Developing a soil health testing protocol for arable cropping systems in Saskatchewan

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ABSTRACT

Farmers are looking for appropriate tools and methods for assessing and interpreting the health status of their soils; however, for Saskatchewan there is no standardized and prairie-based soil health test available. As such, I focused on developing a soil health testing protocol for arable cropping systems in Saskatchewan by building off of the Comprehensive Assessment of Soil Health (CASH) framework developed in the USA. In Sept and Oct 2018, soil samples (0-15, 15-30, and 30-60 cm depths) were collected from 55 arable fields across Saskatchewan—along with a couple native prairie samples. Various soil chemical, physical, and biological attributes were measured (23 attributes in total). Based on the data distribution for each attribute, I developed scoring functions. The results from multivariate analyses were used to determine the weighting factors needed to integrate the individual scores from each soil attribute into a single Saskatchewan Soil Health Score (SSHS). Soil C and N indices (soil organic C, active C, total N, and soil protein) and total P produced the highest weighting factors. I also tested if there were linkages between the soil health scores and crop productivity by assessing the cereal yield trends for the past 10 yrs from the same rural municipalities where the soil samples were collected. A positive relationship between soil health and yields was most apparent during dry years; thus, I recommend further research to explore this linkage at a finer scale. Overall, this research forms the foundation of a promising tool for Saskatchewan producers who are interested in tracking soil health and using the results to inform management practices.

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LIST OF ABBREVIATIONS

BCA assay	Bicinchoninic acid assay
BSA	Bovine serum albumin
CASH	Comprehensive Assessment of Soil Health
CV	Coefficient of variance
e	Eigenvector
EC	Electrical conductivity
FC	Field capacity
IQR	Interquartile range
p	Proportion of explained variance
PCA	Principal component analysis
PMN	Potentially mineralizable N
PC	Principal component
R^2	R square
RMSE	Root mean square error
SOC	Soil organic carbon
SSHS	Saskatchewan Soil Health Score
TC	Total carbon
TN	Total nitrogen
w	Weighting factor
WAS	Wet aggregate stability

1 INTRODUCTION

Soil degradation limits agricultural productivity, resulting in economic losses and contributing to food insecurity. On the Canadian Prairies, one of the historic drivers of soil degradation was wind erosion, exacerbated by periods of drought and frequent tillage operations which exposed the soil to loss and resulted in the Dust Bowl of the 1930s. Since then, soil conservation practices have been adopted in this region to protect the soil and increase agricultural productivity—with (70%) of the cultivated Canadian prairies under no-till management (Clearwater et al. 2016), and only 5% summer-fallowed (Clearwater et al. 2016). In Saskatchewan, the risk of soil erosion is now considered very low (Clearwater et al. 2016). This history clearly demonstrates how improved soil management can minimize the risk of soil degradation. However, there are new concerns on the horizon which are largely brought about by climate change and the intensification of agricultural production. Moving forward, we must continue to identify the soil constraints and work towards supporting the continued functioning of agroecosystems.

Soil health is defined as "the capacity of soil to function as a vital living system, within the ecosystem and land-use boundaries, to sustain plant and animal productivity, maintain or enhance water and air quality, and promote plant and animal health" (Doran and Zeiss, 2000). This description considers soil as an ecosystem. By fulfilling complex functions, soil contributes to ecosystem services and highlights the linkages between soil health and human health. As such, monitoring and tracking the soil health status over time will aid in identifying soil constraints, and in adapting management practices for sustained soil functioning. To do this, however, robust soil health testes are needed in the toolbox.

Farmers and scientists are looking for an appropriate tool to interpret their soil's health status, so the assessment must be comprehensive. No single measurement can quantify soil health, but holistic measures of soil health are challenging because one must integrate biological, chemical, and physical properties, processes and interactions (Karlen et al., 1997). Ideally, a set of comprehensive soil indicators should also be conceptually related to soil function and ecosystem processes, practical to sample and measure, responsive to changes in management, and comparable to a baseline for a meaningful interpretation (Bunemann et al., 2018).

Currently, various soil health tests are in widespread use in many countries, including the USA (Andrews et al., 2002), China (Li et al., 2013; Chen et al., 2013, Zhou et al., 2020, Bi et al., 2013), Turkey (Karaca et al., 2021), UK (Cooper et al., 2020), India (Purakayastha et al., 2018) etc. One on the most comprehensive soil health tests was developed in the USA at Cornell University. Their Comprehensive Assessment of Soil Health (CASH) provides standardized information about the soil's physical and biological constraints, covering approximately 20 soil attributes that include the biological, physical, and chemical properties (Moebius-Clune et al., 2016). Each attribute is scored, and the overall score reflects the 'soil health status' as an unweighted average of all individual indicator's scores. Farmers and researchers are using CASH to estimate their soil health status and improve the management decision. Research showed that CASH was sensitive to various management practices in New York State (Idowu et al., 2009). The CASH provides a useful framework for integrating all the soil attribute into a visualized soil health score. However, the CASH is not always suitable for regions where the soil is different from those used to develop the scoring system used by CASH (i.e., soils outside the northeast region of the USA). For example, when used in locations outside the region of development, the CASH lacked consistent responses across the southeast region of the USA (Roper et al. 2017). Climate and parent material are the major factors that affect the soil formation and using the soil test developed from other regions may lose its meaning when applied to other regions. Numerous researchers recommend developing and using a regionally adapted soil health test to gain the most meaningful interpretation of soil health and functioning (Congreves et al. 2015; Roper et al. 2017; Forst et al. 2019; Chu et al. 2019). Since soil is a living ecosystem with its characteristics, a fixed measuring system may not be useful everywhere; rather, a regional soil health test may be most meaningful to farmers.

On the Canadian prairies—an agriculturally important region of Canada—there is no standardized prairie-based soil health test available. Our objective is to develop a soil health testing protocol, tailored to Saskatchewan soils—one that integrates biological, physical, and chemical indicators; transforms soil attribute values into meaningful scores, and uses a relevant weighting system to calculate the overall soil health score.

2 OBJECTIVES AND HYPOTHESES

The objective of this project is to identify the soil properties that best characterize soil health in the semi-arid prairie, explore the inter-link between attributes, and develop Saskatchewan Soil Health Test tailored to Saskatchewan's semi-arid climate and major soil zones. I hypothesized that the carbon and nitrogen indices are the most important components in determining soil health status.

3 LITERATURE REVIEW

Maintaining and building soil health is an essential component of long-term sustainable agriculture. Soil health can be defined as the capacity of a soil to function, which reflects sustained biological productivity, environmental quality, and plant health (Karlen et al., 1997; Doran and Zeiss, 2000). Important indicators of soil health often reflecting changes to soil structure, infiltration, plant available water, soil acidity, salinity, plant nutrients, organic matter, microbial biomass, and microbial diversity (Allen et al., 2011). A healthy soil will produce high crop yields under favorable weather conditions, and also have a strong capacity to withstand extreme weather events and reduce nutrient loss. Therefore, soil health is crucial for increasing the adaptability and resiliency of agroecosystems to climate change. Research must focus on methods of maintaining/improving soil health and provide appropriate protocols for interpreting soil health status.

No single measurement can quantify soil health; rather it must be inferred by using a framework for integrating many soil attributes (Carter et al., 1997). Holistic measurements of soil health are complex because one must integrate biological, chemical, and physical properties, processes and interactions (Karlen et al., 1997). Cornell University developed their Comprehensive Assessment Soil Health (CASH) in New York, USA to provide farmers with a tool to assess their soil health status. It integrates measurements of numerous soil attributes (approximately 20), including aggregate stability, organic matter, active C, nutrient levels, texture, etc. (Gugino et al., 2009; Moebius-Clune et al., 2016). Using CASH, individual soil attributes are scored (0–100%), the higher the better, providing an easily interpretable metric for characterizing overall soil health.

However, the simple un-weighted average approach of CASH can result in a biased soil health score due to extreme values from individual soil attributes. Consequently, improvements to the way in which the overall soil health score is calculated are required for a more meaningful representation of overall soil health (Van Eerd et al., 2014; Congreves et al., 2015). Furthermore, CASH was developed from soils in northeastern USA which are typically not as rich and fertile as the Chernozemic soils that we have in Saskatchewan. Consequently, Saskatchewan farmers who use the CASH test get exaggerated CASH scores that are not meaningful when deciding on

management practices. The Soil Health Institute recommends that different regions develop their own scoring functions based on local soils, for a regionally representative soil health score. This is because, to be useful, soil health indicators must be clearly interpreted and ideally expressed relative to reference values. Reference values for soil attributes could be derived from an undisturbed soil or a soil with superior primary productivity and environmental performance. As a framework for soil health scoring, soil health indicators at any given site can be compared to either a reference soil (i.e., native prairie), or to the statistical distribution frequency of the given indicator.

3.1 Agriculture challenges

Achieving global food security is one of the most significant challenges in the 21st century. The challenge directly puts pressure on agriculture and the environment, necessitating the production of more food from the land available and leading to agricultural intensification. About 95% of food—directly and indirectly—comes from soil (Bot and Benites, 2018), and the impact of agricultural intensification on soil will, in turn, impact food production. Although agricultural intensification necessary, the productivity of soil is limited. Soil disturbance by tillage operations and traffic, and the application of fertilizers and herbicides will influence soil organic matter dynamics and potentially lead to soil degradation (Acosta-Martínez et al., 2010; Massah and Azadegan, 2016). Once degraded, soil can take decades to recover (Kim et al., 2018; Gao et al., 2019); soil health must be maintained or improved to sustain food production over the long term, and is considered a top priority for the future of agriculture.

Besides the pressure of food production, climate change also brings challenges to agriculture and soil. Climate change is expected to bring periods of drought and floods, warming, and more extreme weather events, which will affect plant growth and nutrient cycling. Increasing temperatures may either increase or decrease crop yield, depending on the crop type and region. Modelling research in Canada suggested that a warmer climate will increase the crop yield of spring wheat, winter wheat, and corn but increase soil N_2O emissions when the potential extreme event was not accounted in the model analysis (Smith et al., 2013). In contrast, American research found warming scenarios predict to cause crop yield declines for soybean, corn, and cotton (Schlenker and Roberts, 2009). Higher atmospheric CO_2 concentrations may increase crop yields

by a greater water use efficiency through increasing photosynthetic rate as well as decreasing stomatal conductance (Parvin et al., 2019). Climate change is expected to not only change crop yields but also alter soil nutrient cycling. For example, the capacity for nitrogen fixation during later growth stages is expected to decline due to the combined effect of CO_2 and drought (Parvin et al. 2019) which will impact soil nutrient availability. Also, atmospheric CO_2 levels are known to impact the stoichiometry of C3 plants, thereby influencing the quality of residues that are returned to the soil, for example, research showed that artificial CO_2 enrichment increased C3 plants foliar C:N and C:P ratios by 22% and 38%, respectively (Sardans et al., 2012). Increased temperatures can influence the quality and chemical composition of plant tissues, i.e., when grown at a higher temperature spring barley had increased levels of some amino acids, but reduced levels of total non-structural carbohydrates, starch, fructose and aluminum (Högy et al., 2013). Further, the quality and nutrient composition of grain is affected by warmer temperatures and altered precipitation patterns. Although the overall effect of climate change on crop production is uncertain and hard to predict (creating difficulty in management planning), it is clear that climate change affects the primary productivity and internal nutrient composition of plants in by various mechanisms (temperature, CO_2, soil nutrient cycling, etc.).

Saskatchewan cropping systems are predominantly rainfed (rather than irrigated) relying on sufficient precipitation for crop production, so soil water storage and supply is a critical component of crop production. Scientists project climate change will lead to periods of drought or intensive rainfall for many regions (Jentsch et al., 2007). With rising temperature and higher evaporation rates, the risk of water deficiency increases. Based on current models and patterns, Canadian climate is projected to become warmer and warmer and increase the severity of extreme weathers such as drought and wildfire risk, and Saskatchewan expected to have less precipitation in winter (Bush and Lemmen, 2019). Drought is one of the major constraints for agriculture. The shortage of soil moisture will seriously restrict plant growth and crop yield. To adapt to increased temperature and drought, plants often accelerate growth to reach reproduction, as was observed for wheat and the shortened period of grain filling and seed reproduction (Altenbach, 2012). As such, the risk of yield loss is expected to increase in the future. For the world's four major crops, is it highly probable that exceptional drought will reduce yields: by over 70% for soybean and maize, by 68% for wheat, and by 64% for rice (Leng and Hall, 2019). Other researchers found that

drought conditions reduced wheat root biomass, shoot biomass, plant biomass and grain yield by 4%, 20%, 11%, 28%, respectively; although root has plasticity to adapt the drought, not all root system response same since the plasticity of the root system is limited by genes (Ehdaie et al., 2012). Besides, extreme weather, such as intensive rainfall, also negatively impacts crop production. The soil moisture not only directly affects plant growth during the growing season, it also influences the nutrient availability and soil microbial community composition during plant establishment and growth. Arbuscular-mycorrhiza symbiosis is one plant strategy to optimize nutrition and promote plant growth, but high soil moisture after intensive rainfall can profoundly influence the arbuscular-mycorrhizal colonization, and therefore influence the capacity for plant nutrient acquisition via mycorrhizal fungi (Cavagnaro, 2016). Overall, periods of decreased or increased soil water caused by climate change will dramatically affect crop growth.

With the rapidly changing climate and the food demand, humans must develop sustainable agriculture practices that not only boost crop yield but that are also highly adaptable and resilient. Soil is the foundation of agriculture; thus, supporting the soil's capacity to function is foundational to increasing agriculture's capacity and the functioning of ecosystem services. Healthy soil has numerous benefits, including regulating water infiltration and retention, supporting nutrient cycling, microorganism activity, biodiversity, and provisioning food. Maintaining or improving the capacity of soil to function goes hand-in-hand with supporting the capacity and resiliency of agriculture production.

3.2 The importance of soil in agriculture and soil health

Humans have a long history of growing crops and raising livestock, and these farming activities are inextricably linked to the soil. Soil is a unique layer of Earth's surface comprised of mineral, organic matter, microorganisms, liquid, and gases. It is a dynamic and diverse ecosystem that supports a wide range of flora and fauna across the Earth. The soil ecosystem provides food, fuel and fibre, clean air and water, shelter, habitat and more. This provisioning demonstrates the valuable and critical role that soil serves for all humans.

Soil organic matter is an essential component of soil health as it supplies nutrients, energy and carbon (C). Soil contains about two-thirds of the total C in the terrestrial ecosystem, and the amount

of C in the soil exceeds the amount of C in plants and the atmosphere (Amundson, 2001; Scharlemann et al., 2014). Greenhouse gases (GHG) contribute to global warming, and agriculture accounts for 20% of global GHG emissions—one of the most significant contributors to global GHG emissions (Lokupitiya and Paustian, 2006). Carbon dioxide is naturally produced and emitted from soil and plants, during microbial and plant respiration, decomposition of organic matter, and acids' reaction with the carbonates. In turn, CO_2 in the atmosphere is taken by plants through photosynthesis, and it is also absorbed by water. The C cycle has been influenced by urbanization and industrialization—anthropogenic activities such as fossil fuel combustion and land-use change. Agriculture soil could be a net sink or source for C, depending on the management practices and their relative effect on C input. Utilizing the capacity of soil to store C could be an efficient method to mitigate the greenhouse effect. No-till, organic amendments, conservation reserves, crop rotation, and improved fertilizer use are practices that tend to increase C storage in the soil (Liang et al., 2020; Nunes et al., 2020). Minasny et al. (2017) concluded that total national GHG emissions were reduced by 2% due to decreasing summer fallow and increasing the no-till acreage on the prairies in 2013. Having continuous living cover helps to sink atmospheric CO_2 into the soil, increase the C sequestration, promote microbial activity through the active nutrient exchange, and prevent soil erosion or runoff caused by the extreme weather (Lal, 2015). Thus, agriculture has potential to store atmospheric CO_2 in the soil, via appropriate soil management.

Soil degradation may limit agricultural productivity, resulting in economic losses. Historically, wind erosion and soil erosion were major threats to prairie farming due to improper management and intensive cropping. With the increasing awareness and adoption of soil conservation practices, the risk of soil erosion has significant decreased across the county since 1981, and the majority of Saskatchewan land is now in the very low-risk class (Clearwater et al., 2016). This improvement is predominantly due to the adoption of no-till practices and by reducing summer fallow. In Saskatchewan, the proportion of farmland under no-till was only 10% in 1991, but it has since expanded—reaching 70% in 2011 (Clearwater et al. 2016). Also as of 2011, only 5% of farmland is summer-fallowed (Clearwater et al. 2016). These changes demonstrate how improved soil management can minimize or even remove the risk of soil degradation. Moving forward, it is

crucial to identify other soil constraints and continue to apply practices that support the functioning of agroecosystems.

The future of agriculture is intertwined with the future of soil resources. Soil is a complex ecosystem in which living microorganisms and plant roots combine with mineral particles and organic matter to form a dynamic structure regulating water, air, and nutrients. Fulfilling its ecosystem service and capacity is key to a successful agronomic practice, and future agriculture should place value in supporting soil health. Today, the terms "soil health" and "soil quality" are considered synonymous and can be used interchangeably. In 1987, the definition of soil quality provided by Soil Science Society of America was “Inherent attributes of soils that are inferred from soil characteristics or indirect observations” (Cited in Doran and Parkin, 1994). Although the definition of soil quality emphasised the inherent characteristics of soil, soil quality had started involved the biological properties in 1990s (Gregorich et al., 1994; Doran and Parkin, 1994). Soil quality was used to describe the inherent properties and agronomic usefulness of soil, but it is synonymous with soil health in agriculture perspective was used to emphasize the ecological system functioning, as well as the inherent soil properties (Magdoff, 2001). Today, both terms are used to describe the role of soil in supporting both agronomic and ecological functions. The most common definition of soil health is "the continued capacity of the soil to function as a vital living ecosystem that sustains plants, animals and humans" (Natural Resources Conservation Services).

Sustainable soil management should consider economic, environmental, and social perspectives. Thus, maintaining soil health is beneficial to farmers and also the environment and wider population. In evaluating soil health through an ecological perspective, various ecological principles are considered when developing sustainable agricultural practices. Soil management strategies aligned with sustainable farming include maximizing biodiversity, minimizing disturbance, maximizing soil cover, and maximizing living root (Stewart et al., 2018). Individual soil health strategies have multiple effects on the agroecosystem. For example, the cover crop grown in the fall will keep the soil covered with living vegetation, help maintain the activity of microorganisms, aid in cycling nutrients, prevent the nitrate leaching into the deeper depth, and provide weed suppression in Fall and Early spring (Lawley et al., 2012; O’Reilly et al., 2012; Ruark et al., 2018). The senesced plant residue from cover crops provides an excellent surface

cover that protects the soil from erosion or runoff, helps with moisture infiltration. The residues will also decompose, supplying nutrients to the soil and further contributing to nutrient cycling (Jahanzad et al., 2016). Jahanzad et al. (2017) demonstrated radish residue with fast decomposing rate provided sufficient N for early planting potato and improved the tuber yield and N use efficiency in Massachusetts. However, some studies stated radish had no significant N contribution to subsequent crop yield (Ruark et al., 2018), and also concerned that the cover crop may lower the available soil water for the subsequent cash crop when comparing with the Fall fallow (Khan and McVay, 2019). Although the improvement of the cover crop on the subsequent crop's yield still be controversial, the benefit of cover crop in Fall is effective and consistent. The successful cover crop application should account for the site characteristics, planting data, and the matching capacity of subsequent crop. Thus, a sustainable soil program should be designed and monitored to facilitate the functioning of ecosystem services (Smith et al., 2016). Healthy soil will maintain/increase crop yield sand quality by supporting plant growth, but also improve water and nutrient retention, reduce the potential of soil degradation such as erosion, compaction, and crusting. Moreover, soil as an ecosystem has the resilience to buffer the outside pressure. Increasing soil biodiversity will increase the stability of the ecosystem's productivity (Isbell et al., 2015). Keeping the soil healthy will sustain the multifunctionality of soil.

3.3 Soil health assessment and research

In the 1990s, Doran and Parkin (1994) introduced the notion of soil health, and Acton and Gregorich (1995) framed the role of soil health in supporting agriculture. Acton and Gregorich described soil health as "the soil's fitness to support crop growth without becoming degraded or otherwise harming the environment." Scientists have since tried to assess the quality and health of soil, and the effect of management practices on soil and environment. The connection between soil health, plant health, and human health was acknowledged by Doran and Zeiss (2000), as they defined soil health as "the capacity of soil to function as a vital living system, within the ecosystem and land-use boundaries, to sustain plant and animal productivity, maintain or enhance water and air quality, and promote plant and animal health". This description considers soil as an ecosystem, and by fulfilling complex soil functions, it contributes to ecosystem services and highlights the linkage between soil health and human health. In the 2010s, with climate change awareness and increasingly extreme weather events that lead to soil degradation, experts have emphasized soil's

continued capacity in the definition of soil health (Natural Resources Conservation Services). This updated description of soil health reflects the public's increasing awareness and interest in soil health.

The development of soil health tests aligns with the movement of defining soil health. The definition and quantification of soil health is complicated, and no single value or measurement can directly assess soil health. Traditional soil tests focused on fertility and measured the nutrient contents and soil pH to improve crop productivity. With the increasing awareness of soil health, researchers have integrated biochemical indicators to study the dynamic soil processes such as earthworm activity, N mineralization, microbial biomass, and C/N ratios (Doran and Zeiss, 2000; Andrews and Carroll, 2001). The ever-increasing number of indicators created more complexity and difficulty in conducting and interpreting a soil test. The cost of tests that have several different indicators also restricted widespread implementation of soil health tests. The minimum data set (MDS) approach was proposed to reduce the number of indicators that were analyzed on a given collection of samples, limited to those most sensitive to management change or most meaningful to farmers. The Haney Soil Health Test (HSHT) is an example of a MDS approach that involved expert-selected indicators (soil respiration, and water-extractable organic C and N) in measuring the soil health status (Haney et al., 2018). To narrow the list of soil health indicators for a MDS, research have employed multivariate analyses (such as principal component analysis, PCA) (Andrews and Carroll, 2001) and regression analyses (Li et al., 2013). Several researchers have use PCA to develop a MDS and to ascertain the weight of individual soil indicators (Andrews et al., 2002; Bi et al., 2013; Chen et al., 2013; Congreves et al., 2015; Purakayastha et al., 2019; Zhou et al., 2020; Karaca et al., 2021). Generally, the variable with the highest eigenvector on one PC is included in the minimum data set. For multi-indicator soil health tests, the importance of each individual indicator (their weight) have been derived from PCA (Andrews et al., 2002; Congreves et al., 2015). Alternatively, Pearson correlation and correlations-sum have been used to reduce the redundancy and number of variables (Andrews and Carroll, 2001; Chen et al., 2013; Li et al., 2013). The cost of MDS-based soil health tests will decrease by reducing number of soil indicators, thus, improving the chances of user uptake. However, the MDS approach runs the risk of over-expressing certain components of soil health (i.e., biological component) while neglecting soil chemical and physical components.

Currently, soil health tests are in widespread use in many countries, including the USA (Idowu et al., 2008; Moebius-Clune et al., 2016; Haney et al., 2018), China (Bi et al., 2013; Chen et al., 2013; Li et al., 2013; Zhou et al., 2020), Turkey (Karaca et al., 2021), UK (Cooper et al., 2020), India (Purakayastha et al., 2019) etc. Farmers and scientists are looking for an appropriate tool to interpret their soil's health status, so the assessment must be comprehensive. The Comprehensive Assessment of Soil Health (CASH) was developed to provide standardized information about the soil's physical and biological constraints, covering approximately 20 soil attributes that include the biological, physical, and chemical properties (Moebius-Clune et al., 2016). Each attribute is scored based on measurement, and the overall score reflects the 'soil health status' as an unweighted average of all individual indicator's scores. Farmers and researchers are using CASH to estimate their soil health status and improve the management decision. Research showed that CASH was sensitive to various management practices in New York State (Idowu et al., 2009).

The CASH provides a useful framework for integrating all the soil attribute into a visualized soil health score. However, the CASH is not applicable or suitable for other regions since the database was developed in New York and the Northeast region. For example, when used in locations outside the region of development, the CASH and HSHT lacked consistent responses (Roper et al., 2017). It was concluded that either test did not have the capacity for soil health determination among agronomic practices in the southeast region of USA (Roper et al., 2017). Moreover, the un-weighted average system may over-simplify the soil health status and ignore the relationship among different soil attributes. In Ontario, the Ontario Soil Health Assessment (OSHA) was developed by accounting for the relationships among soil attributes, based off of the CASH approach (Congreves et al., 2015). Since soil is a living ecosystem with its characteristics, a fixed measuring system may not be useful everywhere; rather, a regional soil health test may be most meaningful to farmers.

The advancement of the soil biology discipline in recent years has provided a wider selection of soil health indicators. Soil protein (or autoclaved citrate extractable protein) was found to be a sensitive soil N indicator, reflecting a functionally relevant organic N pool impacted by management (Hurisso et al., 2018). Further, phospholipid fatty acid analysis (PLFA) measuring

the bacteria, fungi and protozoa in soil provided a snapshot of microbial biomass and the community composition (Mann et al., 2019). Monitoring soil bacteria at the community-level has been used to determine the management effect on microbial community structure, as well as substrate utilization and diversity indices (Gałązka et al., 2017). Soil organic C storage is an essential aspect of land use and management planning, and active C is susceptible to disturbance (Weil et al., 2003). As such, active C, also called permanganate-oxidizable C, is the portion of C that is readily available for soil microbial community to utilize as food and energy source, reflecting the change of microbial activity with management practices.

No single measurement can quantify soil health, but holistic measures of soil health are challenging because one must integrate biological, chemical, and physical properties, processes and interactions (Karlen et al., 1997). Ideally, a set of comprehensive soil indicators should also be conceptually related to soil function and ecosystem processes, practical to sample and measure, responsive to changes in management, and comparable to a baseline for a meaningful interpretation (Bünemann et al., 2018). Still, there is no standardized and prairie-based soil health test available. Climate and parent material are the major factors that affect the soil formation and using the soil test developed from other regions may lose its meaning when applied to other regions. Numerous researchers recommend developing and using a regionally adapted soil health test to gain the most meaningful interpretation of soil health and functioning (Roper et al., 2017; Congreves et al., 2015; Frost et al., 2019; Chu et al., 2019).

4 MATERIALS AND METHODS

Soil samples from the 0-15, 15-30, and 30-60 cm depths were collected from 55 fields (26 sites) across Saskatchewan in Sept and Oct 2018 (Fig. 4-1). The sample from each site was a composite sample (5-7 individual samples) collected using a flat shovel. The selected sites represented various Agri-Arm sites, producer fields, and AAFC long-term sites. Native prairie samples were also collected for comparison. Soil samples were air dried and sieved (2 mm) prior to all analyses described below.

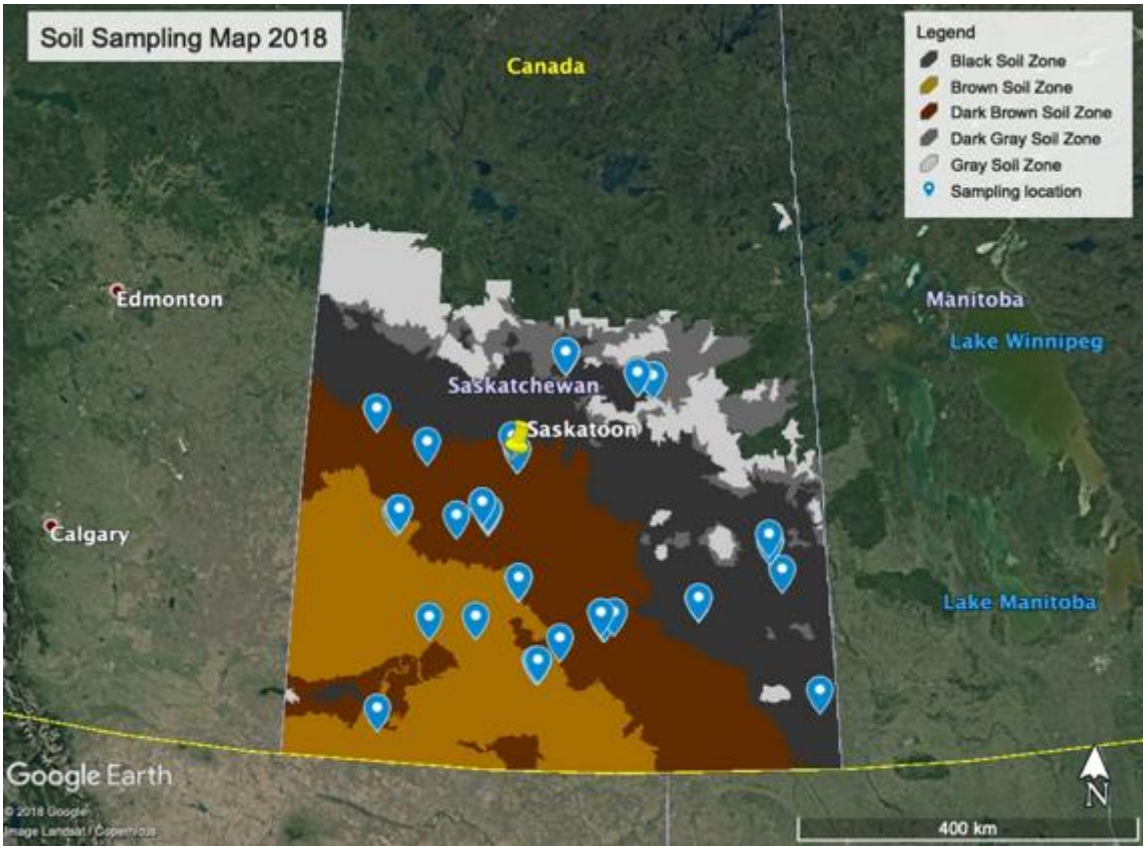

Figure 4-1. Soil sampling locations across Saskatchewan. The points are created based on the GPS coordinates. The soil sampling map overlay with Saskatchewan soil zones, the map resource retrieved from https://open.canada.ca/data/en/dataset/ac6a1e51-9c70-43ab-889f-106838410473.

4.1 Soil chemical attributes

Soil pH and EC

Soil pH and EC were determined by 1:2 soil water slurry, where 10 g of soil was mixed with 20 mL of deionized water and analyzed using a pH meter (Fisher Scientific™, AE 150) and EC meter (Hanna Instrument, HI763100).

Soil nutrient and carbon concentrations

Total concentrations of soil phosphate, potassium, sodium, magnesium, calcium, manganese, iron, copper, zinc, boron, and sulfur were measured by the Natural Resources Analytical laboratory (Edmonton, AB). Briefly, 0.7 g of soil were digested by HNO_3 at 185°C for 10 min, and dissolved metals were analyzed by Inductively Coupled Plasma-Optical Emission Spectroscopy (Thermo iCAP 6000 series).

Soil nitrate (NO_3^-) and ammonium (NH_4^+) were extracted using 25 mL 2.0 M potassium chloride from 5 g of soil, shaken for 30 min at 160 rpm and filtered by Whatman No. 42 filter papers (Maynard et al., 2007). The filtered extracts were stored at -20 °C until analysis, whereupon the extracts were thawed to room temperature and sub-samples (~1 mL) were analyzed for NO_3^- and NH_4^+ concentrations using air segmented, continuous flow colorimetric method with a SEAL AA3 HR chemistry analyzer (SEAL analytical Kitchener Ontario).

To determine soil organic C, soil sub-samples were ball-ground for 3 min to achieve a powdery texture, and 0.8 g of soil was placed in a nickel boat liner inside a ceramic combustion boat. Boats were placed on top of a heater, with a temperature lower than 70°C. Approximate 1 mL of deionized water was added to each boat to moisten the sample. Samples were pre-treated to remove carbonates, following the method of Skjemstad and Baldock (2007); briefly, 6% sulfurous acid was added to each boat until no effervescence was observed, at which point an additional 1 mL of 6% sulfurous acid was added to confirm complete carbonate removal. Thereafter, samples were dried in an oven at 60°C for 48 hours. The carbonate-free samples were analyzed for organic C (%) using a C632 LECO Carbon Analyzer at 1440 °C.

Total C and N was determined by dry combustion (Rutherford et al., 2007; Skjemstad and Baldock, 2007). Sub-samples of ball-ground soil (1.00 g) were placed in a nickel liner inside of a ceramic combustion boat, and analyzed for total C and N by a TruMac CNS analyzer (LECO) at 1350 °C.

Potentially mineralizable N

The determination of potentially mineralizable N is followed the anaerobic incubation method from Curtin and Campbell (2007). Sub-sample soil (5.00 g) was incubated with 10 mL distilled water and place in an incubator with 37 °C for 7 days. Then, NH_4^+ was extracted with 15 mL of potassium chloride (3.33 M) and shaken for 30 min at 120 rpm. The extracts were filtered by Whatman No. 42 filter papers and stored at -20 °C until analysis. The amount of potentially mineralizable N is determined by subtracting pre-incubation (initial) ammonium N from that determined at the end of the incubation.

4.2 Soil physical attributes

Soil texture

Soil texture was determined by using the hydrometer method (Kroetsch and Wang, 2007). Briefly, 25.0 g soil was soaked overnight with 50 mL of 0.082 M sodium hexametaphosphate solution and 200 mL of deionized water. In the morning, the solution was mixed by hand to complete the dispersion. Buoyancy readings of were recorded after mixing, at: 40 sec and 6:52 hrs.

Field capacity

Field capacity (FC) was determined using a modified long column method (Reynolds and Topp, 2007). Soil samples (5 g) were packed in a column with 5.5 ± 0.3 cm height and 0.17 cm diameter and wetted to saturation by placing the column in a beaker filled with water (the water level in breaker was equal to soil surface in the column). Once saturated, the soil-filled column was placed on a fine sand bed, and allowed to drain by gravity for 24 hrs until drainage stopped, reflecting FC. At this point, the weight of the soil and water inside the column was determined by recording the moist weight and dry weight of the soil inside the column (after oven drying at 105 °C for 24 hrs). The FC was expressed as percent by weight.

Wet aggregate stability

Wet aggregate stability (WAS) was measured by using a Wet Sieving Apparatus (Eijkelkamp Soil and Water), operating under the principle that unstable aggregates break down easier and faster than stable aggregates in water. Briefly, 4 g of soil was placed on a sieve, enclosed inside a container filled with distilled water. The apparatus moved up and down for 3 min, and the unstable aggregates were collected in the enclosed container. The sieve with remaining aggregates was place on a new clean water-filled container. The material remaining inside the sieve were considered stable aggregates, disrupted by Ultra Sonic Probe (Branson Sonifier 250), collected, oven-dried overnight at 120 °C. The proportion of water stable aggregate was determined using the dry-weights of the stable and unstable aggregates (Angers et al., 2007).

4.3 Soil biological attributes

Soil protein

Soil protein was extracted and quantified according to the Bicinchoninic acid (BCA) assay, as recommended by Wu et al. (*under review*). Briefly, 1 g of soil was extracted with 8 mL 20 mM sodium citrate (pH=7), shaken at 120 rpm for 5 min, autoclaved at 121°C and 15 psi for 30 min, cooled to room temperature, and thereafter centrifuged at 10,000 x g for 5 min. Subsequently, 25 µL of the supernatant was pipetted into microplate wells (96-well flat-bottomed microplate), and 200 µL of the BCA working reagent was added. After a 30 min incubation in the dark at 37°C and (followed by 15 min cooling period), an absorbance reading was recorded at 562 nm using a microplate spectrophotometer (Bio Tek, Epoch™ 2). Soil extraction and analytical replication was conducted in triplicate and duplicate for each soil sample, respectively.

Active carbon

Active C was measured using the permanganate oxidization approach (Weil et al., 2003). Soil sub-samples (2.5 g) was mixed with 18 mL deionized water and 2 mL 0.2 M potassium permanganate ($KMnO_4$) solution. The mixture was shaken for 2 min at 120 rpm, and left to settle for 8 min. The supernatant was collected, and a 0.5 mL aliquot was diluted with 49.5 mL of deionized water. The amount of active C was calculated after the solution was analyzed by a spectrophotometer at 550 nm.

Soil respiration and nitrous oxide production

A modified "burst" test was conducted to determine soil respiration (CO_2) and nitrous oxide (N_2O) production. Plastic petri dishes with 53 mm of diameter and 13 mm of height, were filled with dry soil samples, and moisture was adjusted to 75% water filled pore space by adding deionized water, the amount of which was calculated from the targeted gravimetric moisture. The petri dish with moist soil was immediately placed in a 1 L mason jar and sealed. The sealed soil sample was incubated at 22 ± 1 ° in the lab for 24 hr, upon which a 20 mL of gas sample was collected and analyzed for CO_2 and N_2O by gas chromatography (Rochette and Bertrand, 2007).

4.4 Data analysis and development of scoring functions

Data were analyzed using SAS (SAS Institute, Inc., university edition, Cary, NC). PROC MEANS was used for descriptive statistics, PROC UNIVARIATE for testing normality, and PROC CORR for evaluating the correlation of soil health score and scoring models. Data was visualized using R studio (R core Team, 2019) and CoPlot (Version 6.45).

4.4.1 Transformations

A Shapiro-Wilk test was conducted in SAS to determine if the data was normally distributed for each soil attribute. There were several cases where the data was not normally distributed; yet, achieving a normal distribution for each soil attribute was a prerequisite for computing the soil health scores. A log transformation resulted in normality for all cases, except for pH and sand which were subjected to a square root transformation to achieve normality (Table A-1). The data of Fe from 30-60 cm depth failed to reach normality via any transformation (be it log, ln, square root, etc.); thus, Fe in 30-60 cm depth were not included in the soil health scoring. Outliers were removed if detected by the interquartile range (IQR) where the value out of the range from (Quartiles 1 – 1.5*IQR) to (Quartile 3 + 1.5*IQR).

4.4.2 Scoring functions for individual soil attributes

Three different types of soil scoring functions were used: i) *more is better*, ii) *optimum is best*, and iii) *less is better*. Each soil attribute was assigned to a scoring function type, based on previous literature as well as author consensus (Table 4-1).

Table 4-1. Different scoring functions as assigned to each soil attribute.

Indicator	Attribute	Scoring function
Chemical	Soil organic C (SOC) and total C	More is better
	Soil total N	More is better
	Inorganic N (nitrate and ammonium)	Optimum is best
	Total phosphorous, potassium, sulfur, calcium, sodium, magnesium, manganese, iron, zinc,	Optimum is best
	pH	Optimum is best
	Electrical conductivity (EC)	Less is better
Biological	Active carbon	More is better
	Soil respiration (CO_2)	More is better
	Soil nitrous oxide (N_2O)	Less is better
	Potentially mineralizable nitrogen (PMN)	More is better
	Soil extractable protein	More is better
Physical	Texture (sand, silt, clay)	Optimum is best
	Wet aggregate stability	More is better
	Field capacity	Optimum is best

Standardized scoring functions were developed to express the score for each soil attribute on a scale of 0 to 100 (Fig. 4-2). The mean, standard deviation, and Z-scores from the normal distribution of each soil attribute were used to develop these scoring functions, following the logic: for any normally distributed dataset, Z-values range from -3 to 3, and a Z-value of 0 corresponds to the observed mean. Therefore,

i) for the *more is better* shape, the health scores are positively related to the Z-scores; the score is highest when Z-value is 3, and lowest when Z-value is -3.

ii) for the *less is better* shape, the health scores are negatively related to the Z-scores; the score is highest when Z-value is -3, and lowest when Z-value is 3.

iii) for the *optimum is best* shape, the health scores are positively related to the Z-scores between the Z-values of -3 to 0, and thereafter negatively related to the Z-scores between Z-values of 0 and 3. As such, the health score is highest when Z-value is 0, and lowest when the Z-value is -3 or 3.

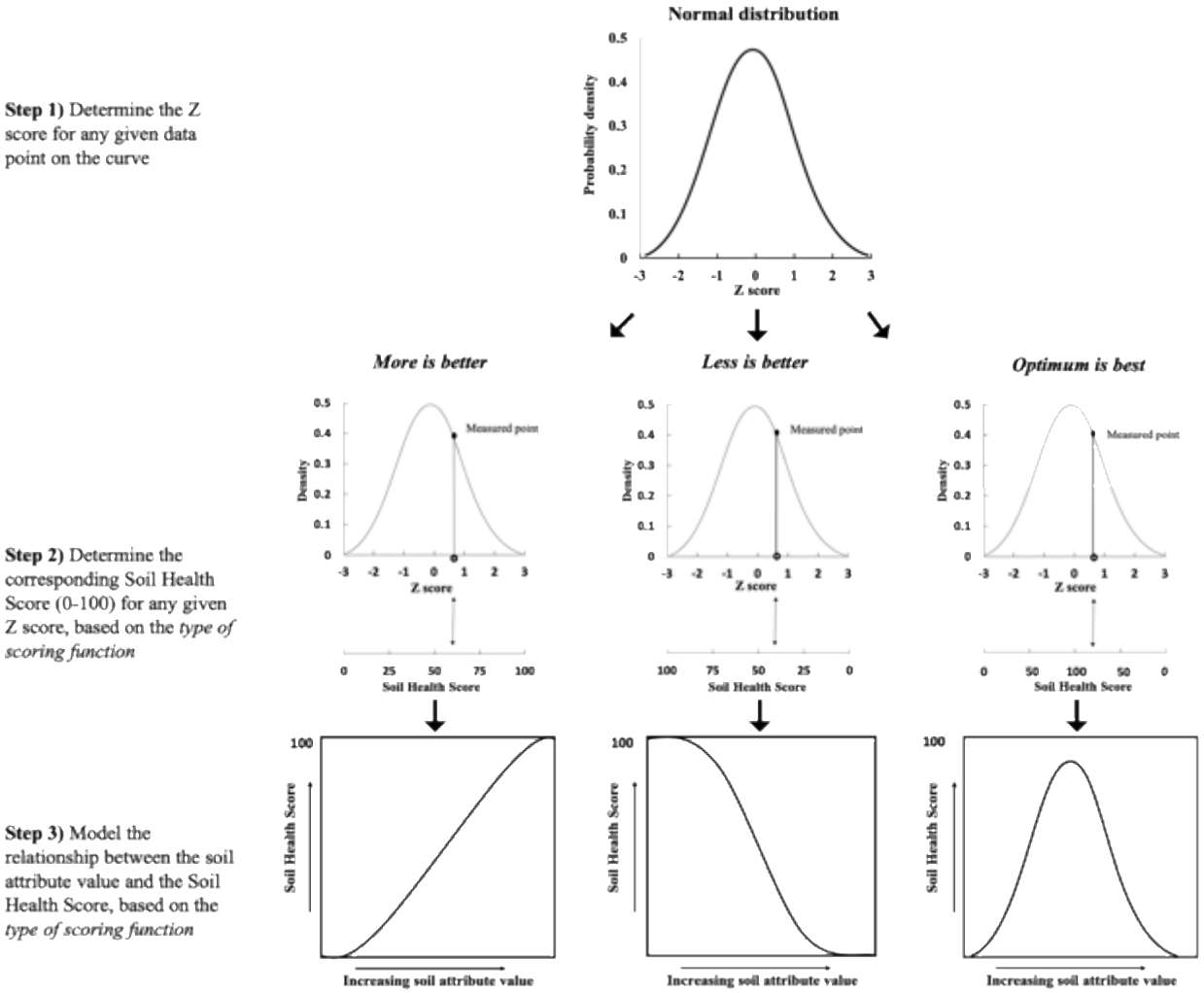

Figure 4-2. Graphical depiction of the development of the Saskatchewan Soil Health Score.

Once the scores were computed for each soil attribute, predictive models were also developed based on the relationship between the soil attribute measurement and score. To do this, several non-linear regressions were tested to determine the best-fit between the measurement and scores, including a second order polynomial regression with and without intercepts, power regression, inverse power regression, square root regression, Hoerl's model, logarithmic regression, and a first order polynomial regression. The R square (R^2) and root mean square error (RMSE) were used to select the best-fit regression, with one additional criterion: the model must not have an inflection point that underestimated scores at the high-end of the scale, which would have erroneously predicted the top score (Tables A-2 and A-3).

4.4.3 Overall soil health scoring

The individual soil health scores were combined into a single overall soil health score using a weighted average approach. Weighting factors were developed by analyzing the patterns in our large dataset, via principal component analysis (PCA). The PCA was conducted using "FactoMineR" package from R studio; data were grouped by soil depth. Soil attributes which explained more variation in the dataset were assigned greater weights, using principal component (PC) eigenvalues, eigenvectors, and the percentage of variance explained. This information was used to develop the weighting factors (*w*) or each attribute, and treated each depth increment separately (Eq. 1):

$$\text{Weighting factor (w)} = \sum_1^k (e_k \times p_k) \qquad \text{(Eq. 1)}$$

where the *e* is the eigenvector of the soil attribute on each PC (*k*); and where p_k is the proportion of explained variance. All PCs were considered up until the cumulative percent variance reached over 80% and p_k reached over 1. Negative weighting factors were set to zero. The overall soil health score was computed according to Eq. 2, separately for each depth increment:

$$\text{Saskatchewan Soil Health Score (SSHS)} = \frac{\sum_1^k (s_k \times w_k)}{\sum_1^k (w_k)} \qquad \text{(Eq. 2)}$$

where *s* represents the soil health score (0-100) for each individual soil attribute; *w* is corresponding weighting factor. Then, the score for the three depth increments were averaged for a single, overall Saskatchewan Soil Health Score (SSHS). The SSHS was normalized from 0 to 100, and the higher SSHS expresses a better soil health status.

To determine whether or not the SSHS produces higher or lower estimates of soil health than the CASH approach, the individual and overall SSHS scores were compared to those from the CASH.

4.4.4 Relationship between soil health score and crop yields

Regional yield data for wheat and barley crops collected from the Saskatchewan AGR RM yield database (http://applications.saskatchewan.ca/agrrmyields) for each of the last 10-yrs from 2009 to 2019, and we also computed the 5-year and 10-year average yields. The yields derived from the rural municipalities were matched to the same rural municipalities where the soil samples were collected, and a correlation test was conducted.

5 RESULTS

5.1 Sites representative of Saskatchewan agriculture

The sampling sites were representative of Saskatchewan agriculture as most sites were previously cropped with wheat (n = 15) or canola (n =21); whereas a few sites had barley (n= 1), chickpea (n = 1), lentil (n =3), field pea (n =1), soybean (n =2), potato (n = 1), and green manure (n=2) (Fig. 5-1).

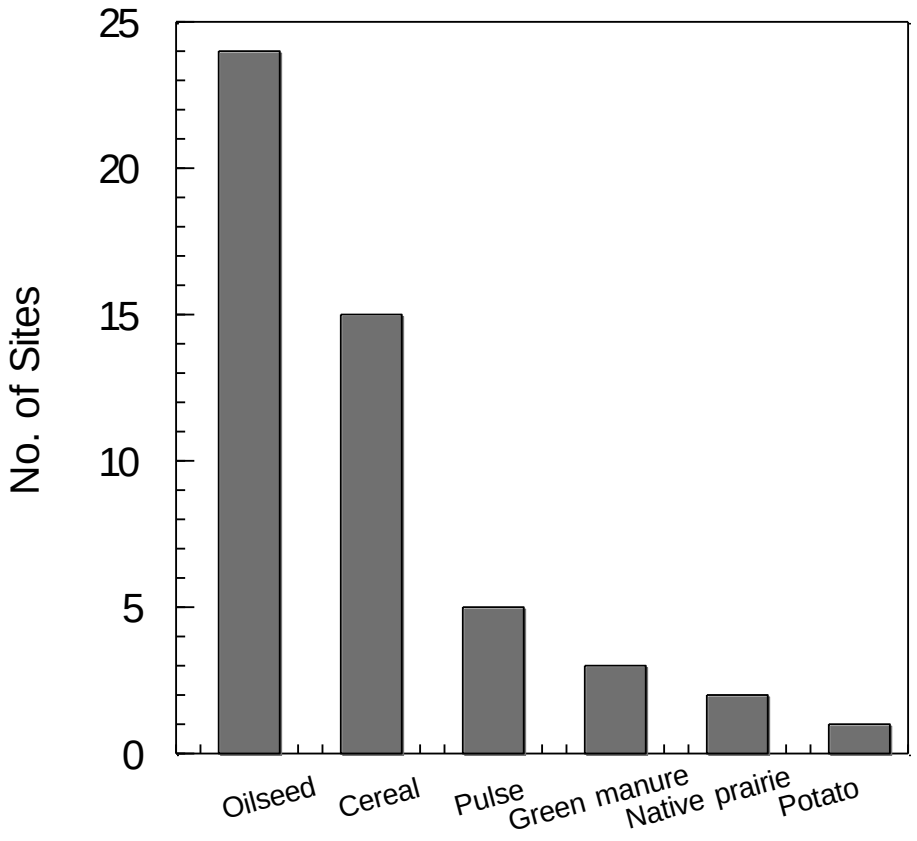

Figure 5-1. The number of sites that were producing each crop type during the year when soil samples were collected (2018).

5.2 Data distributions

Chemical attributes

Soil EC and pH distributions were unimodal regardless of depth, with EC as highly right-skewed and pH as highly left-skewed (Fig. 4A). Soil EC averaged 0.33, 0.31, 0.39 mS cm^{-1} in the 0-15, 15-30, and 30-60 cm depth, respectively; soil pH averaged 7.24, 7.54, 7.93 in the same depth increments, respectively. Soil EC medians did not dramatically differ by soil zone, whereas pH medians were generally higher for the brown soil zone and lower for the gray zone (Fig. 5-2).

Soil TC, SOC, and TN distributions were near normal with some extreme values (Fig. 5-2). For all three attributes, the values decreased with increasing soil depth (Fig. 5-2). Soil TC averaged 26.44, 19.28, 18.21g kg^{-1}, for the 0-15, 15-30, 30-60 cm depths, respectively; whereas for the same respective depth increments, SOC averaged 24.16, 15.52, 12.20 g kg^{-1} and TN averaged 2.32, 1.48, 1.00 g kg^{-1}. Some extremely high SOC values were observed, such as 71.28 g kg^{-1} from the gray soil. For TC, SOC, and TN the difference between medians among soil zones decreased with soil depth (Fig. 5-2). The gray and black soil zones had higher medians in the top 0-15 cm depth, while the gray soil zone had the lowest medians in the 30-60 cm depth. The interquartile range of the gray soil zone was wider than other soil zones for the top 0-15 cm, but sharply reduced with depth (Fig. 5-2).

The shape of NO_3^- and NH_4^+ distributions were unimodal and slightly right-skewed. For NO_3^-, the 0-15 and 15-30 cm depths resulted in flatter distributions than the deeper 30-60cm data (Fig. 5-2). Soil NO_3^- generally decreased with depth, averaging 12.33, 9.31, 4.78 ug g^{-1} in 0-15, 15-30, and 30-60cm, respectively (Fig. 5-2). Soil NH_4^+, on the other hand, showed little variation by soil depth, averaging 4.39, 3.61, 3.77 ug g^{-1} in the 0-15, 15-30, and 30-60 cm, respectively (Fig. 5-2). Noticeably, the gray soil has the lowest NO_3^- values, while the dark brown soil zone had the widest variation. Soil NH_4^+ medians remained fairly consistent among soil zones (Fig. 5-2).

Soil Na, P, and Mn were near normally distributed, with some outliers (Fig. 5-3). Soil Na averaged 90.19, 87.97, 135.63 mg kg^{-1}, for the 0-15, 15-30, 30-60 cm depths, respectively; whereas for the same respective depth increments, P averaged 532.35, 434.16, 419.41mg kg^{-1} and Mn averaged 482.86, 431.86, 408.58 mg kg^{-1}. Some extremely high Na were observed, such as 850.21 mg kg^{-1} in the surface soil from black soil zone and 838.06 mg kg^{-1} in soil depth 30-60cm from brown soil

zone. The highest Na and P values existed in surface soil, while the highest Mn was in the deeper 30-6 0cm.

Soil Ca, S, and Mg distributions were mostly unimodal and right-skewed regardless of depth. For these three nutrients, the 0-15 cm depth had narrower distributions than the deeper 15-30 and 30-60 cm depths (Fig. 5-3). Soil Ca averaged 10218, 15878, 24799 mg kg^{-1} from the soil in 0-15, 15-30, 30-60 cm depth, respectively, whereas for the same respective depth increments, S averaged 574.08, 645.35, 900.73 mg kg^{-1} and Mg averaged 5398.80, 6607.83, 8510.87 mg kg^{-1}. The black soil had highest median of soil Ca, S and Mg regardless of depth.

Soil Zn, Fe, and K distributions were bimodal with two distinct peaks (Fig. 5-3). Soil Zn and K generally decreased with depth, which averaged 67.40, 63.16, 59.74 mg kg^{-1} of Zn and 3423.23, 2972.79, 2584.71 mg kg^{-1} of K in 0-15, 15-30, 30-60cm depth. Conversely, soil Fe generally increased with depth, averaging 17161, 17736, 17770 mg kg^{-1} in the same respective depth increments. No obvious differences were observed between soil zones by depth.

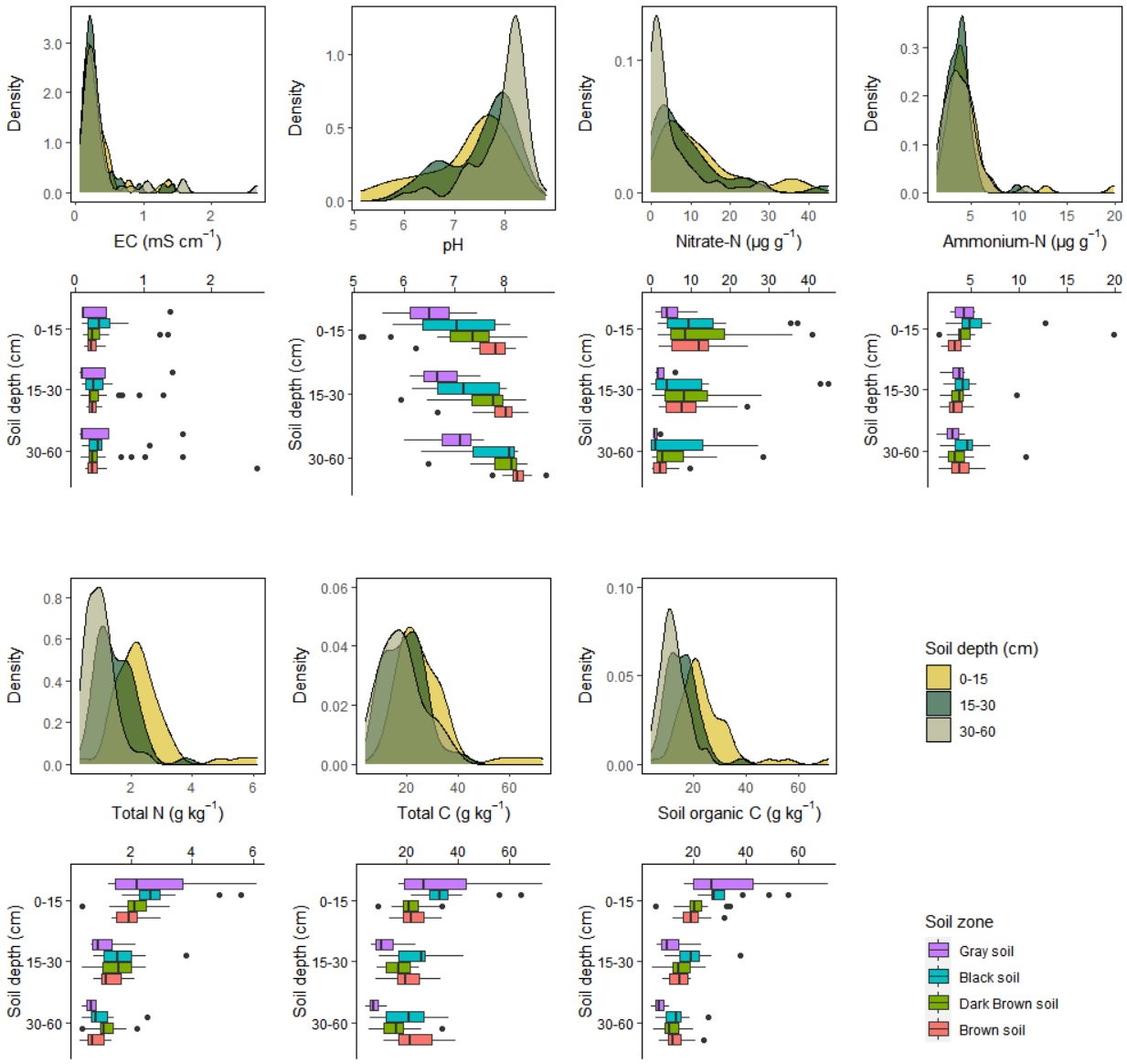

Figure 5-2. The distribution for common soil chemical attributes, presented using density plots. The y-axis is the probability density (kernel estimation) per unit on the x-axis. Box-plots show the interquartile range (solid bar), median (the line inside bars), minimum and the maximum excluding outliers (the extreme line), and outliers (dots) for each soil zone.

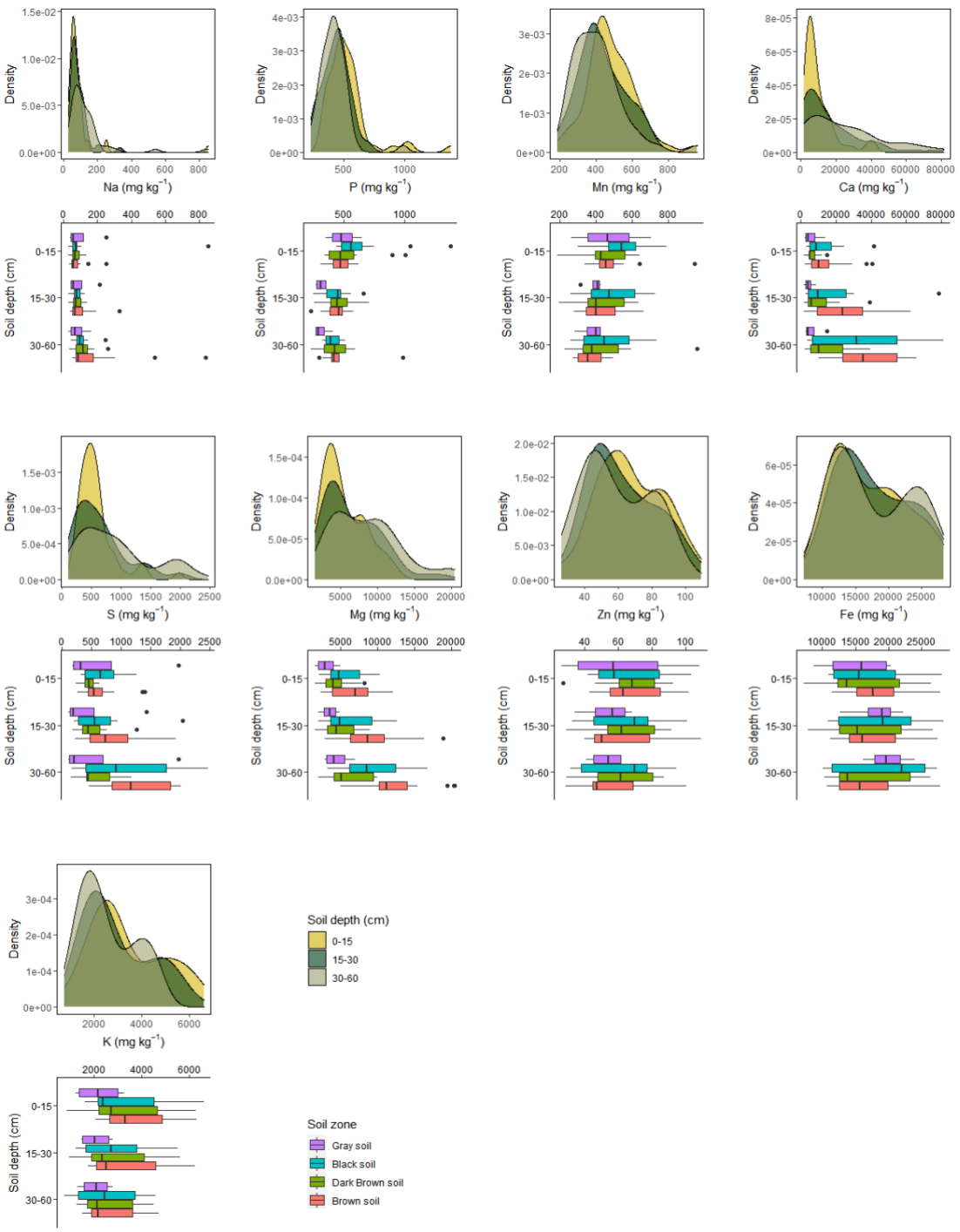

Figure 5-3. The distribution of several soil nutrients as chemical attributes (other than those shown in Figure 5-2, presented using density plots. The y-axis is the probability density (kernel estimation) per unit on the x-axis. Box-plots show the interquartile range (solid bar), median (the line inside bars), minimum and the maximum excluding outliers (the extreme line), and outliers (dots) for each soil zone.

Physical attributes

In our database, the percentage of sand in the soil ranged widely from 1.1% to 81% with a bi-modal distribution—a form that was shared by clay, only to a lesser degree due to the clustering around ~20% and 60% (Fig. 5-4. The silt percentage, on the other hand, showed a unimodal distribution centered around ~40% and was more right skewed with depth (Fig. 5-4). For sand and clay there was a fair amount of overlap in the interquartile range among the soil zones tested, but the soil zones tended to differentiate by silt (Fig. 5-4).

The WAS distribution was unimodal and slightly left-skewed for the 0-15 and 15-30 cm depths, but more uniform for the 30-60 cm depth (Fig. 5-4). The WAS generally decreased with soil depth, averaging 53%, 48%, 44% in 0-15, 15-30, 30-60cm, respectively. For WAS, the dark brown soil and black zone showed wider distributions than the brown or gray soil zones (Fig. 5-4). Soil FC showed a bi-modal distribution at ~40% to 60% with similarities among the soil zones, and little change in distribution with soil depth (Fig. 5-4). Soil FC was nearly consistent among depths, and averaged 46, 44, 43% in 0-15, 15-30, 30-60cm.

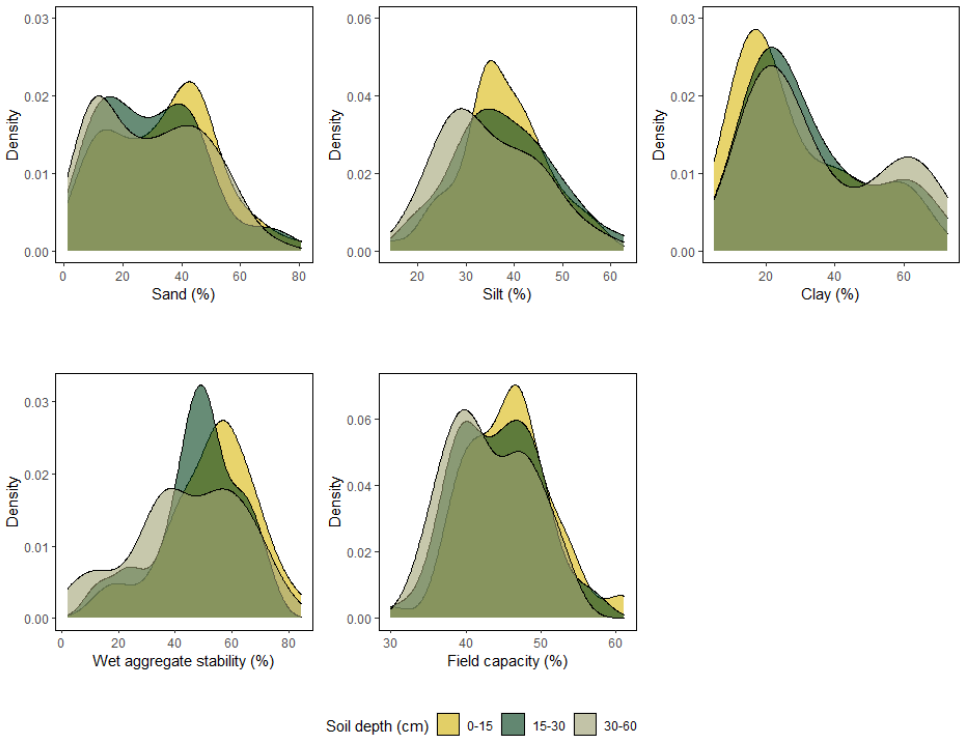

Figure 5-4. The distribution of soil physical attributes presented using density plots. The y-axis is the probability density (kernel estimation) per unit on the x-axis. Box-plots show the interquartile range (solid bar), median (the line inside bars), minimum and the maximum excluding outliers (the extreme line), and outliers (dots) for each soil zone.

Biological attributes

The data distribution for soil active C, CO_2 production, and protein were similar to each other, with unimodal distributions and similar patterns across soil zones (Fig. 5-5). Nitrous oxide production, on the other hand, showed a highly right-skewed unimodal distribution with few differences between soil zones (Fig. 5-5).

Soil protein levels in the 0-15 cm soil ranged from 1 to 17 mg g^{-1}, with a unimodal distribution that is normal and a mean of 6.9 mg g^{-1} (Fig. 5-5). The gray soil zone produced a median protein level that was exceptionally higher than the other soil zones in 0-15 cm depth (Fig. 5-5).

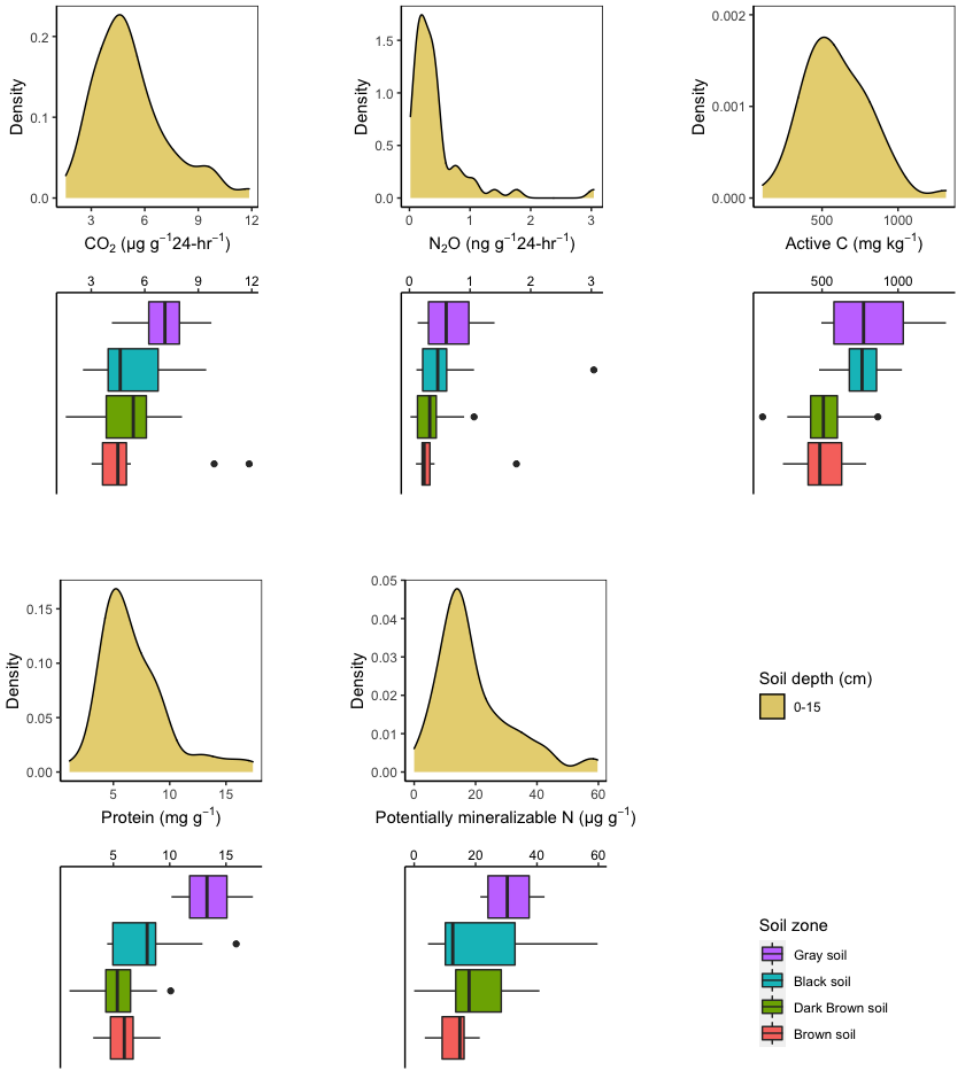

Figure 5-5. The distribution of soil biological indictors in the 0-15 cm depths, presented using density plots. The y-axis is the probability density (kernel estimation) per unit on the x-axis. Box-plots show the interquartile range (solid bar), median (the line inside bars), minimum and the maximum excluding outliers (the extreme line), and outliers (dots) for each soil zone.

5.3 Scoring functions for individual soil attributes

Where the raw data were not normality distributed, transformations ensured normality (Table A-1). The soil health scores following the *more is better, less is better, and optimum is best* scoring functions are shown in relation to the individual soil attribute measurements—along with the predictive models of best fit (Figs. 5-6, 5-7, 5-8, respectively; Tables A-2, A-3, respectively). The formula and threshold limits for each model are also presented herein (Table A-4).

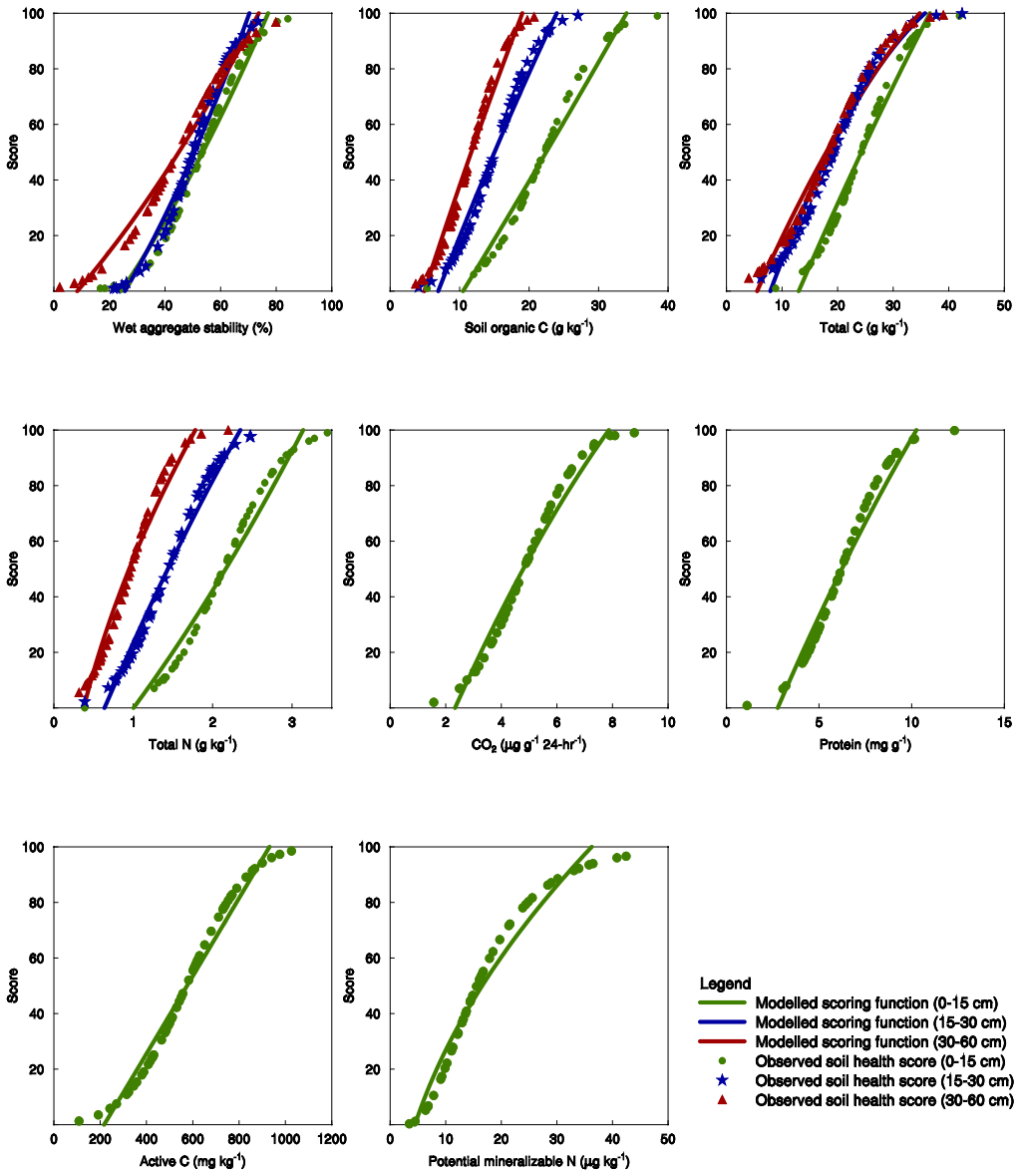

Figure 5-6. The soil health scores for indicators following a "more is better" function (0-15, 15-30, 30-60 cm depth). The coloured symbol indicates the observed soil health score, and the coloured line represents the modelled score.

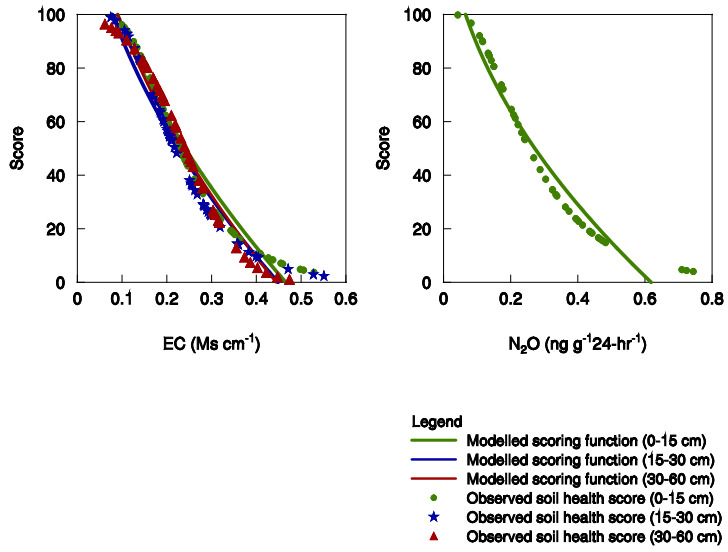

Figure 5-7. The soil health scores for indicators following a "less is better" function (0-15, 15-30, 30-60 cm depth). The coloured symbol indicates the observed soil health score, and the coloured line represents the modelled score.

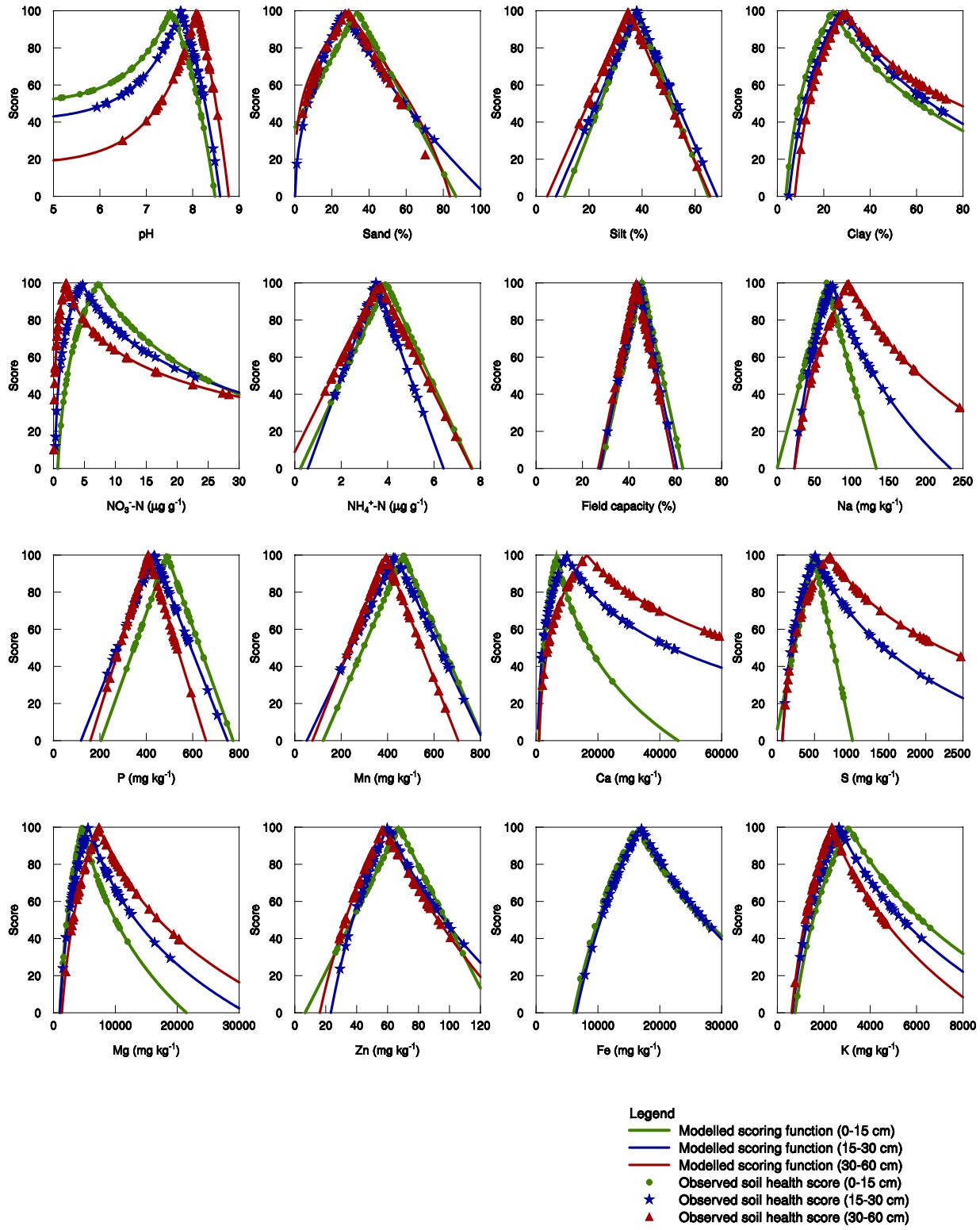

Figure 5-8. The soil health scores for indicators following a "optimum is best" function (0-15 cm, 15-30, 30-60 cm depth). The coloured symbol indicates the observed soil health score, and the coloured line represents the modelled score.

5.4 Towards an overall soil health score

5.4.1 Principal component analysis

The first seven PCs accounted for over 80% of the total variation in the raw data set from 0-15 cm depth, whereas the first five PCs reached this same criterion for the deeper depths (15-30 cm and 30-60 cm) (Table 5-1, Table 5-2, Table 5-3).

For the 0-15 cm soil depth, the PC1 accounted for 30% of the total variation which was predominantly explained by six different soil attributes (i.e., attributes with high positive eigenvectors), including: TC, SOC, TN, WAS, FC, and Zn. The PC2 represented 21% of the total variance and the following attributes had relatively high positive eigenvectors: protein, SOC, active C. The PC3 contributed 11% towards the total variation, with Ca, S, pH, and Mg showing high eigenvectors. The remaining PCs each contributed < 10% of the total variance. Generally, it is observed that different PCs are predominantly explained by indicator type. For example, in the top 15 cm of soil PC1 appears to be explained by soil chemical and physical attributes, whereas PC2 more so by soil biological attributes. Considering all relevant PCs for the 0-15 cm depth, the attributes with the greatest weight (and therefore the most influence on the soil health score) include P, TC, active C, SOC, TN, and N_2O as the top six (Table 5-1).

For the deeper soil depths of 15-30 and 30-60 cm, the first PC accounted for 39% and 35% of the total variance, respectively. Major drivers for this first dimension were clay, Fe, Zn, K, and FC. The PC2 accounted for 20±1% of total variance, predominantly explained by S, Ca, Total C, Mg, and pH. The PC3 explained 11% of the total variance, attributed to TN, SOC, and P. Overall, both soil chemical and physical attributes appeared equally important in these depths (note: biological attributes were not measured in these depths). Taking all relevant PCs for the 15-30 cm depth into account, the attributes that have the most influence on the soil health score are: TC, SOC, FC, P, TN, and WAS (Table 5-2). For the 30-60 cm depth SOC, FC, Mn, TN, Zn, and TC have the greatest influence (Table 5-3).

Table 5-1. Summary of the principal component analysis (PCA) and the resulting eigenvectors for each soil attribute from 0 to 15 cm depth. The eigenvectors and the proportion of explained variance (*pk*) were used to compute the weighting factor (*w*) for the overall soil health score.

Indicator type	Attribute	PC1	PC2	PC3	PC4	PC5	PC6	PC7	w
Chemical	SOC	0.27	0.25	0.08	-0.11	-0.09	-0.1	-0.07	0.14
	Total C	0.27	0.21	0.18	-0.17	-0.09	-0.03	-0.02	0.15
	Total N	0.29	0.22	0.02	-0.1	-0.02	-0.09	-0.09	0.14
	NO_3^--N	-0.02	0.11	0.13	0.38	0.44	0.11	0.21	0.12
	NH_4^+-N	0.13	0.06	-0.06	0.3	0.51	-0.06	-0.1	0.11
	P	0.16	0.2	0.13	0.07	0.22	0.34	-0.12	0.16
	K	0.23	-0.27	-0.15	0.04	0.08	0.03	-0.12	0
	S	0.17	0.01	0.47	-0.1	-0.01	-0.16	0.13	0.12
	Ca	0.03	-0.15	0.49	-0.08	-0.04	0.12	0.18	0.05
	Na	0.21	0.05	-0.02	-0.35	0.19	0.28	0.21	0.09
	Mg	0.13	-0.31	0.29	-0.03	-0.02	0.12	0.05	0.02
	Mn	0.13	-0.09	-0.11	0.32	-0.19	0.44	0.13	0.05
	Fe	0.24	-0.27	-0.17	0	0.02	-0.01	-0.06	0
	Zn	0.27	-0.16	-0.13	-0.04	0.14	-0.19	-0.08	0.04
	pH	0	-0.18	0.35	0.2	-0.15	0.09	-0.01	0.02
	EC	0.16	-0.04	0.22	0.23	0.26	-0.44	0	0.1
Biological	Active C	0.23	0.27	0.04	0.06	-0.14	-0.05	0	0.15
	CO_2	0.14	0.18	-0.05	0.38	-0.27	0.09	-0.19	0.09
	N_2O	0.21	0.17	-0.11	-0.11	0.23	0.34	0.12	0.13
	PMN	0.11	0.15	0.08	0.37	-0.24	-0.07	-0.24	0.08
	Protein	0.17	0.3	-0.11	-0.17	-0.07	-0.04	0.02	0.1
Physical	Sand	-0.25	0.23	0.17	-0.06	0.04	0.12	-0.27	0
	Silt	0.07	0.11	-0.17	0.2	-0.17	-0.2	0.76	0.06
	Clay	0.22	-0.31	-0.08	-0.05	0.05	-0.02	-0.14	0
	WAS	0.26	-0.14	0.02	0.08	-0.2	0.27	0.01	0.07
	FC	0.27	-0.14	-0.1	-0.03	-0.11	-0.19	-0.04	0.03
Eigenvalue		7.88	5.36	3	1.87	1.7	1.18	1	
% variation		30.32	20.6	11.54	7.2	6.54	4.55	3.84	
Cumulative % variation		30.32	50.92	62.47	69.66	76.2	80.75	84.59	
pk		0.36	0.24	0.14	0.09	0.08	0.05	0.05	

Table 5-2. Summary of the principal component analysis (PCA) and the resulting eigenvectors for each soil attribute from 15 to 30 cm depth. The eigenvectors and the proportion of explained variance (*pk*) were used to compute the weighting factor (*w*) for the overall soil health score.

Indicator type	Attribute	PC1	PC2	PC3	PC4	PC5	w
Chemical	SOC	0.19	0.12	0.47	-0.06	-0.2	0.17
	Total C	0.16	0.35	0.26	-0.08	-0.19	0.18
	Total N	0.22	-0.04	0.47	-0.07	-0.18	0.14
	NO_3^--N	-0.09	0.22	0.19	0.4	-0.06	0.06
	NH_4^+-N	0.21	-0.07	0.02	0.43	0.02	0.12
	P	0.03	0.25	0.38	-0.12	0.31	0.14
	K	0.31	-0.09	-0.06	-0.08	-0.05	0.11
	S	0.05	0.46	-0.07	0.09	0	0.13
	Ca	0.03	0.45	-0.18	-0.01	0.02	0.1
	Na	0.19	0.03	-0.24	0.37	0.04	0.09
	Mg	0.17	0.34	-0.24	-0.19	0.06	0.12
	Mn	0.28	-0.09	0.02	0	0.01	0.11
	Fe	0.32	-0.14	-0.12	-0.05	-0.06	0.09
	Zn	0.32	-0.15	-0.02	-0.04	-0.02	0.1
	pH	0.09	0.33	-0.15	-0.05	0.41	0.12
	EC	0.13	0.09	0.02	0.6	-0.18	0.12
Physical	Sand	-0.31	0.11	0.03	-0.04	-0.26	0
	Silt	-0.03	-0.16	0.26	0.21	0.67	0.05
	Clay	0.32	-0.02	-0.18	-0.08	-0.13	0.11
	WAS	0.3	0.02	-0.05	-0.05	0.03	0.14
	FC	0.29	-0.02	0.08	-0.14	0.22	0.15
Eigenvalue		8.14	4.15	2.39	1.35	1.14	
% variation		38.76	19.77	11.36	6.42	5.45	
Cumulative % variation		38.76	58.53	69.89	76.31	81.76	
pk		0.47	0.24	0.14	0.08	0.07	

Table 5-3. Summary of the principal component analysis (PCA) and the resulting eigenvectors for each soil attribute from 30 to 60 cm depth. The eigenvectors and the proportion of explained variance (*pk*) were used to compute the weighting factor (*w*) for the overall soil health score.

Indicator type	Attribute	PC1	PC2	PC3	PC4	PC5	w
Chemical	SOC	0.09	0.3	0.35	-0.04	0.21	0.17
	Total C	0.03	0.42	0.15	-0.07	0.05	0.14
	Total N	0.22	-0.05	0.39	0.11	0.18	0.16
	NO_3^--N	-0.12	0.09	0.23	0.09	0.62	0.05
	NH_4^+-N	0.22	0.01	0.02	0.07	0.24	0.12
	P	-0.08	0.27	0.28	0.21	-0.22	0.08
	K	0.34	0	0	-0.09	-0.05	0.13
	S	-0.04	0.4	-0.24	0.14	0.09	0.08
	Ca	-0.05	0.43	-0.12	-0.1	0	0.07
	Na	0.13	0.03	-0.44	0.34	0.11	0.05
	Mg	0.04	0.43	-0.11	-0.18	-0.08	0.09
	Mn	0.25	-0.01	0.21	0.15	0.2	0.16
	Fe	0.35	-0.06	-0.06	-0.09	-0.05	0.12
	Zn	0.35	-0.04	0.04	0.05	-0.12	0.14
	pH	0	0.33	-0.03	0.18	-0.29	0.08
	EC	0.07	0.02	-0.37	0.49	0.32	0.06
Physical	Sand	-0.34	-0.03	-0.03	-0.09	0.17	0
	Silt	-0.05	-0.06	0.27	0.54	-0.29	0.03
	Clay	0.34	0.06	-0.12	-0.19	0	0.13
	WAS	0.29	0.01	-0.09	-0.25	0.13	0.1
	FC	0.31	0.05	0.07	0.2	-0.15	0.16
Eigenvalue		7.37	4.49	2.22	1.67	1.24	
% variation		35.12	21.39	10.57	7.95	5.93	
Cumulative % variation		35.12	56.51	67.08	75.03	80.96	
pk		0.43	0.26	0.13	0.1	0.07	

5.4.2 The Saskatchewan soil health score

The SSHS averaged 56.97% in the 0-15 cm depth and was lower compared to the 15-30 and 30-60 cm depths, which had average scores of 63.88 and 64.33%, respectively (Fig. 5-9). With scores ranging from 26 to 88% (Fig. 5-9) and a CV of 20%, the top 15 cm soil also had more variation than the deeper depths (with CVs of 15% and 13%, respectively).

The overall SSHS for the 0-60 cm ranged from 41.24 to 77.05%—the highest score belonging to the native prairie soil. No significant difference of overall SSHS across soil zones, and median of overall SSHS were 60.17, 65.68, 62.92, 61.02% in Gray, Black, Dark Brown, and Brown soil zone, respectively.

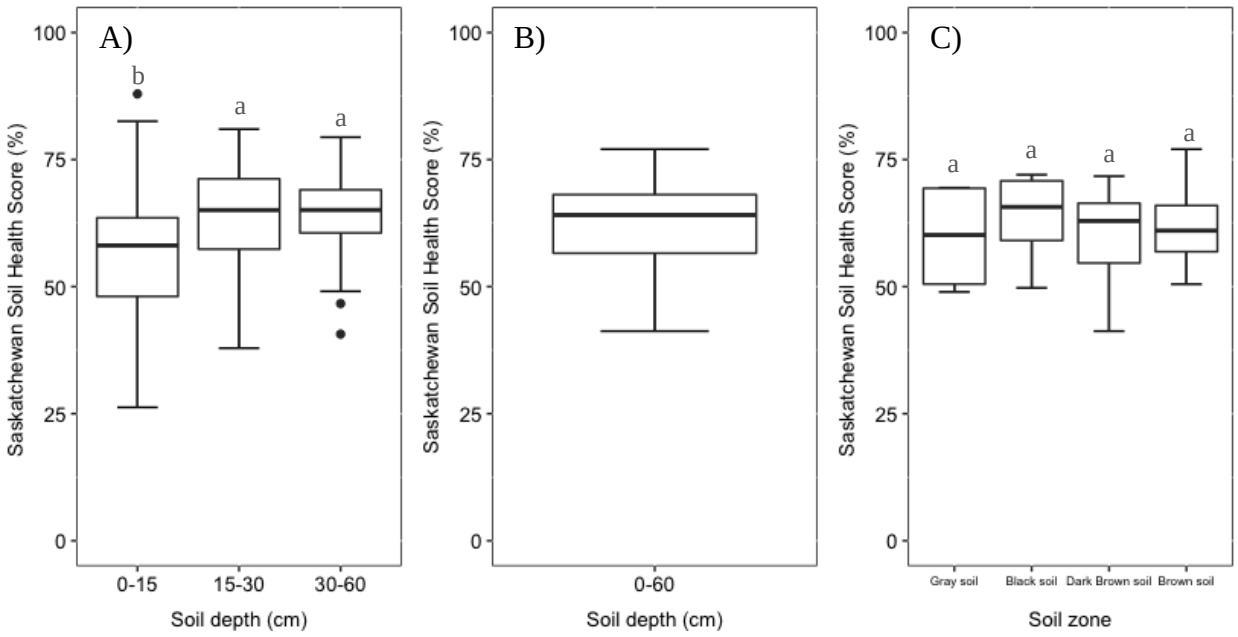

Figure 5-9. The Saskatchewan soil health score **A)** by soil depth increment, **B**) for the full 0-60 cm, and **C**) by soil zone (0-60 cm depth). Boxplots with the same letters are not significantly different ($P > 0.05$) according to Tukey's multiple means comparison.

5.4.3 Saskatchewan Soil Health Score as compared to the Comprehensive Soil Health Assessment

Based on the individual scores, the CASH and SSHS produced similar scores for soil protein and active C; discernable differences for WAS, SOC, and PMN; and very dissimilar scores for soil pH (Fig. 5-10). The CASH overestimated the scores relative to the SSHS for WAS, and also for PMN

and TC (for above-average soils only). For SOC and TN, the divergence between the two approaches is due to how the CASH system adjusts for soil texture. For soil pH, the discrepancy between the two approaches is due to the different 'optimal' ranges used CASH vs the SSHS—the former approach using a more narrow 'optimal' pH range than the SSHS, resulting in many of our Saskatchewan soil samples being assigned a 0 or 100 when using the CASH framework.

Overall, the CASH framework underestimated the soil health status of "below-average to average" Saskatchewan soils, but also had a propensity to overestimate the soil health status of "above-average" SK soils (Fig. 5-10).

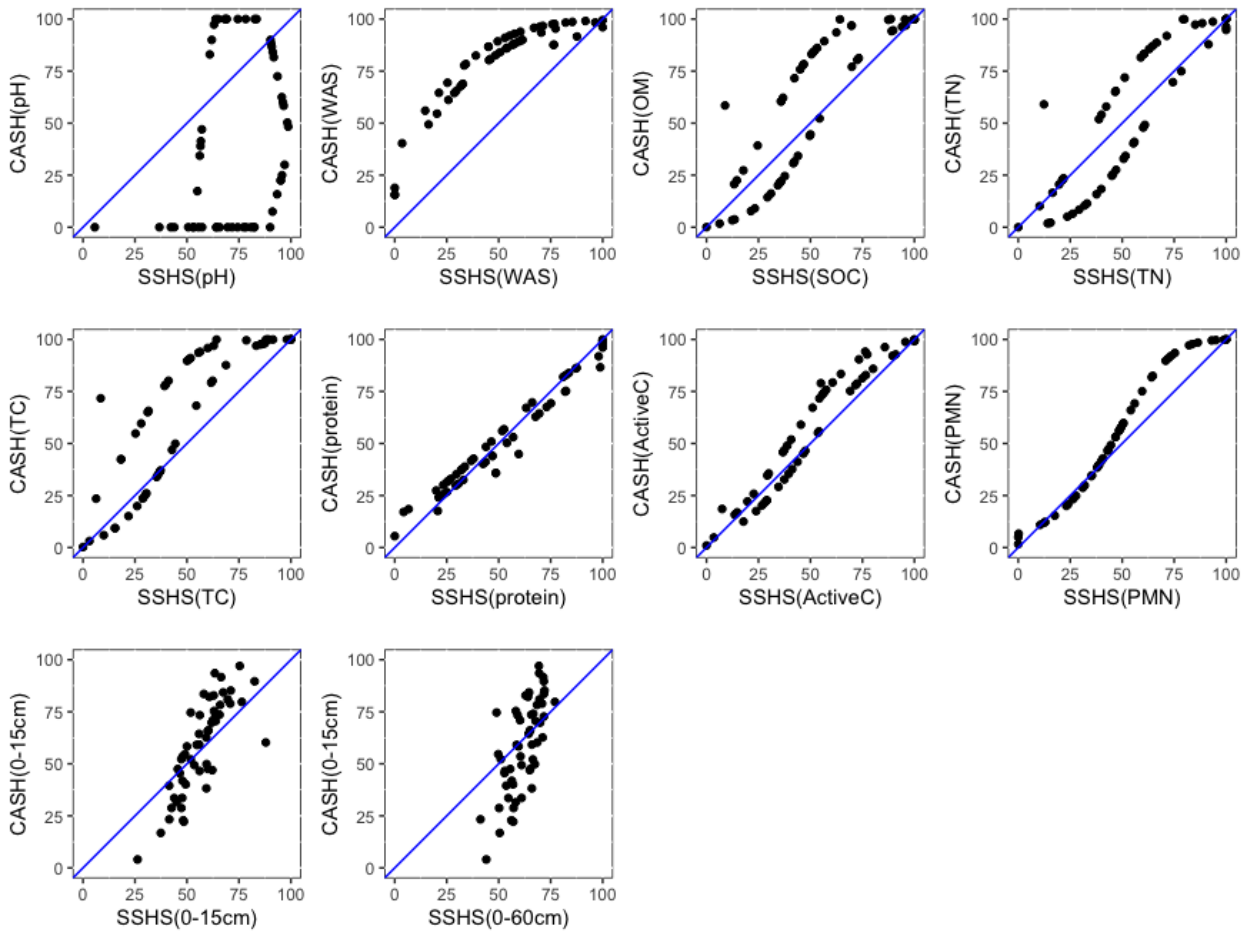

Figure 5-10. Comparison of the Comprehensive Assessment of Soil Health (CASH) and the Saskatchewan Soil Health Score (SSHS). The 1:1 line is presented in addition to the data points that represent the scores from both approaches.

5.4.4 Linking the Saskatchewan Soil Health Score to crop yields

For the most part, cereal crop yields were not well correlated to the SSHS; however, there were two cases in the past 10 years—2009 and 2015—where a positive correlation was detected at $p < 0.05$ (Table 5-2). In both 2009 and 2015, not only were crop yields on the lower end, but precipitation tended to be low as well— especially during the early part of the growing season (Table 5-2). At p values of < 0.1 or $p < 0.15$, the SSHS was positively correlated to 5- or 10-yr average yields (Table 5-2).

Table 5-4. The correlation between the Saskatchewan Soil Health Score (SSHS) and average cereal crop yields obtained from rural municipalities from 2009 to 2019. Significant correlations are indicated at $p < 0.05$ (*), $p < 0.1$ (†) and $p < 0.15$ (‡). Cereal crop yield and precipitation data are included for each year.

	Correlation between cereal crop yield and soil health (Pearson's coefficient)		Crop yields, Mg ha^{-1} (min, median, max)	Average precipitation, mm (annual, Apr-June)
Year	SSHS (0-15 cm)	SSHS (0-60 cm)		
2009	0.64*	0.63*	1.7, 2.4, 3.0	389.6, 108.6
2010	0.09	0.13	2.1, 2.3, 2.7	550.3, 242.0
2011	-0.28	-0.08	2.0, 2.7, 3.3	409.7, 162.7
2012	0.22	0.21	1.8, 2.4, 3.5	446.6, 207.8
2013	0.24	0.26	2.6, 3.6, 3.8	372.8, 139.9
2014	0.37	0.34	2.1, 2.7, 3.2	443.9, 205.4
2015	0.47†	0.65*	2.0, 2.6, 3.2	373.7, 69.0
2016	0.34	0.29	2.3, 3.3, 4.0	478.6, 144.8
2017	0.28	0.21	2.4, 2.9, 3.9	310.0, 108.5
2018	0.43‡	0.32	1.7, 2.8, 3.9	319.0, 104.7
5-yr (2014-2018)	0.47†	0.44‡	2.4, 2.7, 3.4	385.2, 126.5
10-yr (2009-2018)	0.41‡	0.41‡	2.2, 2.8, 3.1	409.5, 149.3

5.5 Case study example

As case studies, the SSHS was applied to three sites of contrasting agricultural management (Table 5-3). Table 5-3 shows the measured value for each soil attribute, as well as the corresponding soil health score for each attribute. The overall SSHS at the bottom of Table 5-3 shows the final weighted score from the 26 different attributes. Soil from native prairie grassland resulted in the greatest number of the attributes considered as optimal status, and produced an overall score of 76% SSHS (Table 5-3). Farm 1 was in the Black soil zone, and it had an overall score of 70% SSHS. This site was managed such that arable crops were grown rotation with a cover crop mixture (Table 5-3). Farm 2, on the other hand, had a much lower score, 48% SSHS (Table 5-3). Although this farm was also in the Black soil zone, it was under intensive potato production.

Table 5-5. Example application of the Saskatchewan Soil Health Test soil health test to three case-study sites (0-15 cm): native prairie grassland (Brown soil zone), Farm 1 (arable crops rotated with summer cover crops from the Black soil zone) and Farm 2 (intensive potato production in the Black soil zone). Red highlighted cells represent poor scores, whereas green highlighted cells represent optimal scores.

		Native prairie		Farm 1		Farm 2	
		Measured value	Score	Measured value	Score	Measured value	Score
Physical	WSA (%)	59.14	61	66.55	76	52.85	49
	FC (%)	48.35	84	45.17	98	37.56	54
	Sand (%)	43.07	82	47.40	74	42.88	82
	Silt (%)	44.72	75	33.46	84	34.34	87
	Clay (%)	12.21	64	19.14	88	22.78	97
Chemical	pH	7.51	99	7.58	97	7.79	82
	EC (mS cm^{-1})	0.22	55	0.79	0	0.77	0
	TN(g kg^{-1})	2.98	92	2.75	79	1.71	29
	TC (g kg^{-1})	33.41	87	36.12	98	33.02	86
	SOC (g kg^{-1})	31.70	90	31.44	89	23.50	54
	$N0_3^-$ - N (µg g^{-1})	3.55	70	15.58	67	37.33	30
	NH_4^+ - N (µg g^{-1})	4.14	95	3.24	81	4.22	93
	P (mg kg^{-1})	540.26	82	591.73	64	563.63	74
	K (mg kg^{-1})	3051.76	99	2232.88	77	1899.51	65
	S (mg kg^{-1})	511.16	96	1058.46	0	1264.26	0
	Na (mg kg^{-1})	39.24	59	52.97	80	107.44	39
	Fe (mg kg^{-1})	13186.74	79	12170.22	71	11561.89	66
	Mg (mg kg^{-1})	3891.75	88	5305.88	92	10073.71	50
	Ca(mg kg^{-1})	5805.82	94	17250.97	50	41359.53	5
	Mn(mg kg^{-1})	418.13	86	541.99	79	478.09	97
	Zn(mg kg^{-1})	55.48	80	41.99	58	45.20	63
Biological	CO_2 (µg g^{-1} 24-hr^{-1})	11.85	100	7.92	100	3.06	16
	N_2O (ng g^{-1} 24-hr^{-1})	0.24	55	0.29	47	0.15	75
	Protein (mg g^{-1})	9.18	88	6.13	49	4.77	30
	Active C (mg kg^{-1})	789.98	80	763.20	76	512.10	41
	PMN-N (µg g^{-1})	14.47	43	56.03	100	9.31	24
	SSHS (SK Soil Health Score)		76		70		48

6 DISCUSSION

6.1 Carbon and nitrogen are key regulators of soil health

Of all the attributes measured, soil protein, active C, total N and C, and SOC explained the greatest amount of variance in the dataset; resulted in greater individual weights for computing the overall score (Table 5-1; Fig. A-3). Unsurprisingly, these C- and N-based attributes were also highly correlated to each other (R^2 of 0.68 to 0.97). Both C and N are key constituents of soil organic matter, which is critical for the functioning of several ecosystem services such as nutrient supply and cycling, water supply and cycling, climate regulation, and supporting plant growth (Lal, 2014, 2016). By having C- and N-based attributes highly weighted in the SSHS framework, the scoring system demonstrates an encouraging linkage to *soil ecosystem functioning*.

Saskatchewan soils hold great potential for C sequestration and storage (McConkey et al., 2003); however, changes in soil organic matter or total C may only be detected in the long-term (5-10-yrs or more) (Simonsson et al., 2014). The conundrum is that soil organic matter is a crucial metric for soil health, but it is a difficult metric to interpret changes in soil health in the short-term. The labile carbon indictors are included to work as the early detector of management practice (Luo et al., 2015; Bongiorno et al., 2019; Miller et al., 2019). By representing both the labile (active C and soil protein) and more stable measures of soil organic matter (total C and N, SOC), the SSHS framework might offer a more useful metric to detect early changes, rather than relying on soil organic matter measures alone.

Total P concentration was highly weighted in the soil health measurement based on the principal component analysis. More than 50% of total P is typically organic P in agricultural soil (Nash et al., 2014), and the correlation analysis found total P was positively correlated to total N, active C, and SOC. Thus, improving soil organic matter goes hand-in-hand with soil P. Rather than available P, total P was more stable as an annual mean for monitor the phosphorus level in soil; for example, phosphorus was greatly influenced by soil pH, more phosphorus tended to fix by calcium since the mean of pH in Saskatchewan is 7.3. Total P may be viewed as a broad indicator of soil P levels for soil health, while available P can be viewed as focusing more on soil fertility.

Some soil health components can be manageable whereas others are more inherent to soil formation. Interestingly, two clusters are observed when evaluating the PCA results, and one was dominated by C and N indices, and another consisted of mainly physical indicators plus some nutrients (Fig. A-6). Thus, it is possible that the PCA results point towards the two distinct components of soil health: a manageable component, and an inherent component. The manageable component is of greater importance when planning agronomic practices aimed at improving soil health, but the inherent component is nonetheless important to consider. Although inherent soil physical attributes like sand, silt, and clay are not changeable with agronomic management, management practices can be designed based on inherent characteristics to maximize efficiency and profitability (i.e., best strategies to improve soil health on a clayey soil will be different than those on a sandy soil).

Different soil zones in Saskatchewan inherently have differences in soil attributes, largely due to differences in soil formation. Although variation in individual soil attributes by soil zone was observed (i.e., see differences in soil organic C by soil zone, Fig. 5-2), there was no significant difference among soil zones when comparing the overall soil health scores across zones (Fig. 5-9). On one hand, further research is recommended to refine the scoring functions by soil zones due to the differences in individual soil attributes among soil zones (and perhaps different soil zones would have different weighting systems); on the other hand, because the overall soil health score did not dramatically differ by zone, it appears to be a suitable foundation for a provincial soil health test for arable cropping systems.

6.2 Consideration of soil depths beyond 0-15 cm

The SSHS framework not only includes the 0-15 cm depth, but also the 15-30 and 30-60 cm depths. Rather than applying the same weighting factors for the 0-15 cm depth to the subsurface depths, the SSHS considers each depth increment independently (i.e., weighting factors are different for each depth increment, as shown in Table 5-1, Figs. A-4, A-5, respectively). If a score for subsurface soil is computed using the same weighting factors as the 0-15 cm depth, the result would mislead users by implying that the subsurface soils "are not as healthy"—when in fact, subsurface *functions* are simply different than those of surface soil. The surface soil is arguably

the most weathered and impacted by agricultural management after the conversion from native grassland to arable cropland; thus, seems logical that the surface soil health score is more variable and numerically lower than the subsurface soils (Fig. 5-9A). This result implies that there is more room for improvement in the surface soil layer than deeper depths, and that management practices aimed at ameliorating the surface conditions such as no-till and crop residue retention might go a long way towards improving soil health overall (Kinoshita et al., 2017). Future work should consider incorporating biological indices for the subsurface soil, along with the physical and chemical indicators.

Clearly, management practices not only influence surface soil attributes but also subsurface soil by influencing the availability and accessibility of soil moisture and nutrients. Efforts to improve subsurface soil for crop production are usually aimed at reducing soil compaction. Subsurface compaction restricts root growth and limits water and nutrients uptake by the plant—leading to reduced crop yields (Vrindts et al., 2005; Obour et al., 2018). Reducing compaction is difficult to achieve in the short-term (Wahlström et al., 2021), but effective practices include: deep tillage, crop residue retention, rotating crops with deep rooted plant such as radish (Kinoshita et al., 2017; Getahun et al., 2018; Wahlström et al., 2021). In some cases, yield increases have been linked to subsoil improvement (Getahun et al., 2018). Soil health testing for subsurface soils might be useful for tracking such changes, but future research is needed.

Globally, soil is the largest C pool—and more than 50% of this C is stored beyond 0-15 cm depth (Rumpel and Kögel-Knabner, 2011). From our PCA analysis, total C and SOC produced high weighting factors even in the subsurface soil. Carbon effects soil aggregation, water retention, and microbial activity. In the long-term, root-derived C and N are incorporated into aggregates during decomposition, which contribute to the formation of stable organic matter (Sanaullah et al., 2011).

6.3 How the Saskatchewan Soil Health Test compares to others?

For meaningful interpretation of soil health and functioning, regional soil health tests are recommended (Frost et al., 2019). Herein, scoring functions and weighting systems were tailored

to Saskatchewan soils—and this soil health test is a distribution-type of test. There are pros and cons when using a distribution-type of soil health test. For example, an advantage of applying the distribution-type of scoring system to the region it was developed from is that the scoring functions and overall scores are representative of the area, hence meaningful for the region. But on the downside, a distribution-type of scoring function might produce a rather narrow range of scores, since the data are required to fit a normal distribution theory. Measured values that are out of the range are directly assigned as 100 or 0 based on this type of scoring function. As such, a rather narrow range of soil health scores might make it difficult to achieve a stellar score (i.e., even the native grassland soil results were in the mid 70s, not 90% or higher).

Compared to the fixed algorithm method developed by Andrews et al (2004), each scoring curve consisted of fixed-number parameters and site-specific parameters with changing numbers as site characteristics such as soil type and climate. An additive approach is the most common and simplest method to integrate each attribute to an overall score, as used by the Comprehensive Assessment of Soil Health (Moebius-Clune et al., 2016) and Soil Management Assessment Framework (Andrews et al., 2004). However, assigning equal weight to each attribute may oversimplify the complex relationship between soil attributes and service in the ecosystem. Other methods integrate several attributes via a weighted average approach. The Haney test (Haney et al., 2018) functions similar to an index by summing several attributes, but only consisted of three soil attributes. Principal component analyses are often used to inform the relative contribution different attributes should contribute to an overall score (Andrews and Carroll, 2001; Bi et al., 2013; Purakayastha et al., 2019; Karaca et al., 2021). This approach involves measuring many different soil attributes, prior to integrating them into a single score. If only a small number of indicators are included in a soil health test, the capacity to detect the soil health conditions from different practices may be limited (Chu et al., 2019).

The Saskatchewan Soil Health Test may not only help to inform management decisions on-farm, but also provide supplementary information when assessing soil capability for agricultural use. For example, Soil Capability information systems already exist and these indicate the soil's capacity for agriculture use—classified based on climate, soil type, and landscape characteristics (Shields et al., 1968). Agricultural capacity classification is also a useful tool to gain the general information

of a land and its inherent properties, resulting from fixed factors, mainly recognized as soil forming factors like: climate, topography, and parent material. Crop production could be designed and developed to minimize adverse impacts of some limitations found in Soil Capability classes, but the soil status would still be changeable over time and require routine soil tests. The Saskatchewan Soil Health test reflects soil health status of the current usage, and the result of test may change over time due to the effect of management practices on soil health status.

No scoring approach is without limitations. It is acknowledged that the SSHS does not consider soil borne disease, nor are there any direct measurements of plant germination and growth—factors that we recommend considering in future efforts to improve soil health scoring. Further, many of the nutrient attributes considered in the SSHS are total concentration rather than available nutrient concentration. On one hand, assessing available nutrient concentrations might be more suitable from the perspective of crop production—but on the other hand, total nutrient concentrations provide a more stable metric that is linked to the potential nutrient supply for crops (only less fluctuation due to environmental conditions and timing of soil sampling). Regardless, the SSHS methodology and scoring system presented herein provides a regional adaptation for a soil health score for one of Canada's most important agricultural regions. Tracking the soil health score over time, together with crop metrics, will provide the information needed to inform and adjust management plans aimed at improving soil health and functioning. Extension tools should be developed to transform farmers' routine soil test data into a soil health score, informed by our scoring approach.

6.4 The link between soil health and crop productivity might be most apparent during suboptimal conditions

Crop yield is one of the most crucial considerations for farmers when deciding on management practices. However, quantitatively linking soil health to crop yield has been an elusive goal (Garland et al 2021). Soil health scoring is aimed at capturing the *capacity of soil to function*; however, supporting crop growth is just one of several functions provided by soil—this likely

contributes to the difficulty in determining an authoritative linkage between soil health scores and crop yields. Despite the challenges, researchers have found relationships between soil health indicators and crop yields, for example, higher soil biological activity corresponded to greater corn yields in United States (Wade et al., 2020). Furthermore, corn and soybean yield were positively associated with soil active C, protein, respiration and Mn in the United States; van Es and Karlen (2019) concluded the labile organic matter—C and N-based indices—is central for linking soil health and crop productivity. Likewise, our SSHS framework prioritizes soil C and N-based attributes and showed promise for linking soil health to crop yield (Table 5-2). Although this is in agreement with others (Lal, 2016; Garcia et al., 2018), certain regions may show tighter relationships between crop yields and organic matter than others (Wood et al., 2018). For example, a global meta-analysis found crop yield positively correlated with SOC when SOC was less than 2%, but the relationship was less clear when SOC was above 2% (Oldfield et al., 2019). Climate and environment play a major role in driving this relationship. The positive relationship between yields and SOC is more apparent in arid regions, but less consistent in semi-arid and humid regions (Sun et al., 2020). Saskatchewan is a semi-arid region, and this may help explain why the soil health scores were positively correlated to crop yields during years with low precipitation only (Table 5-2). It is possible that soil health offers some resiliency for crop production during suboptimal growing conditions. Further research is recommended to link soil health scores to crop yields at a finer-scale (i.e., field-scale), improving upon the regional-scale portrait of crop yield linkages to soil health as presented herein. This would offer more precise information about how different management practices influence soil health scores across Saskatchewan.

6.5 Soil management for improved soil health

In general, the soil from Farmer 1's field produced a relatively high soil health score in the entire regional soil database (Table 5-3). This is likely due to the type of agricultural management that Farmer 1 had practiced: including a summer polyculture cover crop in the rotation with arable crops such as canola and wheat. Further, Farmer 1 also periodically rotates livestock on the field to graze the cover crop. Most of Farmer 1's soil attributes were optimal, with generally high organic carbon, aggregate stability, and active microbial activity. Although the soil EC was in high

range relative to regional soils, the value had not reached a level that would be considered saline but would require careful consideration in the future. Soil S concentrations was relatively high compared to other soils in the region, so the fertilization plan for future crops must take the initial S pool into account. The future fertilization plan should consider the initial S content and EC to avoid over-application and a trajectory towards saline conditions. Accordingly, soil in Farmer 1's field was considered as an example of optimal health status, and indicates that the current practices are helping to maintain soil health.

In contrast to Farm 1, soil health in Farmer 2's field (under potato production) was in the medium to low range of the soil health database (Table 5-3). The physical attributes of Farmer 2's sample were either optimal or normal, so the inherent soil properties were not the limiting factors for productivity. The constraining attributes appeared in the chemical and biological categories, including EC, TN, S, Ca, soil respiration, protein and PMN. The suboptimal nutrient cycling and soil biological activity indicated soil microbial community was under pressure and this may have restricted some key soil functions. As such, restoring the functioning of microbial process should be the first step to improve soil health at this site. Microbial activity is strongly affected by management practice such as tillage (Calderón et al., 2001; Hungria et al., 2009), so reducing soil disturbance would be recommended. However, this is not really an option at this site because the Famer is in the business of potato production. Potato production generally entails tillage to prepare the site and minimize weed pressure, and several soil hilling operations per year to maintain a good seedbed. Still, these practices are likely leading to the degradation of soil health. Cover cropping could be a better alternative to implementing reduced tillage as well as for improving nutrient retention. Research has shown cover cropping effectively suppresses weed emergence in the growing season and provides abundant N for cash crops (Reberg-Horton et al., 2012). White mustard, vetch, and Persian clover are effective for weed suppression in Poland (Kołodziejczyk et al., 2017), and rapeseed and ryegrass effectively reduced weed biomass to less than 1% of the total biomass of crop over in potato field in Italy (Campiglia et al., 2009). Not only weed management but cover cropping also contains many benefits such as erosion prevention, organic matter accumulation, and soil compaction alleviation etc. (Williams and Weil, 2004; Campiglia et al., 2009; Basche et al., 2016; Jahanzad et al., 2016). I recommended the future agronomic research

should be aimed at improving soil health and functionality to increase the sustainability of intensively produced crops.

Healthy soil produces healthy food, and several soil management practices could help farmers to increase soil health. The strategies of building soil health include conservation tillage, compaction reduction, crop and animal diversity, cover cropping, compost and amendments, and continuous living plants (Fig. 6-1). Carbon and N are central in soil health indices, and the strategies mentioned above are targeted at *maximizing carbon gain* and *minimizing carbon loss*.

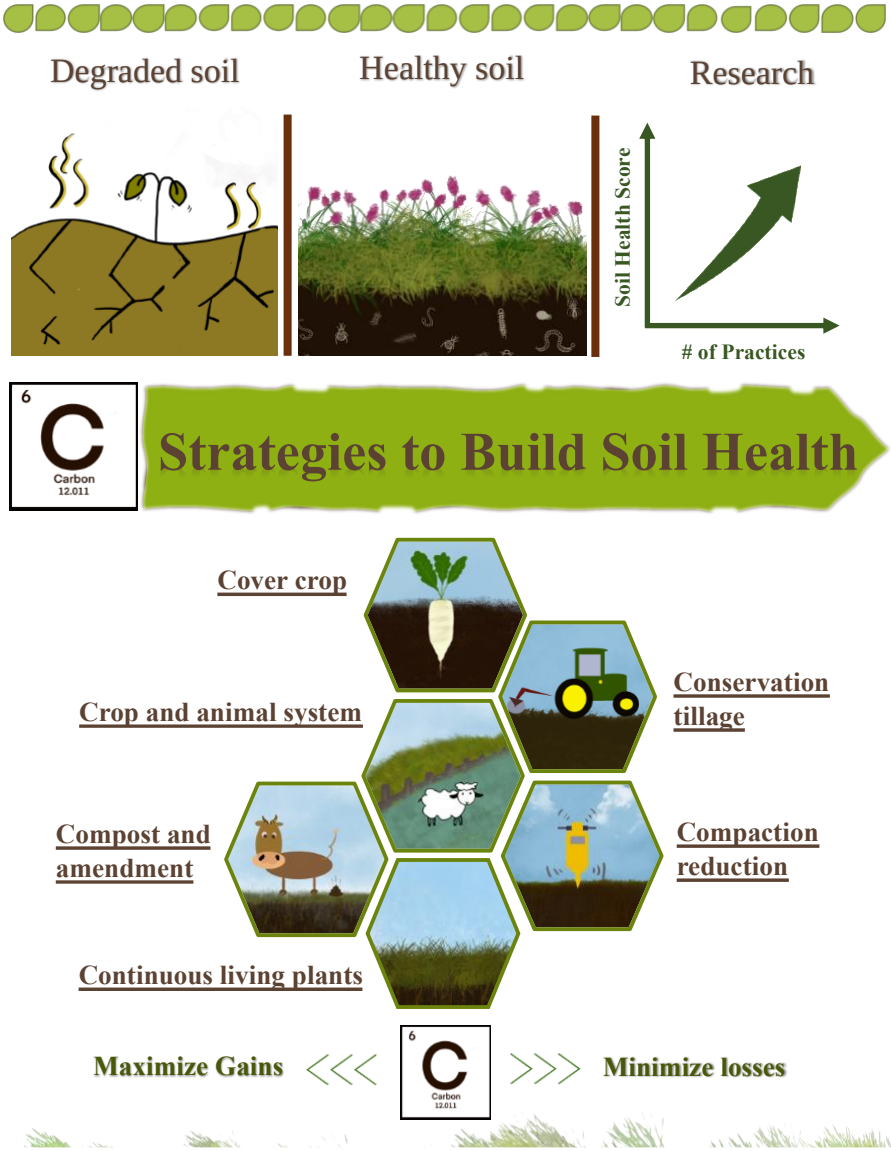

Figure. 6-1. Infographic for strategies to build soil health. Created by Athena Wu, based on Van Eerd et al. *In Press*.

Maximizing carbon gain is important because soil carbon is central for soil health. Improving soil carbon storage is beneficial for nutrient storage, water retention, soil aggregation, C sequestration, and crop productivity. Also, sequestering C into the soil is a practical and effective approach against changing climate. Continuous living plant and cover cropping actively are strategies that expand either time or space of living root system in soil and create favourable habitat for microbial communities, then carbon released through plant residue and dead root decomposition. Beside

contributing to the carbon sequestration, continuous living cover could improve porosity and field capacity, especially in drier climate and sandy soil (Basche and DeLonge, 2017). Moisture is an important factor that limits crop production in Saskatchewan, so improving soil water property could increase the resiliency of agroecosystem under changing climate. The living plant's root system also stabilizes soil from erosion and stimulates soil aggregate formation to build a better soil structure, further reduce C loss by amour soil surface. Also, integrating animals into the cropping system could increase aboveground biodiversity and food web composition, benefiting to the farm and the environment (Lemaire et al., 2014). Manure is a fresh and nutrient-rich source for increasing organic matter and nutrients input, and farmers should be careful to avoid eutrophication when applying manure.

Minimizing carbon loss is important because soil disturbance like tillage and random field traffic can degrades soil carbon by accelerating decomposition (CO_2 loss) and erosion. Saskatchewan farmers have widely accepted no-till practice to avoid soil erosion and increase productivity, but the complete transfer to no-till can be challenging for certain farming systems. For example, some organic production has limited choices for weed control, so tillage is sometimes necessary to implement for weed removal (Wiltshire et al., 2003; Cioni and Maines, 2010). Conservation tillage may be an alternative to minimize the adverse effect of tillage on soil health for farmers who have to till the soil. Conservative tillage had more significant SOC accumulation than traditional tillage (López-Garrido et al., 2011). Other strategies to reduce compaction, growing forage radish, could effectively alleviate soil compaction by accessing soil below the compaction layer through roots that create a channel for the following crop to access nutrients and water (Wahlström et al., 2021). Also, soil functioning is heavily limited in compacted soil, contributing to soil runoff and erosion, nutrient depletion, and drought. Strategies like controlled traffic pathways are necessary to minimize soil compaction (Kingwell and Fuchsbichler, 2011). Minimizing carbon loss is avoids not only unnecessary mechanical activity but also reduces the input cost of farming.

Implementing practices aimed at improving soil health may require time to see the result, but a healthy soil will benefit both farmers and our environment. There is no fixed management to improve soil health, site-specific characteristic could be accounted in the management plan. Strategies could be mixed and combine as a "Soil health cocktail". Research has demonstrated that

the more soil health practices implemented, the healthier the soil (Van Eerd et al., *In Press*). Soil is fundamental for food production, thus guarding soil health is a long-term sustainable development for food security.

7 CONCLUSIONS

Maintaining and improving soil health are central to mitigating the adverse impacts of changing climate on agricultural production, and soil health tests are valuable tools to measure and track soil health over time. Soil health tests can provide the scientific information needed to inform management decisions. The CASH framework provides a roadmap and standardized approach to access soil health status by integrating soil biological, physical, and chemical attributes, but it has not been tailored to Saskatchewan soils—until now. Herein, I present a soil health testing protocol and scoring functions for arable cropping systems in Saskatchewan (the SSHS). My testing protocol and scoring functions provide the foundation for developing extension tools that are capable of transforming farmers' routine soil test data into a soil health score. As an example, a grower-friendly online tool which outputs the SSHS from lab results would be valuable to producers and industry, and I recommend that such a tool be created as the next step. My results indicate C and N-indices primarily drive soil health differences, and therefore indicate that management decisions aimed at improving C and N sequestration will also improve soil health scores. Total P was significantly correlated to C and N-indices, and it provide addition information as an indicator of soil health status as related to P cycling. It is possible that healthier soils may help to safeguard crop yields during sub-optimally dry growing seasons, but further research is recommended to explore this linkage more closely.

8 REFERENCES

Acosta-Martínez, V., Dowd, S.E., Bell, C.W., Lascano, R., Booker, J.D., Zobeck, T.M., and Upchurch, D.R. 2010. Microbial Community Composition as Affected by Dryland Cropping Systems and Tillage in a Semiarid Sandy Soil. Diversity, 2(6): 910–931.

Acton, D.F., and Gregorich, L.J. 1995. The Health of Our Soils: Toward sustainable agriculture in Canada Canadian Cataloguing in Publication Data.

Allen, D.E., Singh, B.P., and Dalal, R.C. 2011. Soil Health Indicators Under Climate Change: A Review of Current Knowledge. p. 25–45. In: Singh B., Cowie A., Chan K. (eds), *Soil Health and Climate Change*. Soil Biology, vol 29. Springer, Berlin, Heidelberg. doi: 10.1007/978-3-642-20256-8_2.

Altenbach, S.B. 2012. New insights into the effects of high temperature, drought and post-anthesis fertilizer on wheat grain development. J. Cereal Sci. 56(1): 39–50. doi: 10.1016/j.jcs.2011.12.012.

Amundson, R., 2001. The carbon budget in soils. Annual Review of Earth and Planetary Sciences, 29(1): 535-562. doi: 10.1146/annurev.earth.29.1.535.

Andrews, S.S., and Carroll, C.R. 2001. Designing a Soil Quality Assessment Tool for Sustainable. Ecol. Soc. Am. 11(6): 1573–1585.

Andrews, S.S., Karlen, D.L., and Cambardella, C.A. 2004. The Soil Management Assessment Framework: A Quantitative Soil Quality Evaluation Method. Soil Sci. Soc. Am. J. 68(6): 1945–1962.

Andrews, S.S., Karlen, D.L., and Mitchell, J.P. 2002. A comparison of soil quality indexing methods for vegetable production systems in Northern California. Agric. Ecosyst. Environ. 90(1): 25–45.

Angers, D.A., Bullock, M.S., and Mehuys, G.R. 2007. Aggregate Stability to Water. p. 845–854. *In* Carter, M., Gregorich, E. (eds.), Soil Sampling and Methods of Analysis. CRC Press.

Basche, A., and DeLonge, M. 2017. The Impact of Continuous Living Cover on Soil Hydrologic Properties: A Meta-Analysis. Soil Sci. Soc. Am. J. 81(5): 1179–1190. doi: 10.2136/sssaj2017.03.0077.

Basche, A.D., Kaspar, T.C., Archontoulis, S.V., Jaynes, D.B., Sauer, T.J., Parkin, T.B., and Miguez, F.E. 2016. Soil water improvements with the long-term use of a winter rye cover crop. Agric. Water Manag. 172: 40–50.

Bi, C., Chen, Z., Wang, J., and Zhou, D. 2013. Quantitative Assessment of Soil Health Under Different Planting Patterns and Soil Types. Pedosphere 23(2): 194–204. doi: 10.1016/S1002-0160(13)60007-7.

Bongiorno, G., Bünemann, E.K., Oguejiofor, C.U., Meier, J., Gort, G., Comans, R., Mäder, P., Brussaard, L., and de Goede, R. 2019. Sensitivity of labile carbon fractions to tillage and organic matter management and their potential as comprehensive soil quality indicators across pedoclimatic conditions in Europe. Ecol. Indic. 99: 38–50.

Bot, A. and Benites, J., 2005. The importance of soil organic matter: Key to drought-resistant soil and sustained food production (No. 80). Food & Agriculture Org..

Bünemann, E.K., Bongiorno, G., Bai, Z., Creamer, R.E., De Deyn, G., de Goede, R., Fleskens, L., Geissen, V., Kuyper, T.W., Mäder, P., Pulleman, M., Sukkel, W., van Groenigen, J.W., and Brussaard, L. 2018. Soil quality – A critical review. Soil Biol. Biochem. 120(September 2017): 105–125. doi: 10.1016/j.soilbio.2018.01.030.

Bush, E., and Lemmen, D.S. 2019. Canada's Changing Climate Report.

Calderón, F.J., Jackson, L.E., Scow, K.M., and Rolston, D.E. 2001. Short-Term Dynamics of Nitrogen, Microbial Activity, and Phospholipid Fatty Acids after Tillage. Soil Sci. Soc. Am. J. 65(1): 118–126. doi: 10.2136/sssaj2001.651118x.

Campiglia, E., Paolini, R., Colla, G., and Mancinelli, R. 2009. The effects of cover cropping on yield and weed control of potato in a transitional system. F. Crop. Res. 112(1): 16–23.

Carter, M.R., Gregorich, E.G., Anderson, D.W., Doran, J.W., Janzen, H.H., and Pierce, F.J. 1997. Chapter 1 Concepts of soil quality and their significance. p. 1–19.

Cavagnaro, T.R. 2016. Soil moisture legacy effects: Impacts on soil nutrients, plants and mycorrhizal responsiveness. Soil Biol. Biochem. 95: 173–179. doi: 10.1016/j.soilbio.2015.12.016.

Chen, Y.D., Wang, H.Y., Zhou, J.M., Xing, L., Zhu, B.S., Zhao, Y.C., and Chen, X.Q. 2013. Minimum data set for assessing soil quality in farmland of northeast china. Pedosphere 23(5): 564–576. doi: 10.1016/S1002-0160(13)60050-8.

Chu, M., Singh, S., Walker, F.R., Eash, N.S., Buschermohle, M.J., Duncan, L.A., and Jagadamma, S. 2019. Soil Health and Soil Fertility Assessment by the Haney Soil Health Test in an Agricultural Soil in West Tennessee. Commun. Soil Sci. Plant Anal. 50(9): 1123–1131. doi: 10.1080/00103624.2019.1604731.

Cioni, F., and Maines, G. 2010. Weed Control in Sugarbeet. Sugar Tech 12(3–4): 243–255. doi: 10.1007/s12355-010-0036-2.

Clearwater, R.L., T. Martin, and T. Hoppe (eds.). 2016. Environmental sustainabilityof Canadian agriculture: Agri-Environmental Indicators Report Series. : 239.

Congreves, K.A., Hayes, A., Verhallen, E.A., and Van Eerd, L.L.. 2015. Long-term impact of tillage and crop rotation on soil health at four temperate agroecosystems. Soil Tillage Res. 152: 17–28. doi: 10.1016/j.still.2015.03.012.

Cooper, R.J., Hama-Aziz, Z.Q., Hiscock, K.M., Lovett, A.A., Vrain, E., Dugdale, S.J., Sünnenberg, G., Dockerty, T., Hovesen, P., and Noble, L. 2020. Conservation tillage and soil health: Lessons from a 5-year UK farm trial (2013–2018). Soil Tillage Res. 202(December 2019): 104648. doi: 10.1016/j.still.2020.104648.

Curtin, D., and Campbell, C.A. 2007. Mineralizable Nitrogen. p. 599–606. *In* Carter, M.R., Gregorich, E.G. (eds.), Soil Sampling and Methods of Analysis.

Doran, J.W., and Parkin, T.B. 1994. Defining and Assessing Soil Quality. p. 1–21. *In* Defining Soil Quality for a Sustainable Environment. wiley.

Doran, J.W., and Zeiss, M.R. 2000. Soil health and sustainability: managing the biotic component of soil quality. Appl. Soil Ecol. 15(1): 3–11.

Ehdaie, B., Layne, A.P., and Waines, J.G. 2012. Root system plasticity to drought influences grain yield in bread wheat. Euphytica 186(1): 219–232.

Fisher Scientific, T. Pierce™ BCA Protein Assay Kit.

Frost, P.S., van Es, H.M., Rossiter, D.G., Hobbs, P.R., and Pingali, P.L. 2019. Soil health characterization in smallholder agricultural catchments in India. Appl. Soil Ecol. 138(October 2018): 171–180.

Gałązka, A., Gawryjołek, K., Grządziel, J., Frąc, M., and Księżak, J. 2017. Microbial community diversity and the interaction of soil under maize growth in different cultivation techniques. Plant, Soil Environ. 63(6): 264–270.

Gao, X., Dong, S., Xu, Y., Wu, S., Wu, X., Zhang, X., Zhi, Y., Li, S., Liu, S., Li, Y., Shang, Z., Dong, Q., Zhou, H., and Stufkens, P. 2019. Resilience of revegetated grassland for restoring severely degraded alpine meadows is driven by plant and soil quality along recovery time: A case study from the Three-river Headwater Area of Qinghai-Tibetan Plateau. Agric. Ecosyst. Environ. 279: 169–177.

Garcia, C., P. Nannipieri, and Hernandez, T. 2018. The future of soil carbon. p. 239–267. *In* The Future of Soil Carbon: Its Conservation and Formation. Elsevier Inc.

Garland, G., Edlinger, A., Banerjee, S., Degrune, F., García-Palacios, P., Pescador, D.S., Herzog, C., Romdhane, S., Saghai, A., Spor, A., Wagg, C., Hallin, S., Maestre, F.T., Philippot, L., Rillig, M.C., and van der Heijden, M.G. 2021. Crop cover is more important than rotational diversity for soil multifunctionality and cereal yields in European cropping systems. Nat. Food 2(1): 28–37. doi: 10.1038/s43016-020-00210-8.

Getahun, G.T., Kätterer, T., Munkholm, L.J., Parvage, M.M., Keller, T., Rychel, K., and Kirchmann, H. 2018. Short-term effects of loosening and incorporation of straw slurry into the upper subsoil on soil physical properties and crop yield. Soil Tillage Res. 184: 62–67.

Gillespie, A.W., Farrell, R.E., Walley, F.L., Ross, A.R., Leinweber, P., Eckhardt, K.U., Regier, T.Z., and Blyth, R.I. 2010. Glomalin-related soil protein contains non-mycorrhizal-related heat-stable proteins, lipids and humic materials.

Gregorich, E.G., Carter, M.R., Angers, D.A., Monreall, C.M., Ellerta, B. 1994. Towards a minimum data set to assess soil organic matter quality in agricultural soils.

Gugino, B.K., Abawi, G.S., Idowu, O.J., Schindelbeck, R.R., Smith, L.L., Thies, J.E., Wolfe, D.W., and van Es, H.M. 2009. Cornell soil health assessment training manual. Cornell University College of Agriculture and Life Sciences.

Haney, R.L., Haney, E.B., Smith, D.R., Harmel, R.D., and White, M.J. 2018. The soil health tool-Theory and initial broad-scale application. Appl. Soil Ecol. 125(July 2017): 162–168. doi: 10.1016/j.apsoil.2017.07.035.

Högy, P., Poll, C., Marhan, S., Kandeler, E., and Fangmeier, A. 2013. Impacts of temperature increase and change in precipitation pattern on crop yield and yield quality of barley. Food Chem. 136(3–4): 1470–1477. doi: 10.1016/j.foodchem.2012.09.056.

Hungria, M., Franchini, J.C., Brandão-Junior, O., Kaschuk, G., and Souza, R.A. 2009. Soil microbial activity and crop sustainability in a long-term experiment with three soil-tillage and two crop-rotation systems. Appl. Soil Ecol. 42(3): 288–296.

Hurisso, T.T., Moebius-Clune, D.J., Culman, S.W., Moebius-Clune, B.N., Thies, J.E., and van Es, H.M. 2018. Soil Protein as a Rapid Soil Health Indicator of Potentially Available Organic Nitrogen. Agric. Environ. Lett. 3(1): 180006.

Idowu, O.J., Van Es, H.M., Abawi, G.S., Wolfe, D.W., Ball, J.I., Gugino, B.K., Moebius, B.N.,

Schindelbeck, R.R., and Bilgili, A.V. 2008. Farmer-oriented assessment of soil quality using field, laboratory, and VNIR spectroscopy methods. Plant Soil 307(1–2): 243–253.

Isbell, F., Craven, D., Connolly, J., Loreau, M., Schmid, B., Beierkuhnlein, C., Bezemer, T.M., Bonin, C., Bruelheide, H., De Luca, E., Ebeling, A., Griffin, J.N., Guo, Q., Hautier, Y., Hector, A., Jentsch, A., Kreyling, J., Lanta, V., Manning, P., Meyer, S.T., Mori, A.S., Naeem, S., Niklaus, P.A., Polley, H.W., Reich, P.B., Roscher, C., Seabloom, E.W., Smith, M.D., Thakur, M.P., Tilman, D., Tracy, B.F., Van Der Putten, W.H., Van Ruijven, J., Weigelt, A., Weisser, W.W., Wilsey, B., and Eisenhauer, N. 2015. Biodiversity increases the resistance of ecosystem productivity to climate extremes. Nature 526(7574): 574–577.

Jahanzad, E., Barker, A. V., Hashemi, M., Eaton, T., Sadeghpour, A., and Weis, S.A. 2016. Nitrogen Release Dynamics and Decomposition of Buried and Surface Cover Crop Residues. Agron. J. 108(4): 1735–1741. doi:10.2134/agronj2016.01.0001.

Jahanzad, E., Barker, A. V., Hashemi, M., Sadeghpour, A., Eaton, T., and Park, Y. 2017. Improving yield and mineral nutrient concentration of potato tubers through cover cropping. F. Crop. Res. 212: 45–51.

Jentsch, A., Kreyling, J., and Beierkuhnlein, C. 2007. A new generation of climate-change experiments: Events, not trends. Front. Ecol. Environ. 5(7): 365–374.

Karaca, S., Dengiz, O., Turan, İ. D., Özkan, B., Dedeoğlu, M., Gülser, F., Sargin, B., Demirkaya, S., and Ay, A. 2021. An assessment of pasture soils quality based on multi-indicator weighting approaches in semi-arid ecosystem. Ecol. Indic. 121(September 2020).

Karlen, D.L., Mausbach, M.J., Doran, J.W., Cline, R.G., Harris, R.F., and Schuman, G.E. 1997. Soil Quality: A Concept, Definition, and Framework for Evaluation (A Guest Editorial). Soil Sci. Soc. Am. J. 61(1): 4.

Khan, Q.A., and McVay, K.A. 2019. Productivity and stability of multi-species cover crop mixtures in the northern great plains. Agron. J. 111(4): 1817–1827.

Kim, S., Zang, H., Mortimer, P., Shi, L., Li, Y., Xu, J., and Ostermann, A. 2018. Tree species and recovery time drives soil restoration after mining: A chronosequence study. L. Degrad. Dev. 29(6): 1738–1747. doi: 10.1002/ldr.2951.

Kingwell, R., and Fuchsbichler, A. 2011. The whole-farm benefits of controlled traffic farming: An Australian appraisal. Agric. Syst. 104(7): 513–521.

Kinoshita, R., Schindelbeck, R.R., and van Es, H.M. 2017. Quantitative soil profile-scale assessment of the sustainability of long-term maize residue and tillage management. Soil Tillage Res. 174: 34–44.

Kołodziejczyk, M., Antonkiewicz, J., and Kulig, B. 2017. Effect of living mulches and conventional methods of weed control on weed occurrence and nutrient uptake in potato. International Journal of Plant Production, 11(2): 276-284.

Kroetsch, D., and Wang, C. 2007. Chapter 55: Particle Size Distribution. p. 713–726. *In* Carter, M., Gregorich, E. (eds.), Soil Sampling and Methods of Analysis, Second Edition.

Lal, R. 2014. Societal value of soil carbon. J. Soil Water Conserv. 69(6): 186A-192A.

Lal, R. 2015. Soil carbon sequestration and aggregation by cover cropping. J. Soil Water Conserv. 70(6): 329–339.

Lal, R. 2016. Soil health and carbon management. Food Energy Secur. 5(4): 212–222.

Lawley, Y.E., J.R. Teasdale, and R.R. Weil. 2012. Pest Interactions in Agronomic Systems Published in. Agron. J 104: 1–10.

Lemaire, G., Franzluebbers, A., de Faccio Carvalho, P. C., and Dedieu, B. 2014. Integrated crop-livestock systems: Strategies to achieve synergy between agricultural production and environmental quality. Agric. Ecosyst. Environ. 190: 4–8.

Leng, G., and Hall, J. 2019. Crop yield sensitivity of global major agricultural countries to droughts and the projected changes in the future. Sci. Total Environ. 654: 811–821. Available at https://doi.org/10.1016/j.scitotenv.2018.10.434.

Li, P., Zhang, T., Wang, X., and Yu, D. 2013. Development of biological soil quality indicator system for subtropical China. Soil Tillage Res. 126: 112–118. doi: 10.1016/j.still.2012.07.011.

Liang, B.C., VandenBygaart, A.J., MacDonald, J.D., Cerkowniak, D., McConkey, B.G., Desjardins, R.L., and Angers, D.A. 2020. Revisiting no-till's impact on soil organic carbon storage in Canada. Soil Tillage Res. 198(October 2019): 104529. doi: 10.1016/j.still.2019.104529.

Lokupitiya, E., and Paustian, K. 2006. Agricultural Soil Greenhouse Gas Emissions. J. Environ. Qual. 35(4): 1413–1427.

López-Garrido, R., Madejón, E., Murillo, J.M., and Moreno, F. 2011. Short and long-term distribution with depth of soil organic carbon and nutrients under traditional and

conservation tillage in a Mediterranean environment (southwest Spain). Soil Use Manag. 27(2): 177–185.

Luo, S., Zhu, L., Liu, J., Bu, L., Yue, S., Shen, Y., and Li, S. 2015. Sensitivity of soil organic carbon stocks and fractions to soil surface mulching in semiarid farmland. Eur. J. Soil Biol. 67: 35–42.

Magdoff, F. 2001. Concept, Components, and Strategies of Soil Health in Agroecosystems. Journal of nematology. 33(4):169.

Mann, C., Lynch, D., Fillmore, S., and Mills, A. 2019. Relationships between field management, soil health, and microbial community composition. Appl. Soil Ecol. 144(July): 12–21. doi: 10.1016/j.apsoil.2019.06.012.

Massah, J., and Azadegan, B. 2016. Effect of chemical fertilizers on soil compaction and degradation. AMA, Agric. Mech. Asia, Africa Lat. Am. 47(1): 44–50.

Maynard, D.G., Kalra, Y.P, and Crumbaugh, J.A.. 2007. Nitrate and Exchangeable Ammonium Nitrogen. p. 71–80. *In* Carter, M.., Gregorich, E.G. (eds.), Soil Sampling and Methods of Analysis. Second Edi. CRC Press.

McConkey, B.G., Liang, B.C., Campbell, C.A., Curtin, D., Moulin, A., Brandt, S.A., and Lafond, G.P. 2003. Crop rotation and tillage impact on carbon sequestration in Canadian prairie soils. Soil Tillage Res. 74(1): 81–90.

Miller, G.A., Rees, R.M., Griffiths, B.S., Ball, B.C., and Cloy, J.M. 2019. The sensitivity of soil organic carbon pools to land management varies depending on former tillage practices. Soil Tillage Res. 194: 104299.

Minasny, B., Malone, B.P., McBratney, A.B., Angers, D.A., Arrouays, D., Chambers, A., Chaplot, V., Chen, Z.S., Cheng, K., Das, B.S., Field, D.J., Gimona, A., Hedley, C.B., Hong, S.Y., Mandal, B., Marchant, B.P., Martin, M., McConkey, B.G., Mulder, V.L., O'Rourke, S., Richer-de-Forges, A.C., Odeh, I., Padarian, J., Paustian, K., Pan, G., Poggio, L., Savin, I., Stolbovoy, V., Stockmann, U., Sulaeman, Y., Tsui, C.C., Vågen, T.G., van Wesemael, B., and Winowiecki, L. 2017. Soil carbon 4 per mille. Geoderma 292: 59–86. doi: 10.1016/j.geoderma.2017.01.002.

Moebius-Clune, B.N., Moebius-Clune, D.J, Gugino, B.K., Idowu, O.J., Schindelbeck, R.R., Ristow, A.J., van Es, H., Thies, J., Shayler, H., McBride, M., Wolfe, D., and Abawi, G. 2016. Comprehensive Assessment of Soil Health - The Cornell Framework Manual.

Moragues-Saitua, L., Merino-Martín, L., Stokes, A., and Staunton, S. 2019. Towards meaningful quantification of glomalin-related soil protein (GRSP) taking account of interference in the Coomassie Blue (Bradford) assay. Eur. J. Soil Sci. 70(4): 727–735. doi: 10.1111/ejss.12698.

Nash, D. M., Haygarth, P. M., Turner, B. L., Condron, L. M., McDowell, R. W., Richardson, A. E., Watkins, M., and Heaven, M.W. 2014. Using organic phosphorus to sustain pasture productivity: a perspective. Geoderma, 221, 11-19. doi:10.1016/j.geoderma.2013.12.004.

Natural Resources Conservation Services. Healthy Soil for Life . Available at https://www.nrcs.usda.gov/wps/portal/nrcs/main/soils/health/ (verified 4 April 2021).

Nunes, M.R., Karlen, D.L., Veum, K.S., Moorman, T.B., and Cambardella, C.A. 2020. Biological soil health indicators respond to tillage intensity: A US meta-analysis. Geoderma 369(December 2019): 114335. doi: 10.1016/j.geoderma.2020.114335.

O'Reilly, K.A., Lauzon, J.D., Vyn, R.J., Van Eerd, L.L. 2012. Nitrogen cycling, profit margins and sweet corn yield under fall cover crop systems For personal use only. J. Soil Sci 92: 353–365.

Obour, P.B., Kolberg, D., Lamandé, M., Børresen, T., Edwards, G., Sørensen, C.G., and Munkholm, L.J. 2018. Compaction and sowing date change soil physical properties and crop yield in a loamy temperate soil. Soil Tillage Res. 184: 153–163. doi: 10.1016/j.still.2018.07.014.

Oldfield, E.E.,Bradford, M.A., and Wood, S.A. 2019. Global meta-analysis of the relationship between soil organic matter and crop yields. Soil Biol. Biochem. 5(4): 15–32. doi: 10.1016/j.soilbio.2020.108007.

Parvin, S., Uddin, S., Tausz-Posch, S., Fitzgerald, G., Armstrong, R., and Tausz, M. 2019. Elevated CO2 improves yield and N2 fixation but not grain N concentration of faba bean (Vicia faba L.) subjected to terminal drought. Environ. Exp. Bot. 165(May): 161–173. doi: 10.1016/j.envexpbot.2019.06.003.

Purakayastha, T.J., Pathak, H., Kumari, S., Biswas, S., Chakrabarty, B., Padaria, R.N., Kamble, K., Pandey, M., Sasmal, S., and Singh, A. 2019. Soil health card development for efficient soil management in Haryana, India. Soil Tillage Res. 191(October 2018): 294–305. doi: 10.1016/j.still.2018.12.024.

Reberg-Horton, S.C., Grossman, J.M., Kornecki, T.S., Meijer, A.D., Price, A.J., Place, G.T., and Webster, T.M. 2012. Utilizing cover crop mulches to reduce tillage in organic systems in

the southeastern USA. Renew. Agric. Food Syst. 27(1): 41–48.

Redmile-Gordon, M.A., Armenise, E., White, R.P, Hirsch, P.R., and Goulding, K.W.T. 2013. A comparison of two colorimetric assays, based upon Lowry and Bradford techniques, to estimate total protein in soil extracts. Soil Biol. Biochem. 67: 166–173.

Reyna, D.L., and Wall, L.G. 2014. Revision of two colorimetric methods to quantify glomalin-related compounds in soils subjected to different managements. Biol. Fertil. Soils 50(2): 395–400.

Reynolds, W.D., and Topp, G.C. 2007. Soil Water Desorption and Imbibition: Long Column. p. 1035–1042. *In* Soil Sampling and Methods of Analysis. CRC Press.

Rochette, P., and Bertrand, N. 2007. Soil-Surface Gas Emissions. p. 885–896. *In* Carter, M., Gregorich, E. (eds.), Soil Sampling and Methods of Analysis. CRC Press.

Roper, W.R., Osmond, D.L., Heitman, J.L., Wagger, M.G., and Reberg-Horton, S.C. 2017. Soil Health Indicators Do Not Differentiate among Agronomic Management Systems in North Carolina Soils. Soil Sci. Soc. Am. J. 81(4): 828.

Ruark, M.D., Chawner, M.M., Ballweg, M.J., Proost, R.T., Arriaga, F.J., and Stute, J.K. 2018. Does Cover Crop Radish Supply Nitrogen to Corn? Agron. J. 110(4): 1513–1522. doi: 10.2134/agronj2017.06.0352.

Rumpel, C., and Kögel-Knabner, I. 2011. Deep soil organic matter-a key but poorly understood component of terrestrial C cycle. Plant Soil 338(1): 143–158.

Rutherford, P.M., McGill, W.B., Arocena, J.M., and Figueiredo, C.T. 2007. Total Nitrogen. p. 267–278. *In* Carter, M., Gregorich, E. (eds.), Soil Sampling and Methods of Analysis. CRC Press.

Sanaullah, M., Chabbi, A., Leifeld, J., Bardoux, G., Billou, D., and Rumpel, C. 2011. Decomposition and stabilization of root litter in top- and subsoil horizons: What is the difference? Plant Soil 338(1): 127–141.

Sardans, J., Rivas-Ubach, A., and Peñuelas, J. 2012. The C:N:P stoichiometry of organisms and ecosystems in a changing world: A review and perspectives. Perspect. Plant Ecol. Evol. Syst. 14(1): 33–47.

Scharlemann, J.P.W., Tanner, E.V.J., Hiederer, R., and Kapos, V. 2014. Global soil carbon: Understanding and managing the largest terrestrial carbon pool. Carbon Manag. 5(1): 81–91.

Schlenker, W., and Roberts, M.J. 2009. Nonlinear temperature effects indicate severe damages to U.S. crop yields under climate change.

Shields, J.A., Clayton, J.S. and Rostad, H.P.W. 1968. A guide to soil capability and land inventory maps in Saskatchewan. Saskatchewan Institute of Pedology, University of Saskatchewan.

Simonsson, M., Kirchmann, H., Magid, J., and Kätterer, T. 2014. Can Particulate Organic Matter Reveal Emerging Changes in Soil Organic Carbon? Soil Sci. Soc. Am. J. 78(4): 1279–1290. doi: 10.2136/sssaj2013.12.0533.

Skjemstad, J.O., and Baldock, J.A. 2007. Total and Organic Carbon. p. 253–266. *In* Carter, M.., Gregorich, E.. (eds.), Soil Sampling and Methods of Analysis. CRC Press.

Smith, P., House, J.I., Bustamante, M., Sobocká, J., Harper, R., Pan, G., West, P.C., Clark, J.M., Adhya, T., Rumpel, C. and Paustian, K. 2016. Global change pressures on soils from land use and management. Global change biology, 22(3), pp.1008-1028.

Smith, W.N., Grant, B.B., Desjardins, R.L., Kroebel, R., Li, C., Qian, B., Worth, D.E., McConkey, B.G., and Drury, C.F. 2013. Assessing the effects of climate change on crop production and GHG emissions in Canada. Agric. Ecosyst. Environ. 179: 139–150.

Stewart, R.D., Jian, J., Gyawali, A.J., Thomason, W.E., Badgley, B.D., Reiter, M.S., and Strickland, M.S. 2018. What We Talk about When We Talk about Soil Health. Agric. Environ. Lett. 3(1): 180033. doi: 10.2134/ael2018.06.0033.

Sun, W., Canadell, J.G., Yu, L., Yu, L., Zhang, W., Smith, P., Fischer, T., and Huang, Y. 2020. Climate drives global soil carbon sequestration and crop yield changes under conservation agriculture. Glob. Chang. Biol. 26(6): 3325–3335. doi: 10.1111/gcb.15001.

Van Eerd, L.L., Congreves, K.A., Hayes, A., Verhallen, A., and Hooker, D.C. 2014. Long-term tillage and crop rotation effects on soil quality, organic carbon, and total nitrogen. Can. J. Soil Sci. 94(3): 303–315.

Van Eerd, L. L., Congreves, K. A., Arcand, M. M., Lawley, Y., Halde, C. 2021. Chapter 15: Soil Health and Management. In M. Krzic, F. Walley, A. Diochon, M. Paré & R. Farrell (Eds.), *Digging Into Canadian Soils: An Introduction to Soil Science*. Pinawa, MB: CSSS. https://openpress.usask.ca/soilsciencetest/chapter/soil-health-and-management/.

van Es, H.M., and Karlen, D.L. 2019. Reanalysis Validates Soil Health Indicator Sensitivity and Correlation with Long-term Crop Yields. Soil Sci. Soc. Am. J. 83(3): 721–732.

Vrindts, E., Mouazen, A.M., Reyniers, M., Maertens, K., Maleki, M.R., Ramon, H., and De Baerdemaeker, J. 2005. Management zones based on correlation between soil compaction, yield and crop data. Biosyst. Eng. 92(4): 419–428.

Wade, J., Culman, S.W., Logan, J.A., Poffenbarger, H., Demyan, M.S., Grove, J.H., Mallarino, A.P., McGrath, J.M., Ruark, M., and West, J.R. 2020. Improved soil biological health increases corn grain yield in N fertilized systems across the Corn Belt. Sci. Rep. 10(1): 1–9.

Wahlström, E.M., Kristensen, H.L., Thomsen, I.K., Labouriau, R., Pulido-Moncada, M., Nielsen, J.A., and Munkholm, L.J. 2021. Subsoil compaction effect on spatio-temporal root growth, reuse of biopores and crop yield of spring barley. Eur. J. Agron. 123: 126225.

Weil, R.R., Islam, K.R., Stine, M.A., Gruver, J.B., and Samson-Liebig, S.E. 2003. Estimating active carbon for soil quality assessment: A simplified method for laboratory and field use. Am. J. Altern. Agric. 18(1): 3–17.

Whiffen, L.K., Midgley, D.J., and McGee, P.A. 2007. Polyphenolic compounds interfere with quantification of protein in soil extracts using the Bradford method. Soil Biol. Biochem. 39(2): 691–694.

Williams, S.M., and Weil, R.R. 2004. Crop Cover Root Channels May Alleviate Soil Compaction Effects on Soybean Crop. Soil Sci. Soc. Am. J. 68(4): 1403–1409. doi: 10.2136/sssaj2004.1403.

Wiltshire, J.J.J., Tillett, N.D., and Hague, T. 2003. Agronomic evaluation of precise mechanical hoeing and chemical weed control in sugar beet. Weed Res. 43(4): 236–244.

Wood, S.A., Tirfessa, D., and Baudron, F. 2018. Soil organic matter underlies crop nutritional quality and productivity in smallholder agriculture. Agric. Ecosyst. Environ. 266: 100–108.

Wu, Q., Farrell, R.E., Congreves, K.A. Fine-tuning the methodology for measuring soil protein. Canadian Journal of Soil Science, Submitted Mar 16, 2021 (Under Review).

Wright, S.F., and Upadhyaya, A. 1998. A survey of soils for aggregate stability and glomalin, a glycoprotein produced by hyphae of arbuscular mycorrhizal fungi. Plant Soil 198(1): 97–107.

Zhou, Y., Ma, H., Xie, Y., Jia, X., Su, T., Li, J., and Shen, Y. 2020. Assessment of soil quality indexes for different land use types in typical steppe in the loess hilly area, China. Ecol. Indic. 118(June): 106743. doi: 10.1016/j.ecolind.2020.106743.

9 APPENDICES

9.1 Fine-tuning the methodology for measuring soil protein

This manuscript is submitted to the *Canadian Journal of Soil Science* as a short communication.

9.1.1 Introduction

The soil organic N pool is increasingly recognized as a critical indicator of soil health, and it is currently included as a component of soil health testing (Moebius-Clune et al., 2016). Soil protein represents a large fraction of the soil organic N pool and contributes to quality and quantity of soil organic matter. Thus, it is crucial that the method and procedural details for soil protein extraction and quantification are optimized for precision, accuracy, and reproducibility.

The Bradford method for measuring protein in solution was first applied to soil extracts with the intent of quantifying glomalin by Wright & Upadhyaya (1998), and later found to extract a rather large range of soil proteins, not just glomalin (Gillespie et al., 2010; Hurisso et al., 2018). Today, despite its widespread use for the quantification of autoclaved-citrate extractable *soil protein* (*ACE protein*), the Bradford method can be challenging to work with. It often produces inconsistent results (Whiffen et al., 2007) and co-extracted compounds—which vary by soil—can interfere with the results (Moragues-Saitua et al., 2019). The Bicinchoninic acid (BCA) method has been suggested as a better option for soil protein quantification (Reyna and Wall, 2014), but different sources report different procedural protocols (Fisher Scientific, n.d.; Hurisso et al., 2018; Moebius-Clune et al., 2016; Redmile-Gordon et al., 2013) and it is unclear which procedural details should be followed. Our objectives were to compare the BCA and Bradford methods to determine which is more reliable and fine-tune the procedural details for standardization.

9.1.2 Materials and Methods

9.1.2.1 Soil protein extraction

Soils (n = 5) representing a range of textures and organic matter contents were obtained from four soil zones in Saskatchewan (i.e., the Brown, Dark Brown, Black, and Gray zones). The soils were characterized as (i) a Brown Chernozem (clay texture, pH 6.2, 16.12 g kg^{-1} organic C, and 1.58 g kg^{-1} total N); (ii) a Dark Brown Chernozem (DB1: sandy loam texture, pH 8.0, 12.67 g kg^{-1} organic

C, and 1.31 g kg^{-1} total N; (iii) a Dark Brown Chernozem (DB2: loam texture, pH of 7.8, 14.79 g kg^{-1} of organic C, and 1.52 N g kg^{-1} total N); (iv) a Black Chernozem (loam texture, pH 5.9, 21.62 g kg^{-1} organic C, and 1.31 N g kg^{-1} of total N); and (v) a Dark Gray Chernozem (clay loam texture, pH 7.5, 71.28 g kg^{-1} organic C, and 6.10 g kg^{-1} total N). Composite soil samples were collected to a depth of 15 cm after harvest (Sept–Oct 2018) using a 2.5-cm (i.d.) auger. Soils were air-dried and sieved through a 2-mm mesh screen.

The standard ACE protein extraction followed the protocol originally developed by Wright & Upadhyaya (1996). Briefly, 1.0 g samples of air-dried soil were weighed into 50-mL Falcon tubes to which 8 mL of 20 mM sodium citrate (pH = 7) was added and the tubes capped and shaken at 120 rpm for 5 min. The caps were then loosened and the suspensions autoclaved at 121°C and 15 psi for 30 min then cooled to room temperature and centrifuged at 5,000 × g for 15 min. A sub-sample of the supernatant was then transferred into a microplate well and analysed immediately (although the samples could be stored at 4°C overnight). Centrifugation speeds up to 10,000 × g have been reported, thus we also examined the effect of a faster/shorter centrifugation step (i.e., 10,000 × g for 5 min).

9.1.2.2 Standard preparation and method comparison

A model protein, bovine serum albumin (BSA), was used as the standard—purchased as ready-to-use solutions (Thermo Fisher cat:23208) having BSA concentrations of 0, 125, 250, 500, 750, 1000, 1500, 2000 µg mL^{-1}. Calibration curves (absorbance *vs*. BSA concentration) were prepared following standard protocols for the Bradford (Wright and Upadhyaya, 1996) and BCA (Hurisso et al., 2018) microplate analyses.

In general, the Bradford method involved transferring an aliquot (5–25 µL) of the BSA standard into a well in the microplate, diluting the BSA solution with phosphate buffered saline (PBS) to a volume of 200 µL, adding 50 µL Coomassie Brilliant Blue G-250 dye to each well, incubating the microplate at room temperature for 5 min, and measuring the absorbance at 590 nm using a microplate spectrophotometer (Bio Tek, Epoch™ 2). Given the range of procedures reported in the literature, we tested five different ratios (v/v) of BSA to PBS (i.e., 1:3, 1:5, 1:13, 1:20, and 1:40). All reagents were supplied with the Bio Rad Protein Assay kit (Bio Rad Laboratories).

The BCA method was performed using a Pierce™ BCA Protein Assay kit (Thermo Fisher Scientific Inc.). In general, the BCA method involved transferring an aliquot (10 µL) of the BSA standard into a well in the microplate, adding 200 µL of the working reagent (WR: a 50:1 v/v mixture of Reagent A and Reagent B) and mixing by shaking for 30 s on a plate shaker, incubating the plate at 37°C for 30 min, and cooling the plate to room temperature before measuring the absorbance at 562 nm using the microplate spectrophotometer. Reagents A (a mixture of sodium carbonate, sodium bicarbonate, bicinchoninic acid and sodium tartrate in 0.1M sodium hydroxide) and Reagent B (4% cupric sulfate) were supplied with the BCA protein assay kit. In addition to the standard procedure described above, the protocol was modified to evaluate a BSA:WR ratio of 25:200 (v/v) and a different set of incubation conditions (i.e., 60°C for 60 min).

Variations of the two methods were evaluated to determine how the calibrations were affected by operational conditions. Calibration curves were obtained by plotting absorbance *vs*. BSA concentration and curve-fitting the data using first- and second-order polynomial regression in SAS v. 9.4. Based on the results of the method comparison, the most reliable method was selected and used to quantify the extractable protein in the reference soils.

9.1.3 Results

9.1.3.1 Method selection

Calibration curves for the Bradford and BCA methods are presented in Figure A-1. The Bradford method was restricted to a narrow range of protein concentrations (i.e., up to ca. 30–50 µg ml-1) and was sensitive to sample dilution (Fig. A-1A). Moreover, the absorbance readings for the Bradford test were very sensitive to the time allowed for color development (data not shown), which contributed an additional source of "between run" (i.e., analytical) variability.

The BCA method was much less sensitive to dilution, yielding calibration curves that spanned a much wider range of BSA concentrations (Fig. A-1B). Post-extraction incubation conditions, on the other hand, had a significant effect on calibration. That is, increasing the temperature and time of the incubation from 37°C for 30 min to 60°C for 60 min resulted in stronger color development (i.e., an increase in absorbance) and an increase in the sensitivity of the response (i.e., the slope of the curve)—though the calibration curve itself was best described using a second-order polynomial (Fig. A-1B). Based on these results, ACE protein assays were conducted using the BCA method.

9.1.3.2 Soil protein measurement and variability as influenced by procedural factors

Whereas the BCA calibration curves were unaffected by dilution, ACE protein concentrations in the reference soils showed a significant dilution effect (Fig. A-2A). In lowering the dilution of the soil extract from 1:20 to 1:8, the ACE protein result was less controlled by incubation temperature and time (Fig. A-2A, note the slope closer to 1) and less variable (Fig A-2B, note the lower CVs). At the same time, changing centrifuge conditions during the protein extraction procedure had no significant effect on ACE protein concentration ($p=0.691$) (Fig. A-2A) nor did the time allotted for color development ($p=0.918$; data not shown).

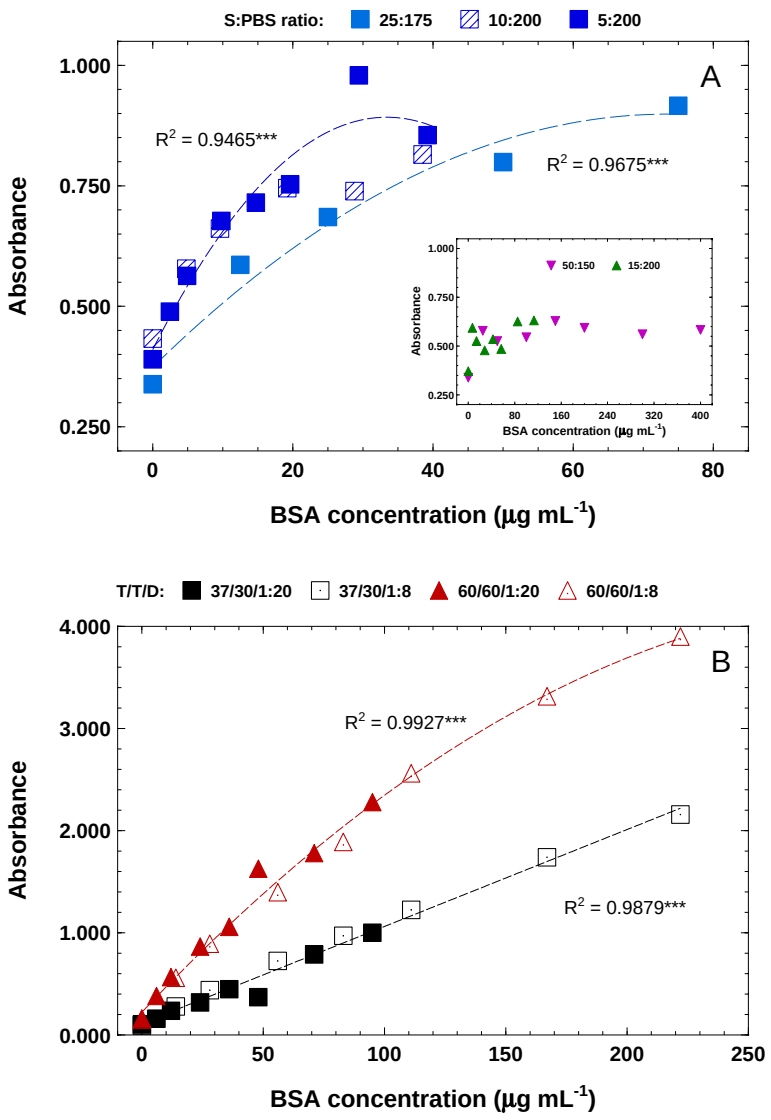

Figure A-1. Standard protein (bovine serum albumin, BSA) calibration curves obtained using the Bradford method (**A**) and the Bicinchoninic acid (BCA) method (**B**). For the Bradford method, different dilution ratios (v/v) of standard to PBS (S:PBS) were tested. For the BCA method, different incubation temperatures and times (T/T), and dilution ratios (D; standard:working reagent, v/v) were tested. Measurements are indicated by markers, and significant regressions are indicated by lines. ***, $p<0.001$.

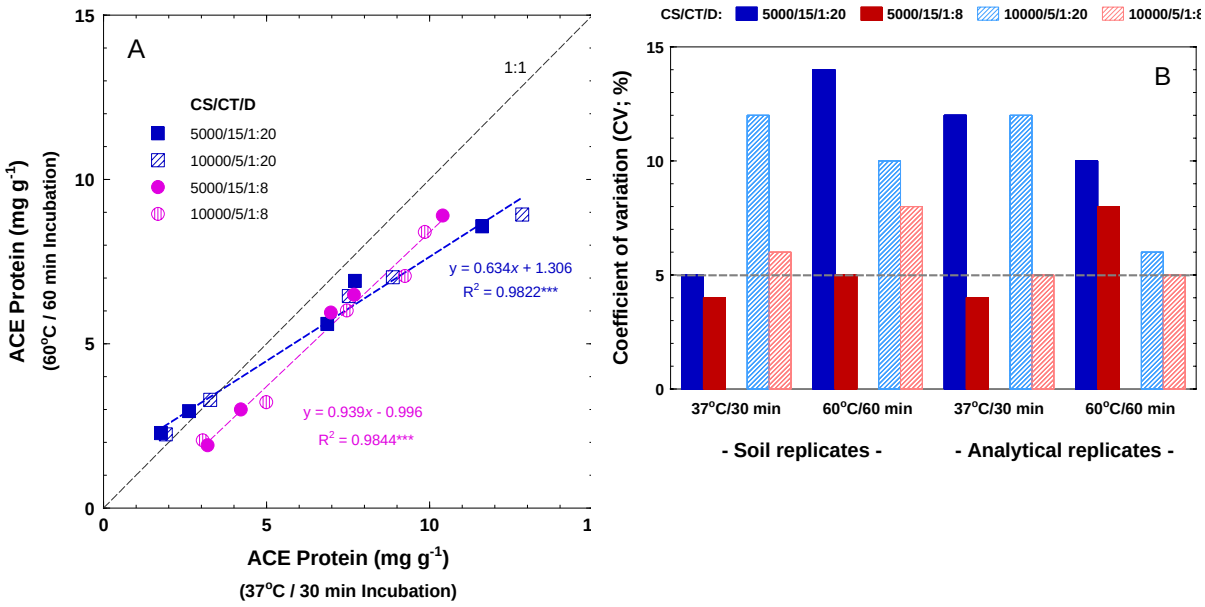

Figure A-2. (**A**) Extractable protein concentrations of five soils determined using different incubation conditions of 37°C / 30 min *vs*. 60°C / 60 min; different centrifuge conditions (centrifuge speed, CS; centrifuge time, CT) of 5000 × *g* for 15 min and 10000 × *g* for 5 min; and dilution ratios (D) of 1:20; 10:200 µL and 1:8; 25:200 µL. The dashed line represents a 1:1 relationship. (**B**) Coefficients of variation (CV) for the analytical and soil replicates for each centrifuge condition and dilution ratio, grouped by incubation temperature/time. Different colours represent the different dilution ratios; different patterns represent the different centrifuge conditions. The horizontal dashed line set at 5% CV to show the criteria of procedure selection. Soil replicates was protein measurement from duplicate soil extractions.

9.1.4 Discussion

In our study, the BCA method produced stronger calibration curves than the Bradford method, thereby enabling soil ACE protein to be quantified more precisely and reproducibly. It is recommended that researchers follow the BCA method instead of the Bradford method to measure soil protein. Our results align with those from others studies where the BCA method was more precise (Reyna and Wall, 2014) and more accurate (Redmile-Gordon et al., 2013) compared to the Bradford method. There are three reasons for why the Bradford method can produce inconsistent results—one being the difficulty attaining a strong and significant calibration curve as it is

sensitive to the dilution ratio used; the second being that the spectrophotometer reading must be immediately collected (within 10 min) otherwise there is an accumulation of precipitate in the sample that interferes with the accuracy of the reading (Redmile-Gordon et al., 2013). Thirdly, the Bradford method is susceptible to confounding artefacts, where the color development is influenced by the polyphenol concentration i.e., phenolic compounds are co-extracted with protein during the soil extraction process (Gillespie et al., 2010).

In using the BCA method on soil samples, our results showed robust measures of ACE protein regardless of post-extraction differences in centrifuge condition or absorbance reading time. These results suggest that minor differences in the procedure are unlikely to influence the resulting protein concentrations. However, the dilution ratio (i.e., soil extract supernatant:WR) did affect the ACE protein estimates in our study—depending on the incubation temperature and time. This is an interesting finding, because the calibration curve was not influenced by the dilution ratio (standard:WR). There is no real 'end-point' for the BCA reaction as the color continues to develop with time (only that it changes less rapidly after a certain point). The extraction process might be more prone to co-extracting other compounds from soil (interfering with color development) depending on the dilution and incubation conditions used. In our study, the 1:8 dilution and low temperature incubation produced more consistent results for the reference soils than the 1:20 dilution.

We included soil and analytical replicates when quantifying protein in the soil extracts and found the lowest CVs (≤ 5%) were generally associated with the 1:8 soil extract:WR dilution and the 37°C/30 min incubation. Further, selecting these operational conditions for soil ACE protein analyses of 160 soil samples for a soil health survey in Saskatchewan yielded average CVs of 3.5% for soil replicates and 5.8% for analytical replicates (*unpublished*). Coefficients of variation for the analytical replicates were somewhat greater than those reported by Hurisso et al. (2018), which suggests that some other factor (e.g., reproducibility of pipetting small volumes) was contributing to this variability. This just emphasises the need for researchers to examine the repeatability in their own labs, because variability is user and instrument dependent. Considering that soil protein measurements are included as a component of soil health testing, an inter-laboratory comparison of soil protein analysis would be a worthwhile next step to further standardize the methodology and best practices.

9.1.5 Conclusion

For quantifying soil ACE protein, the BCA method produced much more reliable calibration curves and should be used rather than the Bradford method. Using the BCA method, researchers should carefully consider the i) dilution ratio when mixing the soil extract supernatant with the working reagent and the ii) incubation temperature and time because both influence the protein estimate and the repeatability of the measurement. I recommend using a 1:8 dilution and an incubation at 37°C for 30 min.

9.2 Appendix Tables and Figures

Table A-1. Shapiro-Wilk probability values indicating the distribution normality for each soil attribute. Where P values are < 0.05, a log or square root transformation was applied to improve normality.

Attributes	Dataset	Soil depth (cm)		
		0-15	15-30	30-60
Wet aggregate stability (%)	Original	0.38	0.30	0.16
Soil organic C (g kg^{-1})	Original	0.48	0.73	0.78
Total C (g kg^{-1})	Original	0.73	0.08	0.14
Total N (g kg^{-1})	Original	0.22	0.07	0.09
Protein (mg g^{-1})	Original	0.15	0.73	0.96
Active C (mg kg^{-1})	Original	0.93	-	-
CO_2 (µg g^{-1} $24hr^{-1}$)	Original	0.53	-	-
EC (mS cm^{-1})	Original	0.00	0.01	0.58
	Log transformation	0.16	0.43	
N_2O (ng g^{-1} $24hr^{-1}$)	Original	0.00	-	-
	Log transformation	0.20		
pH	Original	0.00	0.00	0.00
	Square root	0.05	0.04	0.08
Sand (%)	Original	0.06	0.04	0.00
	Square root		0.45	
Silt (%)	Original	0.82	0.69	0.22
Clay (%)	Original	0.00	0.00	0.00
	Log transformation	0.06	0.14	0.02
NO_3^{-1}-N (µg g^{-1})	Original	0.00	0.00	0.00
	Log transformation	0.22	0.04	0.73
NH_4^+-N (µg g^{-1})	Original	0.70	0.24	0.50
PMN (µg $g^{-1)}$	Original	0.01	-	-
	Log transformation	0.38	-	-
Field capacity (%)	Original	0.49	0.66	0.18
Na (mg kg^{-1})	Original	0.15	0.04	0.04
	Log transformation		0.58	0.56
P (mg kg^{-1})	Original	0.43	0.58	0.73
Mn (mg kg^{-1})	Original	0.90	0.27	0.14
Ca (mg kg^{-1})	Original	0.00	0.00	0.00
	Log transformation	0.67	0.08	0.05
S (mg kg^{-1})	Original	0.33	0.00	0.00
	Log transformation		0.84	0.07

Mg (mg kg^{-1})	Original	0.00	0.00	0.00
	Log transformation	0.29	0.28	0.26
Zn (mg kg^{-1})	Original	0.45	0.04	0.01
	Log transformation		0.33	0.03
Fe (mg kg^{-1})	Original	0.01	0.02	0.00
	Log transformation	0.07	0.11	0.00
K (mg kg^{-1})	Original	0.00	0.00	0.00
	Log transformation	0.11	0.14	0.05

- , data not available because only the surface-most depth increment was analyzed

Table A-2. Model selection for predicting soil health scores from soil attribute measurements according to the "more is better" function. For all models, x is the observed soil score and y is the modelled soil health score. Models are selected based on R^2 and root mean square error (RMSE), indicated in bold.

					Model					
Soil depth (cm)	**Attributes**		**Polynomial with intercept (order =2)**	**polynomial without intercept (order =2)**	**polynomial (order=1)**	**Power**	**Inverse power**	**Square root**	**Hoerl's**	**Logarithmic**
0-15	Wet aggregate stability (%)	R^2	**0.96**	0.95	-	0.86	0.98	0.92	0.97	0.85
		RMSE	**5.59**	6.60	-	10.72	3.80	8.33	4.76	11.29
	Soil Organic C (g kg $^{-1}$)	R^2	**0.96**	0.94	0.96	0.89	-	0.93	0.95	0.85
		RMSE	**5.68**	7.45	5.69	9.95	-	7.64	6.27	11.36
	Total C (g kg $^{-1}$)	R^2	**0.97**	0.93	0.97	0.87	-	0.96	1.00	0.92
		RMSE	**5.34**	7.82	5.41	11.06	-	6.24	1.56	8.50
	Total N (g kg $^{-1}$)	R^2	**0.95**	0.94	-	0.88	-	0.89	-	0.77
		RMSE	**6.26**	7.42	-	10.24	-	9.55	-	14.21
	Protein (mg g^{-1})	R^2	**0.95**	0.91	0.95	0.85	-	0.92	0.95	0.81
		RMSE	**6.60**	8.99	6.82	11.19	-	8.34	6.83	12.67
	CO_2 (mg g^{-1} 24hr^{-1})	R^2	**0.97**	0.92	0.96	0.86	0.98	0.96	0.99	0.92
		RMSE	**5.04**	8.31	5.58	11.02	4.18	5.80	3.02	8.05
	Active C (mg kg $^{-1}$)	R^2	**0.97**	0.95	0.97	0.92	-	0.94	0.97	0.86
		RMSE	**5.05**	6.77	5.05	8.52	-	7.20	5.16	11.29
	Potential mineralizable N (ug g^{-1})	R^2	-	0.94	0.76	0.71	0.96	**0.94**	0.96	0.91
		RMSE	-	6.87	14.01	15.37	5.61	**7.15**	5.97	8.81

15-30	Wet aggregate stability (%)	R^2	**0.98**	0.96	0.97	0.87	0.97	0.94	1.00	0.90
		RMSE	**4.47**	5.47	5.09	10.32	4.87	6.97	0.97	9.36
	Soil Organic C (g kg $^{-1}$)	R^2	**0.97**	0.93	0.97	0.88	0.99	0.95	0.98	0.90
		RMSE	**5.49**	8.01	5.60	10.56	3.71	6.74	4.50	9.76
	Total C (g kg $^{-1}$)	R^2	**0.98**	0.91	0.94	0.83	0.98	0.96	0.99	0.95
		RMSE	**4.43**	9.08	6.99	12.34	4.01	5.58	2.71	6.77
	Total N (g kg $^{-1}$)	R^2	**0.98**	0.95	0.98	0.92	0.99	0.97	0.98	0.92
		RMSE	**4.65**	7.20	4.82	8.61	3.15	5.78	4.14	8.76
	Protein (mg g $^{-1}$)	R^2	**0.97**	0.94	0.97	0.88	0.98	0.95	0.99	0.91
		RMSE	**5.10**	7.34	5.13	10.16	3.80	6.33	2.32	9.13
30-60	Wet aggregate stability (%)	R^2	**0.98**	0.98	0.97	0.98	-	0.90	0.94	0.73
		RMSE	**4.24**	4.69	4.83	4.26	-	9.38	7.41	15.64
	Soil Organic C (g kg $^{-1}$)	R^2	**0.98**	0.94	0.97	0.90	0.99	0.96	0.99	0.91
		RMSE	**4.75**	7.12	4.83	9.43	3.30	5.98	3.09	8.87
	Total C (g kg $^{-1}$)	R^2	**0.98**	0.95	0.97	0.93	-	0.97	0.98	0.91
		RMSE	**4.19**	6.49	5.18	8.05	-	5.35	4.05	8.79
	Total N (g kg $^{-1}$)	R^2	0.98	0.91	0.95	0.85	0.98	**0.97**	0.99	0.95
		RMSE	4.23	8.61	6.88	11.37	3.79	**5.43**	2.70	6.78
	Protein (mg g $^{-1}$)	R^2	**0.96**	0.94	0.96	0.92	0.92	0.92	0.93	0.81
		RMSE	**5.56**	6.98	5.56	8.39	8.56	8.17	7.55	12.74
	Average	R^2	**0.97**	0.94	*	0.89	*	0.94	*	0.88
		RMSE	**5.10**	7.31	*	9.78	*	6.94	*	10.23

-, the curve created by particular model doesn't follow the scoring type; *, the model is not applicable for all selected attributes.

The model with bolding values is the selected model for the attributes in table. Polynomial with intercept (order =2), $y=a+bx+cx^2$. Polynomial without intercept (order =2), $y=ax+bx^2$. Polynomial with intercept (order =1), $y=a+bx$. Power, $y=ax^b$. Inverse power, $y=a*e^{(b/x)}$. Square root, $y=a+b*\sqrt{x}$. Hoerl's, $y=a*x^b*e^{(C*x)}$. Logarithmic, $y=a+b*In(x)$.

Table A-3. Model selection for predicting soil health scores for each soil attribute of "less is better" type in the 0-15, 15-30, and 30-60 cm depth, based on R^2 and root mean square error (RMSE). For all models, x is the observed soil health score and y is the modelled soil health score. Bolded R^2 and RMSE values indicate the selected model.

Soil depth (cm)	Attributes		Polynomial with intercept (order =2)	Polynomial without intercept (order =2)	Polynomial (order=1)	Power	Modal Inverse power	Square root	Hoerl's	Logarithmic
0-15	EC (mS cm $^{-1}$)	R^2	-	-	-	-	-	**0.97**	-	0.99
		RMSE	-	-	-	-	-	**5.30**	-	3.75
	N_2O (ng g^{-1} $24hr^{-1}$)	R^2	-	-	-	-	-	**0.95**	-	0.95
		RMSE	-	-	-	-	-	**6.48**	-	6.77
15-30	EC (mS cm $^{-1}$)	R^2	-	-	0.89	0.72	0.51	**0.95**	-	-
		RMSE	-	-	9.59	15.41	20.39	**6.24**	-	-
30-60	EC (mS cm $^{-1}$)	R^2	-	-	0.97	-	-	**0.96**	-	0.90
		RMSE	-	-	5.21	-	-	**5.98**	-	9.32
Average		R^2	*	*	0.93	*	*	**0.96**	*	0.95
		RMSE	*	*	7.51	*	*	**5.62**	*	6.38

-, the curve created by particular model doesn't follow the scoring type;

*, the model is not applicable for all selected attributes.

Polynomial (order=1), $y=a+bx$. Polynomial with intercept (order =2), $y=a+bx+cx^2$. Polynomial with intercept (order =2), $y=ax+bx^2$. Power, $y=ax^b$. Inverse power, $y=a*e^{(b/x)}$. Square root, $y=a+b*\sqrt{x}$. Hoerl's, $y=a*x^b*e^{(C*x)}$. Logarithmic, $y=a+b*In(x)$;

Table A-4. The formulas and threshold limits that correspond to the models presented in Figures 5-2 to 5-5 (x is measured value, and y is corresponded score.

	0-15 cm depth			15-30 cm depth			30-60 cm depth		
Attribute	**Equation**	**Upper threshold**	**Lower threshold**	**Equation**	**Upper threshold**	**Lower threshold**	**Equation**	**Upper threshold**	**Lower threshold**
				More is better					
Wet aggregate stability (%)	$y=-30.752+1.077*x+0.008*x^2$	84.16	16.69	$y=-36.408+1.130*x+0.011*x^2$	73.53	21.58	$y=-9.442+1.071*x+0.006*x^2$	79.94	2.12
Soil organic C (g kg^{-1})	$y=-42.350+3.967*x+0.006*x^2$	3.85	0.54	$y=-46.456+6.950*x-0.035*x^2$	27.06	4.11	$y=-38.912+8.107*x-0.042*x^2$	20.74	3.69
Total C (g kg^{-1})	$y=-62.579+5.014*x-0.016*x^2$	4.19	0.89	$y=-46.464+6.388*x-0.064*x^2$	42.41	6.24	$y=-25.786+4.864*x-0.036*x^2$	39.04	4.04
Total N (g kg^{-1})	$y=-34.953+30.982*x+3.820*x^2$	0.34	0.04	$y=-44.239+72.271*x-4.622*x^2$	2.48	0.39	$y=-84.735+138.353*\sqrt{x}$	2.20	0.32
Protein (mg g^{-1})	$y=-44.708+16.897*x-0.272*x^2$	12.31	1.11	$y=-47.697+27.304*x-0.363*x^2$	6.33	1.16	$y=-23.544+32.625*x+0.110*x^2$	4.14	0.36
Active C (mg kg^{-1})	$y=-30.213+0.139*x$	1026.02	108.67						
CO_2 (µg g^{-1} $24hr^{-1}$)	$y=-56.012+25.727*x-0.751*x^2$	8.79	1.57						

Potential mineralizable N (μg g^{-1})	$y=-54.072+25.5811\sqrt{x}$								
Less is better									
EC (mS cm^{-1})	$y=178.487-261.560*\sqrt{x}$	0.53	0.10	$y=172.103-256.986*\sqrt{x}$	0.55	0.07	$y=182.305-273.560*\sqrt{x}$	0.47	0.06
N_2O (ng g^{-1} $24hr^{-1}$)	$y=148.330-188.642*\sqrt{x}$	0.74	0.04						
Optimum is best									
pH	$y=(\frac{3-\lvert\frac{\sqrt{10^x}-5815.680)}{3855.037}\rvert}{3})*100$	8.45	5.15	$y=(\frac{3-\lvert\frac{\sqrt{10^x}-7380.540)}{4133.261}\rvert}{3})*100$	8.48	5.94	$y=(\frac{3-\lvert\frac{\sqrt{10^x}-11095.846)}{4452.383}\rvert}{3})*100$	8.82	6.49
Sand (%)	$y=(\frac{3-\lvert\frac{x-33.507)}{17.774}\rvert}{3})*100$	80.59	4.06	$y=(\frac{3-\lvert\frac{\sqrt{x}-5.180)}{1.670}\rvert}{3})*100$	75.18	1.09	$y=(\frac{3-\lvert\frac{\log_{10}(\lvert\log_{10}x/100\rvert)+0.261)}{0.238}\rvert}{3})*100$	70.30	4.37
Silt (%)	$y=(\frac{3-\lvert\frac{x-37.820)}{9.040}\rvert}{3})*100$	58.86	14.45	$y=(\frac{3-\lvert\frac{x-37.918)}{10.122}\rvert}{3})*100$	62.79	18.29	$y=(\frac{3-\lvert\frac{x-34.954)}{10.244}\rvert}{3})*100$	60.72	16.27
Clay (%)	$y=(\frac{3-\lvert\frac{\log_{10}x-1.378)}{0.271}\rvert}{3})*100$	66.25	4.96	$y=(\frac{3-\lvert\frac{\log_{10}x-1.445)}{0.250}\rvert}{3})*100$	71.60	4.98	$y=(\frac{3-\lvert\frac{\log_{10}(\lvert\log_{10}x/100\rvert)-0.165)}{0.074}\rvert}{3})*100$	72.76	10.03
NO_3^--N (μg g^{-1})	$y=(\frac{3-\lvert\frac{log_{10}x-0.856)}{0.342}\rvert}{3})*100$	25.11	1.19	$y=(\frac{3-\lvert\frac{log_{10}x-0.660)}{0.461}\rvert}{3})*100$	22.89	0.28	$y=(\frac{3-\lvert\frac{log_{10}x-0.311)}{0.632}\rvert}{3})*100$	28.36	0.04
NH_4^+-N (μg g^{-1})	$y=(\frac{3-\lvert\frac{x-3.943)}{1.233}\rvert}{3})*100$	7.08	1.56	$y=(\frac{3-\lvert\frac{x-3.490)}{0.974}\rvert}{3})*100$	5.54	1.71	$y=(\frac{3-\lvert\frac{x-3.632)}{1.328}\rvert}{3})*100$	6.93	1.31
Field capacity (%)	$y=(\frac{3-\lvert\frac{x-45.603)}{5.880}\rvert}{3})*100$	61.12	29.99	$y=(\frac{3-\lvert\frac{x-44.157)}{5.531}\rvert}{3})*100$	56.84	30.85	$y=(\frac{3-\lvert\frac{x-43.173)}{5.459}\rvert}{3})*100$	53.51	32.94

Na (mg kg^{-1})	$y=(\frac{3-\left\|\frac{x-66.472)}{22.268}\right\|}{3})*100$	113.29	29.16	$y=(\frac{3-\left\|\frac{log_{10}x-1.864)}{0.168}\right\|}{3})*100$	152.52	28.82	$y=(\frac{3-\left\|\frac{log_{10}x-1.979)}{0.204}\right\|}{3})*100$	245.63	32.20
Ca (mg kg^{-1})	$y=(\frac{3-\left\|\frac{log_{10}x-3.816)}{0.282}\right\|}{3})*100$	24698.46	1615.49	$y=(\frac{3-\left\|\frac{log_{10}x-3.991)}{0.432}\right\|}{3})*100$	78616.06	1842.94	$y=(\frac{3-\left\|\frac{log_{10}x-4.216)}{0.425}\right\|}{3})*100$	81258.63	2099.80
P (mg kg^{-1})	$y=(\frac{3-\left\|\frac{x-489.637)}{94.889}\right\|}{3})*100$	747.99	315.01	$y=(\frac{3-\left\|\frac{x-434.159)}{105.232}\right\|}{3})*100$	706.88	232.08	$y=(\frac{3-\left\|\frac{x-408.124)}{82.978}\right\|}{3})*100$	592.75	230.86
S (mg kg^{-1})	$y=(\frac{3-\left\|\frac{x-490.313)}{174.303}\right\|}{3})*100$	892.06	178.02	$y=(\frac{3-\left\|\frac{log_{10}x-2.713)}{0.296}\right\|}{3})*100$	2043.56	100.63	$y=(\frac{3-\left\|\frac{log_{10}x-2.840)}{0.337}\right\|}{3})*100$	2471.25	105.64
Mg (mg kg^{-1})	$y=(\frac{3-\left\|\frac{log_{10}x-3.669)}{0.221}\right\|}{3})*100$	11970.17	1526.70	$y=(\frac{3-\left\|\frac{log_{10}x-3.751)}{0.248}\right\|}{3})*100$	18870.92	1535.62	$y=(\frac{3-\left\|\frac{log_{10}x-3.865)}{0.244}\right\|}{3})*100$	20371.58	1970.63
Zn (mg kg^{-1})	$y=(\frac{3-\left\|\frac{x-67.397)}{20.225}\right\|}{3})*100$	108.65	26.35	$y=(\frac{3-\left\|\frac{log_{10}x-1.779)}{0.137}\right\|}{3})*100$	109.31	29.26	$y=(\frac{3-\left\|\frac{log_{10}x-1.751)}{0.151}\right\|}{3})*100$	100.57	28.72
Fe (mg kg^{-1})	$y=(\frac{3-\left\|\frac{log_{10}x-4.211)}{0.146}\right\|}{3})*100$	28111.60	7152.07	$y=(\frac{3-\left\|\frac{log_{10}x-4.227)}{0.138}\right\|}{3})*100$	28392.45	7879.71	*	27860.79	10050.85
K (mg kg^{-1})	$y=(\frac{3-\left\|\frac{log_{10}x-3.490)}{0.202}\right\|}{3})*100$	6593.32	866.90	$y=(\frac{3-\left\|\frac{log_{10}x-3.426)}{0.204}\right\|}{3})*100$	6213.33	991.55	$y=(\frac{3-\left\|\frac{log_{10}x-3.370)}{0.194}\right\|}{3})*100$	4724.46	763.12
Mn (mg kg^{-1})	$y=(\frac{3-\left\|\frac{x-468.261)}{115.276}\right\|}{3})*100$	791.37	183.10	$y=(\frac{3-\left\|\frac{x-431.861)}{126.577}\right\|}{3})*100$	728.37	195.35	$y=(\frac{3-\left\|\frac{x-390.575)}{104.663}\right\|}{3})*100$	960.86	223.63

*, The data of Fe from 30-60 cm depth failed to reach normality via any transformation (be it log, ln, square root, etc.); thus, Fe in 30-60 cm depth were not included in the soil health scoring.

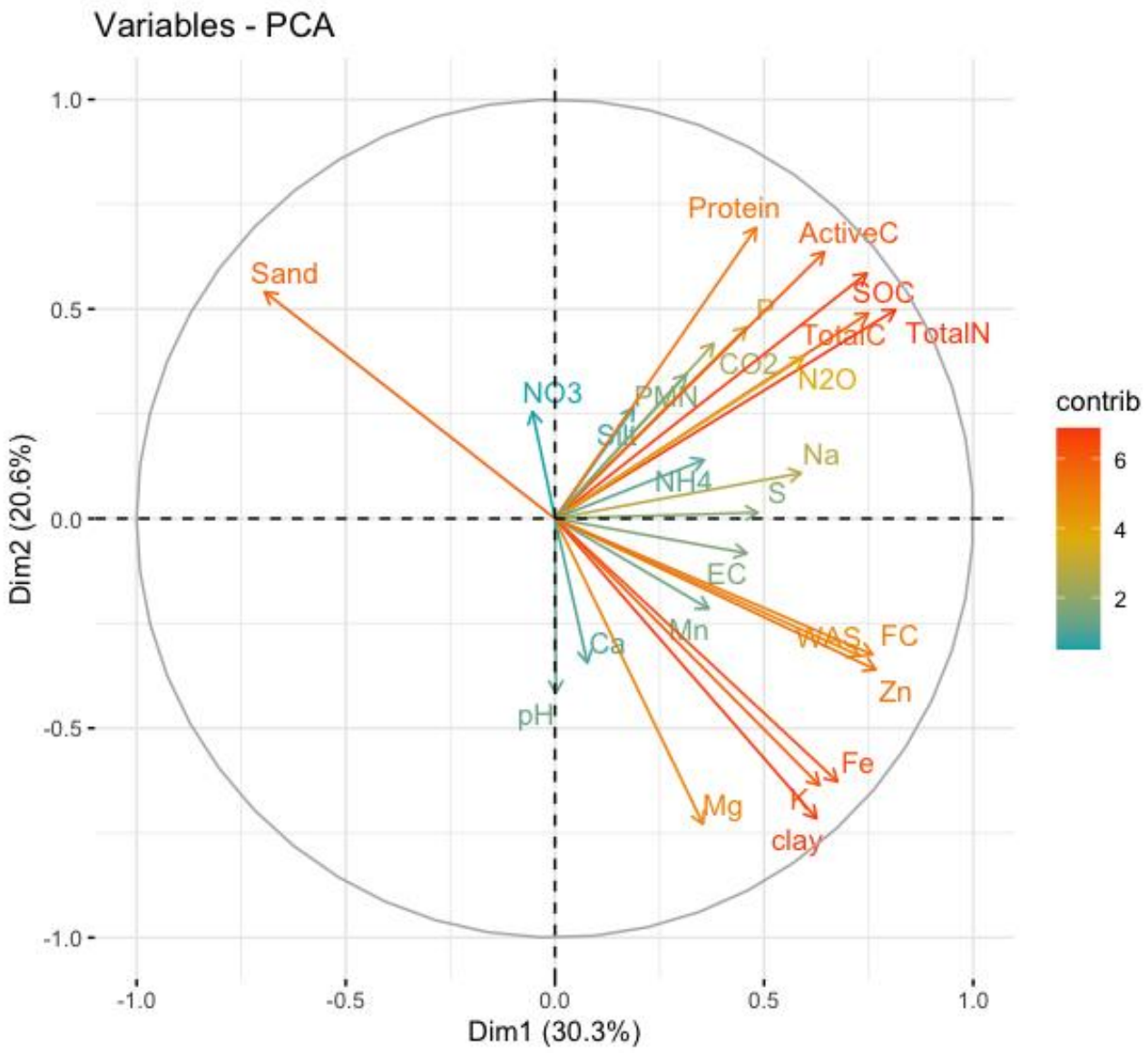

Figure A-3. The correlation of variables in first two principal components computed from principal component analysis. Positive correlated variables point to the same side of the plot. Negative correlated variables point to opposite sides of the graph. The color the variables by its contribution.

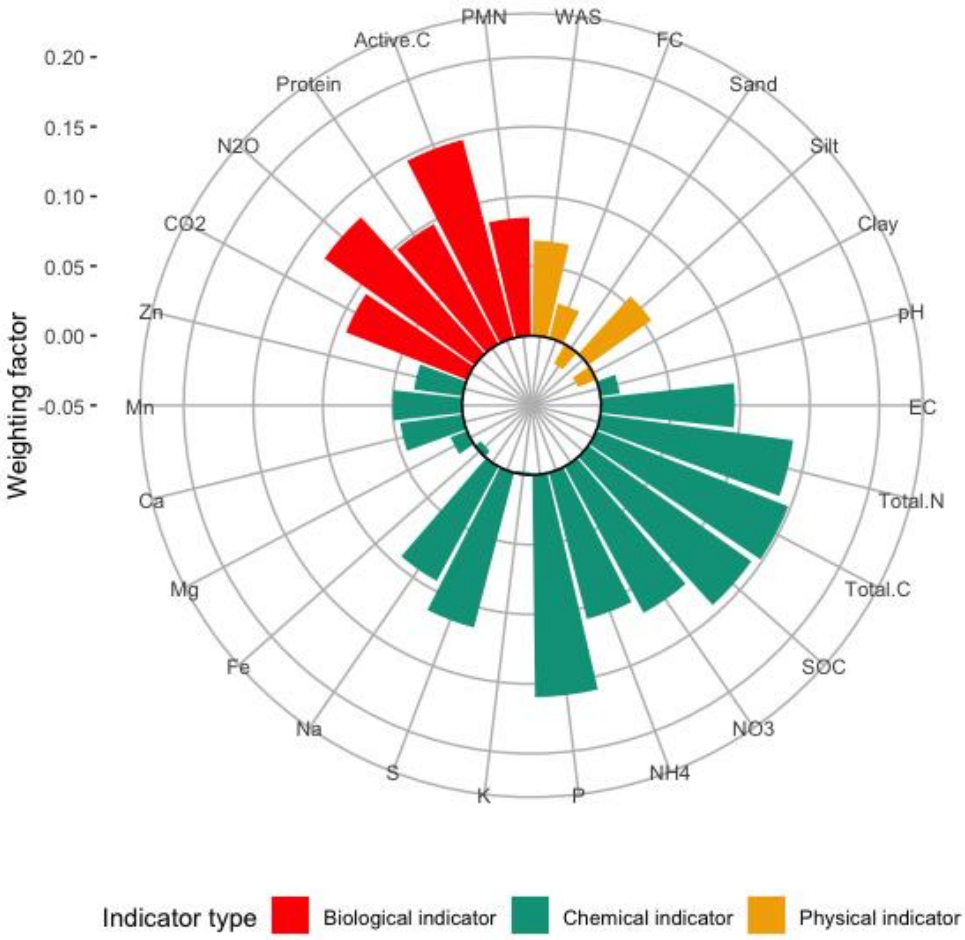

Figure A-4. The weighting factor of each soil attributes in Saskatchewan Soil Health Testing Protocol for soil from 0-15 cm depth.

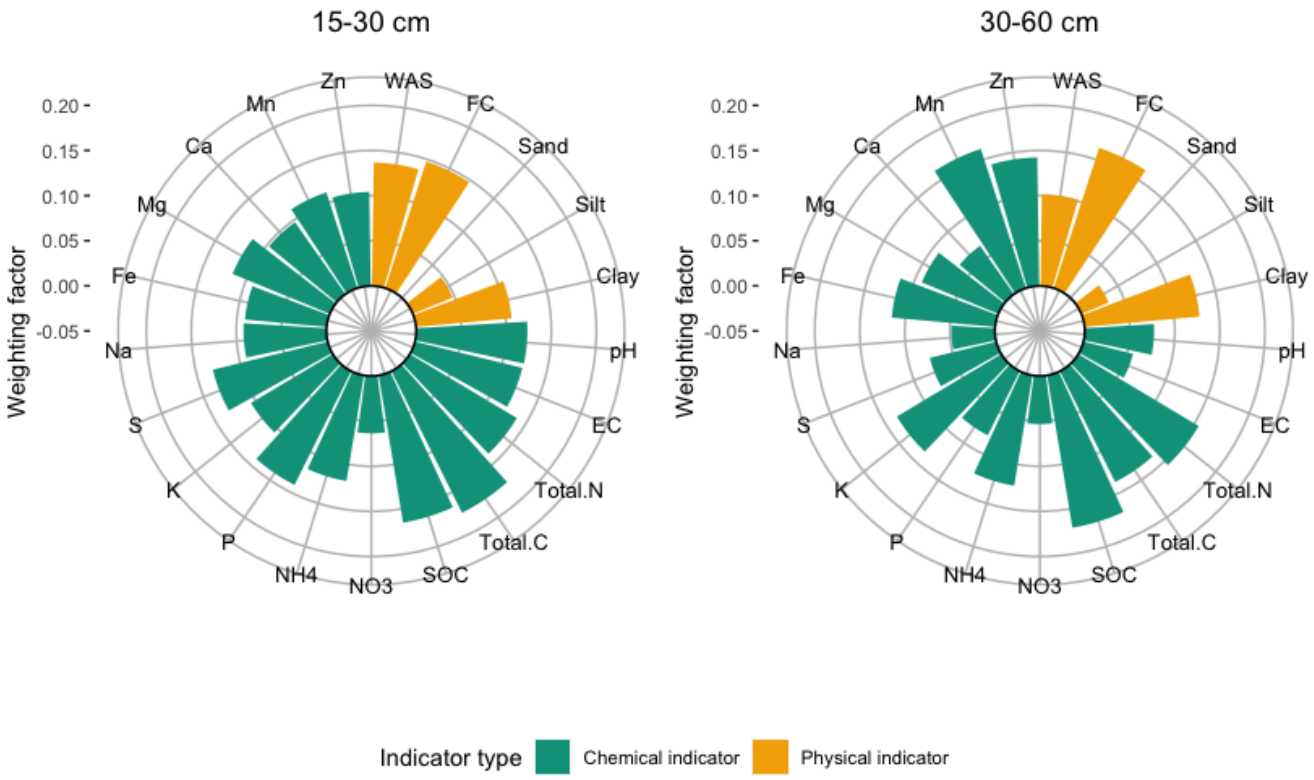

Figure A-5. The weighting factor of each soil attributes in Saskatchewan Soil Health Testing Protocol for soil from 15-30 and 30-60 cm depth, respectively.

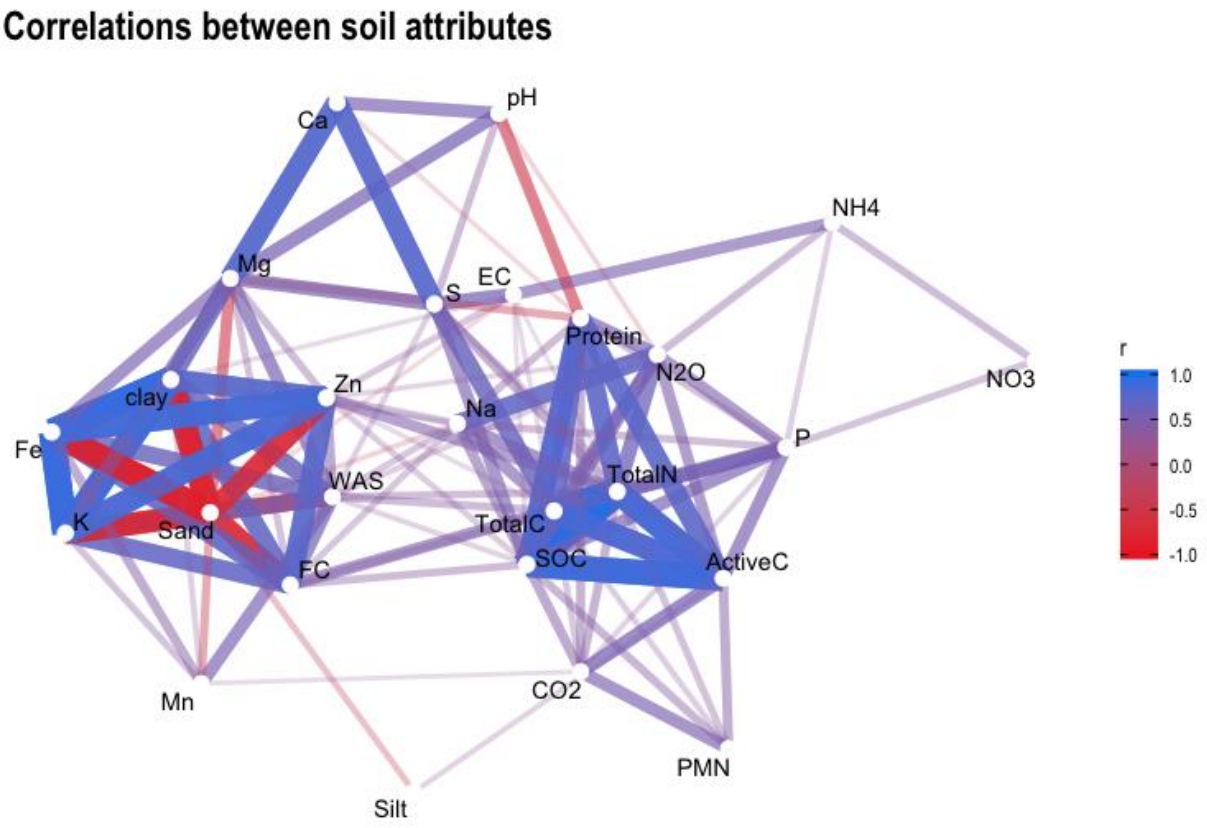

Figure A-6. The correlation network of soil attributes in Saskatchewan Soil Health Testing Protocol for soil from 0-15 cm depth.