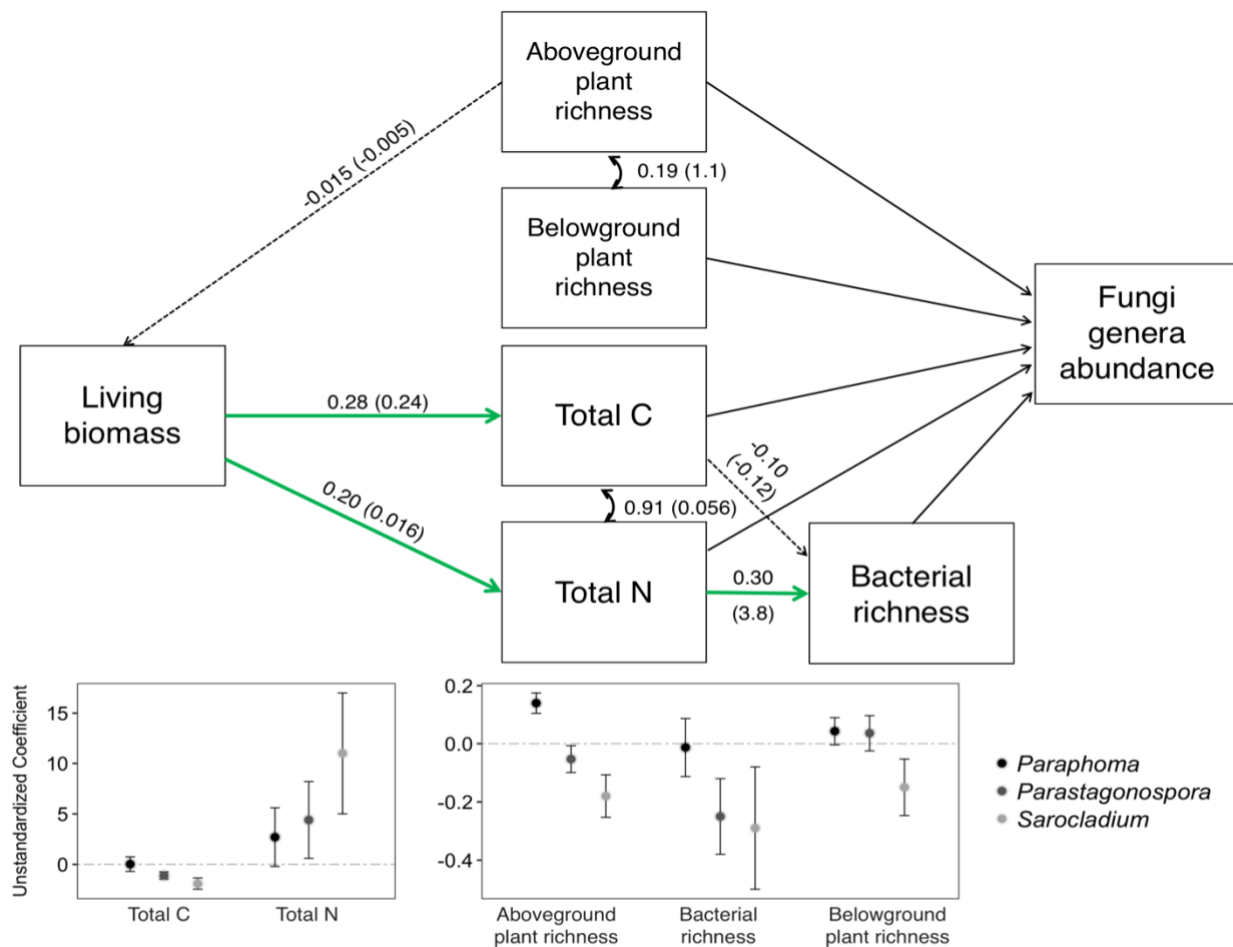
**Fig. 3.12**: Structural equation models (SEM) using the significant fungal genera abundance across the edge of both sites combined. Standardized partial path coefficients and unstandardized partial coefficients in parentheses are next to pathway arrows; these coefficients are representative of the individual models ran with different fungal genera as the coefficients were the same regardless of genus. Unstandardized partial path coefficients to the fungal genera are displayed in a dot plot below the model and correspond to the straight solid black arrows in the diagram above. In the dot plot, the y-axis represents unstandardized partial path coefficient values and the x-axis represents the five variables. Standard error bars are also included on the plot. Colour of the dots correspond to the fungal genera; *Paraphoma* (black), *Parastagonospora* (dark gray), *Sarocladium* (light gray). Solid green arrows are significant positive pathways ($p < 0.1$). The curved arrows represent covariant relationships and the dashed arrows are non-significant pathways.

Sites were also analyzed separately to address site differences. Nine fungal genera were found to be significantly different across the edge at SDNWA (Table 3.5). Three of those genera were significant when both sites were analyzed together, *Clonostachys*, *Parastagonospora*, and *Sarocladium. Parastagonospora* and *Sarocladium* were excluded in SDNWA SEMs to avoid redundancy. *Clonostachys* was included for the SDNWA models, because previously it had poor model fit ($p < 0.05$) when both sites were analyzed together. Using the same previous pathway structure, the seven genera were used in SEMs with fungal genera abundance in place of fungal richness. Aboveground plant richness had significant positive relationships to *Acrostalamus* and *Clonostachys* and a negative relationship with *Devriesa* (Fig. 3.14). *Acrostalagmus* was the only genus to have a significant negative relationship with belowground plant richness (Fig. 3.14) and was most abundant in cropland. *Arthrinium*, *Cistella*, and *Devriesia* had positive relationships with total C (Fig. 3.14) and were more abundant in the perennial grassland. *Acrostalagmus,* *Clonostachys,* and *Schizothecium* had negative relationships with total C; *Schizothecium* was most abundant in the cropland while *Clonostachys* was most abundant at the edge. *Acrostalagmus*, *Arthrinium,* and *Devriesia* had significant negative relationships with bacterial richness, while *Coprinopsis* had a significant positive relationship with bacterial richness (Fig. 3.14). *Coprinopsis* was most abundant in the cropland. *Schizothecium* also had a positive relationship to bacterial richness but was not significant.

Only one significant genus was found at CLC, *Olpidium,* which was significantly greater at the edge than the perennial grassland ($p = 0.045$), and cropland to edge ($p = 0.049$). *Olpidium* had a significant negative relationship with total C and a positive relationship with total N.

**Table 3.5:** Fungal genera abundance that were significant ($p < 0.05$) across the edge of a cropland and perennial grassland at St. Denis National Wildlife Area, determined by Welch's $t$-test using abundance values after centered log-ratio transformation to obtain compositional abundance. *=cropland-grassland, †=edge-grassland, ‡=edge-cropland.

| Phylum | Subphyla | Class | Order | Family | Genus (*p*-value) |
| --- | --- | --- | --- | --- | --- |
| Ascomycota | Pezizomycotina | Sordariomycetes | Glomerellales | Plectosphaerellaceae | *Acrostalagmus* (0.007*, 0.094†) |
| Ascomycota | Pezizomycotina | Sordariomycetes | Xylariales | Apiosporaceae | *Arthrinium* (0.049*) |
| Ascomycota | Pezizomycotina | Leotiomycetes | Helotiales | Hyaloscyphaceae | *Cistella* (0.003*) |
| Ascomycota | Pezizomycotina | Dothideomycetes | Pleosporales | Phaeosphaeriaceae | *Clonostachys* (0.001†) |
| Basidiomycota | Agaricomycotina | Agaricomycetes | Agaricales | Psathyrellaceae | *Coprinopsis* (0.036*) |
| Ascomycota | Pezizomycotina | Dothideomycetes | Capnodiales | Teratosphaeriaceae | *Devriesia* (0.001*, 0.009†) |
| Ascomycota | Pezizomycotina | Dothideomycetes | Pleosporales | Phaeosphaeriaceae | *Parastagonospora* (<0.001*, 0.001‡) |
| Ascomycota | Pezizomycotina | Sordariomycetes | Hypocreales | Nectriaceae | *Sarocladium* (<0.001*) |
| Ascomycota | Pezizomycotina | Sordariomycetes | Sordariales | Lasiosphaeriaceae | *Schizothecium* (0.034*) |
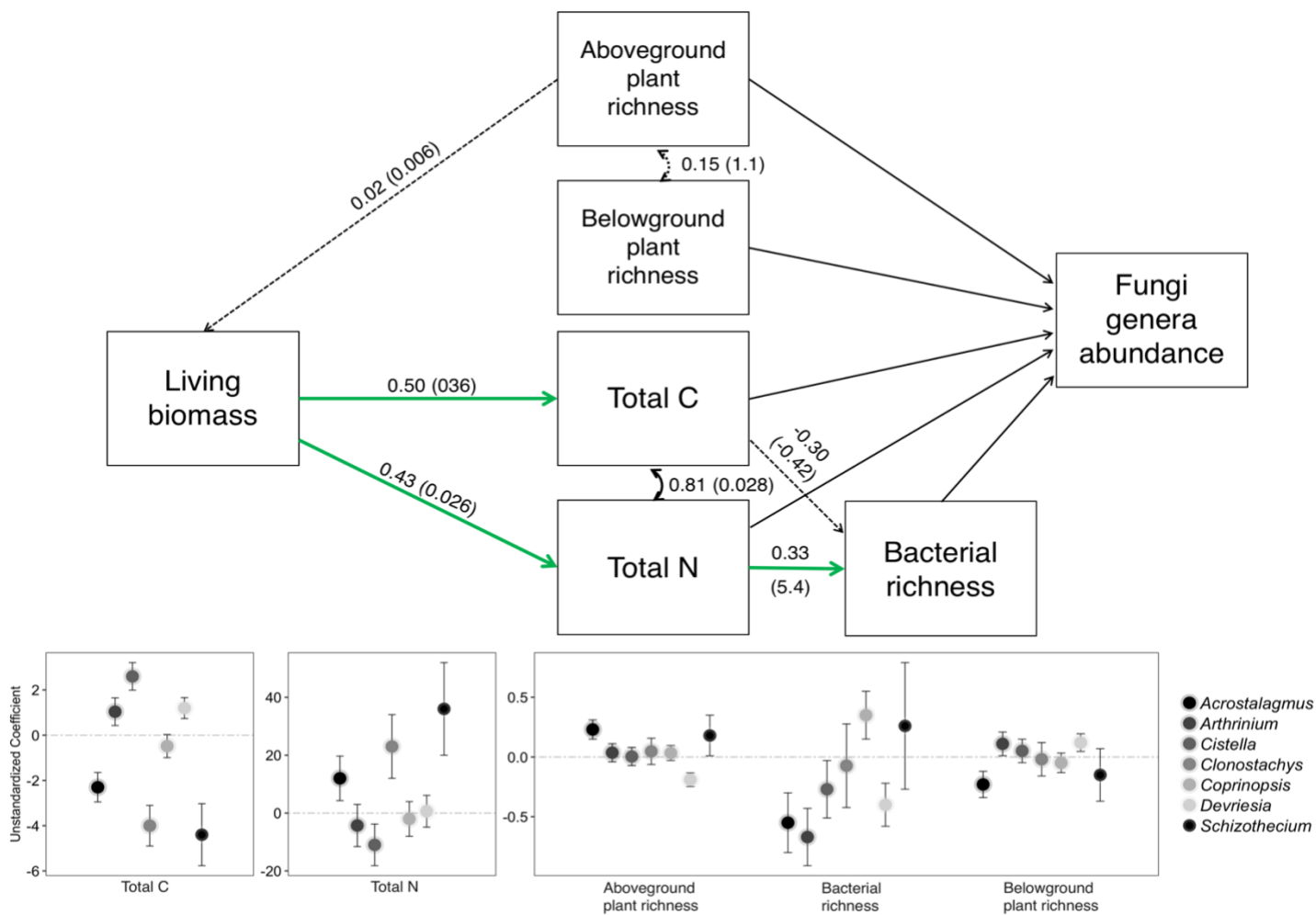
**Fig. 3.13**: Structural equation models (SEM) using the significant fungal genera abundance across the edge at the St. Denis National Wildlife Area. Standardized partial path coefficients and unstandardized partial coefficients in parentheses are next to pathway arrows; these coefficients are representative of the individual models ran with different fungal genera as the coefficients were the same regardless of genus. Unstandardized partial path coefficients to the fungal genera are displayed in a dot plot below the model and correspond to the straight solid black arrows in the diagram above. In the dot plot, the y-axis represents unstandardized partial path coefficient values and the x-axis represents the five variables. Standard error bars are also included on the plot. Colour of the dots correspond to the fungal genera; *Acrostalagmus* (black), *Arthrinium* (dark gray), *Cistella* (dark-medium gray), *Clonostachys* (medium gray), *Coprinopsis* (light-medium gray), *Devriesia* (light gray), and *Schizothecium* (solid black). Solid green arrows are significant positive pathways ($p < 0.1$). The curved arrows represent covariant relationships and the dashed arrows are non-significant pathways.
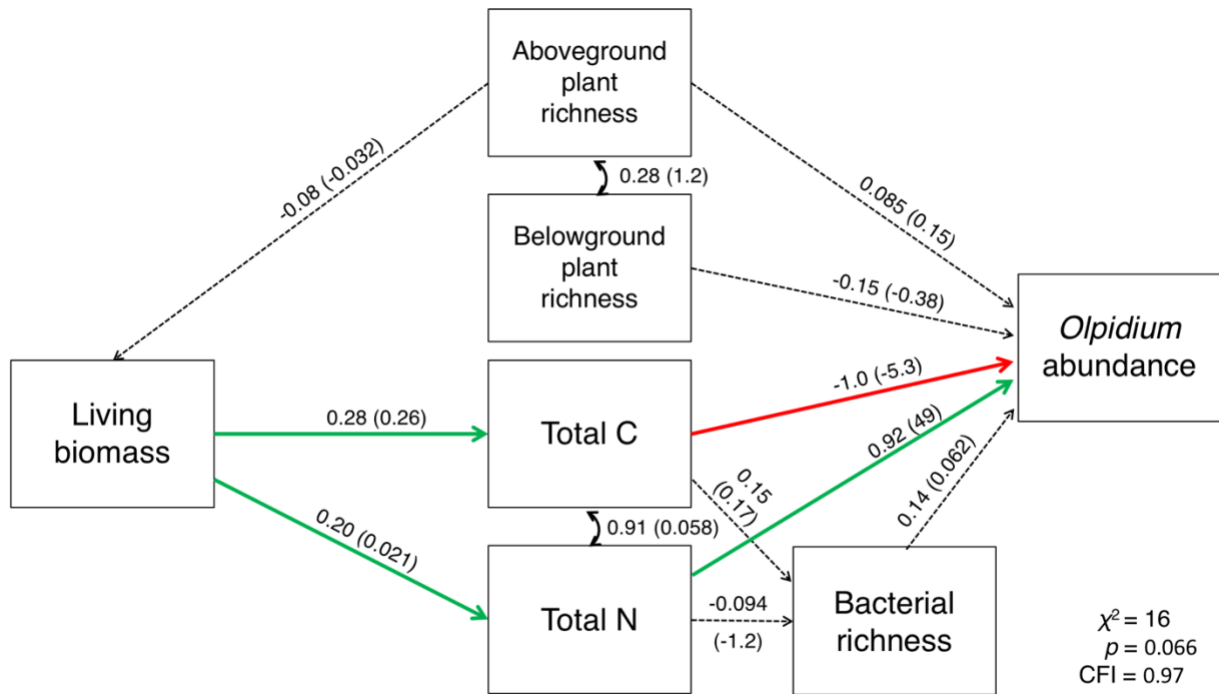
**Fig. 3.14:** A structural equation model using the significant genus across the edge at the Conservation Learning Centre, *Olpidium*. Other observed variables aboveground plant richness, belowground plant richness, living biomass, soil total N, and soil total C. Significant pathways ($p < 0.1$) are denoted by solid arrows and non-significant pathways denoted by dashed arrows. The standardized partial path coefficients and unstandardized partial path coefficients in parentheses are next to pathway arrows and the unstandardized coefficients are in paraentheses. Green arrows represent significant positive pathways while red arrows represent significant pathways. Curved arrows represent covariant relationships.

**3.4 DISCUSSION**

Land use appeared to be primary factor influencing properties across the edge, as observed in the plant community, soil properties, and soil microbial community. The perennial grassland-annual cropland edges gave way to three distinct plant communities. At the edge, weed prevalence increased leading to the greatest plant richness and diversity there. Weed dominated edges are concerning because they may act as a conduit for weeds to disperse into the cropland or grassland and may also host pests or pathogens. We observed soil property differences across the edge, which included a gradient of high total C and total N in the perennial grasslands to low C and N in the cropland. Changes in the soil microbial community also occurred across the edge; fungal community changes were stronger than bacterial community changes. Aboveground and belowground characteristics were linked, with aboveground biomass influencing soil properties that in turn influenced the soil microbial community. Exploring fungi at the genus level revealed that different genera had varying relationships with total C, total N, bacteria, and plant richness. Edges are important in the agroecosystems as they represent places of biodiversity but also vulnerability; edges in this study were dominated by weeds that potentially may spread into the surrounding habitat.

*3.4.1 Vegetation and Soil Biodiversity Across the Edge*

Land use affected plant community composition, richness, and biomass across the edge. Three different vegetation communities were observed; the perennial grassland, the edge (~1 m in width), and the cropland. Perennial grasslands were dominated by brome species (*B. inermis* and *B. bieberstenii*), while vegetation at the edge was predominately annual weedy forbs, such as *G. tetrahit* and *G. aparine*. Grasslands at SDNWA were seeded with non-native mixtures (1977-1983) to be harvested for hay (Hogan and Conly, 2002) that included *B. inermis* in the seed mix. Grasslands at CLC were also seeded at some point, however the exact mix and date is unknown. While biomass production has been positively linked with plant diversity (Tilman et al., 2006) we found that the perennial grasslands had generally lower plant species richness than the edges. Living biomass was greatest in the perennial grasslands which we hypothesize is due to the presence of *B. inermis* likely outcompeting other plants. Not surprisingly, vegetation composition

in the croplands was strongly influenced by the crop seeded; *B. napus* at CLC and *L. usitatissimum* at SDNWA.

The prevalence of *B. inermis* in both perennial grasslands may heavily influence ecosystem function. *B. inermis* is often considered an invasive, is highly competitive, captures resources quickly, and can effectively invade an area and displace native species (Otfinowski et al., 2007; Piper et al., 2015). Invasive species can influence plant communities by increasing net primary production and altering availability of N and N fixation rates (Ehrenfeld, 2003; Vinton and Goergen, 2006). *B. inermis* produces large quantities of biomass and this, along with high forage quality, accounts for the common practice of using *B. inermis* in pastures and hay production (Malhi et al., 2008). Large amounts of litter produced by *B. inermis* can affect N cycling in grassland systems, creating a positive feedback loop for itself, which allows it to outcompete native plant species (Dillemuth et al., 2009). Specifically, *B. inermis* produces large quantities of biomass that promote N mineralization by soil microbes (Piper et al., 2015). Total N was significantly greater in the perennial grassland at SDNWA where *B. inermis* is most abundant. Pressure of *B. inermis* at the grassland edge may result in more abrupt, as opposed to gradual, vegetation change at the edge. Farming disturbances, such as the type of equipment used (e.g., seeders, sprayers, or combines), create a habitat where perennial plants may not establish as well compared to annual plants. Even a highly competitive plant like *B. inermis* cannot tolerate frequent mechanical disturbances, as demonstrated by its decline at the edge.

Plant richness and diversity were highest at the edge due to an increase of annual weeds. Many annual weeds only occurred at the edge. For example, hemp nettle (*G. tetrahit*) was never recorded at distances greater than 1 m away from the edge on the perennial grassland side and was only present 2 m away from the edge in the cropland. Other annual plants were more persistent in the cropland; for instance, *G. aparine* was present 33 m into the cropland at SDNWA. Increased annual weed richness at the edge was also reflected in significantly greater forb biomass at SDNWA edges than in perennial grassland. Annual weeds that were abundant at the edge were primarily ruderal plant species (Grime, 1979). Traits of ruderals include rapid growth, phenotypic plasticity, and high fecundity. Disturbances from farming in the cropland create conditions of high nutrient availability and open space at agricultural edges (Radosevich et al., 2007). While these conditions are ideal habitat for plants, they will have to tolerate disturbances to persist. These plants quickly capitalize on resources and outcompete other

species; leading to higher plant richness at the edge (Aavik et al., 2008; Nagy et al., 2017). This adds to the concern that weeds can compete with the crop and potentially reduce yield (Oerke and Dehne, 2004). Plant-soil feedbacks may also be altered by weeds; as they can promote native microbial pathogens that reduce native plant species, aiding the weed to outcompete native plant species (Mangla and Callaway, 2007).

We examined the viability of using richness data determined through *trn*L sequencing of belowground plants to accurately predict aboveground richness, determined through observation of plant percent cover in a 1 m2.  Directly comparing the relationship between aboveground plant richness and belowground plant richness was variable and weak. The scale of each sample may contribute to discrepancies (Lamb et al., 2016), as well as the taxonomic resolution of *trn*L (Li et al., 2018). The aboveground plant richness was determined by 1 m2 percent cover plots, and the belowground plant richness was determined by sequencing 1 g root-soil subsample from a larger 2 cm diameter by 10 cm core. Generally, as sample size increases, richness will increase, resulting in richness variation (Pärtel et al., 2012). Studies using molecular techniques to describe belowground plant community are relatively new and often find greater plant species richness belowground than aboveground (Pärtel et al., 2012; Oñatibia et al., 2017) but one reported lower belowground richness than aboveground (Kesanakurti et al., 2011). In our study there was an inconsistent trend as to whether richness was greater aboveground or belowground; other studies using *trn*L have also found an inconsistent relationship between aboveground and belowground plant species richness (Price et al., 2012; Träger et al., 2019). Overall, 34 plant species were detected belowground and 55 plant species were recorded aboveground. The second cause of variation may be due to taxonomic resolution. Numerous sequences were assigned only at the family level, the three most common families were Asteraceae, Poaceae, and Brassicaceae. Other research has also reported relatively low taxonomic assignments at the species level for multiple plant barcode markers (Braukmann et al., 2017) and others report low assignment to the genus level specifically using *trn*L for mixed environmental samples (Mallott et al., 2018). Improved resolution may greatly improve the efficacy of using belowground richness determined from sequencing data to predict aboveground richness, and may reveal a greater richness belowground than aboveground.

### 3.4.2 Soil Properties Across the Edge

Living biomass was greatest in the perennial grassland at SDNWA, where soil total C and total N were also the greatest. Compared to CLC, where total C and N were relatively consistent across the edge. Although, perennial grasslands had more C and N than croplands; concordant with other studies that found poorer soil quality in cultivated land than non-cultivated land (Hebb et al., 2017; Cade-Menun et al., 2017; Panico et al., 2018). Perennial grasslands at both sites had plant species with relatively high-quality litter that likely influenced soil properties. For example, *B. inermis*, *M. stavia*, and *M. officinale* have litter that is relatively fast degrading, high in N content and a low C:N (Redin et al., 2014; Lardner et al., 2015). The cropland is relatively productive; however, the majority of aboveground biomass is removed. Removal of aboveground biomass disrupts the natural nutrient cycles in the system and thus soil C and N will decrease overtime. Belowground vegetation inputs via roots are also important for soil C and N (Bolinder et al., 2007). In the cropland, annual root systems are much smaller than perennial root systems (Schenk and Jackson, 2002; Roumet et al., 2006). Reduced root biomass can affect soil structure leading to compaction, which can produce more runoff, leading to loss of C and other soil nutrients (Pennock et al., 2011). Currently croplands at both sites are no-till but were previously tilled, time of conversion to no-till is unknown. Conventional tillage in the prairies caused topsoil erosion, and combined with a hummocky landscape, such as at SDNWA, soil C loss can be extensive (Li et al., 2008).

Another management practice, applying soil amendments, can affect soil properties. $NO_3$ was significantly higher in croplands than in perennial grasslands. At SDNWA, granular fertilizer was applied at a rate of 80 lbs N/acre and at CLC anhydrous fertilizer was applied at 100 lbs N/acre. Long-term application of N fertilizer promotes nitrification and $H_+$ production that causes acidification (Rosenzweig et al., 2016). At CLC, pH was significantly lower in the cropland and the edge compared to the perennial grassland; this drop in pH may be due to N fertilizer addition. The form of fertilizer may also be a factor in how much acidification occurs, as anhydrous fertilizer was applied at CLC. A previous study conducted in Saskatchewan found that anhydrous ammonia acidified soil more than urea fertilizer over a 10-year period (Biederbeck et al., 1996). At SDNWA, pH across the edge was relatively consistent, which may in part be due to lower amounts of N fertilizer and the granular form. Another factor that could explain pH similarity across the edge at SDNWA is the redistribution of topsoil. The undulating hummocky landscape

at SDNWA, combined with previous conventional tillage, resulted in topsoil erosion from upper slopes and deposition into lower lying areas (Papiernick et al., 2005; Helgason et al., 2014).

### *3.4.3 Soil Microbial Community Across the Edge*

Agroecosystem land use and land management practices can affect soil bacterial and fungal community composition (Papiernick et al., 2005; Helgason et al., 2014). In our study, land use appeared to have a stronger influence on fungal community composition rather than bacterial community composition, as consistent differences of fungal community composition were found at both sites. Similarly, when comparing between a no-till cropland, a hayed grassland, and a native prairie, little change in bacterial community composition was found (DuPont et al., 2010). Alternatively, one study did find compositional differences for both bacteria and fungi when analyzing a meadow, mowed pasture, and a grazed pasture (Schöps et al., 2018). Specifically in Saskatchewan, arbuscular mycorrhizal fungi (AMF) community composition was found to be influenced by land use rather than soil type when comparing croplands, roadsides, and grasslands (Bainard et al., 2015). This supports finding differences in fungal community composition across the edge at both perennial grassland-annual cropped sites.

Soil properties can also directly affect the soil microbial community and may explain, in part, the stronger differences in bacterial community composition at SDNWA than at CLC. Soil total C and total N were significantly different across all edge locations at SDNWA, and this trend was weak for total C and total N at CLC. Studies have reported that bacteria are more influenced by soil properties than fungi (Lauber et al., 2008; Wakelin et al., 2016) and may be related to total N. Specifically, total N fluctuations have frequently been accompanied with bacterial community changes (Mueller et al., 2015; Sun et al., 2018; Zhao et al., 2019); thus, total N may contribute to the bacterial community composition differences at SDNWA. In the SEM, total N had a significant positive relationship with bacterial richness and is discussed further in section 3.5.4.

Management practices, such as fertilizer and herbicide application, can alter soil nutrient levels and in turn affect the soil microbial community. Long-term application of anhydrous ammonia was found to have negative effects on microbial biomass (Geisseler and Scow, 2014) and can be toxic to some fungi (Smiley, 1970). Fertilizer is likely affecting the bacterial and fungal community in the croplands and the edges. Glyphosate, an herbicide used at SDNWA and

CLC, has also been shown to reduced AMF colonization (Helander et al., 2018). Other nutrients not measured in this study, such as phosphorus (Cruz et al., 2009; Leff et al., 2018), or other measures like electrical conductivity (Chowdhury et al., 2011; Yan et al., 2015), and soil moisture (Fierer et al., 2003) may also influence soil microbial communities.

Plant community composition due to land use appeared to influence the fungal community across the edge. Fungal community composition change was consistent at both sites while bacterial composition was not, indicating that fungi may be more sensitive to plant community changes than bacteria. Most fungi use plant material for energy, hence plant community composition is an important aspect in shaping the soil fungal community (Taylor and Sinsabaugh, 2015). In addition to considering the whole plant community, individual plant species can also influence soil fungal community composition (Osanai et al., 2013; Cong and Eriksen, 2018). Other measures of the plant community have also been linked to the soil fungal community. Increasing plant species richness has been linked with increasing fungal richness (Dassen et al., 2017) and plant diversity was found to have weak correlations with soil fungal communities (Prober et al., 2015). In our study, the highest plant species richness was at the edge and the fungal community composition appeared to be very similar at the edge and the cropland, where the lowest plant species richness occurred. Thus, individual plant species rather than richness may be more important in determining fungal community composition across the edge.

### 3.4.4 Linking Aboveground and Belowground Changes

The aboveground plant community had direct influences on soil properties and fungi, and indirect influences on bacteria. Changes occurring both aboveground and belowground that result in microbial community change may be the direct product of land use (Schöps et al., 2018). Land use in this study was the single largest factor that determined plant community composition; the plant community largely influenced soil properties and together ultimately influenced the soil microbial community. However, the fungal and bacterial communities responded somewhat differently across perennial grassland-annual cropland edges.

Plants can influence the soil microbial community; for example, some plants have symbiotic relationships with certain microbes such as mycorrhizal fungi and nodule forming $N_2$-fixing bacteria. A positive direct influence from belowground plant species richness to fungal richness was observed, however aboveground plant species richness had a weaker, non-

significant relationship with fungi. A study conducted in south-central Saskatchewan, found that AMF richness was strongly correlated with plant richness, but the correlation was even stronger with belowground plant richness (Hiiesalu et al., 2014).The closer physical proximity of fungi with belowground roots may explain part of these observed relationships, as one study found proximity to the root as a stronger predictor of the fungal community (Schlatter et al., 2018). Plants exude numerous compounds through their roots that help shape the microbial community. Plant root exudates can provide nutrients for microbes such as C compounds like various sugars (Badri and Vivanco, 2009), flavonoids that regulate certain bacteria growth (Bertin et al., 2003), amino acids, and hormones that may promote certain microbes (Lareen et al., 2016). Plants can also influence soil and the microbial community through their biomass. More aboveground biomass may increase input of C and N to the soil, but also belowground dead root biomass can also increase input to soil C and N (Rosenzweig et al., 2016). Living biomass was positively linked to soil total C and total N, and total N was positively linked to bacteria, and bacteria was negatively linked to fungi. No direct link of soil properties to overall fungal richness was found in our study. Other studies have found varied responses of fungi to soil N, some have observed effects (Cong and Eriksen, 2018; Veach et al., 2018) whereas others have not (Mueller et al., 2015; Katulanda et al., 2018). Conversely, total C has been observed to consistently influence fungal community composition (Liu et al., 2015) and increase mycorrhizal fungal richness (Yang et al., 2011).

Bacterial richness was positively influenced by soil total N and is concordant with other studies finding increased soil nutrient availability, such as C and N, increased overall microbial richness (George et al., 2019) and increased microbial biomass (Yao et al., 2000). Inorganic N forms, such as those found in fertilizers, can significantly alter the bacterial community (Ramirez et al., 2012). However, the greatest amount of inorganic N was found in the croplands where total N was lower.

While overall fungal richness was not significantly influenced by total N and total C, many of the fungal genera had significant relationships with these soil properties. For example, *Arthrinium* at SDNWA was positively influenced by total C; higher total C represents more resources for fungi. *Arthrinium* includes species that are saprotrophs, thus higher total C may support more saprophytic fungi (Müllenborn et al., 2008). Some fungal genera, such as *Parastagonospora*, *Sarocladium*, and *Olipidium*, had negative relationships with total C which

may reflect where these genera were most abundant, in the cropland or the edge, where there was lower total C. These genera also contain plant pathogens, including *Sarocladium* (Giraldo et al., 2015) and *Parastagonospora* containing a major wheat pathogen *P. nodorum* (Gao et al., 2015). These fungal pathogens may be most abundant where host plants are most abundant in the cropland and edge areas.

Interactions within the soil microbial community also shapes the microbial community itself. We found overall bacterial richness negatively influenced fungal richness. Relationships in the microbial community are complex and previous research has indicated that bacteria may influence fungi (Ait Barka et al., 2002; Rousk et al., 2008; Kai et al., 2009; Mamet et al., 2017). In contrast, studies also observe that fungi have influence over bacteria (Folman et al., 2008; Moore et al., 2015). At the genus level, bacteria had mostly negative relationships with fungal genera at SDNWA; specifically, *Devriesia,* was negatively influenced by bacteria and this has also been reported in another study (Kim et al., 2011). However, *Coprinopsis* had a positive relationship with bacterial richness which highlights the complexity of the microbial community interactions. Another layer of complexity is within kingdom interactions, such as fungi interacting with other fungi. The genus *Clonostachys* at SDNWA, contained species level identification including *Clonostachys rosea,* a well-known and studied mycoparasite (Vega et al., 2008; Karlsson et al., 2018). Analyzing the microbial community at a finer taxonomic level may reveal important relationships and interactions not found at the kingdom level, or captured in metrics such as diversity or richness.

Investigating the fungal community at the genera level revealed differences in relationships with plants. Certain fungi may have specific relationships with certain plants that are not necessarily captured in plant species richness. For example, *Paraphoma* that had a positive relationship with plant richness; *Paraphoma* are common soil fungi and frequently associate with monocots (Boerema, 2004). One species of *Paraphoma* found was a plant pathogen, *P. chrysanthemicola* (Hay et al., 2015; Ge et al., 2016) and is known to affect plants in the Asteraeae and Rosaceae families (Kowalik and Sagan, 2005). Many Asteraceae plants occurred at the edge and may explain why *Paraphoma* was also most abundant at the edge. Some genera had negative relationships with plant richness, such as *Sarocladium. Sarocladium* was most abundant in the cropland and it may be partly explained by many members of the genus are plant pathogens (Giraldo et al., 2015). Aboveground plant species richness had a significant

relationship with *Sarocladium*, *Sarocladium* was most abundant in the cropland where aboveground plant species richness was the lowest. Plant pathogenic fungi illustrate the close relationship of plants and fungi. The only significant genus at CLC, *Olpidium,* contained one species, *O. brassicae*, that is a well-known pathogen to members of the Brassicaceae family (Lay et al., 2018). Canola was likely the host plant as it was grown during sampling at CLC. As these pathogens can cause extensive damage in croplands, it highlights the importance of understanding these relationships between soil microbes and plants. Further understanding of these relationships may help prevent infestations and better understanding of soil microbial–plant feedback.

## 3.5 CONCLUSION

Aboveground and belowground edge effects were observed across perennial grassland-annual cropland edges. Aboveground, the plant community changed distinctly at the edge and was dominated by annual weeds. Belowground, soil properties also changed across the edge, the most apparent were total C and total N. In addition, the soil microbial community also changed across the edge with the fungal community differing between the perennial grassland and the annual cropland. Changes aboveground were linked to belowground changes; living biomass directly influenced soil properties which in turn influenced bacteria and fungi. Fungi were also directly influenced by the belowground plants; however, further exploration at the genus level revealed differences in relationships to soil properties and the bacteria. Edge effects in the agroecosystem do occur, influencing the plant and soil microbial community. How soil microbial community changes affect ecosystem processes and services at the agroecosystem scale is not fully understood and more research is needed. Left alone, these semi-natural edge areas are vulnerable and can develop undesirable characteristics such as cultivating a predominately annual weed community that may later have adverse effects on crops. While edges will always be present, understanding how they influence the adjacent areas may lead to more sustainable agricultural management practices.

# 4.0 SYNTHESIS AND CONCLUSIONS

To investigate edge effects in agroecosystems, we examined aboveground and belowground attributes across perennial grassland-annual cropland edges in central Saskatchewan. The first objective was to characterize the aboveground and belowground properties across the edge. Aboveground plant communities shifted from perennial plants to annual weedy plants at the edge. Soil properties also changed across the edge, total C and total N were both greater in the grassland and decreased across the edge. Croplands had high levels of $NO_3$, likewise edges had some locations with high $NO_3$, reflecting the management practice of applying fertilizer. We also observed changes belowground with differences in microbial community composition across the edge, specifically the perennial grassland fungal community was compositionally different from the cropland or edge fungal communities.

The second objective was to understand and identify key factors across the edge that influenced the soil microbial community. Aboveground plants, represented by living biomass, had an indirect influence on bacteria and fungi through positively influencing total C and total N. Belowground, plants had a positive direct influence on fungal richness. There was also an indirect pathway of plants on fungi through living biomass that positively influenced soil N which in turn positively influenced bacteria that negatively affected the fungi. These factors that influence the microbial community and plant community at the edge may be altered through adjustment of existing or new management practices. Reduced weed populations and an increase in native plant diversity can improve ecosystem services, such as pollination and pest/pathogen control, improving overall ecosystem health and resilience within these agricultural landscapes.

## 4.1 Edges in the Agroecosystem

Edges in the agroecosystem will become more important as agriculture expands and intensifies. Landscape simplification will likely result in fewer edges causing reduced biodiversity and a loss of ecosystem services. Loss of edges in the agroecosystem have occurred extensively in Britain, where hedgerows along field edges were more common (Marshall and Moonen, 2002). Loss of edges from landscape simplification has caused population declines of various biota due to loss of edge habitat, including butterflies, spiders, birds, and hares (Šálek et

al., 2018). Other consequences of edge loss and landscape simplification include increased generalist plant species and decreased specialist plant species populations. Heterogeneous landscapes typically have greater biodiversity and biodiversity can increase resiliency in these ecosystems, recovery from environmental disturbances, such as droughts or severe weather events (Kremen and Miles, 2012).

Edges can affect plant and soil conditions, which can influence biodiversity in the landscape. A specific edge type in the prairies, shelterbelts, were once prevalent and shelterbelt removal has occurred more recently to create larger fields. Shelterbelts were originally established to prevent wind erosion of soil, but additional benefits include sequestering C in woody biomass, capturing sediment and nutrients in runoff, protecting biodiversity, and improving air and water quality (Kulshreshtha and Kort, 2009). Other perennial vegetation surrounding field edges can support the benefits listed above and also provide habitat for pollinators and natural enemies to pests (Martens et al., 2015).

### 4.2 Managed Edges

Managing field edges is becoming more common and in some cases mandated, specifically planting vegetation at cropland edges to reduce runoff that would pollute waterways in Canada and the U.S. (Gene et al., 2019). Unfortunately, edges receive little consideration when negative edge effects are occurring in adjacent terrestrial ecosystems. In our study, edges had no management and were dominated by weeds. While edges are often known to increase overall biodiversity, landscapes with higher edge-to-area ratio landscapes typically have more invasive species (Wilkerson, 2013). Field edges are highly concerning because of their status as a reservoir for invasive weeds and other undesirable microbial pathogens (Boutin and Jobin, 1998) As discussed previously, elevated nutrient levels at field edges may promote non-native plant species. Elevated N levels from fertilizer applied in adjacent fields can change the plant community at the edge by providing N for disturbance tolerant plants, giving them a competitive advantage over other plants (Schmitz et al., 2014).

Planted vegetation along field edges may promote native non-weedy biodiversity in agroecosystems and provide important ecosystem services. For example, 3 m wide flower strips were planted around potato field edges in Switzerland to promote natural enemies of aphids. Aphid density was reduced by 75% in the potato fields with planted flower strips versus those

without (Tschumi et al., 2016). Another study also conducted in Switzerland found an increase of wild crop pollinators in managed, floristically enhanced field edges (Sutter et al., 2017). Woody vegetation at edges can also have positive effects. Two types of hedgerows along wheat fields in northern Italy were investigated and found that overall hedgerow cover positively influenced pollinators in the landscape (Dainese et al., 2017). The authors of that study also conclude that in order to support more pollinators, incorporating biodiversity on the local scale is not effective and needs to be done on the landscape level. Local efforts in managing field edges may be a small improvement but are the most effective on a larger scale. Edge management practices may help increase native plant diversity and ecosystem services. However, implementing new management practices introduces assorted challenges and would require necessary education and support for large-scale change.

### *4.3 Belowground Edge Effects in the Agroecosystem*

Previous studies on edge effects primarily focused on aboveground vegetation; the current study provides insight into edge effects belowground and what factors are causing changes in the soil microbial community. As discussed above, land use was a large factor in determining plant community composition and these plant community changes subsequently influenced the fungal community. Land use also influenced soil nutrient status that affected the microbial community. Although soil C did not appear to significantly influence the soil microbial community in the current study, C is extremely important as it supports many important ecosystem functions and influences nutrient status and dynamics. In the current study, cropland and edge had significantly less C than the perennial grassland at SDNWA. Soil C and soil organic matter (SOM) help hold nutrients and water in the soil and are strongly influenced by plant productivity and the soil microbial community (Carter, 2002). The proportion of aboveground and belowground inputs can affect the amount C; typically deep-rooted perennial species like grasses and shrubs allocate more to their roots resulting in more C and SOM in these soils (Jackson et al., 2017). The annual plant community at edges in our study will likely not contribute to SOM as much as a perennial plant community. A study on wooded field edges compared to herbaceous field edges found greater SOM and total C at wooded than herbaceous edges (D'Acunto et al., 2014). The soil microbial community is important in soil C cycling, as microbes are responsible for decomposition and transformation of C (Six et al., 2006). It is generally thought that fungi-

dominated systems may amass more soil C because fungi produce more recalcitrant compounds and have a higher carbon use efficiency than bacteria. Fungi also help form macroaggregates in the soil that protect C from being decomposed by microorganisms (Jackson et al., 2017). Maintaining perennial grasslands in agroecosystems may be important for building and retaining soil C.

Increased soil C and SOM, can also influence N dynamics (Carter, 2002). The perennial grasslands had more total N than the edge or cropland, but the form of N may be more important when concerning the soil microbial community. The effects of N containing amendments were discussed in the previous section. In the current study, bacteria appeared to be influenced more by N than fungi. However, excessive inorganic N amendments and spillover from the cropland, can have effects on both bacteria and fungi. These amendments may stimulate select microbes and may alter composition, such as promoting ammonia oxidizing bacteria (Du et al., 2019). Additionally, N amendments have been shown to reduce fungal diversity (Treseder, 2008).

### 4.4 Edges and Plant Pathogens

In the current study, notable fungal genera that were identified across the edge included genera known for containing plant pathogenic members. Fungal genera containing pathogens were most abundant in the cropland, but one, *Olpidium,* was most abundant at the edge. Weeds, most abundant at the edge, can influence pathogen prevalence by helping spread diseases and serve as alternate hosts other than crops (Wisler and Norris, 2005). Soil N as may also influence pathogen presence, one study showed that increasing inorganic N fertilizers changed plant community composition in a meadow, with the highest levels of fertilizer resulting in reduced plant species richness and increased the prevalence of pathogens in the community (Liu et al., 2017).

Pathogens are an important part of the soil microbial community, but in an agroecosystem they can cause major crop yield losses (Mendes et al., 2013). It is estimated that 70-80% of plant diseases are caused by fungi (Zeilinger et al., 2016) and agriculture can exacerbate pathogens, such as successive monocultures building up specific pathogens (Berendsen et al., 2012). Typical methods currently administered to combat fungal pathogens are various fungicides and fumigation; however, these are not the most successful in some cases and may adversely affect other organisms in the ecosystem (Raaijmakers and Mazzola, 2016).

In a natural setting, plants can select or stimulate soil microbes in their rhizosphere by root exudates (Berendsen et al., 2012). Plants can produce compounds that promote certain bacteria, these bacteria in turn, can help protect the plant against fungal pathogens (Hol et al., 2014). Plants can also defend themselves against pathogens by excreting toxic compounds like citric acid, succinic acid, malic acid, and salicylic acid (Berendsen et al., 2012). Other compounds like tannins and polyphenols deter soil fauna and prevents initial breakdown of plant material and the subsequent fungal decomposition (Hättenschwiler and Vitousek, 2000). Microbes can also secrete compounds; isolated fluorescent pseudomonads in one study were found to produce compounds including proteases and siderophores that help suppress fungi. A greenhouse trial in the same study, testing bean plants inoculated with the isolated bacteria, found reduced pathogen prevalence of *Rhizoctonia solani* on the inoculated bean plants (Ahmadzadeh and Sharifi Tehrani, 2009). Mycorrhizae are also important in protecting plants, as their networks may sense pathogens and send signal molecules to the plant as a warning (French, 2017).

Overall plant productivity and protection of crops from soil-borne pathogenic fungi can be facilitated by promoting disease resistant soils, specifically by influencing the rhizosphere (Berendsen et al., 2012; Schlatter et al., 2017; Hayden et al., 2018). Disease suppressive soils have lower incidences pathogens (Berendsen et al., 2012). Developing and cultivating disease suppressive soil rather than targeting one pathogen may be a more sustainable approach to use in agroecosystems (Zeilinger et al., 2016). Methods that reduce incidence of disease are already implemented, such as, crop rotations and organic amendments (Janvier et al., 2007). Other potential management strategies that need further development and experimentation are introducing or inoculating the soil with beneficial microbes, simulating beneficial soil microbes already present, and developing crop cultivars with rhizosphere microbiomes that have pathogen suppressing bacteria (Mendes et al., 2013). Directly managing for pathogens at edge soils may be critical in promoting disease resistant soils in agroecosystems.

### 4.3 Study Limitations

Many types of edges exist in the landscape and this study was specifically limited to edges of perennial grasslands and annual croplands in central Saskatchewan. Both land use types are under anthropogenic management; grassland composition was determined by managers and was seeded based on plant species suited for hay harvesting. More natural land uses with

predominately native vegetation may experience different edge effects. Additionally, sampling occurred at a single time point, therefore, seasonal dynamics were not considered in this study. Moisture and temperature do affect soil microbial community composition and function, and should be considered as edge effects may change over the year. Topography of the landscape can also significantly affect microbial community and function and was not explored in this study.

When creating the SEMs, not all soil properties were measured nor all measured properties included. Omission of these variables could influence model results and lead to interpretation that does not accurately reflect the system (Tomarken and Waller, 2005). For example, archaea were not measure in this study. Archaea are an important group in the soil microbial community as they are also involved in C and N cycling (Deveau et al., 2018). Including more variables like archaea, may help understand the complex interactions occurring across the edge. Many assumptions were made creating the *a priori* model. It was chosen to have bacteria influence fungi in the model, rather than fungi influence bacteria or as covariates. In reality, various interactions between bacteria and fungi occur simultaneously that are positive and negative (Deveau et al., 2018). Another assumption made in the *a priori* model was that plants influenced only fungi and not bacteria. Bacteria too can also be influenced by plants and their root exudates.

Both SEM and multivariate analyses do not directly test mechanisms, it can only be assumed from previous studies what may be actually occurring in these systems. For example, soil N appears to be an important factor for soil bacterial composition, but the mechanisms affecting the bacteria cannot be determined by SEM or multivariate analysis in this observational study. These analyses though can help develop new questions and guide future studies examining and testing these relationships (Paliy and Shankar, 2016).

While next-generation sequencing is a powerful tool, it does have some disadvantages and drawbacks. Obtaining a true, complete, community composition for soil microbes is difficult to achieve for many reasons. Different protocols used to extract DNA can significantly affect the results of molecular analysis, such as bead beating samples used in the current study, could reduce DNA yield (Halwachs et al., 2017). Amplifying DNA during PCR also introduces biases and some DNA may not be readily amplified, therefore the end result after sequencing may not be a true representation of the soil microbial community (Zhou et al., 2015). After amplification and sequencing, the data must be processed to obtain representative species, commonly either

OTUs or ASVs. Numerous algorithms create and classify OTUs slightly different, and there is debate around what percent similarity is considered a species (97% is most commonly used); however, this may not accurately reflect all species (Leavitt et al., 2016). This may influence further analysis on the microbial community such as determining richness, diversity, and evenness. Despite the shortcomings of next-generation sequencing, it is the most powerful tool to analyze microbial communities at finer taxonomic levels than other characterization methods (Segata et al., 2012).

### 4.4 Future Research

Future research on edges should investigate different types of edges and locations, such as native grasslands, grazed grasslands, and other crops, which will provide a more holistic understanding of edges in the agroecosystem. Measuring the function of the soil microbial community may also help further understand the importance of microbial community composition changes across the edge. Studies investigating both microbial community composition and function will greatly improve our understanding of how community composition and function are linked, but also improve our understanding of plant-soil feedbacks.

Research on the role of edges in promoting biodiversity in agroecosystems should also be conducted. Studies on how biodiversity increases ecosystem function may aid in determining best management practices to sustain these functions. Potential edge management strategies, such as planting vegetation along cropland edges could be investigated further to determine their effectiveness on improving ecosystem services, such as pollination or pest control. Monitoring edges, such as the ones examined in this study, may also help understand how edges change overtime. Changes in management in the croplands may also influence edges and edge effects. Studies exploring management practices on unmanaged edges, may reveal which practices contribute to the greatest change at edges and edge effects. How different types of soil amendments and rates influence the presence of fungal pathogens in croplands and edges may be useful in guiding management practices that reduce potential pathogen spillover at edges. Further knowledge of the interactions between the soil microbial community, soil properties, plants, and edges may help develop more sustainable agricultural practices and overall build a more resilient agroecosystem.

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**Fig. A.1:** Edge at one sampling location at the Conservation Learning Centre, July 2017.

**Fig. A.2:** Edge at one sampling location at the St. Denis National Wildlife Area, August 2018.

**Fig. A.3**: Aerial view of edge at one sampling location at the Conservation Learning Centre, July 2017.

*Pilot Study*

A pilot study examining preparation techniques of root-soil samples (2cm diameter x 15 cm depth each) for molecular analysis was conducted using Illumina MiSeq sequencing to determine plant root communities. The purpose is to examine the influence of i) root picking and ii) seed bank on the detection of plant species within samples. A subset of five samples was randomly selected and six additional samples were then selected that had similar plant communities in the perennial field at SDNWA (Table B.1). For each sample, half was sieved (2 mm) and roots were picked out (root samples) while the other half was not sieved nor were the roots picked out (root-soil samples). Soil from the samples where the roots were picked out was also saved and sequenced (soil samples). Foreign seed (*Polygonum alaskanum*) was added to the root-soil samples (soil, root, and seed samples). The root, root-soil and leftover soil samples were freeze-dried for 24 hours. The root-soil samples were subsampled (5g), and the entire root samples were ball ground for 5 minutes at the frequency 22.5 Hz (Retsch Mixer Mill, Hann, Germany).

**Table B.1:** Six additional samples selected for the pilot study based on various aboveground indices (species richness, Shannon's diversity, Simpson's dominance, Pielou's evenness, Simpson's evenness) in the perennial grassland at St. Denis National Wildlife Area

| Distance from Edge | Richness | Shannon's Diversity | Simpson's Dominance | Evenness (Pielou's) | Simpson's Evennnes |
|---|---|---|---|---|---|
| 33m | 5 | 1.1 | 2.5 | 0.67 | 0.50 |
| 16m | 4 | 0.99 | 2.2 | 0.72 | 0.55 |
| 16m | 6 | 1.2 | 2.4 | 0.67 | 0.41 |
| 6m | 3 | 0.42 | 1.3 | 0.38 | 0.42 |
| 16m | 5 | 1.1 | 2.4 | 0.70 | 0.48 |
| 6m | 6 | 0.79 | 1.5 | 0.44 | 0.25 |

DNA was extracted (DNeasy PowerPlant Pro Kit, Qiagen, Germany) from the picked roots, root-soil samples, leftover soil samples, and the root-soil samples with foreign seed. Plant root DNA was amplified with the *trn*L primer set from Lamb et al. (2016). The PCR was performed with total volume of 25µL; 2 µL of template DNA, 20 µL of Platinum Blue SuperMix (Invitrogen, Massachusetts, USA), 1.5 µL of forward primer and 1.5 µL reverse primer. PCR cycles began 95°C for 5 mins, 35 cycles of 95°C 30 s, 55°C 45 s, 72°C 60 s, and 72°C for 7 mins. The PCR products were visualized on a 1.5% agarose gel to confirm amplification of target region. Products were purified using the NucleoMag NGS Clean-up and Size Select magnetic beads (Macherey-Nagel, Germany) following protocol for single size selection with the exception of reduced drying time after the second ethanol wash (2 minutes).

A 'mock community' was used as a positive. The positive control was created with amplified DNA extracted from plant specimens that were collected from the field. Species included were among the most abundant in the perennial field at SDNWA; *Bromus inermis, Cirsium arvensis, Medicago sativa* and *Elymus repens* (Table B.2). Library preparation for Illumina MiSeq followed protocol, using Nextera XT Index Kit v2 Adapters (Illumina, San Diego, USA). The final library concentration was 8 pM with a 25% spike of PhiX (Illumina, San Diego, USA) and 300-cycle MiSeq v2 kit (Illumina, San Diego, USA) was used. Sequencing was performed at the Agriculture and Agri-Food Canada's research centre at the University of Saskatchewan.

**Table B.2:** Plant species used to create the 'mock community' positive control for sequencing. Average cover and frequency was calculated for the perennial field at St. Denis National Wildlife Area (0.25 m-33 m).

| Species | Frequency | Average Cover (%) |
|---|---|---|
| *Elymus repens* | 0.40 | 5.8 |
| *Bromus inermis* | 0.86 | 17 |
| *Cirsium arvense* | 0.90 | 5.8 |
| *Medicago sativa* | 0.26 | 9.45 |

*Bioinformatics and Statistics*

The sequences were processed with the UPARSE pipeline (Edgar, 2013). First, forward and reverse sequences were merged, trimmed and quality filtered. OTUs were created at 97% similarity and chimeras were removed. Taxonomic assignment was carried out by using BLAST and assigning taxonomy to OTUs followed the process of (Leff et al., 2018); taxonomic assignment was only given if it matched the reference sequence identity $> 95\%$. Also, if there were many matches for an OTU and a common level of genus or family, it would be assigned taxonomy at that common level.

The OTU table was imported into R and the *vegan* package (Oksanen et al., 2018) was used to determine diversity (Shannon's *H'*) and species richness. An ANOVA was conducted on richness to determine any significant differences between treatments using the 'aov' function. Post hoc testing was completed using the 'TukeyHSD' function.

*Results and Discussion*

Land use appears to be different with sample preparation, as the perennial grassland sample treatments appeared to have a more consistent pattern, while cropland samples were more variable among treatments (Fig. B.1). Roots that were picked out generally had higher richness than the other treatments, but no significant difference was found between the root treatment and the root-soil treatment ($p = 0.54$). This suggests that extracting and sequencing combined root-soil samples should accurately represent the belowground plant community. The leftover soil treatment and root treatment were significantly different ($p = 0.02$). The spiked seed, *P. alaskanum*, was detected in all samples it was added to, demonstrating that seed DNA can be picked up and sequenced with *trn*L. When using the root-soil approach, the resulting plant community may extend beyond just the roots; other plant parts, such as seeds, can be sequenced. Care should be taken in assuming that all plant materials sequenced with *trn*L represent plant roots and where a persistent seedbank could confound results, removal of roots from mixed samples prior to DNA extraction may be required.

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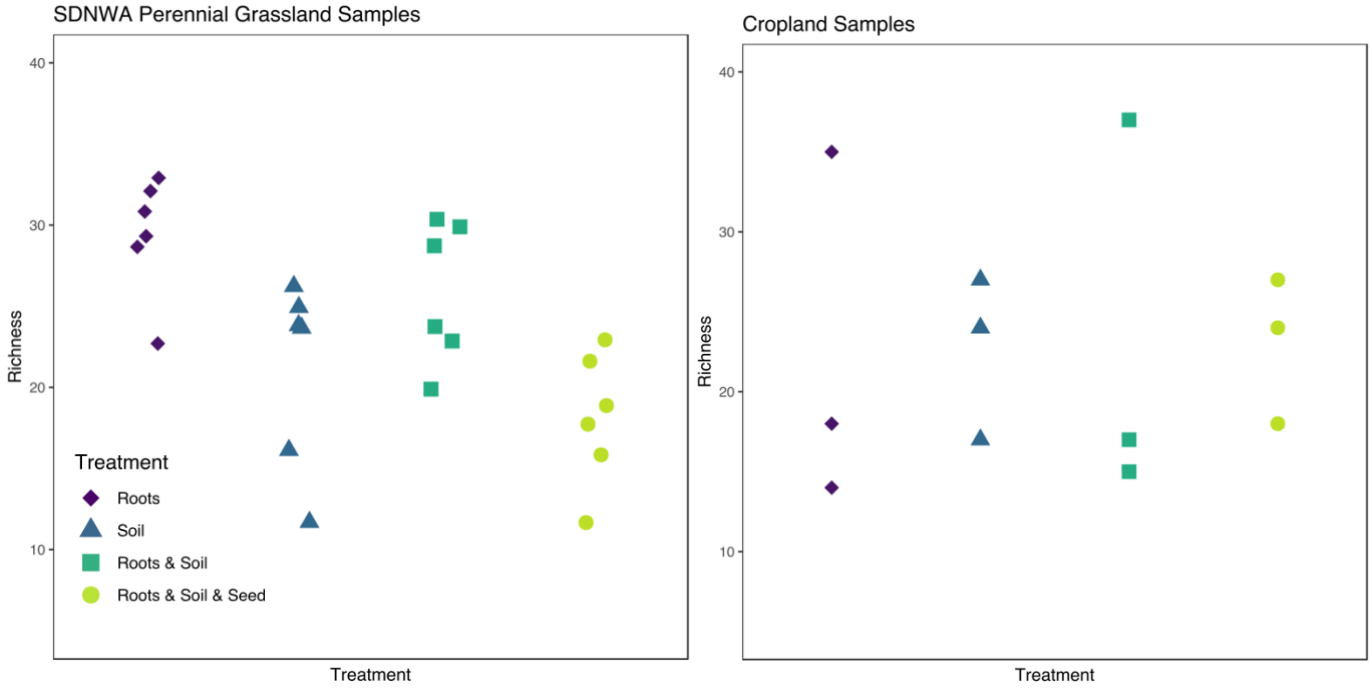
**Fig. B.1:** Plant species richness (number of OTUs) for each sample from the perennial grassland at St. Denis National Wildlife Area and cropland samples from both St. Denis National Wildlife Area and the Conservation Learning Centre. Shape and colour represent the treatment, purple diamonds are the picked roots, blue triangles are leftover soil, teal squares are samples with roots and soil, and the green circles are root and soil with added *Polygonum alaskanum* seed. Points are jittered to make points more visible.

Code used in R for statistics; separated into analysis sections staring with aboveground plant analysis.

```
#Aboveground Vegetation###########################################

#load packages
library(vegan)
library(ggplot2)
library(dplyr)
library(viridis)
library(Cairo)

#Richness, evenness and diversity:

#load data, vegetation cover matrix and corresponding metadata. Note: Rows in matrix and meta data must be the same
veg <- read.csv("/Volumes/BedardHaughn/NSERC_Agroeco/Mariah/Statistics/Veg/data used/Cover.csv",header=T) #cover data
md <- read.table("/Volumes/BedardHaughn/NSERC_Agroeco/Mariah/Statistics/Veg/data used/MasterData.txt", header=TRUE, sep="\t")

#Data preparation
veg[is.na(veg)] <-0 ##Replaced NA values with zero

SD_veg <- veg[c(1:90),] ##Split into each site
CLC_veg <- veg[c(91:180),]


#remove "empty" columns, a.k.a. plants that are not at SD (but at CLC)
SD_veg$BROMRIP <- NULL
SD_veg$unkn_collect <- NULL
SD_veg$ACHIMIL <- NULL
SD_veg$PLANMAJ <- NULL
SD_veg$FESTSAX <- NULL
SD_veg$EQUIARV <- NULL
SD_veg$VICIA <- NULL
SD_veg$EURYCON <- NULL
SD_veg$FRAGVIR <- NULL
SD_veg$SALIBEB <- NULL

#remove "empty" columns, a.k.a. plants that are not at CLC (but at SD)
CLC_veg$TRAGDUB <- NULL
CLC_veg$LEPIDRA <- NULL
CLC_veg$ELYMTRA <- NULL
CLC_veg$PASCSMI <- NULL
CLC_veg$ERIGCAN <- NULL
CLC_veg$THLAARV <- NULL
CLC_veg$LINUUSI <- NULL
CLC_veg$ERYSCHE <- NULL
CLC_veg$SONCOLE <- NULL
CLC_veg$DESCSOP <- NULL
CLC_veg$unknown_herb_3 <- NULL


#shannon's H
sd.h <- diversity(SD_veg)

#richness
sd.Sp <- specnumber(SD_veg)
sd.SP <- as.data.frame(sd.Sp) #need it in data frame

#evenness
sd.even <- (sd.h/log(sd.SP))

#combine into one data frame
sd.plant.att <- cbind(sd.h, sd.SP, sd.even)

#Do same for second site
```

```r
#shannon's H
clc.h <- diversity(CLC_veg)

#richness
CLC.Sp <- specnumber(CLC_veg)
CLC.SP <- as.data.frame(CLC.Sp) #need it in data frame

#evenness
CLC.even <- (CLC.h/log(CLC.SP))

#combine into one data frame
CLC.plant.att <- cbind(CLC.h, CLC.SP, CLC.even)

#Export the attributes and put into the master data file
write.table(CLC.plant.att, "clc-plants.txt", sep="\t")
write.table(SD.plant.att, "sd-plants.txt", sep="\t")

#re-load the master data file to make plots:
data <- read.table("/Volumes/BedardHaughn/NSERC_Agroeco/Mariah/Statistics/MasterData_text.txt", header=TRUE, sep="\t")

#Checking site names, changing SD to SDNWA
print(levels(datat$Site))
levels(datat$Site) <- c("CLC", "SDNWA")

#Changing the X, Y, Z to their actual location names:
print(levels(datat$Group))
levels(datat$Group) <- c("Edge", "Perennial Grassland", "Cropland")

#Ordering distances that they match the transect
print(levels(datat$Distance))
datat$Distance <- factor(datat$Distance, levels(datat$Distance)[c(11,3,15,9,5,13,7,1,6,12,4,8,14,2,10)])

#Creating vector of distance labels, to be used in plotting:
distlabels <- c("33", "16", "6", "2", "1", "0.50", "0.25", "0", "0.25", "0.50", "1", "2", "6", "16", "33")

#example plot, plant richness:
plantrichness <- ggplot(data, aes(y=Sp, x=Distance, color=Group, shape=Group))+
                geom_point(size=3)+
                facet_wrap(~Site,ncol=1, scale="free")+   #Create facets for each site
                scale_shape_manual(values=c(17, 15, 16))+ #Change the point shape
                theme_bw()+
                scale_color_viridis_d(option="D", begin=0.05, end=0.90)+ #Viridis color package
                theme(strip.background=element_blank(), strip.text.y=element_blank())+ #Changing facet lable format and location
                theme(strip.placement="top")+
                theme(panel.spacing = unit(1, "lines"))+
                theme(strip.text = element_text(hjust = 0))+
                theme(panel.grid.major = element_blank(), panel.grid.minor = element_blank())+ #Removing grid lines
                theme(legend.title=element_blank())+
                theme(legend.position = c( 0.88, 0.90), legend.title=element_blank(), legend.background = element_blank(),
                        legend.box.background = element_rect(colour = "black"))+   #Changing legend position
                ylab("Plant Species Richness")+
                xlab("Distance from Edge (m) ")+
                scale_x_discrete(labels=distlabels)+  #The vector of distance labels here
                theme(axis.title.x = element_text(vjust=-0.5))


#Export the plot, using the Cairo package:
CairoPNG("plantrichplot.png", height=6, width=8, units="in", dpi=300)
plantrichness
dev.off()



#NMDS for aboveground vegetation, from cover:
#packages used:
library(ggplot2)
library(vegan)
library(cowplot)

#use master data from previous and the seperated cover matrix for each site.
#split master data into the two sites:
SD_md <- md[c(1:90),]
CLC_md <- md[c(91:180),]

#Hellinger Transformation
SD_veg_hel<-decostand(SD_veg, "hellinger")
```

```
#convert to distance matrix
SD_veg_dist<-vegdist(SD_veg_hel, method="euclidean")

#running NMDS using different number of K dimensions
SD_nmds1 <- metaMDS(SD_veg_dist, k = 1, trymax=500)
SD_nmds2 <- metaMDS(SD_veg_dist, k = 2, trymax=500)
SD_nmds3 <- metaMDS(SD_veg_dist, k = 3, trymax=500)
SD_nmds4 <- metaMDS(SD_veg_dist, k = 4, trymax=500)
SD_nmds5 <- metaMDS(SD_veg_dist, k = 5, trymax=500)
SD_nmds6 <- metaMDS(SD_veg_dist, k = 6, trymax=500)
SD_nmds7 <- metaMDS(SD_veg_dist, k = 7, trymax=500)

#bind stress values
can.stress.df <- data.frame(cbind(N.dim = c(1,2,3,4,5,6,7),
Stress = c(SD_nmds1$stress, SD_nmds2$stress, SD_nmds3$stress,
        SD_nmds4$stress, SD_nmds5$stress, SD_nmds6$stress,
        SD_nmds7$stress)))

#plot stress values
plot(can.stress.df$N.dim, can.stress.df$Stress, ylim = c(0,0.5), type = "b")

#Remove soil variables that are not going to be on the plot, remove any columns not wanted on the plot:
SD_md$Total_Biomass <- NULL
SD_md$Forbs <- NULL
SD_md$Grass <- NULL
SD_md$Litter <- NULL
SD_md$Organic_C <- NULL
SD_md$Organic_N<- NULL
SD_md$Inorganic_Carbon<- NULL
##..ect more columns removed

#fit soil correlations, using nmds with least stress:
ef <- envfit(SD_nmds7, SD_prop, permu=999)

#checking it out:
ef
plot(SD_nmds7, display="sites")
plot(ef, p.max=0.1)

##need to put results into a data frame for ggplot
SD.scores <- as.data.frame(scores(SD_nmds7, display = "sites")) #scores() is a vegan function
SD.ef <- as.data.frame(scores(ef, display = "vectors"))
SD.ef <- cbind(SD.ef, Species = rownames(SD.ef))

#plot
SD.nmds.plot <- ggplot(SD.scores) +
  geom_point(mapping = aes(x = NMDS1, y = NMDS2, colour = SD_md$Distance, shape=factor(SD_md$Group, labels=c("Edge", "Perennial", "Cropland"))), size=4) +
  scale_shape_manual(values=c(17, 15, 16)) +
  coord_fixed() +
  geom_segment(data = SD.ef, aes(x = 0, xend = NMDS1, y = 0, yend = NMDS2),
          arrow = arrow(length = unit(0.25, "cm")), colour = "grey25") +
  geom_text(data = SD.ef, aes(x = NMDS1, y = NMDS2, label = Species, fontface="bold"),
          size = 4, hjust = 0.5, vjust = 3, position="jitter")+
  theme_bw() +
  scale_color_viridis(discrete=TRUE)+
  guides(color=guide_legend("Distance"), shape=guide_legend("Edge Location"))+
  annotate("text", label="stress=0.0454", x=-0.6, y=-0.45) +
  theme(panel.grid.major = element_blank(), panel.grid.minor = element_blank())+
  labs(title="SDNWA")

SD.nmds.plot

#Repeat for CLC and combine both NMDS plots into one image:
#Extract legend from ggplot (cowplot package)
lg.v <-get_legend(SD.nmds.plot)

#Combing both plots and legend:
plots <- plot_grid(CLC.nmds.plot + theme(legend.position="none"), SD.nmds.plot+theme(legend.position="none"), labels=c("A", "B"),
                                        align='vh', hjust=-1, nrow=1)
plots.export.v <- plot_grid(plots, lg.v, rel_widths=c(2, .25))

#Export plot
CairoPNG("nmds.png", height=4, width=12.5, units="in", dpi=300)
plots.export.v
dev.off()
```

```r
#Indicator species:
#packages:
library(indicspecies)

#using same seperated site cover matrix and master data file:
groups <- SD_md$Group
fields <- SD_md$Field
site <- SD_md$Site

#indicator analysis:
sdi <- multipatt(SD_veg, groups, control=how(nperm=999))
summary(sdi)
summary(sdi, indvalcomp=TRUE)

#export summary:
SDout <- capture.output(summary(sdi))
cat("SD-veg-indic", SDout, file="SD_indicator.txt", sep="\t", append=TRUE)

#repeat for CLC

#To check sequence quality#################################################################################

#packages, you will need to install bioconductor first, then install qrqc:
BiocManager::install("qrqc")
library(qrqc)

#WARNING: this is a sort of loop; it will produce quality plots for ALL fasta files in the directory.
#Set directory to a folder containing the sequences:
setwd("/Users/Desktop/Bioinformatics/PilotStudy/Sequences")

#Create list of files in directory:
fqlist <- list.files(getwd())

#Start of lapply
qPlot_fq <- function(fastq) {
  s.fastq <- readSeqFile(fastq, hash=FALSE, kmer=FALSE)
  tiff(paste(fastq, ".tiff"), width = 7, height = 5, units = 'in', res = 300)
  ag<- qualPlot(s.fastq)
  print(ag)
  dev.off()
}

lapply(fqlist, qPlot_fq)


#To inspect single files:
s.fastq <- readSeqFile("2_S295_L001_R1_001.fastq", hash=FALSE, kmer=FALSE) #put file name in
graph1 <- qualPlot(s.fastq)
graph1


#Microbial diversity, evenness, and richness#################################################################
#packages needed:
library(dplyr)
library(ggplot2)
library(phyloseq)
library(Cairo)
library(vegan)
library(CoDaSeq)
library(zCompositions)
library(scales)

#import biom files to phyloseq:
bacteria <- import_biom("/Volumes/BedardHaughn/NSERC_Agroeco/Mariah/Molecular/Pipelines/Bacteria_Qiime2/bacteriaGGp.biom")

#load metadata:
metadata <- read.table("/Volumes/BedardHaughn/NSERC_Agroeco/Mariah/Statistics/SEM/data/masterdata.txt", sep="\t", header=TRUE)

#add row names to metadata so they match sample names from the biom file:
xx <- paste0("sample", 1:180)
xxx <- sort(xx)
row.names(metadata) <- xxx

#import metadata to phyloseq object:
samp <- sample_data(metadata)
```

```
#merge sample data to the phyloseq object:
bac.phylo <- merge_phyloseq(bacteria, samp)

#Remove archaea, mitochondria, and chloroplast seqs:
B.Filt <- bac.phylo %>%
  subset_taxa(
    Rank1 == "k__Bacteria" &
    Rank5  != "f__mitochondria" &
    Rank3  != "c__Chloroplast"
  )

B.Filt

#remove samples that had poor sequencing:
bacteria.rm = subset_samples(B.Filt, sample_names(B.Filt)!="sample80")
bacteria.rm1 = subset_samples(bacteria.rm, sample_names(bacteria.rm)!="sample171")
bacteria.rm3 = subset_samples(bacteria.rm1, sample_names(bacteria.rm1)!="sample136")

#extract otu table from phyloseq object:
bact.otu <- as.data.frame(as(otu_table(bacteria.rm3), "matrix"))

#transpose first to get samples as rows
bact.t <- t(bact.otu)

B.H <- diversity(bact.t) #Diversity
B.S <- specnumber(bact.t) #Evenness
B.J <- B.H/log(B.S) #Evenness

as.data.frame(B.H)
as.data.frame(B.S)
as.data.frame(B.J)

b.att <- cbind(B.H,B.S,B.J) #bind

write.table(b.att, "bacteria.attributes.txt", sep="\t")

#do for fungi:
fungi <- import_biom("/Volumes/BedardHaughn/NSERC_Agroeco/Mariah/Molecular/Pipelines/Fungi_Qiime2/SNV/fungiSNV.biom")

#make phyloseq object:
fungi.phylo <- merge_phyloseq(fungi, samp)

#Remove plants from fungi:

Fun <- subset_taxa(fungi.phylo, !Rank1=="k__Plantae")

#remove bad samples from fungi (did not sequence):
fungi.phylo.rm = subset_samples(Fun, sample_names(Fun)!="sample118")
fungi.phylo.rm.1 = subset_samples(fungi.phylo.rm, sample_names(fungi.phylo.rm)!="sample119")
fungi.phylo.rm.2 = subset_samples(fungi.phylo.rm.1, sample_names(fungi.phylo.rm.1)!="sample29")

#extract otu
fun.otu <- as.data.frame(as(otu_table(fungi.phylo.rm.2), "matrix"))

#transpose first to get samples as rows
fun.t <- t(fun.otu)

F.H <- diversity(fun.t) #Diversity
F.S <- specnumber(fun.t) #Evenness
F.J <- F.H/log(F.S) #Evenness

as.data.frame(F.H)
as.data.frame(F.S)
as.data.frame(F.J)

f.att <- cbind(F.H,F.S,F.J) #bind

write.table(f.att, "fungi.attributes.newest.txt", sep="\t")

#Phylum graphs:
#create colors for graphs (these are rainbow colors), fungi have 11 phyla (c4), bacteria are plotting top 10 phyla (c3):
c3 <- c("#e6194b", "#f58231", "#ffd8b1", "#ffe119", "#3cb44b","#bfef45", "#42d4f4", "#4363d8","#e6beff", "#911eb4")
c4 <- c( "#e6194b","#f58231", "#ffd8b1", "#ffe119", "#3cb44b","#bfef45", "#42d4f4", "#469990", "#4363d8", "#e6beff","#911eb4")
show_col(c4)
show_col(c3)
```

```r
#using dplyr to extract the aggergated phyla, for faster plotting:
bact_phylum <- bacteria.rm3 %>%
  tax_glom(taxrank = "Rank2") %>%              # agglomerate at phylum level
  transform_sample_counts(function(x) {x/sum(x)} ) %>% # Transform to rel. abundance
  psmelt() %>%                     # Melt to long format
  filter(Abundance > 0.02) %>%               # Filter out low abundance taxa
  arrange(Rank2)                   # Sort data frame alphabetically by phylum

fungi_phylum <- fungi.phylo.rm.2 %>%
  tax_glom(taxrank = "Rank2") %>%              # agglomerate at phylum level
  transform_sample_counts(function(x) {x/sum(x)} ) %>% # Transform to rel. abundance
  psmelt() %>%                     # Melt to long format
  filter(Abundance > 0.02) %>%               # Filter out low abundance taxa
  arrange(Rank2)                   # Sort data frame alphabetically by phylum


#fixing phyla names, removing the p___:
bact_phylum$Rank2 <- gsub("[p__]", "", bact_phylum$Rank2)
fungi_phylum$Rank2 <- gsub("[p__]", "", fungi_phylum$Rank2)

#reordeing the groups and re-naming them:
print(levels(bact_phylum$Group))
bact_phylum$Group <- factor(bact_phylum$Group, levels(bact_phylum$Group)[c(2,1,3)])
levels(bact_phylum$Group) <- c("Grassland", "Edge", "Cropland")

print(levels(fungi_phylum$Group))
fungi_phylum$Group <- factor(fungi_phylum$Group, levels(fungi_phylum$Group)[c(2,1,3)])
levels(fungi_phylum$Group) <- c("Grassland", "Edge", "Cropland")

#renaming SD to SDNWA:
print(levels(bact_phylum$Site))
levels(bact_phylum$Site) <- c("CLC", "SDNWA")

print(levels(fungi_phylum$Site))
levels(fungi_phylum$Site) <- c("CLC", "SDNWA")

bact.plot <- ggplot(bact_phylum, aes(x=Group, y=Abundance, fill = forcats::fct_rev(Rank2))) +
  facet_wrap(~Site, scale="free") + theme_bw() +
  geom_bar(stat = "identity") +
  theme(strip.placement="top")+
  theme(panel.grid.major = element_blank(),
      panel.grid.minor = element_blank(),
      strip.background = element_blank(),
      panel.border = element_rect(colour = "black"))+
  theme(strip.text = element_text(hjust = 0))+
  theme(strip.text = element_text(hjust = 0))+
  guides(fill = guide_legend(reverse = FALSE, keywidth = 1, keyheight = 1)) +
  guides(fill=guide_legend(title="Phylum"))+
  scale_y_continuous(breaks=seq(0,30,by=7.5), limits=c(0,30), labels=c("0", "0.25", "0.50", "0.75", "1.00")) +
  scale_fill_manual(values=c3) +
  ylab("Relative Abundance") +
  xlab("")+
  ggtitle("Bacteria ") +
  theme(panel.spacing.x=unit(2, "lines"))+
  theme(plot.title=element_text(hjust=0, vjust=0.5, face="plain"))


fun.plot <- ggplot(fungi_phylum, aes(x=Group, y=Abundance, fill = forcats::fct_rev(Rank2))) +
  facet_wrap(~Site, scale="free") + theme_bw() +
  geom_bar(stat = "identity") +
  theme(strip.placement="top")+
  theme(panel.grid.major = element_blank(),
      panel.grid.minor = element_blank(),
      strip.background = element_blank(),
      panel.border = element_rect(colour = "black"))+
  theme(strip.text = element_text(hjust = 0))+
  theme(strip.text = element_text(hjust = 0))+
  guides(fill = guide_legend(reverse = FALSE, keywidth = 1, keyheight = 1)) +
  guides(fill=guide_legend(title="Phylum"))+
  scale_y_continuous(breaks=seq(0,30,by=7.5), limits=c(0,30), labels=c("0", "0.25", "0.50", "0.75", "1.00")) +
  scale_fill_manual(values=c4) +
  ylab("Relative Abundance") +
  xlab("")+
  ggtitle("Fungi") +
  theme(panel.spacing.x=unit(2, "lines"))+
```

```
                 theme(plot.title=element_text(hjust=0, vjust=0.5, face="plain"))


CairoPNG("BacteriaPlot.png", height=5, width=9, units="in", dpi=500)
bact.plot
dev.off()

CairoPNG("FungiPlot.png", height=5, width=9, units="in", dpi=500)
fun.plot
dev.off()

#Microbial NMDS
#Packages needed:
library(vegan)
library(dplyr)

#use extracted otu tables:

#Split the otu table into the sites
fungi.sd <- dplyr::select(fun.otu, num_range("sample", 1:90))
fungi.clc <- dplyr::select(fun.otu, num_range("sample", 91:180))

#transpose otu table
sd.t.f <- as.data.frame(t(fungi.sd))
clc.t.f <- as.data.frame(t(fungi.clc))

#remove bad samples from fungi (did not sequence):
#"sample29"
#"sample118", "sample119"

#actually removing them from df:
fungi.sd.rm <- sd.t.f[-c(29), ]
fungi.clc.rm <- clc.t.f[-c(28, 29), ]

#Hellinger transformation
sd.f.hel <- decostand(fungi.sd.rm, "hellinger")
clc.f.hel <- decostand(fungi.clc.rm, "hellinger")

#convert to distance matrix
sd.f.dist <- vegdist(sd.f.hel, method="euclidean")
clc.f.dist <- vegdist(clc.f.hel, method="euclidean")

#run nmds
sd.nmds.f <- metaMDS(sd.f.dist, method="NMDS", distance="euclidean", k = 5, noshare=0.1, trymax=500)
clc.nmds.f <- metaMDS(clc.f.dist, method="NMDS", distance="euclidean", k = 5, noshare=0.1, trymax=500)

#shepard plot:
stressplot(sd.nmds.f)
stressplot(clc.nmds.f)

#make df for plotting:
MDS1 = sd.nmds.f$points[,1]
MDS2 = sd.nmds.f$points[,2]
nmds.sd.fungi = data.frame(MDS1 = MDS1, MDS2 = MDS2)

MDS1C = clc.nmds.f$points[,1]
MDS2C = clc.nmds.f$points[,2]
nmds.clc.fungi = data.frame(MDS1 = MDS1C, MDS2 = MDS2C)

#make metadata fit for each site
meta.sd <- metadata[metadata$Site=="SDNWA", ]
meta.clc <- metadata[metadata$Site=="CLC", ]

#remove samples not in fungi:
meta.sd.rm <- meta.sd[-c(29), ]
meta.clc.rm <- meta.clc[-c(28, 29), ]

#reorder the nmds df to match metadata df:
sort.nmds.sd.f <- nmds.sd.fungi[ order(row.names(nmds.sd.fungi)), ]
sort.nmds.clc.f <- nmds.clc.fungi[ order(row.names(nmds.clc.fungi)), ]

#plot NMDS
sd.f.plot <- ggplot(sort.nmds.sd.f , aes(x=MDS1, y=MDS2, col=meta.sd.rm$Group, shape=meta.sd.rm$Group)) +
                     geom_point(size=4) +
                     scale_shape_manual(values=c(15, 17, 16)) +
                     scale_color_manual(values=c("#238A8DFF","#481467FF", "#BBDF27FF")) +
```

```
                        theme_bw() +
                        theme(panel.grid.major = element_blank(), panel.grid.minor = element_blank())+
                        theme(legend.title=element_blank()) +
                        annotate("text", x=-0.55, y=-0.5, label= "stress = 0.10") +
                        theme(legend.position = "none") +
                        ggtitle("SDNWA Fungi")

clc.f.plot <- ggplot(sort.nmds.clc.f , aes(x=MDS1, y=MDS2, col=meta.clc.rm$Group, shape=meta.clc.rm$Group)) +
                        geom_point(size=4) +
                        scale_shape_manual(values=c(15, 17, 16)) +
                        scale_color_manual(values=c("#238A8DFF","#481467FF", "#BBDF27FF")) +
                        theme_bw() +
                        theme(panel.grid.major = element_blank(), panel.grid.minor = element_blank())+
                        theme(legend.title=element_blank()) +
                        annotate("text", x=-0.55, y=-0.55, label= "stress = 0.11") +
                        theme(legend.position = c(0.2, 0.85), legend.title=element_blank(), legend.background = element_blank(),
                        legend.box.background = element_rect(colour = "black")) +
                        ggtitle("CLC Fungi")


CairoPNG("SD_fungiNMDS.png", height=6, width=6, units="in", dpi=500)
sd.f.plot
dev.off()

CairoPNG("CLC_fungiNMDS.png", height=6, width=6, units="in", dpi=500)
clc.f.plot
dev.off()

#repeat for bacteria, plots were combined in terminal (bash shell) using magick

#Microbial PCA plots:

#use extracted otu table from phyloseq:
#seperate by site
fungi.sd <- dplyr::select(fungi.otu2, num_range("sample", 1:90))
fungi.clc <- dplyr::select(fungi.otu2, num_range("sample", 91:180))

bact.sd <- dplyr::select(bact.otu2, num_range("sample", 1:90))
bact.clc <- dplyr::select(bact.otu2, num_range("sample", 91:180))


#replace 0 values with an estimate
bact.na <- cmultRepl(t(bact.otu),  label=0, method="CZM", output="counts")
b.sd.na <- cmultRepl(t(bact.sd),  label=0, method="CZM", output="counts")
b.clc.na <- cmultRepl(t(bact.clc),  label=0, method="CZM", output="counts")

fungi.na <- cmultRepl(t(fungi.otu),  label=0, method="CZM", output="counts")
fungi.sd.na <- cmultRepl(t(fungi.sd),  label=0, method="CZM", output="counts")
fungi.clc.na <- cmultRepl(t(fungi.clc),  label=0, method="CZM", output="counts")

min(fungi.na)
min(fungi.sd.na)
min(fungi.clc.na)

min(bact.na)
min(b.sd.na)
min(b.clc.na)

#log ratio
fungi.clr <- codaSeq.clr(fungi.na + 0.3, samples.by.row=TRUE)
sd.clr <- codaSeq.clr(fungi.sd.na + 1, samples.by.row=TRUE)
clc.clr <- codaSeq.clr(fungi.clc.na + 1, samples.by.row=TRUE)

bact.clr <- codaSeq.clr(bact.na + 1.26, samples.by.row=TRUE)
b.sd.clr <- codaSeq.clr(b.sd.na + 1, samples.by.row=TRUE)
b.clc.clr <- codaSeq.clr(b.clc.na + 4, samples.by.row=TRUE)

dist.f.sd <- dist(sd.clr)
dist.f.clc <- dist(clc.clr)
dist.b.sd <- dist(b.sd.clr)
dist.b.clc <- dist(b.clc.clr)

#PCA
fungi.pc <- prcomp(fungi.clr)
fungi.sd.pc <- prcomp(sd.clr)
fungi.clc.pc <- prcomp(clc.clr)
```

```
bact.pc <- prcomp(bact.clr)
b.sd.pc <- prcomp(b.sd.clr)
b.clc.pc <- prcomp(b.clc.clr)

plot(bact.pc$x[,1:2])
plot(b.sd.pc$x[,1:2])
plot(b.clc.pc$x[,1:2])

plot(fungi.pc$x[,1:2])
plot(fungi.sd.pc$x[,1:2])
plot(fungi.clc.pc$x[,1:2])

#permanova
adonis(dist.b.sd~Group, data=b.sd.gg, permutations=999)
adonis(dist.b.clc~Group, data=b.clc.gg, permutations=999)
adonis(dist.f.sd~Group, data=f.sd.gg, permutations=999)
adonis(dist.f.clc~Group, data=f.sd.gg, permutations=999)

#take scores and move to new data frame for plotting
fungi.plot.data <- as.data.frame(fungi.pc$x[,1:2])
sd.plot.data <- as.data.frame(fungi.sd.pc$x[,1:2])
clc.plot.data <- as.data.frame(fungi.clc.pc$x[,1:2])

bact.plot.data <- as.data.frame(bact.pc$x[,1:2])
bact.sd.plot.data <- as.data.frame(b.sd.pc$x[,1:2])
bact.clc.plot.data <- as.data.frame(b.clc.pc$x[,1:2])

#re ordering data to match metadata order:
order.f.sd <- sd.plot.data[ order(row.names(sd.plot.data)), ]
order.f.clc <- clc.plot.data[ order(row.names(clc.plot.data)), ]
order.b.sd <- bact.sd.plot.data[ order(row.names(bact.sd.plot.data)), ]
order.b.clc <- bact.clc.plot.data[ order(row.names(bact.clc.plot.data)), ]

#add metadata (master data) to PCA results
f.sd.gg <- cbind(order.f.sd , f.sd.md)
f.clc.gg <- cbind(order.f.clc, f.clc.md)
fungi.gg <- cbind(fungi.plot.data, md)

b.sd.gg <- cbind(order.b.sd, b.sd.md)
b.clc.gg <- cbind(order.b.clc, b.clc.md)
bact.gg <- cbind(bact.plot.data, b.md)

#Re-order distance for plots
print(levels(f.sd.gg$Distance))
f.sd.gg$Distance<-factor(f.sd.gg$Distance, levels(f.sd.gg$Distance)[c(1,4,7,3,5,8,2,6)])

print(levels(f.clc.gg$Distance))
f.clc.gg$Distance<-factor(f.clc.gg$Distance, levels(f.clc.gg$Distance)[c(1,4,7,3,5,8,2,6)])

print(levels(fungi.gg$Distance))
fungi.gg$Distance<-factor(fungi.gg$Distance, levels(fungi.gg$Distance)[c(1,4,7,3,5,8,2,6)])

print(levels(b.sd.gg$Distance))
b.sd.gg$Distance<-factor(b.sd.gg$Distance, levels(b.sd.gg$Distance)[c(1,4,7,3,5,8,2,6)])

print(levels(b.clc.gg$Distance))
b.clc.gg$Distance<-factor(b.clc.gg$Distance, levels(b.clc.gg$Distance)[c(1,4,7,3,5,8,2,6)])

print(levels(bact.gg$Distance))
bact.gg$Distance<-factor(bact.gg$Distance, levels(bact.gg$Distance)[c(1,4,7,3,5,8,2,6)])

#Rename groups
print(levels(f.clc.gg$Group))
levels(f.clc.gg$Group) <- c("Edge", "Grassland", "Cropland")

print(levels(f.sd.gg$Group))
levels(f.sd.gg$Group) <- c("Edge", "Grassland", "Cropland")

print(levels(fungi.gg$Group))
levels(fungi.gg$Group) <- c("Edge", "Grassland", "Cropland")

print(levels(b.clc.gg$Group))
levels(b.clc.gg$Group) <- c("Edge", "Grassland", "Cropland")

print(levels(b.sd.gg$Group))
```

```r
levels(b.sd.gg$Group) <- c("Edge", "Grassland", "Cropland")

print(levels(bact.gg$Group))
levels(bact.gg$Group) <- c("Edge", "Grassland", "Cropland")

print(levels(bact.gg$Site))
levels(bact.gg$Site) <- c("SDNWA", "CLC")

print(levels(fungi.gg$Site))
levels(fungi.gg$Site) <- c("SDNWA", "CLC")

#plot fungi:
sd.fungi.pca.group <- ggplot(f.sd.gg, aes(x=PC1, y=PC2, shape=Group, color=Group))+
                             geom_point(size=4)+
                             scale_shape_manual(values=c(17, 15, 16))+
                             theme_bw() +
                             theme(panel.grid.major = element_blank(), panel.grid.minor = element_blank())+
                             guides(legend.title="Edge Location") +
                             theme(legend.title=element_blank())+
                             scale_color_viridis(discrete=TRUE, option="D", begin=0.05, end=0.90)+
                             labs(title="Fungi", subtitle="SDNWA", x="PC1 (7.83%)" , y= "PC2 (4.45%)")


clc.fungi.pca.group <- ggplot(f.clc.gg, aes(x=PC1, y=PC2, shape=f.clc.gg$Group, color=f.clc.gg$Group))+
                             geom_point(size=4)+
                             scale_shape_manual(values=c(17, 15, 16))+
                             theme_bw() +
                             theme(panel.grid.major = element_blank(), panel.grid.minor = element_blank())+
                             guides(legend.title="Edge Location") +
                             scale_color_viridis(discrete=TRUE, option="D", begin=0.05, end=0.90) +
                             theme(legend.title=element_blank())+
                             theme(legend.position = c(0.85, 0.12), legend.title=element_blank(), legend.background = element_blank(),
                             legend.box.background = element_rect(colour = "black"))+
                             labs(title="Fungi", subtitle="CLC", x="PC1 (7.37%)" , y= "PC2 (4.19%)")


#plot bacteria, not shown
#combine all PCA plots (cowplot):
PCA.Group <- plot_grid(clc.b.pca.group, sd.b.pca.group + theme(legend.position="none"),
                             clc.fungi.pca.group + theme(legend.position="none"), sd.fungi.pca.group + theme(legend.position="none"),
                             nrow=2, labels=c("A", "B", "C", "D"),
                       axis="l", align="vh", hjust=-1)

CairoPNG("site.pca.png", height=4, width=12, units="in", dpi=300)
PCA.Group
dev.off()




##Fungi Genera#########################################

#both sites combined first

#filter taxa that do not are not at least in 20% of samples
fungi.filt <- phyloseq_filter_prevalence(fungi.phylo.rm.2, prev.trh = 0.2, abund.trh=NULL)

fungi.na <- cmultRepl(otu_table(fungi.filt), label=0, method="CZM", output="counts")
min(fungi.na) #no corrected values, 0.013

#centered log ratio transformation
fungi.clr <- codaSeq.clr(fungi.na + 0.02, samples.by.row=TRUE)
min(fungi.clr)  #min -6.24

#load into phyloseq object:
fungi.clr.table <- otu_table(fungi.clr, taxa_are_rows=TRUE)

#replace the otu table in phyloseq object:
otu_table(fungi.filt) <- fungi.clr.table

#Get genus
fungi.gen <- tax_glom(fungi.filt, taxrank="Rank6")

##extract the otu table and tax table:
otu.table.gen = as(otu_table(fungi.gen), "matrix")
```

```
tax.table.gen = as(tax_table(fungi.gen), "matrix")

#corece tax table to data frame:
tax = as.data.frame(tax.table.gen)

#chekc out genera
unique(tax$Rank6)  #50 genera (49 genera - unknown)

#merge tax table and otu table based on row names
fungi.me <- merge(otu.table.gen, tax, by="row.names")
fungi.me <- as.data.frame(fungi.me)

#remove columns
fungi.genus <- dplyr::select(fungi.me, -c("Row.names", "Rank1", "Rank2", "Rank3", "Rank5", "Rank7"))

#Clean up genus names
fungi.genus1 <- gsub("[g__]", "", fungi.genus$Rank6)

#create new genus column, add the clean genus names to data frame
fungi.genus$Genus <- fungi.genus1

#remove the old genus column
fungi.genus.clean <- dplyr::select(fungi.genus, -Rank6)

#transpose
fungi.t <- as.data.frame(t(fungi.genus.clean))

indx <- sapply(fungi.t, is.factor)
fungi.t[indx] <- lapply(fungi.t[indx], function(x) as.numeric(as.character(x)))

#warnings come from NAs becasue of genus column, re-add:
#change column names
colnames(fungi.t) <- fungi.genus1

#remove genus row
nrow(fungi.t) #178 rows, remove last row:
fungi.t.rm <- fungi.t[-178,]

#sort by the new column name
sort.md <- metadata[order(rownames(metadata)),]

#remove samples that are not in bacteria data
md1 <- sort.md[!rownames(sort.md) %in% "sample29", ]
md2 <- md1[!rownames(md1) %in% "sample118", ]
md3 <- md2[!rownames(md2) %in% "sample119", ]

head(md3)
fungi.t.rm$Groups <- md3$Group

write.table(fungi.t.rm, "fungi-genera-abund.txt", sep="\t", col.names=TRUE)

#add column to bacteria
fungi.t.rm$Group <- f.ex$Group

#remove unidentified genera, first remove multiple unidentified columns:
temp <- fungi.t.rm[, !duplicated(colnames(fungi.t.rm))]

#then remove column
fungi.t.rm <- subset(temp, select=-c(unidentified))

#create 3 dataframes for each group comparison
crop.grass <- fungi.t.rm[(fungi.t.rm$Group=="Z")| (fungi.t.rm$Group=="Y"), ]
edge.grass <- fungi.t.rm[(fungi.t.rm$Group=="X")| (fungi.t.rm$Group=="Y"), ]
edge.crop <- fungi.t.rm[(fungi.t.rm$Group=="X")| (fungi.t.rm$Group=="Z"), ]


#T TEST -welch
crop.grass.ttest <- lapply(crop.grass[,1:49], function(x) t.test(x ~ crop.grass$Group))
edge.grass.ttest <- lapply(edge.grass[,1:49], function(x) t.test(x ~ edge.grass$Group))
edge.crop.ttest <- lapply(edge.crop[,1:49], function(x) t.test(x ~ edge.crop$Group))

#get p values
crop.grass.pval <- sapply(crop.grass.ttest, '[[', 'p.value')
edge.grass.pval <- sapply(edge.grass.ttest, '[[', 'p.value')
edge.crop.pval <- sapply(edge.crop.ttest, '[[', 'p.value')
```

```
#adjust p-values
crop.grass.p <- p.adjust(crop.grass.pval, method="bonferroni", n=147)
edge.grass.p <- p.adjust(edge.grass.pval, method="bonferroni", n=147)
edge.crop.p <- p.adjust(edge.crop.pval, method="bonferroni", n=147)

write.table(crop.grass.p, "FUNGIcrop.grass.p-ttestn.txt", sep="\t", col.names=TRUE)
write.table(edge.grass.p, "FUNGIedge.grass.p-ttestn.txt", sep="\t", col.names=TRUE)
write.table(edge.crop.p, "FUNGIedge.crop.p-ttestn.txt", sep="\t", col.names=TRUE)

##Seperate sites:

#extract otu table
fun.otu <- as.data.frame(as(otu_table(Fun), "matrix"))

#seperate by site
fun.sd <- dplyr::select(fun.otu, num_range("sample", 1:90))
fun.clc <- dplyr::select(fun.otu , num_range("sample", 91:180))

#create two phyloseq objects:
f.sd <- otu_table(fun.sd, taxa_are_rows=TRUE)
f.clc <- otu_table(fun.clc, taxa_are_rows=TRUE)

otu_table(Fun) <- f.sd
fun.sd.phylo <-Fun

fun.sd.rm = subset_samples(fun.sd.phylo, sample_names(fun.sd.phylo)!="sample29")

##re produce the "B" subset:
Fun <- subset_taxa(fungi.phylo, !Rank1=="k__Plantae")  #Remove plants

otu_table(Fun) <- f.clc
fun.clc.phylo <- Fun

fun.clc.rm = subset_samples(fun.clc.phylo, sample_names(fun.clc.phylo)!="sample118")
fun.clc.rm2 = subset_samples(fun.clc.rm, sample_names(fun.clc.rm)!="sample119")

#filter taxa that do not are not at least in 20% of samples
fun.filt.sd <- phyloseq_filter_prevalence(fun.sd.rm, prev.trh = 0.2, abund.trh=NULL)
fun.filt.clc <- phyloseq_filter_prevalence(fun.clc.rm2, prev.trh = 0.2, abund.trh=NULL)

fun.na.sd <- cmultRepl(otu_table(fun.filt.sd), label=0, method="CZM", output="counts")
fun.na.clc <- cmultRepl(otu_table(fun.filt.clc), label=0, method="CZM", output="counts")
min(fun.na.sd)  #0.009
min(fun.na.clc) #0.007

fun.clr.sd <- codaSeq.clr(fun.na.sd + 0.01, samples.by.row=TRUE)
fun.clr.clc <- codaSeq.clr(fun.na.clc + 0.01, samples.by.row=TRUE)
min(fun.clr.sd)  #-6.71
min(fun.clr.clc) #-6.3922

#load into phyloseq object:
fun.clr.table.sd <- otu_table(fun.clr.sd, taxa_are_rows=TRUE)
fun.clr.table.clc <- otu_table(fun.clr.clc, taxa_are_rows=TRUE)

#replace the otu table in phyloseq object:
otu_table(fun.sd.rm) <- fun.clr.table.sd
otu_table(fun.clc.rm2) <-fun.clr.table.clc

#get genera
fun.gen.sd <- tax_glom(fun.sd.rm, taxrank="Rank6")
fun.gen.clc <- tax_glom(fun.clc.rm2, taxrank="Rank6")

##extract the otu table and tax table:
otu.table.gen.sd = as(otu_table(fun.gen.sd), "matrix")
tax.table.gen.sd = as(tax_table(fun.gen.sd), "matrix")

otu.table.gen.clc = as(otu_table(fun.gen.clc), "matrix")
tax.table.gen.clc = as(tax_table(fun.gen.clc), "matrix")

#corece tax table to data frame:
tax.sd = as.data.frame(tax.table.gen.sd)
tax.clc = as.data.frame(tax.table.gen.clc)

#check out genera
unique(tax.sd$Rank6)
unique(tax.clc$Rank6)
```

```r
#merge tax table and otu table based on row names
fun.me.sd <- merge(otu.table.gen.sd, tax.sd, by="row.names")
fun.me.clc <- merge(otu.table.gen.clc, tax.clc, by="row.names")

#extract genera names from dataframe:
genera.sd <- fun.me.sd$Rank6
genera.clc <- fun.me.clc$Rank6

#remove columns that are  not genus, OMG HAVE TO specify DPLYR
fun.genus.sd <- dplyr::select(fun.me.sd, -c(Row.names, Rank1, Rank2, Rank3, Rank4, Rank5, Rank7))
fun.genus.clc <- dplyr::select(fun.me.clc, -c(Row.names, Rank1, Rank2, Rank3, Rank4, Rank5, Rank7))

#Clean up genus names
fun.genus1.sd <- gsub("[g__]", "", fun.genus.sd$Rank6)
fun.genus1.clc <- gsub("[g__]", "", fun.genus.clc$Rank6)

#create new genus column, add the clean genus names to data frame
fun.genus.sd$Genus <- fun.genus1.sd
fun.genus.clc$Genus <- fun.genus1.clc

#remove the old genus column
fun.genus.clean.sd <- dplyr::select(fun.genus.sd, -Rank6)
fun.genus.clean.clc <- dplyr::select(fun.genus.clc, -Rank6)

#transpose to get samples as rows
fun.t.sd <- as.data.frame(t(fun.genus.clean.sd))
fun.t.clc <- as.data.frame(t(fun.genus.clean.clc))

#transposing makes everything werid, FIX IT:
indx.f.sd <- sapply(fun.t.sd, is.factor)
fun.t.sd[indx.f.sd] <- lapply(fun.t.sd[indx.f.sd], function(x) as.numeric(as.character(x)))

indx.f.clc <- sapply(fun.t.clc, is.factor)
fun.t.clc[indx.f.clc] <- lapply(fun.t.clc[indx.f.clc], function(x) as.numeric(as.character(x)))

#change column names
colnames(fun.t.sd) <- fun.genus1.sd
colnames(fun.t.clc) <- fun.genus1.clc

#remove genus row
nrow(fun.t.sd) #90
nrow(fun.t.clc) #89

fun.t.rm.sd <- fun.t.sd[-90,]
fun.t.rm.clc <- fun.t.clc[-89,]

#sort by the new column name
md.sd <- metadata[metadata$Site=="SD",]
md.clc <- metadata[metadata$Site=="CLC",]

#remove corresponding samples that are not in fungal data:
sd.m.rm <- md.sd[!rownames(md.sd) %in% "sample29", ]

md.clc.rm <- md.clc[!rownames(md.clc) %in% "sample118", ]
md.clc.rm2 <- md.clc.rm[!rownames(md.clc.rm) %in% "sample119", ]

#sort by row names to match order of genera df samples
fun.t.rm.sd.sort  <- fun.t.rm.sd[ order(row.names(fun.t.rm.sd)), ]
fun.t.rm.clc.sort <- fun.t.rm.clc[ order(row.names(fun.t.rm.clc)), ]

groups.sd <- sd.m.rm$Group
groups.clc <- md.clc.rm2$Group

#add column to fungi
fun.t.rm.sd.sort$Group <- groups.sd
fun.t.rm.clc.sort$Group <- groups.clc

#check it
head(fun.t.rm.sd.sort)
head(fun.t.rm.clc.sort)

#remove unidentified genera, first remove multiple unidentified columns:
tempsd <- fun.t.rm.sd.sort[, !duplicated(colnames(fun.t.rm.sd.sort))]
tempclc <- fun.t.rm.clc.sort[, !duplicated(colnames(fun.t.rm.clc.sort))]
```

```
#then remove column
fun.t.rm.sd.sort <- subset(tempsd, select=-c(unidentified))
fun.t.rm.clc.sort <- subset(tempclc, select=-c(unidentified))

write.table(fun.t.rm.sd.sort, "FUNGIgenera-abundSD.txt", sep="\t", col.names=TRUE)
write.table(fun.t.rm.clc.sort, "FUNGIgenera-abundCLC.txt", sep="\t", col.names=TRUE)

#create 3 dataframes for each group comparison
crop.grass.sd <- fun.t.rm.sd.sort[(fun.t.rm.sd.sort$Group=="Z")| (fun.t.rm.sd.sort$Group=="Y"), ]
edge.grass.sd <- fun.t.rm.sd.sort[(fun.t.rm.sd.sort$Group=="X")| (fun.t.rm.sd.sort$Group=="Y"), ]
edge.crop.sd <- fun.t.rm.sd.sort[(fun.t.rm.sd.sort$Group=="X")| (fun.t.rm.sd.sort$Group=="Z"), ]

crop.grass.clc <- fun.t.rm.clc.sort[(fun.t.rm.clc.sort$Group=="Z")| (fun.t.rm.clc.sort$Group=="Y"), ]
edge.grass.clc <- fun.t.rm.clc.sort[(fun.t.rm.clc.sort$Group=="X")| (fun.t.rm.clc.sort$Group=="Y"), ]
edge.crop.clc <- fun.t.rm.clc.sort[(fun.t.rm.clc.sort$Group=="X")| (fun.t.rm.clc.sort$Group=="Z"), ]

##T TEST -welch

crop.grass.ttest.sd <- lapply(crop.grass.sd[,1:58], function(x) t.test(x ~ crop.grass.sd$Group))
edge.grass.ttest.sd <- lapply(edge.grass.sd[,1:58], function(x) t.test(x ~ edge.grass.sd$Group))
edge.crop.ttest.sd <- lapply(edge.crop.sd[,1:58], function(x) t.test(x ~ edge.crop.sd$Group))

crop.grass.ttest.clc <- lapply(crop.grass.clc[,1:45], function(x) t.test(x ~ crop.grass.clc$Group))
edge.grass.ttest.clc <- lapply(edge.grass.clc[,1:45], function(x) t.test(x ~ edge.grass.clc$Group))
edge.crop.ttest.clc <- lapply(edge.crop.clc[,1:45], function(x) t.test(x ~ edge.crop.clc$Group))

#get p values
crop.grass.pval.sd <- sapply(crop.grass.ttest.sd, '[[', 'p.value')
edge.grass.pval.sd <- sapply(edge.grass.ttest.sd, '[[', 'p.value')
edge.crop.pval.sd <- sapply(edge.crop.ttest.sd, '[[', 'p.value')

crop.grass.pval.clc <- sapply(crop.grass.ttest.clc, '[[', 'p.value')
edge.grass.pval.clc <- sapply(edge.grass.ttest.clc, '[[', 'p.value')
edge.crop.pval.clc <- sapply(edge.crop.ttest.clc, '[[', 'p.value')

#adjust p-values
crop.grass.p.sd <- p.adjust(crop.grass.pval.sd, method="bonferroni", n=174)
edge.grass.p.sd <- p.adjust(edge.grass.pval.sd, method="bonferroni", n=174)
edge.crop.p.sd <- p.adjust(edge.crop.pval.sd, method="bonferroni", n=174)

crop.grass.p.clc <- p.adjust(crop.grass.pval.clc, method="bonferroni", n=135)
edge.grass.p.clc <- p.adjust(edge.grass.pval.clc, method="bonferroni", n=135)
edge.crop.p.clc <- p.adjust(edge.crop.pval.clc, method="bonferroni", n=135)


write.table(crop.grass.p.sd, "FUNGI.SDcrop-grass-ttestn.txt", sep="\t", col.names=TRUE)
write.table(edge.grass.p.sd, "FUNGI.SDedge.grass.p-ttestn.txt", sep="\t", col.names=TRUE)
write.table(edge.crop.p.sd, "FUNGI.SDedge.crop.p-ttestn.txt", sep="\t", col.names=TRUE)

write.table(crop.grass.p.clc, "FUNGI.CLCcrop-grass-ttestn.txt", sep="\t", col.names=TRUE)
write.table(edge.grass.p.clc, "FUNGI.CLCedge.grass.p-ttestn.txt", sep="\t", col.names=TRUE)
write.table(edge.crop.p.clc, "FUNGI.CLCedge.crop.p-ttestn.txt", sep="\t", col.names=TRUE)


#SEM

###########################################################################
# "merge.data" is master data file with fungal genera abundances in it


#checking what relationships look like:
plot(merge.data$LiveBio ~ merge.data$Total_C)
abline(lm(merge.data$LiveBio ~ merge.data$Total_C))

plot(merge.data$LiveBio ~ merge.data$Total_N)
abline(lm(merge.data$LiveBio ~ merge.data$Total_N))

plot(merge.data$S ~ merge.data$Total_N)
abline(lm(merge.data$S ~ merge.data$Total_N))

plot(merge.data$S ~ merge.data$Total_C)
abline(lm(merge.data$S ~ merge.data$Total_C))

plot(merge.data$F.S ~ merge.data$Total_N)
abline(lm(merge.data$F.S ~ merge.data$Total_N))
```

```
plot(merge.data$F.S ~ merge.data$Total_C)
abline(lm(merge.data$F.S ~ merge.data$Total_C))

plot(merge.data$F.S ~ merge.data$S)
abline(lm(merge.data$F.S ~ merge.data$S))

plot(merge.data$F.S ~ merge.data$trnl.S)
abline(lm(merge.data$F.S ~ merge.data$trnl.S))

plot(merge.data$F.S ~ merge.data$Sp)
abline(lm(merge.data$F.S ~ merge.data$Sp))

#centre data:
merge.data$bio.z <- scale(merge.data$Total_Biomass, center=TRUE, scale=TRUE)
merge.data$LiveBio.z <- scale(merge.data$LiveBio, center=TRUE, scale=TRUE)
merge.data$S.Z <- scale(merge.data$S, center=TRUE, scale=TRUE)
merge.data$Litter.z <- scale(merge.data$Litter, center=TRUE, scale=TRUE)
merge.data$F.S.Z <- scale(merge.data$F.S, center=TRUE, scale=TRUE)

merge.data <- as.data.frame(merge.data)

#Test genera, richness for plants and bacteria:

mod <-  '
        F.S.Z ~ Total_N + Total_C + Sp + trnl.S + S.Z
        S.Z ~ Total_N + Total_C
        Total_C ~ LiveBio.z
        Total_N ~ LiveBio.z
        LiveBio.z ~ Sp
        Total_C ~ ~ Total_N
        Sp ~ ~ trnl.S
        '

mod.fit <- sem(mod, data=merge.data, missing="ML")
summary(mod.fit, standardized=TRUE, fit.measures=TRUE)

capture.output(summary(mod.fit, fit.measures=TRUE, rsquare=TRUE, standardized=TRUE), file="wholeRichness.txt")

## Chalastospora, Clonostachys, Gibberella, Paraphoma, Parastaonospora, Sarocladium

mod1 <-  '
        Chalastospora ~ Total_N + Total_C + Sp + trnl.S + S.Z
        S.Z ~ Total_N + Total_C
        Total_C ~ LiveBio.z
        Total_N ~ LiveBio.z
        LiveBio.z ~ Sp
        Total_C ~ ~ Total_N
        Sp ~ ~ trnl.S
        '

mod1.fit <- sem(mod1, data=merge.data, missing="ML")
summary(mod1.fit, standardized=TRUE, fit.measures=TRUE)

capture.output(summary(mod1.fit, fit.measures=TRUE, rsquare=TRUE, standardized=TRUE), file="Chala.txt")

mod2 <-  '
        Clonostachys.z ~ Total_N + Total_C + Sp + trnl.S + S.Z
        S.Z ~ Total_N + Total_C
        Total_C ~ LiveBio.z
        Total_N ~ LiveBio.z
        LiveBio.z ~ Sp
        Total_C ~ ~ Total_N
        Sp ~ ~ trnl.S
        '

mod2.fit <- sem(mod2, data=merge.data, missing="ML")
summary(mod2.fit, standardized=TRUE, fit.measures=TRUE)

capture.output(summary(mod2.fit, fit.measures=TRUE, rsquare=TRUE, standardized=TRUE), file="Clono.txt")

mod3 <-  '
        Gibberella ~ Total_N + Total_C + Sp + trnl.S + S.Z
        S.Z ~ Total_N + Total_C
        Total_C ~ LiveBio.z
        Total_N ~ LiveBio.z
        LiveBio.z ~ Sp
```

```
                Total_C ~ ~ Total_N
                Sp ~ ~ trnl.S
                '

mod3.fit <- sem(mod3, data=merge.data, missing="ML")
summary(mod3.fit, standardized=TRUE, fit.measures=TRUE)

capture.output(summary(mod3.fit, fit.measures=TRUE, rsquare=TRUE, standardized=TRUE), file="Gibb.txt")

mod4 <- '
                Paraphoma ~ Total_N + Total_C + Sp + trnl.S + S.Z
                S.Z ~ Total_N + Total_C
                Total_C ~ LiveBio.z
                Total_N ~ LiveBio.z
                LiveBio.z ~ Sp
                Total_C ~ ~ Total_N
                Sp ~ ~ trnl.S
                '

mod4.fit <- sem(mod4, data=merge.data, missing="ML")
summary(mod4.fit, standardized=TRUE, fit.measures=TRUE)

capture.output(summary(mod4.fit, fit.measures=TRUE, rsquare=TRUE, standardized=TRUE), file="Paraphoma.txt")

mod5 <- '
                Parastaonospora ~ Total_N + Total_C + Sp + trnl.S + S.Z
                S.Z ~ Total_N + Total_C
                Total_C ~ LiveBio.z
                Total_N ~ LiveBio.z
                LiveBio.z ~ Sp
                Total_C ~ ~ Total_N
                Sp ~ ~ trnl.S
                '

mod5.fit <- sem(mod5, data=merge.data, missing="ML")
summary(mod5.fit, standardized=TRUE, fit.measures=TRUE)

capture.output(summary(mod5.fit, fit.measures=TRUE, rsquare=TRUE, standardized=TRUE), file="Parastag.txt")

mod6 <- '
                Sarocladium ~ Total_N + Total_C + Sp + trnl.S + S.Z
                S.Z ~ Total_N + Total_C
                Total_C ~ LiveBio.z
                Total_N ~ LiveBio.z
                LiveBio.z ~ Sp
                Total_C ~ ~ Total_N
                Sp ~ ~ trnl.S
                '

mod6.fit <- sem(mod6, data=merge.data, missing="ML")
summary(mod6.fit, standardized=TRUE, fit.measures=TRUE)

capture.output(summary(mod6.fit, fit.measures=TRUE, rsquare=TRUE, standardized=TRUE), file="Sarocladium.txt")

MI<-modificationIndices(mod2.fit)
subset(MI, mi>5)

##site specific SEM, first SD:
##load abudance table:

sd.ab <- read.table("/Volumes/BedardHaughn/NSERC_Agroeco/Mariah/Statistics/SEM/data/SDmetadataSEM.txt", sep="\t", header=TRUE)

clc.ab <- read.table("/Volumes/BedardHaughn/NSERC_Agroeco/Mariah/Statistics/SEM/data/CLCmetaSEM.txt", sep="\t", header=TRUE)

#centre data:

sd.ab$LiveBio.z <- scale(sd.ab$LiveBio, center=TRUE, scale=TRUE)
sd.ab$bio.z <- scale(sd.ab$Total_Biomass, center=TRUE, scale=TRUE)
sd.ab$S.Z <- scale(sd.ab$S, center=TRUE, scale=TRUE)
sd.ab$F.S.Z <- scale(sd.ab$F.S, center=TRUE, scale=TRUE)
sd.ab <- as.data.frame(sd.ab)

#centre data:

clc.ab$LiveBio.z <- scale(clc.ab$LiveBio, center=TRUE, scale=TRUE)
clc.ab$bio.z <- scale(clc.ab$Total_Biomass, center=TRUE, scale=TRUE)
```

```
clc.ab$S.Z <- scale(clc.ab$S, center=TRUE, scale=TRUE)
clc.ab$F.S.Z <- scale(clc.ab$F.S, center=TRUE, scale=TRUE)
clc.ab <- as.data.frame(clc.ab)


sd1 <- '
        Devriesia ~ Total_N + Total_C + Sp + trnl.S + S.Z
        S.Z ~ Total_N + Total_C
        Total_C ~ LiveBio.z
        Total_N ~ LiveBio.z
        LiveBio.z ~ Sp
        Total_C ~~ Total_N
        Sp ~~ trnl.S
        '
sd1.fit <- sem(sd1, data=sd.ab, missing="ML")
summary(sd1.fit, standardized=TRUE, fit.measures=TRUE)

capture.output(summary(sd1.fit , fit.measures=TRUE, rsquare=TRUE, standardized=TRUE), file="Devriesia.txt")


sd2 <- '
        Schizothecium ~ Total_N + Total_C + Sp + trnl.S + S.Z
        S.Z ~ Total_N + Total_C
        Total_C ~ LiveBio.z
        Total_N ~ LiveBio.z
        LiveBio.z ~ Sp
        Total_C ~~ Total_N
        Sp ~~ trnl.S
        '
sd2.fit <- sem(sd2, data=sd.ab, missing="ML")
summary(sd2.fit, standardized=TRUE, fit.measures=TRUE)

capture.output(summary(sd2.fit , fit.measures=TRUE, rsquare=TRUE, standardized=TRUE), file="Schizotheciumsd.txt")


sd3 <- '
        Arthrinium ~ Total_N + Total_C + Sp + trnl.S + S.Z
        S.Z ~ Total_N + Total_C
        Total_C ~ LiveBio.z
        Total_N ~ LiveBio.z
        LiveBio.z ~ Sp
        Total_C ~~ Total_N
        Sp ~~ trnl.S
        '
sd3.fit <- sem(sd3, data=sd.ab, missing="ML")
summary(sd3.fit, standardized=TRUE, fit.measures=TRUE)

capture.output(summary(sd3.fit , fit.measures=TRUE, rsquare=TRUE, standardized=TRUE), file="Arthriniumsd.txt")

sd4 <- '
        Coprinopsis ~ Total_N + Total_C + Sp + trnl.S + S.Z
        S.Z ~ Total_N + Total_C
        Total_C ~ LiveBio.z
        Total_N ~ LiveBio.z
        LiveBio.z ~ Sp
        Total_C ~~ Total_N
        Sp ~~ trnl.S
        '
sd4.fit <- sem(sd4, data=sd.ab, missing="ML")
summary(sd4.fit, standardized=TRUE, fit.measures=TRUE)

capture.output(summary(sd4.fit , fit.measures=TRUE, rsquare=TRUE, standardized=TRUE), file="Coprinopsissd.txt")

sd5 <- '
        Cistella ~ Total_N + Total_C + Sp + trnl.S + S.Z
        S.Z ~ Total_N + Total_C
        Total_C ~ LiveBio.z
        Total_N ~ LiveBio.z
        LiveBio.z ~ Sp
        Total_C ~~ Total_N
        Sp ~~ trnl.S
        '
sd5.fit <- sem(sd5, data=sd.ab, missing="ML")
summary(sd5.fit, standardized=TRUE, fit.measures=TRUE)

capture.output(summary(sd5.fit , fit.measures=TRUE, rsquare=TRUE, standardized=TRUE), file="Cistellasd.txt")
```

```
sd6 <- '
        Acrostalamus ~ Total_N + Total_C + Sp + trnl.S + S.Z
        S.Z ~ Total_N + Total_C
        Total_C ~ LiveBio.z
        Total_N ~ LiveBio.z
        LiveBio.z ~ Sp
        Total_C ~ ~ Total_N
        Sp ~ ~ trnl.S
        '
sd6.fit <- sem(sd6, data=sd.ab, missing="ML")
summary(sd6.fit, standardized=TRUE, fit.measures=TRUE)

capture.output(summary(sd6.fit , fit.measures=TRUE, rsquare=TRUE, standardized=TRUE), file="Acrostalamussd.txt")

sd7 <- '
        Clonostachys ~ Total_N + Total_C + Sp + trnl.S + S.Z
        S.Z ~ Total_N + Total_C
        Total_C ~ LiveBio.z
        Total_N ~ LiveBio.z
        LiveBio.z ~ Sp
        Total_C ~ ~ Total_N
        Sp ~ ~ trnl.S
        '
sd7.fit <- sem(sd7, data=sd.ab, missing="ML")
summary(sd7.fit, standardized=TRUE, fit.measures=TRUE)

capture.output(summary(sd7.fit , fit.measures=TRUE, rsquare=TRUE, standardized=TRUE), file="Clonostachyssd.txt")

clc1 <- '
        Olpidium  ~ Total_N + Total_C + Sp + trnl.S + S.Z
        S.Z ~ Total_N + Total_C
        Total_C ~ LiveBio.z
        Total_N ~ LiveBio.z
        LiveBio.z ~ Sp
        Total_C ~ ~ Total_N
        Sp ~ ~ trnl.S
        '
clc1.fit <- sem(clc1, data=clc.ab, missing="ML")
summary(clc1.fit, standardized=TRUE, fit.measures=TRUE)

capture.output(summary(clc1.fit , fit.measures=TRUE, rsquare=TRUE, standardized=TRUE), file="Olpidium.txt")

# Mixed Modelling ###########################################################################################


#packages used:
library(lme4)
library(stargazer)
library(lmerTest)
library(emmeans)
library(MuMIn)
library(Rmisc)
library(ggplot2)
library(knitr)
library(cowplot)

#Use the master data sheet as previously used:

#load data
full_data <- read.csv("/Volumes/BedardHaughn/NSERC_Agroeco/Mariah/Statistics/MasterData.csv", header=TRUE, sep=",")

#Treat columns as categorical  values
full_data$Distance <- as.factor(full_data$Distance)
full_data$Transect <- as.factor(full_data$Transect)
full_data$ID <- as.factor(full_data$ID)

#Checking the data out...
par(mfrow=c(5,3))
hist(full_data$Total_N)
hist(full_data$Total_C)
hist(full_data$NO3)
hist(full_data$NH4)
hist(full_data$Organic_C)
hist(full_data$pH)
hist(full_data$Litter)
```

```r
hist(full_data$Grass)
hist(full_data$Forbs)
hist(full_data$B.H)
hist(full_data$B.S)
hist(full_data$B.J)
hist(full_data$F.H)
hist(full_data$F.S)
hist(full_data$F.J)

#rename variables
print(levels(full_data$Group))
full_data$Group<-factor(full_data$Group, levels(full_data$Group)[c(2,1,3)], labels=c("Grassland", "Edge", "Cropland"))
full_data$Site <- factor(full_data$Site, levels(full_data$Site), labels=c("CLC", "SDNWA"))

#scale up biomass to grams per m2
full_data$Grass_m2 <- (full_data$Grass * 10)
full_data$Litter_m2 <- (full_data$Litter * 10)
full_data$Forbs_m2 <- (full_data$Forbs * 10)
full_data$TotBio_m2 <- (full_data$Total_Biomass * 10)
full_data$livingbio <- (full_data$TotBio_m2 - full_data$Litter_m2)

##ggplot boxplots
TC <- ggplot(full_data, (aes(y=Total_C, x=Group, fill=Group)))+
        stat_boxplot(geom="errorbar", size=0.5, width = 0.3)+ ##This puts "feet" on the whiskers
        geom_boxplot(color="black", outlier.color="gray35")+ #changing the boxplot color
        theme_bw()+
        facet_wrap(~Site, strip.position="bottom")+
        scale_fill_manual(values=c("gray45", "gray80", "white"))+
        labs(y="Total Carbon (%)", x="", fill="Field")+ #change y and x axis labels
        theme(panel.grid.major = element_blank(), panel.grid.minor = element_blank())+ #remove grid marks
        theme(axis.text.x = element_blank(), axis.ticks.x= element_blank())+ #remove grid marks
        theme(strip.background = element_blank())+ #facet labels
        guides(fill = guide_legend(title= NULL))+ #remove legend title
        theme(legend.position=c(0.85, 0.82))    #legend position

##Do for all properties


#Mixed models

#Models soil
m1.1 <-lmer(Total_N ~ Group*Site + (1|SiteTransect/Subtransect), data=full_data)
m2.1 <-lmer(Total_C ~ Group*Site + (1|SiteTransect/Subtransect), data=full_data)
m3.1 <-lmer(trans_NH4 ~ Group*Site + (1|SiteTransect/Subtransect), data=full_data)
m4.1 <-lmer(trans_NO3 ~ Group*Site + (1|SiteTransect/Subtransect), data=full_data)
m5.1 <-lmer(pH ~ Group*Site + (1|SiteTransect/Subtransect), data=full_data)
m6.1 <-lmer(Organic_C~ Group*Site + (1|SiteTransect/Subtransect), data=full_data)

#Models veg
m7.1 <-lmer(Total_Biomass~ Group*Site + (1|SiteTransect/Subtransect), data=full_data)
m8.1 <-lmer(trans_Grass~ Group*Site + (1|SiteTransect/Subtransect), data=full_data)
m9.1 <-lmer(trans_Forbs~ Group*Site + (1|SiteTransect/Subtransect), data=full_data)
m10.1 <-lmer(trans_Litter~Group*Site + (1|SiteTransect/Subtransect), data=full_data)
m11.1 <- lmer(livingbioT ~ Group*Site + (1|SiteTransect/Subtransect), data=full_data)



#models micro
m12 <-lmer(F.S~ Group*Site + (1|SiteTransect/Subtransect), data=full_data)
m13 <-lmer(B.S~ Group*Site + (1|SiteTransect/Subtransect), data=full_data)

#lmerTest ANOVA
anova(m1.1)
anova(m2.1)
anova(m3.1)
anova(m4.1)
anova(m5.1)
anova(m6.1)
anova(m7.1)
anova(m8.1)
anova(m9.1)
anova(m10.1)
anova(m11.1

#post hoc tukey
emmeans(m1.1, list(pairwise~Group*Site), adjust="tukey")
emmeans(m2.1, list(pairwise~Group*Site), adjust="tukey")
```

```
emmeans(m3.1, list(pairwise~Group*Site), adjust="tukey")
emmeans(m4.1, list(pairwise~Group*Site), adjust="tukey")
emmeans(m5.1, list(pairwise~Group*Site), adjust="tukey")
emmeans(m6.1, list(pairwise~Group*Site), adjust="tukey")
emmeans(m6.1, list(pairwise~Group*Site), adjust="tukey")
emmeans(m12, list(pairwise~Group*Site), adjust="tukey")
emmeans(m13, list(pairwise~Group*Site), adjust="tukey")
emmeans(m10.1, list(pairwise~Group*Site), adjust="tukey")
emmeans(m11.1, list(pairwise~Group*Site), adjust="tukey")

#r-squared values
r.squaredGLMM(m1.1)
r.squaredGLMM(m2.1)
r.squaredGLMM(m3.1)
r.squaredGLMM(m4.1)
r.squaredGLMM(m5.1)
r.squaredGLMM(m6.1)
r.squaredGLMM(m7.1)
r.squaredGLMM(m8.1)
r.squaredGLMM(m8.1)
r.squaredGLMM(m10.1)
r.squaredGLMM(m11.1)

#favorite color packages:
library(LaCroixColoR)
library(wesanderson)
library(viridis)
library(scico)
library(NineteenEightyR)
```
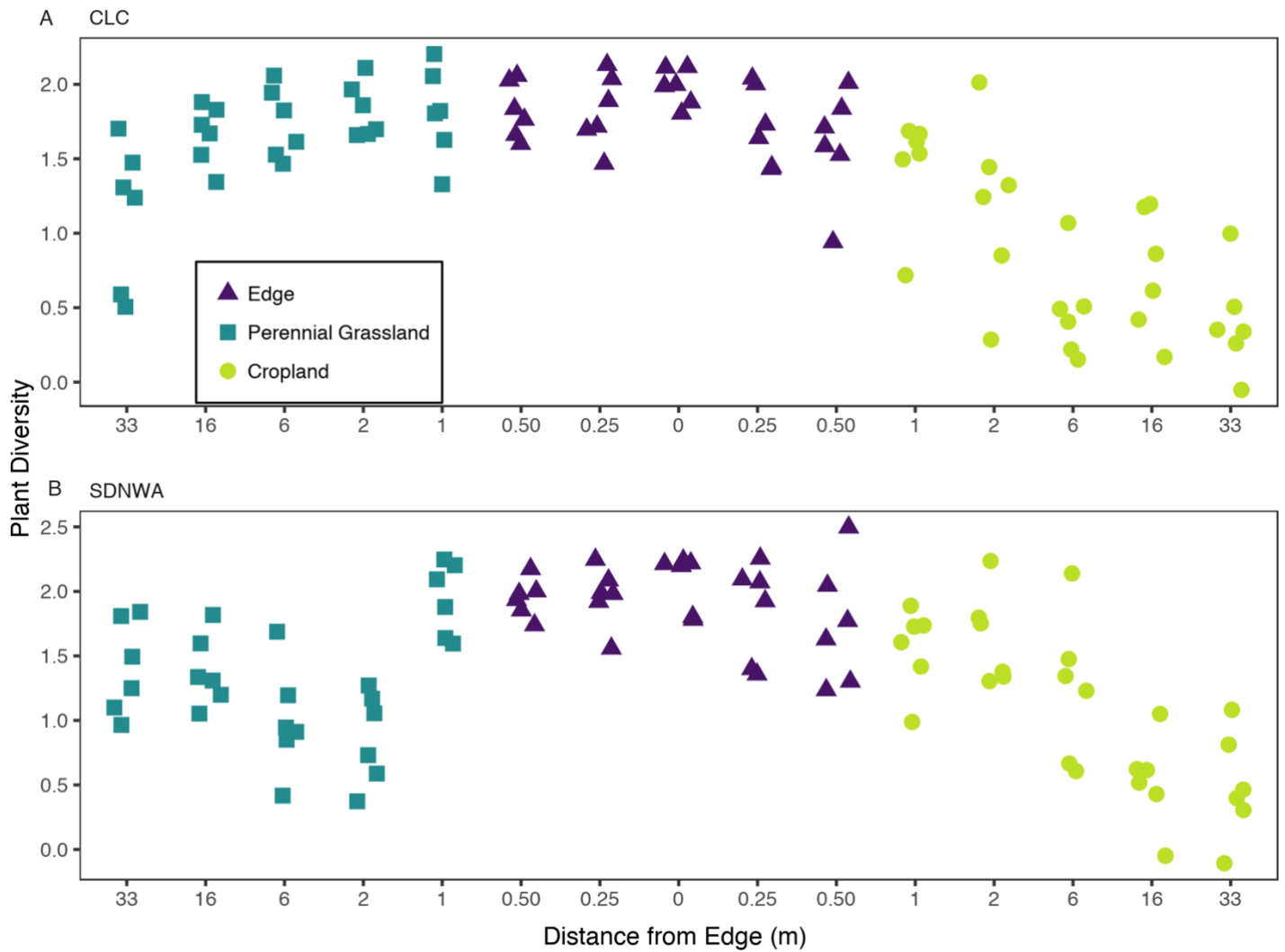
**Fig D.1**: Plant diversity (Shannon's *H'*) across the edge at the Conservation Learning Centre (a) and the St. Denis National Wildlife Area (b). Teal squares are perennial grassland points, purple triangles are edge points, and the green circles are cropland points.
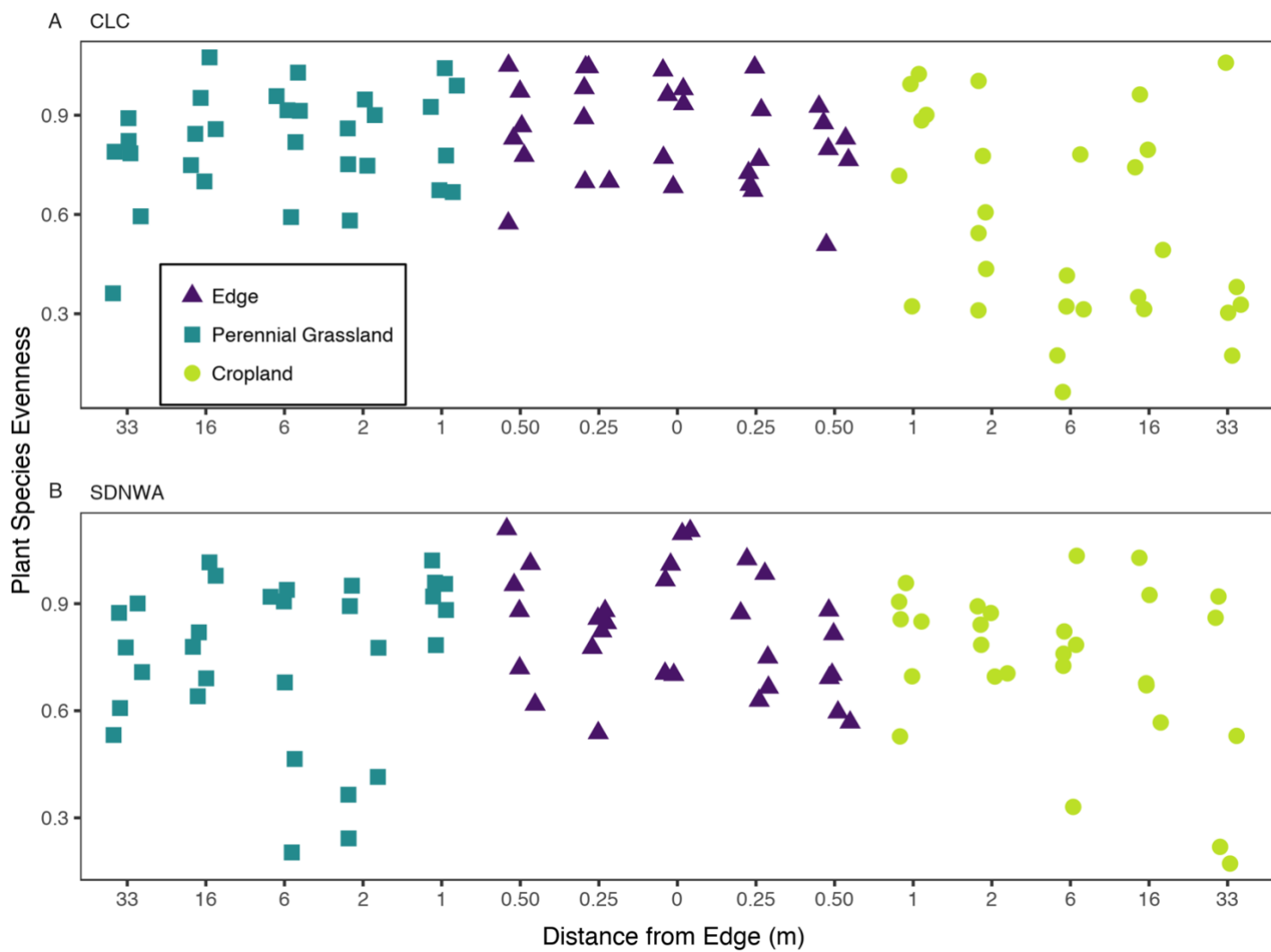
**Fig D.2**: Plant species evenness across the edge at the Conservation Learning Centre (a) and the St. Denis National Wildlife Area (b). Teal squares are perennial grassland points, purple triangles are edge points, and the green circles are cropland points.
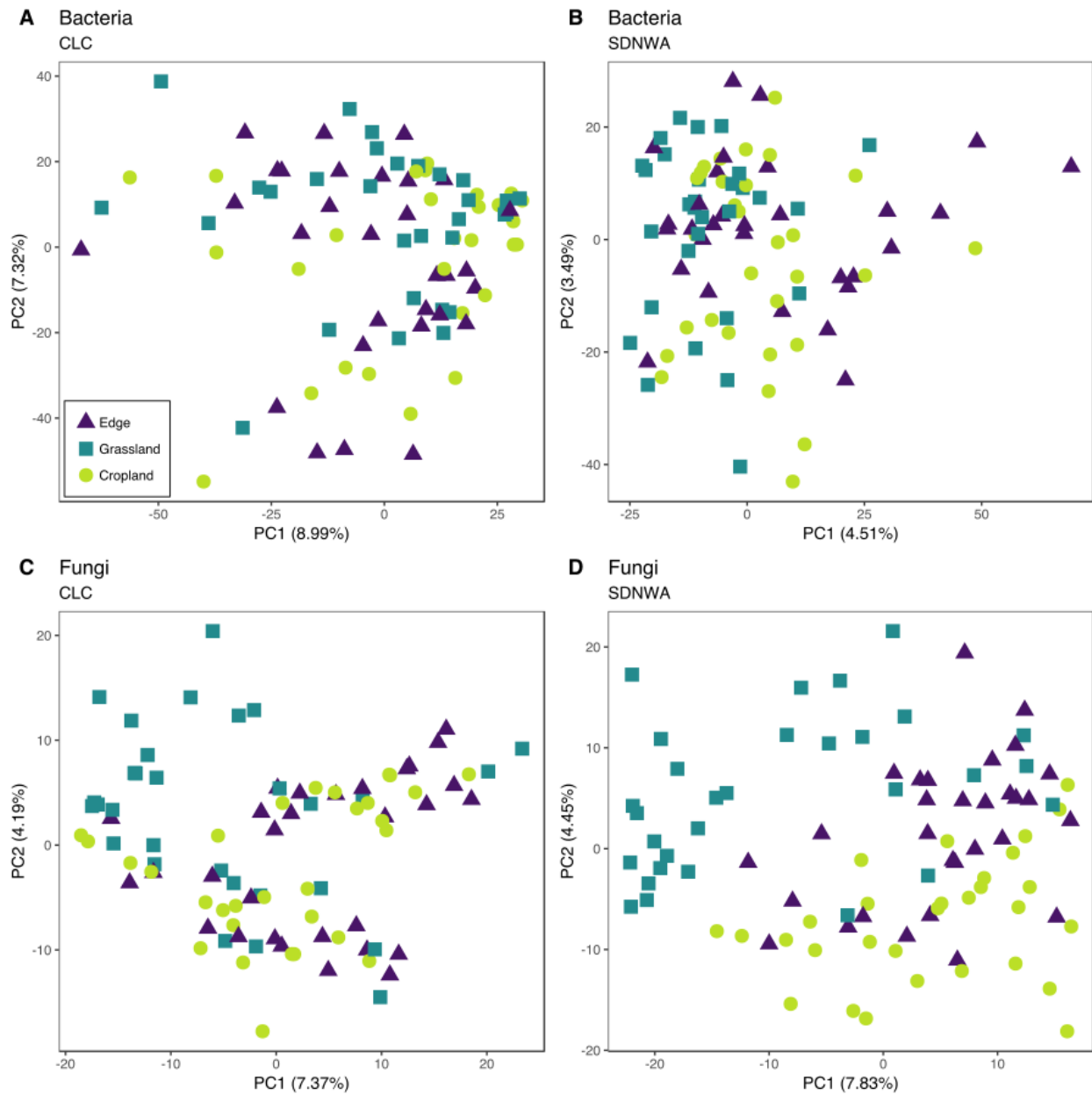
**Fig. E.1:** Principal components analysis on the soil microbial community at the Conservation Learning Centre (CLC) and the St. Denis National Wildlife Area (SDNWA) using the compositional data approach, where data was centered log ratio transformed before the PCA. (a) Bacteria at CLC (b) Bacteria at SDNWA (c) Fungi at CLC (d) fungi at SDNWA.
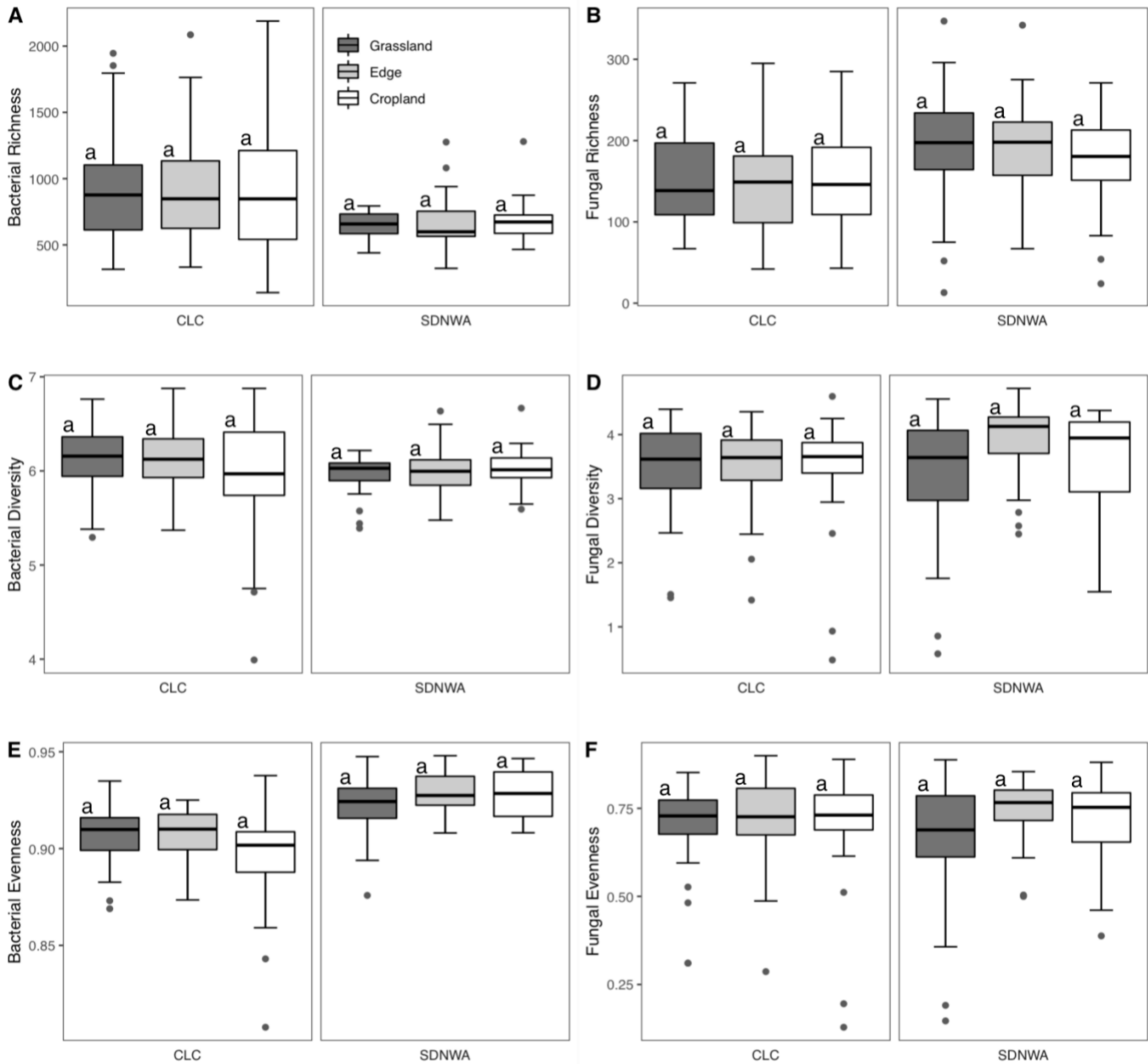
**Fig. F.1**: Microbial metric boxplots with linear mixed model significance at the Conservation Learning Centre (CLC) and the St. Denis National Wildlife Area (SDNWA). Boxes encompass 25-75% quantiles of the data, while whiskers encompass 5-95%. The median is indicated by the black horizontal line, and outliers are shown as dots. Different letters indicate a significant difference (*p*-value < 0.05) between edge locations determined by Tukey-HSD post-hoc tests on the linear mixed models. Colour of the boxplots represent edge location, dark gray = grassland, light gray = edge and white = cropland. (a) bacterial richness (b) fungal richness (*J'*) (c) bacterial diversity (*H'*) (d) fungal diversity (e) bacterial evenness (*J'*) (f) fungal evenness (*H'*).

**Table G.1:** Average percent cover for plant species found at St. Denis National Wildlife Area and the Conservation Learning Centre. The first column is average cover for the entire site; the following columns reflect average aover for fields at each site: the perennial grassland (33 m -1 m), the edge (0.5 m – 0.5 m), and the annual cropland (1 m – 33 m). Asterisks denote plants that were present with 1 m of centre point, but fell outside the cover quadrat (1 m2).

| Plant | Average Cover | Perennial Grassland | Annual Cropland | Edge |
|---|---|---|---|---|
| St. Denis National Wildlife Area | | | | |
| *Bromus inermis* | 15 | 20 | 0 | 3.3 |
| *Poa pratensis* | 2.2 | 2.2 | 0 | 0 |
| *Elymus repens* | 5.4 | 4.1 | 7.1 | 0 |
| *Hordeum jubatum* | 2 | 2.0 | 0 | 0 |
| *Elymus lanceolatus* | 4.5 | 4.5 | 0 | 0 |
| *Elymus trachycaulus* | 3.6 | 3.6 | 0 | 0 |
| *Pascopyrum smithii* | 5 | 5.0 | 0 | 0 |
| *Unknown grass* | 0.6 | 0 | 0.50 | 0.75 |
| *Cirsium arvense* | 5.3 | 5.4 | 5.6 | 3.5 |
| *Taraxacum officinale* | 3.0 | 3.3 | 2.1 | 2.0 |
| *Medicago satvia* | 9.5 | 9.5 | 0 | 0 |
| *Crepis tectorum* | 6.2 | 8.0 | 3.6 | 6.5 |
| *Tragopogon dubis* | 1.5 | 0 | 0 | 1.5 |
| *Chondrilla juncea* | 1.7 | 0.50 | 2.0 | 1.8 |
| *Solidago canadensis* | 4.0 | 1.5 | 0.75 | 6.1 |
| *Erigeron canadensis* | 2.6 | 2.7 | 2.0 | 2.9 |
| *Erysimum cheiranthoides* | 2.8 | 1.1 | 3.0 | 3.2 |
| *Capsella bursa-pastoris* | 1.5 | 0 | 1.5 | 0 |
| *Polygonum convulvus* | 2.7 | 0.63 | 2.9 | 3.0 |
| *Silene latifolia* | 3.9 | 0 | 4.3 | 3.8 |
| *Galium aparine* | 3.4 | 2.0 | 3.00 | 3.7 |
| *Galeopsis tetrahit* | 1.9 | 4.0 | 1.2 | 2.0 |
| *Lepidium draba* | 1.3 | 0 | 0.5 | 1.4 |
| *Sonchus arvensis* | 4.3 | 6.2 | 1.5 | 3.6 |
| *Amaranthus retroflexus* | 1.0 | 0 | 0.94 | 1.0 |
| *Thlaspi arvensis* | 1.4 | 0 | 1.0 | 1.5 |
| *Silene alba* | 2 | 0 | 0 | 2 |
| *Chenopodium berlandieri* | 3 | 3 | 3 | 0 |
| *Linum usitatissimum* | 7.0 | 2.6 | 9.6 | 5.7 |
| *Descurania sophia* | 5.4 | 4.3 | 6.3 | 4.9 |
| *Sonchus oleraceus* | 3 | 3 | 0 | 0 |
| *Crepis* | 2.8 | 3.0 | 2.5 | 2.9 |
| *Uknown 1* | 0.58 | 0 | 0.58 | 0 |
| *Unknown 2* | 3.5 | 0 | 3.5 | 0 |
| *Melilotus officinale** | | | | |

*Artemisia absinthium\**
*Lactuca serriola\**

| Conservation Learning Centre | | | | |
|---|---|---|---|---|
| *Bromus inermis* | 8.8 | 10.3 | 0 | 2.8 |
| *Bromus beiberstanii* | 9.2 | 12 | 0 | 4.0 |
| *Poa pratensis* | 4.4 | 4.4 | 5.0 | 0 |
| *Elymus repens* | 3.3 | 4.5 | 0 | 1.8 |
| *Hordeum jubatum* | 4.4 | 2.2 | 8.0 | 5.4 |
| *Elyumus lanceolatus* | 3 | 3 | 0 | 0 |
| *Festuca saximontana* | 6.6 | 8.3 | 0 | 2.7 |
| *Unknown grass* | 2.5 | 3.5 | 2.3 | 2.8 |
| *Cirsium arvense* | 3.6 | 3.9 | 5.0 | 3.4 |
| *Taraxacum offiniale* | 5.1 | 6.0 | 2.4 | 4.9 |
| *Medicago stavia* | 4.9 | 5.3 | 0 | 2.9 |
| *Crepis tectorum* | 2.5 | 0 | 0 | 2.5 |
| *Chondrilla juncea* | 1.5 | 0 | 0 | 1.5 |
| *Solidago canadensis* | 2.3 | 2.3 | 2.2 | 0 |
| *Capsella bursa-pastoris* | 3 | 3 | 0 | 0 |
| *Polygonum convulvus* | 2.4 | 0.75 | 2.0 | 2.7 |
| *Silene latifolia* | 1 | 1 | 0 | 0 |
| *Galium aparine* | 1.3 | 0.50 | 1.2 | 1.3 |
| *Sonchus arvensis* | 3.9 | 5.5 | 2.8 | 3.2 |
| *Amarantheus retroflexus* | 0.5 | 0 | 0.5 | 0 |
| *Equisetum arvensis* | 6.5 | 3.7 | 11 | 4.9 |
| *Silene alba* | 5 | 5 | 0 | 0 |
| *Eurybia conspicua* | 2.7 | 2.7 | 0 | 0 |
| *Achiella millefolium* | 0.5 | 0.5 | 0 | 0 |
| *Fragaria virgiana* | 3 | 3 | 0 | 0 |
| *Artemisis absinthium* | 1.0 | 2.5 | 0.90 | 0.67 |
| *Vicia Americana* | 2.2 | 2.6 | 0 | 1.2 |
| *Chenopodium berlanderi* | 0.67 | 0 | 0 | 0.67 |
| *Plantago major* | 1.02 | 1.0 | 0.78 | 1.3 |
| *Salix bebbiana* | 2.8 | 2.8 | 0 | 0 |
| *Brassica rapa* | 15.1 | 2.1 | 8.3 | 24 |
| *Galium boreale* | 8 | 8 | 0 | 0 |
| *Crepis* | 1.6 | 0 | 0 | 1.6 |
| *Unknown herb* | 2.1 | 0 | 0 | 2.1 |
| *Melilotus officnale\** | | | | |
| *Astragalus cicer\** | | | | |
| *Populus tremuloides\** | | | | |
| *Platanthera aquilonis\** | | | | |
| *Agrostis scabra\** | | | | |
| *Setaria viridis\** | | | | |

**Table H.1**: Results from linear mixed modelling for soil properties across the edge. Estimate listed first and standard error is in parenthesis. Intercept is the edge. Parameter esitmates derived from the *lme4* R package using the 'lmer' function using restricted maximum likelihood option.

| | *Dependent variable:* | | | | |
| --- | --- | --- | --- | --- | --- |
| | Total C | Total N | NH4† | NO3† | pH |
| Grassland | 4,000*** | 320*** | 0.020 | -0.48*** | 0.45*** |
| | (1400) | (120) | (0.10) | (0.11) | (0.066) |
| Cropland | -1,800 | -120 | -0.084 | 0.028 | -0.084 |
| | (1,400) | (120) | (0.10) | (0.11) | (0.066) |
| SiteSDNWA | -5,200 | -610 | 0.58*** | 0.15 | 1.4*** |
| | (5,900) | (660) | (0.18) | (0.11) | (0.12) |
| Grassland: SiteSDNWA | 2,700 | 120 | 0.67*** | -0.012 | -0.60*** |
| | (1900) | (170) | (0.14) | (0.16) | (0.093) |
| Cropland: SiteSDNWA | -3,800** | -250 | -0.086 | 0.12 | 0.048 |
| | (1,900) | (170) | (0.14) | (0.16) | (0.093) |
| Intercept | 40,000*** | 3,500*** | 2.07*** | 2.6*** | 5.7*** |
| | (4,200) | (470) | (0.13) | (0.078) | (0.082) |
| Observations | 180 | 180 | 180 | 180 | 180 |
| Log Likelihood | -1,800 | -1,300 | -94 | -110 | -22 |

*Note*: Asterisks denote *p*-value, *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$

**Table H.2**: Results from linear mixed modelling for biomass across the edge. Estimate listed first and standard error is in parenthesis. Intercept is the edge. Parameter esitmates derived from the *lme4* R package using the 'lmer' function using restricted maximum likelihood option.

| | *Dependent variable:* | | | |
|---|---|---|---|---|
| | Living Biomass† | Grass† | Forbs† | Litter† |
| Grassland | 1.1*** | 2.4*** | -0.14 | -0.25 |
| | (0.24) | (0.34) | (0.26) | (0.17) |
| Cropland | 0.47 | -0.54 | 0.71*** | -0.34* |
| | (0.24) | (0.34) | (0.26) | (0.17) |
| SiteSD | 0.90** | -0.47 | 1.06*** | -0.19 |
| | (0.27) | (0.40) | (0.28) | (0.40) |
| Grassland:SiteSD | -0.32 | 1.6*** | -1.6*** | 0.64*** |
| | (0.34) | (0.47) | (0.37) | (0.25) |
| Cropland:SiteSD | -1.4*** | -0.76 | -1.3*** | 0.15 |
| | (0.34) | (0.47) | (0.37) | (0.25) |
| Intercept | 4.1*** | -0.13 | 1.4*** | 3.7*** |
| | (0.19) | (0.28) | (0.20) | (0.28) |
| Observations | 180 | 180 | 180 | 180 |
| Log Likelihood | -250 | -300 | -260 | -190 |

*Note*: Asterisks denote *p*-value, *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$

**Table I.1:** Soil property means ± standard deviations for perennial grasslands, edges, and annual croplands at the St. Denis National Wildlife Area (SDNWA) and the Conservation Learning Centre (CLC).

| Site | | Total C (%) | Total N (%) | NH₄ (µg/g soil) | NO₃ (µg/g soil) | pH |
|---|---|---|---|---|---|---|
| SDNWA | grassland | 4.1 ± 0.73 | 0.34 ± 0.077 | 8.3 ± 3.1 | 5.1 ± 5.5 | 7.03 ± 0.20 |
| | edge | 3.4 ± 0.28 | 0.29 ± 0.028 | 3.5 ± 1.1 | 12 ± 10.0 | 7.2 ± 0.10 |
| | cropland | 2.9 ± 0.42 | 0.25 ± 0.039 | 3.05 ± 2.4 | 16 ± 11.0 | 7.2 ± 0.17 |
| CLC | grassland | 4.4 ± 0.94 | 0.39 ± 0.083 | 7.4 ± 2.7 | 3.2 ± 1.05 | 6.2 ± 0.36 |
| | edge | 3.9 ± 0.69 | 0.35 ± 0.07 | 7.8 ± 5.6 | 9.3 ± 7.1 | 5.7 ± 0.21 |
| | cropland | 3.8 ± 0.85 | 0.34 ± 0.089 | 8.7 ± 12.0 | 11 ± 12.0 | 5.7 ± 0.41 |

**Table I.2:** Biomass (grams per 20 x 50 cm plot) means ± standard deviations for perennial grasslands, edges, and annual croplands at the St. Denis National Wildlife Area (SDNWA) and the Conservation Learning Centre (CLC).

| Site | | Grass (g) | Forbs (g) | Litter (g) | Living Biomass (g) |
|---|---|---|---|---|---|
| SDNWA | grassland | 31 ± 15 | 3.1 ± 5.8 | 51 ± 20 | 35 ± 14 |
| | edge | 3.6 ± 8.8 | 13.0 ± 8.0 | 38 ± 25 | 17 ± 11 |
| | cropland | 0.01 ± 0.071 | 9.3 ± 7.6 | 34 ± 23 | 9.3 ± 7.6 |
| CLC | grassland | 15 ± 9.2 | 6.1 ± 6.7 | 35 ± 17 | 22 ± 10 |
| | edge | 2.6 ± 4.5 | 6.2 ± 6.6 | 48 ± 29 | 8.9 ± 7.9 |
| | cropland | 1.5 ± 5.3 | 11 ± 10 | 38 ± 36 | 13 ± 10 |

**Table J.1** Model fit parameters for the structural equation models. Listed is the chi squared value, degrees of freedom (df), *p*-value, Comparative Fit Index (CFI), Root Mean Square Error of Approximation (RMSEA), and the Standardized Root Mean Square Residual (SRMR). Fungi genera significant at both sites are denoted by *, St. Denis National Wildlife Are genera denoted by †, and the Conservation Learning Centre genus denoted by ‡.

| Model | Description | $X_2$ | df | *p* - value | CFI | RMSEA | SRMR |
|-------|-------------|-------|----|-----------|-----|-------|------|
| 1 | Fungal Richness | 14 | 9 | 0.13 | 0.99 | 0.054 | 0.047 |
| 2 | *Paraphoma*∗ | 14 | 9 | 0.13 | 0.99 | 0.054 | 0.045 |
| 3 | *Parastagonospora*∗ | 16 | 9 | 0.059 | 0.98 | 0.068 | 0.045 |
| 4 | *Sarocladium*∗ | 16 | 9 | 0.067 | 0.98 | 0.066 | 0.047 |
| 5 | *Acrostalagmus*† | 9.2 | 9 | 0.42 | 0.99 | 0.015 | 0.062 |
| 6 | *Arthrinium*† | 13 | 9 | 0.18 | 0.98 | 0.067 | 0.066 |
| 7 | *Cistella*† | 16 | 9 | 0.069 | 0.96 | 0.093 | 0.070 |
| 8 | *Clonostachys*† | 9.4 | 9 | 0.40 | 0.99 | 0.023 | 0.062 |
| 9 | *Coprinopsis*† | 11 | 9 | 0.30 | 0.99 | 0.046 | 0.063 |
| 10 | *Devriesia*† | 14 | 9 | 0.13 | 0.97 | 0.078 | 0.068 |
| 11 | *Schizothecium*† | 14 | 9 | 0.12 | 0.97 | 0.079 | 0.068 |
| 12 | *Olpidium*‡ | 16 | 9 | 0.066 | 0.97 | 0.094 | 0.067 |

**Table J.2** Path coefficients for model 1, using fungal richness as an observed variable. The significant paths are bolded ($p < 0.1$). This model is presented in Fig. 3.11.

| Response Variable | Predictor Variable | Estimate | Std.err | Z-value | P(>\|z\|) | Std.all |
|---|---|---|---|---|---|---|
| Fungal richness | Total N | 1.2 | 2.2 | 0.57 | 0.57 | 0.099 |
| | Total C | -0.093 | 0.20 | -0.46 | 0.65 | -0.079 |
| | Aboveground plant richness | 0.026 | 0.026 | 0.97 | 0.33 | 0.073 |
| | **Belowground plant richness** | **0.081** | **0.035** | **2.3** | **0.022** | **0.17** |
| | **Bacterial richness** | **-0.18** | **0.075** | **-2.4** | **0.016** | **-0.18** |
| Bacterial richness | **Total N** | **3.8** | **2.2** | **1.8** | **0.078** | **0.30** |
| | Total C | -0.11 | 0.21 | -0.56 | 0.56 | -0.097 |
| Total C | **Living biomass** | **0.24** | **0.06** | **3.9** | **0.00** | **0.28** |
| Total N | **Living biomass** | **0.016** | **0.006** | **2.7** | **0.007** | **0.20** |
| Living biomass | Aboveground Plant richness | -0.005 | 0.026 | -0.21 | 0.84 | -0.015 |
| Total C | **Total N** | **0.056** | **0.006** | **9.0** | **0.00** | **0.91** |
| Aboveground plant richness | **Belowground plant richness** | **1.1** | **0.45** | **2.5** | **0.013** | **0.19** |

**Table J.3** Path coefficients for model 2, using *Paraphoma* as an observed variable. The significant paths are bolded ($p < 0.1$). This model is displayed in Fig. 3.12.

| Response Variable | Predictor Variable | Estimate | Std.err | Z-value | P(>|z|) | Std.all |
|---|---|---|---|---|---|---|
| *Paraphoma* | Total N | 2.7 | 2.9 | 0.94 | 0.35 | 0.16 |
| abundance | Total C | 0.028 | 0.27 | 0.10 | 0.92 | 0.017 |
| | **Aboveground plant richness** | **0.14** | **0.035** | **4.0** | **0.00** | **0.90** |
| | Belowground plant richness | 0.043 | 0.047 | 0.90 | 0.37 | 0.065 |
| | Bacterial richness | -0.13 | 0.10 | -1.2 | 0.22 | -0.092 |
| Bacterial richness | **Total N** | **3.8** | **2.2** | **1.8** | **0.078** | **0.30** |
| | Total C | -0.12 | 0.21 | -0.58 | 0.57 | -0.10 |
| Total C | **Living biomass** | **0.24** | **0.06** | **3.9** | **0.00** | **0.28** |
| Total N | **Living biomass** | **0.016** | **0.006** | **2.7** | **0.007** | **0.20** |
| Living biomass | Aboveground Plant richness | -0.005 | 0.026 | -0.21 | 0.84 | -0.015 |
| Total C | **Total N** | **0.056** | **0.006** | **9.0** | **0.00** | **0.91** |
| **Aboveground plant richness** | **Belowground plant richness** | **1.1** | **0.45** | **2.5** | **0.013** | **0.19** |

**Table J.4** Path coefficients for model 3, using *Parastagonospora* as an observed variable. The significant paths are bolded ($p < 0.1$). This model is displayed in Fig. 3.12.

| Response Variable | Predictor Variable | Estimate | Std.err | Z-value | P(>\|z\|) | Std.all |
|---|---|---|---|---|---|---|
| *Parastagonospora* abundance | Total N | 4.4 | 3.8 | 1.2 | 0.24 | 0.19 |
| | **Total C** | **-1.1** | **0.36** | **-3.0** | **0.002** | **-0.47** |
| | Aboveground plant richness | -0.053 | 0.046 | -1.1 | 0.25 | -0.082 |
| | Belowground plant richness | 0.036 | 0.061 | 0.59 | 0.56 | 0.042 |
| | **Bacterial richness** | **-0.25** | **0.13** | **-1.9** | **0.052** | **-0.14** |
| Bacterial richness | **Total N** | **3.8** | **2.2** | **1.8** | **0.078** | **0.31** |
| | Total C | -0.12 | 0.21 | -0.60 | 0.60 | -0.10 |
| Total C | **Living biomass** | **0.27** | **0.06** | **3.9** | **0.000** | **0.28** |
| Total N | **Living biomass** | **0.016** | **0.006** | **2.7** | **0.007** | **0.20** |
| Living biomass | Aboveground Plant richness | -0.005 | 0.026 | -0.21 | 0.84 | -0.015 |
| Total C | **Total N** | **0.056** | **0.006** | **9.0** | **0.000** | **0.91** |
| Aboveground plant richness | **Belowground plant richness** | **1.1** | **0.45** | **2.5** | **0.014** | **0.19** |

**Table J.5** Path coefficients for model 4, using *Sarocladium* as an observed variable. The significant paths are bolded ($p < 0.1$). This model is displayed in Fig. 3.12.

| Response Variable | Predictor Variable | Estimate | Std.err | Z-value | P(>\|z\|) | Std.all |
|---|---|---|---|---|---|---|
| *Sarocladium* | **Total N** | **11** | **6.0** | **1.9** | **0.06** | **0.31** |
| abundance | **Total C** | **-1.9** | **0.56** | **-3.5** | **0.001** | **-0.56** |
| | **Aboveground plant richness** | **-0.18** | **0.073** | **-2.5** | **0.013** | **-0.18** |
| | Belowground plant richness | -0.15 | 0.097 | -1.6 | 0.11 | -0.11 |
| | Bacterial richness | -0.29 | 0.21 | -1.4 | 0.16 | -0.10 |
| Bacterial richness | **Total N** | **3.8** | **2.2** | **1.8** | **0.078** | **0.30** |
| | Total C | -0.12 | 0.21 | -0.57 | 0.57 | -0.10 |
| Total C | **Living biomass** | **0.24** | **0.06** | **3.9** | **0.000** | **0.28** |
| Total N | **Living biomass** | **0.016** | **0.006** | **2.7** | **0.007** | **0.20** |
| Living biomass | Aboveground Plant richness | -0.005 | 0.026 | -0.21 | 0.83 | -0.015 |
| Total C | **Total N** | **0.056** | **0.006** | **9.0** | **0.000** | **0.91** |
| Aboveground plant richness | **Belowground plant richness** | **1.1** | **0.45** | **2.5** | **0.014** | **0.19** |

**Table J.6** Path coefficients for model 5, using *Acrostalagmus* as an observed variable. The significant paths are bolded ($p < 0.1$). This model is displayed in Fig. 3.13. St. Denis National Wildlife Area specific fungi.

| Response Variable | Predictor Variable | Estimate | Std.err | Z-value | P(>\|z\|) | Std.all |
|---|---|---|---|---|---|---|
| *Acrostalagmus* | **Total N** | **12** | **7.7** | **1.6** | **0.099** | **0.29** |
| abundance | **Total C** | **-2.3** | **0.65** | **-3.5** | **0.00** | **-0.60** |
| | **Aboveground plant richness** | **0.23** | **0.081** | **2.9** | **0.004** | **0.27** |
| | **Belowground plant richness** | **-0.23** | **0.11** | **-2.2** | **0.03** | **-0.20** |
| | **Bacterial richness** | **-0.55** | **0.25** | **-2.2** | **0.031** | **-0.20** |
| Bacterial richness | **Total N** | **5.4** | **3.2** | **1.7** | **0.091** | **0.33** |
| | Total C | -0.42 | 0.27 | -1.5 | 0.13 | -0.30 |
| Total C | **Living biomass** | **0.36** | **0.066** | **5.4** | **0.00** | **0.50** |
| Total N | **Living biomass** | **0.026** | **0.006** | **4.5** | **0.00** | **0.43** |
| Living biomass | Aboveground Plant richness | 0.006 | 0.034 | 0.19 | 0.85 | 0.02 |
| Total C | **Total N** | **0.028** | **0.005** | **5.9** | **0.00** | **0.81** |
| Aboveground plant richness | Belowground plant richness | 1.1 | 0.80 | 1.4 | 0.16 | 0.15 |

**Table J.7** Path coefficients for model 6, using *Arthrinium* as an observed variable. The significant paths are bolded ($p < 0.1$). This model is displayed in Fig. 3.13. St. Denis National Wildlife Area specific fungi.

| Response Variable | Predictor Variable | Estimate | Std.err | Z-value | P(>\|z\|) | Std.all |
|---|---|---|---|---|---|---|
| *Arthrinium* | Total N | -4.3 | 7.3 | -0.59 | 0.55 | -0.11 |
| abundance | **Total C** | **1.04** | **0.61** | **1.7** | **0.091** | **0.31** |
| | Aboveground plant richness | 0.036 | 0.076 | 0.47 | 0.64 | 0.047 |
| | Belowground plant richness | 0.11 | 0.099 | 1.1 | 0.26 | 0.11 |
| | **Bacterial richness** | **-0.67** | **0.24** | **-2.8** | **0.005** | **-0.28** |
| Bacterial richness | **Total N** | **5.4** | **3.2** | **1.7** | **0.09** | **0.33** |
| | Total C | -0.42 | 0.27 | -1.5 | 0.13 | -0.30 |
| Total C | **Living biomass** | **0.40** | **0.066** | **5.4** | **0.000** | **0.50** |
| Total N | **Living biomass** | **0.026** | **0.006** | **4.5** | **0.000** | **0.43** |
| Living biomass | Aboveground Plant richness | 0.006 | 0.034 | 0.19 | 0.85 | 0.02 |
| Total C | **Total N** | **0.028** | **0.005** | **5.9** | **0.000** | **0.81** |
| Aboveground plant richness | Belowground plant richness | 1.1 | 0.80 | 1.4 | 0.16 | 0.15 |

**Table J.8** Path coefficients for model 7, using *Cistella* as an observed variable. The significant paths are bolded ($p < 0.1$). This model is displayed in Fig. 3.13. St. Denis National Wildlife Area specific fungi.

| Response Variable | Predictor Variable | Estimate | Std.err | Z-value | P(>\|z\|) | Std.all |
|---|---|---|---|---|---|---|
| *Cistella* | Total N | -11 | 7.2 | -1.5 | 0.13 | -0.26 |
| abundance | **Total C** | **2.6** | **0.61** | **4.2** | **0.00** | **0.71** |
| | Aboveground plant richness | 0.004 | 0.076 | 0.057 | 0.96 | 0.005 |
| | Belowground plant richness | 0.051 | 0.098 | 0.52 | 0.60 | 0.048 |
| | Bacterial richness | -0.27 | 0.24 | -1.1 | 0.25 | -0.11 |
| Bacterial richness | **Total N** | **5.4** | **3.2** | **1.7** | **0.091** | **0.33** |
| | Total C | -0.42 | 0.27 | -1.5 | 0.13 | -0.30 |
| Total C | **Living biomass** | **0.40** | **0.066** | **5.4** | **0.00** | **0.50** |
| Total N | **Living biomass** | **0.026** | **0.006** | **4.5** | **0.00** | **0.43** |
| Living biomass | Aboveground Plant richness | 0.006 | 0.034 | 0.19 | 0.85 | 0.02 |
| Total C | **Total N** | **0.028** | **0.005** | **5.9** | **0.000** | **0.81** |
| Aboveground plant richness | Belowground plant richness | 1.1 | 0.80 | 1.4 | 0.16 | 0.15 |

**Table J.9** Path coefficients for model 8, using *Clonostachys* as an observed variable. The significant paths are bolded ($p < 0.1$). This model is displayed in Fig. 3.13. St. Denis National Wildlife Area specific fungi.

| Response Variable | Predictor Variable | Estimate | Std.err | Z-value | P(>\|z\|) | Std.all |
|---|---|---|---|---|---|---|
| *Clonostachys* | **Total N** | **23** | **11** | **2.2** | **0.027** | **0.36** |
| abundance | **Total C** | **-4.0** | **0.90** | **-4.4** | **0.00** | **-0.72** |
| | **Aboveground plant richness** | **0.047** | **0.11** | **4.2** | **0.00** | **0.37** |
| | Belowground plant richness | -0.019 | 0.14 | -0.13 | 0.89 | -0.012 |
| | Bacterial richness | -0.073 | 0.35 | -0.21 | 0.83 | -0.018 |
| Bacterial richness | **Total N** | **5.4** | **3.19** | **1.7** | **0.091** | **0.33** |
| | Total C | -0.42 | 0.27 | -1.5 | 0.13 | -0.30 |
| Total C | **Living biomass** | **0.36** | **0.066** | **5.4** | **0.000** | **0.50** |
| Total N | **Living biomass** | **0.026** | **0.006** | **4.5** | **0.000** | **0.43** |
| Living biomass | Aboveground Plant richness | 0.006 | 0.034 | 0.19 | 0.85 | 0.02 |
| Total C | **Total N** | **0.028** | **0.005** | **5.9** | **0.000** | **0.81** |
| Aboveground plant richness | Belowground plant richness | 1.1 | 0.80 | 1.4 | 0.16 | 0.15 |

**Table J.10** Path coefficients for model 9, using *Coprinopsis* as an observed variable. The significant paths are bolded ($p < 0.1$). This model is displayed in Fig. 3.13. St. Denis National Wildlife Area specific fungi.

| Response Variable | Predictor Variable | Estimate | Std.err | Z-value | P(>\|z\|) | Std.all |
|---|---|---|---|---|---|---|
| *Coprinopsis* | Total N | -2.03 | 6.0 | -0.34 | 0.74 | -0.065 |
| abundance | Total C | -0.48 | 0.51 | -0.93 | 0.35 | -0.18 |
| | Aboveground plant richness | 0.033 | 0.063 | 0.52 | 0.60 | 0.054 |
| | Belowground plant richness | -0.049 | 0.082 | -0.60 | 0.55 | -0.062 |
| | **Bacterial richness** | **0.35** | **0.20** | **1.8** | **0.08** | **0.18** |
| Bacterial richness | **Total N** | **5.4** | **3.2** | **1.7** | **0.091** | **0.33** |
| | Total C | -0.42 | 0.27 | -1.5 | 0.13 | -0.30 |
| Total C | **Living biomass** | **0.36** | **0.066** | **5.4** | **0.000** | **0.50** |
| Total N | **Living biomass** | **0.026** | **0.006** | **4.5** | **0.000** | **0.43** |
| Living biomass | Aboveground Plant richness | 0.006 | 0.034 | 0.19 | 0.85 | 0.02 |
| Total C | **Total N** | **0.028** | **0.005** | **5.9** | **0.000** | **0.81** |
| Aboveground plant richness | Belowground plant richness | 1.1 | 0.80 | 1.4 | 0.16 | 0.15 |

**Table J.11** Path coefficients for model 10, using *Devriesia* as an observed variable. The significant paths are bolded ($p < 0.1$). This model is displayed in Fig. 3.13, St. Denis National Wildlife Area specific fungi.

| Response Variable | Predictor Variable | Estimate | Std.err | Z-value | P(>\|z\|) | Std.all |
|---|---|---|---|---|---|---|
| *Devriesia* | Total N | 0.62 | 5.5 | 0.11 | 0.91 | 0.019 |
| abundance | **Total C** | **1.2** | **0.46** | **2.5** | **0.012** | **0.42** |
| | **Aboveground plant richness** | **-0.19** | **0.057** | **-3.3** | **0.001** | **-0.30** |
| | Belowground plant richness | 0.12 | 0.074 | 1.5 | 0.12 | 0.14 |
| | Bacterial richness | -0.40 | 0.18 | -2.2 | 0.025 | -0.20 |
| Bacterial richness | **Total N** | **5.4** | **3.2** | **1.7** | **0.09** | **0.33** |
| | Total C | -0.42 | 0.27 | -1.5 | 0.13 | -0.30 |
| Total C | **Living biomass** | **0.40** | **0.066** | **5.4** | **0.000** | **0.50** |
| Total N | **Living biomass** | **0.026** | **0.006** | **4.5** | **0.000** | **0.43** |
| Living biomass | Aboveground Plant richness | 0.006 | 0.034 | 0.19 | 0.85 | 0.02 |
| Total C | **Total N** | **0.028** | **0.005** | **5.9** | **0.000** | **0.81** |
| Aboveground plant richness | Belowground plant richness | 1.1 | 0.80 | 1.4 | 0.16 | 0.15 |

**Table J.12** Path coefficients for model 11, using *Schizothecium* as an observed variable. The significant paths are bolded ($p < 0.1$). This model is displayed in Fig. 3.13. St. Denis National Wildlife Area specific fungi.

| Response Variable | Predictor Variable | Estimate | Std.err | Z-value | P(>\|z\|) | Std.all |
|---|---|---|---|---|---|---|
| *Schizothecium* | **Total N** | **36** | **16** | **2.3** | **0.024** | **0.43** |
| abundance | **Total C** | **-4.4** | **1.37** | **-3.2** | **0.001** | **-0.61** |
| | Aboveground plant richness | 0.18 | 0.17 | 1.05 | 0.30 | 0.11 |
| | Belowground plant richness | -0.15 | 0.22 | -0.67 | 0.50 | -0.068 |
| | Bacterial richness | 0.26 | 0.53 | 0.48 | 0.63 | 0.049 |
| Bacterial richness | **Total N** | **5.4** | **3.2** | **1.70** | **0.09** | **0.33** |
| | Total C | -0.42 | 0.27 | -1.5 | 0.13 | -0.30 |
| Total C | **Living biomass** | **0.356** | **0.066** | **5.4** | **0.000** | **0.50** |
| Total N | **Living biomass** | **0.026** | **0.066** | **4.50** | **0.000** | **0.43** |
| Living biomass | Aboveground Plant richness | 0.006 | 0.034 | 0.19 | 0.85 | 0.02 |
| Total C | **Total N** | **0.028** | **0.005** | **5.9** | **0.000** | **0.81** |
| Aboveground plant richness | Belowground plant richness | 1.1 | 0.80 | 1.4 | 0.16 | 0.15 |

**Table J.13** Path coefficients for model 12, using *Olpidium* as an observed variable. The significant paths are bolded ($p < 0.1$). This model is displayed in Fig. 3.14. Conservation Learning Centre specific fungi.

| Response Variable | Predictor Variable | Estimate | Std.err | Z-value | P(>\|z\|) | Std.all |
|---|---|---|---|---|---|---|
| *Olpidium* | **Total N** | **49** | **14** | **3.6** | **0.00** | **0.92** |
| abundance | **Total C** | **-5.3** | **1.3** | **-4.1** | **0.00** | **-1.04** |
| | Aboveground plant richness | 0.15 | 0.18 | 0.83 | 0.40 | 0.085 |
| | Belowground plant richness | -0.38 | 0.27 | -1.5 | 0.14 | -0.15 |
| | Bacterial richness | 0.062 | 0.43 | 1.4 | 0.16 | 0.14 |
| Bacterial richness | Total N | -1.2 | 3.5 | -0.33 | 0.74 | -0.094 |
| | Total C | 0.17 | 0.33 | 0.52 | 0.60 | 0.15 |
| Total C | **Living biomass** | **0.26** | **0.087** | **2.9** | **0.003** | **0.29** |
| Total N | **Living biomass** | **0.021** | **0.008** | **2.6** | **0.01** | **0.26** |
| Living biomass | Aboveground Plant richness | -0.032 | 0.042 | -0.75 | 0.45 | -0.08 |
| Total C | **Total N** | **0.058** | **0.009** | **6.3** | **0.00** | **0.92** |
| Aboveground plant richness | **Belowground plant richness** | **1.2** | **0.50** | **2.5** | **0.013** | **0.28** |
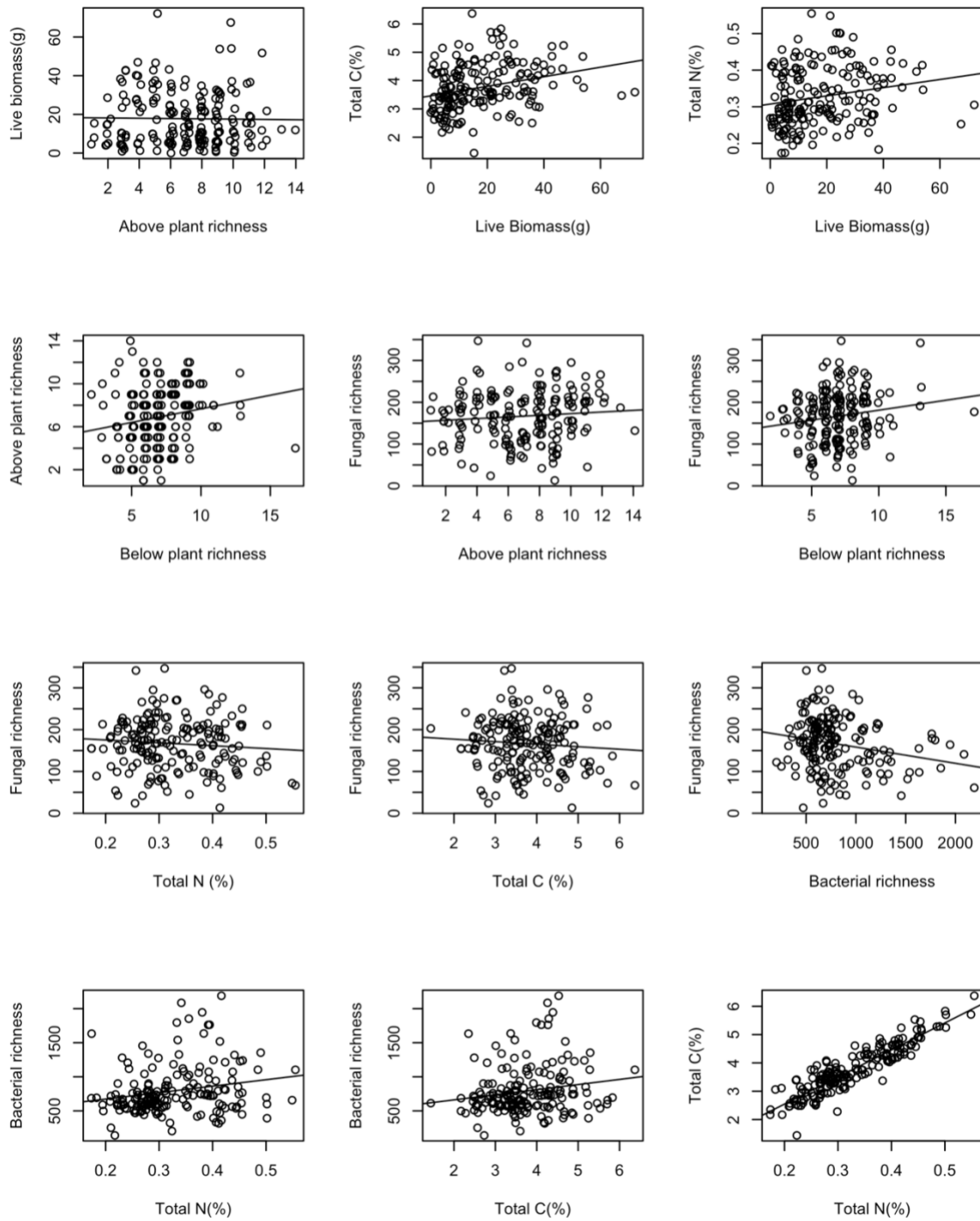
**Fig. K.1**: Bivariate plots for relationships between variables used in the structural equation models for the combined sites.