The long-term effects of crop rotation and fertilizer applications on soil health and crop productivity in Alberta

by

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ABSTRACT

Long-term agricultural management practices affect soil health. Five long-term rotations at the University of Alberta Breton Plots were sampled as part of the Soil Heath Institute (SHI) North American Project to Evaluate Soil Health Measurements (NAPESHM) in 2019: (1) check (no fertilizer addition), NPKS and manure fertility treatments of a wheat–fallow (WF) rotation; (2) check, NPKS and manure fertility treatments of a 5 yr cereal–forage rotation (with and without lime); (3) continuous forage (CF) receiving NPKS fertilizer; (4) continuous grain (CG) receiving NPKS fertilizer; and (5) an 8-yr "agro-ecological" rotation of barley, faba beans and forages receiving manure. In addition to the >25 soil health indicators measured as part of NAPESHM, soil moisture retention curves (SMRC), phospholipid fatty acid (PLFAs) profile, size distribution of water-stable aggregates and total C, N, ^{13}C and ^{15}N within each class of water-stable aggregates were measured on additional samples taken in 2020. These soil health indicators were used to calculate a site-specific soil health index (SPSHI) using methods similar to those used to develop the Cornell comprehensive assessment of soil health (CASH). Multivariate permutational multivariate analysis of variance (PERMANOVA) and non-metric multidimensional scaling (NMDS) were used to assess the significance of long-term crop rotation, fertilization and their interactions on the soil health indicators used to develop the SPSHI. The indicators in the SPSHI equation included autoclave-citrate-extractable (ACE) protein, pH, available P, Na, available water holding capacity (AWHC), the proportion of total carbon in aggregates (PTCA) and Phosphomonoesterase. The higher the SPSHI value, the better the soil health. The SPSHI values of each rotation-fertilizer treatment from high to low are 8-yr with manure (0.802), 5-yr cereal-forage with manure and lime (0.79), WF manure (0.686), 5-yr with manure (0.674), 5-yr NPKS with lime (0.633), CG NPKS (0.507), 5-yr check with lime (0.477), 5-yr NPKS (0.432), 5-yr check

(0.418), WF with NPKS (0.403), CF with NPKS (0.389), and WF with check (0.38). The PERMANOVA results indicated significant effects of fertilizer treatments (p-value =0.0064), rotation treatments (p-value =0.0482) and their interaction (p-value =0.0095) on the soil health indicators. The primary difference in SPSHI values was caused by the difference of C and N input to soils, PTCA and pH in response to fertilizer, manure and rotations. The positive correlation between SPSHI values and crop yield is only weak to moderate, mainly because manure has a greater improvement on soil health than crop yield, whereas NPKS fertilizers had the opposite effect.

PREFACE

I was responsible for the refinement of the research concept, data collection and analysis as well as the manuscript composition. Dr. Miles Dyck assisted with the concept formation, data collection and analysis, and manuscript revision. Dr. Sylvie Quideau assisted with the data analysis, and manuscript revision.

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% TN/TC 0.5/2/4	Percentage of total nitrogen/carbon in aggregates of 0.25~0.5 mm/0.5~2 mm/2~4 mm
a_act_c or AC	Active carbon
a_awhc or AWHC	Available water holding capacity
a_gwc_wet	Gravimetric moisture of composite bulk density as sampled
a_ni/pb/na	Modified Morgan extract Nickel (Ni)/Plumbum (Pb)/Sodium (Na)
ACE protein	Autoclaved-citrate extractable soil protein
AE	Absolute aeration energy
aggs	Aggregates
aggs 2	Aggregates of 0.5~2 mm
AWC	Available water capacity
b_p/na_m3	Mehlich 3 extract Phosphorus (P)/Sodium
BD	Bulk density
C or TC	Carbon or Total carbon
CASH	Comprehensive Assessment of Soil Health
CO2-96 hours	Carbon dioxide of microbial 96h-respiration
CSHA	The Cornell Soil Health Assessment
d_icap_mg	H3A extract magnesium (Mg)
DTC/DTN	Carbon/nitrogen mass in non-water stable aggregates (or non-aggregated soil)
FAMEs	Fatty acid methyl esters
Fe	Iron
hm	Matric potentials

K	Potassium
LFC/N	Light fraction carbon/nitrogen
MBC	Microbial biomass carbon
Mg	Magnesium
Mn	Manganese
MWD	Mean weight diameter
N or TN	Nitrogen or Total nitrogen;
Na	Sodium
Ni	Nickel
NMDS	Non-metric multidimensional scaling
OM	Organic matter
P	Phosphorus
PAWHC	Plant available water holding capacity
Pb	Lead
PCA	Principal Component Analysis
PERMANOVA	Permutational multivariate analysis of variance
PLFAs	Phospholipid Fatty Acid Analysis
PME	Acid phosphomonoesterase
PMN	Potentially mineralizable nitrogen
Prop TC in aggs or PTCA	Proportion of TC in aggregates
RE	Absolute retention energy
SMRC	Soil moisture retention curve
SOC	Soil organic carbon
SPE	Solid phase extraction
SPSHI	Site-specific soil health index
WSA	Water-stable aggregates

Ⅰ. GENERAL INTRODUCTION

1.1 BACKGROUND

1.1.1 Soil health and soil health assessment

Soil is formed through a series of physical, chemical, and biological processes acting on parent geological material. From this perspective, the soil is a renewable resource. However, soil is regarded as a non-renewable resource, especially within the span of human life, because once soil degrades, its regeneration is an extremely slow process (Bai et al., 2018). How to maintain and improve soil health is an increasingly important issue in North America and globally.

Soil health and soil quality are often as assumed to be synonymous in the literature, although they differ as explained below. Soil quality can be conceptualized as the soil intrinsic quality and dynamic quality (Carter et al., 1997). Soil intrinsic quality is an integral part of land quality, and it is not affected by human management. In contrast, its dynamic quality refers to the part that can be affected by soil management. Soil health is equivalent to dynamic soil quality, which refers to soil properties that change due to soil use and management over the human time scale (Moebius-Clune, 2016). Different from soil quality, the idea of soil health is that soil is an ecosystem full of life (Moebius-Clune, 2016). Therefore, concepts applicable to entire ecosystems, such as function, process, attribute, and index, can describe soil health (Carter et al., 1997; Moebius-Clune, 2016).

According to the definition from the United States Department of Agriculture, soil health refers to the continued functioning of soil as an important ecosystem for the survival of plants, animals, and humans. The soil contains living organisms that perform the functions required to produce food and fiber for human beings. Healthy soil is an ecosystem that provides nutrients for plant growth, absorbs, and retains rainwater during periods of drought, filters and buffers potential pollutants away from farmland, provides a solid foundation for agricultural activities, and provides a habitat for soil microbes to multiply and diversify to keep ecosystems functioning (Natural Resources Conservation Service, 2020). These are soil functions that can be used to evaluate the health of the soil.

In general, soil health assessment is complex, so commercial soil health testing is rare. The Cornell Soil Health Assessment (CSHA) is an example of a commercially available soil health test. However, this index is sensitive to extreme values of soil properties, and it is often necessary to adjust the existing assessment when evaluating soil health in a specific area (Congreves et al., 2015). It is necessary to measure soil physical, chemical and biological properties to assess soil health (Altieri and Nicholls, 2003). Healthy soil ensures the quality, yield and safety of plants. The yield and quality of plants is one of the most vital indicators of soil health.

1.1.2 Soil health and agricultural management practices

Soil health is greatly influenced by human management and land-use decision-making (Kremer, 2017). The biochemical and physical properties of soil depend largely on the quantity and quality of crop residues returned to the soil (Campbell et al., 1997). Therefore, it is worth noting how the various crop management systems affect crop residue input, which is a function of crop production (Campbell et al., 1997). Farmers can choose management practices that improve soil health.

Some agricultural management practices can cause soil degradation, for instance, the loss of organic matter (Campbell et al., 1997). Intensive agriculture can lead to soil erosion, which due to the combination of long-term farming and inadequate soil management. A study in western Russia and Siberia has shown that the combined effects of tillage erosion and the sloped gully terrain lead to much soil loss (Golosov et al., 2020). For such obvious topographic factors, soil degradation can be slowed by external improvements. For cultivated land, the depletion of soil nutrients is the main concern. The application of fertilizer and rotation practice is the common management practice for improving soil health (Campbell et al., 1997; Ma,2016).

Long term monitoring and research are needed to assess the impact of natural and man-made processes on soil health attributes. For example, the long-term soil study at the University of Alberta at the Breton plots, and several studies of soils in western Canada by Campbell and other scientists (Campbell et al., 1997; Dyck et al., 2012). Campbell and other scientists conducted a comprehensive study on the changes in soils in Canada prairie areas in 1997. Their research involved a variety of soils: Brown and Dark Brown Chernozemic soils, and Gray Luvisols; a variety of agricultural management measures: fertilization, crop rotation, different tillage methods; a variety of soil health measurement indicators: soil fertility and soil aggregation, nitrate leaching and crop yield. The study showed that fertilization, legume crops and frequent planting can improve soil fertility and soil aggregation, nitrate leaching and crop yield. On the contrary, summer fallow has negative impacts on these indicators (Campbell et al., 1997).

1.1.3 Long-term fertilization and soil health indicators

Fertilization is potentially one of the most significant agricultural practices affecting soil health and sustainable utilization of soil. After fertilization, the increase of soil nutrients is an inevitable trend in the short term and may also enhance soil fertility in the long-term (Campbell et al., 1997). However, the long-term effects of fertilization on soil health and plant quality and quantity are not fully understood. For sustaining soil health, it is necessary to study the long-term impact of agricultural measures and the mechanisms behind the impact. Long-term soil experiments have important scientific value in studying the evolution of soil fertility, fertilizer effect, nutrient cycle, and the relationship between fertilization and the environment (Li et al., 2019). Many studies have been carried out on this aspect in recent years (Altieri and Nicholls, 2003; Dyck and Puurveen, 2020; Fan et al., 2005; Jiang et al., 2018; Kiani et al., 2017).

Long-term fertilization in an important practice to maintain the nutrients and organic matter in the soil (carbon (C), nitrogen (N), phosphorus (P), sulfur (S), and potassium (K)) removed by harvesting crops. By providing the elements needed for growth, the long-term use of some fertilizer combinations can significantly increase crop yields. Balanced fertilization tends to improve crop yields and quality better than other combinations of fertilizers and no-fertilizer applications (Dyck and Puurveen, 2020). In the study carried out by Dyck and Puurveen in 2020, unbalanced fertilizer combinations, including manure, as well as controls without fertilizer, resulted in lower grain and straw yields and lower crop N uptake, compared to balanced fertilizers (NPKS). For some indicators of microbial biomass, the situation may be affected by soil type and climate. In terms of soils in the cold prairie, manure worked better on improving microbial biomass carbon (MBC) than balanced chemical fertilizers or no fertilizer treatment (Kiani et al., 2017). In terms of yellow paddy soils, balanced application of chemical fertilizer (NPK) increased MBC more than other inorganic and organic fertilizer combinations (Li et al., 2019). For microbial biomass phosphorus (MBP) in yellow paddy soils, both inorganic phosphate fertilizer and organic fertilizer improved it (Li et al., 2019). In addition, other studies found organic fertilizers work better to improve the health of plants because excessive use of inorganic fertilizers can lead to the nutrient imbalance and lower pest resistance of plants (Altieri and Nicholls, 2003).

In addition to the benefits of long-term fertilization, there are also potential problems that need to be addressed - for example, the emission of greenhouse gases from the soil such as CO_2 and N_2O. Long-term application of chemical fertilizer or manure increased N_2O emission in the growing season (Giweta et al., 2017).

1.1.4 Long-term crop rotation and soil health indicators

Crop rotation is one of the management practices significantly affecting soil C and N. Recent studies have shown that crop rotation, combined with conservation tillage enhanced soil health, improved seasonal N availability, and provided N input through symbiotic N fixation of legumes (Ma, 2016). Crop rotation can also be a tool to manage soil nutrient levels and changes in soil mycorrhizal numbers, managing pest populations, increasing root activity, reducing disease severity, enhancing biodiversity, and reducing greenhouse gas emissions per unit area or C footprint per unit yield (Ma, 2016).

Effects of crop rotation are often site-specific. Planting legumes more frequently did not necessarily increase soil organic C or N reserves (Bell et al., 2012). A forage-grain rotation showed a higher microbial metabolic quotient (qCO_2) than annual grain rotation (Braman et al., 2016). Crop types may be more important than total crop diversity in improving soil health (Congreves et al., 2015). Different crop varieties may affect soil fertility because of differences in rooting depth, root branching, root turnover, and the amount and composition of root exudates (Congreves et al., 2015). Even though diverse crop rotations can improve soil health, re-establishment of perennial grassland without cultivation may improve it even more (Bell et al., 2012).

1.1.5 The interaction of long-term fertilization and crop rotation on soil health indicators

In farming systems, fertilizer is often used in combination with crop rotation. So it is crucial to understand the interaction between the two for practical applications, which is also an objective of this research. Fertilizer and crop rotation may have very different interactions on soil due to local characteristics (Braman et al., 2016; Dyck and Puurveen, 2020; Giweta et al., 2017). For example, compared with conventional management, organic management (organic fertilizer applied) improved the MBC of forage rotation, but decreased the MBC of annual rotation (Braman et al., 2016). Crop rotation influenced the increase of qCO_2 level, but fertilization type had no significant influence on it (Braman et al., 2016). In terms of the research carried out at

Breton Plots, scientists have found that for cumulative N_2O emissions in the growing season, there was little interaction between crop rotation and fertilizer: crop rotation had a significant effect, while all different fertilizers increased N_2O emissions (Giweta et al., 2017). A large amount of biologically-immobilized N by crop rotation reduces the crop's response to fertilizers with N (Dyck and Puurveen, 2020).

1.1.6 Carbon in soil aggregates

Aggregates are composed of particles with obvious structure and have a certain size and shape (Hillel, 1998). Large inter-aggregate pores favoring high infiltration rates and unrestricted aeration and small intra-aggregate pores increase levels of plant-available water (Hillel, 1998). The stability of soil aggregates is mainly affected by internal organic matter and external disturbance (Congreves et al., 2015; Oades, 1984.). Macroaggregates are surrounded by living or decomposed plant roots and are sensitive to management and increase in number when grasses are grown and the soil is left undisturbed (Oades, 1984). The stability of micro aggregates is influenced by complex organic acids and polysaccharide binding produced by plants and microorganisms, and polyvalent cations (Oades, 1984).

Long-term fertilization can supplement the content of each element in cultivated soil. Crop rotation can also balance or increase nutrients in the soil. However, the distribution of nutrients in aggregates, influenced by long-term fertilizer application or crop rotation in the soil, are not well known. In 2018, Sarker and other scientists carried out a study about the impact of long-term agricultural management practices on C and nutrient concentrations in soil aggregates in Australia (Sarker et al., 2018). They found that soil disturbances (by management practices), climate type, and soil type can influence the amount of organic C and nutrients in soil aggregates and that the latter two factors may outweigh the effects of human management. However, the agricultural management practices of their study only relate to the type of tillage.

Total carbon (TC) in soils includes inorganic C and organic C. TC can improve soil structure formation and tillage capacity by improving soil fertility, quality and water retention, ultimately maintaining and increasing crop yields, and it is related to atmospheric C sequestration and climate change (Wang et al., 2015). The availability of N in soils has become a decisive influencing factor of crop yield in modern agriculture (Sinclair and Rufty, 2012). C and N are highly positively correlated in soils (E. Sheng-zhe et al., 2018), thus they were usually discussed together in this study.

Global climate change is one of the most closely watched contemporary environmental issues. The IPCC predicts that atmospheric carbon dioxide levels and global temperatures will continue to rise over the next several decades, while precipitation will change by at least 20% (Allen et al., 2011). Climate change can have a profound impact on ecosystem C and N cycles, such as affecting soil C storage (Song Bing et al., 2012). Light fraction carbon (LFC) and light fraction nitrogen (LFN) are sensitive to changes in temperature and management practices, so it is a good indicator of environmental changes in soil health. In addition, the natural abundance stable isotopes are also sensitive to changes in soils and can indicate the input source. Carbon-13 (^{13}C) was widely used to determine the change in vegetations with different photosynthetic pathways (Boutton et al., 1998; Martin et al., 1990; Tang et al., 2012). Nitrogen-15 (^{15}N) has played a vital role in identifying putative N_2 fixation and estimating the relative contribution of biological N_2 fixation to plant N nutrition (Chalk and Craswell, 2018).

1.1.7 Soil Moisture Retention Curve

The soil moisture retention curve (SMRC) presents the relationship between volumetric water content and pore water matric potential when the soil is equilibrated with a reference reservoir (Hillel, 1998). The pore size distribution of soil is closely related to SMRC (Wang et al., 2002). The SMRC is usually used to estimate plant-available water holding capacity and aeration. Many SMRC related studies have been conducted on environmental issues, such as the movement of solutes that are harmful to soil water (Achieng, 2019; Wang et al., 2002). However, soil water, besides harmful solutes, also transports water and nutrients for plant growth, so research on SMRC is also an important indicator for soil health in agriculture (Armindo and Wendroth, 2016; Dexter 2004). Measuring and analyzing the moisture curve changes under the influence of long-term fertilization and crop rotation is one of the objectives of this research.

1.1.8 Soil microbial biomass and microbial community structure

The biological properties of soil are mainly related to soil microorganisms. Soil microbial biomass is very sensitive to the change in environmental factors and human disturbance and can reflect the change in soils in time (Li et al., 2019). Many studies have measured MBC, microbial biomass phosphorus(MBP), and microbial metabolic quotient (qCO_2) to reflect microbial biomass in soil (Braman et al., 2016; Li et al., 2019). Phospholipid fatty acid analysis (PLFAs) can be used to describe the structure of the soil microbial community, detect changes caused by various factors, distinguish between bacteria and fungi in the soil, and analyze their respective biomass (Frostegård and Bååth, 1996). Thus, PLFAs is a more comprehensive analysis of soil microbial change.

In Denmark, a soil experiment showed that the application of manure increased the richness and diversity of the bacterial community compared to inorganic fertilizers (van der Bom et al., 2018). Different levels of nutrient inputs lead to different changes in the microbial community structure or abundance. The influence of the N input on the structure of the bacterial community was greater than that of phosphorus or potassium and copiotroph-dominated bacterial groups were encouraged by high nutrient input levels (van der Bom et al., 2018). Gautam et al. also found some similar results in 2020. Compared with mineral fertilizer application, long-term fertilization strategies based on different nutrient requirements, especially high fertilizer treatment, are beneficial to improve soil biochemical and biological indexes (Asmita Gautam et al., 2020).

In terms of the combination of fertilizer application and rotation practices, the research by Soman et al. in 2017 indicates that functional redundancy plays a role in how management practices. influence group structure and function in agricultural soil microbiomes. Throughout the taxa whose relative abundance is influenced by crop-rotations, microbial functions related to substrate usage can be redundant (Soman et al., 2017). Conversely, microbial functional diversity related to substrate-use can play a greater role in shaping the structure of the microbial community between fertilizer treatments (Soman et al., 2017). There have been a few studies on the comparison of farmland with various forms of rotation, such as multiple crops, continuous grain, and continuous forage, more studies concentrate on rotation practices with multiple crops.

1.2 RESEARCH OBJECTIVES

Since soil health is related to various factors, it is meaningful to study the soil in specific areas. The measurement of soil health can be regarded as a technology, or applied science, to solve problems related to soil management, and can be regarded as the key to sustainable land management (Carter et al., 1997). Therefore, this research project aims to compare and analyze the effects of different long-term fertilizer applications, crop rotations and their interactions on multiple soil health indicators, and parts of the study will be the literature review. In summary, the soil health indicators studied in this project included SMRC and related physical properties, and PLFAs in soils, TC, TN, LFC, LFN, $\delta 13C$, $\delta 15N$ in soil aggregates, as well as other soil properties provided by the Soil Heath Institute (SHI) North American Project to Evaluate Soil Health Measurements (NAPESHM) in 2019.

To compare different agricultural management practices, another objective was to generate a modified method of measuring soil health that was developed from the previous studies, such as CHSA. Besides the specific adjustment according to local conditions, another obstacle to assessing soil health is the lack of standardization associated with "critical limits" (Carter et al., 1997), which was also be taken into consideration in the quantified process of assessing soil properties in this project.

Ⅱ. QUANTIFICATION AND VISUALIZATION OF SOIL HEALTH UNDER LONG-TERM FERTILIZER AND ROTATION TREATMENTS IN ALBERTA

2.1 INTRODUCTION

Healthy soils help strike a balance between productivity, environmental quality and animal and plant health, all of which are greatly affected by human management and land-use decisions (Kremer, 2017). There is an increasing number of studies on the effects of long-term agricultural management practices on soil health or soil quality worldwide (Jiang et al., 2018; Norris et al., 2020).

Fertilizer management and crop rotation have been shown to be agricultural management practices that influence soil health. Previous studies have found that the long-term application of both manure and chemical fertilizers improved soil health and crop yield (Fan et al., 2005; Kiani et al., 2017). Compared to chemical fertilizers, manure had a greater positive effect on nutrient cycling (Kiani et al., 2017), soil water-holding capacity (Fan et al., 2005), aggregate water retention ability (Liu et al., 2011), soil water retention and hydraulic conductivity (Zhang et al., 2006), organic C in water-stable aggregates (WSA) (Chai et al., 2019), carbon (C) and nitrogen (N) in soils (Gai et al., 2018). The combination of manure and chemical fertilizers enhanced crop yield more than chemical fertilizers alone in China (Gai et al., 2018; Zhang et al., 2022). Manure alone also increased crop yield, and was particularly beneficial for crops that are sensitive to pH (Barth et al., 2021). However, it was also reported that manure did not significantly increase the yield of wheat or beans (Barth et al., 2021). A meta-analysis based on 20 long-term experiments across China showed that the application of chemical fertilizers might significantly increase grain yield but had minor impacts on soil organic carbon (SOC) sequestration (Jiang et al., 2018). Conversely, compared to chemical fertilizers, the combined use of manure and chemical fertilizers only slightly increased grain yield but significantly improved SOC sequestration (Jiang et al., 2018). Whether chemical fertilizer alone would have achieved similar effect is unknown. To sum up, in the abovementioned studies, manure did not significantly improve crop yield, but improved soil health. Manure application in each region required optimization to achieve better results.

Compared to conventional cropping, the application of legumes and grass hay in complex rotations could improve microbial biomass carbon (MBC) (Kiani et al., 2017), C inputs (Angers et al., 1999) and N inputs (Chalk and Craswell, 2018) to soils. Therefore, in terms of nutrient input and crop yield, rotations with legumes or grass hay have the same trend as applying fertilizer. The more frequent inclusion of legumes in complex rotations, the better the improvement effect (Angers et al., 1999). Whether the use of organic and inorganic fertilizers in complex rotations improves soil health by the same magnitude and whether this corresponds precisely to changes in crop yield has not been studied in detail. Although continuous cropping was reported to degrade soil structure and fertility (Dou et al., 2017; Zou et al., 2018), balanced chemical fertilizer applications significantly reduced the damage in this type of cropping system (Zhang et al., 2006).

Soil health assessment integrates and optimizes the chemical, physical, and biological activities in soils rather than focussing on a single property (Moebius-Clune, 2016). Because multiple soil health indicators or properties are involved, soil health assessment is complex, and commercial soil health testing is rare. The Cornell Soil Health Assessment (CSHA) is an example of a commercially available soil health test. However, the index in CSHA is sensitive to extreme values of soil properties, and it is often necessary to adjust the existing assessment when evaluating soil health in a specific area (Congreves et al., 2015). CSHA is also called Comprehensive Assessment of Soil Health (CASH) and had some updates in 2017 (Fine et al., 2017). Using the Principal Component Analysis (PCA) to set up the equation was a relatively more straightforward way to assess soil health for specific locations (Parra-González and Rodriguez-Valenzuela, 2017). When a large dataset of soil health indicators is involved, the PCA and equation can be a more suitable framework for quantifying soil health. At the same time, scoring soil health indicators by types in the CASH method is also worthy of reference, which was used to standardize indicators to improve the accuracy of soil health assessment. To sum up, this study aims to combine some characteristics of these two methods to produce a modified method - site-specific soil health index (SPSHI) to represent soil health.

Soil health is closely related to crop yield. That is one of the main reasons farmers and scientists alike are concerned about soil health. Crop yield is constrained more by environmental conditions and the availability of N and water resources than by crop genetics in modern agriculture (Sinclair and Rufty, 2012). However, there may be some bias in comparing crop yields of different crop rotations due to different plant species. Due to the easy leaching and loss of N in soils, residual soil N was seldom considered an N source for crops (Lenka et al., 2013). The external N addition from agricultural management practices was strongly related to the crop N recovery (Lenka et al., 2013). Therefore, N recovery may be a more accurate indicator than crop yield when comparing the effects of different crop rotations on soil health and crops.

Over the last 20 – 30 years, increased intensification and diversity of crop rotations, along with increasingly higher yielding crop cultivars on the Northern Great Plains, has increased nutrient removal from cropping systems, but it also increased crop residues returned to the soil, affecting soil nutrient cycling, soil C, and nutrient balances (Grant et al., 2002; Janzen et al., 1998; Lafond et al., 2012; Lemke et al., 2012a, 2012b; Schlegel et al., 2005). The impact of these management trends on soil health is not clear, but there are concerns that the continued incremental increase in crop yields and harvest nutrient removals may negatively impact soil health. Quantifying the link between soil health, crop productivity, crop rotation and nutrient management is important because soil health is an increasingly important consideration in famer's management decisions and consumers' purchasing decisions. Therefore, the objectives of this study are to: (1) quantify and compare the effects of long-term rotation and fertilizer management on soil health using the SPSHI and the CASH score; (2) link SPSHI values of each treatment with crop yield (10-year average above and below biomass or only above biomass, kg/ha) and crop N recovery (10-year average annual crop N recovery plus roots or only above biomass, kg N/ha); and (3) visualize the long-term effects of various treatments on multiple soil health indicators using non-metric multidimensional scaling (NMDS) graphs and using permutational analysis of variance (PERMANOVA) analysis for statistical comparisons. To achieve these objectives, soil health indicators from five long-term rotations with a variety of fertilizer management histories at the University of Alberta, Breton Plots were measured and compared.

2.2 MATERIALS AND METHODS

2.2.1 Study area and experimental design

The University of Alberta Breton Plots, located approximately 100 km southwest of Edmonton, AB, near the town of Breton (53°07′N, 114°28′W), were established on Gray Wooded/Luvisolic soils developed on glacial till material in 1929 by the Department of Soils at the University of Alberta (Dyck et al., 2012; Dyck and Puurveen, 2020). The Breton Plots hosts 2 long-term crop rotation experiments. The Breton Classical Plots (est. 1929), consist of 8 fertility treatments superimposed on two crop rotations 1) wheat-fallow; and 2) 5-year cereal-forage. The Hendrigan Plots (est. 1980) consist of 3 rotation-fertility management systems: 1) continuous forage with chemical fertilizers; 2) continuous grain (cereals) with chemical fertilizers; and 3) 8-year cereal-forage-pulse with manure and chemical fertilizers. Selected plots covering the range of crop rotations and fertility management were sampled as part of the Soil Heath Institute (SHI) North American Project to Evaluate Soil Health Measurements (NAPESHM) in 2019 (Norris et al., 2020). Additional samples from the same plots were collected in 2020 for measurement of soil health indicators that were not measured by SHI.

A summary of the rotation-fertility treatments sampled in 2019 and 2020 is presented in Table 1. For statistical experimental design, the treatments are not randomized, and the rotations are not fully phased (replicated). Soils change over a long period of time, this study compared soil changes over time rather than rates of change.

2.2.2 Soil sample processing and analysis

SHI assessed more than 20 soil health indicators, and sampling details are summarized in Norris et al., 2020. Measurements on the samples collected in 2020 included soil moisture retention curve (SMRC), C and N in aggregates, and Phospholipid Fatty Acid Analysis (PLFAs).

2.2.2.1 Soil moisture retention curve

Moisture retention curves were measured on soil core samples (diameter = 8 cm, height = 5 cm) by measuring soil moisture content following equilibration at known potentials using a pressure plate extractor (Ceramic Plate Extractor, Sanata Barbara, CALIF; Reynolds and Topp 2007). External surfaces of the soil cores were first trimmed to remove any excess soil. To ensure hydraulic contact between the soil in the core and pressure plates, a known weight of saturated soil paste with a known moisture content, made from the Bt horizon from Ellerslie Research Center in Edmonton, was applied to the base of the soil core and covered with nylon mesh secured with elastic bands. The cores were weighed, placed on a ceramic extractor plate and transferred to a water bath for overnight saturation. The cores were then sequentially equilibrated for 3 – 7 days at pressures of 7, 50, 150, 500, 1000, 5000, and 15000 cm H_2O. The weight of the core samples was measured after equilibration at the first three pressures.

Prior to equilibration at 500, 1000, 5000 and 15000 cm H_2O, the nylon mesh and additional saturated paste were removed from the cores, and soil was removed from the cores and divided into 5 subsamples, with 1 subsample used to assess gravimetric moisture content by oven-drying after equilibration at 150 cm H_2O and the other 4 placed on separate extractor plates for equilibration at each of the four remaining pressures. At this time, the weight of the core, nylon mesh and elastic bands were also measured After equilibration, gravimetric moisture content of the subsamples was estimated by oven-drying at 105^{o}C for 48 hours.

Moisture contents at the first three pressures were estimated using the core weights collected following equilibration at 7, 50 and 150 cm H_2O in combination with the gravimetric moisture content measured following equilibration at 150 cm and correcting for the weight of the core, nylon mesh, elastic bands and saturated paste. The total weight of the dry soil in the core was estimated using the core weight and soil gravimetric water content following equilibration at 150 cm, and bulk density (BD) was estimated by dividing the dry weight by the volume of the soil calculated using the core internal diameter and height of the soil in the core. Volumetric water content at all pressures was estimated by multiplication of BD and gravimetric moisture content, even though the subsamples for the highest four pressures were disturbed. Because moisture retention at high pressures is most affected by soil texture, it was assumed that the gravimetric moisture content of the disturbed subsamples was representative of what might have been measured on the undisturbed core.

The SMRC was made by plotting volumetric water contents as a function of the matric potentials (hm). The double exponential model was fit to the measured SMRC (Equation 1; Table 2) in Microsoft Excel Version 16.53 using SOLVER.

$$\boldsymbol{\theta_v(h) = C + A_1 e^{\left(-\frac{h}{h1}\right)} + A_2 e^{\left(-\frac{h}{h2}\right)}} \quad [1]$$

Several important soil water indicators were also derived from the curves, including plant available water holding capacity (Equation 2), air energy/absolute aeration energy (AE, Equation 3) and retention energy/absolute water retention energy (RE, Equation 4).

$$PAWHC = \theta_{fc} - \theta_{pwp} \quad [2]$$

In which PAWHC is plant available water holding capacity, θ_{fc} is the volumetric water content at field capacity ($m^3 \cdot m^{-3}$; hm = 150 cm), θ_{pwp} is the volumetric water content at the permanent wilting point ($m^3 \cdot m^{-3}$; hm = 15000 cm).

$$AE = \int_{\theta_{fc}}^{\theta_s} hm(\theta)\delta\theta \quad [3]$$

$$RE = \int_{\theta_{pwp}}^{\theta_{fc}} hm(\theta)\delta\theta \quad [4]$$

In which AE is the absolute aeration energy (hPa $m^3 \cdot m^{-3}$), RE is the absolute retention energy (hPa $m^3 \cdot m^{-3}$), θ_s is the saturated volumetric water content ($m^3 \cdot m^{-3}$), and $hm(\theta)$ is the inverse SMRC (Armindo and Wendroth, 2016).

2.2.2.2 Carbon and nitrogen in aggregates

A soil slab of about 15cm ×15cm ×15cm was collected in each plot to measure wet aggregate stability, mean weight diameter, and C and N in aggregates. The wet-sieving method was used to obtain aggregates of the following four sizes: 0 – 0.25mm, 0.25 – 0.5mm, 0.5 – 2mm, 2 – 4mm, following (Angers et al., 2007). The wet-sieving apparatus consisted of four water reservoirs in which a sieve stack was gently oscillated up and down by motor-driven crankshaft. Firstly, field-moist aggregates were gently crumbled by hand to pass an 8mm sieve, 40 grams of which was placed on a stack of sieves with- 4mm, 2mm, 0.5mm and 0.25mm orifices. The sieve stack was placed in the wet-sieving apparatus and gently submerged in water for 10 mins to allow the aggregates to slake. Following slaking, an electric motor was energized and moved the sieve up and down 3 cm, 29 times over a 10 min period. Aggregates retained on each sieve were transferred in a pre-weighed and labelled aluminum tray for drying. Each fraction of aggregates was dried at 105 °C for three days, and the dried samples were weighed (w_2) and transferred to 125 ml polypropylene Nalgene bottles. 50 mL of 5 g/L sodium hexametaphosphate solution was added to each bottle containing the transferred dried subsamples and shaken on a reciprocating shaker for 45 minutes. The dispersed sample was then filtered through the corresponding sized sieve to remove coarse fragments and sand. The recovered sand and coarse fragments, as well as the rest of the sample with coarse fragments removed, were oven-dried at 105 °C for two days and weighed. Coarse fragments and sand were discarded, and the remaining sample was initially ground by hand with a mortar and pestle until the texture became homogenized, and then ground using a Retsch Oscillating Mill MM400 (German, 2016) for one and a half minutes. After grinding, samples were sent to the University of Alberta Natural Resources Analytical Laboratory for total carbon (TC), total nitrogen (TN), $\delta 13C$ and $\delta 15N$ analysis, which were measured on a ThermoScientific Flash 2000 Organic Elemental Analyzer, coupled to a Delta V Advantage Isotope Ratio Mass Spectrometer. The data recorded in the process were also used to calculate properties related to soil aggregates, including WSA (Equation 5) and mean weight diameter (MWD) (Equation 6), TC or TN mass in total aggregates or aggregates of four sizes, proportion of TC or TN in aggregates, and TC or TN mass in non-water stable aggregates (non-aggregated soils).

$$WSA_i = \frac{w_{2i} - w_{3i}}{\frac{w_1}{1+wc} - \sum_{i=1}^{4} w_{3i}} \quad [5]$$

$$MWD = \sum_{i=1}^{4} x_i \, WSA_i \quad [6]$$

In which, i= 1, 2, 3, 4 and corresponds to each size fraction. x_i is the mean diameter of each size fraction.

Light fraction carbon (LFC) and light fraction nitrogen (LFN) were also extracted from subsamples from the bulk soil slabs (Carter and Gregorich, 2007). Field moist soil was passed through 2mm sieves and air-dried for several days. A 15g subsamples for each plot was weighed into 125 ml Nalgen bottle and capped after adding 40 mL 1.7 $g \cdot cm^3$ NaI solution. The bottles were shaken 30 minutes on a reciprocating shaker. The suspension was allowed to settle at room temperature for 48 hours. After settling, the first few centimeters of the solution and light fraction were removed with a syringe and filtered using vacuum filtration with through a 0.45 mm (4.75 cm diameter) filter disk. The recovered light fraction retained on the filter disk was washed 3 times under vacuum with 0.01 M $CaCl_2$ to remove the residual NaI. The filter disk and recovered light fraction were dried at room temperature for several days. The recovered light fraction was then gently brushed from the filter disk, weighed, and transferred to a capped vial and sent to the University of Alberta Natural Resources Analytical Laboratory to measure TC and TN in the light fraction.

2.2.2.3 Phospholipid Fatty Acid Analysis

At each plot, a sterilized soil knife was inserted into the soil, and a soil sample of approximately 15cm × 3cm × 1cm was removed, placed into a whirlpack bag, and sent to the University of Alberta Soil Biogeochemistry Research Laboratory for testing. This PLFAs test procedure followed the rules in the study of Quideau (Quideau et al., 2016). In brief, freeze-dried samples were extracted with a modified Bligh and Dyer extractant, using PC(19:0/19:0) nonadecanoate as a surrogate standard. Lipids were separated and purified using solid phase extraction (SPE) silica columns, followed by alkaline methanolysis to form fatty acid methyl esters (FAMEs). Identification and quantification of FAMEs was achieved with an Agilent 6890N Series capillary gas chromatograph coupled to a FID detector, and the Sherlock Microbial Identification System Version 6.3 (MIDI, Inc., Newark, DE).

2.2.3 SPSHI equation development

Development of a site-specific SPSHI equation in this study followed the methods described in Fine et al., 2017; Parra-González and Rodriguez-Valenzuela, 2017. All measured soil health indicators were transformed into Z scores (subtraction of the mean and division by the standard deviation) and then a PCA was performed using the prcomp function in the package “stats” in R Version 4.0.2. A scree test was used to screen out the soil health indicators accounting for most of the variability in the entire dataset. Only the principal components (PC) that explained ≥ 5% of the total variance with eigenvalues > 1 were selected for the next step.

In each selected PC, the indicator with the highest absolute value (A1) and those with a difference of 0.01 or less with A1 was first reserved. Then, Pearson correlation analysis was performed between every two indicators. When the absolute value of the Pearson Correlation coefficient is larger than 0.5, the correlation was considered high, and the indicator with the higher absolute value was reserved as the “Critical” indicator in each analysis pair. When the correlation was low, both indicators in the analysis pair were reserved as “Critical” indicators for the SPSHI equation. In addition, there are two exceptional cases: (1) If there is only one indicator with the highest value in the selected PC and there is no similar value, then this indicator was considered to be the only “Critical” indicator in this PC for SPSHI equation. (2) If there is more than one A1 in the selected PC, they were both reserved as “Critical” indicators if they were not highly correlated with each other. If they were highly correlated, the choice of which indicator to preserve was determined according to which indicator resulted in the maximum correlation between the SPSHI and crop yield or total crop N recovery. Combining with the result of PCA, the SPSHI equation can be generated [Equation 7].

$$SPSHI = (\frac{p_1}{x} * PC_1[\mathrm{SI}_1 + \ldots + SI_x] + \frac{p_2}{x} * PC_2[\mathrm{SI}_1 + \ldots + SI_x] + \cdots + \frac{p_n}{x} * PC_n[\mathrm{SI}_1 + \ldots + SI_x]) / \sum_1^n p_i \quad [7]$$

In which p_n is the percentage of explained variance for the nth principal component in the Scree test. x means the number of “Critical” indicators in each PC. PC_n means the nth principal component in PCA, the values of PC_n. SI means standardized values of “Critical” indicators.

Prior to SPSHI estimation, the original data were standardized between values of 0 and 1 to eliminate the influence of the different value ranges. Like Fine et al. in 2017, one of three types of standardization functions were assigned to each indicator: (1) more is better, the cumulative normal distribution can be used; (2) less is better, 1 - the cumulative normal distribution can be used; (3) there is an optimum/peak value, the normal density distribution can be used.

2.2.4 Statistical Analysis

After the standardized data were substituted into the SPSHI equation to obtain specific values, scatter plots were drawn using ggplot function in the package "ggplot2" in R Version 4.0.2 to show the relationship between SPSHI and the crop/forage yield or total crop N recovery in each plot. Moreover, linear equations and coefficient of determination R^2 were displayed simultaneously.

The CASH method (Fine et al., 2017) was used to obtain CASH scores. CASH method assigns scores to four physical indicators (Wet Aggregate Stability, Available Water Capacity, Penetration Resistance 0- to 15-cm, and Penetration Resistance 15- to 45-cm), five biological indicators (contents of organic matter, Active Carbon (AC), and Autoclaved-Citrate Extractable Protein (ACE protein), Soil Respiration, and the Root Health Rating), and seven chemical properties (pH and Modified Morgan Extractable phosphorus (P), potassium (K), magnesium

(Mg), iron (Fe), manganese (Mn), and zinc (Zn)) according to the textural category of the soil (fine medium or coarse). All soil samples in this study were classified a medium soil texture. The CASH scores represent the mean of the scores for physical and biological indicators and pH, P, and K scores, and a combined score for micronutrients.

The Pearson correlation test was carried out between SPSHI values and CASH scores was also assessed in a scatter plot in R Version 4.0.2

Like PCA, NMDS is a multivariate method that allows the reduction of datasets, and NMDS is more widely applicable. NMDS is usually used to visualize the dissimilarity of microbial communities in soils or environmental factors among sites (Sun et al., 2022; Yokobe et al., 2020). NMDS plots and PERMANOVA were used together to visualize the ordination of soil health indicators involved in the SPSHI equation or CASH method among sites and indicate whether there are significant differences among treatments. NMDS was performed using the metaMDS function in the package “vegan” in R Version 4.0.2.

2.3. RESULTS AND DISCUSSION

2.3.1 Quantifying the long-term effects of rotation and fertilization on soil health using PCA and the SPSHI equation

2.3.1.1 Scree test

The Scree test in Figure 1 shows the percentage of the total dataset variance explained by each of the principal components (PCs). The first six PCs explained more than 5% of the variance individually (> 80% cumulatively) with eigenvalues > 1 (Table 3). The percentage values of the first six PCs were used as the coefficients for the SPSHI equation and accounted for 86.8% of the total variability in the raw data set.

2.3.1.2 Selection of "Critical" indicators and composing SPSHI equation

In PC1, ACE protein, TN, TC, C, and N in aggregates of 0.25~0.5 mm/0.5~2 mm/2~4 mm, AC, gravimetric moisture of composite BD, and BD were considered as important factors. Both ACE protein and TN had the highest absolute value and were correlated with each other. ACE Protein was selected as the first "Critical" indicator because it resulted in the maximum correlation between the SPSHI and crop yield or total crop N recovery. All important factors in PC1 are highly correlated with ACE protein (Table 7), which are greatly affected by N or C in soil. TC and TN are highly positively correlated in soils (E. Sheng-zhe et al., 2018) and aggregates (Pearson correlation = 0.99). In PC1, only ACE protein is chosen as the "Critical" indicator composing the SPSHI equation.

PC2 had four important factors: pH, Mg, nickel (Ni), and lead (Pb). pH had the highest absolute value, and other factors were highly correlated with it (Table 7). Thus, only pH was chosen as the “Critical” indicator for the SPSHI equation. PC3 only had one important factor, so P is this PC's “Critical” indicator. In PC4, sodium (Na) extracts by the two methods were important factors, and they were highly correlated (Table 7), so the one with the higher absolute value (Modified Morgan extract) was chosen as the "Critical" indicator. In PC5, available water holding capacity (AWHC) and the proportion of TC in aggregates (PTCA) had the highest absolute value. Because these two indicators were not highly correlated, both of them were choses as “Critical” indicators for the SPSHI equation the weight for PC5 was split evenly between them PC6 only has one important factor, so acid phosphomonoesterase (PME) was chosen as the “Critical” indicator for PC6.

To sum up, the “Critical” indicators chosen were ACE protein, pH, P, Na, AWHC, PTCA, and PME. The coefficient of "Critical" indicators in the SPSHI equation is determined by the percentage of the explained variances of the PC where they are located. PC5 has two "Critical" indicators, so their coefficient is the percentage of explained variances multiplied by 1/2. SPSHI equation is formed basing on these results [Equation 8].

$$SPSHI = (0.365 * ACE\ Protein + 0.204 * pH + 0.099 * P + 0.084 * Na + 0.032 * AWHC + 0.032 * PTCA + 0.052 * PME)/0.868 \qquad [8]$$

2.3.1.3 Standardization of data

Through standardization, the range of all data of “Critical” indicators was transformed to values between 0~1 (Figure 2). Except for Na and pH, other “Critical” indicators are considered to be “the more the better” type and standardized with the cumulative normal distribution. An example of the “more is better” standardization for ACE Protein is presented in Figure 2 (a). ACE protein is positively correlated with N. N, P, and C are beneficial and essential for plant growth (E. Sheng-zhe et al., 2018; Liu and Chen, 2014). Although Na is also an element that can be used for plant growth, it is not scarce. When the concentration of Na is too high, it is toxic to plants and may make the land salinized and reduce the soil health (Bazihizina et al., 2012). 0~10 mM Na is a low-salinity environment for plants (Bazihizina et al., 2012), and the Na concentration in this study were all above 20 mM, so it was identified as an indicator of the "less is better" type standardized with a reversed cumulative normal distribution (Figure 2 (b)). For the pH in this study, pH=6 was set as the optimal value (USDA Natural Resources Conservation Service, 1998) and values above and below scoring lower according to a scaled normal probability density distribution. The higher the distance from pH=6, the lower the normalized value (Figure 2 (c)). Standardized values of “Critical” indicators are presented in Table 4.

2.3.1.4 ”Critical” indicators and soil health

ACE protein and Potentially mineralizable nitrogen (PMN) are related to the ability of the soil to make N available for plants by mineralization (Geisseler et al., 2019). C and N can positively affect the amount of organic matter in soils, and more organic matter means better soil structure, such as higher porosity (Oades, 1984). Porosity and BD are related by porosity = 1 – BD/PD, where PD = particle density. Higher porosity may also indicate more water storage space and higher moisture. In addition, among the important factors of PC1, the size of the aggregates involved is not less than 0.25mm, which may be because most of the organic C or N is stored in macroaggregates (Atere et al., 2020; Liu et al., 2018). AWHC is one of the vital soil hydraulic properties, especially in the water-constrained environment (Wang et al., 2017). PME has become an important biochemical soil health indicator in recent years, and it is mainly affected by soil organic matter and pH (Kiboi et al., 2018; Wade et al., 2021). Soil organisms secrete catalytic enzymes that participate in various biochemical reactions in soil, such as PME, N-acetyl β -glucosaminidase, and Arylsulfatase.

2.3.1.5 Quantification of soil health by SPSHI equation

Standardized values of "Critical" indicators (Table 4) were put into the SPSHI equation, and SPSHI values of each treatment were shown in Table 5. More than half of the "Critical" indicators are closely related to C or N. TN, or ACE protein accounted for most of the total variability (36.5%) among all "Critical" indicators. Therefore, it can be said that most of the difference in SPSHI is the difference in C or N input of various treatments in soils. According to the SPSHI values, conventional farming (WF rotation without fertilizer) has the lowest soil health index. In contrast, 8-yr rotation with manure has the best soil health index. Both legumes and fertilizer applications in rotation can increase N or soil organic matter, so this result is expected. For the two complex rotations, the SPSHI value of 8-yr (0.802) is higher than that of WOBHH (with manure, 0.674), suggesting that the higher frequency of legumes planting in rotations could increase N or C input and organic matter supplement in soils. This is similar to the results of a previous study in 1999 (Angers et al., 1999), in that the more frequent the legumes, the more C input over a long period. Grass hay has been used in the WOBHH rotation to increase crop diversity, which is not used in the 8-yr rotation. Grass hay can also improve C input, but much less than legumes (Angers et al., 1999). Compared to complex rotations, WF has no extra C input excepting a little from crop residues.

Without fertilizers, WOBHH (0.418) has higher soil health index than WF (0.380). However, when manure is applied, WOBHH (0.674) has a similar SPSHI value to WF (0.686). The Manure significantly compensated for the nutrients in the WF rotation. Without fertilizers, the ACE protein of the WOBHH rotation is 9 times more than the WF rotation. By contrast, with manure application, the ACE protein of the WOBHH rotation is only 1.4 times more than the WF rotation. Meanwhile, WF has less soil disturbance, resulting in better retention of the aggregates and the C and organic matter in the soil (Sarker et al., 2018).

In 2018, a long-term study in China showed that both manure and inorganic fertilizers could improve the content of C and N in soils, and manure has greater benefits (Gai et al., 2018). In this study, manure has the highest soil health index for the same rotation type (WF or WOBHH), followed by NPKS and Check. Under the NPKS treatment, CG has the highest soil health index, followed by WOBHH, WF, and CF. The influence of NPKS or manure on the relationship between SPSHI values of WOBHH and WF is different.

CG and CF both grow similar crops continuously. Continuous grain cropping was reported degrading soil structure and fertility (Dou et al., 2017; Zou et al., 2018). CF involves both grass hay and legume hay. Although the usage of legumes and grass hay in crop rotations was reported to enhance C input (Angers et al., 1999), in this study, CF (0.389) has lower soil health index than CG (0.507) when NPKS is applied. This suggests that N input is not the most significant factor in the difference between the two rotations. As shown from table 3, CF only has ACE protein, AWHC, and PME higher than CG, and the difference in ACE protein is slight. The pH, P, Na, and PTCA of CF were lower than those of CG, especially the difference in PTCA or Na was huge. Balanced chemical fertilizer can improve soil physical structure in continuous grain cropping (Zhang et al., 2006). As an essential part of soil structure, stable aggregates are the primary storage place for C or N (Zhang et al., 2021). When NPKS increases the content of stable aggregates in CG, it may also increase PTCA. However, NPKS did not have this benefit in CF, which may be due to the type of plants. A similar situation was observed in comparing CG and WOBHH when NPKS was applied (Table 4, Table 5). The PTCA and pH of WOBHH were greatly lower than those of CG (Table 4). Grass hay and legume hay are involved in both CF and WOBHH, increasing C input, and C is a critical factor of aggregates forming and stability (Oades, 1984). However, grass hay has minimal improvement in C input (Angers et al., 1999) and legume hay only significantly improve aggregate stability when the high concentration of manure is applied (Hurisso et al., 2013). Using conventional cropping (WF with NPKS) as the evaluation criteria, CF reduced the optimum values of pH and Na less, increased rather than decreased PTCA, compared with WOBHH and CF (Table 4). Although the soil health index of CG is better than that of complex rotation when NPKS is applied, crop diversity can improve crop yield and prevent infections of some diseases from monocropping (Tounkara et al., 2020). When other treatments are the same, the application of lime improves the soil health index, especially when NPKS is applied, which is because lime can improve pH and soil physical properties (Sojka et al., 2005).

2.3.2 Comparison between SPSHI values and CASH scores

CASH scores are also presented in Table 5. The CASH texture of all sites in the Breton Plots is medium; thus, all treatment has similar scores for this part. According to correlation analysis in Table 8, CASH scores are greatly impacted by scores of carbon dioxide of microbial 96 h-respiration (CO2-96 hour, stands for microbial biomass), AC, ACE protein, WSA, and organic matter. The correlation between SPSHI values and CASH scores is highly positive (Pearson correlation = 0.75, Table 7), and the correlation can be explained by 56% (Figure 3).

The distribution of soil CASH scores under different treatments is partly consistent with SPSHI values, and there are also some differences. In WOBHH rotations, NPKS has a lower CASH score than Check. NPKS increased ACE protein and organic matter but negatively impacted microbial biomass and AC. A study in 2021 by Saini et al. showed that the application of lime and potash improved microbial biomass and increased AC consumption by microorganisms (Saini et al., 2021). However, this is partly the opposite of our results. For WOBHH rotations, NPKS reduced both microbial biomass and AC, but when lime was applied, NPKS improved the two indicators (Table 6). Similar to the results of Saini et al., in WOBHH without fertilizer, after receiving lime, microbial biomass and AC are negatively related. The coexistence of these two phenomena may be because treatments increase the ratio of microorganisms and AC differently. When using both fertilizer and lime in complex rotation, the increase in AC is much more than its consumption by increased microbial organisms. Under the NPKS treatment, CF has the highest CASH score, followed by CG, WOBHH, and WF. This was because the CO2-96 hour, AC, and organic matter of CF were much higher than those of the other three rotations (Table 6), indicating that planting legume hay and grass hay increased the input of soil nutrients and microbial biomass. Similar to the situation in SPSHI "critical" indicators, CG is the highest for improving aggregate-related properties (WAS). Massive roots of forages may contribute to the increase in micro-aggregate content and the decrease in WSA, which can be improved by a short fallow period (Udom and Omovbude, 2019). Under the manure treatment, WOBHH has a higher CASH scores than WF, which is consistent with our hypothesis because the complex rotation can provide more N inputs.

The CASH method includes more chemical indicators than the SPSHI equation, such as Mn, Fe, Mg, and K. The availability of these elements is greatly affected by pH (USDA Natural Resources Conservation Service, 1998). If no soil amendment is applied, no fertilizer treatment or manure treatment has a more optimal pH value than NPKS treatment (Table 4). It was also reported that the long-term application of manure could maintain pH while inorganic fertilizer decreased pH (Ozlu and Kumar, 2018). The presence of carbonates and bicarbonates and carboxyl and phenolic hydroxyl in manure can work as a pH buffer (Liang et al., 2012). To sum up, the CASH method is greatly affected by both C or N input and pH.

2.3.3 Linking quantified soil health with crop yield or crop N recovery

Correlation analysis showed that both SPSHI values and CASH scores were positively correlated with crop yield or crop N recovery (Table 7, Figure 4). In the linear fitting of the correlation between soil health and crop outcome, the results can be summarized as follows: (1) crop N recovery performs better than crop yield (Figure 4 (a),(b)); (2) outcome of total biomass performs better than the biomass only above ground (Figure 4 (b), (c)); (3) SPSHI values perform better than CASH scores (Figure 4 (b), (d)). As an indicator of crop outcome, crop N recovery is less affected by different crop types than crop yield. Soil health affects crops, including the part above the ground and the roots. Due to the influence of regional climate, soil type, and others, the soil health indicators chosen by the CASH method may not be the most suitable for quantifying the soil health in the Breton Plots and its correlation with crop outcome. PCA and SPSHI equation developed a more specific and appropriate method to quantify the soil health of the Breton Plots.

The correlation between SPSHI values and crop yield or crop N recovery exists, but it is not strong. This phenomenon may be caused by the inconsistent changes in SPSHI values and crop yield/crop N recovery under different fertilizer treatments in the same crop rotation group (Table 5, Figure 4). According to SPSHI values, manure led to higher soil health than NPKS when other treatments were the same. However, in the WOBHH rotation, manure and NPKS have similar crop yield/crop N recovery. In the WOBHH rotation with lime addition, NPKS has a higher crop yield/crop N recovery than manure. NPKS has a much higher crop yield/crop N recovery than manure in the WF rotation, which is particularly evident in Figure 4 (a).

Although manure can improve many soil health indicators or properties, its influence on crop yield is not always consistent with the improvement. The different effects of manures on crop yield may be due to the interactions between crop types and the environment (Wankhede et al., 2021), or the interaction between chemical fertilizer and manures is much greater than their respective effects on crops. The yield gained by manure addition for maize may be much more than that of wheat, oats and, soybeans (Barth et al., 2021; Gai et al., 2018; Zhang et al., 2021). For crops like wheat, it was reported that manure could improve more in soil properties while chemical fertilizers can improve more in crop yield, especially when single cropping (Jiang et al., 2018). This is similar to the result of this study. In Figure 4 (a), although the SPSHI value of WF rotation with NPKS is relatively low among all treatments, its crop yield is very high, which exceeds almost all treatments. Complex rotations can reduce the difference to some extent. In WOBHH rotations, NPKS also greatly increased crop yield, but in the end, it was only similar to the crop yield under manure treatment.

2.3.4 Visualize, statistical analysis, and compare the long-term effects of various treatments on multiple soil health indicators

Figure 5 (a) visualizes the “Critical” indicators applied in the SPSHI equation in multivariate NMDS space. The stress of NMDS analysis is 0.04, so the ordination fit is “good”. Significant effects exist in these soil indicators under fertilizer treatments (p-value = 0.0064), rotation treatments (p-value = 0.0482), and the interaction of rotation and fertilizer treatments (p-value = 0.0095). The directivity of "Critical" indicators in SPSHI is distributed around. CF rotation with NPKS is in a central position. The dispersion of each treatment is similar to the quantified soil health. NPKS treatment greatly impacts P, and manure has a great impact on ACE protein and PTCA. Check, and manure have a better effect on pH than NPKS. For soil with organic or inorganic fertilizers, lime can effectively improve soil properties, but the effect of lime is negligible for soil without fertilizers.

Soil health indicators of the CASH method are visualized in Figure 5 (b). Some indicators in the CASH methods adopt the direct scoring method, so they are not shown in the figure. The stress of NMDS analysis is 0.04, so the ordination is good fitted. There is a significant effect under fertilizer treatments (p-value = 0.0031). Indicators in the CASH method are mainly lateral separation. The horizontal differences of various treatments are explained in 3.3.1 and are mainly influenced by the indicators to the left in Figure 5 (b).

Analyses of NMDS plots and PERMANOVA of the SPSHI and CASH indicators convey different messages. However, all soil health indicators came from the same sampling land. This suggests that crop rotation types, fertilizer types, or their interactions all significantly affect soil health properties.

2.4. CONCLUSION

Based on quantified soil health values, soil health improvement by management measures is mainly determined by the input of nutrients in the long term. Long-term application of both organic and inorganic fertilizers could improve soil health in the same rotation, but the improvement of manure was more significant than that of NPKS. In the same fertilizer treatment, long-term application of 8-yr, WOBHH, and CG rotations increased soil health while CF rotation decreased, even though CF rotation enhanced the C or N input. This may be because forage plants have fewer benefits on aggregate-related properties than crops. Increasing the planting frequency and diversity of legumes in complex rotations can improve various soil properties and soil health. Although CG rotation alone seems to have the possibility of soil degradation, the application of NPKS fertilizer can significantly make up for this harm so that the soil health of CG rotation was even higher than that of WOBHH rotation. The biggest reason may be that CG rotation with NPKS has high PTCA and more optimum soil pH. On the contrary, under NPKS treatment, CF rotation reduced a lot of PTAC, so when compared with conventional cropping, CF reduced soil health.

When correlated with crop yield or crop N recovery, the improved SPSHI equation outperformed the CASH method in quantifying soil health in the Breton plot. When SPSHI values were linked with crop yield or crop N recovery, the correlation strength was weak to medium because soil health improvement is not necessarily proportional to crop yield and quality. In the case of Gray Luvisols in Alberta, manure could significantly improve soil health but not crop yield, while

NPKS fertilizer could significantly improve crop yield but not soil health. The visualization results suggest that crop rotation types, fertilizer types, or their interactions in the long term all have significant effects on soil health properties.

For sustainable development and efficiency, combining organic fertilizer and balanced chemical fertilizer is the best way. If sufficient additional nutrients are available, CG is better than WF when crop residue remains in soils. The combined application of fertilizers and complex rotation can be the best way to improve soil health and crop yield when the frequency of legumes in the rotation is high enough. In addition, it is better to choose the crop legume rather than the forage legume for complex rotations.

The limitation of this study is the shortage of samples from different areas. If possible, soil samples under similar long-term agricultural management in Gray Luvisol in other areas should be analyzed together to see if there are differences in results.

Table 1. Description of treatments

Experiment	Rotation	Fertility Treatment	Current nutrient Application Rates: N-P-K-S (kg ha-1)	Nutrient Sources	On-site Plot[a] Number	Additional information available from
Classical Plots	2-yr wheat (*Triticum aestivum L.*) – fallow **(WF)**	Check (no fertilizer)	0-0-0-0	NA	E5	Dyck et al. 2012; Dyck and Puurveen 2020
		NPKS	90-22-46-20	N[b]: Urea; P[c]: phosphate; K[c]: potash; S[d]: elemental sulfur	E2	
		Manure	—	Locally sourced, composted cattle manure with straw bedding [e]	E3	

	5-yr WOBHH **w**heat (*T. aestivum L.*) – **o**ats (*Avena sativa*) – **b**arley (*Hordeum vulgare L.*) – alfalfa (*Medicago sativa*) – brome (*Bromus tectorum*) **h**ay **(WOBHH)**	Check (no fertilizer)	0-0-0-0	NA	F5E (with lime); F5W (without lime)	
		NPKS	__-22-46-20	N[b]: Urea; P[c]: phosphate; K[c]: potash; S[d]: elemental sulfur	F3E (with lime); F3W (without lime)	
		Manure	__	Locally sourced, composted cattle manure with straw bedding [e]	F2E (with lime); F2W (without lime)	

Hendrigen Plots	Continuous forage - red fescue (*Festuca rubra L.*), tall fescue (*Festuca arundinacea Schreb.*) and white "Dutch" clover (*Trifolium repens L.*) **(CF)**	NPKS		N: N-fixing legumes; P: phosphate; K: NA; S: elemental sulfur	B14[f]	Dyck et al. 2012; Ross et al. 2008
	Continuous grain – barley (*Hordeum vulgare L.*) **(CG)**	NPKS	90-22-46-20	N: Urea; P: phosphate; K: potash; S: elemental sulfur	B15	
	8-yr "agro-ecological" (8-yr) rotation – cereal (barley, *Hordeum vulgare L.*) – cereal (barley, *Hordeum vulgare L.*) – feba bean (*Vicia faba*) –	Manure	__-22-46-20	Locally sourced, composted cattle manure with straw bedding [g]	B16	

	cereal (barley, *Hordeum vulgare L.*) /brome (*Bromus tectorum*) – alfalfa (*Medicago sativa*) /brome (*Bromus tectorum*) – alfalfa (*Medicago sativa*) /brome (*Bromus tectorum*) – alfalfa (*Medicago sativa*) /brome (*Bromus tectorum*) – alfalfa (*Medicago sativa*) /brome (*Bromus tectorum*) **(8-yr)**					

[a]**:** "Plot" refers to the experiment's initial design (Dyck et al. 2012) and is aligned with the fertility treatments' physical position as the Breton Plots.

[b]: N (applied as urea) rate depends on the crop and its place on rotation: wheat after forage (50 kg N ha−1), oat or barley after wheat (75 kg N ha−1), barley under seeded to hay: 50 kg N ha−1, and legume–grass forages: 0 kg N ha−1.
[c]: Rates are the rates of the nutrient element rather than P_2O_5 and K_2O convention. Phosphorus (P) is applied as triple super phosphate (0-46-0), and potassium (K) is applied as muriate of potash (0-0-62).
[d]: S is applied as elemental S at a rate of 5.5 kg ha−1 from 1980 to 2007 and 20 kg ha−1 from 2007 to present.
[e]: Composted cattle manure with bedding straw incorporated; N application via manure depends on crop rotation, i.e., wheat–fallow: 90 kg N ha−1 during cropped years, and cereal crops in wheat–oat–barley–hay–hay rotation: 175 kg N ha−1 every 5-year applied in two equal applications. Actual manure rate is calculated using % TN measured on composite samples.
[f]: The system is based on nitrogen supply through N-fixing legumes (white clover) and low amounts of added P and S fertilizers.
[g]: Composted cattle manure with bedding straw incorporated, the manure rates are calculated using the assumption that grazing animals would return 70% of the nitrogen they consume in the forage as manure.

Table 2. Description of the double exponential model parameters (Dexter et al., 2008)

Parameter	Description	Parameter	Description
$\boldsymbol{\theta_v}$	Soil volumetric water content (cm^3/cm^3)	A_2	Matrix porosity
C	Residual porosity	h_1	Suction to empty structural pores (hPa)
A_1	Structural porosity	h_2	Suction to empty matrix pores (hPa)

Table 3. Eigenanalysis and eigen values for the first six principal components of the 168 soil health indicators in the dataset

	PC1	PC2	PC3	PC4	PC5	PC6
Eigenvalue	**61.3**	**34.4**	**16.7**	**14.1**	**10.8**	**8.7**
Indicator						
ACE Protein	**-0.125**	-0.006	0.016	-0.004	-0.021	0.030
TN	**-0.125**	-0.008	-0.017	-0.044	-0.004	-0.006
TN mass Mg ha	**-0.124**	-0.017	-0.002	-0.019	0.032	-0.034
TC	**-0.122**	-0.003	-0.029	-0.048	0.024	-0.021
% TN 2	**-0.120**	-0.028	-0.001	-0.022	-0.076	-0.004
a_act_c	**-0.119**	-0.037	-0.007	-0.045	0.039	-0.038
a_gwc_wet	**-0.119**	0.035	-0.020	0.038	-0.027	0.028
% TN 4	**-0.119**	-0.029	0.017	0.005	-0.083	-0.028
TC mass Mg ha	**-0.118**	-0.010	-0.018	-0.021	0.062	-0.051
% TC 2	**-0.118**	-0.032	0.002	-0.035	-0.080	-0.012
TN mass in total aggs	**-0.116**	-0.050	0.017	-0.005	-0.073	-0.030
% TN 0.5	**-0.116**	-0.046	0.008	-0.034	-0.077	0.008
% TC 4	**-0.116**	-0.034	0.016	-0.016	-0.095	-0.026
PMN	**-0.116**	-0.024	-0.066	-0.016	0.012	0.034
TC mass in aggs 2	**-0.115**	-0.005	-0.032	-0.011	-0.085	-0.004
TN mass in aggs 2	**-0.115**	0.000	-0.035	0.003	-0.079	0.002
Bulk Density	**0.115**	-0.039	0.043	0.046	-0.030	-0.054
pH	0.023	**-0.154**	-0.054	0.007	-0.001	0.050
d_icap_mg	-0.036	**-0.150**	0.011	-0.031	0.076	-0.022
a_ni	0.017	**0.144**	0.093	-0.082	0.026	-0.022
a_pb	-0.004	**0.144**	0.056	-0.067	0.091	0.007
b_p_m3	-0.039	-0.010	**0.212**	-0.067	0.001	-0.068
a_na	-0.056	0.052	-0.023	**-0.195**	0.038	-0.008
b_na_m3	-0.021	0.086	-0.042	**-0.190**	0.082	0.054
Prop TC in aggs	-0.050	-0.080	0.040	-0.004	**-0.215**	0.003
a_awhc	-0.018	-0.045	-0.030	0.096	**0.225**	-0.140
DTC soil aggs	-0.058	0.050	-0.052	-0.009	**0.215**	-0.048
DTN soil aggs	-0.070	0.054	-0.036	-0.035	**0.208**	-0.022
Phosphomonoesterase	-0.055	-0.043	-0.028	-0.007	0.015	**0.228**

This table only presents variables with the highest absolute value (±0.01). Soil properties measured in this study are bolded in the Indicator column. Important factors in each PC are bolded.

Abbreviations: ACE protein, Autoclaved-citrate extractable soil protein; TN, total nitrogen; TC, total carbon; % TN/TC 0.5/2/4, parentage of total nitrogen/carbon in aggregates of 0.25~0.5 mm/0.5~2 mm/2~4 mm; a_act_c, active carbon; a_gwc_wet, gravimetric moisture of composite bulk density as sampled; aggs, aggregates; PMN, potentially mineralizable nitrogen; aggs 2, aggregates of 0.5~2 mm; d_icap_mg, H3A extract magnesium (Mg); a_ni/pb/na, Modified Morgan extract Nickel (Ni)/Plumbum (Pb)/Sodium (Na); b_p/na_m3, Mehlich 3 extract Phosphorus (P)/Sodium; Prop, proportion of; a_awhc, available water holding capacity; DTC/DTN, carbon/nitrogen mass in non-water stable aggregates (or non-aggregated soil); Phosphomonoesterase, acid phosphomonoesterase.

Table 4. Standardized values of "Critical" indicators in site-specific soil health index (SPSHI) equation under each treatment (See Tables 1 for management history and treatment explanations). -L means lime addition.

Plot	Fertilizer	Rotation	ACE protein	pH	P	Na	AWHC	PTCA	PME
B14	**NPKS**	**CF**	0.49	0.41	0.36	0.004	0.49	0.08	0.39
B15	**NPKS**	**CG**	0.44	0.65	0.67	0.422	0.31	0.88	0.11
B16	**Manure**	**8-yr**	0.96	0.85	0.91	0.158	0.08	0.99	0.64
E2e	**Manure**	**WF**	0.55	0.90	0.95	0.543	0.98	0.50	0.46
E3e	**NPKS**	**WF**	0.08	0.77	0.55	0.893	0.48	0.36	0.13
E5e	**Check**	**WF**	0.03	0.92	0.15	0.761	0.08	0.54	0.67
F2w	**Manure**	**WOBHH**	0.74	0.65	0.19	0.899	0.81	0.63	0.79
F3w	**NPKS**	**WOBHH**	0.51	0.12	0.90	0.471	0.25	0.25	0.33
F5w	**Check**	**WOBHH**	0.27	0.74	0.13	0.713	0.94	0.08	0.17
F2e	**Manure**	**WOBHH-L**	0.92	1.00	0.20	0.417	0.65	0.63	0.98
F3e	**NPKS**	**WOBHH-L**	0.72	0.50	0.73	0.527	0.41	0.22	0.91
F5e	**Check**	**WOBHH-L**	0.32	0.97	0.12	0.555	0.33	0.64	0.18

Abbreviation: ACE protein, autoclaved-citrate extractable soil protein; P, phosphorus; Na, sodium; AWHC, available water holding capacity; PTCA, proportion of total carbon in aggregates; PME, acid phosphomonoesterase.

Table 5. Site-specific soil health index (SPSHI) values, comprehensive assessment of soil health (CASH) scores, crop yield and crop nitrogen (N) recovery in soils under each treatment (See Tables 1 for management history and treatment explanations). -L means lime addition.

Plot	Fertilizer	Rotation	SPSHI values	CASH scores	Crop yield	Crop N recovery
B14	**NPKS**	**CF**	0.389	80.80	4695.6	68.1
B15	**NPKS**	**CG**	0.507	70.66	6438.8	107.4
B16	**Manure**	**8yr**	0.802	88.10	10054.1	168.6
E2e	**Manure**	**WF**	0.686	71.25	5635	77.9
E3e	**NPKS**	**WF**	0.403	59.83	9287.4	109.3
E5e	**Check**	**WF**	0.380	49.83	2636.7	27.8
F2w	**Manure**	**WOBHH**	0.674	85.68	7489.3	128.3
F3w	**NPKS**	**WOBHH**	0.432	60.47	7506.3	121.6
F5w	**Check**	**WOBHH**	0.418	72.86	3952.5	58
F2e	**Manure**	**WOBHH-L**	0.790	95.22	8339.1	132.9
F3e	**NPKS**	**WOBHH-L**	0.633	76.05	9129.3	148.8
F5e	**Check**	**WOBHH-L**	0.477	74.36	4122.8	58.2

Table 6. Scores of indicators highly correlated with CASH scores (See Tables 1 for management history and treatment explanations). -L means lime addition.

Plot	Fertilizer	Rotation	CO2-96 hour	AC	ACE Protein	WSA	Organic matter
B14	**NPKS**	**CF**	95	73	49	64	99
B15	**NPKS**	**CG**	34	49	44	88	38
B16	**Manure**	**8-yr**	77	94	96	88	84
E2e	**Manure**	**WF**	34	84	55	55	55
E3e	**NPKS**	**WF**	10	10	8	4	12
E5e	**Check**	**WF**	1	2	3	2	4
F2w	**Manure**	**WOBHH**	78	66	74	73	61
F3w	**NPKS**	**WOBHH**	39	21	51	45	40
F5w	**Check**	**WOBHH**	57	30	27	44	29
F2e	**Manure**	**WOBHH-L**	85	85	92	84	84
F3e	**NPKS**	**WOBHH-L**	58	62	72	27	46
F5e	**Check**	**WOBHH-L**	49	39	32	59	34

Abbreviation:CO2-96 hour, carbon dioxide of microbial 96h-respiration ; AC, acitive carbon; ACE protein, autoclaved-citrate extractable soil protein; WSA, water-stable aggregates.

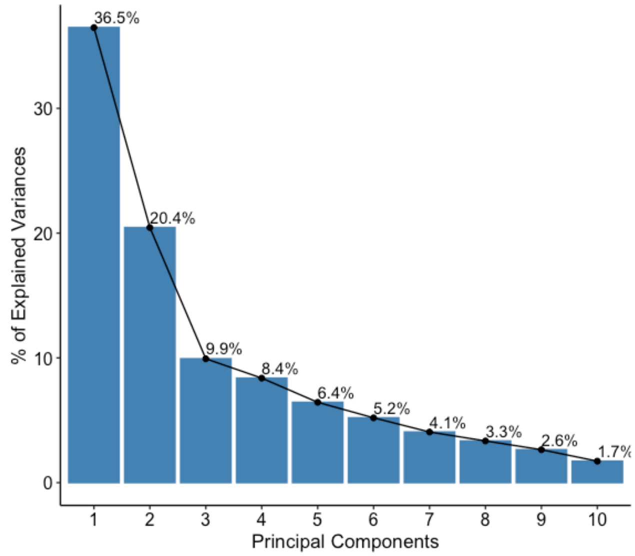


Figure 1. Scree test for PCA

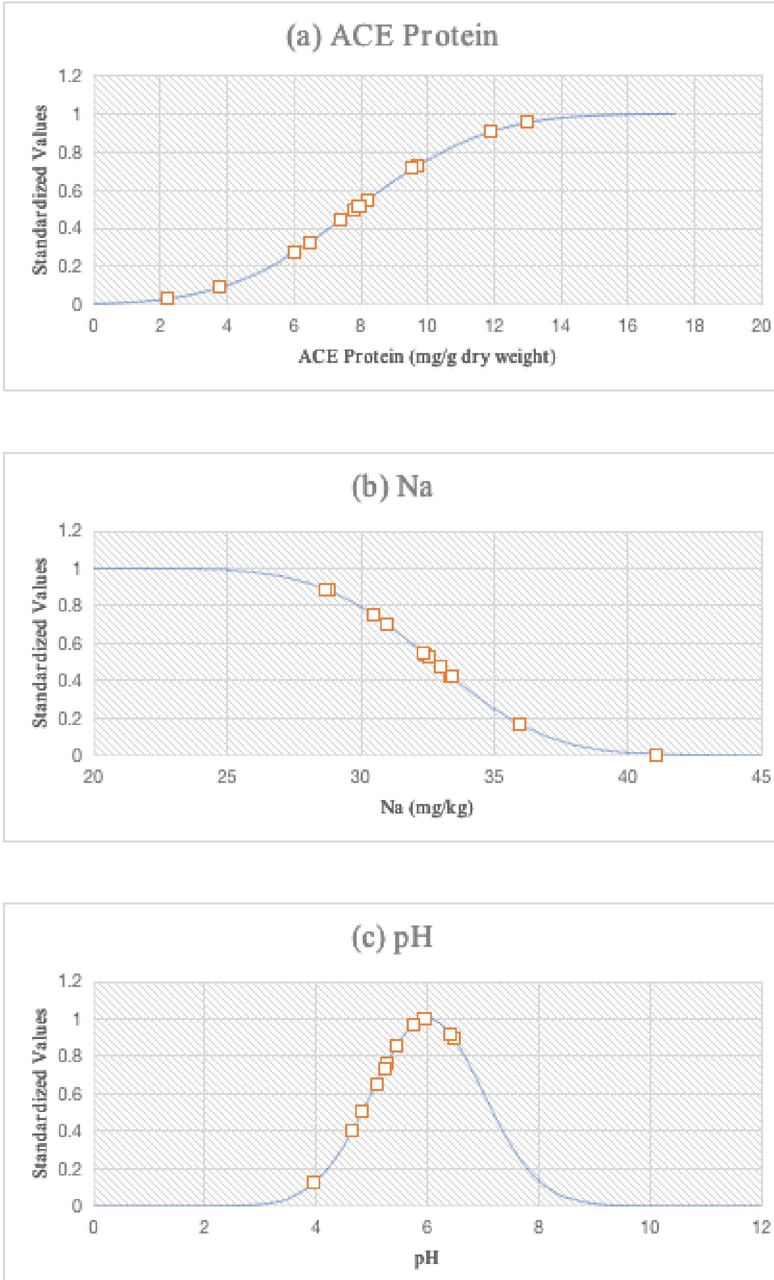


Figure 2. Standardized function of "Critical" indicators. (a) Standardization of autoclaved-citrate extractable soil protein (ACE Protein), representing "the more the better" type. (b) Standardization of sodium (Na), representing "the less the better" type. (c) Standardization of pH, representing "optimum value" type. The x value of each point on the figure represents the original measured value of this indicator in each treatment, and the corresponding y value is its standardized value.

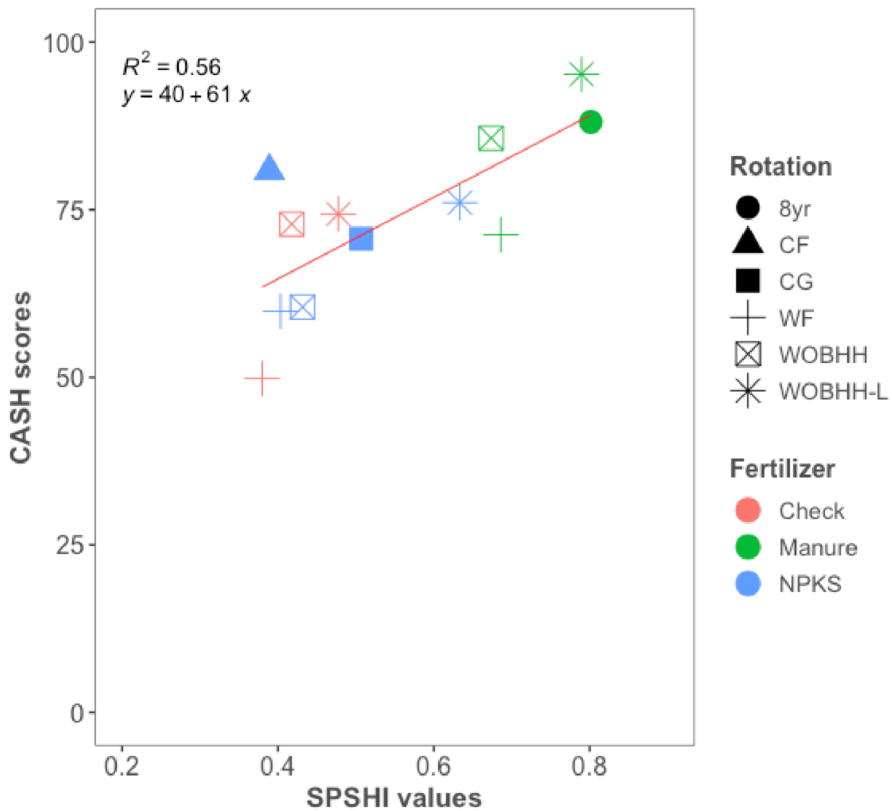


Figure 3. The positive correlation between site-specific soil health index (SPSHI) values and comprehensive assessment of soil health (CASH) scores (See Tables 1 for management history and treatment explanations). -L means lime addition.

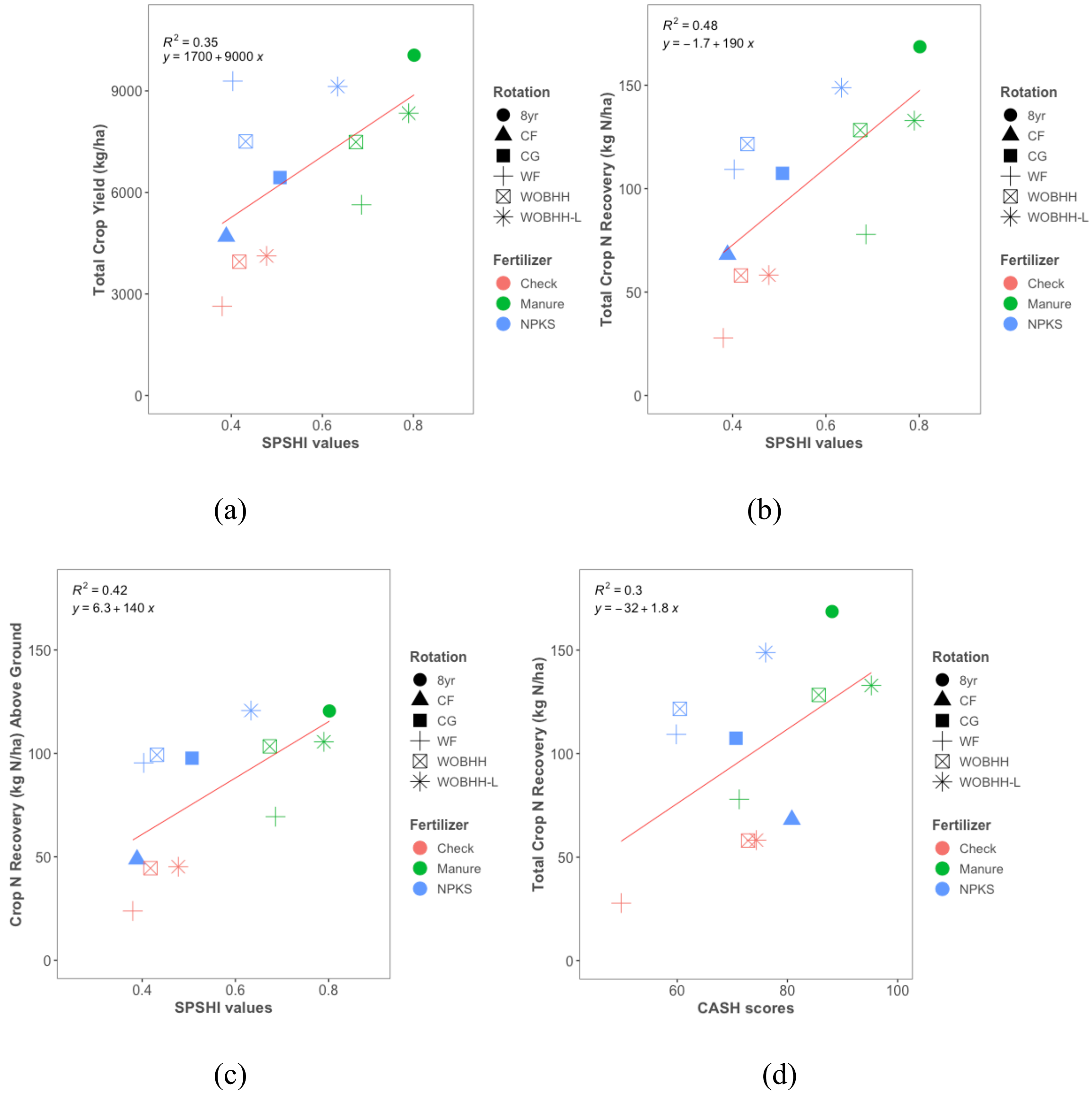
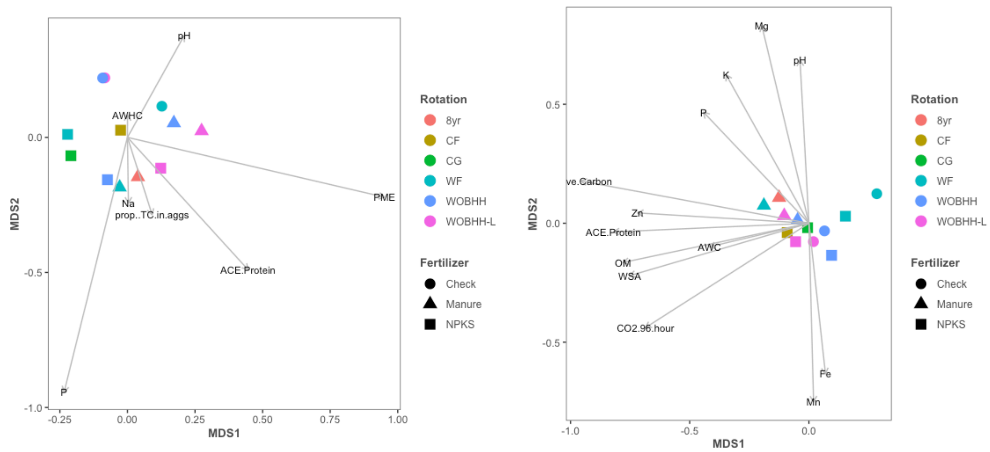


Figure 4. Correlation between qualified soil health and crop yield or crop nitrogen (N) recovery under each treatment (See Tables 1 for management history and treatment explanations). -L means lime addition. (a) Correlation between site-specific soil health index (SPSHI) values and average annual crop yield-total (the whole plant including roots). (b) Correlation between SPSHI values and average annual crop N recovery-total (the whole plant including roots); for wheat-fallow (WF) rotation, NPKS has lower SPSHI values and higher crop N recovery-total than manure. (c) Correlation between SPSHI values and average annual crop N recovery-above ground. (d) Correlation between comprehensive assessment of soil health (CASH) scores and average annual crop N recovery-total. According to R^2, average annual crop N recovery-total fits better than average annual crop yield-total and average annual crop N recovery-above ground; SPSHI values fit better than CASH scores.



(a) (b)

Figure 5. Non-metric multidimensional scaling (NMDS) analysis of indicators in site-specific soil health index (SPSHI) equation and comprehensive assessment of soil health (CASH) method (See Tables 1 for management history and treatment explanations). (a) NMDS analysis of soil health indicators in SPSHI equation (see table 4 for indicators explanation); NPKS has great impacts on phosphorus (P); manure has great impacts on proportion of total carbon in aggregates (prop TC in aggs) and autoclaved-citrate extractable soil protein (ACE protein); there are significant differences under rotation treatments (p-value = 0.0482), fertilizer treatments (p-value = 0.0064) and their interaction effects (p-value = 0.0095). (b) NMDS analysis of soil health indicators in CASH method (see table 6 for indicators explanation); treatments mainly affected the indicators to the left; there are only significant differences under fertilizer treatments (p-value = 0.0064. -L in the legend means the addition of lime, treatment with lime addition does not mean a difference in crop rotation type, so it is only labelled in NMDS graphs for differentiation and is not used in Permutational multivariate analysis of variance (PERMANOVA).

Ⅲ. SUMMARY

3.1 RESEARCH SUMMARY

The overall objective of this study is to assess the long-term effects of various rotations and fertilization on soil health in Alberta. Chapter 2 explored this objective.

The first step was to measure soil properties not included in Soil Heath Institute (SHI) North American Project to Evaluate Soil Health Measurements (NAPESHM)'s shared data through experiments. The measurement of samples was comprehensive, involving physical properties: soil moisture retention curve (SMRC) and related properties, biological properties: phospholipid fatty acids (PLFAs), and chemical properties: carbon (C), nitrogen (N), light fraction carbon (LFC), light fraction nitrogen (LFN), and in aggregates.

The second step was setting up the site-specific soil health index (SPSHI) equation and obtaining modified SPSHI values for each treatment. "Critical" indicators were selected through principal component analysis (PCA). Based on "Critical" indicators and the result of the Scree test, the SPSHI equation was established. Then the "Critical" indicators are given different function distributions and standardized values according to their characteristics. Finally, the standardized values of "Critical" indicators were put into the SPSHI equation to obtain the SPSHI values.

The third step was comparing and analyzing SPSHI values among treatments. Linking the ranking of SPSHI values to each "Critical" indicator and previous studies explained many mechanisms behind the results.

The fourth step analyzed the correlation between SPSHI and crop yield or crop nitrogen recovery.

The final step was to visualize "Critical" Indicators through Non-metric multidimensional scaling (NMDS) analysis to show the differences between processes. The visualized results show more about how crop rotation and fertilizers interact.

In addition, SPSHI values were compared with the Cornell comprehensive assessment of soil health (CASH) scores from the third to last step. The former was more strongly correlated with crop yield or crop nitrogen recovery for the Breton Plots. SPSHI also reflected more comprehensive treatments effects on soils than CASH.

3.2 POSSIBLE IMPLICATIONS

The results from the Breton Plots were consistent with the meta-analysis result based on 20 long-term experiments across China in 2018. Within a given rotation manure additions improved soil health more than chemical fertilizers, but chemical fertilizers could improve crop yield significantly more than manure. This phenomenon was evident in the conventional 2-year rotation. However, the gap in crop yield between manure and fertilizer was reduced in complex rotations. This suggests that it is best to use a mixture of organic and inorganic fertilizers for rotations with only annual grain crops to improve soil health while increasing crop yield. For complex rotations, if only one fertilizer is applied, manure is more suitable than chemical fertilizer. Although the yield of manure is slightly lower than that of chemical fertilizer, it has dramatically improved the soil health, conducive to sustainable development.

In general, complex rotations including legumes with higher frequency and more diversity improved soil health and crop yield more than rotations with continuous monocropping. Forage (including legumes and grasses hay) has been used to improve soil nutrients in rotations. However, it should be noted that forage roots tend to miniaturize soil aggregates in continuous fescue/clover cropping, which reduces soil structure to some extent. In addition, grass hay improved soil health much more minor than legumes, Therefore, legumes are more recommended to increase crop diversity in rotations. Continuous monocropping is better than conventional rotation for maintaining soil health and improving yield as long as there are sufficient supplemental nutrients.

This study's modified soil health quantification method can be applied :(1) when a large amount of data is available; (2) want to carry out regional quantification more accurately. As external factors such as climate are relatively consistent, when applying the SPSHI method, the SPSHI value of this paper may be used as a reference for Gray Luvisol in Alberta.

SPSHI values can also be correlated with greenhouse gas emission values to know the possible impact of various management practices on the environment or climate. However, because SPSHI is mainly affected by the content of nutrients (nitrogen), the greenhouse gases here may be limited to nitrogen related, such as nitrous oxide.

3.3 PROJECT LIMITATIONS

A primary limitation of this study is the lack of sample duplication. It is better to measure the same type of soil under similar climates and similar treatment in different areas. Long-term soil experiments are hardly all in one area and managed by the same organization. So when samples are from different locations, there will always exist differences. Due to practical reasons, we cannot collect qualified soil samples from other regions.

The specific value of root health properties in the CASH method was missing, so it may affect the correlation analysis of various properties in CASH scores and the CASH method to a certain extent.

The samples measured in this study were all from only 0 ~ 20 cm soils. Long-term agricultural management measures can affect the distribution of nutrients or aggregates at different depths in the soil. We did not explore this aspect in this study.

3.4 FUTURE RESEARCH

As mentioned in the limitation part, future research could examine more soils of the same type under similar treatment from different sites, making the results more general and more reliable. More sites from different regions could be included to construct a more widely applicable SPSHI equation.

In addition to increasing the sample replication, samples could be taken from greater depths. Since it is a long-term study of agricultural management practices, the soil will also be affected at deeper levels. This may make more sense for sustainable development, as some measures may improve the soil in a different direction. For example, when comparing manure with chemical fertilizer, samples from different soil depths may indicate why manure improves soil health but has less crop yield than chemical fertilizer.

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APPENDIX A

Table 7. Pearson correlation analysis among "Critical" indicators, site-specific soil health value (SPSHI) values, comprehensive assessment of soil health (CASH) Scores, crop yield and total crop nitrogen (N) recovery

	ACE Protein	TN	TN mass Mg/ha	TC	%TN-2	a_act_c	a_gwc_wet	%TN-4	TC mass Mg/ha	%TC-2	TN in aggs	%TN-0.5	%TC-4	PMN	TC mass 2	TN mass 2	Bulk Density	pH SWEL (1:2)	d_icap_mg	a_ni	a_pb	b_p_m3	a_na	b_na_m3	prop. TC in aggs	a_awhc	DTCsoil-aggs	DTNsoil-aggs	Phosphomonoesterase	SPSHI values	CASH scores	Crop yield	Total crop N recovery
ACE Protein	1																																
TN	0.957286	1																															
TN mass Mg/ha	0.941699	0.978656	1																														
TC	0.935656	0.980907	0.973145	1																													
%TN-2	0.937637	0.95371	0.915161	0.896602	1																												
a_act_c	0.90925	0.944193	0.950522	0.951169	0.885878	1																											
a_gwc_wet	0.921646	0.883552	0.861175	0.85468	0.839176	0.76993	1																										
%TN-4	0.933323	0.920473	0.894989	0.86181	0.986241	0.873049	0.836827	1																									
TC mass Mg/ha	0.894182	0.932916	0.970332	0.974648	0.827005	0.936412	0.806867	0.805942	1																								
%TC-2	0.918986	0.942823	0.899401	0.890946	0.995004	0.890519	0.794207	0.980435	0.818454	1																							
TN in aggs	0.908548	0.912397	0.906975	0.868675	0.963585	0.885189	0.793781	0.966224	0.836825	0.961374	1																						
%TN-0.5	0.921937	0.935164	0.905153	0.883643	0.985614	0.881311	0.783809	0.964495	0.824162	0.984949	0.975517	1																					
%TC-4	0.916315	0.913973	0.877524	0.855601	0.988746	0.868324	0.792768	0.994049	0.789346	0.991356	0.964815	0.975851	1																				
PMN	0.882909	0.934563	0.908194	0.900612	0.888963	0.850997	0.862518	0.845172	0.846014	0.860537	0.828919	0.864673	0.825984	1																			
TC mass 2	0.874336	0.919928	0.862632	0.849876	0.964955	0.792142	0.851034	0.941032	0.760689	0.951061	0.889092	0.921217	0.936935	0.904502	1																		
TN mass 2	0.871483	0.910583	0.858945	0.836279	0.948574	0.768452	0.877539	0.92588	0.75139	0.924919	0.869631	0.899615	0.913146	0.914573	0.994958	1																	
Bulk Density	-0.85928	-0.90023	-0.85019	-0.87861	-0.79284	-0.81346	-0.90037	-0.73954	-0.8005	-0.76301	-0.69397	-0.73736	-0.7172	-0.868	-0.80838	-0.82172	1																
pH SWEL (1:2)	-0.16754	-0.13029	-0.09694	-0.13961	-0.04185	0.025626	-0.34486	-0.0671	-0.10187	-0.01562	0.083964	0.073517	-0.03237	-0.0268	-0.14956	-0.17811	0.310396	1															
d_icap_mg	0.258527	0.345662	0.414456	0.31977	0.370083	0.479918	0.032497	0.351255	0.373963	0.390208	0.488829	0.45937	0.366932	0.404271	0.258983	0.230529	-0.07893	0.725177	1														
a_ni	-0.13603	-0.14777	-0.17747	-0.12354	-0.26531	-0.26101	-0.01459	-0.27074	-0.15177	-0.27271	-0.35149	-0.32705	-0.27688	-0.30242	-0.20693	-0.19252	-0.06311	-0.86002	-0.69698	1													
a_pb	-0.01231	0.020819	0.009791	0.051086	-0.15886	-0.12879	0.151477	-0.1949	0.039092	-0.18366	-0.25521	-0.2277	-0.21848	-0.06569	-0.06214	-0.03163	-0.25875	-0.84459	-0.56404	0.92355	1												
b_p_m3	0.350123	0.282196	0.326742	0.251067	0.32165	0.357369	0.112945	0.371984	0.27551	0.34978	0.357837	0.350738	0.391734	0.015308	0.161975	0.125856	-0.11079	-0.21017	0.182355	0.304243	0.158008	1											
a_na	0.383482	0.523645	0.456725	0.585537	0.363811	0.504344	0.313989	0.265697	0.518039	0.400053	0.312101	0.367198	0.310836	0.336529	0.334347	0.29043	-0.58379	-0.25647	-0.00907	0.375027	0.442498	0.223127	1										
b_na_m3	0.132774	0.247017	0.156659	0.324914	0.049982	0.215597	0.124423	-0.05115	0.242359	0.080568	-0.06127	0.02539	-0.01421	0.138263	0.074285	0.043591	-0.43728	-0.4019	-0.25904	0.549035	0.61456	0.023346	0.884599	1									
prop. TC in aggs	0.423836	0.39344	0.321884	0.276052	0.61569	0.374741	0.316923	0.644365	0.177192	0.635638	0.677449	0.644972	0.673273	0.335338	0.553621	0.518629	-0.18108	0.330612	0.388459	-0.44146	-0.54579	0.261307	-0.03093	-0.34083	1								
a_awhc	0.073004	0.107359	0.277392	0.186459	-0.03707	0.26753	0.071876	0.005463	0.365318	-0.06375	0.049868	-0.04103	-0.05652	0.188113	-0.07845	-0.04875	-0.03677	0.164566	0.412088	-0.29286	-0.1109	-0.05178	-0.21446	-0.25352	-0.42413	1							
DTCsoil-aggs	0.398159	0.450693	0.536254	0.578464	0.190933	0.485209	0.421062	0.156242	0.67629	0.168792	0.167247	0.16618	0.117538	0.423858	0.177554	0.196672	-0.51572	-0.30539	0.015052	0.18733	0.418018	-0.00693	0.447563	0.45347	-0.59755	0.635472	1						
DTNsoil-aggs	0.484173	0.562326	0.621679	0.630915	0.320563	0.547982	0.511728	0.26909	0.683652	0.288295	0.233946	0.275269	0.231395	0.554915	0.337839	0.365519	-0.67199	-0.37992	0.047644	0.244013	0.497218	0.088778	0.473883	0.475572	-0.51682	0.547585	0.926847	1					
Phosphomonoesterase	0.563967	0.440006	0.402658	0.446115	0.41128	0.417758	0.459692	0.371132	0.399425	0.385465	0.375815	0.464257	0.368703	0.553159	0.330841	0.342015	-0.43438	0.207577	0.224371	-0.32315	-0.21678	-0.06732	0.041402	0.068856	0.123522	-0.01608	0.223582	0.230582	1				
SPSHI values	0.862431	0.782787	0.790382	0.737022	0.846945	0.816293	0.695122	0.863872	0.717869	0.834326	0.874401	0.879887	0.854887	0.778424	0.726942	0.718327	-0.58608	0.273198	0.554438	-0.53527	-0.45921	0.315198	0.040322	-0.23347	0.59357	0.156157	0.133016	0.198378	0.674341	1			
CASH scores	0.872034	0.914643	0.891358	0.915822	0.848884	0.878731	0.873766	0.817418	0.873187	0.824421	0.799967	0.815032	0.794776	0.951125	0.848116	0.854707	-0.87545	-0.0273	0.298179	-0.32377	-0.12151	-0.06563	0.374536	0.171629	0.28274	0.247681	0.506792	0.569895	0.518331	0.749984	1		
Crop yield	0.621543	0.531465	0.495971	0.435928	0.62508	0.441268	0.537029	0.659276	0.361617	0.599316	0.517102	0.593943	0.655538	0.483868	0.594042	0.605762	-0.44642	-0.32793	-0.01779	0.090474	0.015541	0.520838	-0.03692	-0.16131	0.369448	-0.13607	-0.03547	0.183236	0.341497	0.593915	0.408146	1	
Total crop N recovery	0.782167	0.665952	0.625685	0.590042	0.745166	0.571942	0.715635	0.786733	0.515427	0.719672	0.659955	0.707365	0.77386	0.594315	0.705843	0.714187	-0.5764	-0.38777	-0.04082	0.089803	0.04252	0.505145	0.055968	-0.09259	0.435531	-0.14996	0.053196	0.217001	0.431693	0.695735	0.544258	0.952376	1

Table 8. Pearson correlation analysis between scores of indicators in comprehensive assessment of soil health (CASH) method and CASH scores

	WSA	OM	CO2-96 hour	Active Carbon	ACE Protein	pH	P	K	AWC	Secondary scores (Fe, Zn, Mg, Mn)	CASH Score
WSA	1										
OM	0.739104	1									
CO2-96 hour	0.704611	0.903813	1								
Active Carbon	0.758452	0.882794	0.761487	1							
ACE Protein	0.730455	0.810951	0.762097	0.874314	1						
pH	0.068688	-0.10507	-0.12891	0.163171	-0.04327	1					
P	-0.02269	-0.06344	0.1839	-0.3333	-0.05018	-0.23441	1				
K	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	1			
AWC	0.119278	0.151738	0.249063	0.273152	0.094704	0.130053	-0.50933	#DIV/0!	1		
Secondary scores (Fe, Zn, Mg, Mn)	0.07766	0.093472	0.132864	0.305101	-0.01153	0.711127	-0.09091	#DIV/0!	0.242271	1	
CASH Score	0.787605	0.844576	0.905328	0.864519	0.850252	0.189218	0.061424	#DIV/0!	0.306672	0.325021223	1

Since K content of all plots is greater than 74ppm, their K scores are same (100). Therefore, the correlation between other terms and K is not available (see table 6 for indicators explanation).