Impact of Farming Practices on Soil Bacterial Community Composition, Diversity, and Interactions in the Main Agricultural Regions of Alberta

by

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ABSTRACT

Soil health refers to “the capacity of soil to function as a vital living system”, implying not only the capacity of soil to providing services of human interest, but also its capacity to provide ecological services, which makes ecosystems sustainable for the long term. The assessment of soil health in agricultural systems is crucial for the sustainability of both agriculture and soil. While agriculture depends on soil resources and their ability to support plant growth and crop productivity, the intensification of management in these systems represents a stress to the soil environment and can lead to soil degradation. The assessment of soil health could provide insights about the impact of the different agricultural practices on soil attributes. Given that soil health is a non-directly quantifiable feature that reflects multiple soil physical, chemical, and biological attributes, measurable indicators are used as proxies of the soil condition and integrated into single-score indexes. However, many of the developed indexes overlook the role of microbial communities in the soil health. Microorganisms play a crucial role in soil functionality, are involved in biogeochemical cycling, and contribute to the availability of nutrients in soil. They form symbiotic relationships with plants and are basal feeders of trophic networks in these systems. Moreover, microbial communities are sensitive to the changes in the soil environment. Changes in soil microbial communities should therefore provide information regarding shifts in processes occurring in soils from different natural and managed systems. Though, due to functional redundancy in microbial communities, shifts in their structure may be independent from changes in soil processes.

In Alberta, the Soil Quality Monitoring Program (SQMP) conducted from 1997 to 2007 aimed to characterize soil quality over time from benchmark sites across the province by evaluating multiple soil physico-chemical parameters. However, no biological indicators of the soil quality were included at the time. Given that agricultural practices could affect the soil environment and microbial communities are sensitive to environmental changes, I revisited the SQMP benchmark sites and evaluated bacterial communities from soils undergoing different agricultural practices (i.e., tillage intensity, crop type, herbicide use, and fertilization method). I assessed the effect of agricultural practices on bacterial community composition, heterogeneity, diversity, and co-occurrence via high-throughput sequencing of the 16S rRNA marker gene and statistical and multivariate analyses. pH and ecoregion were important drivers of bacterial community composition. Agricultural practices influenced the heterogeneity and evenness of bacterial communities but did not play a major role in shaping their composition. On the other hand, co-occurrence network analyses revealed that the complexity and behavior of interactions among the members of the soil bacterial community are altered by different agricultural practices. These changes in the community dynamics could indicate changes in microbial functionality, and the capacity of the community to overcome environmental stress, which in turn could influence the functionality of the soil system. Altogether, my results indicate that bacterial community composition is insensitive to different agricultural practices such as tillage, fertilization, crop type and herbicide usage. However, co-occurrence network metrics may be promising indicators of soil health in agricultural systems.

PREFACE

The research project included as part of this thesis is a section of the project "Revisiting the soil quality benchmark sites to assess the effects of agronomic practices on soil biology as it relates physicochemical parameters and a quality index", conducted in collaboration with Alberta Agriculture and Forestry. This research project received research ethics approval from the University of Alberta Research Ethics Board, Project Name "Soil Quality Monitoring Program", No. Pro00092032, August 13th, 2019.

The thesis is an original work by Angelica Maria Aguirre Monroy. The literature review in Chapter 1, the data collection and analysis referred to in Chapter 2, and the concluding analysis in Chapter 3 correspond to my original work.

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1.1. Soil health

1.1.1 Soil health concept

Soil underpins multiple natural and managed systems on earth by supporting the delivery of multiple ecosystem services (i.e., multifunctionality) (Lal, 2016). Ecosystem services are defined as "the benefits that humans derive from ecosystems" (Bünemann et al., 2018; Costanza et al., 2017). Namely, soil is habitat for biodiversity, and it sustains plant productivity by providing the nutrients needed for their growth and development (Lal, 2016). Soil also harbors hydrological cycles and helps moderate global climate through C sequestration and the control of gaseous fluxes (Lal, 2016). Moreover, soil supports multiple human needs by being the source of industrial and pharmaceutical raw materials, and an archive of climate, nature, and human history (Lal, 2016).

To identify the condition and capabilities of soil to provide ecosystem services, several concepts, and definitions regarding the status of soil have been proposed over the past decades (Amacher et al., 2007). These concepts are considered primarily from an agricultural perspective where the principal objective has been to optimize crop productivity. From *soil fertility* to *land quality,* to *soil quality,* to *soil health*, these concepts have been replaced and, in some cases, used interchangeably (Bünemann et al., 2018). For instance, *soil fertility* refers directly to the nutrients and water available in soil for plant growth, disregarding other functions and aspects of the change on soil condition over time and space (Bünemann et al., 2018). *Land quality* was proposed in the context of soil monitoring and aimed to integrate soil physical and chemical characteristics over time, as well as the vegetation supported in soil (Bünemann et al., 2018).

Later, the concept of *soil quality* was introduced as "the soil's fitness to support crop growth without resulting in soil degradation or otherwise harming the environment" (Acton et al., 1995; Bünemann et al., 2018). This definition was proposed from an agricultural view that ignored any soil attributes and functions that did not relate directly to crop productivity. Hence, the concept was criticized, and as a means to encompass different soil-ecosystems and dynamism, differentiation between *soil quality* and *soil health* was proposed (Bünemann et al., 2018; Pankhurst & CAB International, 1997). The latter term has been redefined as "the continued capacity of soil to function as a vital living system to sustain biological productivity, maintain environmental quality and promote plant, animal and human health" (Bünemann et al., 2018; Pankhurst & CAB International, 1997). Despite the two terms overlapping to some extent, a scientific argument has been raised around how the terms differ and must not be interchanged, given that *soil health* refers not only to functions that are of human interest but also provides a holistic ecological view that considers the interaction of multiple soil functions and perceives soil as a bi-directional living resource that influences life, and at the same time is influenced by time, space and the diversity of life (Bünemann et al., 2018; Lal, 2016; Norris et al., 2020; Pankhurst & CAB International, 1997; Pawlett et al., 2021). Hence, soil health integrates soil biological attributes, and considers their contribution to soil functionality in different ecosystems or under different land-uses, characteristics that were overlooked in the previous concepts (Bünemann et al., 2018).

1.1.2. Soil health indicators and indexes

The concept of a soil health index was first introduced by Rust et al. (1972) in response to the rising concerns regarding soil and water toxicity and contamination as a result of the excess of N fertilizer use in agricultural systems (Rust et al., 1972). The index was developed as a tool

to achieve a N input equal to the N output, which considered as input all organic and inorganic amendments as well as organic matter mineralization and plant residue, and as output nitrogen losses processes and plant uptake (Rust et al., 1972). Since then, the concept has evolved to include various physical, chemical, and biological soil properties assessed using a plethora of statistical tools that have produced many proposed soil health indexes relative to reference soils (Lal, 1998). A soil health index integrates multiple soil properties into a single value that implies the condition of soil (Amacher et al., 2007). Given that soil health is a non-directly quantifiable feature that reflects multiple and variable soil attributes, inherent parameters of soil (physical, chemical, and biological) are used as proxy indicators of soil health (Adetunji et al., 2017; Pankhurst & CAB International, 1997). A soil health indicator must be a measurable (group of) characteristic(s) that is(are) able to reveal the capacity of a soil to provide ecosystem functions (Fierer et al., 2021; Pankhurst & CAB International, 1997). Ideal indicators are both sensitive and rapid in response to environmental or management changes (Adetunji et al., 2017; Pankhurst & CAB International, 1997). Most soil health indexes assess the condition of soil relative to baseline values for soil health indicators in reference soils, which allow the identification of a soil health gap in cultivated land (Lal, 1998; Maharjan et al., 2020). Under an agricultural framework, reference soils should be native, uncultivated, and undisturbed soils located in an agroecosystem that resembles climatic and environmental conditions to those of the evaluated site (Maharjan et al., 2020).

Soil systems are complex, meaning that (i) isolated indicators are not an accurate representation of the entire soil condition and processes, and (ii) single indicators may not be equally relevant for the system functioning and health (Norris et al., 2020). For this reason, indicators are rarely assessed individually, and multiple indicators are commonly integrated as

part of soil health indexes (Adetunji et al., 2017; Amacher et al., 2007; Fine et al., 2017; Laishram et al., 2012; Lal, 2016; Pankhurst & CAB International, 1997; R. Xue et al., 2019). In these indexes, a minimum data set of indicators is identified, then scaled up and integrated into a single score (Laishram et al., 2012). A soil health index for forest soils was proposed using 19 different measurements of soil physical and chemical attributes, which included bulk density, pH, total organic C in mineral soils, total N, exchangeable Na, and several exchangeable cations (Amacher et al., 2007). A threshold was assigned to each parameter and a score of -1, 0, 1, or 2 was assigned correspondingly (Amacher et al., 2007). The weight given to each parameter for the overall score was equivalent and assumes that all soil attributes are equally relevant to soil health and disregards any possible correlations between them (Amacher et al., 2007).

Lal (1998) proposed an index using a scoring scheme relative to baseline or threshold values for each indicator in native reference sites, with the concept that native soil functioning represents the full soil functional potential. Indicators corresponding to "critical soil functions" for different soil uses were assigned a higher weight for the overall index score (Lal, 1998). The index consisted of a minimum data set that included soil texture, depth of plant rooting, infiltration and bulk density, water holding capacity, organic matter, pH, EC, extractable N, P, K, microbial biomass C and N, potentially mineralizable N or N in organic residues, and soil respiration (Lal, 1998). Physical indicators considered were mostly related to soil water retention and transport, and soil erosion; chemical attributes were related to soil fertility and thresholds for microbial activity and biological indicators were related to potential microbial activity (Lal, 1998).

More recently, The Comprehensive Assessment of Soil Health (CASH) index was developed by Cornell University using 15 physical, chemical, and biological indicators. These

were: soil texture, wet aggregate stability, available water capacity, root penetration resistance, active carbon, extractable protein, and root health rating (Fine et al., 2017; Moebius-Clune, 2016). For the index, the scoring weight of each indicator was calculated as a cumulative normal distribution considering their mean and variation and derived from a Gaussian function (Fine et al., 2017; Moebius-Clune, 2016).

Commonly used physical indicators include saturated hydraulic conductivity and porosity, which also provide insights into the soil compaction and aeration, which are also a reflection of management practices or ecological events (Norris et al., 2020; Pankhurst & CAB International, 1997). Chemical indicators include feature most pertinent to soil suitability and toxicology for plant growth, such as Cation Exchange Capacity (CEC), major elements, nutrients availability, heavy metals, and for arid regions, Sodium Adsorption Ratio (SAR), (Pankhurst & CAB International, 1997). Biological indicators include enzymatic activity, abundance of microorganisms, abundance of soil fauna, root disease, soil biodiversity, food web structure, plant growth and plant diversity among others, which accounts for the above-ground and below-ground organisms in soil, at micro and macro scales (Pankhurst & CAB International, 1997).

1.1.2.1. Limitations of soil health indexes

Despite the notable advantages of soil health indexes, there is bias in their interpretation and use. For instance, most soil quality indexes do not explicitly include biological factors (Amacher et al., 2007; Lal, 1998). Biological attributes are often inferred from physico-chemical indicators (Amacher et al., 2007; Lal, 1998). However, the variability of these attributes may differ since biological indicators may respond differently to perturbations (Pérez-Valera et al., 2015). Furthermore, the resolutions of biological parameters largely depend on the scale of the measurements, which could lead to inaccurate estimation of these attributes (Meyer et al., 2018).

Some of the methods used for the assessment of biological indicators only account for specific functions of soil and for a limited and selective proportion of the entire biological communities (Lal, 1998). Moreover, there is no consensus on how soil health is assessed, how each soil health indicator should be interpreted, and how indicative each type of soil attribute is for the overall soil health (Xue et al., 2019).

Also, identifying the spatiotemporal variability of each soil health indicator is needed to determine its suitability to reveal both short-term and long-term changes in soil health, and also to ensure the repeatability of the use of each indicator, by excluding those with low and large variability (Carini et al., 2020; Fierer et al., 2021; Hurisso et al., 2018). Considering the variation of soil health indicators in time and space is also crucial to guarantee they are representative of the real status of an entire landscape or region (Carini et al., 2020; Doran & Parkin, 1994a; Fierer et al., 2021; Lal & Soil and Water Conservation Society (U.S.), 1994). However, discrepancies still exist regarding sampling scale and method required for the use of each index, ranging from sampling a single point in a land space, to large plots or transects, and from single moments in time to seasonal or yearly sampling events (Lal, 1998).

1.1.3. History of soil health in North America

In North America, particular interest in soil quality was awakened after the droughts of the 1930s or the so-called “Dust Bowl" years, in which dust storms, soil erosion (i.e., susceptibility to wind action), crop failure, and the complete loss of topsoil were common in the southern Great Plains, leading to economic depression and raising concerns about soil degradation (Baveye et al., 2011; Hansen & Libecap, 2004; Montanarella, 2015). During these years, the temperatures of the sea-surface and the atmospheric circulations caused intensified droughts in the Southern Great Plains (Amacher et al., 2007; Lee & Gill, 2015). Added to the

droughts, the dry techniques (non-irrigated farming in arid regions) and the intensive tillage practiced for agriculture at the time caused loss of soil cohesion (i.e., force that binds particles together) and land cover, which in turn lead to soil erodibility (Lee & Gill, 2015). In response to the "Dust Bowl" years, soil has been recognized as a non-renewable resource that requires attention for its conservation (Montanarella, 2015). Therefore, governments and scientific research to evaluate and monitor soil condition as well as to promote sustainable agricultural practices that ensure the conservation of the soil resource are widespread (Baveye et al., 2011; Cathcart et al., 2008; Hansen & Libecap, 2004; Montanarella, 2015). To prevent soil erosion, irrigation systems were implemented, as were deep plowing techniques to replace clay to the soil surface after being translocated by water percolation (i.e., water movement through the soil matrix) (Lee & Gill, 2015). Tillage intensity was reduced and has been performed with crop residue on the land surface (Lee, 2015). Since the 1930s the governments started motivating landowners to plant native grasslands as part of soil conservation programs, some included economic incentives such as the 'Soil Bank Program' started in the 1950s in the U.S., and the 'Conservation Reserve Program' started in 1985 to the current date in Canada (Lee, 2015).

Another threat for soil conservation is desertification (i.e., land degradation and loss of soil fertility) of the Great Plains due to the interaction and change of the urban, agricultural, and animal use of soil (Le Houérou, 1996). In other words, desertification occurs due to an abuse in the use of the resource (Le Houérou, 1996). The destruction of perennial vegetation leads to a cascade of effects that negatively impact soil health and functional capacity (Le Houérou, 1996). The loss of perennial vegetation causes a change in the input of organic matter to soil, which modifies (i) the physical structure of soil by destabilizing soil aggregates, leading to soil compaction and changing the water retention, drainage capacity, porosity, and oxygen

availability; (ii) the nutrients availability and chemical properties of soil, reducing biological activity and hindering biogeochemical cycles important for soil functionality and fertility (Le Houérou, 1996). These changes make soil more vulnerable to destruction and the phenomenon could be exacerbated by global climate change (Hatfield et al., 2014; Le Houérou, 1996). More frequent extreme weather events coupled with climatic changes could lead to increased soil erosion, soil temperature changes, reduced water availability, changes in soil organic matter content, reduced crop productivity and defense against new pathogenic organisms that benefit from the changing climate (Hatfield et al., 2014). All these changes together force human activity to modify patterns, including timing, location, and farming management practices, which in turn have an important impact on global food markets and economies (Hatfield et al., 2014).

1.2. Soil health and agriculture

Agricultural productivity is dependent on the soil's ability to provide ecosystem services and serve as a plant growth medium (Food and Agriculture Organization of the United Nations, 2017; Lal, 2016). Agriculture comprises the domestication of animals and plants for human consumption and is recognized as a key factor for the development of human civilizations (Harris & Fuller, 2014; "World Development Report 2008: Agriculture for Development," 2008). Agriculture is important because there is an increasing demand for food production as global human population continues to growth (Food and Agriculture Organization of the United Nations, 2017). Agricultural productivity is often associated with economic growth and poverty reduction (The World Bank, 2008). In contrast, low agricultural productivity prevents the industrial growth of a nation and this is reflected in a lower per capita income (Gollin et al., 2002).

By 2050 the global population is expected to reach 9 billion people that need to be fed (Hatfield et al., 2014). While agriculture benefits from soil, the increasing population along with the increasing consumption patterns and need for food, fiber and fuel production are imposing more and more stress and degradation on soil and water, both non-renewable resources, essential for agricultural production (Hatfield et al., 2014). Human demands have led to the conversion of natural ecosystems into large agricultural lands (Tardy et al., 2015). For instance, the global forest cover loss caused by the shift to croplands is around 5 million hectares a year, which corresponds to 24% of the total global annual forest cover loss (Curtis et al., 2018). In North America, grasslands are the most common land cover affected by land transformation, where almost 220 thousand km^2 of the Great Plains were converted to croplands from 2009 to 2015 (Gaworecki, 2016). It is estimated that more than 50% of the original temperate grassland had been affected by land conversion as of 2016 and keeps declining (Kraus, 2016).

Temperate grasslands, which include the Canadian Great Plains are crucial for biodiversity conservation (Kraus, 2016). This ecosystem is habitat of more than sixty animal species in Canada and play an important role in carbon sequestration and water filtration for several water streams (Kraus, 2016). In 2008, the temperate grassland ecosystem was identified as the *world's most endangered ecosystem* and the most affected by land use (Kraus, 2016). Beyond land conversion, land use intensification, mechanization, and the development of new agricultural practices also influence and alter soil properties and can contribute to soil degradation (Hatfield et al., 2014).

Under an agricultural framework, *soil health* is relevant because it determines farming sustainability and productivity to a large extent. Therefore, it is fundamental to determine and identify management practices that are sustainable in the long term, promote soil resource

conservation and help overcome climate change impact (Doran & Parkin, 1994b; Hatfield et al., 2014; Lal, 2016).

1.3. Soil microbiology

Microorganisms, specifically bacteria and fungi, are the most abundant belowground organisms in terms of both biomass and individuals per gram of soil, and they play important roles in many ecosystem functions (Coleman et al., 2018; Tardy et al., 2015; Trivedi et al., 2016). Microorganisms influence nutrient cycling and ensure the availability of nutrients for plant uptake by being involved in nitrification and fixation of N, solubilization of P, reduction of S, C sequestration, and decomposition of organic matter (Coleman et al., 2018; Fierer et al., 2021). Further, soil microorganisms can produce and promote the production of auxins that favor plant growth, largely by stimulating root development (Gusain et al., 2015; A. L. Khan et al., 2016). Some bacteria and fungi can establish symbiotic relationships with plants and protect them from diseases through antagonistic relationships with pathogenic bacteria, fungi, and other pathogenic organisms (Coleman et al., 2018; Saia et al., 2015; Trivedi et al., 2017). Additionally, microbes are important for the establishment of trophic relationships among soil organisms, principally by being a food source for nematodes and protozoans (Coleman et al., 2018). Given the numerous interactions of microbes with other above and below-soil organisms, the structure and diversity of microbial communities is influenced by them (Coleman et al., 2018; Gusain et al., 2015; A. L. Khan et al., 2016). Moreover, different microbial species have specific physicochemical requirements and limitations for their growth and metabolic activity. For instance, microorganisms require specific conditions of air and water in soil, specific substrates, and specific pH and temperature ranges (Coleman et al., 2018). Therefore, the environmental characteristics that shape the soil microbial habitat also influences the structure and diversity of

microbial communities (Bowles et al., 2014; Kuzyakov & Blagodatskaya, 2015; Tardy et al., 2015; Trivedi et al., 2016; Habig & Swanepoel, 2015).

1.3.1. Microbial community diversity, structure, interactions, and ecosystem functionality

Multifunctionality is understood as multiple ecosystem functions or services that occur simultaneously in the system (Delgado-Baquerizo et al., 2016). In order to examine the relationship between microbial diversity and ecosystem services in soil, a multifunctionality index was developed derived from the plant productivity, net nitrogen mineralization in soil, concentration of nitrate, ammonium, DNA and available phosphorus (Delgado-Baquerizo et al., 2016). Results from the study suggest a positive correlation between overall microbial diversity and ecosystem multifunctionality for both bacteria and fungi in different ecosystems including, grasslands, woodlands, and arable lands (Delgado-Baquerizo et al., 2016). As biodiversity increases, the ecosystem functionality increases. Biodiversity could refer to phylogenetic and morphological diversity, species richness (i.e., number of species) or evenness (i.e., species relative abundance) (Purvis & Hector, 2000). The correlation between biodiversity and ecosystem functionality is due to three main reasons: (i) Different species have different resource needs; therefore, communities with higher richness utilize more of the overall soil resource, increasing productivity in turn (i.e. resource partitioning) (Bell et al., 2005; Loreau & Hector, 2001; Louca et al., 2018). (ii) Given that some species have a higher contribution to the ecosystem functionality (i.e., Keystone taxa), communities with higher species richness have a higher chance of including keystone taxa in its composition (Bell et al., 2005; Loreau & Hector, 2001). (iii) Individual ecosystem functions are shared by different taxa (i.e., functional

redundancy). Consequently, communities with higher diversity have a higher chance of maintaining ecosystem functions after environmental changes (Yachi & Loreau, 1999).

While diversity of bacterial communities has been clearly correlated to ecosystem functionality, shifts in the structure of microbial communities is not consistently associated with changes in ecosystem functionality (Bell et al., 2005; Bissett et al., 2011; Jeanbille et al., 2016; Yachi & Loreau, 1999). Some studies indicate that functional redundancy implies communities with different structure not necessarily differ in their functionality (Bissett et al., 2011), while other studies have reported that shifts in the structure of microbial communities are associated with physico-chemical factors that reflect ecosystem functionality or below-ground processes (Jeanbille et al., 2016). For instance, when evaluating archaeal, fungal, and bacterial community structures form different farmed soil systems and unfarmed soils in Australia, multivariate analysis of Terminal restriction fragment length polymorphism (T-RFLP) data revealed significantly different microbial communities in farmed and unfarmed systems (Bissett et al., 2011). However, community level physiological profiling (CCLPP) exhibited no differences in the functionality of microbial communities from agricultural sites compared to that of communities in unfarmed soils (Bissett et al., 2011). Moreover, actual and potential N transformation evaluated through targeted extra-cellular enzyme assays (EEA) before and after fertilizer addition respectively, revealed only significant differences in the nitrification potential, which was significantly higher in unfarmed sites compared to farmed soils (Bissett et al., 2011). These results indicate the ecosystem functionality does reflect the microbial community structure differences between farmed and unfarmed soils, despite N transformation potential differing between the two communities (Bissett et al., 2011). In contrast, clear association of the structure of microbial community and ecosystem functionality was identified under the influence of soil

pH (Jeanbille et al., 2016). 16S rRNA marker gene analyses indicated changes in pH across topographic sequences under the same land cover led to changes in microbial community structure (Jeanbille et al., 2016). Structural changes of the community included differences in the abundance of Keystone taxa correlated the metabolic potential of polysaccharide and monosaccharides (Jeanbille et al., 2016).

The contradictory results in the numerous studies regarding the relationship between soil bacterial community composition and ecosystem functionality could be attributed to some level to the sensitivity and accuracy of the methods used for assessing functionality, or to the level of patchiness in the different systems (Pérez-Valera et al., 2015). The relationship between edaphic parameters, overall microbial diversity and ecosystem functionality may only be evident in highly heterogeneous systems, whereas in systems with low heterogeneity, the microbial community structure may drive soil functionality to a greater extent (Pérez-Valera et al., 2015). Given the sensitivity of bacterial communities to the changing environment, the behavior of microbial communities may present high variation between different systems and sites (Pérez-Valera et al., 2015). Overcoming those limitations and revealing the association of microbial dynamics with microbial and ecosystem functionality remains a challenge that requires both experimental and modeling approaches combined (Widder et al., 2016).

Coexistence of microbial species reflects the environmental processes that shaped their communities (Pérez-Valera et al., 2015). Thus, the interactions between the members of a microbial community provide insights regarding the community functionality (Bissett et al., 2011; Cardona et al., 2016; Faust & Raes, 2012). It has been reported that in non-patchy systems, the coexistence of different taxa is a better predictor of soil microbial functionality when compared to overall diversity (Pérez-Valera et al., 2015). Changes in microbial interaction can

occur before species exclusion and can sooner reveal variation in microbial communities that diversity parameters cannot (Karimi et al., 2017). Co-occurrence network analysis are used to recognize patterns in coexistence and interactions of microbial communities; and also allow the identifications keystone taxa link to environmental processes (Banerjee et al., 2019; Karimi et al., 2017; Ma et al., 2018; Zheng et al., 2018). Altogether, the structure, diversity and dynamics of soil microbial communities are important for determining and preserving essential functions in soil, though in specific systems one may play a major role than the other (Girvan et al., 2005; Pérez-Valera et al., 2015; Tardy et al., 2015). Therefore, it is important to determine how the three aspects of microbial communities are affected by the influence of environmental changes in different systems and by the influence of other soil organisms (Bowles et al., 2014; Coleman et al., 2018; Delgado-Baquerizo et al., 2016; Kuzyakov & Blagodatskaya, 2015; Louca et al., 2018; Pérez-Valera et al., 2015; Tardy et al., 2015; Trivedi et al., 2016).

1.3.2 Soil physico-chemistry and soil microbial community association

Several studies suggest the composition of microbial communities is regulated by different physico-chemical parameters in different ecosystems, these parameters include soil organic carbon content, pH, water content, soil salinity, and soil porosity and compaction (Delgado-Baquerizo et al., 2016; Fierer & Jackson, 2006; Jeanbille et al., 2016; Lin et al., 2019; Rousk et al., 2010). However, among these parameters, pH is the only to be consistent across studies and has shown a clear correlation with soil microbial community composition at both large and small scales in different ecosystems (Fierer & Jackson, 2006; Rousk et al., 2010; Delgado-Baquerizo, 2017). Soil pH influences microbial growth, diversity, structure, and activity, which in turn affects soil functionality (Cai et al., 2018; Chen et al., 2018; Fierer & Jackson, 2006; J. Liu et al., 2018; Oehl et al., 2017; Rousk et al., 2010; Sheng & Zhu, 2018; C.

Wang et al., 2018; Zhou et al., 2017). Delgado-Baquerizo et al, (2017) suggest that the influence of microbial community composition and diversity on soil multifunctionality can be controlled by changes in soil pH. For instance, when evaluating the relationship between soil pH and microbial carbon cycling in acidic soils subjected to different land use intensification levels, where liming is applied, two different microbial mechanisms for SOC accumulation were identified (Malik et al., 2018): (i) when soil pH increases above an identified threshold of 6.2, acidic stress is alleviated leading to an increase in carbon use efficiency (CUE) as microbial biomass is synthesized (Malik et al., 2018). (ii) Below a pH of 6.2 microbial growth and organic matter decomposition are reduced, leading to unutilized organic C accumulation (Malik et al., 2018).

1.3.3. Studying soil microbial communities

For years, the characterization and quantification of soil microbial community composition relied on low-resolution techniques that offered broad and limited information. For instance, only a small proportion of the known microorganisms are culturable and can be recovered with culture-dependent techniques such as enrichment and isolation cultures (Coleman et al., 2018), which can lead to underestimation of microbial diversity in the system. Other methods based on substrate-induced respiration select only for fast growing members of the microbial communities with metabolic pathways specific for the evaluated substrates, and therefore, underestimating the metabolic diversity of complex systems (Coleman et al., 2018; Elsas et al., 1997; Vieira & Nahas, 2005).

More recently, DNA-based analysis introduced a promising alternative to study those under-looked microbial communities and their functionality in soil (Fierer et al., 2021; Norris et al., 2020). There is still no consensus on the use of genomic methods as indicators of soil health;

however, some approaches have been proposed. For example, high-throughput sequencing of marker genes (e.g., 16S rRNA gene for the study of bacterial and archaeal communities), which are genes that are present in all organisms from a same phylogenetic lineage but variable enough to allow identify different taxonomic groups, have been broadly used to assess microbial community structure, and diversity (Armalytė et al., 2019; Cai et al., 2018; Chen et al., 2018; Lupatini et al., 2017; Merloti et al., 2019; Oehl et al., 2017; Ren et al., 2016; Wolińska et al., 2017). Marker genes are used to infer the abundance of microbial taxa groups and allow to evaluate the correlation between shift in community composition and other soil health indicators (Fierer et al., 2021). Microbial taxa are obtained from the high-throughput sequencing of marker genes as Amplicon Sequence Variants (ASVs), which are sequences that vary in single nucleotides, and represent microbial species based on their DNA (Callahan et al., 2017). Taxonomy is assigned to each individual specie by comparing to a taxonomic database (Callahan et al., 2017). High throughput sequencing of marker genes is also used to identify indicator species, which are species associated to certain soil conditions, for example to a specific pH or temperature, or to specific soil functions, such as nitrifying organisms, P solubilizers, OM decomposers, methanogens (Fierer et al., 2021). Thus, indicator species can be used to infer soil functions in a fast and low-cost manner, providing a practical way for decision-making and land management (Fierer et al., 2021). Although barcoding of amplicon sequences alone allows for thorough characterization of microbial community structure, it does not provide information about soil microbial functionality (Fierer et al., 2021). Also, this method relies heavily in complex multivariate analysis, such as ordinations used to compare the composition of different microbial communities, which have complex interpretations.

-Omic- approaches are the different disciplines aiming to obtain a holistic view of collective communities based on different biological molecules, and they could be used to assess soil microbial functionality (Fierer et al., 2021; Prosser, 2015). For instance, to characterize microbial communities with metagenomic approaches, all microbial genomes in an environmental sample are studied, and by evaluating gene composition of the metagenomes, the metabolic potential of microbial communities is assessed, providing information about soil potential functionality (Kroeger et al., 2018; Manoharan et al., 2017; Miura et al., 2019). Metagenome analyses could be either genome- or gene-centric (Nissen et al., 2021; Taş et al., 2021). Genome-centric metagenomics consist in the reconstruction of entire genomes by "grouping metagenomic sequences of an organisms of origin" (Nissen et al., 2021), which allow the functional characterization of unknown organisms (Nissen et al., 2021). On the other hand, gene-centric approaches are targeted and focus on specific genes by annotation of short sequences, and it is used to quantify gene abundance (Taş et al., 2021).

Other "-omic" approaches are useful to assess direct functionality of microbial communities in environmental samples and better understand processes occurring in soil (Baraniya et al., 2018; Malik et al., 2018; Nair & Raja, 2017; Vailati-Riboni et al., 2017). For example, meta-transcriptomics and meta-proteomics are used to study messenger RNA molecules and proteins of entire communities, respectively, providing insight into the actual contribution of biological communities to the system functionality (Dubey et al., 2020).

1.3.4. Microbial communities in agricultural systems

The contribution of soil bacterial and fungal communities to soil functionality has been broadly studied (Delgado-Baquerizo et al., 2016; Louca et al., 2018; Pérez-Valera et al., 2015). For instance, in agricultural systems microbial functionality contributes to soil fertility, which

affects crop yields (Coleman et al., 2018). Every cultivated crop or plant requires soil nutrients for growth that microbes make available (Coleman et al., 2018). Overall, N and P are the most studied limited nutrients among agricultural soil (Brady & Weil, 2010). These nutrients are mineralized while organic matter is decomposed by microbes (Coleman et al., 2018). To satisfy C needs microbes access organic matter, while obtaining organic N and P from this source, which are nutrients needed for the synthesis of proteins and amino acids (Coleman et al., 2018). By the effect of enzymatic activity, N and P excess is released to the soil matrix and made available for plants or microbial transformations in form of ammonium and phosphates (inorganic forms of these nutrients), respectively (Coleman et al., 2018). Agricultural practices could potentially shape microbial communities with different potential metabolic capacities to cycle C and mineralize N and P (Schimel et al, 2012; Bissett et al., 2011; Habig & Swanepoel, 2015). When comparing till vs. no-tillage systems, Habig & Swanepoel (2015) reported that no-tillage increased microbial diversity and the associated capacity to decompose organic matter, and mineralize N and P. The structure of microbial communities also drives C cycling in soil (Habig & Swanepoel, 2015).

1.3.4.1. Impact of agricultural practices on microbial community parameters: previous insights

Many soil functions are the result of biological processes performed by belowground organisms (Brady & Weil, 2010; Coleman et al., 2018). The intensification of land use imposed by agriculture affects those processes either directly by the loss of biodiversity or indirectly by changing physical and chemical environmental conditions that consequently affect the functional groups of microorganisms (Cai et al., 2018; Chen et al., 2018; Merloti et al., 2019). For instance, microorganisms, earthworms, and microarthropods in soil are affected by the broad use of fertilizers and pesticides, high intensity tillage, and low plant diversity (Bardgett & Cook, 1998;

Doran & Werner, 1990; Edwards et al., 2020; F. Miura et al., 2008). The increasing use of heavy machinery in arable land causes soil compaction, which in turn leads to the loss of soil functions due to decreased hydraulic conductivity and mechanical inhibition of root growth (de Lima et al., 2017; Keller et al., 2019; Shah et al., 2017; Stoessel et al., 2018). The disruption of root growth causes a reduction in crop yield and affects the microbial environment (de Lima et al., 2017; Keller et al., 2019; Shah et al., 2017; Stoessel et al., 2018).

Different farming systems can shape soil physico-chemical properties and the composition of microbial communities (Habig & Swanepoel, 2015). For instance, when comparing soil physicochemical parameters and microbial composition under different un-fertilized or fertilized systems, and systems with either chemical or biological plant protection, both nutrient content and pH vary across the different farming systems (Hartmann et al., 2015). Interestingly, unfertilized soils differ the most among all the treatments in that study (Hartmann et al., 2015). Bacterial and fungal communities differ between fertilized and unfertilized soils, where 49% of the taxa identified are associated with the management system and 10% of these taxa determined the differences among the soil microbial communities (Hartmann et al., 2015). Overall, crops biologically protected and fertilized with compost harbor the highest microbial diversity, whereas mineral fertilization and chemical plant protection harbor bacterial and fungal communities with the least diversity (Hartmann et al., 2015). Likewise, a study evaluating the influence of organic fertilization, crop rotation and tillage systems on soil microbial diversity suggests that zero-tillage and highly fertilized systems promote soil microbial diversity, while different cropping systems could either promote or reduce diversity (Habig & Swanepoel, 2015). Crops represent an important C source for soil microorganisms (Habig & Swanepoel, 2015). Different crop rotations could ensure the input of multiple C types, which along with the direct

input of SOM from organic fertilization and the low disturbance in zero-tillage systems create new niches, increasing the number of microbial species that can utilize the available nutrients (Habig & Swanepoel, 2015).

Several studies have addressed the impact of agricultural practices on microbial communities using high throughput sequencing (Cai et al., 2018; Chen et al., 2018; Merloti et al., 2019; Oehl et al., 2017; Wolińska et al., 2017). For instance, barcoding of the ITS and 16S rRNA marker genes revealed that fungal diversity is affected by farming systems to a larger extent than bacterial diversity when evaluating the long-term impact of different cropping systems on microbial communities (Chen et al., 2018). Both bacterial and fungal communities in farming systems were different from those found in forest plantation and abandoned agricultural sites considered as natural succession ecosystems (Chen et al., 2018). Agricultural systems play an important role in regulating the abundance of habitat-specific taxa in both fungal and bacterial communities, the mechanism of which is proposed to be through the influence of varying soil pH and N from different fertilization methods (Chen et al., 2018). To evaluate the impact of land conversion to agriculture, a study conducted in China compared microbial communities under different land uses at several stages of forest succession and in agricultural land (Cai et al., 2018). Changes in the differential abundance of the dominant taxa were observed, and site-specific genera were identified for both bacterial and fungal communities (Cai et al., 2018). However, a significant reduction in fungal richness and an increase in bacterial richness was detected in agricultural soils compared to forest succession soils, with no differences in overall microbial diversity across any of the sites (Cai et al., 2018). Likewise, shifts in microbial communities as a result of forest conversion into croplands in the Amazon have been reported as a consequence of the subsequent changes in the soil chemistry (Merloti et al., 2019). For

example, qPCR results revealed a higher abundance of nitrifying and denitrifying bacteria in agricultural soils when compared to forest soils (Merloti et al., 2019).

Furthermore, arbuscular mycorrhizal fungi (AMF) richness decreases in croplands compared to grasslands; and patterns in the distribution of specific species have been identified (Oehl et al., 2017). For instance, some species are associated with land use intensity, others with nutrient availability, and others with parameters related to soil pH, which indicates the potential of specific species as indicators of soil functions (Oehl et al., 2017). In one particularly clear example of using microbial markers of soil function, a comparative analysis of the abundance of *Bacteroidetes* in arable soils and wetlands showed a reduction of the phylum abundance in arable soils, and a preference of its members to colonize soils originated from loess material and particles from standing water over soils originating from limestone parent material (Wolińska et al., 2017). Given the sensitivity of *Bacteroidetes* species to land use, and their association with specific soil types, the phylum is proposed as a suitable indicator of these two soil characteristics (Wolińska et al., 2017).

When comparing the microbial community of soils under conventional management with tillage, conventional management with no-tillage, and organic management systems with moldboard plough tillage, no significant differences were identified in the overall microbial diversity of the three systems (Banerjee et al., 2019). However, the three farming systems harbor different bacterial communities, indicating a significant influence of the agricultural practices on community structure, but not on the overall diversity (Banerjee et al., 2019). These differences were further studied through co-occurrence network analysis, revealing that organic practices among all farming systems promote the highest number of interacting species and interactions between them (Banerjee et al., 2019). The authors argue that organic management of soils

represents a lower land use intensity with higher resilience (i.e., capacity to overcome environmental stress, without the loss of functionality in the system) than conventionally managed systems, which is reflected in the complexity of the network connectivity and the higher abundance of keystone taxa in organic soils (Banerjee et al., 2019).

In recent years, the study of the effect of organic and conventional agricultural practices on microbial communities have received particular attention (Armalytė et al., 2019; Habig & Swanepoel, 2015; Lupatini et al., 2017). Using high throughput sequencing of the 16S rRNA marker gene, the structure, diversity, and richness of bacterial communities were evaluated under conventional and organic managed farmlands with chemical or non-chemical pathogenic control practices (Lupatini et al., 2017). Results from this study show higher richness and more heterogeneous and diverse microbial communities under non-chemical fertilization practices regardless of the pathogenic control method used (Lupatini et al., 2017). However, chemical, and non-chemical pathogen control practices do not affect the heterogeneity or diversity of microbial communities and only change the community composition, with overrepresentation of specific taxa under non-chemical fertilization and control practices (Lupatini et al., 2017). Aligned with these results, soils with similar structure and pH, show stable and similar microbial communities under conventional and organic farming systems, with no significant differences in any of the highly abundant taxa at the phylum level (Armalytė et al., 2019). However, at the genus level, there are small differences in the abundance of the least prevalent bacterial genera; while *Rhodanobacter* is found exclusively in conventional systems (Armalytė et al., 2019).

Individual agricultural practices could have different impacts on soil microbial communities (Habig & Swanepoel, 2015). The direct input of organic fertilizers, such as compost or manure increases soil organic matter and therefore, triggers an increase in the

number of microorganisms that can utilize the available substrates (Habig & Swanepoel, 2015). Furthermore, the replacement of tillage with less disruptive plant protection methods, such as manual weed removal or biological control, allows more vulnerable microorganisms to proliferate and thrive in the environment, thereby contributing to increased soil microbial diversity (Lupwayi et al., 1998). In long-term studies, crop rotation systems have also revealed an impact on microbial community composition (Chavarría et al., 2016). For instance, soil undergoing monoculture for decades showed lower microbial diversity than those subjected to plant rotation systems (Chavarría et al., 2016). This difference is likely due to the single type of C input from monoculture sources, which over time narrows the niche to specialized microorganisms (Chavarría et al., 2016). Moreover, the crop type heavily influences fungal diversity in agricultural systems, primarily by the establishment of symbiotic relationships of fungi with specific plant families (Coleman et al., 2018; Hartmann et al., 2015).

1.4. Regenerative agriculture

Agricultural systems drastically change soil environments and represent a threat to biodiversity conservation compared to natural ecosystems (Rodrigues et al., 2013). For example, the transformation of forest to agricultural lands results in the homogenization of soil microbial communities (Rodrigues et al., 2013). Therefore, considering that some agricultural practices represent more disruption to the soil environment than others, regenerative agriculture aims to improve approaches and reduce the conflict between productive land use and biodiversity conservation in agricultural lands (Bender et al., 2016).

Regenerative agriculture involves an alternative land management approach, which includes the use of less disruptive practices to promote conservation of more diverse and

functional microbial communities (Bender et al., 2016). Under a sustainable agricultural framework, crop management promotes the elimination of tillage and the selection of plants to ensure diversity through the implementation of crop rotation, intercropping systems, or N-fixing legumes alternation (Bender et al., 2016). These cropping practices can stimulate the formation and input of organic matter and the creation of new habitats for diverse microbial communities, while it also enhances the availability of growth-limiting nutrients (Bender et al., 2016). Further, sustainable agricultural management considers soil bio-augmentation practices, which consists of the genetic manipulation and direct addition of functional microbial organisms to soil (Bender et al., 2016). Genetic manipulation refers to plant breeding and to the direct transformation of microbial-derived ecosystem functions to enhance nutrient cycling capacity, or to suppress denitrification mechanisms (Bender et al., 2016). In addition, the inoculation of beneficial organisms to soil, such as mycorrhiza or plant growth-promoting bacteria, could enhance nutrient uptake by plants, reduce nutrient loss, and stimulate C accumulation; contributing to soil health and plant productivity (Bender et al., 2016). Altogether, sustainable soil management strategies could also maximize OM accumulation and SOC storage by increasing microbial CUE. Through the selection of crop types and conservation strategies the input of OM increases to either generate positive feedback on microbial growth efficiency and biomass incorporation (Malik et al., 2018) or decrease the decomposition rates.

1.5. Soil Quality Monitoring Program (SQMP)

The Soil Quality Monitoring Program (SQMP) was an initiative started in 1997, aiming to: (i) determine and monitor soil quality across the province of Alberta, (ii) evaluate the impact of the common farming practices on soil health, and (iii) identify and promote sustainable agricultural practices that ensure the conservation of soil (Cathcart et al., 2008). The program

consisted of nine consecutive years of sampling, from 1998 to 2006, in which soils from forty-two established benchmark sites, representing the farming practices in each of the seven agricultural ecoregions of the province, were collected and analyzed. Pedological characterization of the sites was conducted as a first stage of the project (Cathcart et al., 2008). Soil sampling was conducted yearly, after harvest and before the soil reached 5 ℃, consisting of composite samples at three slope positions along a catena (Upper, Middle and Lower) and two depths (0–15cm and 15–30cm) at each site (Cathcart et al., 2008). Soil physical and chemical indicators considered included: soil texture, bulk density, organic matter, pH, electro conductivity, ammonium, nitrates, phosphates, potassium, sulfates, organic carbon, light fraction of carbon, and light fraction of nitrogen (Cathcart et al., 2008).

Over the course of the SQMP, the most common farming practices across Alberta consisted of annual cultivations, increasing forage usage in rotation, and reduced tillage, with irrigation practices in the Mixed Grassland ecoregion (Cathcart et al., 2008). Agricultural practices remained without major changes for the duration of the study (Cathcart et al., 2008). The temporal factor was mostly correlated with bulk density, which decreases over time, and to phosphorus concentrations which increases over time (Cathcart, 2008). The authors claimed that these two parameters are an important reflection of the long-term effect of farming practices (Cathcart et al., 2008).

Overall, the Northern regions of the province exhibit a higher content of organic carbon than the Southern regions, with lower carbon content in the upper slope position (Cathcart et al., 2008). The nutrient profile showed high levels of variation and no specific patterns (Cathcart et al., 2008). Soil texture, pH, cation exchange capacity and $CaCO_3$ differ between different ecoregions of Alberta (Cathcart et al., 2008). These differences can be attributed to soil

formation processes and location-specific agricultural practices, even though the association of the latter with the mentioned physico-chemical parameters and soil health was not directly evaluated in the study (Cathcart et al., 2008).

Survey data on farming practices was gathered for each benchmark site; however, this information was not integrated with the physical and chemical soil health indicators (Cathcart et al., 2008). Instead, main agricultural practices were grouped by ecoregion (Cathcart et al., 2008). Due to the high variation in physical and chemical soil health indicators, this approach did not allow the identification of patterns relevant to management practices (Cathcart et al., 2008). No biological indicators of soil health were included in the study (Cathcart et al., 2008; Chavarría et al., 2016; Habig & Swanepoel, 2015; Lupwayi et al., 1998). However, the data from the study remains available and soil samples collected were archived for future usage (Cathcart et al., 2008).

1.5.1. Agricultural Ecoregions of Alberta considered for the SQMP

National ecological classification of Alberta's territory consists of ecozones, ecoregions, and ecodistricts (from highest to lowest hierarchy) (Ecological Stratification Working Group, 1996). Ecozones correspond to units with common biotic and abiotic characteristics at a subcontinental level; within ecozones, ecoregions are characterized by shared climatic conditions and dominant vegetation; and within ecoregions, ecodistricts share land relief, soil type, and land use (Ecological Stratification Working Group, 1996). New classification systems have been proposed provincially, with different hierarchical levels that consist of natural regions and subregions that differ from those first introduced nationally (Downing & Pettapiece, 2006).

Most agricultural activity in the province of Alberta is located across seven ecoregions: Mixed Grasslands, Moist Mixed Grasslands, Aspen Parklands, Fescue Grasslands, Peace Lowland, Boreal Transition, and Mid-Boreal Uplands (Cathcart et al., 2008).

1.5.1.1. Mixed Grasslands ecoregions

As part of the Prairies ecozone, the Mixed Grasslands (MG) ecoregion is semi-arid and is dominated by short and mid-sized grasses and sedges, accounting for approximately 95% of the above-ground vegetation, with no tree species found, except for valleys were shading and limited growth of deciduous trees occurs (Downing & Pettapiece, 2006). In Alberta, this ecoregion extends across the southern border with the United States, covering around 4.5 million hectares of land, with a mean elevation of 795 meters above sea level (m.a.s.l.) (Cathcart et al., 2008; Downing & Pettapiece, 2006). The Mixed Grasslands have mean annual temperatures (MAT) of 5°C, 17.9°C for summer and -12.8°C during winter (Cathcart et al., 2008; Downing & Pettapiece, 2006). The mean annual precipitation fluctuates between 314 mm and 363 mm and moisture insufficiency is common during the summertime (Cathcart et al., 2008). The dominant soils in this ecoregion are Brown Chernozemic, Brunisolic and Solonetzic, predominantly with loamy texture, from glacial till, Cretaceous, lacustrine, and eolian deposits (Downing & Pettapiece, 2006). These conditions together mainly support the production of cereal grains; practicing fallow rotations is common in the region (Downing & Pettapiece, 2006).

1.5.1.2. Moist Mixed Grasslands

This ecoregion consists of the northern grasslands of the Prairies ecozone, with characteristic semi-arid conditions and a mean elevation of 880 m.a.s.l. (Cathcart et al., 2008; Downing & Pettapiece, 2006). The dominant vegetation are short and mid-sized grasses and deciduous shrubs. Deciduous trees can be found in valleys and river terraces (Downing &

Pettapiece, 2006). The dominant soils found in this ecoregion are Brown and Dark Brown Chernozems, and high occurrence of Solonetzic soils with sandy to clayey texture from glacial till and Cretaceous and lacustrine sediments (Downing & Pettapiece, 2006). The Moist Mixed Grasslands ecoregion presents a MAT of 2.5°C, with summer temperature of 16.9°C and winter temperature of -10.8°C (Cathcart et al., 2008; Downing & Pettapiece, 2006). Mean annual precipitation fluctuates between 368 mm and 422 mm (Cathcart et al., 2008). About 80% of the ecoregion is used for cultivation with focus on cereal grains and oilseeds; fallow rotations and minor irrigation in the southern region are standard agricultural practice (Downing & Pettapiece, 2006).

1.5.1.3. Aspen Parklands

In Alberta, this ecoregion as part of the Prairies ecozone extends throughout the northern apex of central Alberta and represents the transition between the Prairies and the boreal forests (Downing & Pettapiece, 2006). It is located in the transitional grassland climatic zone with a mean elevation of 775 m.a.s.l. (Cathcart et al., 2008; Downing & Pettapiece, 2006). Dominant vegetation consists of deciduous trees, mixed- tall shrubs, and fescue grasslands. Black Chernozemic and Gleysolic soils with loamy texture are predominant in the area, with Cretaceous shale, glacial till, lacustrine and fluvioglacial deposit parent material (Downing & Pettapiece, 2006). MAP fluctuates between 391 mm and 478 mm (Cathcart et al., 2008). The Aspen Parklands present a mean annual temperature of 1.5°C degrees, 16.5°C during summer, and –14.3°C during winter (Cathcart et al., 2008; Downing & Pettapiece, 2006). Soil conditions and fertility favors the productivity of several crops, such as a variety of cereal grains, oilseeds, forages, and specialty crops; continuous cropping of grain crops is common (Downing & Pettapiece, 2006).

1.5.1.4. Fescue Grasslands

This ecoregion consists of 14,926 km^2 and is found in the Chinook belt climatic zone in southwestern Alberta bordering the Rocky Mountain Foothills and is part of the Prairies ecozone, with a mean elevation of 1100 m.a.s.l. (Cathcart et al., 2008; Downing & Pettapiece, 2006). Dominant vegetation includes fescue grasslands, forbs, shrubs, and deciduous trees adjacent to watercourses in shaded locations (Downing & Pettapiece, 2006). Dark Brown and Black Chernozemic soils are predominant with loamy to clayey texture originating from shale, glacial till and lacustrine sediments (Downing & Pettapiece, 2006). Mean annual precipitation fluctuates between 427mm and 537mm, and the MAT is approximately 3.5°C, with 15.6°C during summer and -9.5°C during winter (Cathcart et al., 2008; Downing & Pettapiece, 2006). Agricultural practices include grazing, as well as grain and oilseed production, with tillage happening in the cultivated areas mostly in the northern portion of the ecoregion (Downing & Pettapiece, 2006).

1.5.1.5. Peace Lowland

As part of the Boreal Plains ecozone, this ecoregion extends across north-central Alberta and shows a sub-humid climate, with a mean elevation of 536 m.a.s.l. (Cathcart et al., 2008; Downing & Pettapiece, 2006). Dominant vegetation consists of deciduous trees, mixed-tall shrubs, and herbs. In Alberta Gray Luvisols, Solonetzic and Dark Gray Chernozemic soils are predominant, with clayey to sandy texture from till, and lacustrine and fluvial sediments (Downing & Pettapiece, 2006). This ecoregion presents a mean annual precipitation ranging from 435 mm to 517 mm and a MAT of 0.5°C, with 13.3°C during summer and -17.2°C during winter (Cathcart et al., 2008; Downing & Pettapiece, 2006). Agricultural activity in the region focuses on annual cropping of small grains and grasses (Downing & Pettapiece, 2006).

1.5.1.6. Boreal transition

This ecoregion encompasses central Alberta and is part of the Boreal Plains ecozone, with a sub-humid climate and a mean elevation of 697 m.as.l. (Cathcart et al., 2008; Downing & Pettapiece, 2006). Dominant vegetation consists of deciduous boreal forest, along with mixed herbs, tall shrubs, and sedges (Downing & Pettapiece, 2006). Gray Luvisolic and Dark Gray Chernozemic soils are predominant in the area, with Cretaceous shale glacial till and lacustrine sediments parent material (Downing & Pettapiece, 2006). Mean annual precipitation in the region fluctuates between 428 mm and 535 mm and presents a MAT of 1°C, 15.9°C during summer, and -15°C during winter (Cathcart et al., 2008; Downing & Pettapiece, 2006). Agricultural activity is important in the region with approximately 70% of the land designated to farming, dominated by cereal grains, oilseeds, and hay production (Downing & Pettapiece, 2006).

1.5.1.7. Mid-Boreal uplands

The Mid-Boreal Uplands consist of multiple separate upland areas. As part of the Boreal Plains ecozone, in Alberta this ecoregion extends throughout the Alberta Plateau from the north-central region of the province to the Rocky Mountains foothills. It has a sub-humid climate and a mean elevation of 640 m.a.s.l. (Cathcart et al., 2008; Downing & Pettapiece, 2006). Vegetation in these areas consists of mixed-boreal coniferous and deciduous forest (Downing & Pettapiece, 2006). Permafrost can rarely be found in peatlands and Gray Luvisolic soils are predominant, with low occurrence of Gleysolic and Brunisolic soils (Downing & Pettapiece, 2006). These soils exhibit mainly a loamy to clayey texture from Cretaceous shales, glacial till and lacustrine sediments, and coarse-textured soils from fluvioglacial sediments can also be found (Downing & Pettapiece, 2006). The mean annual precipitation in the region fluctuates between 400mm and

550mm, and the MAT is between -1°C and 1°C, with 15.5°C during summer and -16.4°C during winter (Cathcart et al., 2008; Downing & Pettapiece, 2006). Agricultural activity occurs in the southern portion of the ecoregion but is limited in the northern portion given the short growing season (Downing & Pettapiece, 2006).

1.6. Research project

1.6.1. Scientific problem

Agricultural practices and land intensification represent major changes and perturbations to the soil environment. These practices alter soil physical, chemical, and biological attributes, thereby affecting soil functionality and overall health. In Alberta, there are approximately 210,000 km^2 of land used for agricultural purposes and impacted by farming practices. Therefore, assessing soil health becomes fundamental to ensure the long-term sustainability of agriculture as well as the conservation of the soil resource. Using soil physical and chemical indicators, the SQMP attempted to establish baseline data to be used as part of a long-term monitor program to assess changes in soil health across the different land slope positions and agricultural ecoregions of Alberta. However, the soil attributes measured by the SQMP did not allow the identification of major patterns (Cathcart et al., 2008) and the differences in soil condition were mainly attributed to the slope position. No biological attributes of soil were included in the SQMP and the relationships between the findings and the agricultural practices were not assessed, leaving a knowledge gap regarding whether farming practices in the province are related to soil biological degradation. The challenge of this study therefore is to identify soil biological characteristics that can be measured in order to evaluate the impact of agricultural practices in the soil health

1.6.2. Research questions and hypothesis

Biological indicators of soil health were not included in the SQMP, in part due to the low practicability, and the high level of expertise required to do so (Cathcart et al., 2008). Considering the rapid growth of microorganisms along with their sensitivity to changes in soil physico-chemical properties and environmental conditions, such as those caused by agricultural practices, microbial communities appear to be suitable indicators of soil health in farming systems (Bowles et al., 2014; Tardy et al., 2015; Trivedi et al., 2016). Moreover, the SQMP findings indicate slope position and ecoregions may as well alter the soil physico-chemical environment in which microbes inhabit, possibly influencing microbial community structure and diversity in turn.

Promoting microbial diversity in agricultural soils is crucial to enhance system functionality, and in turn to promote soil health (Habig & Swanepoel, 2015). High-throughput sequencing techniques currently available bring an affordable and practical opportunity to assess microbial communities in soil. Therefore, in this study I addressed the following questions:

1. ***Do slope positions and ecoregions influence soil bacterial diversity and the structure of soil bacterial communities?***
2. ***How do agricultural practices in Alberta affect soil microbial community composition and diversity?***

I hypothesize that both slope position and ecoregions shape soil bacterial community structure and diversity. I also hypothesize that land management practices are important drivers of bacterial community structure and diversity. Hence, practices that are more disruptive to the

soil environment such as high tillage or chemical herbicide use, reduce soil microbial diversity and exhibit different microbial community structure than that of microbial communities undergoing more sustainable practices.

2. Chapter 2: Drivers of soil bacterial community composition in agricultural systems of Alberta and impact of farming practices on bacterial community structure, diversity, and dynamics.

2.1. Introduction

Soil provides multiple ecosystem functions (Coleman et al., 2018; Lal, 2016). Namely, soil supports biological productivity, maintains environmental quality, and promotes plant, animal, and human health (Lal, 2016). The continued capacity of soil to perform these functions as a "vital living system" is known as *soil health* (Lal, 2016). Soil health is a non-directly quantifiable feature that reflects multiple soil physical, chemical, and biological attributes. Thus, in order to assess and quantify soil health, several indexes have been developed, commonly overlooking soil biological attributes fundamental for soil functioning (Adetunji et al., 2017; Amacher et al., 2007; Fine et al., 2017; Laishram et al., 2012; Lal, 2016; Pankhurst & CAB International, 1997; Xue et al., 2019).

A soil health index is a single value that represents an integrative measure of the capacity of soil to perform ecosystem functions that imply the condition of soil (Adetunji et al., 2017; Bünemann et al., 2018; Pankhurst & CAB International, 1997). Given that soil health is a non-directly quantifiable feature that considers multiple soil attributes, it is assessed using soil physical, chemical and biological attributes as measurable proxy indicators of the soil condition at a point in time and space (Adetunji et al., 2017; Bünemann et al., 2018; Pankhurst & CAB International, 1997). Single indicators are not representative of the entire soil condition and processes and are nor equally relevant for soil health (Norris et al., 2020). Therefore, to achieve a holistic and more sensitive and accurate view of soil health, indicators for all three type of

attributes must be considered (Adetunji et al., 2017; Amacher et al., 2007; Fine et al., 2017; Laishram et al., 2012; Lal, 2016; Pankhurst & CAB International, 1997; Xue et al., 2019).

Some agricultural practices could potentially alter the soil's physical and chemical environment (Coleman et al., 2018; Lal, 2016). For instance, some heavy tillage practices cause physical disruption of the soil structure, could cause changes in soil aeration and drainage, and induce soil compaction in the long term (Ampoorter et al., 2007). The overuse of fertilizers, herbicides, and other chemical agents, can cause changes in the soil physicochemical properties; the availability, type, and amount of soil nutrients; and can trigger the accumulation of soil contaminants in soil (Carbonetto et al., 2014). Different crop types could also change soil chemistry (Habig & Swanepoel, 2015; Z. Li et al., 2021). Exudates from plants could potentially alter soil pH (Amacher et al., 2007; X. Li et al., 2014; Q. Liu et al., 2021; Niu et al., 2020), and different crops have different nutritional needs, which changes nutrients uptake from the soil (X. Li et al., 2014; Q. Liu et al., 2021; Niu et al., 2020). In agricultural systems, cover crops represent an important input of C to the soil (Duval et al., 2016). Different crops can provide different types of C, meaning that monocultures restrict the soil environment to a single C type (Duval et al., 2016). These physico-chemical and biological changes stress microbial communities living in soil and alter their composition and dynamics (Leff et al., 2015; Merloti et al., 2019).

In Alberta, the Soil Quality Monitoring Program (SQMP) was initiated in 1997 and aimed to monitor soil conditions across the province, assess the impact of the most common agricultural practices (Cathcart et al., 2008), and provide guidelines for management to landowners (Cathcart et al., 2008). The program was conducted over nine consecutive years with a yearly soil sampling of benchmark sites paired with a pedological characterization and a survey

about agricultural practices at each site (Cathcart et al., 2008). Throughout the province, there was a decrease in soil bulk density and an increase in soil phosphorus concentrations over time (Cathcart et al., 2008). Ecoregions also differed in their pH, cation exchange capacity (CEC), and $CaCO_3$ content; and these differences were attributed to different soil formation processes (Cathcart et al., 2008). The high variation in the nutrient profiles of the different soils of the province did not allow the identification of patterns or differences across ecoregion or slope positions (Cathcart et al., 2008). Organic C content was lower in the upper slope position of the landscape when compared to middle and lower slopes, and organic C was also higher in the northern regions of the province (Cathcart et al., 2008). The impact of the agricultural practices on the soil physical and chemical parameters was not assessed but information from the survey remains in a database (Cathcart et al., 2008).

Microorganisms are crucial for soil functioning (Coleman et al., 2018; Fierer et al., 2021; Gusain et al., 2015; A. L. Khan et al., 2016; Tardy et al., 2015; Trivedi et al., 2016). They are basal feeders of trophic relationships (Coleman et al., 2018), form symbiotic interactions with plants (Coleman et al., 2018), and they can promote or produce auxins that stimulate root growth (A. L. Khan et al., 2016). Finally, they are involved in nutrient cycling, making the latter available for plant uptake (Coleman et al., 2018; Fierer et al., 2021; Gusain et al., 2015; A. L. Khan et al., 2016; Tardy et al., 2015; Trivedi et al., 2016). However, different soil microorganisms require specific physical and chemical conditions for their growth and activity; and the structure of microbial communities is also influenced by above-ground and below-ground organisms (Bowles et al., 2014; Coleman et al., 2018; Kuzyakov & Blagodatskaya, 2015; Tardy et al., 2015; Trivedi et al., 2016). The contribution of microbial communities to soil

functionality along with their sensitivity to changes to the soil environment, make them suitable biological indicators of soil health in farming systems (Fierer et al., 2021).

No biological indicators were included in the original SQMP assessment, in part due to the lack of methods available for their accurate assessment at the time and the high level of expertise required to analyze results (Cathcart et al., 2008). Modern techniques such as high-throughput sequencing could provide a high level of resolution at a relatively low cost to assess microbial communities in soil (Fierer et al., 2021). Given the influence of the land slope position, ecoregions (Cathcart et al., 2008), and farming practices on the soil environment (Coleman et al., 2018; Lal, 2016), in addition to the potential sensitivity of microbial communities to changes in soil, this study aimed to address the following questions:

1. ***Do slope positions and ecoregions influence soil bacterial diversity and the structure of soil bacterial communities?***
2. ***How do agricultural practices in Alberta affect soil microbial community composition and diversity?***

The overall goal of the study is to identify soil biological characteristics that can be measured to evaluate the impact of agricultural practices on soil health. To address the objective, I revisited benchmark sites from the SQMP undergoing different crop types, tillage intensities, herbicides, and fertilization methods. Soils from these sites were distributed across the main agricultural ecoregions of Alberta and were evaluated at three different slope positions. Then, I characterized the physico-chemical environment and the bacterial communities of the soils using high-throughput sequencing of the 16S rRNA marker gene. I hypothesized that both slope position and ecoregions shape soil bacterial community structure and diversity. Also, I

hypothesized that agricultural practices are drivers of bacterial community structure and diversity; hence, (i) different crop types produce different soil microbial communities, (ii) physically disruptive tillage methods reduce soil microbial diversity and produce different microbial community structure than low till or no till methods, (iii) different fertilization methods produce different soil microbial communities, and (iv) chemical herbicide use reduces soil microbial diversity and produces different soil microbial communities than low or low herbicide usage.

2.2. Materials and methods

2.2.1. Soil sampling

Soil samples were collected from benchmark sites of the SQMP during the fall of 2019 after harvest once soil temperature reached ~5°C (Cathcart et al., 2008) (Figure S.1). Separate samples were collected for physico-chemical characterization and for microbial analyses. Twenty-four sites out of thirty-eight were sampled in 2019 due to the early onset of winter and soils freezing. The remaining 18 sites were sampled in fall 2020, following the same sampling protocols described here. Three soil samples (0–15 cm in depth) were collected for microbial analysis from each of three slope positions along a catena: upper (U), middle (M), and lower (L). For microbial analyses nine soil samples were collected per site using a soil sample probe of 5 cm diameter. Replicates of soil samples were collected as three parallel soil cores at each landscape position. For physico-chemical analyses, composite samples were collected as a mixture of ten core samples in a three-meter radius from a central point at each slope position, for a total of three composite samples per site. Samples for bulk density were collected using a density core sampler of 10 cm height × 10 cm diameter. All samples were placed in separate labelled plastic bags inside a cooler with blue ice for same-day transportation to the laboratory.

2.2.2. Soil physico-chemical parameters

Routine soil testing was performed by Element Materials Technology in Edmonton, AB by standard methods. Physical and chemical parameters measured included: bulk density, moisture content, pH, total carbon (C), total nitrogen (N), available ammonium (NH_4^+), available nitrate (NO_3^-), available phosphate PO_4^{-3}, base saturation (BS), total exchange capacity (TEC), sodium (Na^+), calcium (Ca^{+2}) carbon to nitrogen ratio (C:N), total organic matter content (OM), percentage of silt content (0.05 mm–2 mm particle size), percentage of clay content (< 2 mm particle size), percentage of sand content (2.0-0.05 mm particle size), electrical conductivity (EC), cation exchange capacity (CEC), and chloride (Cl^-).

2.2.3. Samples preparation, DNA extractions and sequencing

All soil samples for microbial analyses were homogenized by sieving at 4 mm, roots were removed by hand, and then soils were frozen at -80°C until DNA extraction was performed. Microbial DNA extractions were conducted in duplicates from 0.25 g of the sieved samples using the DNeasy PowerSoil Pro® kit (QIAGEN, Toronto, Canada), according to the manufacturer's instructions. Samples were processed in batches of seven or less along with a blank. Duplicates of the extraction products for each sample were combined and the DNA concentration of the combined samples was measured with Qubit™ dsDNA HS assay kit according to the manufacturer's protocol (Thermo Fisher Scientific, Ottawa, Canada). A mock community was created using DNA from 10 known different bacterial species as a positive control. Soil DNA samples, positive controls, and extraction blanks were sent to Microbiome Insights (Vancouver, Canada) for high throughput sequencing. Primers 515F and 806R targeting the V4 region of bacterial 16S rRNA were used to PCR-amplify the DNA of each sample (Caporaso et al., 2016). The resulting amplicon was sequenced by the ILLUMINA MiSeq

platform using the 250-bp paired-end kit ((V2 500-cycle PE Chemistry, Illumina, USA) based on the protocol recommended by the Earth Microbiome Project (EMP).

2.2.5. Bioinformatics

The quality profile of the demultiplexed sequences received from Microbiome Insights was evaluated separately for forward and reverse reads to determine the trimming and filtering parameters required, using the DADA2 package (Callahan et al., 2016) in Rstudio V. 1.3.959 (RStudio Team, 2020). The last ten nucleotides of the forward reads were trimmed at 240 bp, and the last thirty nucleotides of the reverse reads were trimmed at 220 bp. No 'N' nucleotides were allowed for further generation of Amplicon Sequence Variants (ASVs), and the parameters maxEE and truncQ were set as default (Callahan et al., 2016). ASVs consisted of inferred unique sequences from the core sample inference algorithm clustered at 100% of similarity in DADA2 and were considered as analogous to bacterial species in this study (Callahan et al., 2016). Pair-end reads were merged and mapped to the ASVs previously generated into a table including reads count (Callahan et al., 2016). Following this, chimeras were removed, and taxonomy was assigned using the Silva database v. 132 in DADA2 (Callahan et al., 2016). Given the large size of the combined data from 2019 and 2020, the initial steps of the sequences cleaning and the generation of ASVs were performed separately for the two years to improve the computational power for data processing. Identification of contaminant ASVs was conducted using the *Decontam* package (Davis et al., 2018), with combined methods of prevalence and frequency, and with batch assignation. Indicated contaminants were removed accordingly, obtaining a total of 13,352 ASVs for the 2019 samples and 10,587 ASVs for the 2020 samples. ASV tables from sampling years 2019 and 2020 were merged, obtaining a total of 18,827 ASVs. Data was normalized by rarefaction to 4522 reads (Supplementary Figure 2), which corresponded to the

number of reads of the sample with the lowest reads among all, using the "total group" algorithm in Mothur v.1.41.3. (Schloss et al., 2009), eliminating blank samples, and obtaining a total of 17,242 ASVs. Total number of reads obtained at the different stages of samples processing can be found in Supplementary Table 1. Finally, using the *phyloseq* package (McMurdie & Holmes, 2013) in R, ASVs corresponding to Archaea and mitochondria were removed.

2.2.3. Agricultural practices survey

Information regarding specific farming practices of the corresponding sampling year (2019 or 2020) was obtained from an online survey based on the original SQMP data (UofA Human Ethics Pro00092032). Surveys were voluntarily filled out by landowners or the producers from each of the sites; information was gathered for twenty-six of the thirty-eight sites (Supplementary Table 2). Information was extracted regarding farming practices, including crop type, tillage intensity, herbicide used, and fertilization methods. For this study, answers related to tillage intensity and herbicide used were assigned to predetermined categories for each practice accordingly as follows (i.) "*zero"* if no tillage practices were conducted, "*low"* if there was only one tillage pass, and "*high"* if there were two or more passes; (ii.) "*Glyphosate"* if the main active agent of the herbicide used was glyphosate, "*other"* if the main active agent of the herbicide used was different to glyphosate, including chlorophenol, ethalfluralin, tribenuron-methyl, and florasulam, or "*none"* if no herbicide was applied. Fertilization methods included fertilization *banded with seed*, *banded without seed*, *broadcast,* and *none*. Crop types included *alfalfa seeds*, *barley*, *canola*, *durum, fallow*, *forage*, *hay*, *livestock*, *sugar beets*, *wheat*, and *none.* Crops under the "*none"* category consisted only of abandoned sites. Given the low number of samples in some of the crop types, for the co-occurrence network analysis *fallow* sites were

combined with *none* sites, and *alfalfa seed* sites were combined with *sugar beets* sites under the "*special crops*" category.

2.2.6. Statistical analyses

Statistical analyses were conducted using RStudio v.1.3.959 (RStudio Team, 2020). Given the low variation in bacterial community structure observed among replicate samples of the same slope position at each site compared to that of samples between sites (Supplementary Figure 3), sample replicates were merged to obtain mean values for read counts of ASVs. After merging, only one mean sample from the upper slope, one mean sample from the middle slope, and one mean sample from the lower slope remained per site.

Since the initial SQMP survey showed differences in the organic matter content of different slope positions and ecoregions, the influence of these two factors on soil bacterial community composition was evaluated (Cathcart et al., 2008). Analysis comparing bacterial communities and physicochemical parameters across slopes and ecoregions was performed using the entire data set. However, for analyses of the agricultural practices data, only the twenty-six sites with complete surveys were considered. Each of the agricultural practices evaluated were treated separately due to low replicability of treatment combinations, i.e. lack of samples that represent all possible combinations of the four agricultural practices.

For each slope position, ecoregion, tillage intensity, crop type, herbicide, and fertilization system, the observed number of ASVs, the Chao1 richness estimator, Inverse Simpson Index, and Pielou's evenness index were calculated using the *phyloseq* (version 1.30.0; McMurdie & Holmes, 2013) and *microbiome* packages in R (version 1.8.0; Lahti & Shetty, 2017). For normally distributed data, an ANOVA test followed by a Tukey pairwise comparison test was performed to evaluate significant differences in α-diversity metrics across slope positions,

ecoregions and the different agricultural practices. If data were not normally distributed, a Kruskal Wallis test followed by a Dunn's pairwise comparison test was performed following a Benjamini & Hochberg adjustment to evaluate significance differences in α-diversity for each parameter tested. Relative abundance of the ASVs was calculated and taxonomic profiles of the different slope positions, ecoregions, and agricultural practices were visualized in stack bar plots generated using *phyloseq* V 1.30.0 and *ggplot2* V 3.3.3 (Wickham, 2016) packages in R. To compare the composition of the soil bacterial community between slopes, ecoregions and agricultural practices, a Hellinger transformation was applied to the ASV read counts table (Legendre & Borcard, 2018), and data were visualized in a non-metric multidimensional scaling ordination (NMDS) using Bray-Curtis dissimilarity in the *Vegan* V 2.5.6 (Oksanen et al., 2019) package in R. An PERMANOVA test was used to evaluate pairwise comparisons between the corresponding ordination clusters to evaluate differences in the composition of bacterial communities across slopes, ecoregions, and agricultural practices (Martinez, 2020). To examine differences in heterogeneity of bacterial communities withing each of the evaluated factors, a Bray-Curtis dissimilarity matrix was generated, and a pairwise-Wilcox rank test was performed on the matrix to examine the significance of the differences.

To visualize soil physico-chemical profiles across slope positions, ecoregions and agricultural practices, physico-chemical data was centered, scaled, and multicollinearity between variables was evaluated through a variance inflation factor test with a threshold of 10, using the *usdm* package V1.1-18 in R (Naimi et al., 2014). Highly correlated variables were excluded for further analysis: total N, total C, Sand content, and Na concentration. Following, data was visualized with a Principal Component Analysis (PCA) using Euclidean distance algorithm for each slope, ecoregion, tillage intensity, crop type, herbicide, and fertilization system. Slopes and ecoregion were compared using a pairwise PERMANOVAs (Martinez, 2020) test based on the

PCA ordination clusters. Differences in the heterogeneity of soil physico-chemical parameters were evaluated with the Euclidean distance matrix paired with a Kruskal-Wallis test followed by a pairwise-Wilcox rank test.

The top twenty Indicator species for each of the agricultural practices tested were identified through random forest modeling for predictor ASVs with 100 iterations, using *phyloseq* and *randomForest* V 4.6.14 (Liaw & Wiener, 2002) packages in R (Supplementary Figure 8). This model identifies species that are specific to an indicated habitat based on its prominence. In this study, indicator ASVs were identified as bacterial species that are affected by specific agricultural practices.

To examine the impact of farming systems on the bacterial community dynamics, interactions among soil bacterial communities undergoing the different agricultural practices were compared through co-occurrence network analysis using CoNet (Faust & Raes, 2016) in Cytoscape V 3.8.0 (Shannon, 2003). Accounting for the different number of samples in each category within an agricultural practice, data subsets were generated randomly based on the minimum samples in a category, e.g., for tillage intensity practices, if only three sites (nine samples) corresponded to high tillage, only three random sites were taken from low tillage and zero tillage for the network analyses. Only ASVs representing > 1% of the total community were included for co-occurrence analyses (Shannon, 2003). Networks were constructed with a 0.75 threshold for both Pearson and Spearman correlation, a 0.2 threshold for Bray Curtis dissimilarity distance, and a Fisher's Z P-value threshold of 0.05 for each subset of read counts table of ASVs, and their respective taxonomy and physico-chemical data (Shannon, 2003). Given the low number of samples in each network, no further filtration was applied, except for a parent-child exclusion (Shannon, 2003). Networks of each category within an agricultural practice were intersected and compared with an ANOVA test in R. Network aspects evaluated

included: (i) size, indicated by the number of nodes, i.e., participating ASVs, (ii) connectivity, indicated by the number of edges, i.e., interactions among the participating ASVs, (iii) behavior, calculated as positive to negative connections rate, (iv) complexity, indicated by the average degree, i.e., edges per node; (v.) modularity, indicated by the average cluster coefficient, i.e., proportion of potential links that are occurring, and (vi) centrality, indicated by the average closeness centrality, i.e., the distance between each pair of nodes (Banerjee et al., 2019; Karimi et al., 2017).

A multivariate regression tree was built with 100 cross validations to determine predictors of the soil bacterial community distribution among all physical, chemical, and agricultural parameters, using the *mvpart* package (version 1.6.2) in R (Therneau & Atkinson, 2014). Variables with positive co-linearity were also removed from the analysis as well as instantaneous moisture content because it is not an inherent attribute of soil (Naimi et al., 2014).

2.3. Results

2.3.1. Impact of slope position and ecoregion on soil attributes: re-evaluating SQMP findings

Non-significant results for the influence of slope position on the different soil attributes are found in the following supplementary material: Supplementary Tables 3 and 4, and Supplementary Figures 4.A, 5.A, and 6.A.

Soil physico-chemical properties and composition of soil bacterial communities did not differ across slope positions (Table 1). However, the variability of both physico-chemical properties and bacterial communities differ across slope positions. Physico-chemical properties of soils at mid-slope were the least variable, followed by soils at the upper slope, and lastly by

soils at the lower slope (Supplementary Figure 4.B). Bacterial communities at the lower slope position had a significantly higher heterogeneity than either middle or upper slope positions (Figure 5.B). Bacterial taxonomic profiles at the order level and alpha diversity indexes showed no differences according to slope. Overall, slope position only influenced the variability of soil bacterial communities and physico-chemical properties, but it did not affect bacterial community composition or diversity (Table 1).

Physical and chemical parameters of soils in the different ecoregions were significantly different (Figure 1.A; Supplementary Table 5); these differences can mostly be attributed to differences in the variability of these parameters. Variability was significantly lower in the Fescue Grasslands ecoregion, followed by Mixed Boreal and Moist Mixed grasslands ecoregion when compared to the other agricultural ecoregions of Alberta considered in this study (Figure 1.B). In a similar manner, bacterial community composition differed across ecoregions (Figure 2.A; Supplementary Table 6); the only exception was the bacterial communities in the Fescue Grasslands and the Moist Mixed Grassland ecoregions, which were not significantly different to each other. Differences in bacterial communities across ecoregions were likely influenced by their heterogeneity (Figure 2.B). There was comparable bacterial community heterogeneity in Aspen Parkland, Boreal Transition, Mixed Grassland, and Peace Lowland ecoregions. Bacterial community heterogeneity was significantly lower in Moist Mixed Grassland, Mixed Boreal, and Fescue Grassland ecoregions; the bacterial community heterogeneity in these three ecoregions did not differ significantly (Figure 2.B). These results follow the same trends found for the physical and chemical parameters, except for the Fescue Grassland ecoregion that had the lowest variability in soil physical and chemical parameters, but the most heterogeneous bacterial community, among all the ecoregions.

Taxonomic profiles of bacterial communities at the phylum level were similar across ecoregions (Figure 3.A). Nevertheless, specific dominant taxa were overrepresented according to the ecoregion, e.g., *Firmicutes* exhibited a higher abundance in the Mixed Boreal ecoregion when compared to all other regions (Figure 3.A). Among all alpha diversity indexes, only richness of bacterial communities was significantly different between the Mixed Grassland ecoregion and the Aspen Parkland ecoregion, as indicated by the Observed Chao1 indexes (Figure 4.A). Altogether, ecoregions influenced soil physico chemical parameters and their heterogeneity as well as bacterial community composition and diversity.

2.3.2. Impact of agricultural practices on soil attributes

Given the similar responses of bacterial community composition and diversity to the various agricultural practices, tillage intensities and crop types are presented as examples through the results and discussion referent to the different farming practices. Results about the impact of herbicide use and fertilizer are found in the following sections of the supplementary material: Supplementary Tables 11-14, and Supplementary Figures 4-7.

2.3.2.1. Impact of agricultural practices on soil physico-chemical parameters

Soil physico-chemical profiles did not differ across the evaluated agricultural practices (Table 1, Supplementary Tables 7, 9, 11 and 13), with the exception of tillage intensity, which exhibited different physico-chemical profiles between zero and no tillage (Figure 1.C; Supplementary Table 7). In all cases, the soil physico-chemical variability was influenced by the different agricultural practices evaluated (Table 1). Variability was significantly lower in sites with high tillage than in sites with zero and low tillage respectively (Figure 1.D). Among crop types, lower variability was observed in sites with hay, forage, or no cover crop (Figure 1.F). Sites where glyphosate was used exhibited significantly lower heterogeneity in the soil physico-chemical

parameters than sites that applied a non-glyphosate-based herbicide type or no herbicide (Supplementary Figure 4.D). Unfertilized sites and sites broadcast fertilized showed similar levels of physico-chemical heterogeneity, which was significantly higher than that of soils fertilized with banded methods (Supplementary Figure 4.F).

2.3.2.2. Impact of agricultural practices on soil bacterial community composition.

Despite bacterial community composition overlapping across the different agricultural practices, significant (i.e., p-value<0.05) and marginal (i.e., p-value between 0.05 and 0.1) differences were observed among them (Table 1; Supplementary Tables 8, 10, 12, 14). These differences were mainly attributed to bacterial heterogeneity. For example, soil bacterial communities under different tillage intensities exhibited significantly lower heterogeneity for *high* tillage compared to *low* and *zero* tillage (Figure 2.D). Across crop types, communities in *fallow* and *alfalfa* sites were distinct from those with other cover crops (Figure 2.E; Supplementary Table 10). Interestingly, bacterial communities in sites without a crop differed only when compared communities in *canola* crops (Supplementary Table 10). Relative to the other crops, *canola* harboured the most different bacterial communities, which were different from all other crop types except for communities in sites with *livestock* (Supplementary Table 10). Following *canola* crops, *barley* and *wheat* crops also exhibited different soil bacterial communities to those found in most crops considered in the study (Supplementary Table 10). Marginal differences were observed in bacterial communities between several crop types (Supplementary Table 10). Overall, differences observed among soil bacterial communities among crop types were also driven by heterogeneity, which was lower in sites with no crop or special crops, and higher in *wheat* crops and sites with *livestock* (Figure 2.F). Across herbicide treatments, significantly lower heterogeneity was observed for bacterial communities undergoing

the use of herbicides regardless of whether it was *glyphosate* or *other*, and when compared to sites with no herbicide use (Supplementary Figure 5.D). Across fertilization methods *fertilization banded without seed* exhibited the least heterogeneous communities followed by those from sites with *fertilization banded with seed*, and then by communities in sites fertilized *broadcast* or unfertilized indistinguishably (Supplementary Figure 5.F).

2.3.2.3. Impact of agricultural practices on bacterial diversity

Taxonomic profiles at the phylum level were similar across the different agricultural practices, with minimum discrepancies in the relative abundance of dominant taxa (Figures 3.B and 3.C; Supplementary Figures 6.B and 6.C). Richness indexes did not differ across any of the agricultural practices (Figures 4.B and 4.C; Supplementary Figures 7.B and 7.C). However, Pielou's index indicated an influence of the different farming practices on the evenness of bacterial communities, meaning that different practices may lead to an overrepresentation of specific taxa in the system. For example, sites with high tillage harbored communities with significantly lower evenness when compared to sites with low and high tillage intensities, with higher abundance of Verrucomicrobia and Gemmatimonadetes, and lower abundance of Bacteroidetes compared to communities under zero tillage (Figure 4.B). Moreover, crop type influenced both soil bacterial evenness and richness (Figure 4.C). For instance, the lowest evenness was found in communities of sugar beet crops compared to all crop types considered. Hay crops exhibited communities with the lowest richness, and the special crops (i.e., alfalfa seeds and sugar beets) showed communities with the highest richness among all crop types considered.

2.3.2.1. Impact of agricultural practices on co-occurrence networks of bacterial communities

Tillage practices altered the centrality, complexity, and behavior of the bacterial community. Co-occurrence network analysis indicated high tillage practices harbor soil bacterial communities with the lowest number of nodes but highest number of connections between them, when compared to low and zero tillage practices (Table 2.A, Supplementary Figure 9). Low tillage exhibited communities with the highest number of nodes among the tillage systems, and zero tillage showed the communities with the lowest number of edges (Table 2.A, Supplementary Figure 9). Positive to negative connections ratio was higher in soil communities with no tillage practices and lower in communities undergoing high tillage practices. Network average degree revealed higher complexity of soil bacterial communities under high tillage practices than that of communities under low or zero tillage (Table 2.A, Supplementary Figure 9). However, the clustering coefficient of bacterial communities only differed between high and low tillage, exhibiting higher centrality in high intensity systems.

Differences in the interactions of bacterial communities in different crop types were identified (Table 2.B, Supplementary Figure 10), indicating cover crop influence the dynamics of soil bacterial communities. Among all crop types, the lowest number of nodes was found in communities from special crops and the highest in communities from sites with livestock (Table 2.B, Supplementary Figure 10). Edges of connectivity were higher in communities from sites with no crops and lower in communities from special crops when compared to all crop types. Across crop types, positive to negative connections were higher in bacterial communities from canola crops and lower in communities from sites with no crop (Table 2.B, Supplementary Figure 10). Complexity of soil bacterial communities in uncultivated sites was significantly higher than in all crops analyzed (Table 2.B, Supplementary Figure 10). Bacterial communities

from special crops and uncultivated sites exhibited significantly higher closeness centrality and communities from canola crops exhibited lower centrality than communities from all other crops (Table 2.B, Supplementary Figure 10).

The different herbicides used influenced the interactions among the bacterial community. For instance, results revealed soil bacterial communities with a similar number of nodes but lower number of connections in sites where glyphosate was applied, when compared to sites that used other or no herbicides (Table 2.C, Supplementary Figure 11). A higher positive to negative connections ratio was also observed in bacterial communities undergoing the use of glyphosate (Table 2.C, Supplementary Figure 11). Moreover, the complexity of the community was significantly lower in sites that used glyphosate-based herbicides, followed by that of communities with no herbicide used (Table 2.C, Supplementary Figure 11). Both complexity and centrality were significantly higher in soil bacterial communities from sites that used non-glyphosate-based herbicides, compared to sites with no herbicide and sites where glyphosate was applied (Table 2.C, Supplementary Figure 11).

Likewise, co-occurrence network analysis indicated the fertilization method affected microbial community dynamics. Among all fertilization systems, soil bacterial communities with the highest number of nodes were found in unfertilized sites, followed by sites with fertilization banded with seed, and by sites with broadcast fertilization and fertilization banded without seed (Table 2.D; Supplementary Figure 12). The interactions of soil bacterial communities from sites where fertilizer was applied with seed were the most different, with the lowest number of edges, the highest number of positive to negative connections ratio, and with significantly lower average degree and closeness centrality than all other fertilization methods (Table 2.D; Supplementary Figure 12).

2.3.3. Predictors of soil bacterial community composition

Among all physico-chemical parameters and farming systems, only pH and ecoregions predicted bacterial community composition, but together, these two parameters only accounted for 14.8% of the biological variation, explaining 11.1% and 3.7% respectively (Figure 5). The multivariate regression tree analysis identified a threshold of 6.35 for the soil pH, at which the bacterial community composition differentially shifted. When soil pH was higher than the threshold, the ecoregion does not influence the bacterial community composition. In contrast, at pH lower than 6.35 bacterial communities from soils in the Mixed grassland and Peace Lowland ecoregions distinctively shifted from those the other ecoregions included in the study, indicating an influence of climate and soil type in biological variability. Contrary to the expected, no agricultural practices were found to be a driver of bacterial community composition in Alberta's soils.

2.4. Discussion

2.4.1. Ecoregion rather than slope position shape soil physico-chemical and biological attributes

I hypothesized that both slope position and ecoregions influence the composition of soil bacterial communities. However, contrary to the expected, my results indicate that slope position is not a major driver of soil bacterial community structure or diversity in Alberta's agricultural land. The influence of slope position on individual soil physico-chemical parameters has been widely reported (Cathcart et al., 2008; Khan et al., 2013; Miheretu & Yimer, 2018). Parameters such as soil bulk density, EC, available phosphorus, organic matter, total C, total N, pH, CEC, sand, clay, and silt content vary by the slope position in the land (Cathcart et al., 2008; F. Khan et al., 2013; Miheretu & Yimer, 2018). However, contrary to these findings, my results suggest

that the effect of landscape undulation on individual soil physico-chemical parameters does not differentiate the soils; instead, it affects the heterogeneity of the soil physico-chemical environment, and in response, the heterogeneity of soil bacterial communities (Table 1), with higher variability at the lower slope. Differences in soil physical and chemical heterogeneity are presumably due to soil and water erosion processes, which remove or move nutrients, ions, and soil particles downwards, from the upper and the middle slope to the lower slope position, accumulating them at the bottom with patchiness (Khan et al., 2013; Miheretu & Yimer, 2018). In line with expected, heterogeneity of bacterial communities reflected the variability of soil physico-chemical parameters, probably due to the response of bacterial communities to changes in the soil environment (Leff et al., 2015; Merloti et al., 2019). In addition, heterogeneity of soil physical, chemical, and biological parameters in this study could also be influenced by different elevations of the slopes in Alberta's soils. That is some of the sampled sites corresponded to undulated landscapes with sharp slopes; however, the representation of the slope was not accurate in flatter sites. Those flat sites may have exhibited more uniform parameters than those with pronounced slopes, which may have affected the heterogeneity found for a specific slope position. Elevation measurements could be included in future studies to assess the correlation between soil parameters and slope.

Ecoregions in this study represent mainly areas with differences in climatic regimes and soil types (Cathcart et al., 2008; Downing & Pettapiece, 2006; Ecological Stratification Working Group, 1996). Therefore, differences among the soil physico-chemical parameters could be related to either regional climatic conditions, as climate is a soil formation factor and could alter soil conditions (Brady & Weil, 2010) (Figure 1.A); or to the origin and composition of different parent materials, which could also influence the formation and evolution of the subsoil and

topsoil layers over time (Brady & Weil, 2010). The influence of parent material on multiple physico-chemical parameters such as pH, EC, nutrient availability, particle size composition, and organic carbon content has been reported previously, as has been the differential response of soil parameters to agricultural management in soils with different parent material (Gruba & Socha, 2016; Hartemink & Bridges, 1995; Orgill et al., 2017; Zhang et al., 2019). For instance, under the same farming system of unfertilized sisal crops, Ferralsolic soils exhibit a drastic loss of fertility when compared to Cambisolic soils, with a drastic acidification and loss of exchangeable bases (Hartemink & Bridges, 1995). Resonating with this study, my findings suggest that differences in soil physico-chemical properties across ecoregions could be associated with different responses of soil type to agricultural practices. Climate differences across ecoregions could also be playing a major role in shaping different soil physico-chemical profiles (Soriano-Soto et al., 1995). However, data obtained in this study is insufficient to differentiate the contribution of climate and soil type to the soil physico-chemical behavior. Furthermore, different ranges of variability across ecoregions may be attributed to: (i) different agricultural practice across sites within the same ecoregion; (ii) differences in the climatic conditions (Orgill et al., 2017; Zhang et al., 2019); namely, ecoregions with a larger land extension could undergo climatic factors that are specific to smaller areas, thus increasing the variability between sites from the same ecoregion; and (iii) different number of sites sampled from each ecoregion.

Quantification of the different metrics used to evaluate physico-chemical and biological attributes of soil may have been affected by the discrepancies in the number of sampled sites withing the different ecoregions. For instance, the variation of physico-chemical parameters and bacterial communities may be underestimated for the Fescue Grasslands as only one site from this ecoregion was included in the study (Figure 1.B). One site is not representative of the

spectrum of the soil condition in the ecoregion, and samples from this site are likely more similar between them than samples from ecoregions that include numerous sites. In a similar manner, other metrics included, such as soil physico-chemical profile, bacterial community composition and diversity could have also been biased by this factor.

Different climatic conditions and soil type may also affect the composition of microbial communities across ecoregions (Barreiro et al., 2022). A recent study using PLFA revealed bacterial biomass and structure of bacterial communities to be more responsive to regional climate than to land-management intensity (Barreiro et al., 2022). Moreover, climatic factors and soil physico-chemical parameters were identified as drivers of bacterial niche differentiation globally (Barreiro et al., 2022). Several studies have shown the relationship between soil type and microbial communities (Trivedi et al., 2017; Ulrich & Becker, 2006; Wagai et al., 2011). In arable soils, the bacterial community structure and composition is influenced by soil texture and parent material (Ulrich & Becker, 2006). Correspondingly, soil bacterial community composition was found to be influenced by ecoregion (Figure 2.A), with the same behavior as the soil physico-chemical parameters. Ecoregions shape bacterial community composition and heterogeneity (Figure 2.B), likely due to the indirect effects of the soil type and climate history on the soil physico-chemical characteristics, which define the resources available and the environment of the bacterial habitat (Canarini et al., 2021; Soriano-Soto et al., 1995). For instance, parent material largely determines soil pH (Alfaro et al., 2017; Gruba & Socha, 2016; Hartemink & Bridges, 1995; Orgill et al., 2017; Zhang et al., 2019), which has been identified in multiple studies as an important predictor of bacterial community composition and diversity (Alfaro et al., 2017; Lauber et al., 2009; Leff et al., 2015).

While ecoregions play an important role in shaping bacterial community composition, they have little influence on the overall bacterial diversity, although diversity was reduced in the Aspen Parkland and increased in the Mixed Grassland relative to other ecoregions. Soils from both ecoregions are predominantly Chernozemic with loamy texture (Cathcart et al., 2008). Additionally, climatic conditions of the two ecoregions have similar mean elevation, mean annual temperatures, and mean precipitation in respect to other ecoregions (Cathcart et al., 2008). Differences in soil bacterial diversity by ecoregion may be therefore attributed to other factors, such as: (i) the different agricultural practices in these two ecoregions, which results indicate have an influence on bacterial diversity. In the Aspen Parkland ecoregion, practices include continuous cropping of cereal grains, while in the Mixed Grassland ecoregion fallow is a common practice among farmers (Downing & Pettapiece, 2006). In my study, fallow sites exhibited an increased overall bacterial diversity than most cultivated sites, which could be contributing to the increased diversity in the Mixed Grassland ecoregion and the decreased diversity in the Aspen Parklands where fallow practices are uncommon (Downing & Pettapiece, 2006). (ii) to the effect of the predominantly lower soil pH observed for the Aspen Parkland ecoregion; as pH has been reported to correlate with bacterial diversity in arable soils and at different spatial scales (Lauber et al., 2009; Rousk et al., 2010).

2.4.2. Agricultural practices have minimal effect on soil bacterial community composition and diversity but affect the dynamics of the community.

I hypothesized that agricultural practices are drivers of bacterial community composition and diversity. Nevertheless, this hypothesis was rejected. Results indicate agricultural practices play a major role determining the variability of soil physico-chemical parameters, which has been previously observed (Ozgoz et al., 2013; Tsegaye & Hill, 1998) (Table 1; Figures 1.D and

1.F). However, differences in the variability of these parameters do not necessarily imply changes in the entire soil physico-chemical profiles. For example, soils undergoing high tillage, had significantly lower physico-chemical heterogeneity than low and zero tillage (Figure 1.C), probably due to the frequency of the soil nutrients redistribution and homogenization process that tillage represents (Le Guillou et al., 2019). However, results suggest that only soil physicochemical profiles under low and zero tillage are different from each other. The lack of distinction of these two profiles from those in high tillage indicate that differences in the physico-chemical profiles are unrelated to the heterogeneity of these parameters. All the other practices, namely, different crop types, herbicide use, and fertilization systems also affected the variability of soil physico-chemical parameters, but unlike tillage, with no effect on the physico-chemical profiles.

Given that bacterial community heterogeneity followed similar patterns to those observed for the physicochemical variability under the different agricultural practices, results suggest the heterogeneity of bacterial communities is indirectly influenced by different practices. For example, high homogeneity of soil microbial communities in highly tilled soils was observed when compared to that of communities undergoing low tillage or zero-tillage practices (Figure 2.D); probably as a reflection of the variability of soil physico-chemical parameters influenced by the different practices. In line with previous reports, physico-chemical variability across crop types was reflected in the heterogeneity of the soil bacterial communities associated with each crop (Doi & Ranamukhaarachchi, 2009), except for soils from alfalfa seeds and sugar cane crops, which exhibited high physico-chemical heterogeneity but low bacterial heterogeneity (Figure 2.F). Nitrogen input from alfalfa (an N-fixer plant) and the sugar-rich roots of sugar beet plants may be enriching for soil bacterial communities that are more suitable for homogeneous

copiotrophic communities which differentiate from those communities found in other crops (Delgado-Baquerizo et al., 2017; Huang et al., 2019; Niu et al., 2020).

Unlike physico-chemical parameters, for which differences in their variability did not imply (or seemed unrelated to) differences the entire soil physico-chemical profile, findings revealed that differences in bacterial heterogeneity under different agricultural practices could contribute to the differentiation of the composition of soil bacterial communities. These findings indicate bacterial community composition exhibit higher resolution than soil physico-chemical parameters for revealing differences in the condition of soils from agricultural systems, as it has previously been reported (Bouchez et al., 2016; Thiele-Bruhn et al., 2020). In most cases, significantly different bacterial communities overlapped in the ordination space but differed in their heterogeneity. This pattern was observed for the different tillage intensities, crop types and fertilization methods (Supplementary Tables 7, 9, and 13). In contrast, different herbicide used doesn't seem to have a major influence in the composition of bacterial communities since only marginal differences were observed between them (Supplementary Figure 11).

The different agricultural practices played a major role in shaping bacterial diversity in this study, mainly by affecting community evenness (Table 1). Across tillage intensities, high tillage reduced bacterial evenness the most, probably because tillage represents physical disturbance to the environment by physical transformation of the soil habitat and the redistribution of nutrients (Le Guillou et al., 2019) (Figure 4.B). The disturbance caused by tillage may affect the abundance of the most sensitive species and favor the abundance of specific taxa; thus, leading to the reduced evenness in the soil bacterial community (Le Guillou et al., 2019). The effect of agricultural practices on soil bacterial abundance and diversity has been previously reported. For instance, Habig & Swanepoel (2015) observed the same trend,

where bacterial diversity in no-tillage systems compared with tilled systems. Le Guillou et al. (2019) reported that while soil microbial biomass decreases with high tillage intensity and pesticide use, and increases with permanent pastures or pasture rotations when compared to annual cropping; tillage intensity and pastures rotation systems influenced soil bacterial richness and evenness more than fertilizer and pesticide use. My results indicated the use of herbicides, either glyphosate or other, may alter the abundance of specific groups of bacteria, which could be evidenced by the reduction in soil bacterial evenness. Lone et al (2013) reported that the use of pesticides and herbicides triggered the accumulation of toxic substances, and changed soil chemical characteristics (Lone et al., 2013). Soil chemical changes and toxicity caused by herbicide application affects soil microbial groups differently (Du et al., 2018; Lone et al., 2013; Singh et al., 2018). For instance, in the short term, growth of fungal and Actinomycete species is inhibited after mesosulfuron-methyl applications, but populations of resistant species from these groups increase over time (Singh et al., 2018). Lone et al. (2013) also observed different responses from microbial groups according to the type and dose of the herbicide used. The abundance of phosphorus solubilizers increased with herbicide use, while Actinomycetes and Azotobacter species have mixed responses (Lone et al., 2013). Moreover, changes in microbial diversity induced by herbicide use have been associated with reduced microbial activity, which may impact soil functionality (Du et al., 2018; Kumar et al., 2020; Shrestha et al., 2019). Among the evaluated fertilization methods, fertilization banded with seed decreased bacterial evenness the most in this study. This fertilization method consists of the direct application of fertilizer to the soil in concentrated strips, and close to the furrow where the plant seed is placed (Alberta Agriculture and Food, 2008). All fertilized sites included in this study consisted of mineral fertilization methods, which favor the presence of copiotrophic organisms that grow in nutrient rich environments, over that of oligotrophic organisms that proliferate in nutrient-poor

environments (Habig & Swanepoel, 2015). Banded fertilization favors the growth of the crop plants, also altering plant-microbe interactions, which could reduce evenness of bacterial communities (Chaparro et al., 2014; Hargreaves et al., 2015; Houlden et al., 2008).

Different crops exhibited both different soil bacterial richness and evenness (Figure 4.C). Differences in these diversity metrics could be attributed to: (i) soil physico-chemical characteristics influenced by different crop types, which affects nutrient availability and the number of species that can coexist in the environment (Q. Liu et al., 2021; Niu et al., 2020), and/or (ii) crop plant root exudates and their root-associations influence microbial communities, often selecting specific members of the community and increasing their abundance (Chaparro et al., 2014; Hargreaves et al., 2015; Houlden et al., 2008). For instance, Niu et al (2020) compared fourteen years of continuous alfalfa crops to annual crops of maize, wheat, potato and millet, after nine years of continuous alfalfa. They observed that continuous cropping decreased organic carbon and N in soils as well as microbial biomass and diversity (Niu et al., 2020). Others have reported that different plant species had a greater impact on less abundant microbial taxa at specific plant developmental stages and rhizodeposition timing (Chaparro et al., 2014; Hargreaves et al., 2015; Houlden et al., 2008; P. Wang et al., 2017).

Several studies have suggested agricultural systems shape the composition and diversity of soil bacterial communities. For instance, when compared to non-agricultural environments such as pastures, forest, and successional ecosystems, agricultural sites harbor the most homogeneous soil bacterial communities (Banerjee et al., 2019; Cai et al., 2018; Karimi et al., 2017; Merloti et al., 2019; R. Xue et al., 2019). Altogether, my study indicated agricultural practices influence bacterial diversity, mainly by changes in bacterial evenness. Moreover, the influence of agricultural practices in the composition of soil bacterial communities, could be

attributed mostly to their effect on bacterial heterogeneity. Findings from my study suggest an indirect effect of agricultural practices in the heterogeneity of the soil bacterial community, as bacterial communities respond to the changes in soil physicochemical variability that different practices cause. Nonetheless, results of the multivariate regression tree indicate none of the agricultural practices were identified as principal drivers of soil bacterial community composition, and instead only pH and ecoregion were found to be important predictors of bacterial community composition.

Nonetheless, in this study, results regarding agricultural practices could have been obscured by the different (sometimes low) number of samples withing each of the categories for each farming practice. For example, within the tillage intensities, only three sites corresponded to high tillage, while low tillage included twelve sites and zero tillage eleven sites. The number of samples within each treatment could have a particularly major impact on the variability quantification of both soil physico-chemical parameters and bacterial communities. Agricultural practices with low number of sites, could have exhibited underestimation of data variability compared to practices with higher number of sites. Alpha diversity indexes could have also been affected by the different number of samples for each of the practices, specially, when evaluating bacterial richness. If treatments with low number of sampled sites also corresponded to sites with similar characteristics or within the same region, these are likely to have more similar species than treatments with high number of samples from a broader geographical distribution, causing underestimation of the number of species within the former treatments (Barreiro et al., 2022).

Recent studies have shown the influence of environmental factors in microbial community stability can be also observed in the changes in interactions between community members (Banerjee et al., 2019; Hernandez et al., 2021), as environmental stresses could explain

51–78% of the variances in co-ocurrence and co-exclusion patterns of bacterial communities (Banerjee et al., 2019; Hernandez et al., 2021). Therefore, in my study, much of the unexplained variation of soil bacterial community composition and influence of environmental factors could be rather assessed through the analysis of the interactions among bacterial taxa at each site (Nunan, 2017; Xue et al., 2022). Results from my study evidenced that soil bacterial interactions vary according to different land management practices (Table 3). All community network parameters evaluated (namely network size, connectivity, complexity, modularity, behaviour, and centrality) were affected by tillage intensity, crop type, herbicide use and fertilization methods (Table 3). These results are supported by previous findings in which the complexity of the soil microbial community in agricultural systems decreased compared to the bacterial community in forest soils and increased with increasing land management intensification (Xue et al., 2020; Xue et al., 2022).

Complexity of microbial communities is often interpreted as interaction diversity and is associated with community resilience to environmental stress and plant success (Banerjee et al., 2019; Karimi et al., 2017; Tao et al., 2018), while centrality is associated with the rate at which a community responds to environmental stress (Faust & Raes, 2012). Modularity of co-occurrence networks is interpreted as the redundant pathways between species and high modularity is associated with more environmental niches (Faust & Raes, 2016; Karimi et al., 2017). A lower positive-to-negative interactions rate indicates a more negative behaviour of the community, which is associated with negative interactions among community members, such as competition and amensalism (Faust & Raes, 2012). Accordingly, when comparing the influence of different tillage intensities on soil bacterial communities in this study, the higher complexity and centrality observed in the soil bacterial communities undergoing high tillage could be attributed

to the frequent physico-chemical disruption of soil, which may create more resilient microbial communities over time (Katulanda et al., 2018; Moreno et al., 2019; Pey & Dolliver, 2020; Philippot et al., 2021). Correspondingly, the lower modularity observed in the high tillage communities indicates that the homogeneity created by high tillage practices may lead to shared niches and increase negative interactions among the members of the community via competition for resources and space in a constantly changing environment (Faust & Raes, 2012).

Across crop types, fallow sites favoured bacterial community complexity the most, as well as the modularity and the centrality of the community. Higher complexity and centrality have been associated with ecosystem functionality and resilience (Banerjee et al., 2019; Faust & Raes, 2012; Karimi et al., 2017); therefore, allowing fields to lay fallow may have a positive effect of soil bacterial communities. During fallow periods, bacterial communities may be able recover from the environmental stress caused by agricultural practices and recover ecosystem functionality (Barrios et al., 2005; Styger & Fernandes, 2006).

While fertilization methods and herbicide use showed little to no significant changes in the composition of bacterial communities, co-occurrence networks revealed some of these agricultural practices affected the interactions between bacteria. Among herbicide use and fertilization methods, both glyphosate-based herbicides and fertilization banded with seed negatively affected interactions of microbial communities, resulting in decreased connectivity, complexity, and centrality. The effect of these practices could reduce the resilience of soil microbial communities and their ability to quickly respond to environmental stress (Faust & Raes, 2012).

Altogether my study showed changes in within-community interactions could provide additional insights about the effects of agricultural practices on microbial communities, and in some instances (e.g. herbicide use and fertilization methods) could reveal alterations that are not evident through indicators such as community composition and overall diversity. Similar findings have been previously reported, in which loss of interactions in the community occur even before major changes in the community composition and diversity can be detected (Banerjee et al., 2019; Karimi et al., 2017). Clusters within microbial networks have been associated with functional groups performing major functions such as C and N cycling (P. Xue et al., 2022); therefore, studying the changes of co-occurrence networks in agricultural systems may be a suitable method to assess changes in soil functioning.

2.4.3. Predictors of soil bacterial community composition

Among all physico-chemical parameters and agricultural practices assessed during my study, only pH and ecoregions were important drivers of soil microbial community composition in agricultural systems of Alberta (Figure 5). These results are similar to previous studies, where pH has been found to regulate community composition at both large and small scales (Fierer & Jackson, 2006) by directly influencing microbial growth and soil nutrient availability (Fierer & Jackson, 2006; Malik et al., 2018; Moebius-Clune, 2016; Xu et al., 2020). However, in my study, pH only explained 11.1% of the soil bacterial community variation and ecoregions only explained 3.7%, leaving most of the variation (85.2%) to be explained by other factors.

2.5. Conclusions

This study revealed the effects of agricultural practices on soil bacterial communities in Alberta's soils. The composition of soil bacterial communities was influenced only indirectly by

the different practices. Agricultural practices determined the variability of soil physico-chemical parameters, which was reflected in the heterogeneity of soil bacterial communities. The heterogeneity of bacterial communities contributed to determine the differences in the composition of the communities under different practices. Among the different categories, bacterial heterogeneity was reduced in soil undergoing high tillage, fertilization banded with seed, herbicide application and in soils with no crop, special crops, hay and forage. Moreover, the different agricultural practices were found to be drivers of bacterial diversity, specifically by altering bacterial evenness. Differences in crop type influenced both soil bacterial richness and evenness. The low differentiation of soils physico-chemical profiles under different agricultural practices, indicates bacterial community composition has a higher resolution for identifying differences in the condition of soils from agricultural system. However, results in this study may have been obscured by the dissimilar and low number of samples in some of the categories assigned for the farming practices in the province.

In contrast to the previously observed results of the SQMP in which differences in individual soil parameters were attributed to the land slope position, this study found no influence of slope position on the various physico-chemical parameters measured or in the composition of soil bacterial communities. A low percentage of bacterial community variability was explained by pH and ecoregions as important drivers of bacterial community composition in agricultural soils.

Given the observed low resolution of soil bacterial community composition for elucidating the impact of agricultural practices in the soil condition, this study provides evidence for the potential use of different bacterial community metrics as biological indicators of soil health. For instance, the use of co-occurrence networks paired with functional analyses to study

the activity of bacterial communities could provide insights about the soil functions that are affected by specific agricultural practices.

Table 1. Summary of the significance of each variable per treatment. Variables were considered significant when at least two categories within treatments differ between them ($p < 0.05$). AP: Aspen Parkland; BT: Boreal transition; FG: Fescue Grassland; MB: Mixed Boreal; MG: Mixed grassland; MM: Mixed Moist Grassland; PL: Peace Lowland

Treatment/Variable	Physico-chemical profile (PCA ordination)	Physico-chemical variability (Euclidean dissimilarity)	Bacterial community composition (NMDS ordination)	Bacterial heterogeneity (Bray-Curtis dissimilarity)	a-diversity indices (richness and evenness)	Co-occurrence network metrics
Slope position: Upper, Middle, Lower	NS	NS	NS	S	NS	.
Ecoregion: AP, BT, FG, MB, MG, MM, PL	S	S	S	S	S	.
Tillage intensity: High, Low, Zero	S	S	S	S	S	S
Crop type: Alfalfa seed, Barley, Canola, Durum, Fallow, Forage, Hay, Livestock,	NS	S	S	S	S	S
Herbicide: Glyphosate, Other, None	NS	S	S	S	S	S
Fertilization method: Banded with seed, Banded without seed, Broadcast, None	NS	S	S	S	S	S

(S) = Significant differences, (NS) = No signinicant differences (.)= Not evaluated

Table 2. Co-occurrence network metrics for soil bacterial communities undergoing different (A) tillage intensities, (B) crop types, (C) herbicide, and (D) Fertilization methods. Different lower-case letters indicate significant differences across categories within the different practices. Nodes correspond to interactive ASVs; Edges correspond to interactions among ASVs; Pos/Neg edges correspond to the rate of positive to negative connections; Average degree corresponds to the mean number of edges per node; Average cluster coefficient corresponds to the proportion of potential links that are occurring; and the Average closeness centrality corresponds to the distance between each pair of nodes

A. Tillage intensity	High	Low	Zero	.	.	.	.
Nodes	149	164	158				
Edges	1752	1667	1487				
Pos/Neg edges	1195/557 (2.1)	1301/366 (3.5)	1220/267 (4.6)				
Average degree	23.52 **a**	20.33 **b**	18.82 **b**				
Average cluster coefficient	0.55 **a**	0.6 **b**	0.59 **ab**				
Average closeness centrality	0.47 **a**	0.42 **b**	0.42 **b**				
B. Crop type	**Barley**	**Hay**	**Livestock**	**No crop**	**Special crop**	**Wheat**	**Canola**
Nodes	140	145	152	142	102	150	149
Edges	2514	2836	2702	3391	1872	2893	2117
Pos/Neg	1631/882 (1.8)	1820/1016 (1.8)	2702/888 (2.0)	2081/1310 (1.6)	1302/570 (2.3)	1960/932 (2.1)	1605/512 (3.1)
Average degree	35.91 **a**	39.12 **a**	35.55 **a**	47.76 **c**	36.71 **a**	38.57 **a**	28.42 **b**
Average cluster coefficient	0.64 **a**	0.66 **a**	0.65 **a**	0.74 **b**	0.67 **a**	0.64 **a**	0.62 **a**
Average closeness centrality	0.55 **a**	0.54 **a**	0.52 **a**	0.59 **c**	0.59 **c**	0.54 **a**	0.49 **b**
C. Herbicide	**Glyphosate**	**Other**	**No herbicide**	.	.	.	.
Nodes	165	163	160				
edges	925	1747	1315				
Pos/Neg	809/116 (7.0)	1363/384 (3.5)	1019/296 (3.4)				
Average degree	11.42 **a**	21.44 **b**	16.44 **c**				
Average cluster coefficient	0.57	0.6	0.57				
Average closeness centrality	0.34 **a**	0.43 **b**	0.39 **c**				
D. Fertilization method	**Banded w/ seed**	**Banded w/o seed**	**Broadcast**	**No fertilizer**	.	.	.
Nodes	164	157	157	167			
Edges	887	1201	1186	1350			
Pos/Neg	775/112 (6.9)	969/232 (4.2)	929/257 (3.6)	1069/281 (3.8)			
Average degree	10.82 **a**	15.3 **b**	15.11 **b**	16.17 **b**			
Average cluster coefficient	0.55	0.52	0.54	0.57			
Average closeness centrality	0.33 **a**	0.39 **b**	0.38 **b**	0.38 **b**			

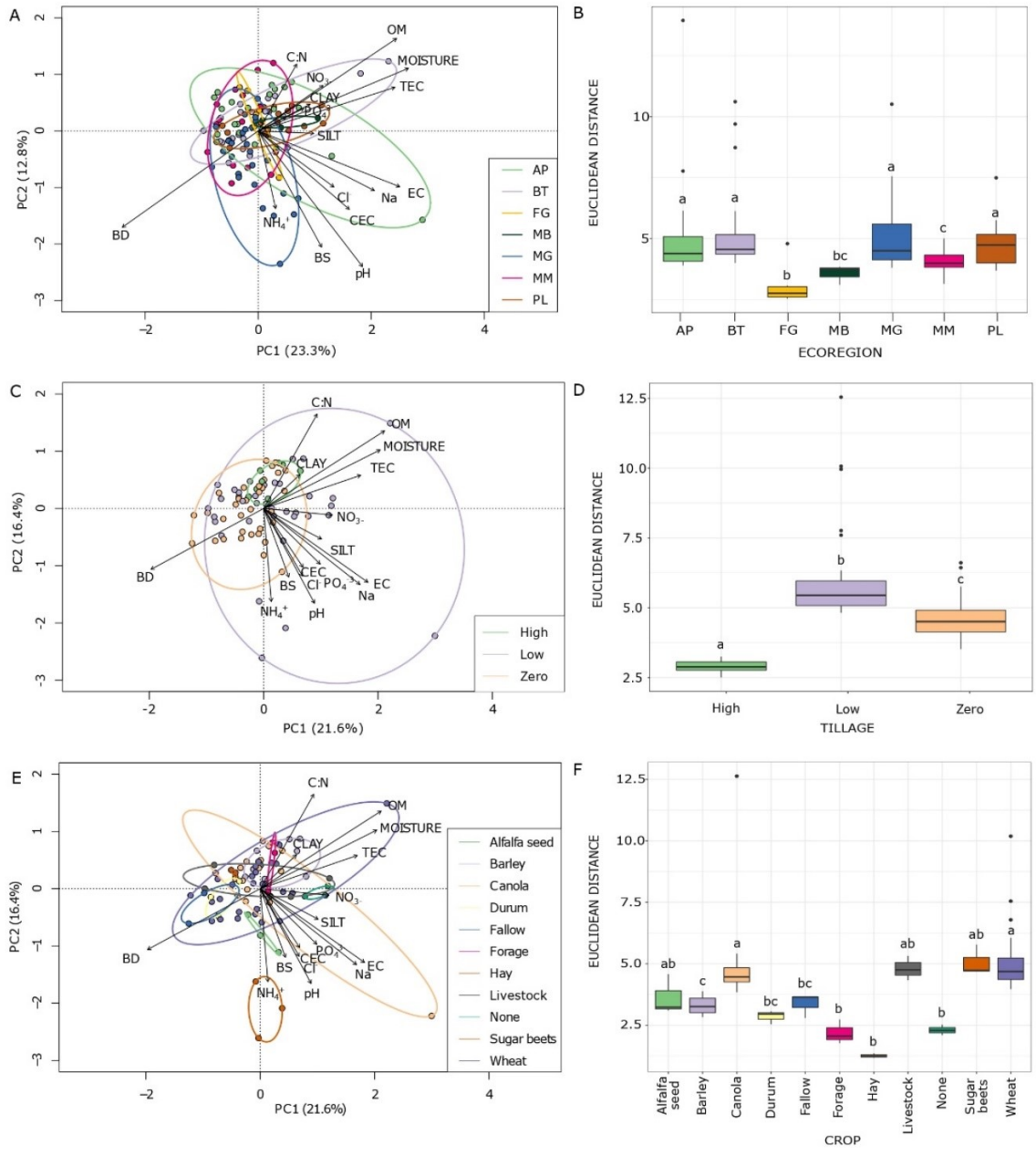

Figure 1. Principal component analysis of soil physico-chemical parameters clustered according to (A) ecoregions ($p < 0.05$; AP: Aspen Parkland; BT: Boreal transition; FG: Fescue Grassland; MB: Mixed Boreal; MG: Mixed grassland; MM: Mixed Moist Grassland; PL: Peace Lowland), (C) tillage intensities ($p < 0.05$), and (E) Crop types ($p < 0.05$). Length of vectors indicate the influence of each parameter in the distribution of the data in the ordination space. Euclidean distance of soil physico-chemical parameters between samples within (B) ecoregions, (D) tillage

intensities, and (F) crop types. Different lower-case letters indicate significant differences according to pairwise Wilcoxon Rank.

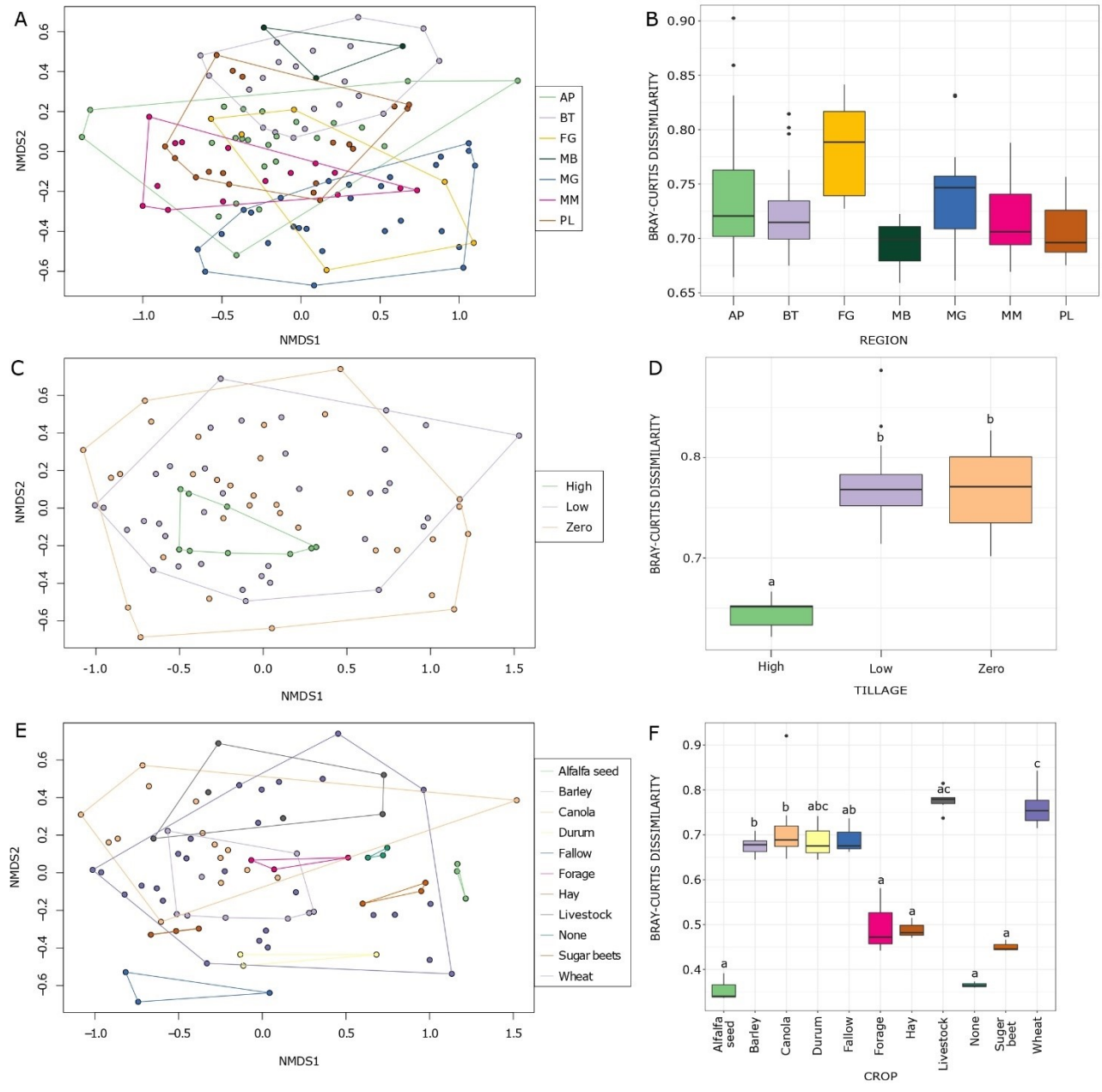

Figure 2. Non-metric multidimensional scaling ordination of bacterial community composition at the ASV level, and clustered according to (A) ecoregions (ordination stress = 0.171282, $p > 0.05$; AP: Aspen Parkland; BT: Boreal transition; FG: Fescue Grassland; MB: Mixed Boreal; MG: Mixed grassland; MM: Mixed Moist Grassland; PL: Peace Lowland), (C) tillage intensities (ordination stress = 0.1546061, $p < 0.05$)., and (E) crop types (ordination stress = 0.1546061, $p < 0.05$). (B) Bray-Curtis Dissimilarity of soil bacterial communities across (B) ecoregions, (D)

tillage intensities, and (F) crop types. Different lower-case letters indicate significant differences according to pairwise Wilcoxon Rank.

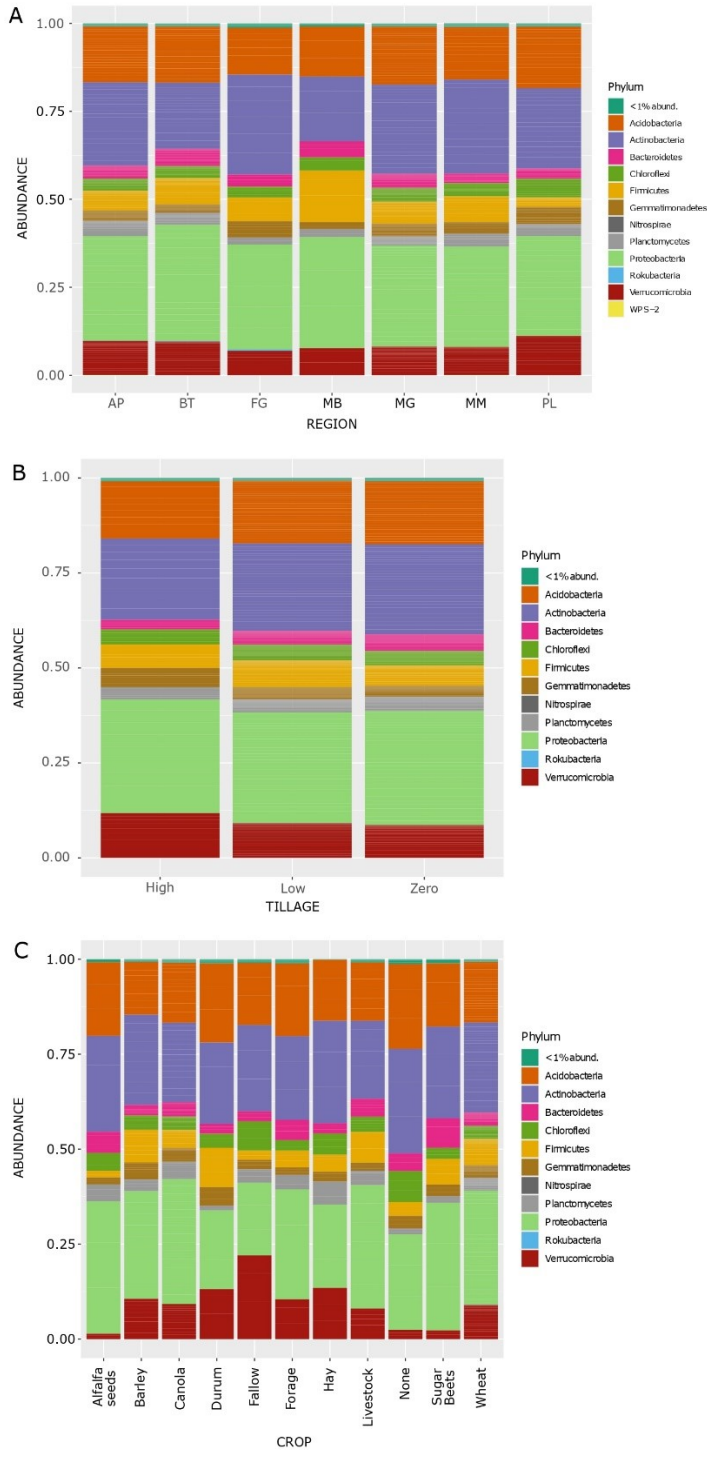

Figure 3. Taxonomic profile of bacterial communities showing the relative abundance of the dominant groups at the Phylum level across (A) ecoregions (AP: Aspen Parkland; BT: Boreal transition; FG: Fescue Grassland; MB: Mixed Boreal; MG: Mixed grassland; MM: Mixed Moist

Grassland; PL: Peace Lowland), (B) tillage intensities, and (C) crop types. Taxa with an abundance < 1% are grouped together.

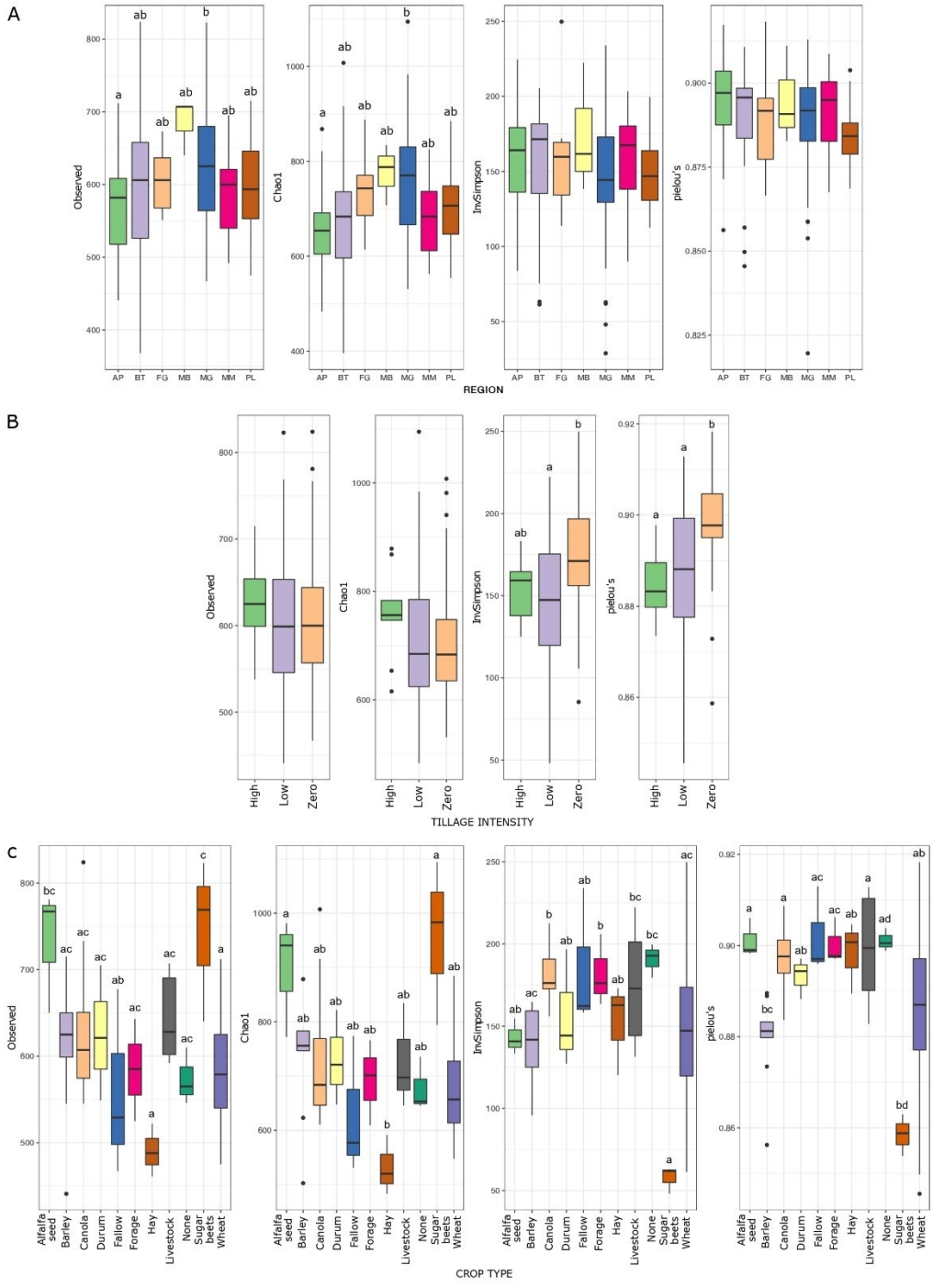

Figure 4. Alpha-diversity metrics of bacterial communities across (A) ecoregion (AP: Aspen Parkland; BT: Boreal transition; FG: Fescue Grassland; MB: Mixed Boreal; MG: Mixed grassland; MM: Mixed Moist Grassland; PL: Peace Lowland), (B) tillage intensities, and (C) crop types. Different lower-case letters indicate significant differences. Measures indexes

included observed number of ASVs, Chao1 index, Inverse Simpson index, and Pielou's evenness; shown in separate plots.

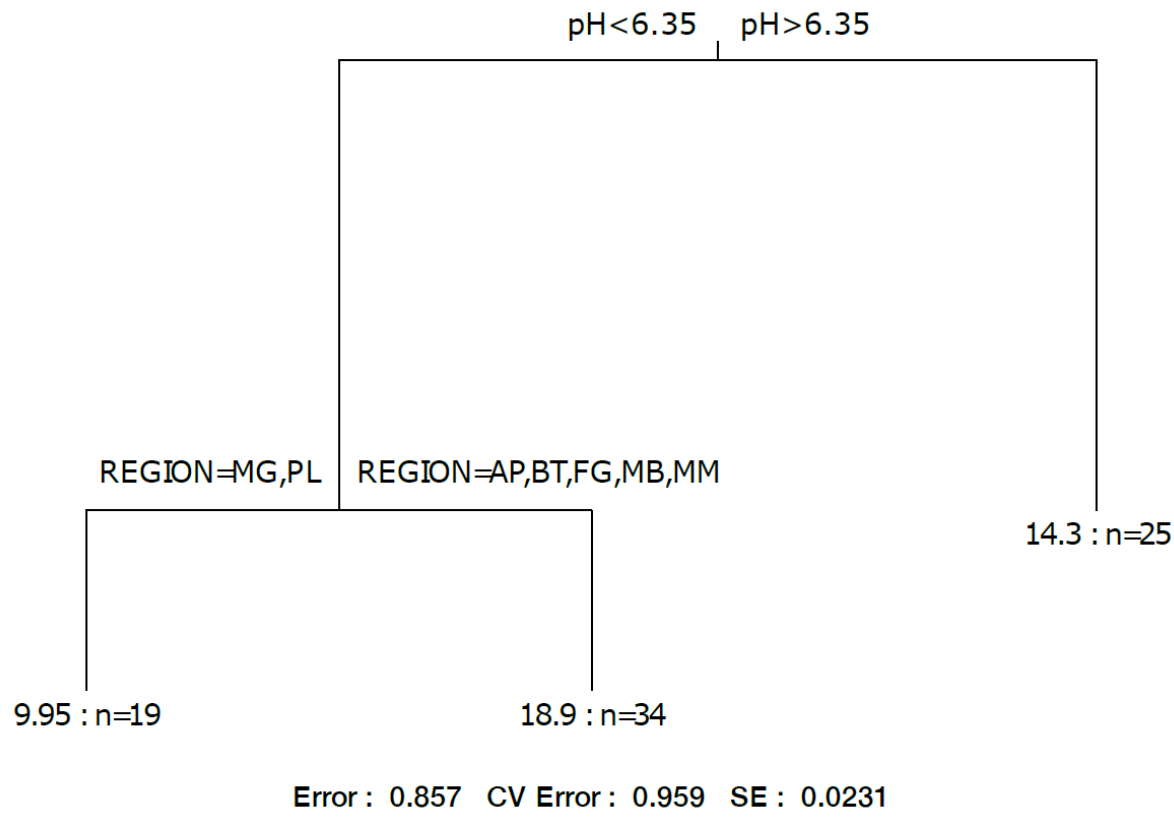

Figure 5. Multivariate regression tree showing the identified predictors of bacterial community composition in the agricultural soils of Alberta. pH and ecoregions explained 11.1% and 3.8% of the variation of bacterial communities respectively. AP: Aspen Parkland; BT: Boreal transition; FG: Fescue Grassland; MB: Mixed Boreal; MG: Mixed grassland; MM: Mixed Moist Grassland; PL: Peace Lowland

3. CHAPTER 3

3.1. Main Findings

Assessing soil health in agricultural systems could provide insights into the impact of agricultural practices on the soil attributes and is key to ensure agriculture and soil sustainability over time (Giri & Varma, 2020; Mijangos Amezaga, 2009). Some efforts have been made to determine a measurement for soil health globally and in different systems (Adetunji et al., 2017; Amacher et al., 2007; Fine et al., 2017; Laishram et al., 2012; Lal, 2016; Maharjan et al., 2020; Moebius-Clune, 2016; Norris et al., 2020; Pankhurst & CAB International, 1997; Rust et al., 1972; R. Xue et al., 2019). However, given the huge variation and the different relevance of system-specific factors affecting soil attributes, these metrics have low applicability. Some soil health metrics, focus on the assessment of soil physico-chemical attributes and the soil ability to provide services of human interest, disregarding soil biological importance and the ecological productivity and functionality implied in soil health (Amacher et al., 2007; Fine et al., 2017; Lal, 1998; Pérez-Valera et al., 2015), thus approaching more the soil quality concept. In Alberta, the Soil Quality Monitoring program was a governmental effort that focused on monitoring and evaluating the physico-chemical attributes of soils across the province (Cathcart et al., 2008). This program set an important step towards the assessment of the soil condition province wide.

Given the important role of microbial communities in soil functionality and their sensitivity to soil physico-chemical changes (Coleman et al., 2018; Fierer & Jackson, 2006; Gusain et al., 2015; Tardy et al., 2015; Trivedi et al., 2017), I evaluated the potential of multiple bacterial community metrics as biological indicators that could help close the gap between soil quality and soil health assessment in agricultural systems. Consequently, I evaluated the impact

of different agricultural practices on soil bacterial community composition, heterogeneity, diversity and on the interactions among the members of the soil bacterial community.

Previous finding from the SQMP indicated differences in individual soil physico-chemical attributes are associated with land undulations and ecoregional climatic and pedological differences (Cathcart et al., 2008). Contrary to my expectations, results from this study indicate that landscape position does not play a major role shaping the soil physico chemical profiles or the composition and diversity of bacterial communities from the agricultural soils of Alberta. While slope position determines individual physico-chemical parameters (Cathcart et al., 2008), these variations are not enough to differentiate the overall geo-physico-chemical parameters. In turn, bacterial communities inhabiting soils with similar physico-chemical characteristics did not differ across slopes. Despite following the same sampling methodology used for the SQMP, in this study the lack of differentiation of soil attributes across landscape positions could be attributed to the high variability caused by differential slope elevation from site to site, which was not considered in this study, and which may have changed over time. While some of the sites had pronounced slope elevation, others lacked them or presented only small undulations in the terrain. The lack of pronounced slopes at most sites sampled could have caused an underestimation of the overall effect of landscape position on both the edaphic and the biological parameters and is an important aspect to consider for future studies. Including measurement of the elevation of each sampling point, could elucidate a more accurate impact of the land topography on soil physical, chemical and biological attributes.

This study revealed different ways in which agricultural practices alter soil microbial communities. Overall, different farming practices influenced the heterogeneity and evenness of the community, as well as the interactions among its members. While tillage intensity, crop type and fertilization methods exhibited small differences on the composition of bacterial

communities, none of the differences in soil bacterial communities could be consistently attributed directly to each of the practices. Instead, agricultural practices influenced the variability of soil physico-chemical parameters, which in turn determine the variability and composition of bacterial communities in soil. These findings are in line with previous studies, in which the heterogeneity of bacterial communities is altered by farming practices and reduced in farming systems compared to native or successional environments, indicating low variability in agricultural systems (Le Guillou et al., 2019; Banerjee et al., 2019; Cai et al., 2018; Karimi et al., 2017; Merloti et al., 2019; Xue et al., 2019). Specific agricultural practices can induce the overrepresentation of individual taxa, which could explain the influence of farming practices on bacterial evenness as observed in this study (Le Guillou et al., 2019; Du et al., 2018; Lone et al., n.d.; (Habig & Swanepoel, 2015).

Despite differences in soil bacterial community composition that were identified under different agricultural practices, the differentiation of communities in the ordination space was not clear. On the other hand, complexity, and behavior of interactions among the bacterial community was clearly affected by the different farming practices and may be correlated to soil functionality and overall health. Hence, this study indicates the co-occurrence network of bacterial communities could be a better biological indicator of soil health than the composition of the community.

Among all agricultural practices and physico-chemical parameters included in the study, only pH and ecoregions were found as important drivers of soil bacterial community composition, despite only explaining ~11% and ~3% of the total variation respectively. The threshold for a shift in bacterial community composition was a soil pH of 6.35. pH has previously been identified as a major regulator of bacterial community composition at small and large scales, by its influence on the availability of soil nutrients and its impact on bacterial

growth (Fierer & Jackson, 2006; Malik et al., 2018; Moebius-Clune, 2016; Xu et al., 2020). Moreover, pH has been found to alter soil bacterial metabolic activity (Malik et al., 2018; Moebius-Clune et al., 2016; Xu et al., 2020). Identifying how bacterial functionality is altered across the pH threshold in response to agricultural practices could provide insights into which practices that favor soil functionality and soil health, which may be an important step toward reevaluating land management policies and strategies in Alberta's agricultural soils in the future.

Bacterial communities in soil with pH < 6.35 were also shaped by ecoregion. Ecoregions in this study represented areas with different soil types and climate. Bacterial community composition is influenced by the soil texture, parent material, and climatic history; these factors ultimately shape the soil environment in which bacterial communities are found (Canarini et al., 2021; Trivedi et al., 2016; Ulrich & Becker, 2006; Wagai et al., 2011).

3.2. Contributions, improvements, and future directions

This project is large-scale study in evaluating microbial communities in a non-controlled environment. Samples were collected from farm operations, considering the most common practices performed in the agricultural region of Alberta. This is an asset, because it evaluates the real applicability of bacterial communities as soil health indicators. Obtaining data from farm representative farm operations allows the development of a framework that is customized, applicable and serves as a guide for land management and regional policy making. However, there were also disadvantages. For instance, isolation of different treatments or variables to evaluate their effect is not possible; there is noise from unmeasured and uncontrolled variables; and there is no control over the number of samples in each of the categories assigned for the agricultural practices given that the information regarding these practices was obtained after sampling collection. For example, having uneven number of sampled sites for the different

tillage intensities could influence the heterogeneity of bacterial communities and soil physico-chemical parameters. Differential response of soil attributes has been reported for different soil types (Gruba & Socha, 2016; Hartemink & Bridges, 1995; Orgill et al., 2017; Ulrich & Becker, 2006), meaning that sites that have similar soil types and are subjected to the same agricultural practice likely have a more similar bacterial communities than sites with different soil types (Ulrich & Becker, 2006). Therefore, if tillage intensities with low number of samples include only soils from closer locations with similar soil types and climatic conditions, low heterogeneity (i.e., underestimated) will be observed in the attributes of these soils. This bias could apply to the heterogeneity of biological and physico-chemical parameters, and to the alpha diversity metrics of bacterial communities under all agricultural practices included, as well as for ecoregions. Future assessment of soil health in agricultural systems of the province could benefit from a preliminary evaluation of farming-practices-survey responses to determine uniformity in the distribution of sites and practices. Paring field analysis with experiments in controlled environments, where different agricultural practices are simulated separately may reduce noise and skew in some of the biological and physico-chemical results. Increasing the number of sites sampled could ensure a minimum number of samples per treatment, allowing to test the hypotheses more accurately. Another alternative to eliminate the effect of uneven number of samples on statistical analyses is to subsample the data randomly selecting a determined number of samples from each treatment to test, and excluding those treatment that do not have enough samples to be tested.

Diversity and co-occurrence metrics to evaluate soil bacterial communities in this study elucidated differences in response to the most common agricultural practices in Alberta. This study represents the first step towards the addition of microbial communities as part of an assessment of soil health province wide. On the other hand, composition of soil bacterial

community alone did not provide enough resolution to clearly differentiate soils undergoing different agricultural practices and thus may not be a suitable indicator for soil health in agricultural systems, perhaps due to the low sensitivity of bacterial communities in these systems, which has been previously reported (Barreiro et al., 2022; Chen et al., 2018). Soil microbial communities are shaped by different drivers, depending on their broad taxonomic affiliation (Barreiro et al., 2022). For example, a recent study shows soil microbial community structure is mainly shaped by the physico-chemical environment. In that study, bacterial communities were more sensitive to region and climate, while soil fungal communities had a stronger response to management intensity (Barreiro et al., 2022).Hence, the assessment of fungal community composition and diversity through marker gene sequencing could better elucidate the impact of different practices on soil biological attributes in agricultural systems than bacterial community composition and diversity.

Based on the 16S rRNA analysis performed in this study, different agricultural practices do not clearly shift soil bacterial community composition. However, agricultural practices did affect activity of community members and their subsequent ability to interact. Thus, elucidating how these changes in the interactions of soil bacterial community members could affect soil functionality and health is the next step. For instance, evaluating the correlation of co-occurrence network analyses metrics with the expression of functional genes from q-PCR analyses could indicate which functions are altered by changes in community activity (Shi et al., 2020). Another alternative is the use of metaproteomics or metatranscriptomics to evaluate enzymatic activity and gene expression of the bacterial community respectively, which could provide information about the metabolic traits and pathways to and associated co-occurrence network configuration (Dubey et al., 2020). Insights into the broad functional potential of a bacterial community

configuration could be evaluated with metagenomics, by characterizing functional traits of the keystone taxa identified from co-occurrence networks (Goel et al., 2017).

Altogether, multiple directions can follow this work. Namely, (i) the assessment of soil fungal community composition, which may exhibit higher sensitivity and resolution than that of bacterial community composition; and may elucidate the impact of agricultural practices on soil biological attributes; (ii) the assessment of soil bacterial community functionality and its association with different configurations and behavior of interactions among the community; which could provide insights into the soil functions affected by the different agricultural practices; (iii) the determination of land undulation influence on soil physico-chemical and biological attributes via the comparison of parameters at different slope positions along a gradient of land elevation; (iv) the preliminary selection of well-distributed and representative sites for future monitoring of soil health based on producers surveys; which may result in more precise and accurate findings; and (V) The comparison between soil attributes of agricultural and reference sites, which may allow the integration of biological indicators into a soil health index.

3.3 Integrating soil biological indicators into a soil health index

The biological data obtained in this study could be integrated along with soil physico-chemical parameters into a soil health index metric that provides a ranking value of the soil condition with respect to the baseline condition of reference soil from undisturbed native environments. Native soils could be used as waypoints to determine how far away (and in what direction) a soil is from the "starting point". For sites that do not have a nearby native area, soils from farmstead or uncultivated lowly disturbed soils from near fences could be used as the reference (Maharjan et al., 2020). Soil health indexes can aid in making decisions regarding land management, based on the score difference between agricultural soil sites and native or naturally

occurring systems, which are considered to be more sustainable on the long term (Lal, 1998). However, under an agricultural framework the distance of a soil to any of these different reference soils proposed does not imply productivity, predictability, and disease suppression capacity, which are all aspect of interest for the agricultural industry. To determine some of these aspects as part of the soil health assessment of agricultural soils, different tests could be performed in controlled conditions (i.e., green house or growth chamber). These tests could include indicators of potential productivity, and disease suppression capacity that could be measured for the reference and the evaluated soils. Indicators of potential productivity could include germination rate per area, plant growth-rate, plant biomass or even foliar nutrient content (Gheysari et al., 2017; Natale et al., 2002; Nikolaychenko et al., n.d.). Indicators for disease suppression capacity could include disease incidence (i.e., proportion of diseased plants) and severity (i.e., proportion of plan area affected per individual) (Seem, 1984), which could be determined for the pathogens already present in the soils or after the soil inoculation with selected pathogens of importance for a specific crop. Integrating these measurements with soil physico-chemical parameters and metrics of soil microbial communities into a single soil health score could provide a more informative and holistic view for land management of agricultural systems.

Soil health indexes are often derived from multivariate analyses, which simultaneously integrate different types of variables, and when introduced in simple arithmetic equations can provide a single numeric score (Beck & Hatch, 2009; Mukhopadhyay et al., 2014). From the results of this study, I propose to develop a soil health index (SHI) that includes ecological and structural aspects of the system. Namely, (i) soil physico chemical parameters that best explain the variation between samples, (ii) soil bacterial community composition derived from the Bray-Curtis dissimilarity with respect to native reference sites, and (iii) the soil bacterial overall

diversity determined from the Inverse Simpson index, which considers both richness and evenness of bacterial communities. Soil physico-chemical parameters to include in the index could be selected via principal component analysis (PCA) (Hogberg et al., 2020). Only principal components (PCs) that have an eigenvalue higher than 1.0 and explain >5.0 % of the total variation would be considered (Hogberg et al., 2020). For each considered PC, the variable with the highest absolute eigenvector would be selected along with variables with eigenvectors within 10% of that value (Hogberg et al., 2020). The raw score for a site would be calculated as the sum of each selected variable value times the eigenvector of the corresponding principal component. The final score for the physico-chemical aspect would be calculated as the difference between the sum for the disturbed site and the sum of the reference site (Hogberg et al., 2020). The equation would be as follows:

i. Physico-chemical contribution = Physico-chemical score $_{\text{natural site}}$ - Physico-chemical score $_{\text{evaluated site}}$

   Physico-chemical contribution = Σ (eigenvector PC $_n$* X variable) - Σ (eigenvector PC $_n$* X variable)

Where n is the number of the significant Principal Component and determines the weight of each physico-chemical variable. And X is the scaled-mean-value of selected variables (e.g., pH) for a site.

Bacterial abundance data could be Hellinger-transformed to avoid double zeros that may lead to misinterpretation of species absence in a site (Legendre & Borcard, 2018). Transformed abundance data could then be used to construct a dissimilarity matrix such as Bray-Curtis, which has been reported to efficiently detect gradients in species composition (Minchin, 1987). The contribution of the bacterial community composition aspect would correspond to the mean dissimilarity value of a site with respect to the reference sites, which results in a value between

zero and 1. The closer the score is to zero the more it would resembles the bacterial community composition of native reference soils.

ii. Contribution of bacterial community composition = mean Bray-Curtis dissimilarity value of a site with respect to the reference native site.

The contribution of bacterial overall diversity could be calculated as the difference between the mean-diversity index of natural sites and the mean-diversity index of the evaluated site.

iii. Alpha diversity contribution = (Inverse Simpson index $_{\text{natural site}}$ - Inverse Simpson index $_{\text{evaluated site}}$)

To integrate the three aspects, the contribution value of each aspect needs to be scaled and the weight of each one needs to be determined. Weight coefficients would be multiplied by each of the scaled aspect-contribution values. The final score would be the absolute value of the following equation, and the closer it is to zero, the more it resembles the health of native reference soils:

SHI = |(coefficient $_{\text{physico-chemical}}$ * Physico-chemical contribution) + (coefficient $_{\text{community composition}}$ * contribution of bacterial community composition) + (coefficient $_{\text{diversity}}$ * contribution of alpha diversity contribution)|

There are still some limitations for the development of this index. Namely, the different nature of the data from each aspect hinders the integration of all datasets in a single matrix that allows the identification of weight coefficients. Moreover, given that results from this study indicated bacterial communities differ from one region to another, reference sites would need to be specific for the region where the evaluated site is located, which ensures the reference soils resemble environmental characteristics of the evaluated site. Furthermore, findings from the study and previous reports suggest bacterial community composition does not have enough

sensitivity to identify differences between soils in agricultural systems, and therefore other bacterial community metrics would be more suitable as biological indicators of soil health. Including metrics derived from cooccurrence network analyses as well as functional traits of bacterial communities could improve the discriminatory power of the index between sites.

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Appendix: Supplementary Material for Chapter 2

Table S.1. Number of reads obtained during data processing for 2019 and 2020 samples. Processing steps include reads at the initial input (input), reads after filtering (filtering), forward reads after denoising (denoisedF), reverse reads after denoising (denoisedR), reads after merging forward and reverse reads (merged), reads after removing chimeras (nonchim)

Year	input	filtered	denoisedF	denoisedR	merged	nonchim
2019	12775484	7904516	7004803	7065818	5163801	2743320
2020	3591879	2149020	1923650	1932631	1564471	721494

Table S.2. Summary of survey responses regarding agricultural practices performed during the corresponding year of sampling (2019 or 2020) at each site. Only questions relevant to this study are included in the table.

Timestamp	Consent	Ecodistrict #	What is your farm operation type?	Tillage disturbance	Name of Herbicide	Fertilizer Application Methods	Crop	If Specialty crop, which?
9/5/2019 9:06:43	YES	586	Annual Cropping	High Disturbance (1 Pass)	Round-up	Banded without seed	Barley	
11/25/2019 15:27:27	YES	740	Annual Cropping	High Disturbance (2 Passes)	None	Banded with seed	Canola	
11/25/2019 15:56:59	YES	746	Hay production	None	None	Broadcast	Forage	
11/25/2019 17:24:48	YES	593	Annual Cropping	Low Disturbance (1 Pass)	Glyphosate	Banded with seed	Wheat	
12/5/2019 11:39:44	YES	812	Annual Cropping	Low Disturbance (1 Pass)	None	None	Alfalfa seed	Alfalfa
12/6/2019 8:51:50	YES	802	Annual Cropping	None	glyphosate	Banded with seed	Wheat	
12/18/2019 9:14:30	YES	793	None	Low Disturbance (1 Pass)	Glyphosate	Banded with seed	Wheat	
12/18/2019 12:42:00	YES	815	Annual Cropping	None	2,4_D Ester 700		Wheat	
3/26/2020 17:10:35	YES	688	Annual Cropping	Low Disturbance (1 Pass)	Prepass	Banded without seed	Canola	
4/9/2020 11:50:45	YES	727	Annual Cropping	Low Disturbance (1 Pass)	Glyphosate	Banded with seed	Barley	
4/16/2020 11:20:07	YES	804	Annual Cropping	None	None	None	Fallow	
4/16/2020 11:51:03	YES	823	Annual Cropping	High Disturbance (3 Passes)	edge	Broadcast	Specialty Crop	sugar beets
4/17/2020 9:20:36	YES	684	Annual Cropping	None	RoundUp	Banded without seed	Canola	
4/21/2020 13:35:01	YES	687	Annual Cropping	Low Disturbance (1 Pass)	Glyphosate	Banded with seed	Wheat	
4/23/2020 23:54:45	YES	703	Annual Cropping	Low Disturbance (1 Pass)	Roundup	Banded with seed	Wheat	
4/27/2020 8:41:29	YES	744	Annual Cropping	Low Disturbance (1 Pass)	express pro	Banded with seed	Wheat	
5/20/2020 12:22:24	YES	800	Annual Cropping	High Disturbance (1 Pass)	roundup	Banded with seed	Barley	
1/27/2021 17:29:51	YES	595	Annual Cropping	Low Disturbance (1 Pass)	No	Banded with seed	Wheat	
1/27/2021 19:15:36	YES	781	Annual Cropping	No till	VP 480	Broadcast	Canola	
1/28/2021 8:23:08	YES	599	Annual Cropping	Low Disturbance (1 Pass)	Glyphosate	Banded without seed	Wheat	
1/28/2021 19:15:30	YES	738	Crop/Livestock	Low Disturbance (1 Pass)	none	Broadcast	hay	
2/2/2021 10:27:33	YES	615	Crop/Livestock	None	Glyphosate	Broadcast	Livestock	
2/2/2021 10:35:08	YES	592	Annual Cropping	Low Disturbance (1 Pass)	None	None	None	
3/4/2021 8:57:42	YES	809	Annual Cropping	Low Disturbance (1 Pass)	Glyphosate	Banded with seed	Durum	
3/4/2021 12:49:37	YES	791	Annual Cropping	Low Disturbance (1 Pass)	Glyphosate	Banded with seed	Canola	
10/2/2020 21:51:04	YES	730	Crop/Livestock	Low Disturbance (1 Pass)	none			

Table S.3. Results from the pairwise PERMANOVA comparing the soil physico-chemical parameters between different slope positions. The F statistic is a measure of effect-size, the R2 indicates the variation explained by the model and the adjusted P-value specifies the overall significance of the comparison.

Pairwise comparison			F	R^2	Adjusted P-value	Significance
L	vs	M	1.968	0.026	0.156	
L	vs	U	2.030	0.027	0.156	
M	vs	U	0.109	0.001	0.994	

Significance code: **<0.01,*<0.05, . <0.1

Table S.4. Results from the pairwise PERMANOVA comparing the bacterial community composition between different slope positions. The F statistic is a measure of effect-size, the R2 indicates the variation explained by the model and the adjusted P-value specifies the overall significance of the comparison.

Pairwise comparison			F	R^2	Adjusted P-value	Significance
L	vs	M	0.703	0.009	0.959	
L	vs	U	1.067	0.014	0.957	
M	vs	U	0.665	0.009	0.959	

Significance code: **<0.01,*<0.05, . <0.1

Table S.5. Results from the pairwise PERMANOVA comparing the soil physico-chemical parameters between different ecoregions. The F statistic is a measure of effect-size, the R2 indicates the variation explained by the model and the adjusted P-value specifies the overall significance of the comparison. AP: Aspen Parkland; BT: Boreal transition; FG: Fescue Grassland; MB: Mixed Boreal; MG: Mixed grassland; MM: Mixed Moist Grassland; PL: Peace Lowland

Pairwise comparison			F	R^2	Adjusted P-value	Significance
MG	vs	PL	2.223	0.049	0.191	
MG	vs	MB	17.775	0.388	0.007	**
MG	vs	BT	2.911	0.059	0.082	.
MG	vs	AP	0.761	0.015	0.581	
MG	vs	MM	3.380	0.078	0.037	*
MG	vs	FG	1.955	0.059	0.189	
PL	vs	MB	18.716	0.496	0.007	**
PL	vs	BT	9.957	0.212	0.007	**
PL	vs	AP	0.210	0.005	0.936	
PL	vs	MM	9.164	0.228	0.007	**
PL	vs	FG	4.470	0.169	0.040	*
MB	vs	BT	57.466	0.723	0.007	**
MB	vs	AP	1.549	0.058	0.189	
MB	vs	MM	62.415	0.796	0.007	**
MB	vs	FG	46.196	0.869	0.037	*
BT	vs	AP	1.318	0.030	0.371	
BT	vs	MM	1.700	0.048	0.201	
BT	vs	FG	1.734	0.065	0.304	
AP	vs	MM	1.050	0.028	0.405	
AP	vs	FG	0.434	0.015	0.487	
MM	vs	FG	0.487	0.025	0.633	

Significance code: **<0.01,*<0.05, . <0.1

Table S.6. Results from the pairwise PERMANOVA comparing the bacterial community composition between different ecoregions. The F statistic is a measure of effect-size, the R2 indicates the variation explained by the model and the adjusted P-value specifies the overall significance of the comparison. AP: Aspen Parkland; BT: Boreal transition; FG: Fescue Grassland; MB: Mixed Boreal; MG: Mixed grassland; MM: Mixed Moist Grassland; PL: Peace Lowland

Pairwise comparison			F	R^2	Adjusted P-value	Significance
MG	vs	PL	5.251	0.109	0.003	**
MG	vs	MB	2.384	0.078	0.006	**
MG	vs	BT	5.810	0.112	0.003	**
MG	vs	AP	5.099	0.094	0.003	**
MG	vs	MM	3.743	0.085	0.005	**
MG	vs	FG	1.841	0.056	0.041	*
PL	vs	MB	2.027	0.096	0.033	*
PL	vs	BT	3.291	0.082	0.003	**
PL	vs	AP	3.298	0.076	0.003	**
PL	vs	MM	3.938	0.113	0.003	**
PL	vs	FG	2.472	0.101	0.016	*
MB	vs	BT	1.600	0.068	0.033	*
MB	vs	AP	2.084	0.077	0.018	*
MB	vs	MM	2.493	0.135	0.018	*
MB	vs	FG	1.776	0.202	0.046	*
BT	vs	AP	2.549	0.056	0.003	**
BT	vs	MM	3.963	0.104	0.003	**
BT	vs	FG	2.015	0.075	0.006	**
AP	vs	MM	1.950	0.050	0.016	*
AP	vs	FG	1.613	0.054	0.046	*
MM	vs	FG	1.319	0.065	0.166	

Significance code: **<0.01,*<0.05, . <0.1

Table S.7. Results from the pairwise PERMANOVA comparing the soil physico-chemical parameters between different tillage intensities. The F statistic is a measure of effect-size, the R2 indicates the variation explained by the model and the adjusted P-value specifies the overall significance of the comparison.

Pairwise comparison			F	R^2	Adjusted P-value	Significance
High	vs	Low	1.607	0.036	0.289	
High	vs	Zero	0.876	0.021	0.406	
Low	vs	Zero	5.392	0.074	0.003	**

Significance code: **<0.01,*<0.05, . <0.1

Table S.8. Results from the pairwise PERMANOVA comparing the bacterial community composition between different tillage intensities. The F statistic is a measure of effect-size, the R2 indicates the variation explained by the model and the adjusted P-value specifies the overall significance of the comparison.

Pairwise comparison	F	R^2	Adjusted P-value	Significance
High vs Low	1.710	0.038	0.036	*
High vs Zero	2.472	0.058	0.018	*
Low vs Zero	1.882	0.027	0.021	*

Significance code: **<0.01,*<0.05, . <0.1

Table S.9. Results from the pairwise PERMANOVA comparing the soil physico-chemical parameters between different crop types. The F statistic is a measure of effect-size, the R2 indicates the variation explained by the model and the adjusted P-value specifies the overall significance of the comparison.

Pairwise comparison			F	R^2	P-value	Significance
Barley	vs	None	2.412	0.194	0.267	
Barley	vs	Wheat	1.390	0.039	0.349	
Barley	vs	Livestock	7.670	0.371	0.088	
Barley	vs	Canola	0.586	0.026	0.799	
Barley	vs	Hay	4.138	0.293	0.073	
Barley	vs	Forage	2.022	0.168	0.275	
Barley	vs	Fallow	2.811	0.219	0.277	
Barley	vs	Durum	2.564	0.204	0.267	
Barley	vs	Alfalfa seed	1.937	0.162	0.301	
Barley	vs	Sugar beets	32.303	0.764	0.073	
None	vs	Wheat	0.981	0.034	0.504	
None	vs	Livestock	2.181	0.238	0.318	
None	vs	Canola	0.195	0.012	0.787	
None	vs	Hay	37.600	0.904	0.275	
None	vs	Forage	8.680	0.684	0.275	
None	vs	Fallow	1.030	0.205	0.598	
None	vs	Durum	11.882	0.748	0.275	
None	vs	Alfalfa seed	1.678	0.295	0.275	
None	vs	Sugar beets	20.105	0.834	0.275	
Wheat	vs	Livestock	8.014	0.205	0.082	
Wheat	vs	Canola	0.830	0.020	0.598	
Wheat	vs	Hay	1.307	0.045	0.309	
Wheat	vs	Forage	0.929	0.032	0.530	
Wheat	vs	Fallow	1.312	0.045	0.313	
Wheat	vs	Durum	1.258	0.043	0.313	
Wheat	vs	Alfalfa seed	0.271	0.010	0.947	
Wheat	vs	Sugar beets	14.924	0.348	0.055	
Livestock	vs	Canola	0.263	0.014	0.791	
Livestock	vs	Hay	3.264	0.311	0.277	
Livestock	vs	Forage	3.033	0.303	0.277	
Livestock	vs	Fallow	0.510	0.068	0.610	

Livestock	vs	Durum	3.031	0.302	0.300
Livestock	vs	Alfalfa seed	1.552	0.181	0.382
Livestock	vs	Sugar beets	1.966	0.219	0.313
Canola	vs	Hay	0.242	0.015	0.313
Canola	vs	Forage	0.248	0.015	0.313
Canola	vs	Fallow	0.040	0.002	0.947
Canola	vs	Durum	0.294	0.018	0.313
Canola	vs	Alfalfa seed	0.077	0.005	1.000
Canola	vs	Sugar beets	0.584	0.035	0.313
Hay	vs	Forage	4.705	0.540	0.275
Hay	vs	Fallow	0.961	0.194	0.947
Hay	vs	Durum	12.429	0.756	0.275
Hay	vs	Alfalfa seed	1.463	0.268	0.512
Hay	vs	Sugar beets	28.468	0.877	0.275
Forage	vs	Fallow	1.081	0.213	0.402
Forage	vs	Durum	3.128	0.439	0.313
Forage	vs	Alfalfa seed	1.214	0.233	0.402
Forage	vs	Sugar beets	25.409	0.864	0.275
Fallow	vs	Durum	1.133	0.221	0.313
Fallow	vs	Alfalfa seed	0.294	0.068	1.000
Fallow	vs	Sugar beets	2.659	0.399	0.313
Durum	vs	Alfalfa seed	2.016	0.335	0.275
Durum	vs	Sugar beets	25.993	0.867	0.275
Alfalfa seed	vs	Sugar beets	9.027	0.693	0.275

Significance code: **<0.01,*<0.05, . <0.1

Table S.10. Results from the pairwise PERMANOVA comparing the bacterial community composition between different crop types. The F statistic is a measure of effect-size, the R2 indicates the variation explained by the model and the adjusted P-value specifies the overall significance of the comparison.

Pairwise comparison			F	R^2	P-value	Significance
Barley	vs	None	4.044	0.288	0.025	*
Barley	vs	Wheat	1.530	0.043	0.095	.
Barley	vs	Livestock	2.041	0.135	0.045	*
Barley	vs	Canola	2.537	0.103	0.014	*
Barley	vs	Hay	2.748	0.215	0.040	*
Barley	vs	Forage	1.902	0.160	0.063	.
Barley	vs	Fallow	2.584	0.205	0.031	*
Barley	vs	Durum	1.652	0.142	0.104	
Barley	vs	Alfalfa seed	5.301	0.346	0.015	*
Barley	vs	Sugar beets	4.014	0.286	0.023	*
None	vs	Wheat	3.018	0.097	0.015	*
None	vs	Livestock	2.547	2.267	0.055	.
None	vs	Canola	4.408	0.216	0.015	*
None	vs	Hay	9.626	0.706	0.104	
None	vs	Forage	6.740	0.627	0.104	
None	vs	Fallow	5.032	0.557	0.104	
None	vs	Durum	4.005	0.500	0.104	
None	vs	Alfalfa seed	11.046	0.734	0.104	
None	vs	Sugar beets	7.288	0.646	0.104	
Wheat	vs	Livestock	1.462	0.045	0.104	
Wheat	vs	Canola	2.066	0.049	0.040	*
Wheat	vs	Hay	2.183	0.072	0.040	*
Wheat	vs	Forage	1.640	0.055	0.104	
Wheat	vs	Fallow	2.001	0.067	0.041	*
Wheat	vs	Durum	1.408	0.048	0.109	
Wheat	vs	Alfalfa seed	3.485	0.111	0.014	*
Wheat	vs	Sugar beets	2.830	0.092	0.014	*

Livestock	vs	Canola	1.757	0.085	0.053	.
Livestock	vs	Hay	2.152	0.235	0.057	.
Livestock	vs	Forage	1.946	0.217	0.059	.
Livestock	vs	Fallow	1.739	0.199	0.063	.
Livestock	vs	Durum	1.563	0.182	0.104	
Livestock	vs	Alfalfa seed	3.596	0.339	0.040	*
Livestock	vs	Sugar beets	2.663	0.276	0.042	*
Canola	vs	Hay	2.400	0.130	0.014	*
Canola	vs	Forage	2.417	0.131	0.014	*
Canola	vs	Fallow	2.340	0.128	0.014	*
Canola	vs	Durum	2.312	0.126	0.040	*
Canola	vs	Alfalfa seed	5.302	0.249	0.014	*
Canola	vs	Sugar beets	4.326	0.213	0.014	*
Hay	vs	Forage	4.899	0.550	0.104	
Hay	vs	Fallow	1.931	0.325	0.104	
Hay	vs	Durum	2.705	0.403	0.104	
Hay	vs	Alfalfa seed	11.298	0.738	0.104	
Hay	vs	Sugar beets	8.123	0.670	0.104	
Forage	vs	Fallow	3.327	0.454	0.104	
Forage	vs	Durum	2.591	0.393	0.104	
Forage	vs	Alfalfa seed	7.674	0.657	0.104	
Forage	vs	Sugar beets	5.748	0.590	0.104	
Fallow	vs	Durum	1.338	0.251	0.200	
Fallow	vs	Alfalfa seed	6.255	0.610	0.104	
Fallow	vs	Sugar beets	4.631	0.536	0.104	
Durum	vs	Alfalfa seed	4.771	0.544	0.104	
Durum	vs	Sugar beets	3.108	0.437	0.104	
Alfalfa seed	vs	Sugar beets	5.424	0.576	0.104	

Significance code: **<0.01,*<0.05, . <0.1

Table S.11. Results from the pairwise PERMANOVA comparing the soil physico-chemical parameters between different herbicides used. The F statistic is a measure of effect-size, the R2 indicates the variation explained by the model and the adjusted P-value specifies the overall significance of the comparison.

Pairwise comparison			F	R^2	P-value	Significance
Glyphosate	vs	None	2.179	0.033	0.318	
Glyphosate	vs	Other	1.071	0.020	0.499	
None	vs	Other	0.625	0.018	0.577	

Significance code: **<0.01,*<0.05, . <0.1

Table S.12. Results from the pairwise PERMANOVA comparing the bacterial community composition between different herbicides used. The F statistic is a measure of effect-size, the R2 indicates the variation explained by the model and the adjusted P-value specifies the overall significance of the comparison.

Pairwise comparison			F	R^2	P-value	Significance
Glyphosate	vs	None	1.725	0.026	0.092	.
Glyphosate	vs	Other	1.482	0.028	0.092	.
None	vs	Other	1.526	0.043	0.092	.

Significance code: **<0.01,*<0.05, . <0.1

Table S. 13. Results from the pairwise PERMANOVA comparing the soil physico-chemical parameters between different fertilization methods. The F statistic is a measure of effect-size, the R2 indicates the variation explained by the model and the adjusted P-value specifies the overall significance of the comparison.

Pairwise comparison			F	R^2	Adjusted P-value	Significance
Banded with Seed	vs	Banded without seed	0.179	0.004	0.881	
Banded with Seed	vs	None	0.161	0.003	0.881	
Banded with Seed	vs	Broadcast	0.385	0.008	0.881	
Banded without seed	vs	None	0.908	0.035	0.864	
Banded without seed	vs	Broadcast	1.652	0.062	0.534	
None	vs	Broadcast	1.757	0.060	0.534	

Significance code: **<0.01,*<0.05, . <0.1

Table S.14. Results from the pairwise PERMANOVA comparing the bacterial community composition between different fertilization methods. The F statistic is a measure of effect-size, the R2 indicates the variation explained by the model and the adjusted P-value specifies the overall significance of the comparison.

Pairwise comparison			F	R^2	Adjusted P-value	Significance
Banded with Seed	vs	Banded without seed	1.664	0.035	0.036	*
Banded with Seed	vs	None	2.800	0.054	0.003	**
Banded with Seed	vs	Broadcast	1.483	0.029	0.067	.
Banded without seed	vs	None	3.429	0.121	0.003	**
Banded without seed	vs	Broadcast	2.018	0.075	0.024	*
None	vs	Broadcast	1.763	0.059	0.067	.

Significance code: **<0.01,*<0.05, . <0.1

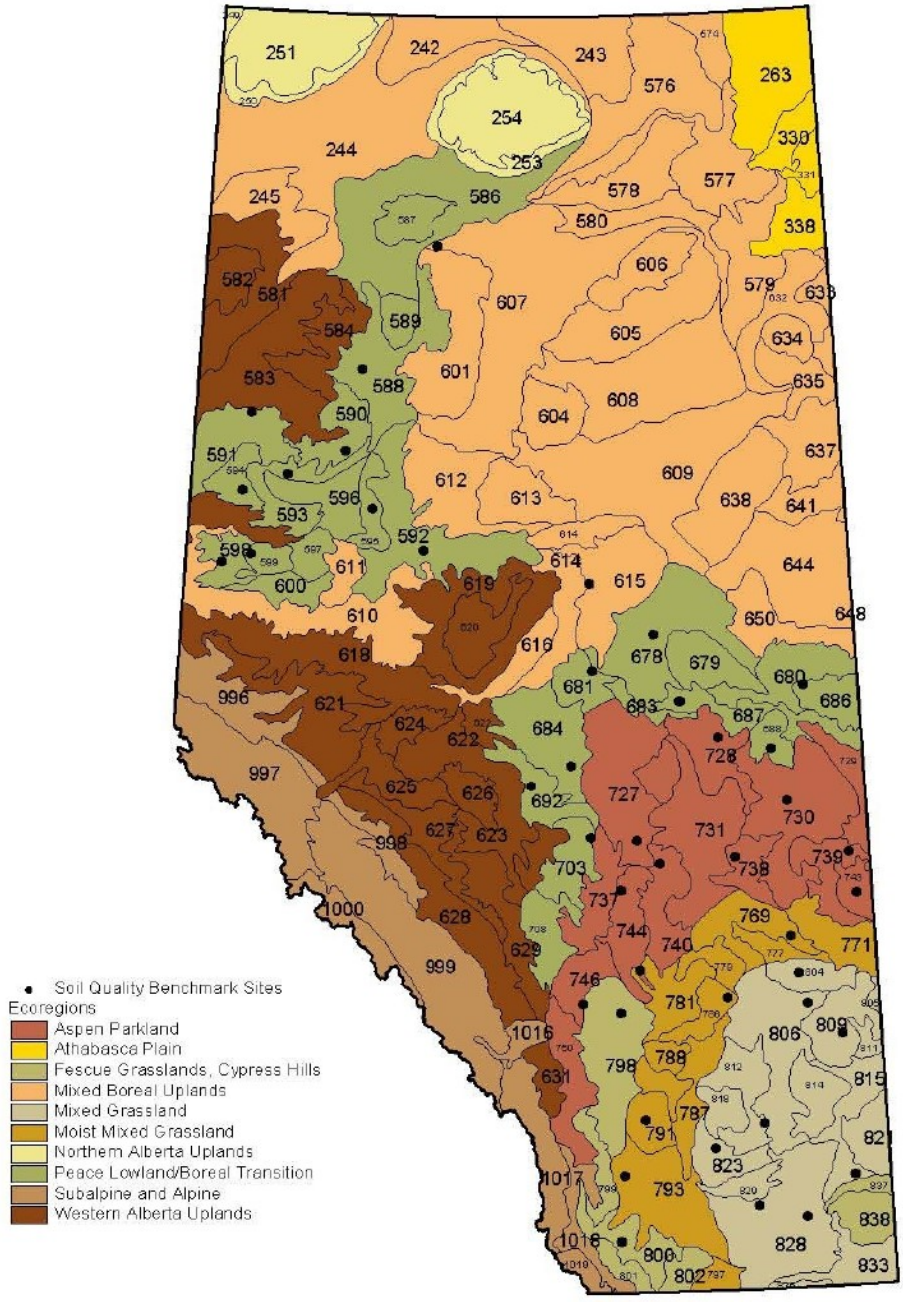

Figure S.1. Location of SQMP benchmark sites. Distribution of sites across the main agricultural ecoregions of Alberta. Numbers indicate the eco-district of the site and colors indicate different ecoregions (Cathcart et al., 2008).

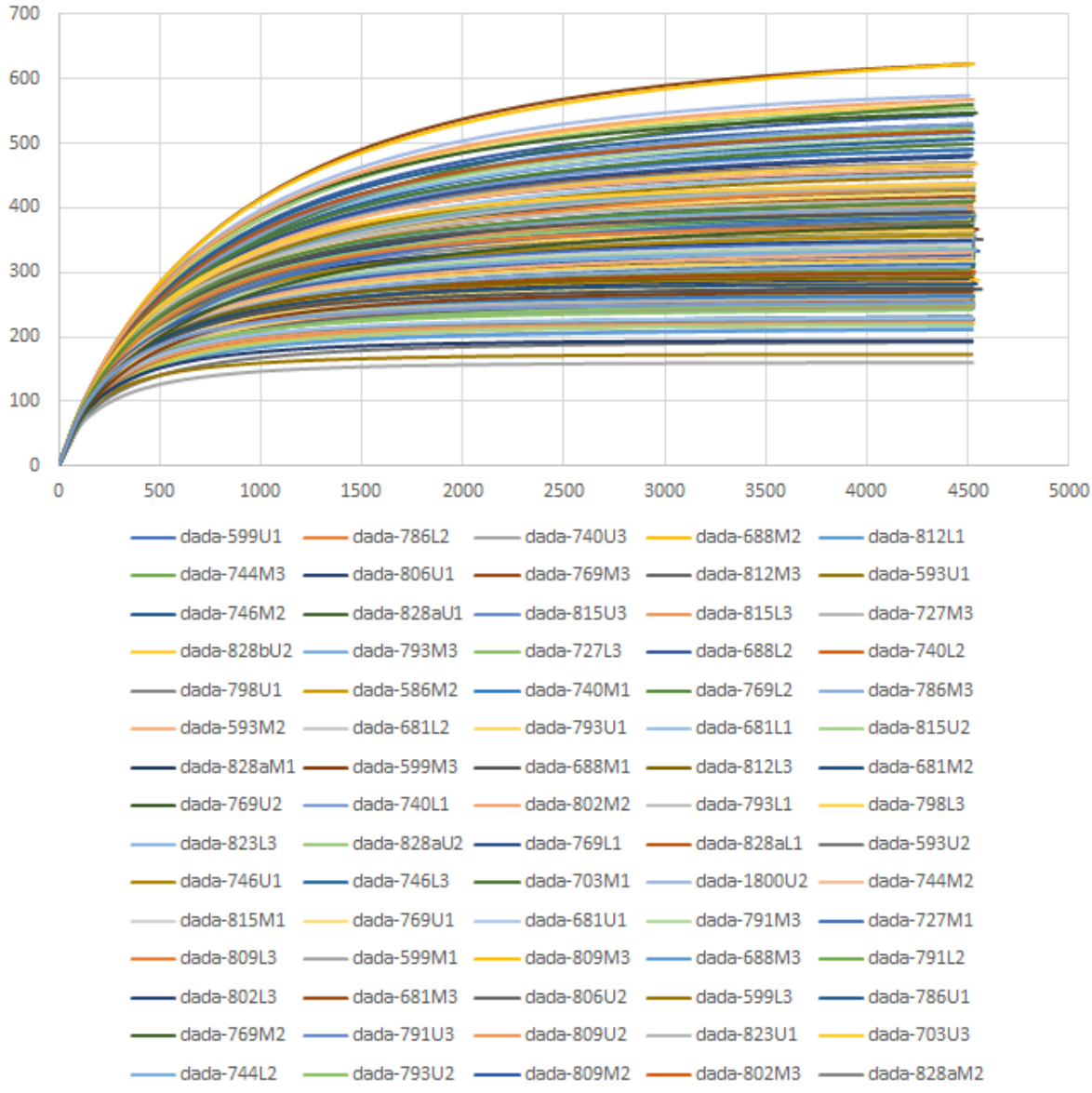

Figure S.2. Rarefaction curves for 254 random samples. Each curve indicates the subsampled richness level at each level of sequencing intensity from 0 to 10,000 sequences. Samples are labeled with the site number followed by the slope letter indicator and the replicate number.

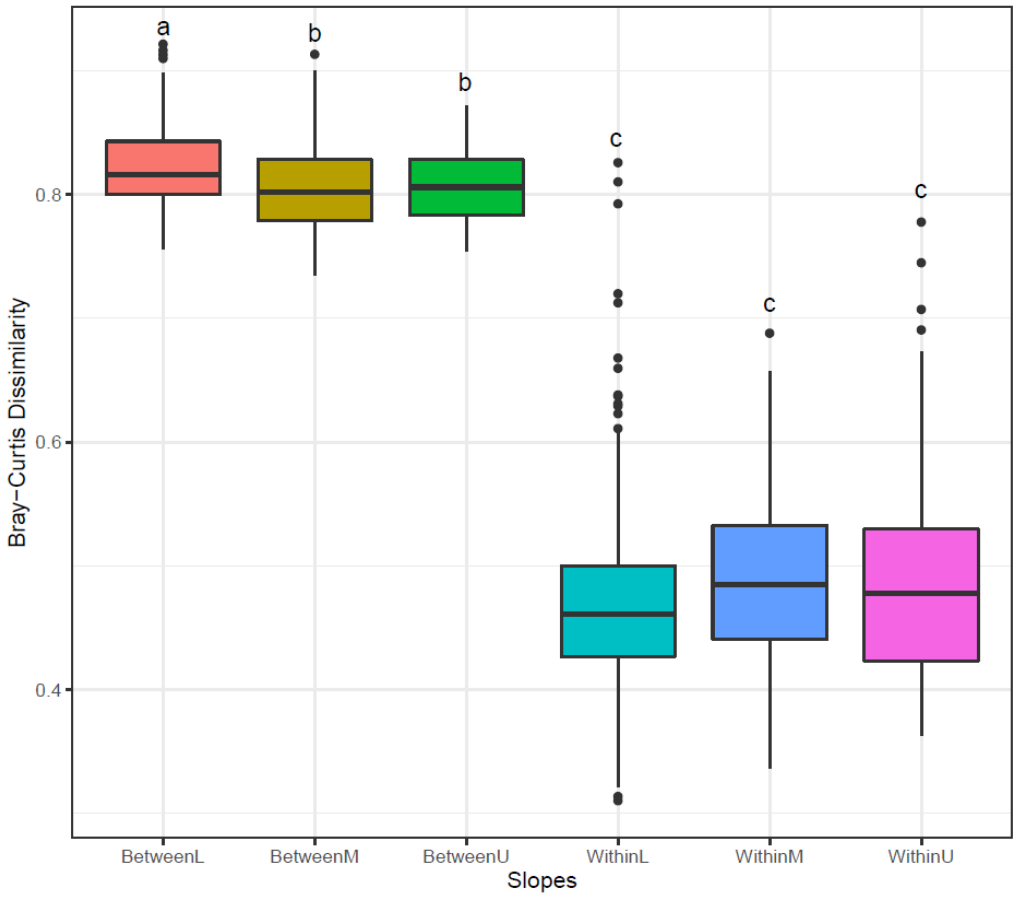

Figure S.3. Bray-Curtis dissimilarity of soil bacterial communities between the same slope position of the different sites, and within slope position replicates for all the sites. Different lower-case letters indicate significant differences according to pairwise Wilcoxon Rank. Mean dissimilarity of the same slope position between sites (0.458) > mean dissimilarity Within Slopes of all sites (0.812).

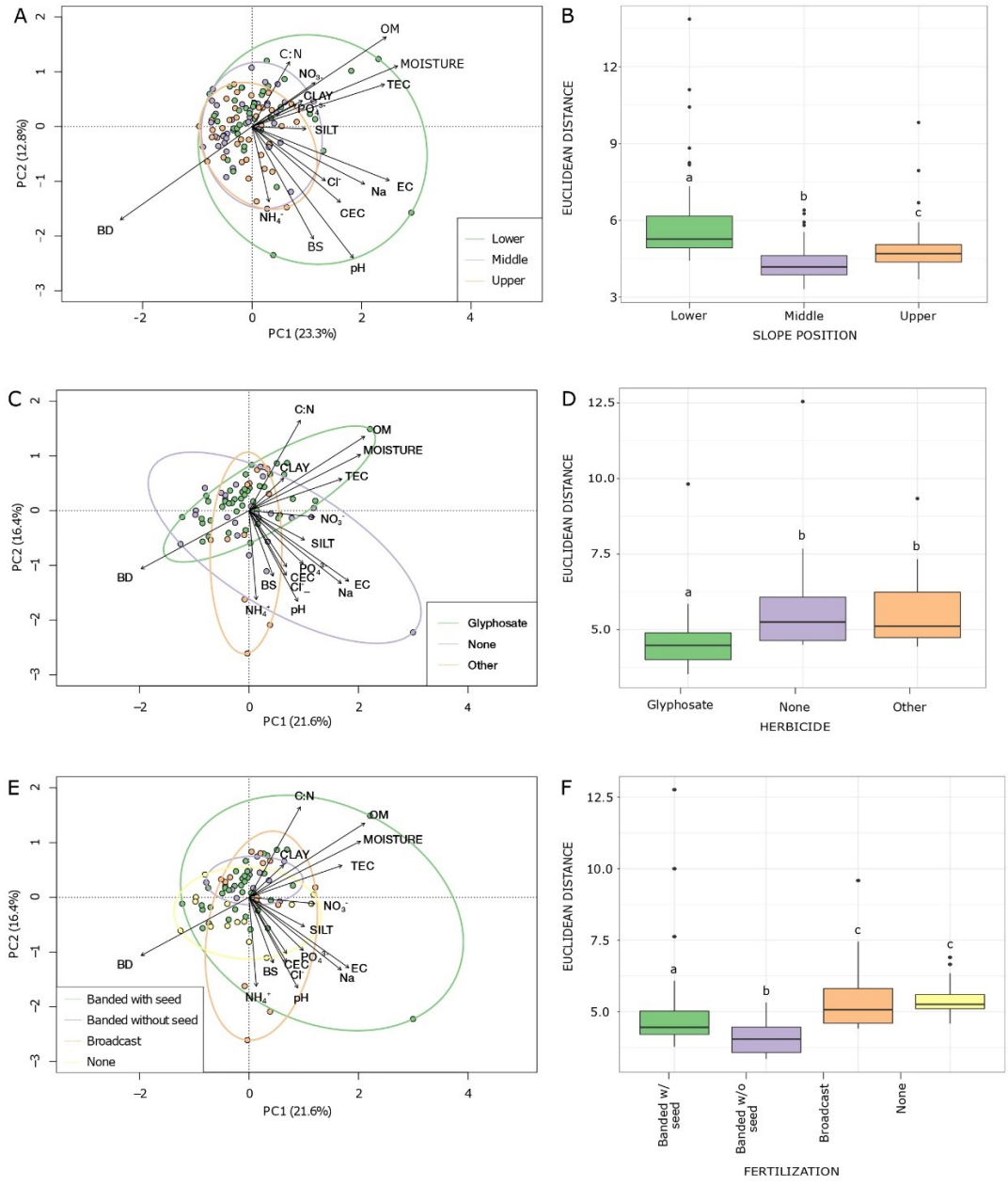

Figure S.4. Principal component analysis of soil physico-chemical parameters clustered according to (A) slope positions ($p > 0.05$), (C) herbicides ($p > 0.05$), and (E) fertilization methods ($p > 0.05$). Length of vectors indicate the influence of each parameter in the distribution of the data in the ordination space. Euclidean distance of soil physicochemical parameters across (B) slope position, (D) herbicides, and (F) fertilization methods. Different lower-case letters indicate significant differences according to pairwise Wilcoxon Rank.

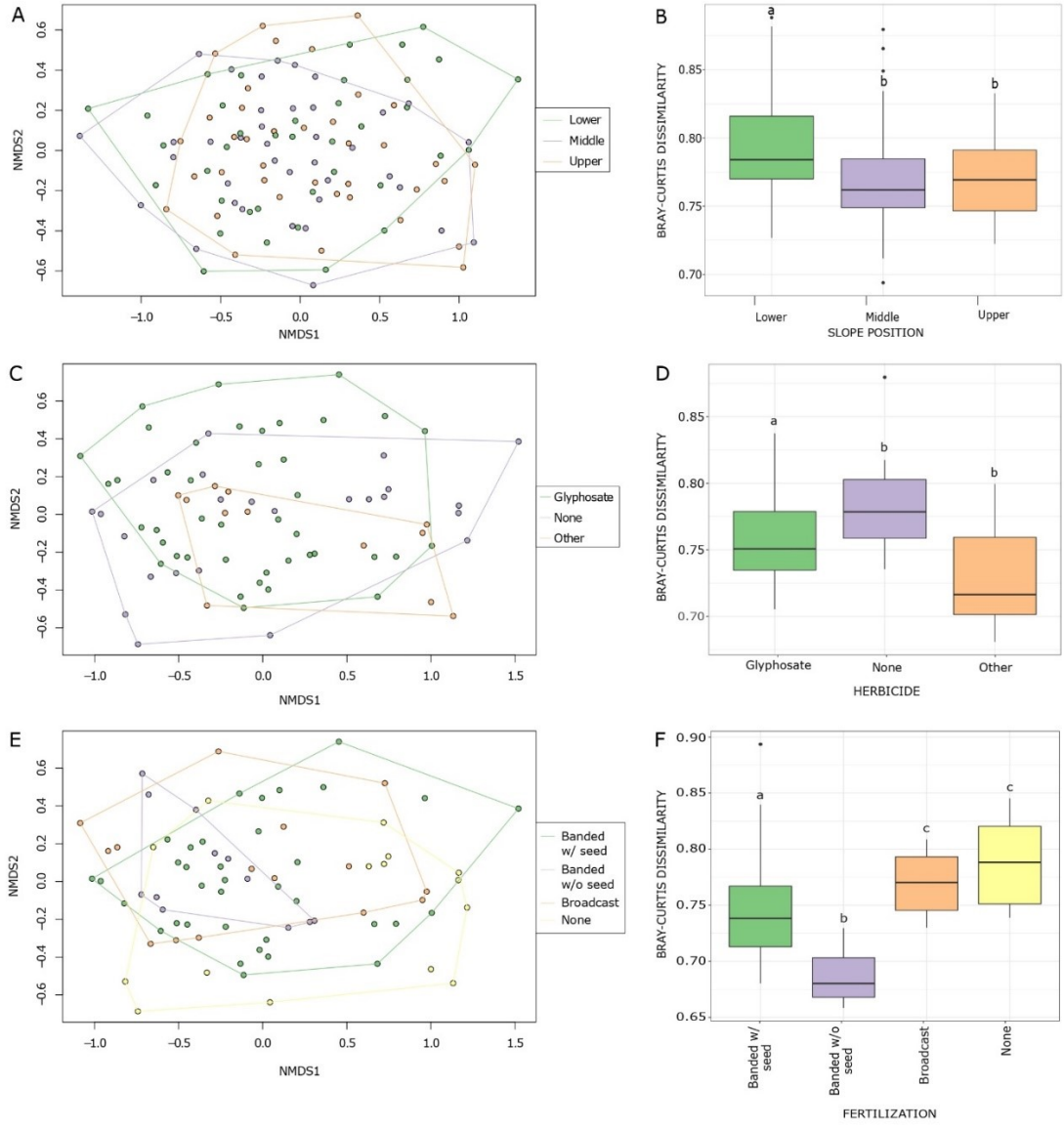

Figure S.5. Non-metric multidimensional scaling ordination of bacterial community composition at the ASV level, and clustered according to (A) slope position (ordination stress = 0.171282, $p > 0.05$), (C) herbicide (ordination stress = 0.1546061, $p > 0.05$)., and (E) fertilization (ordination stress = 0.1546061, $p < 0.05$). (B) Bray-Curtis Dissimilarity of soil bacterial communities across (B) slope positions, (D) herbicides, and (F) fertilization methods. Different lower-case letters indicate significant differences according to pairwise Wilcoxon Rank.

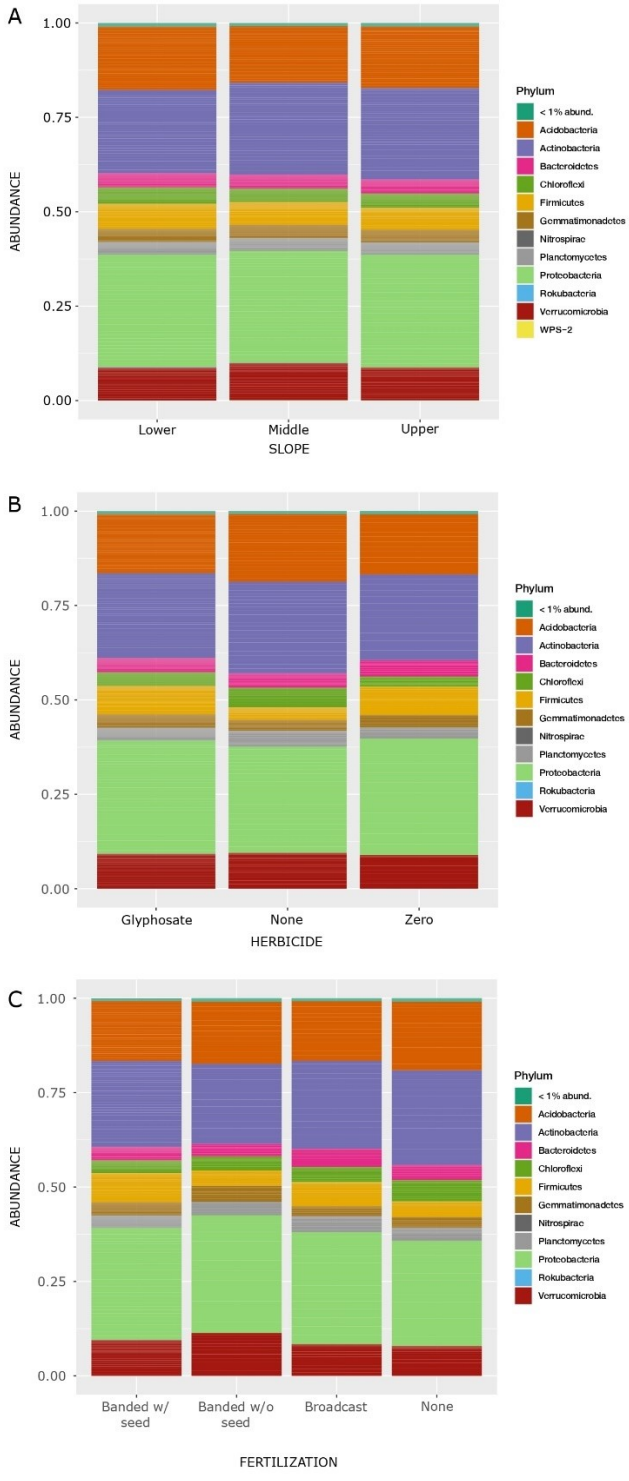

Figure S.6. Taxonomic profile of bacterial communities showing the relative abundance of the dominant groups at the Phylum level across (A) slope positions (B) herbicides, and (C) fertilization methods. Taxa with an abundance < 1% are grouped together.

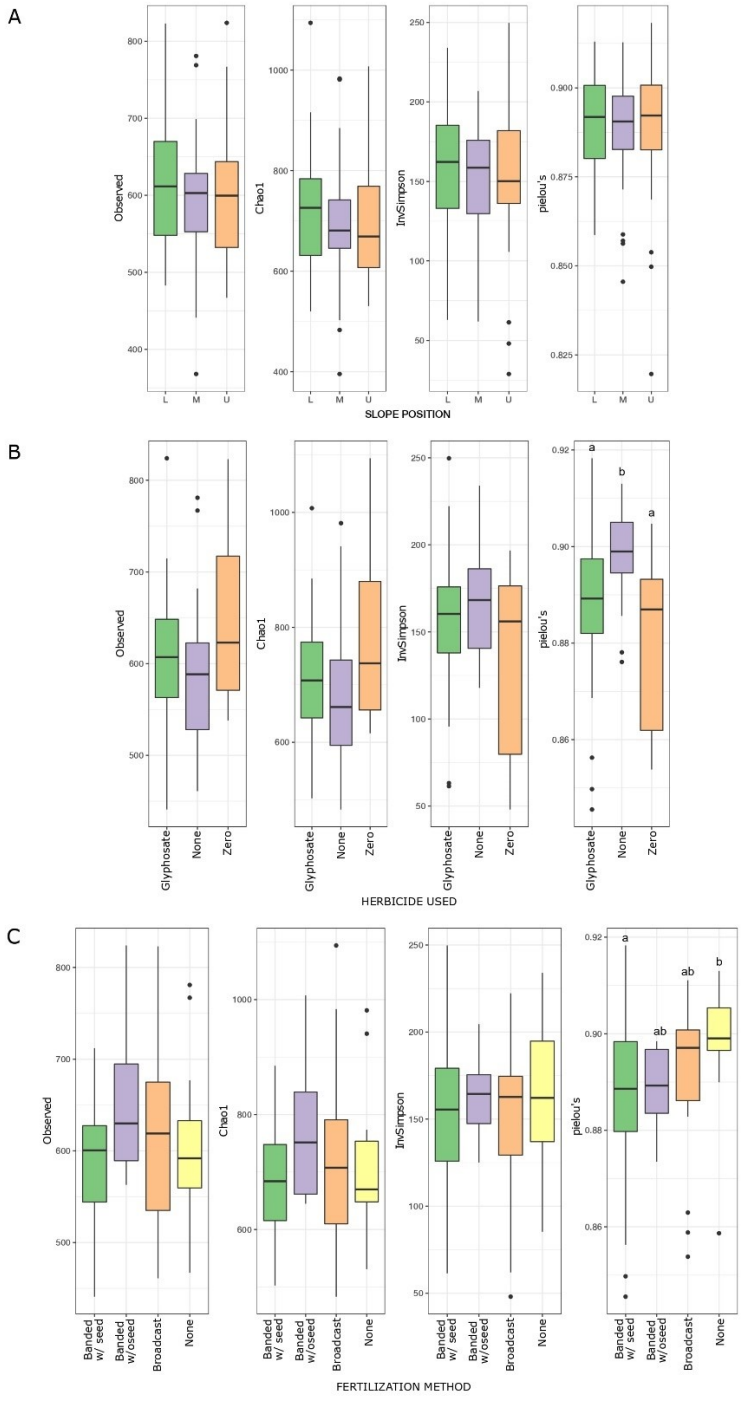

Figure S.7. Alpha-diversity metrics of bacterial communities across (A) slope positions (B) herbicides, and (C) fertilization methods. Different lower-case letters indicate significant differences. Measures indexes included observed number of ASVs, Chao1 index, Inverse Simpson index, and Pielou's evenness; shown in separate plots.

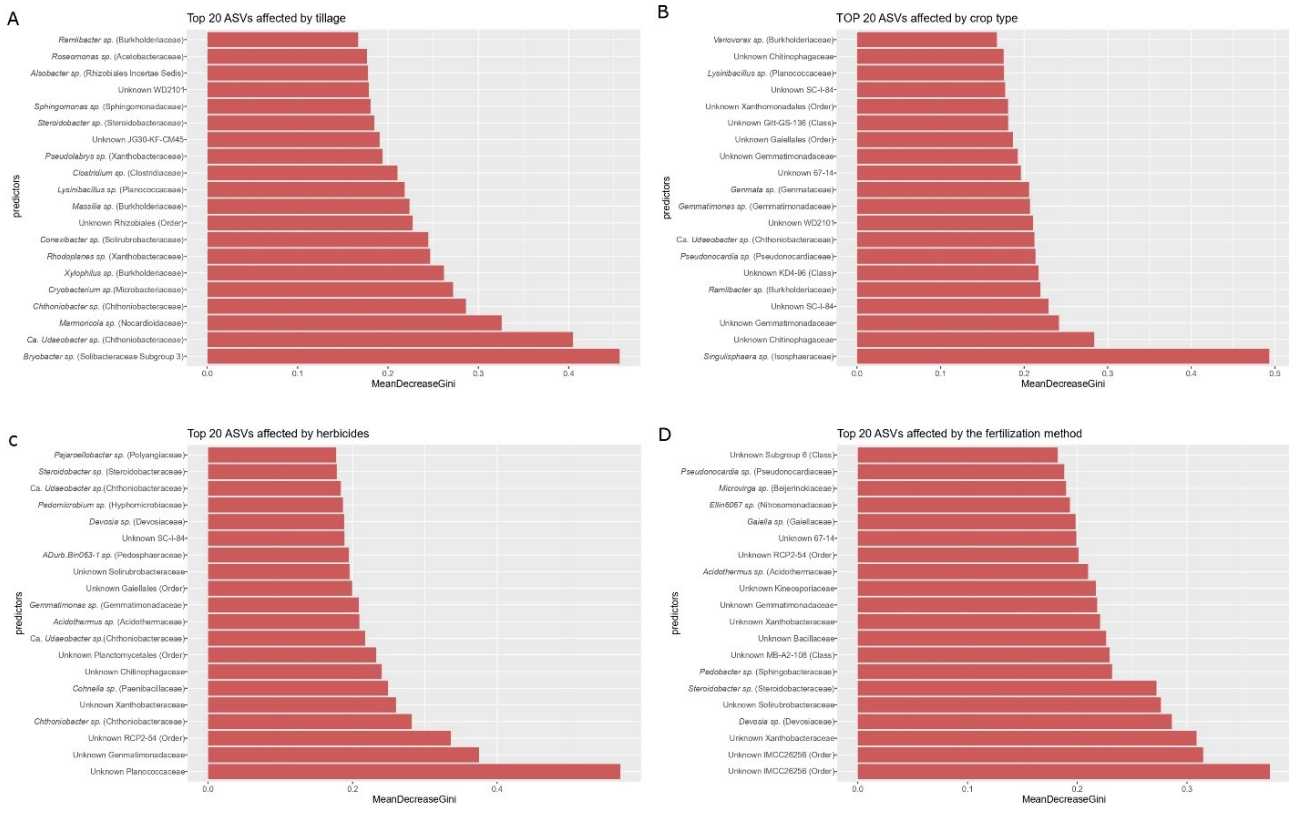

Figure S.8. Top twenty indicator species affected by (A) tillage intensities, (B) crop types, (C) herbicides used, (D) fertilization methods fertilization method, according to Random Forest Modeling. Higher mean Decrease Gini indicated ASVs more affected by the fertilization method.

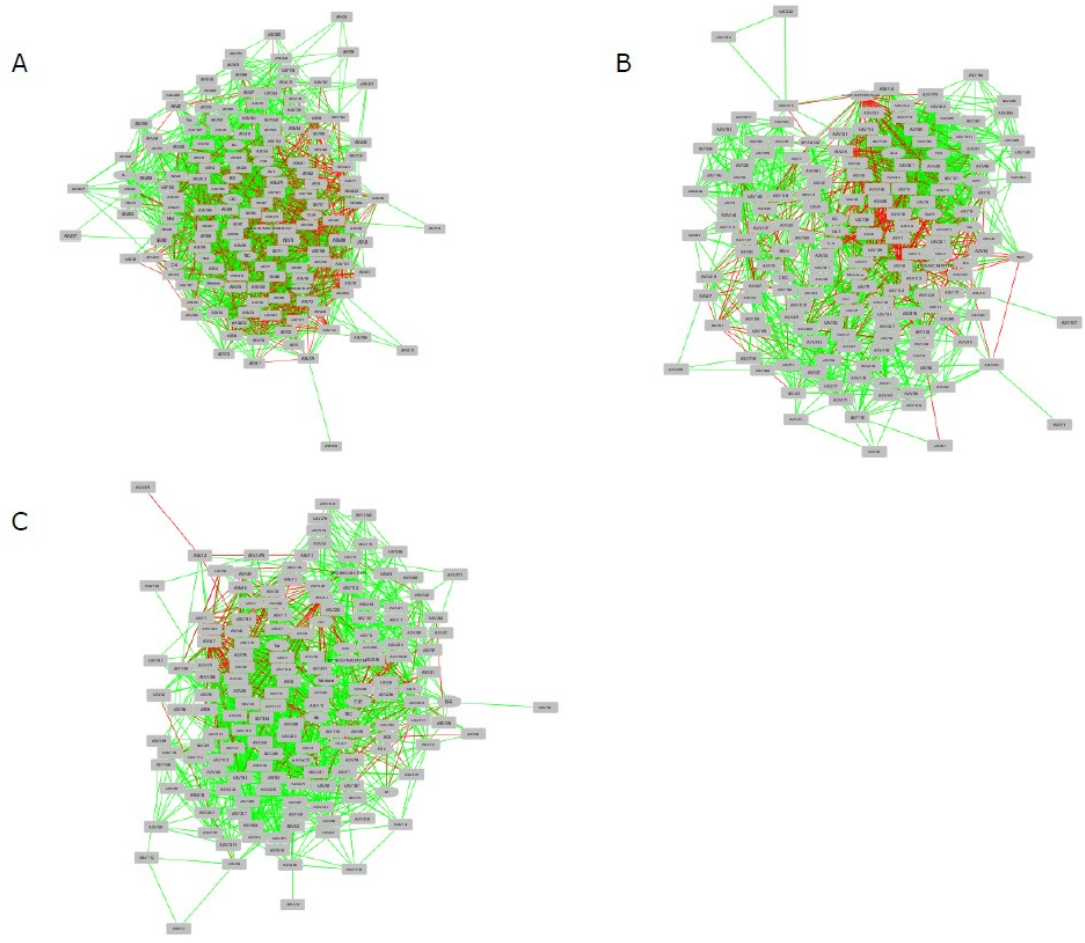

Figure S.9. Co-occurrence network analysis of bacterial communities undergoing (A) high tillage, (B) low tillage, and (C) Zero tillage. Each node represents a bacterial ASV, edges represent Spearman and Pearson correlations higher than 0.75 (green) or less than -0.75 (red), and Bray-Curtis dissimilarity less than 0.2.

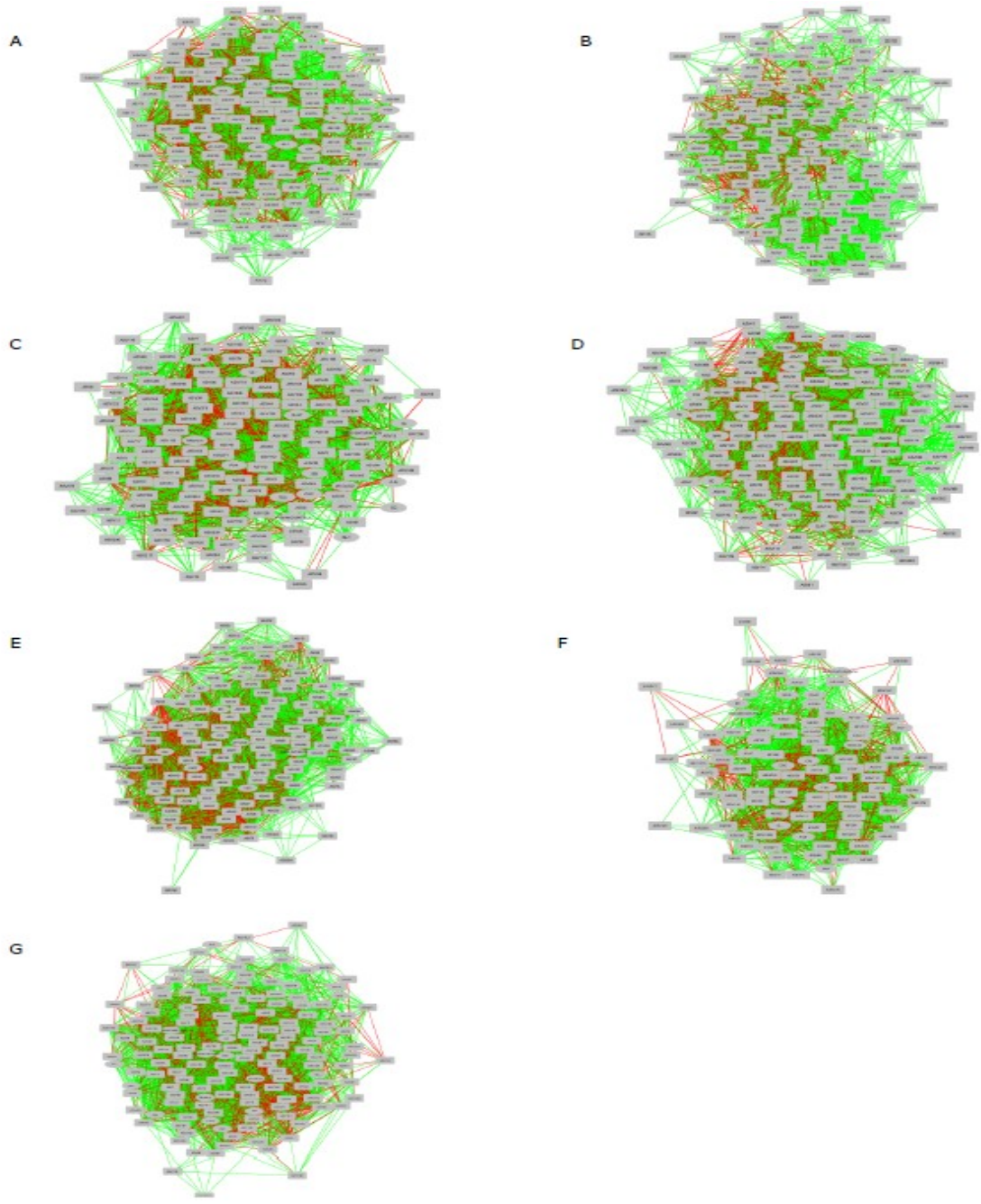

Figure S.10. Co-occurrence network analysis of bacterial communities from different crop types: (A) barley, (B) canola, (C) forage/hay (D) livestock, (E) none/fallow, (F) special crops, and (G) wheat/durum. Each node represents a bacterial ASV, edges represent Spearman and Pearson correlations higher than 0.75 (green) or less than -0.75 (red), and Bray-Curtis dissimilarity less than 0.2.

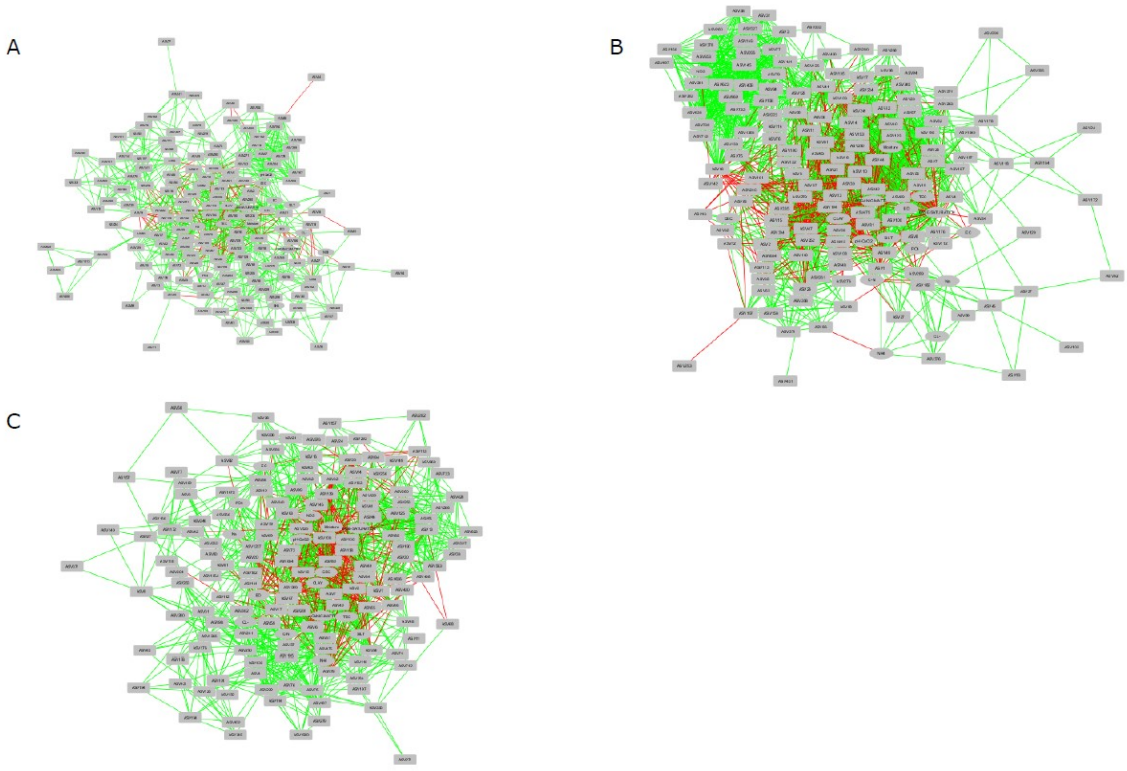

Figure S.11. Co-occurrence network analysis of bacterial communities undergoing the use of different herbicides: (A) glyphosate, (B) other, and (C) no/herbicide. Each node represents a bacterial ASV, edges represent Spearman and Pearson correlations higher than 0.75 (green) or less than -0.75 (red), and Bray-Curtis dissimilarity less than 0.2.

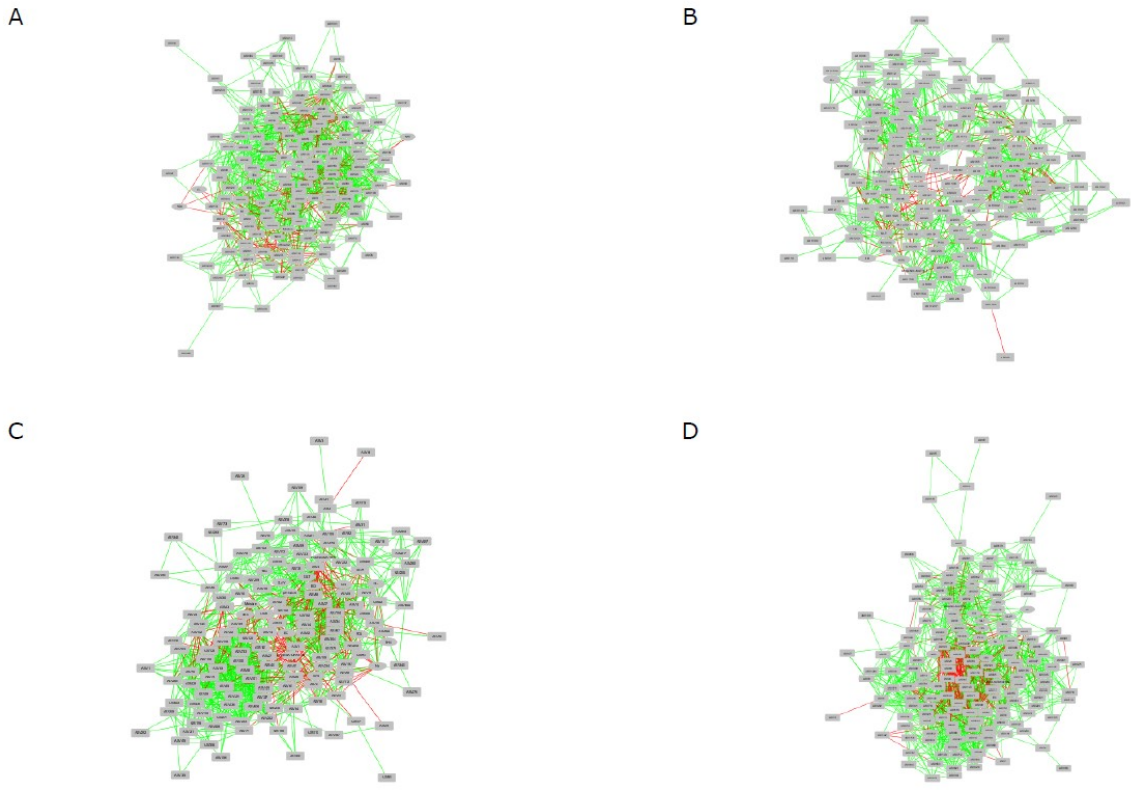

Figure S.12. Co-occurrence network analysis of bacterial communities undergoing different fertilization methods: (A) banded without seed, (B) banded with seed, (C) broadcast, and (D) no-fertilization. Each node represents a bacterial ASV, edges represent Spearman and Pearson correlations higher than 0.75 (green) or less than -0.75 (red), and Bray-Curtis dissimilarity less than 0.2.