SOIL HEALTH ASSESSMENT OF THE SANBORN FIELD

LONG-TERM EXPERIMENTAL STUDY

A Dissertation

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In Partial Fulfillment

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Doctor of Philosophy

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by

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The undersigned, appointed by the Dean of the Graduate School,

have examined the dissertation entitled:

SOIL HEALTH ASSESSMENT OF THE SANBORN FIELD

LONG-TERM EXPERIMENTAL STUDY

Presented by Saranya Norkaew

A candidate for the degree of

Doctor of Philosophy

And hereby certify that, in their opinion, it is worth of acceptance.

Randall J. Miles (Chair)

Stephen H. Anderson (Co-chair)

Peter P. Motavalli

Robert J. Kremer

Allen Thompson

DEDICATION

I dedicate this work to:

the memory of my mom, who inspired my pursuit of soil science,

my dad and my brother, who offer unconditional love and endless support,

and always have been there for me.

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I am tremendously fortunate to have Dr. Randall Miles as my academic advisor. He always provided me with wise guidance, kind assistance and expertise in order to make my study possible. I would like to express the deepest appreciation to Dr. Stephen Anderson, my co-chair, for all his generous help, advice, and encouragement to complete my dissertation. I owe special gratitude to committee members, Dr. Peter Motavalli, Dr. Robert Kremer, and Dr. Allen Thompson, who helped and supported me by providing thoughtful feedback, guidance and their valuable time. I am very thankful to Dr. Russell Dresbach and Donna Brandt for academic support, imparting knowledge and laboratory expertise in this area of study. This work would not have been completed without the financial support of the Sanborn Field funding and Tucker Prairie data from Patricia Quackenbush. I am grateful for a number of friends and colleagues for friendship and pushing me to keep the work going. Lastly, I would have not been here without the Thai government, who gave me a scholarship and opportunity to pursue my PhD degree.

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ABSTRACT

Soil health assessment uses a combination of potential indicators affecting soil processes to comprehensively monitor soil change, caused by cropping systems and soil management. The objectives of the study were to assess the effects of selected cropping systems, soil management and landscape slope positions on the soil health characteristics of the Sanborn Field long-term experimental study in Columbia, Missouri, United States. Soil samples were collected on each of four dates over two years (8^{th} May 2014, 4^{th} September 2014, 1^{st} April 2016, and 18^{th} August 2016) from selected plots to address each objective, and these time samples were used as replications. Soil physical, chemical, and biological characteristics were analyzed in the laboratory for these samples to assess soil health using the Cornell Comprehensive Assessment of Soil Health (CASH) method. To assess soil health in this study, soil health scoring was determined used R-studio version 1.1.149 to relate the interaction of cropping systems, soil management, and slope positions. Most soil resources on Sanborn Field are a poorly-drained claypan soil classified as a Mexico silt loam (fine, smectitic, mesic Vertic Epiaqualf). In addition, soil samples collected from Tucker Prairie was used as a proxy for the original state of Sanborn Field soils. The first study was conducted to evaluate the effects of long-term cropping systems on soil health properties. The results from the characterization indicated

that continuous timothy (*Phleum pretense* L.) and warm season grass treatments were classified with very high soil health scores, and the lowest score was found for continuous corn (*Zea mays* L.). In addition, results showed strong positive linear associations between soil organic carbon, total nitrogen, potentially mineralizable nitrogen, active carbon, microbial biomass, and water stable aggregates; while a strong negative linear correlation existed between each of these properties and bulk density. The second study was conducted to evaluate the effects of long-term annual applications of no fertilizer, full fertilizer, and manure on soil health measurements of selected cropping systems. Different cropping systems, including continuous corn, continuous wheat (*Triticum aestivum* L.), corn-wheat-red clover (*Trifolium pretense* L.) rotation, and corn-soybean (*Glycine max* L.)-wheat rotation treatments were used in this study. Results showed that annual dairy cow *(Bos Taurus)* manure applications had the greatest effect on all soil health indicators and had the largest overall soil health score compared to full fertility and no fertilizer treatments. Moreover, continuous wheat with manure application presented the best combination of effects on soil properties with the largest score for most soil health indicators and an overall health score of 82 out of 100 classified as very high which is the best. The last study evaluated the effects of landscape slope positions on soil health properties of the long-term experiment. Results showed that the summit position had the highest overall soil health score while the lowest score was found on the shoulder position. However, there were no significant differences along the transect slope for water-stable aggregates and bulk density. There were significant differences along the transect for the biological properties such as soil organic carbon, active carbon, potentially mineralizable nitrogen, and microbial biomass. Results of this study illustrate the effect of selected variables on soil health and provide the recent addition of using biological characteristics to account for soil health properties. It is important to remember that this study of the long-term Sanborn Field experiment is just for a small-sized plot area. Future studies of soil management effects on soil health need to account for their own field conditions and their own unique environment.

1 INTRODUCTION, STUDY AREA, LITERATURE REVIEW, AND OBJECTIVES

1.1 INTRODUCTION

Degradation of soil resources is the most serious and widespread threat to humankind. Soil plays an important role in the global environment in various aspects involved with providing food, water infiltration, climate change adaptation, biodiversity, and restoring ecosystems (McBratney et al., 2014). Worldwide research has increased awareness that soil resources have been affected by human activities. In addition, soil is also a vital resource in the ecosystem. It is important to possess a better knowledge and awareness of soil quality by assessing soil properties (Karlen et al., 2003). Therefore, soil quality assessments are periodically needed to determine soil conditions at many scales (Karlen et al., 2008). Additionally, a soil health assessment on a long-term study can provide baseline data on the rapidity and magnitude of soil changes.

The main concept of soil health assessment emphasizes evaluating the soil conditions, yet there is little research focusing on the soil health assessment on a long-term experiment related to the physical, chemical, and biological properties of soil. The results of this type of study can illustrate the long-term effects of cropping systems and soil management on soil health, including demonstrating the benefit of using practices from organic agriculture, such as application of animal manure, versus practices used in conventional agriculture. At present, in many cultivated areas using crop rotation have significantly increased due to their benefits in improving physical, chemical and biological properties of the soil over monoculture systems. The knowledge from this type of study can potentially be used to simplify the effect of long-term cropping systems and soil management in various parts of Missouri. Moreover, these studies not only provide a better understanding of soil health but can be used as decision-making tools for sustainable land management in the future.

The soil science community has discussed for the past few decades the definition of soil quality and identified a potential group of soil quality indicators (Andrews et al., 2003). The discussion has now focused on the idea of soil health in terms of added beneficial soil biological properties. The specific measurement of soil microorganisms will depend on the purpose of the project (McBratney et al., 2014). Armenise et al. (2013) proposed that a robust soil quality index should be responsive to soil management, sensitive to soil changes, and easily measurable. According to the complexities associated with crop rotation and soil management, researchers and land managers need to improve their understanding of soil health and knowledge of land use changes to benefit agriculture and ecosystems services.

1.2 STUDY AREA AND SITE SELECTION

Sanborn Field is the second oldest field of continuous agricultural experimentation in the United States. Dean J.W. Sanborn established the plots in 1888 at the University of Missouri, Columbia, Missouri (Fig. 1−1). The primary purpose of the plots was to observe the benefits of crop rotations and manure on crop yields. The plots had been relatively unchanged in cropping practices and management until 1950 (Upchurch et al., 1985). Since then minimal changes have occurred. There are 44 plots in the operation consisting of 38 plots remaining of the original field and 6 plots for special studies (Fig. 1−2).

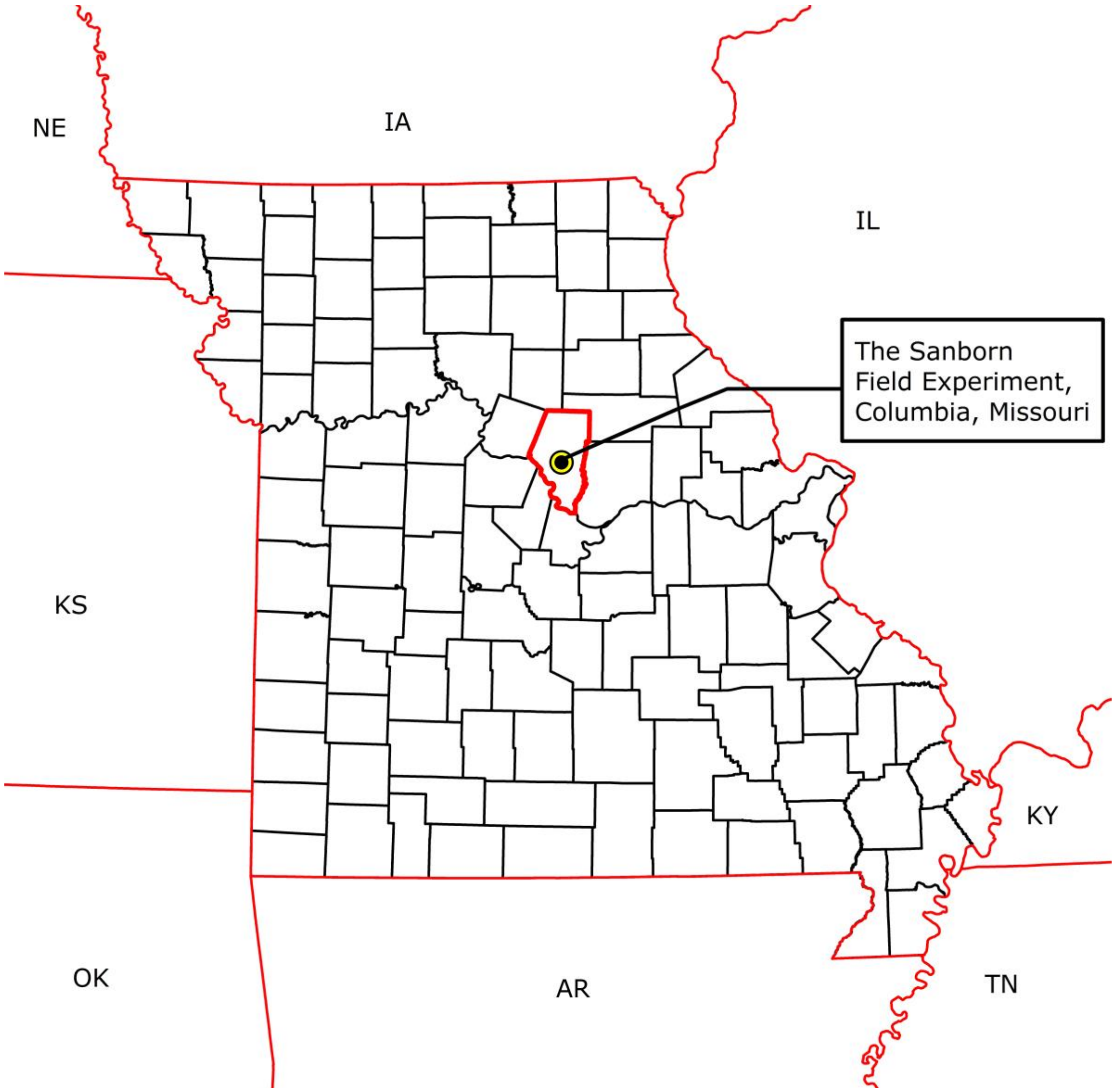


Fig. 1−1. Location of Sanborn Field Experiment at the University of Missouri, Columbia, Missouri, bordered by Nebraska (NE), Kansas (KS), Oklahoma (OK), Arkansas (AR), Tennessee (TN), Kentucky (KY), Illinois (IL), and Iowa (IA).

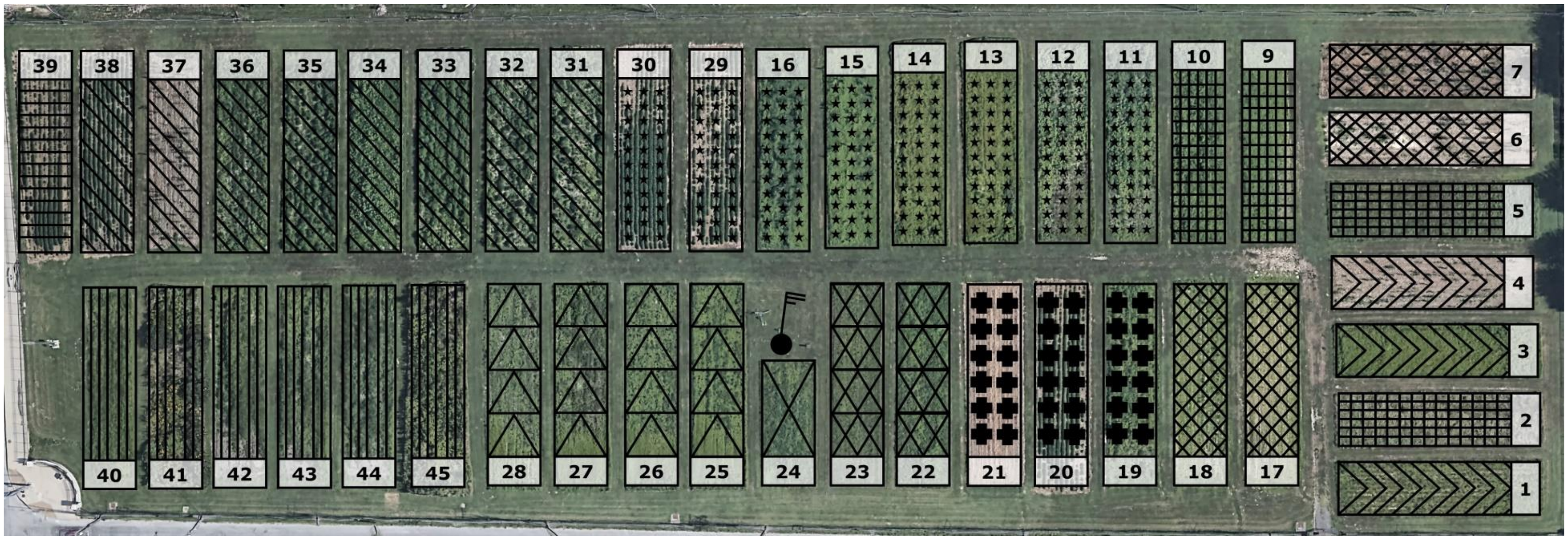
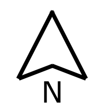


Fig. 1−2. Plot plan of Sanborn Field since 1990; the plan description is shown on the following page.



Continuous Cropping

Corn (ContC)

Plot 6 - Full Fertility and Conventional Tillage
Plot 7 - Full Fertility and No Tillage
Plot 17 - No nutrients inputs
Plot 18 - Manure†

Soybean (ContSb)

Plot 39 - Full Fertility

Timothy (ContT)

Plot 22 - Manure†
Plot 23 - No nutrients inputs

Wheat (ContW)

Plot 2 - Full Fertility
Plot 5 - Manure† plus Nitrogen
Plot 9 - No nutrients inputs
Plot 10 - Manure

Tall Fescue (ContTF)

Plot 24 - Full Fertility

Rotated Cropping

Corn-Soybeans-Wheat (green manure; red clover) (CSbW(Rc))

Plot 31 - Full Fertility
Plot 32 - Full Fertility minus Potassium
Plot 33 - Full Fertility minus Phosphorus
Plot 34 - Manure†
Plot 35 - No nutrients inputs
Plot 36 - Full Fertility (No red clover)
Plot 37 - Full Fertility
Plot 38 - Full Fertility

Corn-Soybean-Wheat-Red clover (CSbWRc)

Plot 11, 13, 16, and 29 - Full Fertility
Plot 12, 14, 15, and 30 - Full Fertility minus Nitrogen

Corn-Wheat-Red clover (CWRc)

Plot 25 - Manure†
Plot 26 - Full Fertility
Plot 27 - No nutrients inputs
Plot 28 - Full Fertility minus Nitrogen

Corn-Wheat-Red clover (since 1950) (CWRc)

Plots 1, 3, and 4 - Full Fertility

Grain Sorghum-Soybean-Wheat (green manure; red clover) (GSSbW(Rc))

Plots 19, 20, and 21 - Full Fertility

Research, Teaching, and Demonstration

Plots 40, 41, 42, 43, and 44
Plot 45 - Warm Season Grass (Burn only)

Weather Station

† Dairy Cattle Manure 13.4 Mg ha^{-1} per year.

In addition, Brown (1994) and Rachman et al. (2003) stated that Sanborn Field had no replication plots since appropriate statistics had not been developed when the field was established. According to the complexities associated with crop rotation and soil management, the field has provided research results and contributed management interpretations to benefit agriculture for 130 years. Studying how soil health has changed under various continuous cropping systems and management for more than a hundred and thirty years will provide not only the yield data but also a baseline of data and information to make educated sustainable agriculture decisions.

This study was conducted on the long-term Sanborn Field experiment, which is on a poorly-drained claypan soil. Most soil resources on Sanborn Field are a poorly-drained claypan soil classified as a Mexico silt loam (fine, smectitic, mesic Vertic Epiaqualf), and the other soils include Mollic Epiaqualfs and Cumulic Epiaquolls (Miles and Hammer, 1989; Miles and Brown, 2011). In general, Sanborn Field has soil derived from loess over glacial till and has a slightly rolling topography (Veum et al., 2013). Well expressed argillic horizons are present in the subsurface which affect the water holding capacity and movement.

1.3 LITERATURE REVIEW

1.3.1 Definition of Soil Health

With the increasing global emphasis on sustainable land use and soil management, the concept of soil quality was first developed throughout the 1990s by soil scientists to provide guidance to improved soil resource management. These soil scientists have worked in the education and assessment area to provide a better understanding and awareness of soil resources (Karlen et al., 2003). Karlen et al. (1997) defined soil quality as “the capacity of a specific kind of soil to function, within natural or managed ecosystem boundaries, to sustain plant and animal productivity, maintain or enhance water and air quality, and support human health and habitation”.

The soil quality concept in the United States gained further attention after an agenda was published for agriculture in soil and water quality (National Research Council, 1993). During that time the National Resources Conservation Service developed a statistical tool for monitoring and assessing soil quality on regional and national scales. In addition, Karlen et al. (2003) stated that assessment tended to determine the inherent dynamic soil properties and processes, focusing on the upper 20−30 cm of soil. They illustrated the current research and evaluated methods, such as the field soil quality test kit developed by Liebig et al. (1996), the field tool of soil health using scorecards developed by Romig et al. (1996), and the Illinois Soil Quality Initiative (ISQI); a farmer-centered and on-farm approach developed by Walter et al. (1997).

1.3.2 Assessment Methods and Indicators

Moebius-Clune et al., (2016) issued a soil health assessment method, the Comprehensive Assessment of Soil Health (CASH), which focuses on the integration of biological, chemical, and physical characteristics consisting of various measurements of soil, including available water capacity, surface hardness, subsurface hardness, aggregate stability, organic matter, soil protein, soil respiration, active carbon, root pathogen pressure rating, potentially mineralizable nitrogen, soil chemical composition, salinity and sodicity, and heavy metals.

An earlier similar study reported by Karlen et al. (2003), stated that a potential soil quality index should consist of appropriate indicators, forming a minimum data set, and scoring the indicators. The potential soil quality indicators were comprised of phosphate sorption capacity, cation exchange capacity, organic matter content, bulk density, water retention, random roughness, porosity, hydraulic conductivity, seal conductivity, saturated hydraulic conductivity, soil productivity, and rooting depth. In contrast to Moebius-Clune et al. (2016), Congreves et al. (2015) applied an initial version of CASH to study the effects of crop rotation and tillage systems (no-till and conventional tillage) called the Ontario Soil Health Assessment (OSHA). Congreves et al. (2015) likewise argued that the OSHA could provide a better overall soil health score and indicators of soil quality characteristics than CASH. Un-weighted overall score which excluded soil compaction, available water capacity (AWC) and iron (Fe) could affect the score. However, they suggested that the minimum dataset for assessment included a light fraction of organic matter, organic matter, microbial biomass, carbon, nitrogen mineralization, soil respiration and aggregation.

Recent research has applied non-linear scoring curves using soil characteristics to assess the effect of soil management practices. Veum et al. (2013) studied the relationship between microbial enzyme activities and the effect of soil management on soil quality using the Soil Management Assessment Framework (SMAF). The SMAF was developed to use in large-scale assessment, conservation planning, and soil health evaluation. It recently added an evaluation of β-glucosidase, which reflects the plant residue decomposition and microbial biomass-C scoring curves (Stott et al., 2010). In this framework, Veum et al. (2013) indicated multiple potential indicators that could be combined into a soil quality index, including biological indicators: microbial biomass carbon and microbial enzyme activities; chemical indicators: soil reaction, soil nutrients, active carbon, and organic matter; and physical indicators: bulk density and aggregate stability.

To study biological indicators, a phospholipid fatty acid (PLFA) measurement has become one of the most common methods that have been used to study microorganisms in the soil. Frostegård et al. (2011) described that there were two approaches to interpret PLFA data: 1) by filtering PLFA patterns through multivariate statistical techniques and 2) by clarifying specific groups of microorganisms. They also mentioned that the PLFA method could not elucidate a response to the rapid changes (turn-over) and calculate the diversity of organisms. Like Frostegård et al. (2011), Blagodatskaya and Kuzyakov (2013) reported the type of microorganisms in soils which were active, potentially active and dormant. Their approach was to evaluate each type of microorganism, especially focusing on active microorganisms, by using the PLFA method. One important point in this study showed that PLFA results indicate only the groups of the microbial community and do not directly point to microbial activity. In addition, the PLFA content cannot be used to predict the soil condition.

1.3.3 Significant Studies

Over the last two decades, several studies have been conducted on Sanborn Field to study the effect of rotation cropping systems and soil management. Drawing back to 1990, according to public concern, water contamination led to low-input and sustainable agriculture techniques (e.g., rotations and annual additions of manure) to reduce water runoff and soil erosion through increased infiltration. Physical properties of bulk density, water retention, saturated hydraulic conductivity, and pore-size distributions were analyzed to study the effect of continuous and crop management of Sanborn Field (Anderson et al., 1990). They found that the manure treatment (13.4 Mg ha^{-1}) had a greater saturated hydraulic conductivity than the unfertilized treatment (9 times) of continuous wheat, continuous corn, continuous timothy and corn-wheat-red clover rotation. Besides, there were slight positive effects on bulk density, water retention, and pore-size distribution. Likewise, they noted that 70 tons ha^{-1} annual additions of manure at Rothamsted (UK) had no measurable effect on soil aggregate stability. Inspired by the results obtained by Anderson et al. (1990), Rachman et al. (2003) determined aggregate stability, soil shear strength, and single-drop rainfall splash detachment and developed a better understanding of cropping systems affecting soil erodibility. Moreover, the authors found that all treatments had the greatest soil aggregate stability in July. The best parameter for soil erodibility evaluation was splash detachment because it was more sensitive than other measures. Aggregate stability of the timothy treatment was three times greater than other treatments (root system), and it had significantly greater soil shear strength 10−27% compared to other treatments.

More recently, Miles and Brown (2011) briefly explained the history and major changes of cropping system and management that have been altered since 1888. The authors showed soil organic carbon data which were collected from 1915, 1938, 1962, and 1988. During these years, the residue was removed up until 1950 with additions and to the plots after 1950 affecting the amount of organic carbon in the soil. They found it would take 30 to 40 years of development to get to the equilibrium level of organic matter in the soil after returning annual residues for the large input treatments (manure and fertility). Marginal treatments did not have much gain in soil organic carbon 30 to 40 years after residues were returned. The measurement of the active carbon level indicated that the manure treatment had a greater benefit than the non-manure treatment. Seasonal instability of temperature, moisture, and active carbon affected microbial activity (Miles and Brown, 2011).

Several microbial assessment methods on Sanborn Field were conducted by Jordan et al. (1995) to show potential indicators of soil quality. The results showed that the effects of cropping systems and management practices were observed on soil microbial biomass, phospholipids, and enzyme activity. Furthermore, enzymatic activity had a strong correlation with soil organic matter. A further example is a study that addressed the effects of selective soil enzyme activities on Sanborn Field by the long-term cropping system, including fertilization, tillage, and crop rotation. Eivazi et al. (2003) studied five selected enzymes in this experiment, acid and alkaline phosphatases, alpha-glucosidase, arylsulfatase, and urease. They indicated that there was a significant and positive correlation between soil organic carbon and the five enzymes ($r = 0.75$; $r = 0.67$; $r = 0.89$ for acid phosphatase, alkaline phosphatase, and sulfatase, respectively). In manure plots, there were significantly greater activities of all five enzymes than for the full fertility (inorganic fertilizers) plots. A three-year rotation plot (corn-wheat-clover) had the largest activity values, and the smallest values were found in the continuous soybean plot.

Jordan et al. (2004) studied earthworm abundance and microbial activity as affected by management practices and cropping systems on Sanborn Field. This study showed that most of the earthworm species and the greatest microbial activity were found during the spring partly due to the soil moisture content. In addition, the greatest earthworm abundance and microbial activity were reported in manure, no-tillage and crop rotation (including legumes) treatments. It was important to note that microbial activity had consistent trends with earthworm density.

In a recent publication, Veum et al. (2013) studied the relationship between microbial enzyme activities and the effect of soil management on soil quality on Sanborn Field. The authors found that perennial vegetation had the greatest soil quality (native prairie, restored prairie, timothy), followed by no-till and conventional tillage of wheat and corn, respectively (soil quality increased with the decreasing level of soil disturbance). For the fertilizer treatment, soil quality decreased in the following sequence: manure, inorganic fertilizer, no fertilizer, respectively. Important similar research was conducted in a 150-year UK grassland to study the effect of nitrogen fertilizer and pH on soil microbial growth. Rousk et al. (2011) found that bacterial growth decreased, and fungal growth increased in acid pH. There were no significant effects of nitrogen fertilizer levels on the growth of fungi and bacteria, but those explicitly affected the PLFA composition in the short term.

In very recent years, there have been several studies conducted on Sanborn Field emphasizing soil physical and chemical properties; however, there is little biological research using the PLFA to evaluate the contribution of microorganisms which is a very significant factor indicating the dynamic processes of change in the soil. Consequently, research will be conducted to assess the effect of long-term cropping systems and soil management on soil health attributes, especially interpreting fundamental PLFA data.

Thus, over the lifetime of the Sanborn Field, researchers have been conducting experiments to study the effects of crop rotation and soil management. This setting provides an excellent opportunity to assess long-term treatment of monocultures and rotations with different treatment inputs on soil properties.

1.4 OBJECTIVES AND HYPOTHESES

The combination of the five factors of soil formation develops soil attributes in the profile in any given place. These factors consist of parent material, climate, biota (vegetation), topography, and time. In response to these factors, soil naturally creates horizons from the surface to the deepest horizon. The primary purpose of this research was to assess the effects of selected cropping systems and soil management employed on Sanborn Field over a hundred and thirty years on soil health properties. The long-term goal for crop production is enhancing land management for sustainable agriculture. Therefore, studies must be first understood the impacts and interactions of cropping systems and soil management on soil health.

1.4.1 First Objective and Hypothesis

The first objective of this study was to assess the long-term effects of cropping systems with no inputs including monoculture, crop rotation, and pasture on soil health properties. The hypotheses related to this objective were that differences in cropping systems cause differences in soil health properties. In addition, crop rotations provide a benefit to the soil by maintaining nutrient capacity and improving soil conditions relative to monoculture. If cropping systems have affected soil health, then soil properties will have altered soil structure, nutrient availability, and biological properties.

1.4.2 Second Objective and Hypothesis

The second objective of this research was to assess the effects of annual fertilizer and manure applications on soil health properties. The hypotheses were that fertilizer and manure applications improve soil nutrient availability and enhance microbial activity. If the applications of fertilizer and manure have affected soil attributes, then nutrient content plus the amount and diversity of soil microbes for fertilizer and manure application treatments would be greater than non-fertilized treatments.

1.4.3 Third Objective and Hypothesis

The third objective of this research was to assess the effects of slope positions on soil health. The hypotheses for this objective were that landscape slope positions have affected soil health properties including the soil distribution pattern within the field. If the slope positions have influenced soil properties, then each position will cause differences in soil properties.

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2 LONG-TERM EFFECTS OF CROPPING SYSTEM ON SOIL HEALTH PROPERTIES OF THE SANBORN FIELD EXPERIMENTAL STUDY

2.1 ABSTRACT

Soil degradation has been a critical problem of cultivation affecting the capacity of food production around the world. Therefore, incorporation of crop rotations into cropping systems has been suggested as an effective practice to improve soil health for sustainable agriculture. This study was conducted on a range of cropping systems with little fertilizer inputs to evaluate the effects of long-term cropping systems on soil health properties in the Sanborn Field study. Soil samples were collected on two dates during two years from each plot; these time samples were used as replications. Soil physical, chemical, and biological characteristics were analyzed in the laboratory for these samples to assess soil health using the Cornell Comprehensive Assessment of Soil Health (CASH) method. Soil samples collected from Tucker Prairie was used as a proxy for the original state of Sanborn Field soils to compare to soil in the different cropping systems. The results from the characterization indicated that continuous timothy (*Phleum pretense* L.) and warm season grass treatments were classified with very high soil health scores, and the lowest score was found for continuous corn (*Zea mays* L.) with continuous wheat and the rotations in the middle. In addition, results showed strong positive linear associations between the following properties: soil organic carbon, total nitrogen, potentially mineralizable nitrogen, active carbon, microbial biomass, and water stable aggregates (r = 0.60 to 0.96). In contrast, these properties had strong negative linear correlations between each of these properties and bulk density (r = -0.59 to -0.71). To obtain an integrated soil

health index, a combination of soil physical, chemical, and biological properties is required to adequately assess the soil health status of selected cropping systems and make potential recommendations for future sustainable management.

2.2 INTRODUCTION

Researchers have reported (Bai et al., 2013) that one-fourth of the world's soil has been affected by soil degradation, reducing the ability of soil to function and serve humans for many land uses. Soil degradation during the past century has affected the capacity of soil to effectively function in many capacities. Moreover, increased intensity of land use not only increases soil degradation but also decreases the diversity of soil biology (Bai et al., 2008). Many studies have suggested that degraded soil may have been caused by various factors, such as soil erosion, nutrient depletion, as well as inappropriate management and practices which diminishes the soil's physical, chemical, and biological properties (Gregory et al., 2005; Jin et al., 2015; Nielsen et al., 2015).

For these reasons, crop rotations have been utilized in the United States and other areas of the world for several decades since these rotations can maintain and improve the quality of soil in various ways. For instance, Benjamin et al. (2007) showed changes of selected physical properties in a 15-year experiment. For example, soil bulk density decreased from 1.39 to 1.25 g cm^{-3} in perennial grass plots, which was different from the decrease over this time period for the annual crops, a decrease of 1.38 to 1.30 g cm^{-3}. They also found that saturated hydraulic conductivity (K_{sat}) increased from 27 to 98 mm h^{-1} in the grass plots and increased from 14 to 35 mm h^{-1} for the annual crops during this 15-year experiment. Equally important, the study of the long-term Morrow plots showed that the crop rotation with full fertility not only produced the greatest yield compared to continuous corn but also retained the greatest content of nitrogen and soil organic carbon (Odell et al., 1984).

Besides evaluating individual soil properties, developing a soil health index (SHI) which integrates physical, chemical, and biological soil properties is useful to evaluate management systems. An SHI can be a useful parameter for assessing sustainable agricultural productivity. Using a soil quality index to assess the results of crop rotations for a corn-soybean (*Glycine max* L.) rotation, continuous corn-cover crop system, and corn-soybean-wheat rotation as well as selected management practices was evaluated by Nakajima et al. (2016). The corn-soybean rotation had a greater soil quality index than the continuous corn-cover crop system and the corn-soybean-wheat rotation. Moreover, they also concluded that the major indicators in soil quality assessment were clay content and organic carbon in soil for their soil-site setting.

Over the years, changes in soil conditions due to differences in cropping systems have been reported. Comparing the influence of cropping systems, such as grain-based crops or forage-based crops and pasture, Jokela et al. (2011) evaluated soil health using the Soil Management Assessment Framework (SMAF). They found that pasture had a significantly greater soil quality index than other cropping systems. In addition, the forage-based systems had greater levels of total nitrogen, soil organic carbon, potentially mineralizable nitrogen, and water-stable aggregates as well as lower bulk density than the grain-based cropping systems, although the soil quality indices were not statistically different. To observe the impact of continuous corn, a corn-soybean rotation, and a corn-soybean-wheat-cowpea rotation, Aziz et al. (2013) indicated biological properties responded to management practices as well as conventional tillage and no-tillage systems. They suggested that soil biological properties can be used as a sensitive indicator of soil quality evaluation according to the strong relationship with soil quality index.

While many studies have focused only on the effects of cropping systems in short-term experiments, research considering long-term experiments may discover the positive and negative effects of these cropping systems through soil health indicators. One site to provide this assessment is the Sanborn Field experimental plots (130 years of continuous management). In order to study the influence of long-term cropping systems on soil properties, a comparison of the treatment effects of continuous corn, continuous wheat (*Triticum aestivum* L.), continuous timothy, corn-wheat-red clover (*Trifolium pretense* L.) rotation, corn-soybean-wheat rotation (red clover as a green manure), and warm season grasses on Sanborn Field was conducted. An additional treatment from the undisturbed Tucker Prairie with similar soils as Sanborn Field serves as a proxy for the original soil and vegetative conditions before Sanborn Field was established was utilized as a reference site. Since all plots were treated the same with no fertilizer input, the objective of the study was to observe the long-term effects of cropping systems on soil health properties which include selected soil physical, chemical and biological properties.

2.3 MATERIALS AND METHODS

2.3.1 Plot Selection

The study was conducted on the long-term Sanborn Field experiment at the University of Missouri-Columbia located in Columbia, Missouri, USA (38°94N, 92°32W). Since establishment, one of the purposes of the study was to observe and measure the effects of manure management on selected cropping systems and rotations (Wagner, 1989). There are different types of cropping systems and management practices for the plots with the historical information given in Table 2−1 (Brown and Wyman, 1989; Miles and Brown, 2011).

The tillage and cropping management for all of the plots on Sanborn Field are as follows. Each plot is moldboard plowed to a depth of 20 cm. After plowing, a disk harrow is used to incorporate any pre-plant fertilizer, agricultural lime, and manure applications to a depth of 5−8 cm while also providing a firm seedbed for planting the designated crop seed. Most soil resources on Sanborn Field are a poorly-drained claypan soil classified as a Mexico silt loam (fine, smectitic, mesic Vertic Epiaqualf), and the other soils include Mollic Epiaqualfs and Cumulic Epiaquolls (Miles and Hammer, 1989; Miles and Brown, 2011). In general, the field is derived from loess over glacial till, and the typical area is slightly rolling in topography (Veum et al., 2013). Subsurface soil includes a well expressed argillic horizon which influences the water holding capacity and rate of water movement.

To study cropping system effects on soil health properties, six plots were chosen as experimental units with no inputs. These were continuous corn (ContC: plot 17), continuous wheat (ContW: plot 9), continuous timothy (ContT: plot 23), corn-wheat-red clover rotation (CWRc: plot 27), corn-soybean-wheat rotation having red clover as a green manure (CSbW(rc): plot 35), warm season grass (WSG: plot 45 − burn only), and Tucker Prairie (TP) used as the reference area of healthy soil.

Table 2−1. Cropping systems and management history of selected treatments from the Sanborn Field experimental study since 1888† and Tucker Prairie as reference area located in Columbia, Missouri, United States. Legend: Cont = continuous; C = corn; O = oat; Rc = red clover; Sb = soybean; T = timothy; TP = Tucker Prairie; W = wheat; Rc/Ls = red clover with lespedeza; W/Sb = wheat with soybean; W(Rc) = wheat (green manure; red clover).

Plot No.	Cropping System	Years	Management and Practices
9	ContW	1888−present	No nutrient inputs
17	ContC	1888−present	No nutrient inputs
23	ContT	1888−present	No nutrient inputs
27	CWRc	1888−1939	No nutrient inputs
	C,W,Rc/Ls	1940−1949	No nutrient inputs
	CWRc	1950−present	No nutrient inputs
35	COWRc	1888−1913	No nutrient inputs
	COWRc	1914−1939	No nutrient inputs
	C,O,W,Rc/Ls	1940−1949	No nutrient inputs
	COWRc	1950−1989	No nutrient inputs
	CSbW(Rc)	1990−present	No nutrient inputs
45	C,Sb,W/Sb	1888−1989	Full Fertility
	Warm season grass	1990−present	No nutrient inputs
TP	Tall grass prairie	1958−present	No nutrient inputs

† Enhancement of Brown and Wyman (1989) and Miles and Brown (2011).

2.3.2 Field Sampling

Four replicate soil samples were collected on each of four dates, 8th May 2014, 4th September 2014, 1st April 2016 and 18th August 2016 from six selected plots resulting in a total of 96 samples. The dimensions of each plot is 30.55 m by 9.42 m (Miles and Brown, 2011). For this study, four samples from the 0−10 cm depth were taken from the representative area, 15.28 m by 3.14 m of a rectangle in the center of each plot, to avoid intra-plot variability (Fig. 2−1). To obtain a representative sample of the plot, sampled points were chosen to have a consistent feature such as avoiding being too wet or too dry and unusual area as much as possible in term of crop types and management practices with distance away for "border effects". Aluminum rings (76 mm diameter) were used to collect soil samples during the selected sampling times during each year. Surface litter on the soil was removed before sampling with the aluminum rings. Following sampling preparation, the core was driven into soil by hammering on a woodblock that was placed on the top of the core. The next steps were digging the core out with a shovel and trimming the protruded soil and plant roots. Finally, the soil sample was placed in a labeled plastic bag to maintain the field moisture content of the sample for determination of gravimetric water content at sampling before laboratory analyses.

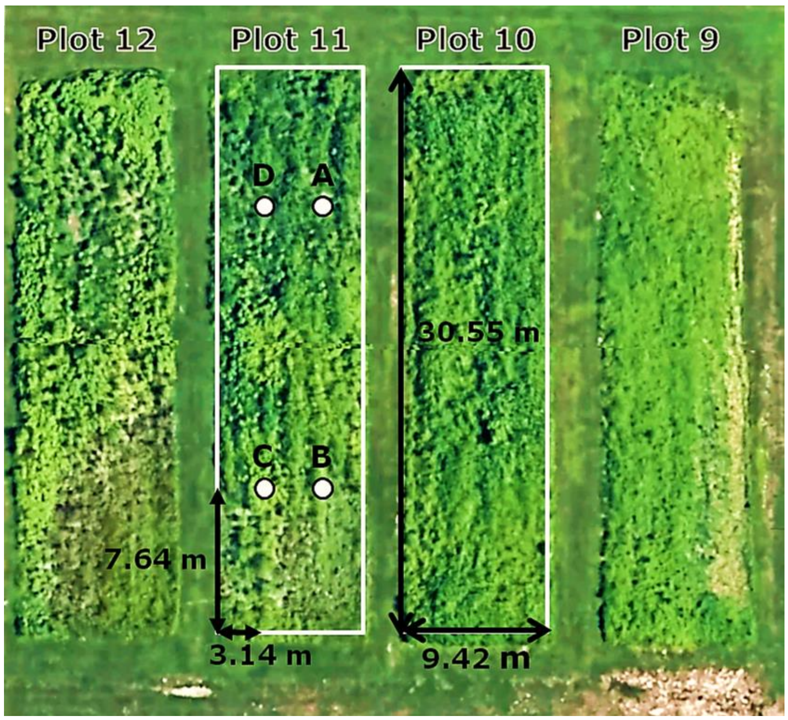

Fig. 2−1. Soil sampling approach for each experimental plot in the study. Points A, B, C, and D were collected to be representative samples of the plot.

2.3.3 Laboratory Analyses

At the Soil Health Assessment Center (SHAC), a soil health testing laboratory located in Columbia, Missouri, soil samples were promptly processed to avoid any time-affected factors in the analysis. The samples were divided into two portions with one portion immediately freeze-dried for phospholipid fatty acid analysis. For laboratory sample preparation, the rest of the soil portions were weighed to determine gravimetric water content at time of sampling. After that, the soil samples were air-dried at room temperature, ground with a mortar and pestle, and passed through a 2-mm sieve for the rest of the analyses (Soil Survey Staff, 2014).

To assess soil physical conditions resulting from past management, bulk density was measured to relate to the level of soil compaction (Keller and Håkansson, 2010). From these known volumes of the core, bulk density was computed by the ratio of oven-dry weight of soil sample to soil volume (Blake and Hartge, 1986). An additional physical indicator was water-stable aggregates, which refers to soil aggregates that were able to resist disruption such as tillage (Kumar et al., 2014) and erosion (Holifield Collins et al., 2015). To determine water-stable aggregates, soil aggregate retention was measured by placing the soil sample on a 0.5-mm sieve, immersing it in water overnight, and agitating the samples (raising and lowering the sieve manually). Afterward, the samples were dried and weighed for the final calculation for the percent of water stable aggregates (Kemper and Rosenau, 1986).

Nutrient analyses were performed to relate to soil nutrient availability status. These analyses included soil pH in water and salt (0.01M $CaCl_2$) which indicates the level of acidity and alkalinity and affects nutrient availability in the soil for phosphorus and potassium (Scanlan et al., 2017). The pH meter (Ross Sure-Flow Electrodes) was used to measure a 1:1 soil:deionized water suspension (Soil Survey Staff, 2014) after shaking for an hour. The Bray I extractant of ammonium fluoride and a dilute solution of hydrochloric acid was used to extract available phosphorus. 2 grams of soil with 25 ml of extracting solution were shaken for 10 minutes. Afterward, the samples were allowed to settle before filtering to take the aliquot. Extracted phosphorus was measured using a spectrophotometer (Spectronic $20D^+$) after the blue color intensity developed from using the molybdate-ascorbic acid reagent (Frank et al., 1998). The determination of total nitrogen content in the samples was performed by a LECO FP-528 nitrogen combustion analyzer. This analyzer involved the assessment of the volume of nitrogen (N_2) content, measured by thermal conductivity (Soil Survey Staff, 2014). The cation exchange capacity (CEC) was determined by the 1M ammonium acetate method at pH 7. The soil samples were saturated with the ammonium acetate solution, which exchanged with the existing exchangeable cations in the soil; after reaction, the quantity of exchangeable ammonium acetate was determined (Soil Survey Staff, 2014).

Soil biological properties were also assessed. First, soil organic carbon content was evaluated by a LECO C-144 carbon analyzer using combustion with measurement of the carbon dioxide (CO_2) mass. The principle of the analysis is that when the soil sample was introduced and combusted in an oxygen atmosphere, the mass of present CO_2 was determined (Soil Survey Staff, 2014). The evaluation of active carbon in the soil was performed by a potassium permanganate method, which is a moderate oxidant (0.2 M potassium permanganate) used to oxidize organic matter in the soil. The change of purple color was measured by a spectrophotometer by the Agilent Cary 60 UV-Vis spectrophotometer (Weil et al., 2003). In addition, an anaerobic method was utilized to estimate the potentially mineralizable nitrogen content, which indicated the available nitrogen in the soil. Approximately 8 grams of soil samples were placed in 50 ml Falcon tubes with deionized water and incubated at 40°C under anaerobic conditions for 7 days. The potentially mineralizable nitrogen contents were measured with the ammonium nitrogen produced after a week of anaerobic incubation using the Spectronic $20D^+$ spectrophotometer (Waring and Bremner, 1964). Phospholipid fatty acids (PLFAs) analysis is one of the most common methods used to provide the information of microbial biomass in soil. The Bligh-Dyer extractant was used to extract lipids from freeze-dried soil samples. Then, the phospholipids were separated using solid phase extraction techniques and transesterification of the PLFAs. Finally, gas chromatography (Agilent Technologies 7890A GC System) was used to measure fatty acid methyl esters (Buyer and Sasser, 2012).

Table 2−2. Summary of soil health laboratory analyses including physical, chemical and biological properties and their analytical methods.

Property	Indicator	Abbrev.	Method	Reference
Physical Properties	Bulk Density	BD	Core method	Blake and Hartge, 1986
	Water Stable Aggregates	WSA	Wet-Sieving	Kemper and Rosenau, 1986
Chemical Properties	Cation Exchange Capacity	CEC	1M ammonium acetate at pH7	Soil Survey Staff, 2014
	Phosphorus	P	Bray 1	Frank et al., 1998
	Potential of Hydrogen	pH	1:1 soil:water	Soil Survey Staff, 2014
	Total Nitrogen	TN	Dry combustion by LECO FP-528 nitrogen analyzer	Soil Survey Staff, 2014
Biological Properties	Active Carbon	AC	Potassium permanganate test	Weil et al., 2003
	Soil Organic Carbon	SOC	Dry combustion by LECO C-144 carbon analyzer	Soil Survey Staff, 2014
	Potentially Mineralizable Nitrogen	PMN	Seven-days anaerobic incubation test at 40°C	Waring and Bremner, 1964
	Microbial Biomass	MB	Bligh-Dyer lipid extraction	Buyer and Sasser, 2012

2.3.4 Statistical Analyses

After all data were obtained from laboratory analyses, the general approach of data analysis consisted of two main steps: 1) calculating the correlation coefficients and doing a Principal Component Analysis (PCA) among all of the indicators (bulk density: BD, water-stable aggregates: WSA, cation exchange capacity: CEC, available phosphorus: P, soil reaction: pH, total nitrogen: TN, active carbon: AC, soil organic carbon: SOC, potentially mineralizable nitrogen: PMN, and microbial biomass: MB) for all treatments, and 2) assessing significant differences for the properties among cropping systems. All steps were performed using the R-studio statistical package (version 1.1.149).

In the beginning, the first step was calculating the Pearson correlations between each pair of properties to observe the trends in relationships. The next step utilized Principal Component Analysis (PCA) to abate the number of variables, but still maintain most of the information in the previous models.

Brown (1994) reported that the experimental plots in Sanborn Field had no replication since it was established in 1888 before Fisher statistical methods with traditional plot replications were used. Repeated measures analysis of variance (Repeated Measures ANOVA), within-subjects ANOVA and hypothesis testing, were used to run significant difference tests among the means of the independent variables since the samples were collected and measured repeatedly over multiple times. The forms of repeated measures ANOVA consisted of one-way repeated measures ANOVA and two-way repeated measures ANOVA, such that the best appropriate model under this objective was one-way repeated measures ANOVA. The assumptions of this model were that the sampling data were repeated, the dependent variable must be a quantitative (interval or ratio) variable, the independent variables were qualitative (nominal or ordinal) variables, the dependent variables should be normally distributed, and the variables should be a sphericity, having equal variance. Additionally, a Shapiro-Wilk method was used to test the normality and a Levene's method was used to test the sphericity. The Shapiro-Wilk test started by creating a null hypothesis: the variable was from a normal distribution, and an alternative hypothesis, the variable was from other distributions. Following these steps, the next step was choosing a 5% significance level or alpha ($\alpha = 0.05$), selecting type I error for the test, and calculating the statistic W and p-value. If the p-value was more than the significance level of 0.05, the null hypothesis was accepted. Similar to the Shapiro-Wilk test, Levene's test also had similar steps like the other test. However, the null hypothesis was changed so variances are equal or homogeneity of variance, and the test statistic was replaced by an F-test. If the F-statistic value was greater than the critical value or the p-value was smaller than the significance level, the alternative hypothesis was accepted. After testing all assumptions, the significant difference test was the last process for the statistical analysis. This process used the Tukey Honest Significant Differences (TuckeyHSD) method through R-studio. Finally, the outcomes presented the significant groups of independent variables.

2.3.5 Soil Health Assessment

To provide a relative soil health assessment of Sanborn Field plots, the approach from the Cornell Comprehensive Assessment of Soil Health (CASH) (Moebius-Clune et al., 2016) was utilized. Before the calculating soil health index for all physical, chemical and biological properties, the first step was creating the cumulative normal distribution (CND) function and curve of each indicator in order to provide the soil health score index by using the raw data from laboratory analyses and results from the statistical program R-studio version 1.1.149. The soil health score ranged from 0 to 100 (with 100 the best), which was divided into five even intervals; scores 0−20, 20−40, 40−60, 60−80, and 80−100. All scales were represented in the diagram by five colors in five meanings; red described as Very Low, orange described as Low, yellow described as Medium, light green described as High, and dark green described as Very High, respectively.

Three scoring types were used in the procedure of the soil health assessment.

1) More is Better

In this case, if the mean value of each measured indicator is large, the score will be large as well. The indicators in this category were comprised of water-stable aggregates, total nitrogen, soil organic carbon, cation exchange capacity, active carbon, potentially mineralizable nitrogen, and microbial biomass.

2) Less is Better

Only bulk density fell into this scoring type. Additionally, less is better means that the smaller the measured value, the larger the score is observed.

3) Optimum Curve

Optimum curve was applied with both pH and available phosphorus. The feature of this curve consists of two parts which are positive scoring curve and negative scoring curve; moreover, the score of 100 must be larger than the 0 unit of measurement but smaller than 100 units of measurement. In other words, the curve has a bell shape regardless of skewness.

Fig. 2−2, for example, showed the cumulative normal distribution curve of active carbon and consisted of the horizontal axis presenting the amount of active carbon (mg kg^{-1}), the vertical axis presenting the score ranging from 0 to 100. If a soil sample has active carbon of 400 mg kg^{-1}, the soil health score will equal to 66 which is considered as high-level score.

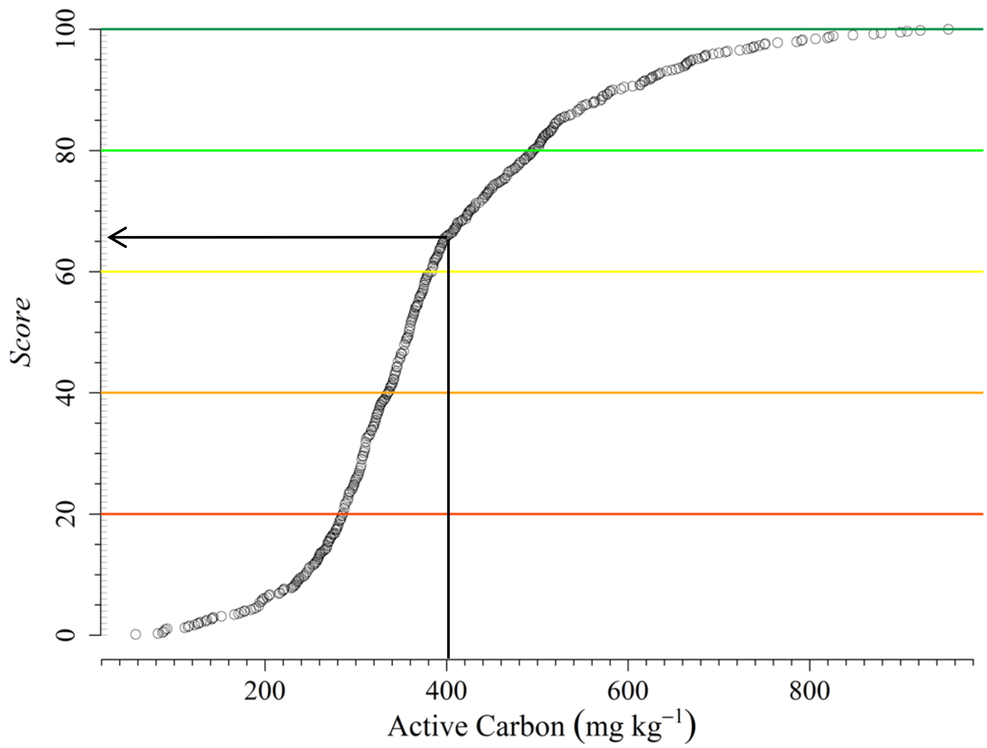

Fig. 2‒2. Cumulative normal distribution curve of active carbon of the collected Sanborn Field and Tucker Prairie soil samples.

Subsequently, an overall soil health score is calculated by summing soil health scores from the cumulative normal distribution function of each indicator and dividing the sum by number of the indicators. In addition, the overall score also used the soil health score range from 0 to 100 as well as the ratings terms of soil health scores.

2.4 RESULTS AND DICUSSION

2.4.1 Physical Properties

The effects of cropping systems with no inputs on physical soil health indicators are shown in table 2‒3. When comparing similar levels of inputs for continuous corn (ContC), continuous timothy (ContT), continuous wheat (ContW), corn-soybean-wheat rotation (CSbW), corn-wheat-red clover rotation (CWRc), and warm season grass (WSG) treatments with Tucker Prairie (TP) as a reference area of having little to no soil disturbance, results showed the percentage of water-stable aggregates ranged from 8 to 83% among all treatments. There were no significant differences for water-stable aggregates between continuous timothy and Tucker Prairie which had 76% and 83% respectively, with continuous timothy having the greatest value followed by warm season grass, continuous wheat, corn-soybean-wheat rotation, and corn-wheat-red clover rotation which had 55, 22, 18, and 12% respectively. In contrast, continuous corn (8%) had almost ten times smaller water-stable aggregate values than continuous timothy and Tucker Prairie.

Table 2−3. Statistical means with significant differences for physical properties in different cropping systems in Sanborn Field as well as Tucker Prairie. Legend: Cont = continuous; C = corn; Rc = red clover; Sb = soybean; T = timothy; W = wheat; WSG = warm season grass; TP = Tucker Prairie. Means followed by lower case letters within the parenthesis indicated the significant differences according to TukeyHSD ($p < 0.05$).

Crop	WSA†	BD†
	%	g cm^{-3}
ContC	8 (a)	1.41 (c)
ContT	76 (d)	1.18 (b)
ContW	22 (b)	1.40 (c)
CSbW	18 (ab)	1.41 (c)
CWRc	12 (ab)	1.38 (c)
WSG	55 (c)	1.20 (b)
TP‡	83 (d)	1.03 (a)

† WSA, Water-stable aggregates; BD, Bulk density.
‡ Source: Data of Patricia Quackenbush.

While continuous timothy did not have different values for water-stable aggregates compared to Tucker Prairie, every cropping system had significantly larger bulk density than the prairie. Based on values in table 2−3, continuous timothy had the smallest bulk density of 1.18 g cm^{-3}, followed by warm season grass, corn-wheat-red clover rotation, and continuous wheat which had 1.20, 1.38, and 1.40 g cm^{-3}, respectively. Additionally, continuous corn and corn-soybean-wheat rotation had the largest values of bulk density at 1.41 g cm^{-3}. However, the differences among corn-wheat-red clover rotation, continuous wheat, corn-soybean-wheat rotation, and continuous corn were not statistically significantly different.

2.4.2 Chemical Properties

Soil reaction (pH), cation exchange capacity (CEC), total nitrogen, and available phosphorus are presented in table 2−4. The data showed that average soil pH ranged from 5.2 to 6.4; this indicates soil pH in the experimental plots were slightly acidic to moderately acidic. Continuous wheat and continuous corn had more acidic pH values, 5.2 and 5.4 respectively, compared to the other treatments while the warm season grass treatment had the least acidic pH value of 6.4.

Table 2−4. Statistical means with significant differences of chemical property in different cropping systems in the Sanborn Field as well as Tucker Prairie. Legend: Cont = continuous; C = corn; Rc = red clover; Sb = soybean; T = timothy; W = wheat; WSG = warm season grass; TP = Tucker Prairie. Means followed by lower case letters within the parenthesis indicated the significant differences according to TukeyHSD ($p < 0.05$).

Crop	pH	CEC†	TN†	P†
		$cmol_c$ kg^{-1}	g kg^{-1}	mg kg^{-1}
ContC	5.4 (ab)	21.0 (cd)	0.85 (a)	6.5 (a)
ContT	6.0 (c)	16.5 (ab)	1.98 (c)	4.0 (a)
ContW	5.2 (a)	18.6 (bc)	1.25 (b)	5.2 (a)
CSbW	5.7 (bc)	17.0 (ab)	1.27 (b)	5.6 (a)
CWRc	5.8 (c)	14.2 (a)	1.22 (b)	4.4 (a)
WSG	6.4 (d)	17.7 (ac)	2.01 (c)	69.0 (b)
TP‡	5.6 (bc)	22.5 (d)	3.19 (d)	3.7 (a)

† CEC, Cation exchange capacity; TN, Total nitrogen; P, Phosphorus.
‡ Source: Data of Patricia Quackenbush.

Another significant soil health indicator was CEC. All soils had CEC values ranging from 14.2 to 21.0 $cmol_c$ kg^{-1}. Results showed continuous corn had the greatest CEC which was not significantly different with Tucker Prairie that had 22.5 $cmol_c$ kg^{-1}.

For soil nutrient content, significant differences were observed among treatments for percentage of total nitrogen in the soil. Warm season grass and continuous timothy treatments had the largest values of total nitrogen, 2.01 g kg^{-1} and 1.98 g kg^{-1} respectively; in contrast, continuous corn had the smallest value, about two times smaller than the largest treatment values. Meanwhile, percentage of total nitrogen for all experimental treatments were smaller than values for Tucker Prairie which had 3.19g kg^{-1} total nitrogen. Additionally, continuous wheat, corn-soybean-wheat rotation, and corn-wheat-red cover rotation treatments were not significantly different.

Similar to soil reaction and total nitrogen, the warm season grass treatment had the greatest available phosphorus content compared to the other treatments. However, available phosphorus exhibited very different patterns compared to the other soil chemical indicators which meant the significances were not observed among the rest of the cropping systems.

2.4.3 Biological Properties

Table 2–5 shows the soil organic carbon (SOC) content for all cropping systems which varied between 7.5 g kg^{-1} and 23.2 g kg^{-1}; significant differences were noticed for Tucker Prairie which had larger SOC compared to the warm season grass treatment. Although the warm season grass treatment had less SOC than the reference treatment, values were still significantly larger compared to the rest of the experimental cropping systems. In addition, there were no significant differences among continuous wheat, corn-soybean-wheat rotation, and corn-wheat-red cover rotation treatments. Continuous corn had the smallest SOC content.

Like soil organic carbon, active carbon (AC) data ranged from 113.1 mg kg^{-1} soil to 616.9 mg kg^{-1} and showed the amount of AC decreased in order of warm season grass, continuous timothy, corn-wheat-red cover rotation, corn-soybean-wheat rotation, continuous wheat, and continuous corn treatments, respectively. In addition, both warm season grass and Tucker Prairie treatments had no significant differences.

In the case of potentially mineralizable nitrogen (PMN), continuous timothy was not significantly different from the soil from Tucker Prairie, and it also showed a significantly greater level of PMN than the warm season grass treatment which had 145.3 mg kg^{-1} and 113.5 mg kg^{-1}, respectively. In contrast, continuous corn showed the smallest PMN value at 19.3 mg kg^{-1} which was smaller than that value for continuous timothy by about 7.5 times. Equally important, there were no significant difference among continuous wheat, corn-wheat-red cover rotation, and corn-soybean-wheat rotation treatments.

For soil microbial biomass, the greatest value was found in Tucker Prairie with 346,700 pmol g^{-1} while the amount of microbes in all cropping systems were smaller than those in Tucker Prairie. In addition, continuous timothy had the largest value for microbial biomass, followed by warm season grass, continuous wheat, corn-soybean-wheat rotation, corn-wheat-red cover rotation, and continuous corn treatments.

Table 2‒5. Statistical means with significant differences of chemical property in different cropping systems in the Sanborn Field as well as Tucker Prairie. Legend: Cont = continuous; C = corn; Rc = red clover; Sb = soybean; T = timothy; W = wheat; WSG = warm season grass; TP = Tucker Prairie. Means followed by lower case letters within the parenthesis indicated the significant differences according to TukeyHSD ($p < 0.05$).

Crop	**SOC†**	**AC†**	**PMN†**	**MB†**
	g kg^{-1}	mg kg^{-1}	mg kg^{-1}	pmol g^{-1}
ContC	7.5 (a)	113.1 (a)	19.3 (a)	48,682 (a)
ContT	20.8 (c)	463.7 (c)	145.3 (d)	177,869 (c)
ContW	11.3 (b)	179.3 (ab)	54.8 (b)	91,951 (b)
CSbW	12.4 (b)	231.3 (b)	59.7 (b)	74,925 (ab)
CWRc	11.6 (b)	254.3 (b)	63.9 (b)	73,818 (ab)
WSG	23.2 (d)	616.9 (d)	113.5 (c)	109,406 (b)
TP‡	33.3 (e)	585.4 (d)	162.4 (d)	346,700 (d)

† SOC, Soil organic carbon; AC, Active carbon; PMN, Potentially mineralizable nitrogen; MB, Microbial biomass (based on total PLFA content).
‡ Source: Data of Patricia Quackenbush.

2.4.4 Correlation and Principal Component Analysis

In this study, R-studio [version 1.1.149 at a 5% significance level (*)] was utilized to analyze the correlation between soil health indicators. Results are provided in pie charts in the upper right portion of the figure and numbers in the lower left portion. These charts represent the size of the relationship between variables or the relevance among interested parameters. In addition, the blue color of the diagram (pie chart and number) indicates a positive correlation while the red color refers to a negative correlation. The guidance intensity of the shade presented in the right side of the figure illustrates how strong the indicators are related.

From Fig. 2−3, bulk density is observed to have a strong negative correlation with most of the indicators (r = -0.59 to -0.71); in contrast, active carbon, soil organic carbon, total nitrogen, potentially mineralizable nitrogen, water-stable aggregates and microbial biomass were positively associated with each other (r = 0.60 to 0.96). However, soil reaction, phosphorus content and cation exchange capacity exhibited no correlation with other variables or a weak association (r = 0.00 to 0.44). Examples of negative correlation, strong and weak positive correlation, and uncorrelated relationships are shown in Fig. 2−4.

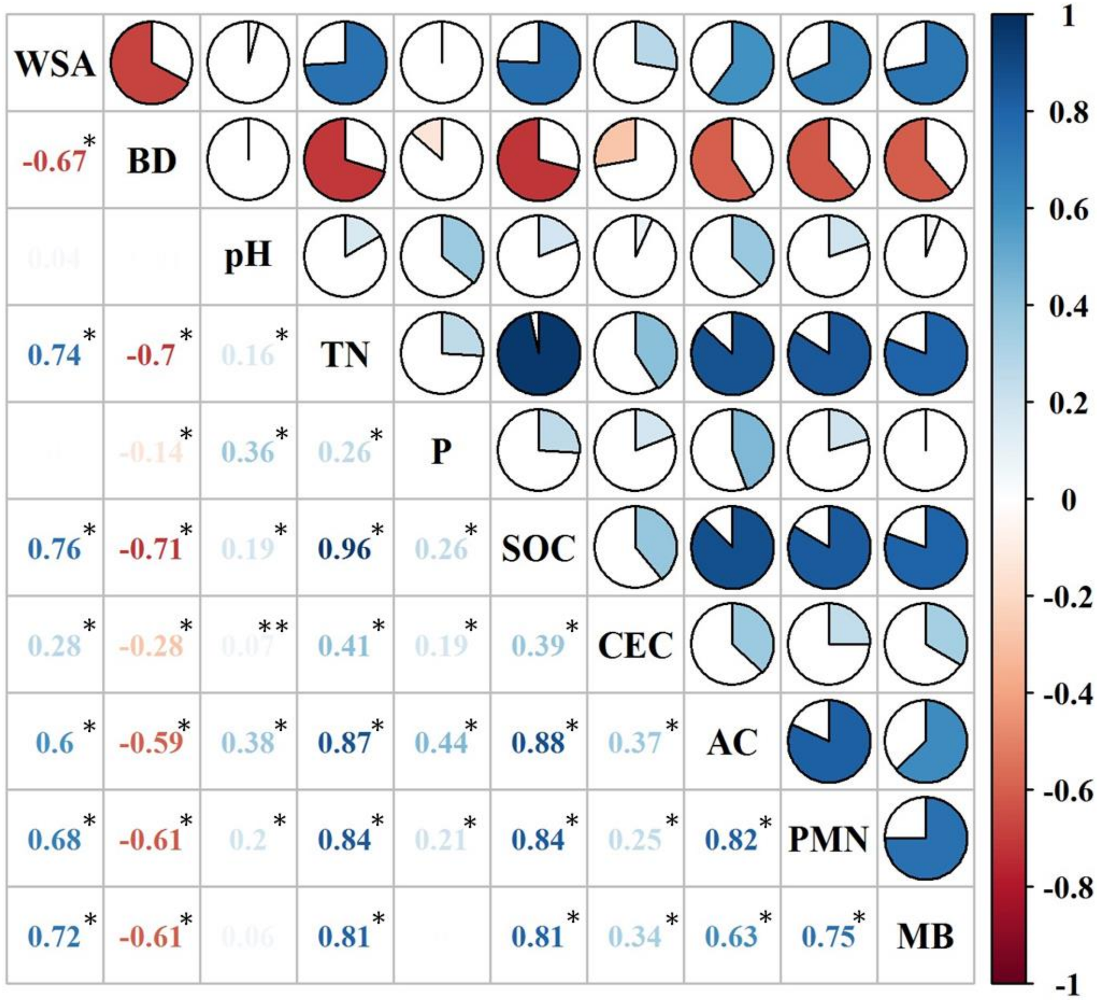

Fig. 2‒3. Correlation coefficients and pie charts for pairs of soil health indicators including water-stable aggregates (WSA), bulk density (BD), soil reaction (pH), total nitrogen (TN), phosphorus (P), soil organic carbon (SOC), cation exchange capacity (CEC), active carbon (AC), potentially mineralizable nitrogen (PMN) and microbial biomass (MB). Using Pearson correlation at 5% significance level.
(* significant at $p < 0.05$; ** significant at $p < 0.10$).

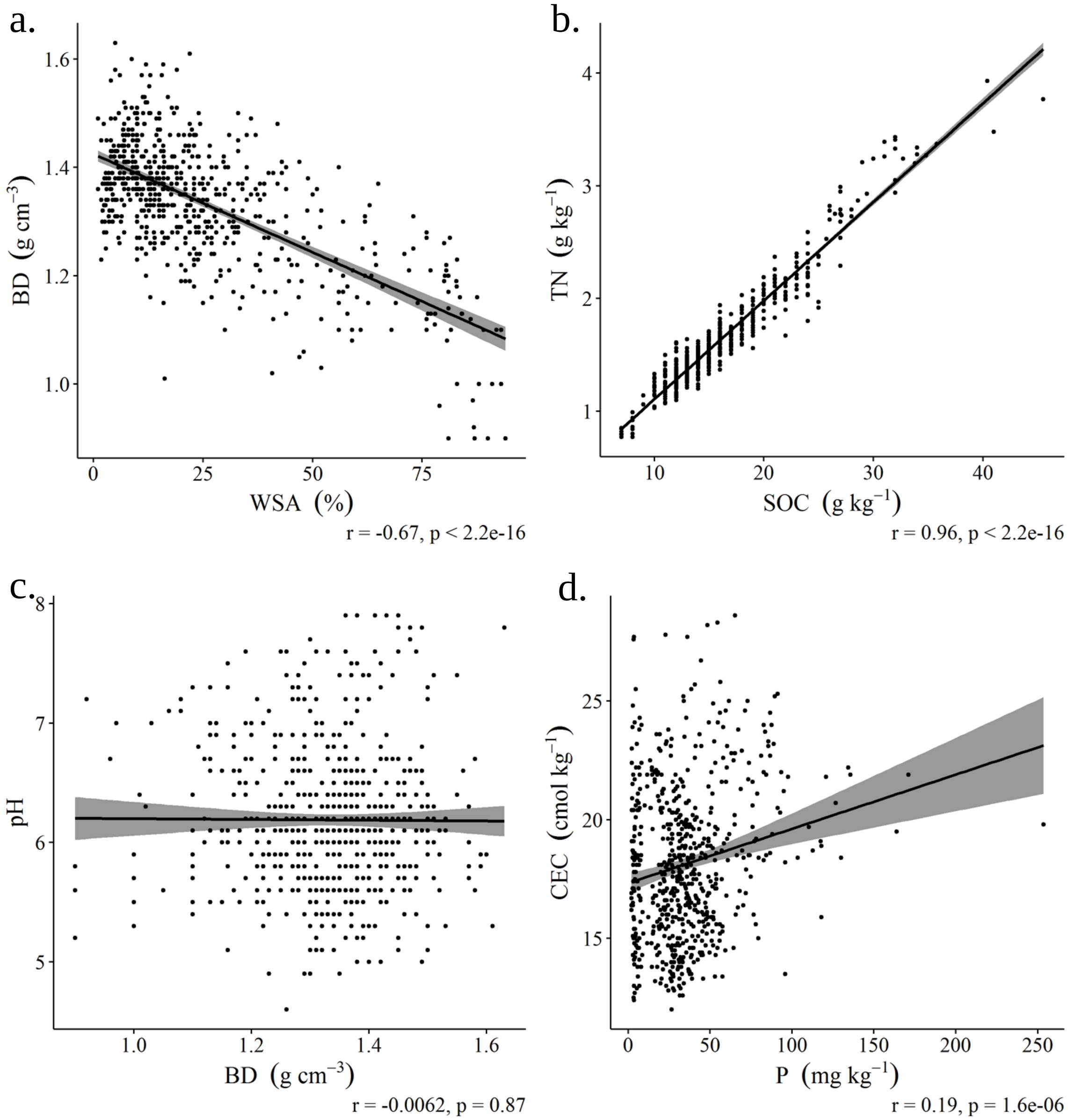

Fig. 2−4. Example of negative linear association between water-stable aggregates (WSA) and bulk density (BD) (a), example of strong positive linear association between soil organic carbon (SOC) and total nitrogen (TN) (b), example of no linear correlation between bulk density (BD) and soil reaction (pH) (c), example of weak positive linear correlation between phosphorus (P) and cation exchange capacity (CEC) (d). Using Pearson correlation at 5% significance level.

To understand the dimension of the correlation matrix, Fig. 2−5 was created using principal component analysis to explain nearly 72% of the total variance. This analysis allowed visualization of the association lines among the variables. The first principal component almost completely correlated with soil organic carbon, total nitrogen, and potentially mineralizable nitrogen. The second component correlated very highly with active carbon, while the third component was highly correlated with water-stable aggregates and microbial biomass. Finally, the last component had a high negative correlation with bulk density. As has been noted, most significantly correlated variables for soil health assessment in this study indicate a focus on water-stable aggregates, bulk density, soil organic carbon, active carbon, potentially mineralizable nitrogen, and microbial biomass.

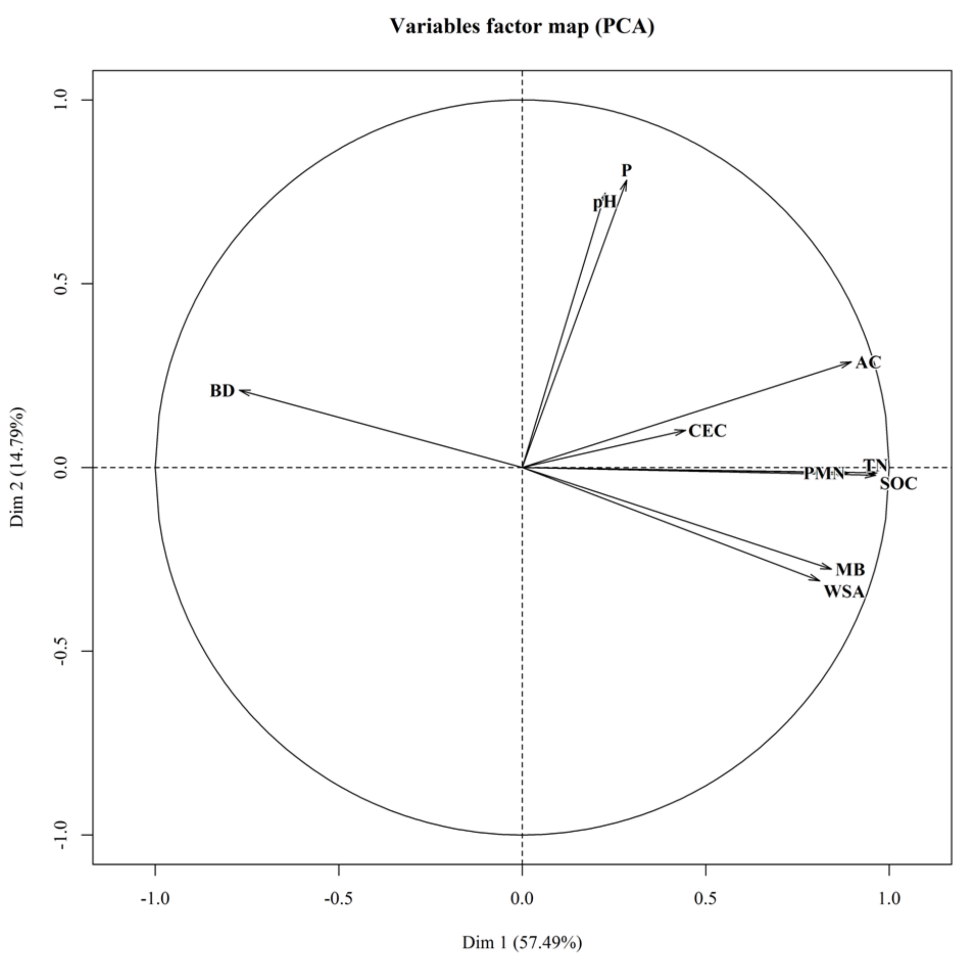

Fig. 2−5. Principal Component Analysis (PCA) map of soil health indicators including water-stable aggregates (WSA), bulk density (BD), soil reaction (pH), total nitrogen (TN), phosphorus (P), soil organic carbon (SOC), cation exchange capacity (CEC), active carbon (AC), potentially mineralizable nitrogen (PMN) and microbial biomass (MB).

2.4.5 Soil Health Scores

Overall soil health scores are presented in Fig. 2−6. After calculating soil health scores in every cropping system from the mean values of selected indicators (water-stable aggregates, bulk density, total nitrogen, soil organic carbon, active carbon, potentially mineralizable nitrogen and microbial biomass), continuous timothy and warm season grass treatments had scores of 88 and 86 which are considered as a very high level and similar to the soil health score of Tucker Prairie which was 97 out of 100 and considered as very high. Subsequently, continuous wheat, corn-wheat-red cover rotation, and corn-soybean-wheat rotation treatments were considered as low. Continuous corn had the lowest soil health score of 7 out of 100, considered as a very low level.

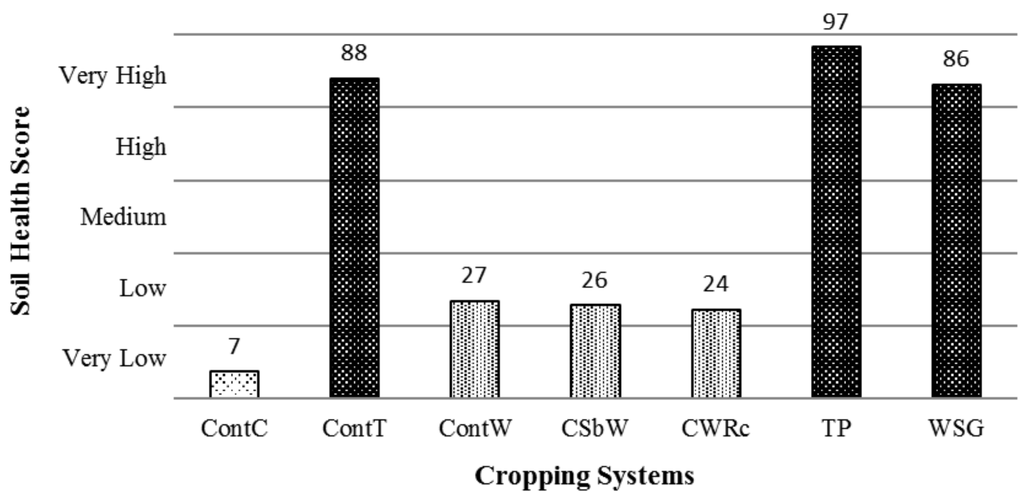

Fig. 2−6. Overall soil health scores ranged from 0 to 100 of selected cropping systems including continuous corn (ContC), continuous timothy (ContT), continuous wheat (ContW), corn-soybean-wheat rotation (CSbW), corn-wheat-red clover rotation (CWRc), warm season grass (WSG), and Tucker Prairie. Overall soil health scores calculated from the CND functions of water-stable aggregates, bulk density, soil organic carbon, active carbon, potentially mineralizable nitrogen, and microbial biomass (Appendix A).

It is not surprising that Tucker Prairie as a reference treatment had the greatest overall soil health score, classified as very high, calculated from all selected physical, chemical, and biological soil health indicators. After considering the results of the long-term effects of cropping systems with few inputs on soil health properties, the cropping systems on the Sanborn Field study could be divided into three groups. The first set had the overall soil health score as very high; it included continuous timothy and warm season grass treatments. The second group was comprised of continuous wheat and the crop rotations having levels of soil health scores at a low level. Continuous corn was the last group with a very low soil health score.

Continuous timothy and warm season grass treatments had the greatest overall soil health scores for cropping systems and were the top two systems having greater average values for each soil measurement. Similar results were obtained by Veum et al. (2013), who indicated that the greatest score of the soil management assessment framework method was found in perennial vegetation. A more current study of Veum et al. (2015) showed that perennial systems had the greatest scores of SMAF (93% − 97%) while annual cropping systems had scores of 78% − 92%. Since perennial vegetation has less disturbance plus greater coverage of the soil surface over time after reaching equilibrium, this treatment has greater stable aggregates and an ability to resist disruptive environmental factors of raindrop impact and air-blown particles directly hitting the soil surface, thus less erosion. Continuous timothy and warm season grass treatments are perennial vegetation treatments. The continuous timothy treatment has been infrequently tilled (usually during reseeding) compared to the other cultivated cropping systems (less disturbance); only tilled for re-planting every 8 to 10 years. The warm season grass treatment has been reserved to hopefully work to achieve a restored prairie plot to observe how well soil is returned to native prairie conditions. Supported by statistical results from this study, the warm season grass treatment is helping to restore soil health; for example, considering the physical properties of continuous timothy and warm season grass treatments, the water-stable aggregate means were 76% and 55% as well as the bulk density means were 1.18 and 1.20 g cm^{-3}, respectively. On the other hand, the other cultivated cropping systems had intermediate values of water-stable aggregates at 22% and a larger value of bulk density at 1.38 g cm^{-3}.

While continuous timothy and warm season grass treatments represented a very high soil health level, continuous corn had the overall soil health score of 7 described as very low. It also had the smallest average values in all soil properties since there were no nutrient inputs for years and the treatment has been plowed regularly and having less continual plant residue input from plant growth soil coverage than the other cropping systems with wheat and clover thus, stimulating erosion. Supporting these results, Tenge et al. (1998) observed that large corn yields have expanded the degree of erosion. In this case, it can be noted for soil biological properties that continuous corn had the microbial levels of only 48,682 pmol g^{-1} which was 7 times smaller than the Tucker Prairie treatment 346,700 pmol g^{-1}. Meanwhile, the previous studies of mono-cropping of corn in Canada showed that monoculture had lesser populations and less species diversity of arbuscular mycorrhiza fungi than the tree-based intercropping system (Bainard et al., 2012). Consequently, this system also affected the biological soil health property values which had smaller quantities of soil organic carbon, active carbon, and potentially mineralizable nitrogen than those of the other cropping systems.

Due to having overall low soil health scores, continuous wheat was categorized similar to the crop rotation treatments including corn-soybean-wheat rotation and corn-wheat-red cover rotation. In the case of continuous wheat, this treatment had small mean values of total nitrogen, potentially mineralizable nitrogen, soil organic carbon, and active carbon; in contrast, the treatment had larger mean values of water-stable aggregates, and microbial biomass. Due to the fibrous root system of the continuous wheat treatment, more continual vegetation/residue cover and being member of the grass system, this treatment promoted soil aggregate stability by binding soil particles, increasing porosity, forming organic matter, and raising microbial activities. Equally important, both crop rotations had similar property values and were often not significantly different in all soil health measurements in this experimental study. However, there were a tendency that the corn-soybean-wheat rotation treatment had greater soil properties than the corn-wheat-red clover rotation treatment because of the nitrogen addition from soybean cultivation from nitrogen fixation and the nitrogen use efficiency of this crop rotation system (Attia et al., 2015).

The limitation of this study was that there were no replicated plots since Sanborn Field was established before replicated field studies began. Therefore, the samples were collected four times to be used as the replication data. For this reason, the approach for collecting samples is a very important issue. To get the best representative sample, it should be planned systemically involving the environmental factors such as timing, season, sampling spot, as well as rotation vegetation at the selected time. Additionally, further studies of the diversity and function of microorganisms on long-term cropping systems can help develop a deep understanding of soil health assessment in the future.

2.5 SUMMARY AND CONCLUSIONS

Cropping systems have a major influence on soil health indicators. This study evaluated the relationship between cropping systems and soil health properties for a long-term experimental study. The results concluded that perennial grasses (continuous timothy and warm season grass) had the best capacity to maintain nutrients in the soil, followed by the crop rotations (corn-soybean-wheat and corn-wheat-red clover rotations) and the monoculture crop (continuous corn), respectively all of which had not nutrient inputs. After calculating simple correlation coefficients and creating the principal component analysis (PCA) diagram of all soil health indicators (water-stable aggregates, bulk density, soil reaction, total nitrogen, phosphorus, soil organic carbon, cation capacity exchange, active carbon, potentially mineralizable nitrogen and microbial biomass), only seven out of ten measurements including water-stable aggregates, bulk density, total nitrogen, soil organic carbon, active carbon, potentially mineralizable nitrogen and microbial biomass had significant linear associations to each other and were responsive soil health indicators for the assessment. Continuous timothy and warm season grass had overall soil health scores near that of the native prairie reference, followed in decreasing order by continuous wheat and all of the crop rotations with continuous corn being last. The results also suggest that discovering the relationship among the soil properties before evaluating the soil health assessment was a useful method to select the soil factors efficiently and accurately; however, a single soil property cannot account for the whole meaning of soil health assessment.

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3 EFFECTS OF LONG-TERM FERTILIZER AND MANURE APPLICATIONS ON SOIL HEALTH FOR CROPPING SYSTEMS OF SANBORN FIELD

3.1 ABSTRACT

Efficient management practices, together with applications of advanced technology inputs are required to intensify crop yields on limited existing agricultural land. Use of manure may improve soil physical, chemical, and biological properties. This study was conducted to evaluate the effects of long-term applications of management practices on soil health measurements by comparing the selected management systems including no fertilizer, full fertilizer, and manure applications. Full fertilizer means input of fertilizer by soil test to obtain a high yield. Different cropping systems, including continuous corn (*Zea mays* L.), continuous wheat (*Triticum aestivum* L.), corn-wheat-red clover (*Trifolium pretense* L.) rotation, and corn-soybean (*Glycine max* L.)-wheat rotation on the 130-year old Sanborn Field were used in this study. Results showed that manure application had the greatest effect on all soil health indicators and had the largest overall positive soil health score compared to full fertility and no fertilizer treatments. Moreover, continuous wheat with manure application presented the best combination of effects on soil properties with the largest score for most soil health indicators and an overall health score of 82 (out of 100), classified as very high. In addition, cropping systems should be considered as a whole since the soil management of each crop had different nutrient requirements. If fertilizer is applied at greater levels than needed, this practice may lead to deterioration of the environment instead of enriching and leaching of stored soil nutrients.

3.2 INTRODUCTION

The continuing increase in world population has led to increased demands for agricultural products and made food security a global issue (Godfray and Garnett, 2014). As a result, efficient management practices together with application of advanced technologies are required to increase crop yields on limited existing agricultural land.

The United States is one of the largest global producers of crops, especially in the row-crop intensive Midwest, in which the major crops are corn, soybean, and wheat. Therefore, large amounts of synthetic fertilizers are added to fields each year to increase or maintain existing crop yields as the soils in these cropped areas have been degraded physically, chemically, and biologically. For instance, Martínez et al. (2017) revealed that the application of synthetic nitrogen fertilizer affected corn yield in a 12-year experiment and recommended using 203 kg N ha^{-1} to gain maximum grain yield of 15 Mg ha^{-1}. However, Zhang et al. (2014) studied corn production using manure and chemical fertilizer. Their study found that after 12 years of a long-term experiment, the manure addition treatments had increased corn yield greater than the chemical fertilizer treatment due to slow nutrient release characteristics. Another recent study also observed that there were increasing corn yields in the first twelve years when N-P-K fertilizers were applied (Li et al., 2017).

While applying fertilizers has a benefit for users to raise their crop yields, an excessive use of this process may also have a negative impact on soil properties. For example, Jafar and Behzad (2016) studied the effects of fertilizers that resulted in soil compaction of wheat fields. This study showed that bulk density changed from 1.34 to 1.80 g cm^{-3} which decreased water permeability by 81.4%, available water by 34%, and yields by 40%. In addition, the change of microbial diversity can be positively affected by nitrogen fertilizer application, especially in fungal species (Sarathchandra et al., 2001).

Currently, there are many different types of soil amendments on the market which users can add to the soil to support plant growth; inorganic and organic. In fact, almost all producers have decided to use chemical fertilizers because of the nutrient content, low-price, immediate crop response and ready availability of the applied nutrients for plant use (Holeplass et al., 2004; Yang et al., 2016). Meanwhile, most manure applications provide a source of slow release nutrients but have additional beneficial impacts on soil. For instance, this system improves soil physical conditions by reducing bulk density, enhancing aggregate stability, and increasing water holding capacity (Haynes and Naidu, 1998).

Sanborn Field is a long-term research experiment which has selected soil management and cropping systems. This site was utilized as the experimental area in this study. This research focused on the effects of long-term manure and fertilizer applications on soil health measurements by comparing these management systems which include no fertilizer, full fertilizer, and manure applications. Cropping systems for this study included continuous corn, continuous wheat, corn-wheat-red clover rotation, and corn-soybean-wheat rotation, so the long-term impact on soil health indicators can be observed for these treatments.

3.3 MATERIALS AND METHODS

3.3.1 Plot Selection

The study was conducted on the long-term Sanborn Field experiment at the University of Missouri-Columbia located in Columbia, Missouri, USA (38°94N, 92°32W). Since establishment, one of the purposes of the study was to observe and measure the effects of manure management on selected cropping systems and rotations (Wagner, 1989). There are different types of cropping systems and management practices for the plots with the historical information given in Table 3−1 (Brown and Wyman, 1989; Miles and Brown, 2011). The tillage and cropping management for all of the plots on Sanborn Field are as follows. Each plot is moldboard plowed to a depth of 20 cm. After plowing, a disk harrow is used to incorporate any pre-plant fertilizer, agricultural lime, and manure applications to a depth of 5−8 cm while also providing a firm seedbed for planting the designated crop seed. Most soil resources on Sanborn Field are a poorly-drained claypan soil classified as a Mexico silt loam (fine, smectitic, mesic Vertic Epiaqualf), and the other soils include Mollic Epiaqualfs and Cumulic Epiaquolls (Miles and Hammer, 1989; Miles and Brown, 2011). In general, the field is derived from loess over glacial till, and the typical area is slightly rolling in topography (Veum et al., 2013). Subsurface soil includes a well expressed argillic horizon which influences the water holding capacity and rate of water movement.

Table 3−1. Historical information of cropping systems and management practices of selected plots from the Sanborn Field established in 1888†. Legend: Cont = continuous; C = corn; O = oat; Sb = soybean; Rc = red clover; W = wheat; Rc/Ls = red clover with lespedeza; W(Rc) = wheat (green manure; red clover).

Plot No.	Cropping System	Years	Management and Practices (N−P−K in kg ha^{-1} per year)
2	ContW	1888−1927	56−9.9−18.6
	ContW	1928−1949	56−9.9−18.6 (1/3 N fall; 2/3 N spring)
	ContW	1950−1989	22−9.9−18.6 (starter); 34−0−0 (topdressed in spring)
	ContW	1990−present	Full Fertility
6	Rc	1888−1913	Manure;13.5 Mg ha^{-1} per year
	Cowpeas	1914−1927	Manure;6.7 Mg ha^{-1} per year
	COWRc	1928−1949	18−36−18 on C and W, 16−0−0 on C, 0−30−0 on O
	ContC	1950−1989	6−24−24 (starter); 112 kg ha^{-1} on N (plowed down), 37 kg ha^{-1} on N (sidedressed)
	ContC	1990−present	Full Fertility
9	ContW	1888−present	No nutrient inputs
10	ContW	1888−present	Manure; 13.4 Mg ha^{-1} per year
17	ContC	1888−present	No nutrient inputs
18	ContC	1888−present	Manure; 13.4 Mg ha^{-1} per year
25	CWRc	1888−1939	Manure; 13.4 Mg ha^{-1} per year
	C,W,Rc/Ls	1940−1949	Manure; 13.4 Mg ha^{-1} per year
	CWRc	1950−1989	Manure; 13.4 Mg ha^{-1} per year
	CWRc	1990−present	Manure; 13.4 Mg ha^{-1} per year with 112−0−0 on C, 37−0−0 on W
26	CWRc	1888−1913	Manure; 13.4 Mg ha^{-1} per year
	CWRc	1914−1927	Manure; 20.2 Mg ha^{-1} per year
	CWRc	1928−1939	Lime, 0−16−0 on C and W
	C,W,Rc/Ls	1940−1949	Lime, 0−16−0 on C and W
	CWRc	1950−present	Full Fertility
27	CWRc	1888−1939	No nutrient inputs
	C,W,Rc/Ls	1940−1949	No nutrient inputs
	CWRc	1950−present	No nutrient inputs
34	COWRc	1888−1939	Manure; 13.4 Mg ha^{-1} per year
	C,O,W,Rc/Ls	1940−1949	Manure; 13.4 Mg ha^{-1} per year
	COWRc	1950−1989	Manure; 13.4 Mg ha^{-1} per year
	CSbW(Rc)	1990−present	Manure; 13.4 Mg ha^{-1} per year
35	COWRc	1888−1913	No nutrient inputs
	COWRc	1914−1939	No nutrient inputs
	C,O,W,Rc/Ls	1940−1949	No nutrient inputs
	COWRc	1950−1989	No nutrient inputs
	CSbW(Rc)	1990−present	No nutrient inputs
37	COWRc	1888−1913	Manure; 13.4 Mg ha^{-1} per year
	COWRc	1914−1927	10−15−11 on C, 7−10−7 on W
	COWRc	1928−1939	9−12−7 on C and W
	C,O,W,Rc/Ls	1940−1949	9−12−7 on C and W
	COWRc	1950−1989	9−12−7 on C and W
	CSbWRc	1990−present	Full Fertility

† Enhancement of Brown and Wyman (1989) and Miles and Brown (2011).

To study soil management effects, the following treatments were evaluated: no fertilizer, full fertility, and manure applications. These treatments were evaluated for soil health properties. Twelve plots were chosen as experimental units. These were continuous wheat (plot 9−no fertilizer, plot 10−manure, and plot 2−full fertility), continuous corn (plot 17−no fertilizer, plot 18−manure, and plot 6−full fertility), corn-wheat-red clover rotation (plot 27−no fertilizer, plot 25−manure, and plot 26−full fertility), and corn-soybean-wheat rotation with red clover as a green manure (plot 35−no fertilizer, plot 34−manure, and plot 37−full fertility), and Tucker Prairie (TP) used as the reference area of healthy soil.

3.3.2 Field Sampling

Four replicate soil samples were collected on each of four dates, 8th May 2014, 4th September 2014, 1st April 2016 and 18th August 2016 from six selected plots resulting in a total of 96 samples. The dimensions of each plot is 30.55 m by 9.42 m (Miles and Brown, 2011). For this study, four samples from the 0−10 cm depth were taken from the representative area, 15.28 m by 3.14 m of a rectangle in the center of each plot, to avoid intra-plot variability (Fig. 3−1). To obtain a representative sample of the plot, sampled points were chosen to have a consistent feature such as avoiding being too wet or too dry and unusual area as much as possible in term of crop types and management practices with distance away for "border effects". Aluminum rings (76 mm diameter) were used to collect soil samples during the selected sampling times during each year. Surface litter on the soil was removed before sampling with the aluminum rings. Following sampling preparation, the core was driven into soil by hammering on a woodblock that was placed on the top of the core. The next steps were digging the core out with a shovel and trimming the protruded soil and plant roots. Finally, the soil sample was placed in a labeled plastic bag to maintain the field moisture content of the sample for determination of gravimetric water content at sampling before laboratory analyses.

Fig. 3−1. Soil sampling approach of the studied plots; point A, B, C and D are the collected points in each experiment. Plot 2 and 6 followed the points on horizontal plots in figure (right side) but other plots followed the points on vertical plots in figure (left side).

3.3.3 Laboratory Analyses

At the Soil Health Assessment Center (SHAC), a soil health testing laboratory located in Columbia, Missouri, soil samples were promptly processed to avoid any time-affected factors in the analysis. The samples were divided into two portions with one portion immediately freeze-dried for phospholipid fatty acid analysis. For laboratory sample preparation, the rest of the soil portions were weighed to determine gravimetric water content at time of sampling. After that, the soil samples were air-dried at room temperature, ground with a mortar and pestle, and passed through a 2-mm sieve for the rest of the analyses (Soil Survey Staff, 2014).

To assess soil physical conditions resulting from past management, bulk density was measured to relate to the level of soil compaction (Keller and Håkansson, 2010). From these known volumes of the core, bulk density was computed by the ratio of oven-dry weight of soil sample to soil volume (Blake and Hartge, 1986). An additional physical indicator was water-stable aggregates, which refers to soil aggregates that were able to resist disruption such as tillage (Kumar et al., 2014) and erosion (Holifield Collins et al., 2015). To determine water-stable aggregates, soil aggregate retention was measured by placing the soil sample on a 0.5-mm sieve, immersing it in water overnight, and agitating the samples (raising and lowering the sieve manually). Afterward, the samples were dried and weighed for the final calculation for the percent of water stable aggregates (Kemper and Rosenau, 1986).

Nutrient analyses were performed to relate to soil nutrient availability status. These analyses included soil pH in water and salt (0.01M $CaCl_2$) which indicates the level of acidity and alkalinity and affects nutrient availability in the soil for phosphorus and potassium (Scanlan et al., 2017). The pH meter (Ross Sure-Flow Electrodes) was used to measure a 1:1 soil:deionized water suspension (Soil Survey Staff, 2014) after shaking for an hour. The Bray I extractant of ammonium fluoride and a dilute solution of hydrochloric acid was used to extract available phosphorus. 2 grams of soil with 25 ml of extracting solution were shaken for 10 minutes. Afterward, the samples were allowed to settle before filtering to take the aliquot. Extracted phosphorus was measured using a spectrophotometer (Spectronic $20D^+$) after the blue color intensity developed from using the molybdate-ascorbic acid reagent (Frank et al., 1998). The determination of total nitrogen content in the samples was performed by a LECO FP-528 nitrogen combustion analyzer. This analyzer involved the assessment of the volume of nitrogen (N_2) content, measured by thermal conductivity (Soil Survey Staff, 2014). The cation exchange capacity (CEC) was determined by the 1M ammonium acetate method at pH 7. The soil samples were saturated with the ammonium acetate solution, which exchanged with the existing exchangeable cations in the soil; after reaction, the quantity of exchangeable ammonium acetate was determined (Soil Survey Staff, 2014).

Soil biological properties were also assessed. First, soil organic carbon content was evaluated by a LECO C-144 carbon analyzer using combustion with measurement of the carbon dioxide (CO_2) mass. The principle of the analysis is that when the soil sample was introduced and combusted in an oxygen atmosphere, the mass of present CO_2 was determined (Soil Survey Staff, 2014). The evaluation of active carbon in the soil was performed by a potassium permanganate method, which is a moderate oxidant (0.2 M potassium permanganate) used to oxidize organic matter in the soil. The change of purple color was measured by a spectrophotometer by the Agilent Cary 60 UV-Vis spectrophotometer (Weil et al., 2003). In addition, an anaerobic method was utilized to estimate the potentially mineralizable nitrogen content, which indicated the available nitrogen in the soil. Approximately 8 grams of soil samples were placed in 50 ml Falcon tubes with deionized water and incubated at 40°C under anaerobic conditions for 7 days. The potentially mineralizable nitrogen contents were measured with the ammonium nitrogen produced after a week of anaerobic incubation using the Spectronic $20D^+$ spectrophotometer (Waring and Bremner, 1964). Phospholipid fatty acids (PLFAs) analysis is one of the most common methods used to provide the information of microbial biomass in soil in soil. The Bligh-Dyer extractant was used to extract lipids from freeze-dried soil samples. Then, the phospholipids were separated using solid phase extraction techniques and transesterification of the PLFAs. Finally, gas chromatography (Agilent Technologies 7890A GC System) was used to measure fatty acid methyl esters (Buyer and Sasser, 2012).

Table 3−2. Summary of soil health laboratory analyses including physical, chemical and biological properties and their analytical methods.

Property	Indicator	Abbrev.	Method	Reference
Physical Properties	Bulk Density	BD	Core method	Blake and Hartge, 1986
	Water Stable Aggregates	WSA	Wet-Sieving	Kemper and Rosenau, 1986
Chemical Properties	Cation Exchange Capacity	CEC	1M ammonium acetate at pH7	Soil Survey Staff, 2014
	Phosphorus	P	Bray 1	Frank et al., 1998
	Potential of Hydrogen	pH	1:1 soil:water	Soil Survey Staff, 2014
	Total Nitrogen	TN	Dry combustion by LECO FP-528 nitrogen analyzer	Soil Survey Staff, 2014
Biological Properties	Active Carbon	AC	Potassium permanganate test	Weil et al., 2003
	Soil Organic Carbon	SOC	Dry combustion by LECO C-144 carbon analyzer	Soil Survey Staff, 2014
	Potentially Mineralizable Nitrogen	PMN	Seven-days anaerobic incubation test at 40°C	Waring and Bremner, 1964
	Microbial Biomass	MB	Bligh-Dyer lipid extraction	Buyer and Sasser, 2012

3.3.4 Statistical Analyses

After all data were obtained from laboratory analyses, the general approach of data analysis consisted of two main steps: 1) calculating the correlation coefficients and doing a Principal Component Analysis (PCA) among all of the indicators (bulk density: BD, water-stable aggregates: WSA, cation exchange capacity: CEC, available phosphorus: P, soil reaction: pH, total nitrogen: TN, active carbon: AC, soil organic carbon: SOC, potentially mineralizable nitrogen: PMN, and microbial biomass: MB) for all treatments, and 2) assessing significant differences for the properties among cropping systems. All steps were performed using the R-studio statistical package (version 1.1.149).

In the beginning, the first step was calculating the Pearson correlations between each pair of properties to observe the trends in relationships. The next step utilized Principal Component Analysis (PCA) to abate the number of variables, but still maintain most of the information in the previous models.

Brown (1994) reported that the experimental plots in Sanborn Field had no replication since it was established in 1888 before Fisher statistical methods with traditional plot replications were used. Repeated measures analysis of variance (Repeated Measures ANOVA), within-subjects ANOVA and hypothesis testing, were used to run significant difference tests among the means of the independent variables since the samples were collected and measured repeatedly over multiple times. The forms of repeated measures ANOVA consisted of one-way repeated measures ANOVA and two-way repeated measures ANOVA, such that the best appropriate model under this objective was one-way repeated measures ANOVA. The assumptions of this model were that the sampling data were repeated, the dependent variable must be a quantitative (interval or ratio) variable, the independent variables were qualitative (nominal or ordinal) variables, the dependent variables should be normally distributed, and the variables should be a sphericity, having equal variance. Additionally, a Shapiro-Wilk method was used to test the normality and a Levene's method was used to test the sphericity. The Shapiro-Wilk test started by creating a null hypothesis: the variable was from a normal distribution, and an alternative hypothesis, the variable was from other distributions. Following these steps, the next step was choosing a 5% significance level or alpha ($\alpha = 0.05$), selecting type I error for the test, and calculating the statistic W and p-value. If the p-value was more than the significance level of 0.05, the null hypothesis was accepted. Similar to the Shapiro-Wilk test, Levene's test also had similar steps like the other test. However, the null hypothesis was changed so variances are equal or homogeneity of variance, and the test statistic was replaced by an F-test. If the F-statistic value was greater than the critical value or the p-value was smaller than the significance level, the alternative hypothesis was accepted. After testing all assumptions, the significant difference test was the last process for the statistical analysis. This process used the Tukey Honest Significant Differences (TuckeyHSD) method through R-studio. Finally, the outcomes presented the significant groups of independent variables.

3.3.5 Soil Health Assessment

To provide a relative soil health assessment of Sanborn Field plots, the approach from the Cornell Comprehensive Assessment of Soil Health (CASH) (Moebius-Clune et al., 2016) was utilized. Before the calculating soil health index for all physical, chemical and biological properties, the first step was creating the cumulative normal distribution (CND) function and curve of each indicator in order to provide the soil health score index by using the raw data from laboratory analyses and results from the statistical program R-studio version 1.1.149. The soil health score ranged from 0 to 100 (with 100 the best), which was divided into five even intervals; scores 0−20, 20−40, 40−60, 60−80, and 80−100. All scales were represented in the diagram by five colors in five meanings; red described as Very Low, orange described as Low, yellow described as Medium, light green described as High, and dark green described as Very High, respectively.

Three scoring types were used in the procedure of the soil health assessment.

1) More is Better

In this case, if the mean value of each measured indicator is large, the score will be large as well. The indicators in this category were comprised of water-stable aggregates, total nitrogen, soil organic carbon, cation exchange capacity, active carbon, potentially mineralizable nitrogen, and microbial biomass.

2) Less is Better

Only bulk density fell into this scoring type. Additionally, less is better means that the smaller the measured value, the larger the score is observed.

3) Optimum Curve

Optimum curve was applied with both pH and available phosphorus. The feature of this curve consists of two parts which are positive scoring curve and negative scoring curve; moreover, the score of 100 must be larger than the 0 unit of measurement but smaller than 100 units of measurement. In other words, the curve has a bell shape regardless of skewness.

Fig. 3−2, for example, showed the cumulative normal distribution curve of active carbon and consisted of the horizontal axis presenting the amount of active carbon (mg kg^{-1}), the vertical axis presenting the score ranging from 0 to 100. If a soil sample has active carbon of 400 mg kg^{-1}, the soil health score will equal to 66 which is considered as high-level score.

Subsequently, an overall soil health score is calculated by summing soil health scores from the cumulative normal distribution function of each indicator and dividing the sum by number of the indicators. In addition, the overall score also used the soil health score range from 0 to 100 as well as the ratings terms of soil health scores.

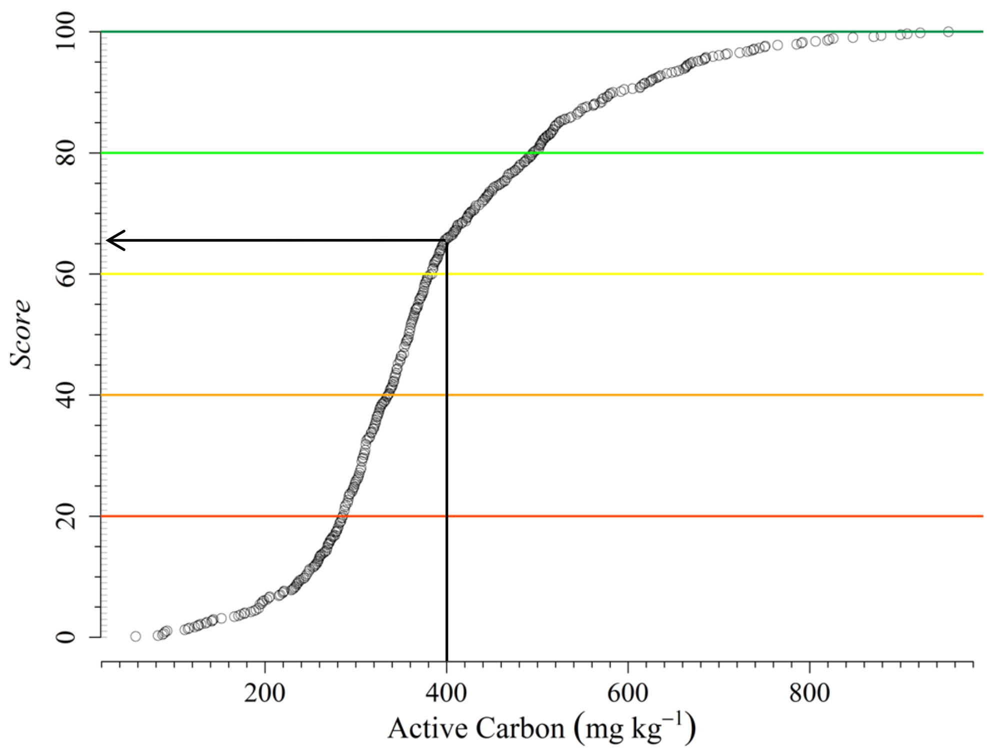

Fig. 3−2. Cumulative normal distribution curve of active carbon of the collected Sanborn Field and Tucker Prairie soil samples.

3.4 RESULTS AND DISCUSSION

The results are divided into two types of tables, the first table in each scenario shows the mean of each measured indicator for the selected management practices: no fertilizer (NF), full fertility (FF) and manure (M), and the second table of each scenario shows the means of these properties for the selected cropping systems: continuous corn, continuous wheat, corn-soybean-wheat rotation, and corn-wheat-red clover rotation treatments.

3.4.1 Physical Properties

The means of soil physical soil indicators including water-stable aggregates and bulk density in selected management practices averaged over cropping systems are shown in table 3−3 while the means regarding each cropping system and fertilizer management system averaged are shown in table 3−4.

Table 3−3. Statistical means with significant differences of physical property in different management practices in the Sanborn Field. Legend: NF = no fertilizer; FF = full fertility; M = manure. Means followed by lower case letters within the parenthesis indicated the significant differences according to TukeyHSD ($p < 0.05$).

Treatment	WSA†	BD†
	%	g cm^{-3}
NF	15 (a)	1.40 (b)
FF	17 (a)	1.36 (ab)
M	25 (b)	1.33 (a)

† WSA, Water-stable aggregates; BD, Bulk density.

The water-stable aggregates values ranged from 15% to 25%. This property was significantly greater difference for the manure treatment compared to the other treatments; the values were greatest in the manure treatment (25%), followed by full fertility and no fertilizer treatments which had 17% and 15%, respectively. When considering the cropping systems (Table 3−4), the manure treatment still had the greatest mean values of water-stable aggregates in every cropping system and significantly different from the others except in the continuous wheat treatment. Meanwhile, the no fertilizer treatment had the smallest mean values in all indicators for all cropping systems, including continuous corn, continuous wheat, and corn-wheat-red clover rotation treatments but in the case of the corn-soybean-wheat rotation with full fertility treatment had the smallest means.

Another measurement of physical soil health indicators was bulk density. Regardless of cropping system, manure had the smallest bulk density of 1.33 g $cm^{-3}$and was significantly smaller than the largest, which was for the no fertilizer treatment with a value of 1.40 g cm^{-3} (Table 3−3). Supported by information in table 3−4, the manure plots of continuous corn, continuous wheat, and corn-wheat-red clover rotation had the smallest value of bulk density. In addition, the means for bulk density of both manure and full fertility management had no significant differences for all cropping systems.

Table 3−4. Statistical means with significant differences of physical property for the selected management practices for each cropping system in Sanborn Field. Legend: NF = no fertilizer; FF = full fertility; M = manure; Cont = continuous; C = corn; Rc = red clover; Sb = soybean; W = wheat. Means followed by lower case letters within the parenthesis indicated the significant differences according to TukeyHSD ($p < 0.05$).

Crop	Treatment	WSA†	BD†
		%	g cm^{-3}
ContC	NF	8 (a)	1.41 (b)
	FF	12 (a)	1.39 (ab)
	M	21 (b)	1.34 (a)
ContW	NF	22 (a)	1.40 (b)
	FF	28 (a)	1.35 (ab)
	M	28 (a)	1.30 (a)
CSbW	NF	18 (ab)	1.41 (a)
	FF	14 (a)	1.37 (a)
	M	22 (b)	1.38 (a)
CWRc	NF	12 (a)	1.38 (b)
	FF	13 (a)	1.33 (a)
	M	29 (b)	1.30 (a)

† WSA, Water-stable aggregates; BD, Bulk density.

3.4.2 Chemical Properties

The mean values of soil reaction (pH), cation exchange capacity (CEC), total nitrogen (TN), and available phosphorus (P), considered as the chemical soil health properties in this experimental study, are shown in table 3−5 (averaged across cropping systems) and table 3−6 (for each cropping system).

Without considering cropping system (Table3−5), the means of the no fertilizer treatment had the smallest values for every chemical indicator; pH of 5.5 as moderately acid, total nitrogen of 1.15 g kg^{-1}, available phosphorus of 5.2 mg kg^{-1} and cation exchange capacity of 17.7 $cmol_c$ kg^{-1}. In contrast, the manure treatment always had the largest soil nutrient content compared to the others such as pH of 6.9 as neutral and total nitrogen of 1.78 g kg^{-1}. Obviously, for available phosphorus, the means had a large interval from 5.2 mg kg^{-1} to 56.2 mg kg^{-1} as the soil test phosphorus mean of manure was ten times greater than the mean of plots without fertilizer.

Table 3−5. Statistical means with significant differences of chemical property in different management practices in the Sanborn Field. Legend: NF = no fertilizer; FF = full fertility; M = manure. Means followed by lower case letters within the parenthesis indicated the significant differences according to TukeyHSD ($p < 0.05$).

Treatment	pH	TN†	P†	CEC†
		g kg^{-1}	mg kg^{-1}	$cmol_c$ kg^{-1}
NF	5.5 (a)	1.15 (a)	5.2 (a)	17.7 (a)
FF	5.7 (a)	1.45 (b)	37.0 (b)	18.0 (b)
M	6.9 (b)	1.78 (c)	56.2 (c)	19.8 (c)

† TN, Total nitrogen; P, Phosphorus; CEC, Cation exchange capacity.

Concerning cropping system as a factor (Table 3−6), soil reaction (pH) ranged from 5.2 to 7.4 or strongly acid to slightly alkaline. No fertilizer treatment gave the smallest pH means for every crop compared to the other fertilizer management treatments, and the soils without any fertilizer were in the strongly acid and moderately acid classes. There were no significant differences between no fertilizer and full fertility treatments in continuous corn. In the case of applying manure, the soil pH means were all greater than the other treatments and the classes of pH (USDA-NRCS) shifted from strongly and moderately acid to slightly acid and slightly alkaline levels. For example, in continuous corn plot without fertilizer there was a pH of 5.4 as strongly acid which was more acid than the pH of 7.4 as slightly alkaline in the plot of corn with manure.

Similar to soil reaction (pH), all total nitrogen means for no fertilizer plots were smaller than those of full fertilizer and manure, respectively, for each cropping system in this study. For instance, the means of corn-wheat-red clover rotation plots were arranged in descending order as 1.79 g kg^{-1} in manure treatment, 1.38 g kg^{-1} in full fertility treatment, and 1.22 g kg^{-1} in no fertilizer. Moreover, there were significant statistical differences among the three treatments for all cropping systems.

Table 3−6. Statistical means with significant differences of chemical property in different management practices in the Sanborn Field regarding to cropping system. Legend: NF = no fertilizer; FF = full fertility; M = manure; Cont = continuous; C = corn; Rc = red clover; Sb = soybean; W = wheat. Means followed by lower case letters within the parenthesis indicated the significant differences according to TukeyHSD (p < 0.05).

Crop	Treatment	pH	TN†	P†	CEC†
			g kg^{-1}	mg kg^{-1}	$cmol_c$ kg^{-1}
ContC	NF	5.4 (a)	0.85 (a)	6.5 (a)	21.0 (ab)
	FF	5.3 (a)	1.39 (b)	36.8 (b)	18.4 (a)
	M	7.4 (b)	1.55 (c)	71.9 (c)	23.3 (b)
ContW	NF	5.2 (a)	1.25 (a)	4.5 (a)	18.6 (a)
	FF	6.0 (b)	1.69 (b)	24.5 (b)	20.8 (b)
	M	6.5 (c)	2.10 (c)	72.2 (c)	22.0 (b)
CSbW	NF	5.7 (a)	1.26 (a)	5.6 (a)	17.0 (a)
	FF	5.9 (b)	1.36 (b)	45.1 (b)	18.4 (a)
	M	7.1 (c)	1.67 (c)	37.4 (b)	16.6 (a)
CWRc	NF	5.7 (a)	1.22 (a)	4.4 (a)	14.2 (a)
	FF	5.9 (b)	1.38 (b)	41.4 (b)	14.7 (a)
	M	7.1 (c)	1.79 (c)	43.4 (b)	17.5 (b)

† TN, Total nitrogen; P, Phosphorus; CEC, Cation exchange capacity.

The third column of table 3−6 shows the soil test phosphorus means of the different cropping systems under different soil fertility treatments. Under a monoculture system, continuous corn and continuous wheat had significant differences of soil test phosphorus among the treatments, means of the manures were the greatest value, followed by plots with full fertility and plots without fertilizer, respectively. Additionally, the largest values were about 66 mg kg^{-1} larger for manure (71.9 mg kg^{-1}) than the smallest values with no fertilizer with 6.5 mg kg^{-1}. On the other hand, soil test phosphorous was significantly smaller for the no fertilizer treatment compared to the other fertilizer treatments for the corn-soybean-wheat rotation and corn-wheat-red clover rotation. The full fertility plots had generally the same soil test phosphorus values as plots with manure.

For cation exchange capacity indicator (CEC), the means were not quite different when compared within the cropping systems, and the values ranged from 14.2 $cmol_c\ kg^{-1}$ to 23.3 $cmol_c\ kg^{-1}$. Besides, all means of continuous corn and continuous wheat were greater than all means of the crop rotations. According to table 3−6, the data showed that all treatments of rotated crops had larger mean values of CEC than continuous crops.

3.4.3 Biological Properties

The measurements of biological soil health properties including soil organic carbon, active carbon, potentially mineralizable nitrogen, and microbial biomass are presented in table 3−7 and table 3−8. Table 3−7 showed the means of each indicator regardless of cropping system, yet table 3−8 showed the means with regard to cropping system for selected soil fertility management.

Table 3−7. Statistical means with significant differences of biological property in different management practices in the Sanborn Field. Legend: NF = no fertilizer; FF = full fertility; M = manure. Means followed by lower case letters within the parenthesis indicated the significant differences according to TukeyHSD ($p < 0.05$).

Treatment	SOC†	AC†	PMN†	MB†
	$g\ kg^{-1}$	$mg\ kg^{-1}$	$mg\ kg^{-1}$	$pmol\ g^{-1}$
NF	10.7 (a)	194.5 (a)	49.4 (a)	72,344 (a)
FF	14.0 (b)	354.6 (b)	76.2 (b)	90,902 (b)
M	17.5 (c)	476.2 (c)	94.7 (b)	121,854 (c)

† SOC, Soil organic carbon; AC, Active carbon; PMN, Potentially mineralizable nitrogen; MB, Microbial biomass (based on total PLFA content).

The average values of each indicator for no fertilizer, full fertility, and manure treatments were arranged in ascending order in terms of active carbon, 194.5 $mg\ kg^{-1}$ in no fertilizer, 354.6 $mg\ kg^{-1}$ in full fertility treatment, and 476.2 $mg\ kg^{-1}$ in manure treatment. Focusing on significant statistical differences, almost all biological soil health indicators, except potentially mineralizable nitrogen, were classified in different groups that were no fertilizer as group a, full fertility as group b, and manure as group c by the mean of biological contents. As for potentially mineralizable nitrogen, there were no significantly different values between full fertility and manure application treatments; in fact, both numerical averages were dissimilar.

Table 3‒8. Statistical means with significant differences of biological property in different management practices in the Sanborn Field regarding to cropping system. Legend: NF = no fertilizer; FF = full fertility; M = manure; Cont = continuous; C = corn; Rc = red clover; Sb = soybean; W = wheat. Means followed by lower case letters within the parenthesis indicated the significant differences according to TukeyHSD ($p < 0.05$).

Crop	Treatment	SOC†		AC†		PMN†		MB†	
		g kg^{-1}		mg kg^{-1}		mg kg^{-1}		pmol g^{-1}	
ContC	NF	7.5	(a)	113.1	(a)	19.3	(a)	48,682	(a)
	FF	12.8	(b)	309.2	(b)	68.5	(b)	70,576	(b)
	M	14.8	(c)	443.9	(c)	77.3	(b)	101,709	(c)
ContW	NF	11.3	(a)	179.3	(a)	54.8	(a)	91,951	(a)
	FF	16.6	(b)	482.7	(b)	102.7	(b)	129,706	(b)
	M	20.4	(c)	531.1	(c)	124.8	(c)	153,746	(c)
CSbW	NF	12.4	(a)	231.3	(a)	59.7	(a)	74,925	(a)
	FF	12.9	(a)	302.1	(b)	59.8	(a)	78,409	(a)
	M	16.5	(b)	440.9	(c)	81.5	(b)	108,131	(b)
CWRc	NF	11.6	(a)	254.3	(a)	63.9	(a)	73,818	(a)
	FF	13.6	(b)	324.4	(b)	74.0	(a)	84,916	(a)
	M	18.3	(c)	488.8	(c)	95.0	(b)	123,831	(b)

† SOC, Soil organic carbon; AC, Active carbon; PMN, Potentially mineralizable nitrogen; MB, Microbial biomass (based on total PLFA content).

The results when cropping system as a factor in this study was added were similar to those with fertilizer and manure input management. The soil organic carbon average ranged from 7.5 g kg^{-1} to 20.4 g kg^{-1}. Two interesting points for soil organic carbon were that continuous corn had the smallest amount compared to most other treatments, and all manure and full fertility treatments were larger compared to no fertilizer treatment for continuous corn, continuous wheat and corn-wheat-red clover rotation. The remaining cropping system, corn-soybean-wheat rotation, with no fertilizer treatment had the same statistically different group as the plot applied full fertility, but it differed significantly from the manure treatment.

Meanwhile for active carbon, there were significant differences among the three fertilizer treatments for each cropping system. By ordering in descending order, the manure treatment gave the greatest means among the treatments of 443.9, 531.1, 440.9 and 488.8 mg kg^{-1} in continuous corn, continuous wheat, corn-soybean-wheat rotation, and corn-wheat-red clover rotation, respectively. After manure treatment, the order was full fertility treatment followed by no fertilizer treatment for active carbon.

Similar to previous biological soil heath indicators, potentially mineralizable nitrogen and microbial biomass also had the distinctive mean order of different treatments as manure treatment, full fertility treatment and no fertilizer treatment; in addition, the largest means of both measurements occurred in the manure treatment. For instance, the potentially mineralizable nitrogen means of continuous wheat ranged from manure of 124.8 mg kg^{-1}, full fertility of 102.7 mg kg^{-1} and no fertilizer of 54.8 mg kg^{-1}, respectively. In sum, continuous corn without any input had the minimum mean of biological soil health properties, but continuous wheat with manure application had the greatest average content of biological soil health properties.

3.4.4 Correlation and Principal Component Analysis

In this study, R-studio [version 1.1.149 at a 5% significance level (*)] was utilized to analyze the correlation between soil health indicators. Results were provided in heatmap in the upper right portion of the figure and numbers in the lower left portion. These charts represent the size of the relationship between variables or the relevance among interested parameters. In addition, the blue color of the diagram (heatmap and number) indicates a positive correlation while the red color refers to a negative correlation. The guidance intensity of the shade presented in the right side of the figure illustrates how strong the indicators are related.

From Fig. 3−3, bulk density is observed to have a strong negative correlation with most of the indicators (r = -0.59 to -0.71); in contrast, active carbon, soil organic carbon, total nitrogen, potentially mineralizable nitrogen, water-stable aggregates and microbial biomass were positively associated with each other (r = 0.60 to 0.96). However, soil reaction, soil test phosphorus content and cation exchange capacity exhibited no correlation with other variables or a weak association (r = 0.00 to 0.44). Examples of negative correlation, strong and weak positive correlation, and uncorrelated relationships are shown in Fig. 3−4.

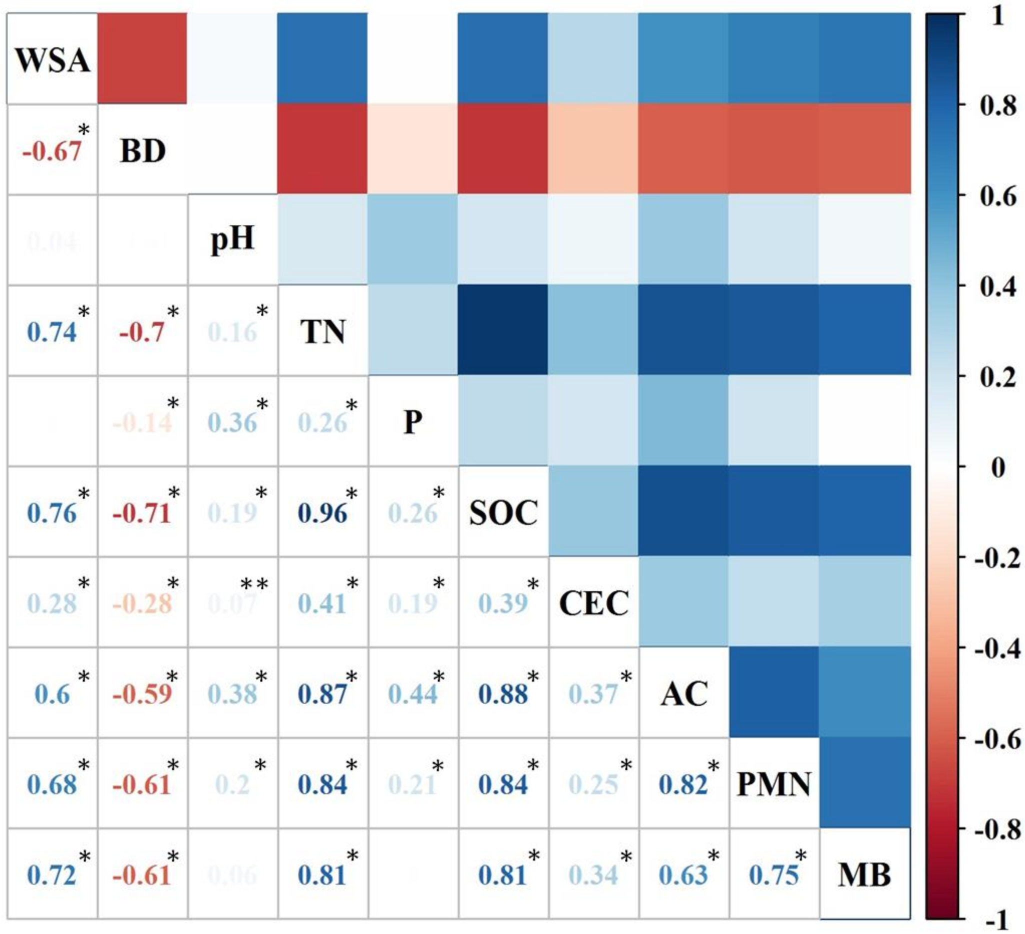

Fig. 3−3. Correlations between pairs of soil health indicators including water-stable aggregates (WSA), bulk density (BD), soil reaction (pH), total nitrogen (TN), phosphorus (P), soil organic carbon (SOC), cation exchange capacity (CEC), active carbon (AC), potentially mineralizable nitrogen (PMN) and microbial biomass (MB). Using Pearson correlation at 5% significance level. (* significant at $p < 0.05$; ** significant at $p < 0.10$).

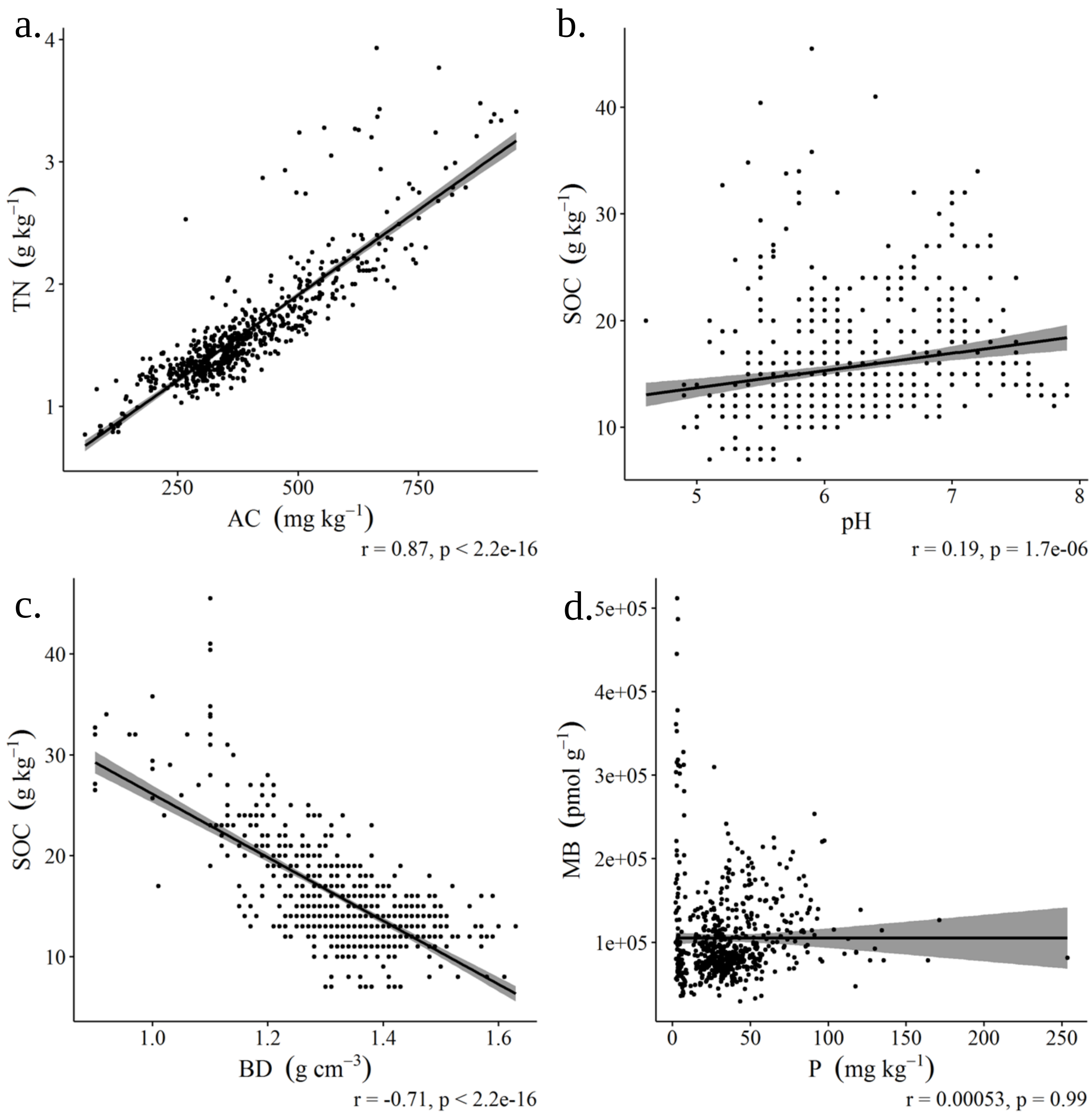

Fig. 3‒4. Example of strong positive linear association between active carbon (AC) and total nitrogen (TN) (a), example of weak positive linear correlation between soil reaction (pH) and soil organic carbon (SOC) (b), example of negative linear association between bulk density (BD) and soil organic carbon (SOC) (c), example of no linear correlation between phosphorus (P) and microbial biomass (MB) (d). Using Pearson correlation at 5% significance level.

To understand the dimension of the correlation matrix, Fig. 3‒5 was created using principal component analysis to explain nearly 72% of the total variance. This analysis allowed visualization of the association lines among the variables. The first principal component almost completely correlated with soil organic carbon, total nitrogen, and potentially mineralizable nitrogen. The second component correlated very highly with active carbon, while the third component was highly correlated with water-stable aggregates and microbial biomass. Finally, the last component had a high negative correlation with bulk density. As has been noted, most significantly correlated variables for soil health assessment in this study indicate a focus on water-stable aggregates, bulk density, soil organic carbon, active carbon, potentially mineralizable nitrogen, and microbial biomass.

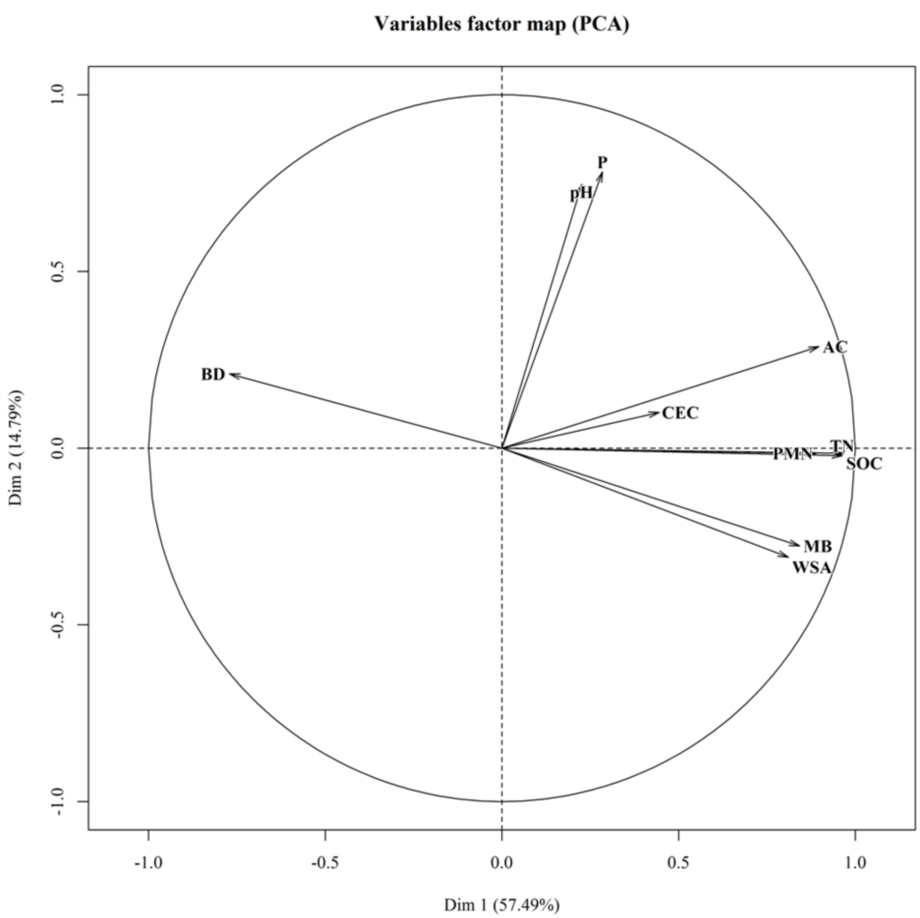

Fig. 3−5. Principal Component Analysis (PCA) map of soil health indicators including water-stable aggregates (WSA), bulk density (BD), soil reaction (pH), total nitrogen (TN), phosphorus (P), soil organic carbon (SOC), cation exchange capacity (CEC), active carbon (AC), potentially mineralizable nitrogen (PMN) and microbial biomass (MB).

3.4.5 Soil Health Scores

After calculating PCA and the scores from all physical, chemical, and biological properties both without and with cropping system as a control factor, the overall soil health scores without the control factor are illustrated in figure 3−6 and the other case is illustrated in figure 3−7. The case of overall soil health scores without the control factor undoubtedly ranged in ascending order as no fertilizer treatment, full fertility treatment, and manure treatment which have scores of 19, 50 and 72, respectively.

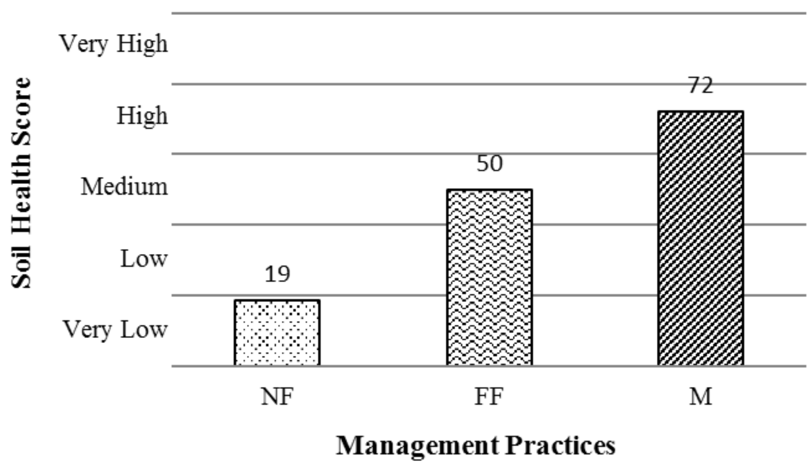

Fig. 3−6. Overall soil health scores ranged from 0 to 100 of selected management practices including no fertilizer (NF), full fertility (FF), and manure (M) and calculated from the CND functions of water-stable aggregates, bulk density, soil organic carbon, active carbon, potentially mineralizable nitrogen, and microbial biomass (Appendix A).

Regarding the cropping systems, the cropping systems consisted of continuous corn, continuous wheat, corn-soybean-wheat rotation and corn-wheat-red clover rotation had the same pattern of overall soil health scores without the control factor condition which ranged from no fertilizer treatment to manure treatment. However, the soil health scores of continuous corn were smaller than those of the continuous wheat and rotations in each treatment. In conclusion, continuous wheat together with manure had the largest overall soil health scores when compared to the others.

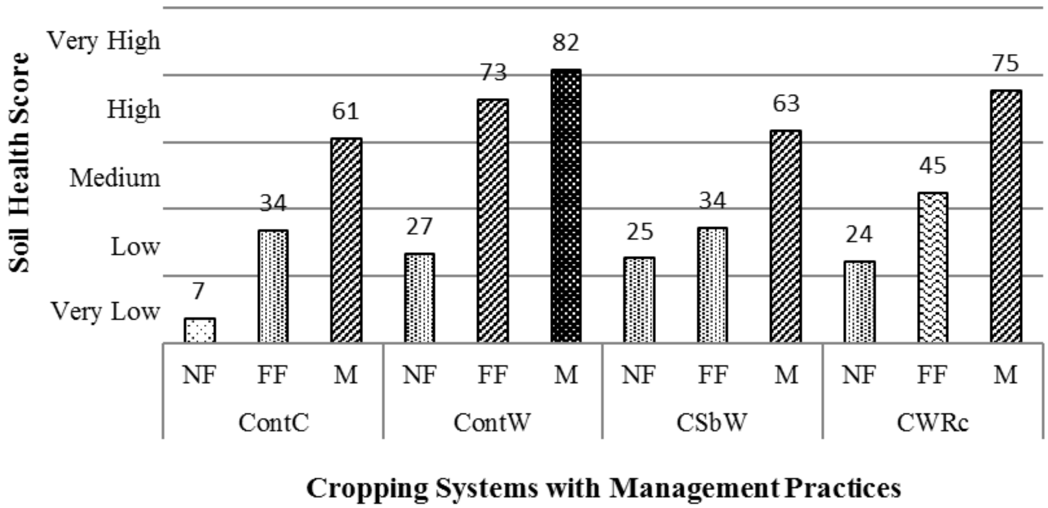

Fig. 3−7. Overall soil health scores ranged from 0 to 100 of selected management practices including no fertilizer (NF), full fertility (FF), and manure (M) regarding to cropping systems; continuous corn (ContC), continuous wheat (ContW), corn-soybean-wheat rotation (CSbW) and corn-wheat-red clover rotation (CWRc). Scores calculated from the CND functions of water-stable aggregates, bulk density, soil organic carbon, active carbon, potentially mineralizable nitrogen, and microbial biomass (Appendix A).

The statistical results showed that management practices including no fertilizer, full fertility, and manure, as well as cropping systems including continuous corn, continuous wheat, corn-soybean-wheat rotation, and corn-wheat-red clover rotation have influenced the soil properties, which was consistent across the experiment. Considering management practices, use of manure had a greater influence to improve all soil physical, chemical, and biological properties than the full fertility treatment and the no fertilizer treatment. Karlen et al., (2014) evaluated the effects of manure on soil quality indicators within five experimental watersheds, and they found that without or with manure application, the SMAF soil quality index ranged from 83% to 84% according to their inherent potential.

Compared to chemical fertilizer, manures may not contain as many immediately available nutrients (including micronutrients) or possess different nutrient composition depending on the material. However, the improved organic matter content may help improve soil structure (Celik et al., 2004), soil available water, and promote growth and maintenance of soil microorganisms. Kong et al. (2011) compared the effects of chemical fertilizer (conventional), fertilizer and cover crop (low input), and composted manure (organic) on soil organic carbon and the microbial community and found the greatest total PLFA biomass in the composted manure treatment. The microbial communities were not different between conventional and low input treatments. The results also showed that continuous wheat had the largest amounts of soil active carbon and organic carbon for full fertility and manure treatments compared to the other cropping systems. Same result of Rasmussen et al. (1998) on continuous wheat of Sanborn Field showed that manure and mineral fertilizer treatments had larger amount of soil organic carbon than no addition treatment due to the increasing amount of crop residue. Soil organic content had increased because of the returning residue back to soil in the early 50s (Buyanovsky and Wagner, 1998; Rasmussen et al., 1998). In addition, Miles and Brown (2011) stated that it may take about 30 to 40 years to develop the level of soil organic carbon to reach an equilibrium level on systems with large fertilizer and manure inputs. On the other hand, Huggins et al. (1998) reported that the treatments of perennial grass and legumes in rotation had potential to have large soil organic carbon contents. Eghball et al. (2004) studied corn production and soil properties affected by manure application and showed that the manure residual effects improved corn yield for one year and had effects on soil properties for a few years after application. In addition, slower nutrient release and nutrient loss reduction of organic material was reported by Nevens and Reheul (2003). They concluded that manure application had the capability to improve use of nutrients over a longer time period in corn monoculture.

However, previous studies also reported that use of chemical fertilizer and organic manure did not significantly alter soil properties. Grover et al. (2009) indicated that the impacts of inorganic fertilizers and manure on corn yield were not different among cropping systems such as continuous corn, corn-soybean rotation, corn-4-year alfalfa. Similarly, a review by Edmeades (2003) concluded that there was no significant differences on soil quality and crop yields by applying manure and fertilizer on long-term plots (20 to 120 years).

According to the positive and negative effects of inorganic and organic amendment, this study suggests that a combination of fertilizer and manure may be a good option to gain better agricultural productivity (Liu et al., 2013; Zhang et al., 2015; Zhang et al., 2016). Several studies have shown that the combination use of chemical and organic fertilizers had a positive response, which resulted in soil properties and productivity enhancement, and can lower the impact induced by chemical fertilizer and improve soil productivity (Kaur et al., 2005; Chand et al., 2006; Murmu et al., 2013; Han et al., 2016).

Soil fertility management along with cropping systems can influence changes in soil physical, chemical, and biological properties. The statistical results showed that continuous wheat had the greatest soil health scores in all selected measurements, followed by corn-wheat-red clover rotation, corn-soybean-wheat rotation and continuous corn, respectively.

Furthermore, if the soil management was considered together with the cropping systems, the best combination of practices to use for soil health was continuous wheat with a manure application; meanwhile, continuous corn without any inputs was the worst treatment for soil health in this study. Moreover, the plots without any inputs in each cropping system had the smallest soil health levels compared to the full fertilizer and the manure treatments, so suitable soil management and fertilizer applications are required to improve and maintain soil health. However, the cropping system should have been considered together with the soil management since each crop had different nutrient requirement. If the application applies more than is needed, this practice may lead to environmental deterioration instead of enriching stored and leachable soil nutrients.

3.5 SUMMARY AND CONCLUSIONS

The study was conducted to evaluate the effects of long-term applications of management practices on soil health measurements by comparing the selected management systems including no fertilizer, full fertilizer, and manure applications. The main purpose of the study was to observe the effects of management practices on long-term cropping systems. The results of the study obviously showed that manure application had the greatest single effect on all soil health indicators and had the largest overall soil health score compared to full fertility and no fertilizer treatments with most cropping systems. In addition, continuous wheat with manure application presented the best combination in this study having the best soil health indicators and had the highest score of 82 (out of 100), classified as very high. Furthermore, this study suggests that combined applications of fertilizer and manure maybe a key option to maintain soil health properties and gain a better balanced performance on sustainable agriculture in the future in that fertilizer inputs provide more immediate plant nutrient availability while manure applications provide slower long-term availability of plant essential nutrients while maintaining stronger physical and biological properties relative to no inputs or just fertilizer inputs.

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4 INFLUENCE OF SLOPE POSITION ON SOIL HEALTH PROPERTIES FOR SANBORN FIELD

4.1 ABSTRACT

Soil characteristic properties vary across the landscape. Topographic position can affect soil physical, chemical, and biological properties in different ways. In this study, water-stable aggregates, bulk density, pH, total nitrogen, phosphorus, organic carbon, cation exchange capacity, active carbon, potentially mineralizable nitrogen, and microbial biomass were determined. The objective of the study was to evaluate the effects of slope positions on soil health properties in the long-term Sanborn Field experiment. Results showed that the summit position had the largest overall soil health score of 61 out of 100 while the smallest score of 17 was found on the shoulder position. The accelerated erosion on the convex shoulder slope is the most plausible explanation for this relationship while the stability of summit accounted for the high score. However, there were no significant differences along the transect slope for water-stable aggregates and bulk density. There were significant differences along the transect for all of the biological properties with values large for the summit than the other positions. Therefore, from these results it is important to consider topographic position associated with long-term agricultural production due to the effects on some soil health properties over the landscape.

4.2 INTRODUCTION

Soil properties vary across the landscape (Cambardella et al., 2004). Topographic position can affect soil physical, chemical, and biological properties in many different ways. Therefore, scientists have shown that variability of soil properties is related to the position on the landscape. One study found soil hydraulic conductivity and bulk density were affected by topographic position (Jiang et al., 2007). Schimel et al. (1985) showed that more carbon, nitrogen and phosphorus were found in the A horizon of the summit position than in the backslope position. They also found an increasing amount of carbon, nitrogen and phosphorus in downslope positions. Similarly, a study by Hartemink et al. (2017) showed that the lowest elevation of the landscape in South Central Wisconsin, USA had the greatest soil organic carbon content and the backslope had the largest soil erosion rate at 16.2 Mg ha^{-1} yr^{-1}. However, the lower part of the backslope contributed the greatest soybean (*Glycine max* L.) yield at 6.3 Mg ha^{-1} which had a positive correlation to the A horizon thickness. The smallest yield of 1.6 Mg ha^{-1} was produced in the summit position due to the sandy soil texture and a pH greater than 7. Furthermore, relationships between crop production and landscape position were determined by Jones et al. (1989) who found that crop yields of corn (*Zea mays* L.), sorghum (*Sorghum bicolor*), and soybean ranked in order of the slope position as follows from high to low: lower interfluve, footslope, upper interfluve, shoulder, upper liner and lower linear, respectively. They concluded that crop yields were significantly influenced by slope, thickness of mollic color, and slope gradient. In addition, the position and slope were factors that influenced corn and sorghum yields, but they did not significantly affect the soybean yield. In addition, Zhu et al. (2015) reported that the lower concave part of the area had the largest corn yield and response to nitrogen application of 8.7 Mg ha^{-1}. This was also supported in the study by Changere and Lal (1997). Slope position significantly influenced corn growth as the footslope position had 3.14 and 3.70 times greater crop yield than the summit and backslope positions due to the soil water movement.

In this study, the long-term plots on Sanborn field were used to study the effects of slope position on soil health properties. To make this comparison, the corn-soybean-wheat-red clover rotation and the corn-soybean-wheat rotation with red clover as a green manure plots of Sanborn Field were utilized. Most soil resources on Sanborn Field are a poorly-drained claypan soil classified as a Mexico silt loam (fine, smectitic, mesic Vertic Epiaqualf), and the other soils include Mollic Epiaqualfs and Cumulic Epiaquolls (Miles and Hammer, 1989; Miles and Brown, 2011). The field has soil derived from loess over glacial till. The area has a slightly rolling topography (Veum et al., 2013). Besides, Hammer and Brown (1989) reported that the slope of Sanborn field ranged from nearly level to 7.5 percent. Considering the landscape of Sanborn field, plots 11, 13, 29, and 37 were selected to study as the summit, shoulder, backslope, and footslope according to the transect data and soil profile of Hammer and Brown (1989) as shown in Fig. 4−1.

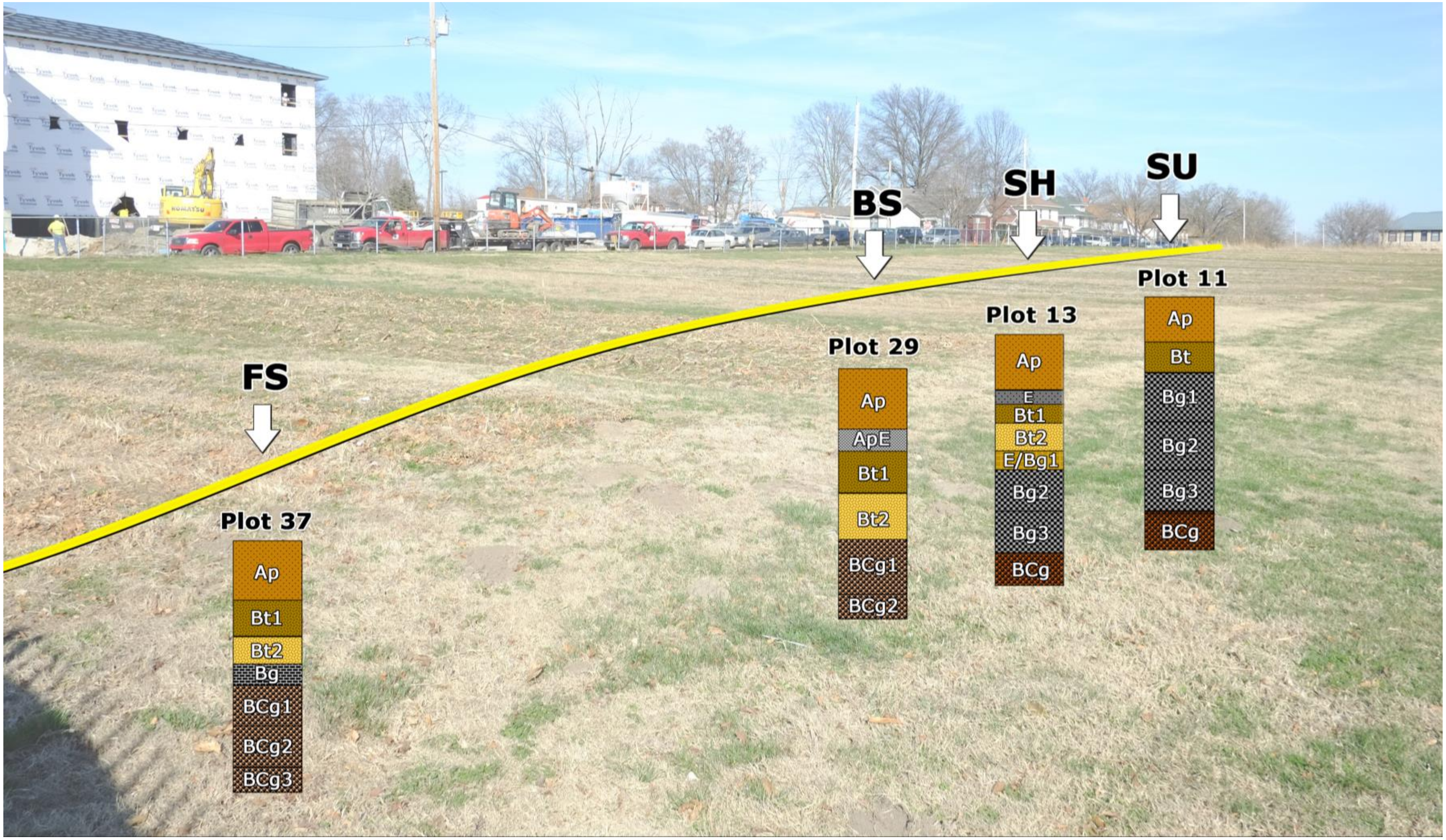

Fig. 4−1. Soil profiles of summit (SU: plot 11), shoulder (SH: plot 13), backslope (BS: plot 29), and footslope (FS: plot 37) positions in the study.

4.3 MATERIALS AND METHODS

4.3.1 Plot Selection

The study was conducted on the long-term Sanborn Field experiment at the University of Missouri-Columbia located in Columbia, Missouri, USA (38°94N, 92°32W). Since establishment, one of the purposes of the study was to observe and measure the effects of manure management on selected cropping systems and rotations (Wagner, 1989). There are different types of cropping systems and management practices for the plots with the historical information given in Table 4−1 (Brown and Wyman, 1989; Miles and Brown, 2011).

The tillage and cropping management for all of the plots on Sanborn Field are as follows. Each plot is moldboard plowed to a depth of 20 cm. After plowing, a disk harrow is used to incorporate any pre-plant fertilizer, agricultural lime, and manure applications to a depth of 5−8 cm while also providing a firm seedbed for planting the designated crop seed. Most soil resources on Sanborn Field are a poorly-drained claypan soil classified as a Mexico silt loam (fine, smectitic, mesic Vertic Epiaqualf), and the other soils include Mollic Epiaqualfs and Cumulic Epiaquolls (Miles and Hammer, 1989; Miles and Brown, 2011). In general, the field is derived from loess over glacial till, and the typical area is slightly rolling in topography (Veum et al., 2013). Subsurface soil includes a well expressed argillic horizon which influences the water holding capacity and rate of water movement. To study slope position effects on soil health properties, four plots were chosen as experimental units. These were summit (plot 11), shoulder (plot 13), footslope (plot 29), and backslope (plot 37), and Tucker Prairie (TP) used as the reference area of healthy soil.

Table 4−1. History of cropping systems and management practices of selected treatments from the Sanborn Field established in 1888†. Legend: Cont = continuous; C = corn; O = oat; Rc = red clover; Sb = soybean; T = timothy; W = wheat; O/Ls = oats with lespedeza; Rc/Ls = red clover with lespedeza; W/Ls = wheat with lespedeza; W(Rc) = wheat (green manure; red clover).

Plot No.	Cropping System	Years	Management and Practices (N−P−K in kg ha^{-1} per year)
11	COWRcTT	1888−1913	Manure
	COWRcTT	1914−1939	Manure; 19.8 Mg ha^{-1} on C, 12.4 Mg ha^{-1} on W, 2nd timothy; (Rock Phosphate 454 kg on C)
	COWRcTT	1940−1949	No manure input on T
	COWRc	1950−1989	Manure; 19.8 Mg ha^{-1} on C, 12.4 Mg ha^{-1} on W, Lime + K based on soil tests, 1.12 Mg ha^{-1} rock phosphate/8th year, 112 kg ha^{-1} N on C, 6−24−24 on C (East ½ starter), 20−20−20 on W (West ½ starter)
	CSbWRc	1990−present	Full Fertility
13	COWRcTT	1888−1989	No nutrient inputs
	CSbWRc	1990−present	Full Fertility
29	ContW	1888−1907	No nutrient inputs
	ContW	1908−1913	Manure; 13.4 Mg ha^{-1} per year
	ContW	1914−1927	10−0−0 $(NH_4)_2SO_4$
	ContW	1928−1939	10−0−0 Fall, 10−0−0 Spring as $(NH_4)_2SO_4$
	W/Ls (1yr)	1940−1949	0-38-0, East ½ lime, West ½ no lime
	C,O/Ls,W/Ls	1950−1989	72−24−24 on C, 20−20−20 on O&W, Lespedeza hay, East ½ lime, West ½ no lime
	CSbWRc	1990−present	Full Fertility
37	COWRc	1888−1913	Manure; 13.4 Mg ha^{-1} per year
	COWRc	1914−1927	10−15−11 on C, 7−10−7 on W
	COWRc	1928−1939	9−12−7 on C and W
	C,O,W,Rc/Ls	1940−1949	9−12−7 on C and W
	COWRc	1950−1989	9−12−7 on C and W
	CSbW(Rc)	1990−present	Full Fertility

† Enhancement of Brown and Wyman (1989) and Miles and Brown (2011).

4.3.2 Field Sampling

Four replicate soil samples were collected on each of four dates, 8th May 2014, 4th September 2014, 1st April 2016 and 18th August 2016 from six selected plots resulting in a total of 96 samples. The dimensions of each plot is 30.55 m by 9.42 m (Miles and

Brown, 2011). For this study, four samples from the 0−10 cm depth were taken from the representative area, 15.28 m by 3.14 m of a rectangle in the center of each plot, to avoid intra-plot variability (Fig. 4−2).

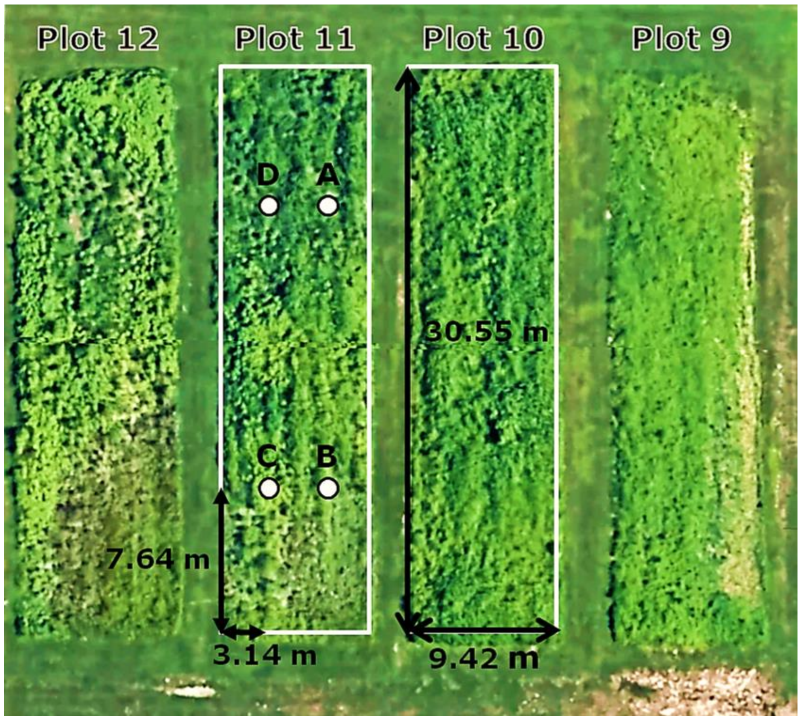

Fig. 4−2. Soil sampling approach for each experimental plot in the study. Points A, B, C, and D were collected to be representative samples of the plot.

To obtain a representative sample of the plot, sampled points were chosen to have a consistent feature such as avoiding being too wet or too dry and unusual area as much as possible in term of crop types and management practices with distance away for "border effects". Aluminum rings (76 mm diameter) were used to collect soil samples during the selected sampling times during each year. Surface litter on the soil was removed before sampling with the aluminum rings. Following sampling preparation, the core was driven into soil by hammering on a woodblock that was placed on the top of the core. The next steps were digging the core out with a shovel and trimming the protruded soil and plant roots. Finally, the soil sample was placed in a labeled plastic bag to maintain the field moisture content of the sample for determination of gravimetric water content at sampling before laboratory analyses.

4.3.3 Laboratory Analyses

At the Soil Health Assessment Center (SHAC), a soil health testing laboratory located in Columbia, Missouri, soil samples were promptly processed to avoid any time-affected factors in the analysis. The samples were divided into two portions with one portion immediately freeze-dried for phospholipid fatty acid analysis. For laboratory sample preparation, the rest of the soil portions were weighed to determine gravimetric water content at time of sampling. After that, the soil samples were air-dried at room temperature, ground with a mortar and pestle, and passed through a 2-mm sieve for the rest of the analyses (Soil Survey Staff, 2014).

To assess soil physical conditions resulting from past management, bulk density was measured to relate to the level of soil compaction (Keller and Håkansson, 2010). From these known volumes of the core, bulk density was computed by the ratio of oven-dry weight of soil sample to soil volume (Blake and Hartge, 1986). An additional physical indicator was water-stable aggregates, which refers to soil aggregates that were able to resist disruption such as tillage (Kumar et al., 2014) and erosion (Holifield Collins et al., 2015). To determine water-stable aggregates, soil aggregate retention was measured by placing the soil sample on a 0.5-mm sieve, immersing it in water overnight, and agitating the samples (raising and lowering the sieve manually). Afterward, the samples were dried and weighed for the final calculation for the percent of water stable aggregates (Kemper and Rosenau, 1986).

Nutrient analyses were performed to relate to soil nutrient availability status. These analyses included soil pH in water and salt (0.01M $CaCl_2$) which indicates the level of acidity and alkalinity and affects nutrient availability in the soil for phosphorus and potassium (Scanlan et al., 2017). The pH meter (Ross Sure-Flow Electrodes) was used to measure a 1:1 soil:deionized water suspension (Soil Survey Staff, 2014) after shaking for an hour. The Bray I extractant of ammonium fluoride and a dilute solution of hydrochloric acid was used to extract available phosphorus. 2 grams of soil with 25 ml of extracting solution were shaken for 10 minutes. Afterward, the samples were allowed to settle before filtering to take the aliquot. Extracted phosphorus was measured using a spectrophotometer (Spectronic $20D^+$) after the blue color intensity developed from using the molybdate-ascorbic acid reagent (Frank et al., 1998). The determination of total nitrogen content in the samples was performed by a LECO FP-528 nitrogen combustion analyzer. This analyzer involved the assessment of the volume of nitrogen (N_2) content, measured by thermal conductivity (Soil Survey Staff, 2014). The cation exchange capacity (CEC) was determined by the 1M ammonium acetate method at pH 7. The soil samples were saturated with the ammonium acetate solution, which exchanged with the existing exchangeable cations in the soil; after reaction, the quantity of exchangeable ammonium acetate was determined (Soil Survey Staff, 2014).

Soil biological properties were also assessed. First, soil organic carbon content was evaluated by a LECO C-144 carbon analyzer using combustion with measurement of the carbon dioxide (CO_2) mass. The principle of the analysis is that when the soil sample was introduced and combusted in an oxygen atmosphere, the mass of present CO_2 was determined (Soil Survey Staff, 2014). The evaluation of active carbon in the soil was performed by a potassium permanganate method, which is a moderate oxidant (0.2 M potassium permanganate) used to oxidize organic matter in the soil. The change of purple color was measured by a spectrophotometer by the Agilent Cary 60 UV-Vis spectrophotometer (Weil et al., 2003). In addition, an anaerobic method was utilized to estimate the potentially mineralizable nitrogen content, which indicated the available nitrogen in the soil. Approximately 8 grams of soil samples were placed in 50 ml Falcon tubes with deionized water and incubated at 40°C under anaerobic conditions for 7 days. The potentially mineralizable nitrogen contents were measured with the ammonium nitrogen produced after a week of anaerobic incubation using the Spectronic $20D^{+}$ spectrophotometer (Waring and Bremner, 1964).

Table 4‒2. Summary of soil health laboratory analyses including physical, chemical and biological properties and their analytical methods.

Property	Indicator	Abbrev.	Method	Reference
Physical Properties	Bulk Density	BD	Core method	Blake and Hartge, 1986
	Water Stable Aggregates	WSA	Wet-Sieving	Kemper and Rosenau, 1986
Chemical Properties	Cation Exchange Capacity	CEC	1M ammonium acetate at pH7	Soil Survey Staff, 2014
	Phosphorus	P	Bray 1	Frank et al., 1998
	Potential of Hydrogen	pH	1:1 soil:water	Soil Survey Staff, 2014
	Total Nitrogen	TN	Dry combustion by LECO FP-528 nitrogen analyzer	Soil Survey Staff, 2014
Biological Properties	Active Carbon	AC	Potassium permanganate test	Weil et al., 2003
	Soil Organic Carbon	SOC	Dry combustion by LECO C-144 carbon analyzer	Soil Survey Staff, 2014
	Potentially Mineralizable Nitrogen	PMN	Seven-days anaerobic incubation test at 40°C	Waring and Bremner, 1964
	Microbial Biomass	MB	Bligh-Dyer lipid extraction	Buyer and Sasser, 2012

Phospholipid fatty acids (PLFAs) analysis is one of the most common methods used to provide the information of microbial biomass in soil. The Bligh-Dyer extractant was used to extract lipids from freeze-dried soil samples. Then, the phospholipids were separated using solid phase extraction techniques and transesterification of the PLFAs. Finally, gas chromatography (Agilent Technologies 7890A GC System) was used to measure fatty acid methyl esters (Buyer and Sasser, 2012).

4.3.4 Statistical Analyses

After all data were obtained from laboratory analyses, the general approach of data analysis consisted of two main steps: 1) calculating the correlation coefficients and doing a Principal Component Analysis (PCA) among all of the indicators (bulk density: BD, water-stable aggregates: WSA, cation exchange capacity: CEC, available phosphorus: P, soil reaction: pH, total nitrogen: TN, active carbon: AC, soil organic carbon: SOC, potentially mineralizable nitrogen: PMN, and microbial biomass: MB) for all treatments, and 2) assessing significant differences for the properties among cropping systems. All steps were performed using the R-studio statistical package (version 1.1.149).

In the beginning, the first step was calculating the Pearson correlations between each pair of properties to observe the trends in relationships. The next step utilized Principal Component Analysis (PCA) to abate the number of variables, but still maintain most of the information in the previous models.

Brown (1994) reported that the experimental plots in Sanborn Field had no replication since it was established in 1888 before Fisher statistical methods with traditional plot replications were used. Repeated measures analysis of variance (Repeated

Measures ANOVA), within-subjects ANOVA and hypothesis testing, were used to run significant difference tests among the means of the independent variables since the samples were collected and measured repeatedly over multiple times. The forms of repeated measures ANOVA consisted of one-way repeated measures ANOVA and two-way repeated measures ANOVA, such that the best appropriate model under this objective was one-way repeated measures ANOVA. The assumptions of this model were that the sampling data were repeated, the dependent variable must be a quantitative (interval or ratio) variable, the independent variables were qualitative (nominal or ordinal) variables, the dependent variables should be normally distributed, and the variables should be a sphericity, having equal variance. Additionally, a Shapiro-Wilk method was used to test the normality and a Levene's method was used to test the sphericity. The Shapiro-Wilk test started by creating a null hypothesis: the variable was from a normal distribution, and an alternative hypothesis, the variable was from other distributions. Following these steps, the next step was choosing a 5% significance level or alpha ($\alpha = 0.05$), selecting type I error for the test, and calculating the statistic W and p-value. If the p-value was more than the significance level of 0.05, the null hypothesis was accepted. Similar to the Shapiro-Wilk test, Levene's test also had similar steps like the other test. However, the null hypothesis was changed so variances are equal or homogeneity of variance, and the test statistic was replaced by an F-test. If the F-statistic value was greater than the critical value or the p-value was smaller than the significance level, the alternative hypothesis was accepted. After testing all assumptions, the significant difference test was the last process for the statistical analysis. This process used the Tukey Honest Significant Differences (TuckeyHSD) method through R-studio.

Finally, the outcomes presented the significant groups of independent variables.

4.3.5 Soil Health Assessment

To provide a relative soil health assessment of Sanborn Field plots, the approach from the Cornell Comprehensive Assessment of Soil Health (CASH) (Moebius-Clune et al., 2016) was utilized. Before the calculating soil health index for all physical, chemical and biological properties, the first step was creating the cumulative normal distribution (CND) function and curve of each indicator in order to provide the soil health score index by using the raw data from laboratory analyses and results from the statistical program R-studio version 1.1.149. The soil health score ranged from 0 to 100 (with 100 the best), which was divided into five even intervals; scores 0−20, 20−40, 40−60, 60−80, and 80−100. All scales were represented in the diagram by five colors in five meanings; red described as Very Low, orange described as Low, yellow described as Medium, light green described as High, and dark green described as Very High, respectively.

Three scoring types were used in the procedure of the soil health assessment.

1) More is Better

In this case, if the mean value of each measured indicator is large, the score will be large as well. The indicators in this category were comprised of water-stable aggregates, total nitrogen, soil organic carbon, cation exchange capacity, active carbon, potentially mineralizable nitrogen, and microbial biomass.

2) Less is Better

Only bulk density fell into this scoring type. Additionally, less is better means that the smaller the measured value, the larger the score is observed.

3) Optimum Curve

Optimum curve was applied with both pH and available phosphorus. The feature of this curve consists of two parts which are positive scoring curve and negative scoring curve; moreover, the score of 100 must be larger than the 0 unit of measurement but smaller than 100 units of measurement. In other words, the curve has a bell shape regardless of skewness.

Fig. 4−3, for example, showed the cumulative normal distribution curve of active carbon and consisted of the horizontal axis presenting the amount of active carbon (mg kg^{-1}), the vertical axis presenting the score ranging from 0 to 100. If a soil sample has active carbon of 400 mg kg^{-1}, the soil health score will equal to 66 which is considered as high-level score.

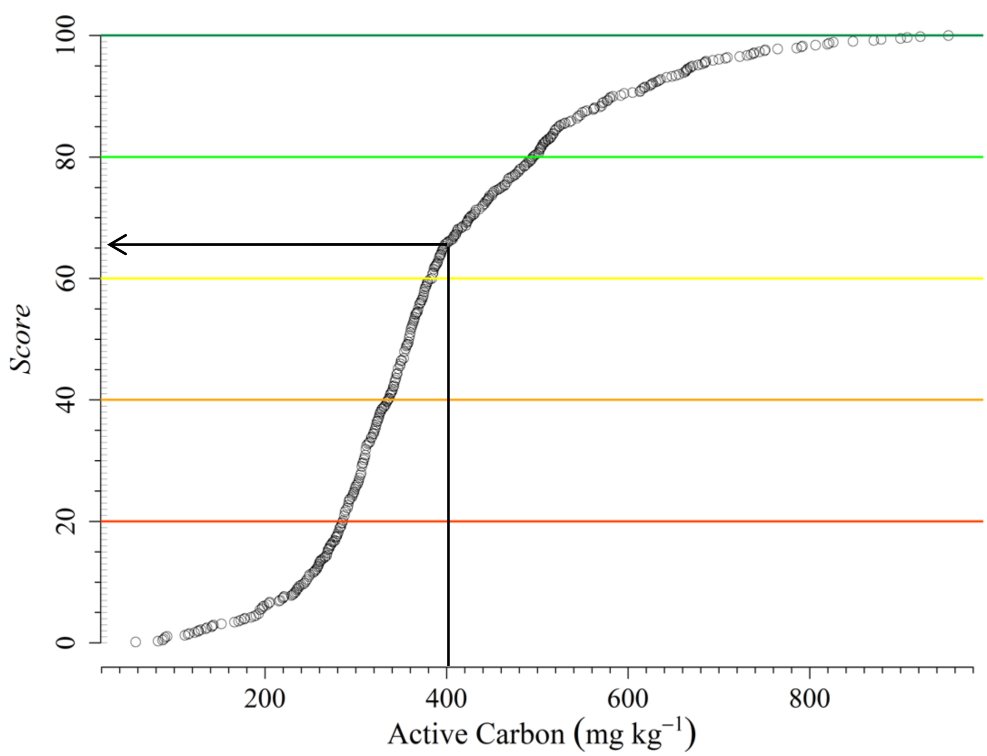

Fig. 4−3. Cumulative normal distribution curve of active carbon of the collected Sanborn Field and Tucker Prairie soil samples.

Subsequently, an overall soil health score is calculated by summing soil health scores from the cumulative normal distribution function of each indicator and dividing the sum by number of the indicators. In addition, the overall score also used the soil health score range from 0 to 100 as well as the ratings terms of soil health scores.

4.4 RESULTS AND DISCUSSION

4.4.1 Physical Properties

Table 4–3 reports the means of soil physical indicators including water-stable aggregates and bulk density in different slope positions: summit (SU), shoulder (SH), backslope (BS), and footslope (FS). The results showed that slope position did not affect changes in soil physical properties. The water-stable aggregate values ranged from 9% to 15%, and the bulk density values were from 1.35 to 1.42 g cm^{-3}. In addition, there were no significant differences among the slope positions of water-stable aggregates and bulk density. However, the shoulder had the smallest percentage of water-stable aggregates of 9% and a high bulk density of 1.39 g cm^{-3} since it was the most easily eroded compared to the other positions (Martz, 1992). Moreover, the slightly smaller bulk density values of summit position could be due to the benefit of soil organic carbon (De et al., 2014).

Table 4–3. Statistical means of physical property in different slope positions for the Sanborn Field. Means followed by lower case letters within the parenthesis indicated the significant differences according to TukeyHSD ($p < 0.05$).

Slope position	WSA†	BD†
	%	g cm^{-3}
Summit	12 (a)	1.35 (a)
Shoulder	9 (a)	1.39 (a)
Backslope	15 (a)	1.42 (a)
Footslope	14 (a)	1.37 (a)

† WSA, Water-stable aggregates; BD, Bulk density.

4.4.2 Chemical Properties

Soil reaction (pH), cation exchange capacity, total nitrogen, and available phosphorus are presented in table 4−4. The soil reaction was moderately acidic with pH values ranged from 5.7 to 6.0. Cation exchange capacity values ranged from 14.3 to 19.6 $cmol_c\ kg^{-1}$. The greater values were found at the summit and footslope positions, but they were not statistically different. The footslope had larger CEC than shoulder and backslope positions, which could be caused by the influences of leaching and water movement of clay and organic matter from a higher position to a lower position (Donald et al., 1993). In contrast, the backslope had the smallest mean values of cation exchange capacity. Similar statistical trends for total nitrogen content were observed. The shoulder position had only 1.24 mg kg^{-1} total nitrogen when compared to the other slope positions. The results showed amounts of total nitrogen for the summit, footslope, and backslope of 1.67, 1.36, and 1.34 mg kg^{-1}, respectively. In addition, the amounts of available phosphorus ranged from 29.6 to 45.1 mg kg^{-1} and there were no differences among the slope positions.

Table 4−4. Statistical means with significant differences of chemical property in different slope positions in the Sanborn Field. Means followed by lower case letters within the parenthesis indicated the significant differences according to TukeyHSD ($p < 0.05$).

Slope position	**pH**	**TN†**	**P†**	**CEC†**
		g kg^{-1}	mg kg^{-1}	$cmol_c\ kg^{-1}$
Summit	6.0 (a)	1.67 (c)	30.5 (a)	19.6 (b)
Shoulder	5.8 (a)	1.24 (a)	29.6 (a)	14.3 (a)
Backslope	5.7 (a)	1.34 (b)	32.7 (a)	15.1 (a)
Footslope	5.9 (a)	1.36 (b)	45.1 (a)	18.4 (b)

† TN, Total nitrogen; P, Phosphorus; CEC, Cation exchange capacity.

4.4.3 Biological Properties

Soil biological properties including soil organic carbon (SOC), active carbon (AC), potentially mineralizable nitrogen (PMN), and microbial biomass (MB) are shown in Table 4−5. The summit position had the largest values for all biological measurements: SOC of 16.2 g kg^{-1}, AC of 399.1 mg kg^{-1}, PMN of 88.6 mg kg^{-1}, and MB of 111,010 pmol g^{-1}. Meanwhile, the shoulder position had the smallest values for SOC (10.7 g kg^{-1}), AC (237.2 mg kg^{-1}), PMN (59.4 mg kg^{-1}), and MB (61,196 pmol g^{-1}).

Table 4−5. Statistical means with significant differences of biological property in different slope positions in the Sanborn Field. Means followed by lower case letters within the parenthesis indicated the significant differences according to TukeyHSD ($p < 0.05$).

Slope position	SOC†	AC†	PMN†	MB†
	g kg^{-1}	mg kg^{-1}	mg kg^{-1}	pmol g^{-1}
Summit	16.2 (c)	399.1 (c)	88.6 (b)	111,010 (c)
Shoulder	10.7 (a)	237.2 (a)	59.4 (a)	61,196 (a)
Backslope	12.4 (b)	325.9 (b)	67.4 (a)	97,003 (bc)
Footslope	12.9 (b)	302.1 (b)	59.8 (a)	78,409 (ab)

† SOC, Soil organic carbon; AC, Active carbon; PMN, Potentially mineralizable nitrogen; MB, Microbial biomass (based on total PLFA content).

4.4.4 Correlation and Principal Component Analysis

In this study, R-studio [version 1.1.149 at a 5% significance level (*)] was utilized to analyze the correlation between soil health indicators. Results were provided in ellipse charts in the upper right portion of the figure and numbers in the lower left portion. These charts represent the size of the relationship between variables or the relevance among interested parameters. In addition, the blue color of the diagram (ellipse chart and number) indicates a positive correlation while the red color refers to a negative correlation. The guidance intensity of the shade presented in the right side of the figure illustrates how strong the indicators are related.

From Fig. 4−4, bulk density is observed to have a strong negative correlation with most of the indicators (r = -0.59 to -0.71); in contrast, active carbon, soil organic carbon, total nitrogen, potentially mineralizable nitrogen, water-stable aggregates and microbial biomass were positively associated with each other (r = 0.60 to 0.96). However, soil reaction, phosphorus content and cation exchange capacity exhibited no correlation with other variables or a weak association (r = 0.00 to 0.44). Examples of negative correlation, strong and weak positive correlation, and uncorrelated relationships are shown in Fig. 4−5.

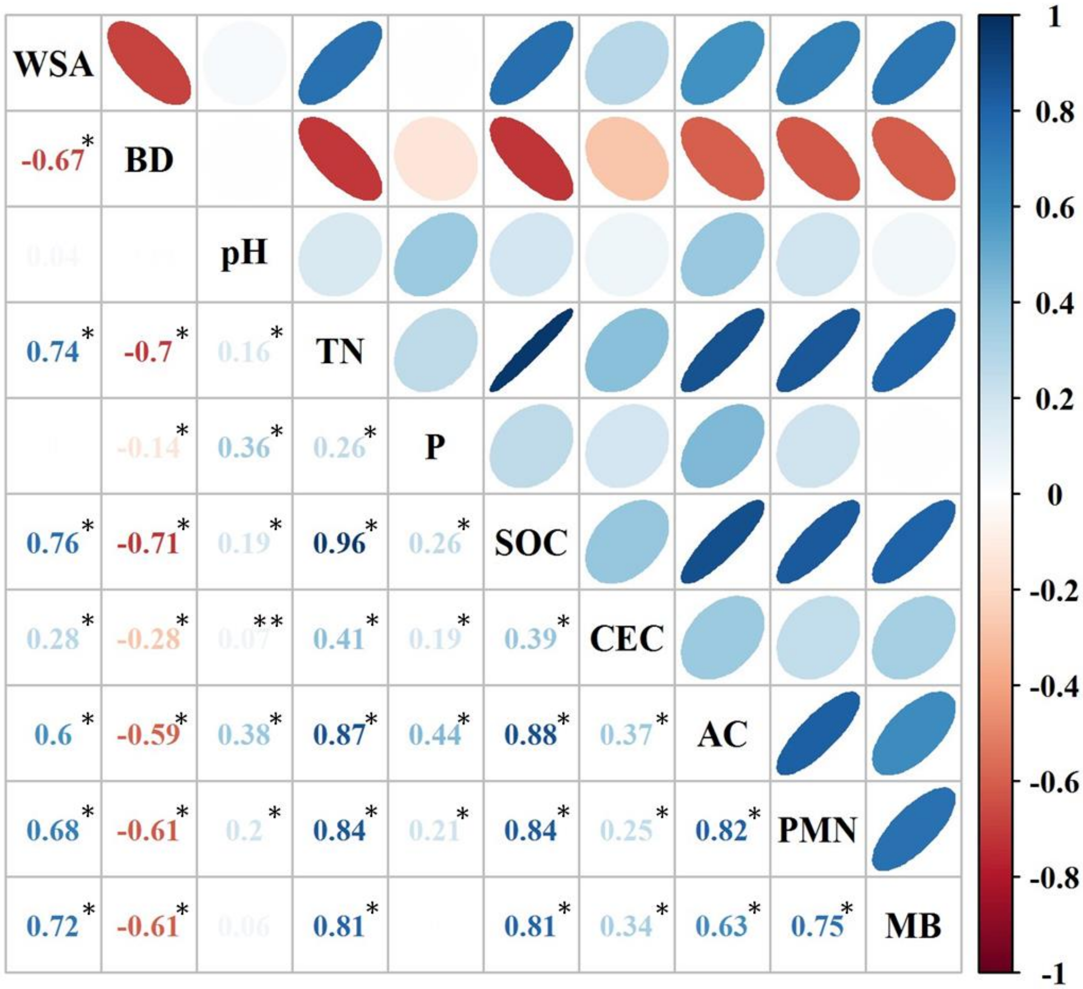

Fig. 4−4. Correlation coefficients and ellipse charts for pairs of soil health indicators including water-stable aggregates (WSA), bulk density (BD), soil reaction (pH), total nitrogen (TN), phosphorus (P), soil organic carbon (SOC), cation exchange capacity (CEC), active carbon (AC), potentially mineralizable nitrogen (PMN) and microbial biomass (MB). Using Pearson correlation at 5% significance level.
(* significant at $p < 0.05$; ** significant at $p < 0.10$).

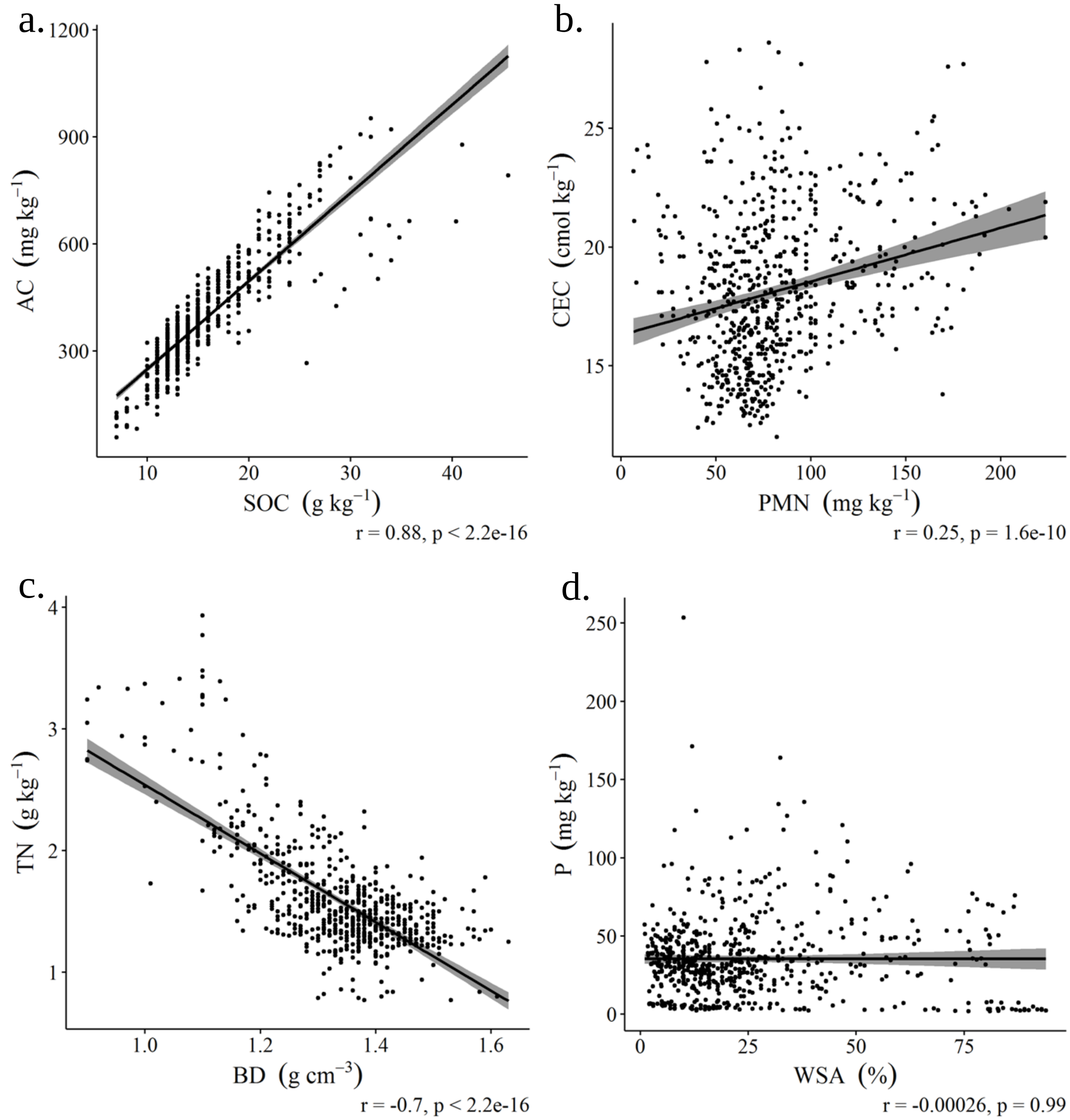

Fig. 4‒5. Example of strong positive linear association between soil organic carbon (SOC) and active carbon (AC) (a), example of weak positive linear correlation between potentially mineralizable nitrogen (PMN) and cation exchange capacity (CEC) (b), example of negative linear association between bulk density (BD) and total nitrogen (TN) (c), example of no linear correlation between water-stable aggregates (WSA) and phosphorus (P) (d). Using Pearson correlation at 5% significance level.

To understand the dimension of the correlation matrix, Fig. 4‒6 was created using principal component analysis to explain nearly 72% of the total variance. This analysis allowed visualization of the association lines among the variables. The first principal component almost completely correlated with soil organic carbon, total nitrogen, and potentially mineralizable nitrogen. The second component correlated very highly with active carbon, while the third component was highly correlated with water-stable aggregates and microbial biomass. Finally, the last component had a high negative correlation with bulk density. As has been noted, most significantly correlated variables for soil health assessment in this study indicate a focus on water-stable aggregates, bulk density, soil organic carbon, active carbon, potentially mineralizable nitrogen, and microbial biomass.

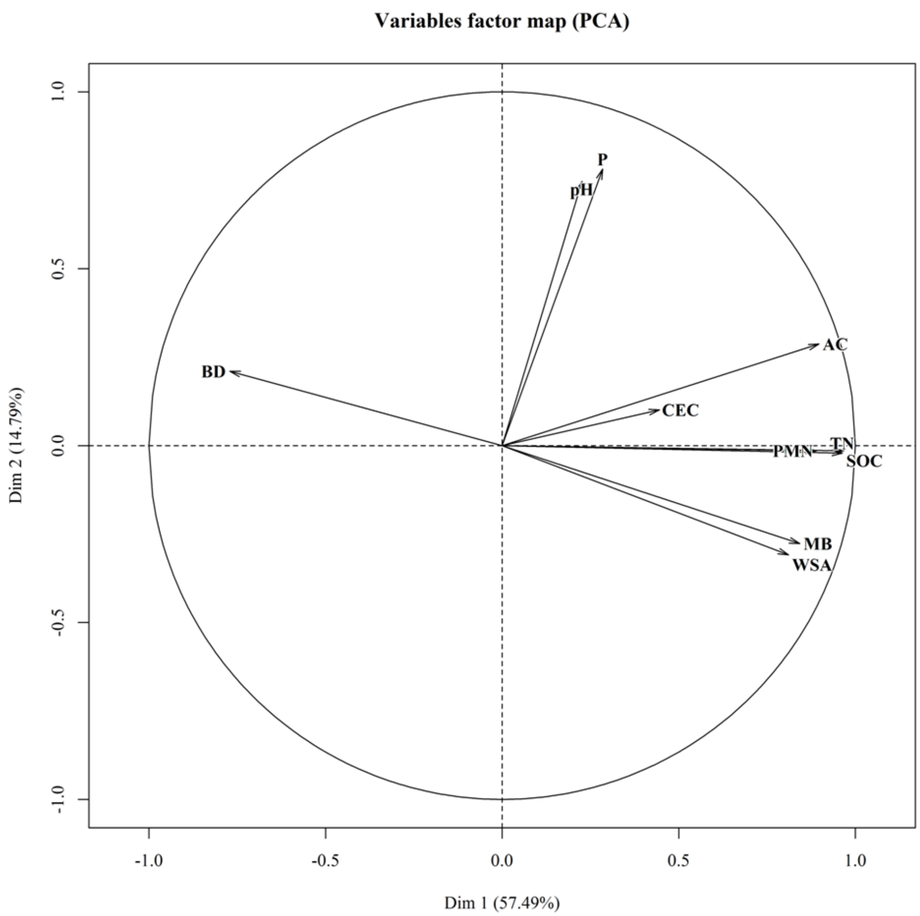

Fig. 4−6. Principal Component Analysis (PCA) map of soil health indicators including water-stable aggregates (WSA), bulk density (BD), soil reaction (pH), total nitrogen (TN), phosphorus (P), soil organic carbon (SOC), cation exchange capacity (CEC), active carbon (AC), potentially mineralizable nitrogen (PMN) and microbial biomass (MB).

4.4.5 Soil Health Scores

The overall soil health scores of different slope positions are presented in Fig. 4−7. The summit position had the greatest soil health score at 61 considered as high while the shoulder position had the lowest score at 17 considered as very low. For backslope and footslope positions, they were considered as at a low level with the scores of 36 and 34, respectively.

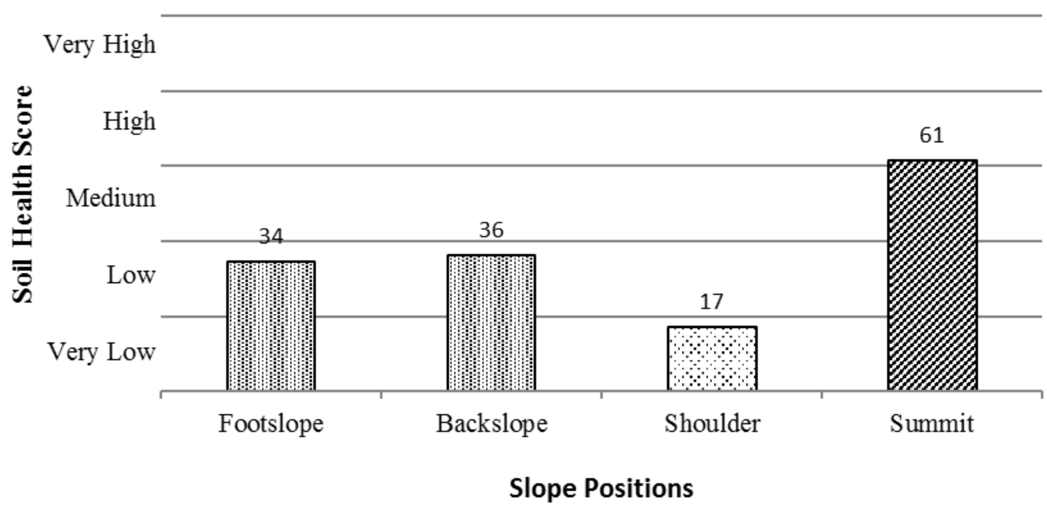

Fig. 4−7. Overall soil health scores of selected slope positions including footslope, backslope, shoulder, and summit positions ranged from 0 to 100. Overall soil health scores calculated from the CND functions or soil health scoring curve of water-stable aggregates, bulk density, total nitrogen, organic carbon, active carbon, potentially mineralizable nitrogen, and microbial biomass (Appendix A).

To determine the influences of slope position on soil health properties in this study, the overall soil health score of the summit position had a larger score than shoulder, backslope, and footslope because the summit position was nearly level and was considered as a relative stable area against erosion. Moreover, the summit soil has been the likely to have been more well developed compared to the others, and it also had a thick soil surface initially than the other positions. Therefore, it leads to increased nutrient storage for plant growth. In contrast, the shoulder position has a thin soil surface because of surface runoff and sediment transport from soil erosion resulting in lesser nutrient and water holding capacity. In addition, the main cause of soil erosion probably was soil tillage from crop cultivation (Papiernik et al., 2009). These soils have been moldboard plowed with disk harrowing for producing a fine seed bed for plant establishment and incorporation of fertilizer and manure inputs. After many of the tillage operations little cover is left on the surface; therefore, a great possibility of raindrop impact and soil erosion. Moreover, the slope aspect of the field is west-facing slopes that lead to potentially drier soil than east or north aspects. This information supports the result that the shoulder position had the smallest overall soil health score in this study.

The slope positions did not influence soil physical properties since there were no significant differences among slope positions for wet aggregate stability and bulk density. The similar long-term tillage practices likely contributed to this lack of differences. The possible reasons could be the inconsistent differences of cropping systems in each slope position and only 3.7% slope difference between the summit position and the footslope position (Gantzer et al., 1990).

4.5 SUMMARY AND CONCLUSIONS

This study examined the effects of slope position on soil health properties of the long-term Sanborn Field experimental study. The selected topographic positions consisted of summit, shoulder, backslope, and footslope. The results concluded that the summit position had the highest soil health scores while the shoulder position had the lowest soil health scores; the summit position was nearly level and was considered as a relative stable area with lesser potential for soil erosion and loss of the silt loam material or mixture of the silt loam into the lower Bt horizon over 125 plus years of continual moldboard plowing. However, there were no significant differences in soil physical properties among the slope positions; in part, due to the long-term tillage regime. Most chemical properties were not significant among slope positions while the biological properties of organic carbon, active carbon, potentially mineralizable nitrogen, and microbial biomass were significantly larger in the summit landscape compared to the other positions. This study suggests that it is necessary to consider topographic position in the field since the differences of some soil health properties were found across the landscape.

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5 CONCLUSIONS

Soil health assessment of major physical, chemical, and biological properties was used in this study to examine the long-term effects of cropping systems (i.e., monoculture crop, rotation crop and perennial grass), soil management practices for selected cropping systems (no fertility, annual fertilizer application, and annual manure application), and topographic positions (summit, shoulder, backslope and footslope) on soil health properties of Sanborn Field, located in Columbia, Missouri, United States. Additionally, the soil health score of these treatments was adapted from the Cornell Comprehensive Assessment of Soil Health (CASH) tool. Most soil resources on Sanborn Field are a poorly-drained claypan soil classified as a Mexico silt loam (fine, smectitic, mesic Vertic Epiaqualf). Tucker prairie, a native prairie with similar soils to those of Sanborn Field was used as a proxy reference to compare to the cultivated soils. Moreover, the integrated information of the study area was processed to observe the relationships among the variables.

The results of this long-term experimental study concluded that cropping system is a factor that causes differences in soil health properties in each experimental plot. The perennial grass treatment was the best cropping system in terms of relative soil health assessment score for Sanborn Field. Besides, this treatment can be one of the most efficient methods to rehabilitate or ameliorate soil conditions because grass has a root system which has the capability to absorb soil nutrients and return soil organic matter and residues to the soil and provide perennial cover for a protection from erosion. As a result, this perennial grass system can be used to restore soil health.

Cropping system is not the only factor affecting soil health, since soil management practices also cause modifications to soil health. Cropping systems together with annual fertilizer and manure applications were examined under the second objective (Chapter 3). Regardless of cropping systems, the annual manure treatment was the best management practice since it produced a greater statistical improvement in soil health than soil management treatments with and without fertilizer, respectively. Additionally, continuous wheat with manure application presented the best combination in this study having the highest soil health score classified as very high.

Last, the experiment showed that the landscape position also had a significant influence on soil health. Specifically, the summit position had the healthiest condition and more abundance of soil nutrients, while the shoulder position showed the opposite condition with the lowest soil health score. Even though Sanborn Field is a small area having a size of only 2.83 hectares, there are variations in soil characteristics. Those variations in the soil will influence crop growth. Therefore, factors such as slope position, slope aspect, slope gradient, and slope length should be considered while assessing soil health for cropping systems.

This study provides the finding effects of cropping systems, management practices, and topographic landscape which can contribute knowledge and a better understanding on long-term agriculture, and the results also can be used as a database for soil health assessment in this area that researchers can use in the future. During the study, there were limitations that may have affected and restricted the results. For example, since Sanborn Field has no replication plots, researchers had to select an appropriate statistical model for the study to avoid any confounding or hidden factors which affect the results. According to the laboratory capacity, this study could not cover all potential soil health indicators which may reflect the status of soil health as well as other selected indicators in this study (i.e. soil water available capacity, soil enzymes, microbial communities).

Suggestions for future research would be in-depth exploration of how types of microorganisms affect soil properties, including the role of soil enzymes, and how water infiltrate and stored in soil may help explaining, interpreting, and enhancing soil health assessment method. Soil assessments to a greater depth (i.e. 1 m) that would reflect root zone soil health should be considered.

The overall purpose of this study was to illustrate the effects of long-term cropping systems, management practices, and topographic positions on soil health properties. To evaluate soil health, the experiment should involve all physical, chemical and biological properties since the soil health index cannot be described by only a single parameter and all of these physical, chemical, and biological components can reflect processes and changes in soil. Furthermore, to interpret the soil health score, the results should provide some soil health score information, limitations, and management recommendations. Finally, this study can apply to other locations, but the local factors of those areas must be taken into account prior to extrapolating the results from this study to other fields.

APPENDIX A. SOIL HEALTH SCORING CURVES

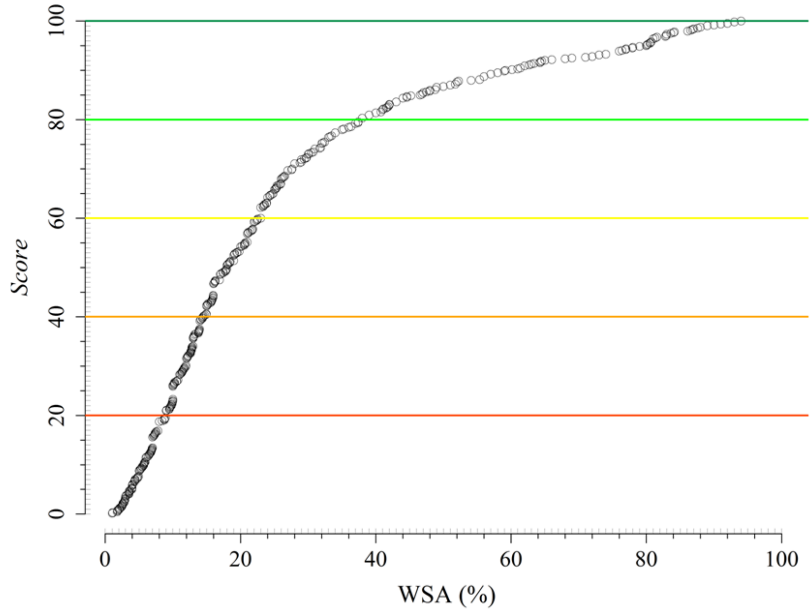

Fig. A−1. Cumulative normal distribution function or soil health scoring curve of water-stable aggregates of the Sanborn Field and Tucker Prairie.

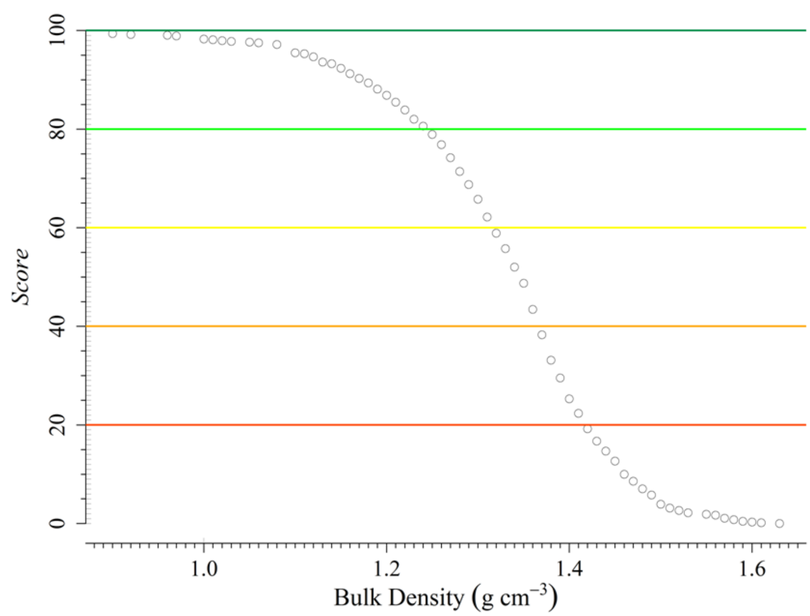

Fig. A−2. Cumulative normal distribution function or soil health scoring curve of bulk density of the Sanborn Field and Tucker Prairie.

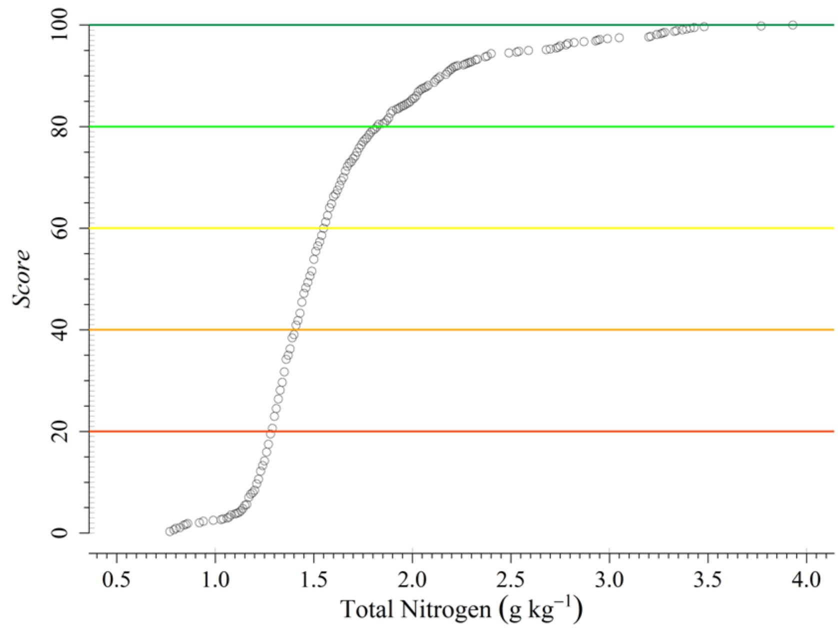

Fig. A−3. Cumulative normal distribution function or soil health scoring curve of total nitrogen of the Sanborn Field and Tucker Prairie.

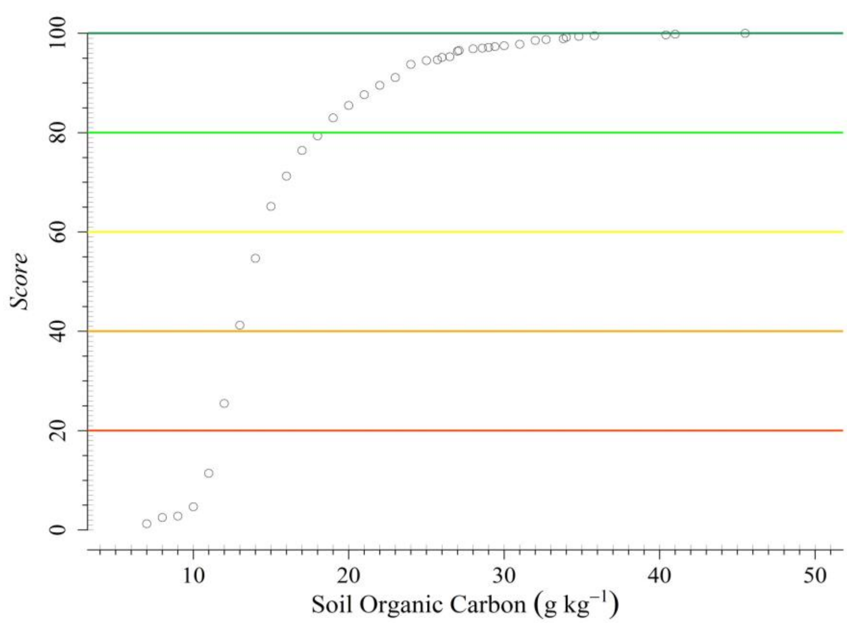

Fig. A−4. Cumulative normal distribution function or soil health scoring curve of soil organic carbon of the Sanborn Field and Tucker Prairie.

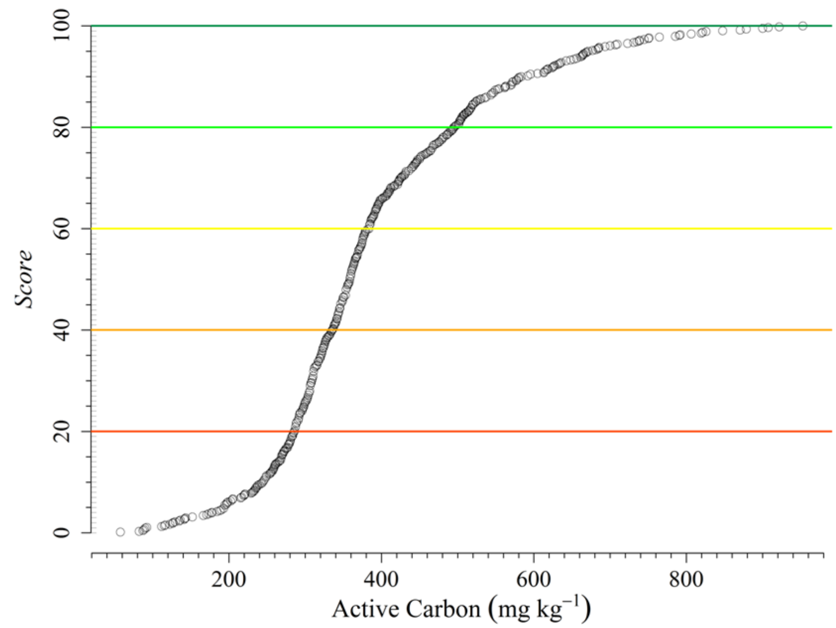

Fig. A–5. Cumulative normal distribution function or soil health scoring curve of active carbon of the Sanborn Field and Tucker Prairie.

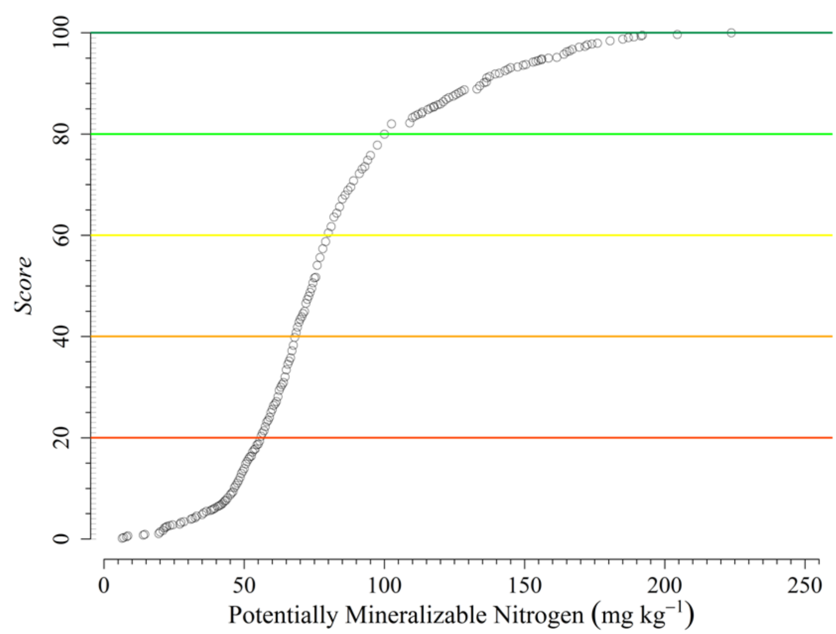

Fig. A–6. Cumulative normal distribution function or soil health scoring curve of potentially mineralizable nitrogen of the Sanborn Field and Tucker Prairie.

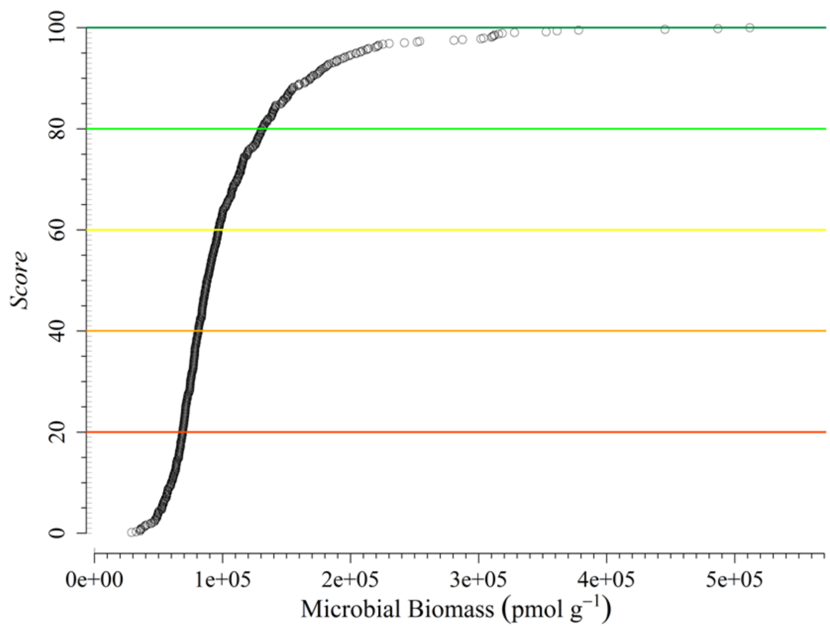

Fig. A−7. Cumulative normal distribution function or soil health scoring curve of microbial biomass of the Sanborn Field and Tucker Prairie.

APPENDIX B. DATA OF SOIL PROPERTY ANALYSES IN EACH OBJECTIVE

Table B−1. Data of soil physical analyses of objective 1 (Chapter 2).

		Water Stable Aggregate				Bulk Density				Water content			
Plot&Point	**Crop**	**R1†**	**R2†**	**R3†**	**R4†**	**R1**	**R2**	**R3**	**R4**	**R1**	**R2**	**R3**	**R4**
		%				g cm^{-3}				%			
9A‡	ContW§	32	18	13	38	1.29	1.42	1.50	1.31	13.0	24.3	24.1	17.5
9B	ContW	13	10	16	36	1.38	1.40	1.39	1.49	16.4	25.5	29.0	14.9
9C	ContW	24	15	12	38	1.42	1.45	1.40	1.37	14.4	26.4	26.1	18.0
9D	ContW	21	18	13	33	1.41	1.35	1.40	1.45	17.0	27.8	26.6	22.2
17A	ContC§	6	2	4	17	1.34	1.30	1.42	1.53	19.3	31.8	27.6	15.8
17B	ContC	5	2	3	8	1.43	1.37	1.38	1.41	19.5	30.4	26.7	20.3
17C	ContC	7	2	3	5	1.41	1.48	1.36	1.58	19.3	27.1	29.4	22.5
17D	ContC	25	5	5	22	1.36	1.31	1.31	1.61	19.9	27.9	28.6	20.0
23A	ContT§	81	81	69	83	1.21	1.27	1.15	1.23	30.5	30.7	38.0	28.1
23B	ContT	83	80	52	81	1.19	1.20	1.12	1.20	27.2	30.3	37.6	31.1
23C	ContT	84	84	56	76	1.13	1.16	1.10	1.27	27.6	33.3	39.0	29.6
23D	ContT	86	88	66	73	1.12	1.16	1.18	1.25	28.4	31.9	38.3	31.2
27A	CWRc§	18	10	13	26	1.33	1.39	1.39	1.27	22.6	26.0	28.1	17.6
27B	CWRc	6	6	8	8	1.40	1.44	1.41	1.36	26.9	25.7	28.9	25.6
27C	CWRc	5	7	13	7	1.41	1.45	1.34	1.37	26.5	25.5	27.4	24.3
27D	CWRc	12	12	28	15	1.40	1.48	1.36	1.33	23.3	24.9	27.4	19.4
35A	CSbW§	22	18	14	20	1.47	1.37	1.41	1.38	15.9	24.3	31.9	14.1
35B	CSbW	21	18	12	16	1.43	1.42	1.24	1.39	21.6	22.8	58.3	10.6
35C	CSbW	19	15	10	20	1.46	1.47	1.50	1.39	19.2	24.0	28.1	12.5
35D	CSbW	18	15	14	30	1.51	1.35	1.41	1.36	16.8	24.0	28.5	15.2
45A	WSG§	47	24	44	74	1.19	1.24	1.13	1.15	31.9	30.5	36.5	21.4
45B	WSG	83	52	19	71	1.18	1.18	1.25	1.24	23.8	35.8	29.2	29.3
45C	WSG	81	80	28	76	1.22	1.26	1.19	1.28	29.0	28.4	33.8	20.4
45D	WSG	45	54	41	57	1.19	1.21	1.12	1.20	34.2	28.9	35.0	16.8
TP1‡	TP§	89	81	76	91	1.10	0.90	1.10	1.00	N/A	N/A	N/A	N/A
TP2	TP	89	90	61	93	1.10	0.90	1.10	1.00	N/A	N/A	N/A	N/A
TP3	TP	92	94	59	83	1.10	0.90	1.10	1.00	N/A	N/A	N/A	N/A
TP4	TP	93	87	68	88	1.10	0.90	1.10	1.00	N/A	N/A	N/A	N/A

† Dates of sample collection. R1, Round 1 (8th May 2014); R2, Round 2 (4th September 2014); R3, Round 3 (1st April 2016); R4, Round 4 (18th August 2016).
‡ 9A, Point A of plot no. 9; TP1, Point 1 of Tucker Prairie.
§ ContW, Continuous wheat; ContC, Continuous corn; ContT, Continuous timothy; CWRc, Corn-wheat-red clover rotation; CSbW, Corn-soybean-wheat rotation with red clover as a green manure; TP, Tucker Prairie.

Table B−2. Data of soil chemical analyses of objective 1 (Chapter 2).

		pH				Cation Exchange Capacity				Total Nitrogen				Phosphorus			
Plot&Point	**Crop**	**R1†**	**R2†**	**R3†**	**R4†**	**R1**	**R2**	**R3**	**R4**	**R1**	**R2**	**R3**	**R4**	**R1**	**R2**	**R3**	**R4**
						cmol$_c$ kg^{-1}				g kg^{-1}				mg kg^{-1}			
9A‡	ContW§	5.4	5.0	5.3	5.2	16.8	17.8	18.1	18.6	1.50	1.04	1.06	1.43	6.6	4.6	3.1	4.0
9B	ContW	5.5	5.0	5.3	5.0	16.1	17.4	17.4	17.1	1.31	1.13	1.25	1.21	8.9	5.3	3.3	3.3
9C	ContW	5.8	5.2	5.3	5.3	18.3	18.4	18.6	19.1	1.44	1.23	1.08	1.39	7.1	4.8	3.3	3.4
9D	ContW	5.3	4.9	5.3	5.1	20.4	21.7	20.4	21.5	1.24	1.22	1.14	1.39	5.9	4.0	2.3	2.7
17A	ContC§	5.3	5.1	5.5	5.3	21.3	20.7	22.2	23.2	0.86	0.79	0.84	0.77	7.3	6.4	6.6	5.3
17B	ContC	5.5	5.4	5.6	5.6	17.1	18.1	18.1	18.5	0.84	0.79	0.77	0.80	6.4	6.6	5.4	5.8
17C	ContC	5.6	5.4	5.8	5.6	19.7	19.4	20.4	21.1	0.92	0.94	0.85	0.84	8.0	7.0	6.4	6.0
17D	ContC	5.2	5.1	5.5	5.3	24.3	23.8	24.0	24.1	0.94	0.82	0.99	0.80	7.0	6.9	8.2	4.1
23A	ContT§	6.1	6.2	6.0	6.0	15.9	13.8	17.1	16.7	1.79	1.89	1.96	2.05	3.3	8.0	2.7	2.1
23B	ContT	6.2	6.2	6.0	5.9	17.4	15.7	16.4	16.4	1.89	1.83	1.99	1.89	3.6	7.6	2.9	2.0
23C	ContT	6.1	6.1	5.9	6.0	17.0	16.6	16.5	16.9	2.18	2.02	2.08	2.02	3.9	7.5	2.9	2.0
23D	ContT	6.0	6.0	6.1	5.9	17.4	16.7	16.5	16.2	2.17	2.06	2.04	1.83	3.2	7.5	2.9	2.1
27A	CWRc§	5.7	5.9	5.7	5.6	17.5	13.4	15.0	15.8	1.17	1.27	1.30	1.31	4.2	6.0	3.3	3.6
27B	CWRc	6.1	5.9	6.1	6.0	13.0	14.9	13.0	12.7	1.07	1.27	1.23	1.17	6.9	5.5	3.5	4.1
27C	CWRc	6.2	6.2	6.0	6.1	12.9	14.6	12.5	12.4	1.13	1.33	1.18	1.21	5.4	5.7	3.3	3.7
27D	CWRc	5.6	6.1	5.8	5.5	15.4	13.9	15.4	15.1	1.15	1.22	1.39	1.16	4.2	3.9	3.7	3.0
35A	CSbW§	5.8	5.3	6.2	5.6	18.4	18.5	17.4	19.6	1.27	1.20	1.37	1.25	5.8	7.9	5.8	4.0
35B	CSbW	5.8	5.7	6.2	5.7	14.6	14.3	14.7	14.8	1.27	1.30	1.31	1.28	5.7	8.7	5.0	3.9
35C	CSbW	5.8	5.6	6.1	5.7	15.0	15.2	15.2	15.0	1.26	1.24	1.28	1.24	6.9	9.2	4.8	4.1
35D	CSbW	5.7	5.4	6.0	5.6	20.1	20.6	19.3	19.7	1.15	1.26	1.28	1.34	4.6	4.8	3.8	4.0
45A	WSG§	6.4	6.3	6.7	6.6	17.9	16.9	18.4	19.7	2.04	1.83	2.11	2.20	72.0	71.8	79.8	53.2
45B	WSG	6.5	6.6	6.9	6.7	17.4	18.3	17.0	17.3	2.01	2.32	2.03	1.97	50.4	60.8	68.2	53.4
45C	WSG	7.0	6.5	7.0	6.7	16.4	15.6	18.5	16.0	1.74	1.67	2.00	1.80	70.4	54.0	84.4	65.8
45D	WSG	6.5	6.0	6.2	5.9	19.4	18.5	18.3	17.6	2.29	2.12	2.12	1.92	88.0	73.6	82.8	75.0
TP1‡	TP§	5.7	5.6	6.1	5.9	21.9	20.4	21.9	22.2	3.20	2.74	3.43	3.37	3.2	3.4	7.2	4.9
TP2	TP	5.4	5.6	5.8	5.5	20.4	18.4	20.5	18.7	3.27	2.75	3.26	2.93	2.7	2.7	7.1	2.8
TP3	TP	5.5	5.2	5.8	5.3	24.8	22.4	25.5	20.9	3.93	3.24	3.28	2.53	3.0	2.4	4.7	2.5
TP4	TP	5.9	5.8	6.4	5.7	27.6	23.9	27.7	23.0	3.77	3.05	3.48	2.87	3.4	2.8	3.7	2.3

† Dates of sample collection. R1, Round 1 (8th May 2014); R2, Round 2 (4th September 2014); R3, Round 3 (1st April 2016); R4, Round 4 (18th August 2016).

‡ 9A, Point A of plot no. 9; TP1, Point 1 of Tucker Prairie.

§ ContW, Continuous wheat; ContC, Continuous corn; ContT, Continuous timothy; CWRc, Corn-wheat-red clover rotation; CSbW, Corn-soybean-wheat rotation with red clover as a green manure; TP, Tucker Prairie.

Table B−3. Data of soil biological analyses of objective 1 (Chapter 2).

		Soil Organic Carbon				Active Carbon				Potentially Mineralizable Nitrogen				Microbial Biomass			
Plot&Point	Crop	R1†	R2†	R3†	R4†	R1	R2	R3	R4	R1	R2	R3	R4	R1	R2	R3	R4
		g kg^{-1}				mg kg^{-1}				mg kg^{-1}				pmol g^{-1}			
9A‡	ContW§	13	10	9	13	249.1	151.9	141.8	219.6	102.5	36.5	21.5	68.5	87,348	97,587	55,196	141,236
9B	ContW	13	11	11	11	241.9	194.4	170.6	122.4	88.0	27.5	83.0	27.5	86,877	87,458	96,571	91,677
9C	ContW	12	12	11	13	220.3	198.7	143.3	177.8	91.0	51.5	54.5	72.0	89,772	100,209	66,140	116,666
9D	ContW	11	10	9	12	187.9	173.5	82.1	193.0	56.0	24.5	23.5	49.5	77,608	82,356	56,440	138,071
17A	ContC§	8	7	7	8	129.6	115.2	88.6	87.1	22.5	20.0	19.5	6.5	49,772	45,941	39,693	59,521
17B	ContC	7	7	7	7	126.0	126.7	57.6	90.0	21.5	27.0	20.0	8.0	47,029	36,066	35,832	61,139
17C	ContC	8	8	7	8	141.1	136.1	111.6	90.0	21.0	21.5	32.5	7.0	48,407	38,871	49,897	63,556
17D	ContC	8	7	8	8	134.6	116.6	166.3	92.2	14.0	14.5	44.0	8.5	49,595	41,169	52,571	59,856
23A	ContT§	19	20	21	20	427.0	466.6	466.6	355.7	102.5	169.5	143.0	116.5	154,004	182,264	204,260	170,810
23B	ContT	20	19	22	19	506.2	457.2	504.0	322.6	143.0	145.0	164.0	123.0	158,285	204,140	175,465	147,538
23C	ContT	23	22	23	21	549.4	523.4	552.2	442.8	167.0	174.0	169.5	134.0	164,535	280,781	209,719	135,460
23D	ContT	21	22	21	19	499.0	493.9	502.6	349.9	171.5	166.0	135.5	100.0	180,284	252,088	126,381	99,897
27A	CWRc§	12	13	10	13	269.3	288.0	194.4	236.9	65.0	80.0	57.5	65.0	67,974	79,306	64,125	72,573
27B	CWRc	11	12	10	12	290.9	285.8	234.7	248.4	68.0	66.0	65.5	45.0	88,092	62,571	78,437	66,891
27C	CWRc	12	12	10	12	288.0	311.0	166.3	243.4	77.0	67.5	68.0	40.5	86,909	70,903	73,975	64,024
27D	CWRc	11	11	13	12	270.0	269.3	277.2	194.4	62.5	71.5	91.0	33.0	70,234	68,816	107,966	58,298
35A	CSbW§	12	12	13	13	248.4	194.4	288.0	197.3	72.5	43.0	94.0	31.0	85,124	53,791	112,361	68,234
35B	CSbW	13	14	12	13	261.4	268.6	235.4	193.0	67.0	72.0	68.0	53.0	75,638	66,957	68,760	66,322
35C	CSbW	12	12	12	12	237.6	257.0	246.2	184.3	68.5	82.0	85.0	41.0	74,572	62,878	63,382	83,988
35D	CSbW	11	11	12	14	202.3	177.1	259.9	249.1	41.5	31.0	59.0	47.0	90,058	49,901	77,848	98,984
45A	WSG§	24	21	24	24	661.0	613.4	627.1	738.7	100.0	82.0	135.5	139.5	125,691	95,625	134,890	78,688
45B	WSG	23	24	20	25	573.1	735.1	577.4	699.8	135.5	149.5	92.0	111.0	154,041	119,070	115,264	67,807
45C	WSG	20	22	22	24	478.1	450.7	612.7	491.0	94.0	95.0	110.0	80.0	132,618	99,015	111,868	64,567
45D	WSG	27	24	23	25	722.9	655.9	661.0	571.7	145.0	122.0	122.0	102.5	141,291	114,169	127,525	68,372
TP1‡	TP§	34	27	32	36	652.3	515.5	668.9	664.6	223.7	154.9	185.0	192.0	378,008	318,420	312,531	310,712
TP2	TP	35	27	31	29	618.5	496.1	625.7	473.0	223.7	172.4	191.5	164.0	445,429	352,649	328,008	287,357
TP3	TP	40	33	34	26	663.1	502.6	554.4	266.4	156.1	117.7	165.0	73.5	511,787	361,165	301,793	221,334
TP4	TP	46	32	41	29	792.0	568.8	878.4	426.2	172.4	136.3	180.5	89.0	486,898	315,510	311,870	303,735

† Dates of sample collection. R1, Round 1 (8th May 2014); R2, Round 2 (4th September 2014); R3, Round 3 (1st April 2016); R4, Round 4 (18th August 2016).

‡ 9A, Point A of plot no. 9; TP1, Point 1 of Tucker Prairie.

§ ContW, Continuous wheat; ContC, Continuous corn; ContT, Continuous timothy; CWRc, Corn-wheat-red clover rotation; CSbW, Corn-soybean-wheat rotation with red clover as a green manure; TP, Tucker Prairie.

Table B−4. Data of soil physical analyses of objective 2 (Chapter 3).

Plot&Point	Crop	Treatment	Water Stable Aggregate				Bulk Density				Water content			
			R1†	R2†	R3†	R4†	R1	R2	R3	R4	R1	R2	R3	R4
			%				$g\ cm^{-3}$				%			
9A‡	ContW§	NF¶	32	18	13	38	1.29	1.42	1.50	1.31	13.0	24.3	24.1	17.5
9B	ContW	NF	13	10	16	36	1.38	1.40	1.39	1.49	16.4	25.5	29.0	14.9
9C	ContW	NF	24	15	12	38	1.42	1.45	1.40	1.37	14.4	26.4	26.1	18.0
9D	ContW	NF	21	18	13	33	1.41	1.35	1.40	1.45	17.0	27.8	26.6	22.2
2A	ContW	FF¶	14	16	24	28	1.42	1.01	1.29	1.40	16.1	30.4	30.0	18.0
2B	ContW	FF	5	18	24	47	1.49	1.33	1.31	1.41	22.3	27.0	29.9	21.0
2C	ContW	FF	41	23	28	42	1.39	1.46	1.32	1.48	13.5	24.9	27.8	17.6
2D	ContW	FF	49	16	34	37	1.26	1.28	1.36	1.36	17.4	26.8	28.4	17.7
10A	ContW	M¶	47	17	12	26	1.16	1.40	1.20	1.29	15.0	25.1	36.2	18.4
10B	ContW	M	33	4	11	32	1.14	1.37	1.38	1.31	17.2	27.1	29.0	24.7
10C	ContW	M	24	13	27	29	1.48	1.36	1.27	1.21	15.3	27.6	31.5	22.7
10D	ContW	M	63	37	26	49	1.33	1.22	1.29	1.38	15.8	28.2	30.4	19.5
17A	ContC§	NF	6	2	4	17	1.34	1.30	1.42	1.53	19.3	31.8	27.6	15.8
17B	ContC	NF	5	2	3	8	1.43	1.37	1.38	1.41	19.5	30.4	26.7	20.3
17C	ContC	NF	7	2	3	5	1.41	1.48	1.36	1.58	19.3	27.1	29.4	22.5
17D	ContC	NF	25	5	5	22	1.36	1.31	1.31	1.61	19.9	27.9	28.6	20.0
6A	ContC	FF	6	6	16	20	1.57	1.31	1.40	1.35	20.8	26.8	28.3	20.6
6B	ContC	FF	6	4	7	15	1.50	1.30	1.52	1.37	24.3	30.1	23.7	14.7
6C	ContC	FF	31	2	7	9	1.30	1.33	1.36	1.46	10.1	28.4	26.4	20.1
6D	ContC	FF	26	12	7	24	1.29	1.29	1.44	1.40	15.7	28.2	28.2	16.5
18A	ContC	M	23	19	19	44	1.37	1.28	1.29	1.28	17.7	27.2	32.7	26.0
18B	ContC	M	16	3	7	23	1.26	1.25	1.45	1.44	21.7	38.2	28.5	26.0
18C	ContC	M	19	8	15	44	1.39	1.38	1.31	1.40	20.2	29.1	30.7	24.4
18D	ContC	M	22	26	22	33	1.30	1.30	1.19	1.50	18.0	26.3	35.9	22.2
27A	CWRc§	NF	18	10	13	26	1.33	1.39	1.39	1.27	22.6	26.0	28.1	17.6
27B	CWRc	NF	6	6	8	8	1.40	1.44	1.41	1.36	26.9	25.7	28.9	25.6
27C	CWRc	NF	5	7	13	7	1.41	1.45	1.34	1.37	26.5	25.5	27.4	24.3
27D	CWRc	NF	12	12	28	15	1.40	1.48	1.36	1.33	23.3	24.9	27.4	19.4
26A	CWRc	FF	12	25	17	10	1.38	1.32	1.40	1.30	21.5	25.6	29.4	25.2
26B	CWRc	FF	13	7	6	14	1.38	1.37	1.38	1.26	19.4	24.9	31.0	26.4
26C	CWRc	FF	12	3	15	17	1.32	1.39	1.32	1.33	23.6	26.0	30.3	29.5
26D	CWRc	FF	8	6	13	23	1.41	1.38	1.16	1.22	20.2	27.1	48.6	21.2
25A	CWRc	M	21	37	20	23	1.31	1.35	1.19	1.38	27.0	27.3	33.9	25.7
25B	CWRc	M	32	41	23	34	1.28	1.30	1.32	1.32	25.4	29.5	34.0	23.9
25C	CWRc	M	25	29	19	40	1.26	1.29	1.37	1.27	20.0	30.7	34.1	25.0
25D	CWRc	M	42	30	25	27	1.23	1.27	1.34	1.29	24.4	29.1	34.6	24.0

			Water Stable Aggregate				Bulk Density				Water content			
Plot&Point	**Crop**	**Treatment**	**R1**	**R2**	**R3**	**R4**	**R1**	**R2**	**R3**	**R4**	**R1**	**R2**	**R3**	**R4**
			%				g cm^{-3}				%			
35A	CSbW§	NF	22	18	14	20	1.47	1.37	1.41	1.38	15.9	24.3	31.9	14.1
35B	CSbW	NF	21	18	12	16	1.43	1.42	1.24	1.39	21.6	22.8	58.3	10.6
35C	CSbW	NF	19	15	10	20	1.46	1.47	1.50	1.39	19.2	24.0	28.1	12.5
35D	CSbW	NF	18	15	14	30	1.51	1.35	1.41	1.36	16.8	24.0	28.5	15.2
37A	CSbW	FF	25	10	6	27	1.28	1.31	1.42	1.38	12.6	26.0	26.0	18.5
37B	CSbW	FF	14	6	8	15	1.31	1.26	1.49	1.49	13.3	26.2	24.9	16.8
37C	CSbW	FF	11	4	4	16	1.27	1.39	1.49	1.40	17.0	26.3	24.8	15.4
37D	CSbW	FF	22	13	10	27	1.34	1.38	1.49	1.26	13.7	25.3	25.8	15.5
34A	CSbW	M	19	25	27	38	1.38	1.36	1.40	1.34	18.5	24.8	26.6	13.4
34B	CSbW	M	11	23	24	23	1.50	1.42	1.42	1.23	24.3	24.6	31.6	12.4
34C	CSbW	M	28	13	33	17	1.24	1.37	1.37	1.36	18.1	23.4	28.7	11.5
34D	CSbW	M	13	10	30	25	1.55	1.38	1.35	1.38	23.0	24.2	29.0	13.0

† Dates of sample collection. R1, Round 1 (8th May 2014); R2, Round 2 (4th September 2014); R3, Round 3 (1st April 2016); R4, Round 4 (18th August 2016).

‡ 9A, Point A of plot no. 9.

§ ContW, Continuous wheat; ContC, Continuous corn; CWRc, Corn-wheat-red clover rotation; CSbW, Corn-soybean-wheat rotation with red clover as a green manure.

¶ NF, No fertilizer; FF, Full fertilizer; M, Manure.

Table B−5. Data of soil chemical analyses of objective 2 (Chapter 3).

Plot&Point	Crop	Trt	pH				Cation Exchange Capacity				Total Nitrogen				Phosphorus			
			R1†	R2†	R3†	R4†	R1	R2	R3	R4	R1	R2	R3	R4	R1	R2	R3	R4
							$cmol_c$ kg^{-1}				g kg^{-1}				mg kg^{-1}			
9A‡	ContW§	NF¶	5.4	5.0	5.3	5.2	16.8	17.8	18.1	18.6	1.50	1.04	1.06	1.43	6.6	4.6	3.1	4.0
9B	ContW	NF	5.5	5.0	5.3	5.0	16.1	17.4	17.4	17.1	1.31	1.13	1.25	1.21	8.9	5.3	3.3	3.3
9C	ContW	NF	5.8	5.2	5.3	5.3	18.3	18.4	18.6	19.1	1.44	1.23	1.08	1.39	7.1	4.8	3.3	3.4
9D	ContW	NF	5.3	4.9	5.3	5.1	20.4	21.7	20.4	21.5	1.24	1.22	1.14	1.39	5.9	4.0	2.3	2.7
2A	ContW	FF¶	6.1	6.4	6.4	6.0	17.1	22.0	22.6	21.2	1.60	1.73	1.82	1.74	28.5	50.8	40.8	27.8
2B	ContW	FF	6.5	5.9	6.1	5.9	13.8	22.5	21.9	22.4	1.57	1.69	1.61	1.67	27.3	13.4	11.6	8.3
2C	ContW	FF	6.1	5.8	5.7	6.0	19.6	21.4	22.0	21.6	1.72	1.50	1.64	1.77	32.9	11.6	16.4	16.2
2D	ContW	FF	6.5	5.8	6.1	5.8	23.4	20.3	21.0	20.4	1.74	1.69	1.78	1.75	26.5	29.7	31.2	19.7
10A	ContW	M¶	7.5	6.0	6.9	6.9	21.8	22.2	22.0	21.9	2.19	1.86	2.17	2.13	120.8	60.2	93.6	84.4
10B	ContW	M	7.0	6.1	7.1	6.9	22.0	20.3	22.7	22.7	2.40	1.71	2.32	2.18	82.8	50.4	86.8	81.2
10C	ContW	M	6.4	6.2	6.9	6.5	18.6	20.5	23.1	22.8	1.94	1.79	2.37	2.59	54.0	50.8	85.6	65.2
10D	ContW	M	6.7	6.6	6.6	6.5	21.6	23.9	22.8	22.6	1.85	1.81	2.03	2.19	59.8	58.0	63.2	58.0
17A	ContC§	NF	5.3	5.1	5.5	5.3	21.3	20.7	22.2	23.2	0.86	0.79	0.84	0.77	7.3	6.4	6.6	5.3
17B	ContC	NF	5.5	5.4	5.6	5.6	17.1	18.1	18.1	18.5	0.84	0.79	0.77	0.80	6.4	6.6	5.4	5.8
17C	ContC	NF	5.6	5.4	5.8	5.6	19.7	19.4	20.4	21.1	0.92	0.94	0.85	0.84	8.0	7.0	6.4	6.0
17D	ContC	NF	5.2	5.1	5.5	5.3	24.3	23.8	24.0	24.1	0.94	0.82	0.99	0.80	7.0	6.9	8.2	4.1
6A	ContC	FF	5.7	5.2	5.7	5.8	15.9	18.4	20.2	21.7	1.35	1.35	1.38	1.38	48.9	39.8	39.5	32.0
6B	ContC	FF	5.1	5.1	5.6	5.4	16.5	16.5	16.5	16.2	1.45	1.26	1.30	1.46	47.2	43.0	38.5	40.6
6C	ContC	FF	4.9	5.4	5.8	5.4	19.7	17.3	16.9	17.3	1.64	1.25	1.31	1.31	21.7	39.0	35.8	37.0
6D	ContC	FF	6.1	4.9	5.6	5.0	18.9	20.3	20.0	21.3	1.54	1.35	1.44	1.54	35.0	26.8	35.6	29.0
18A	ContC	M	7.4	7.2	7.5	7.4	23.7	24.6	24.5	23.2	1.43	1.44	1.55	1.88	83.6	71.5	86.8	88.8
18B	ContC	M	7.6	7.6	7.6	7.4	24.0	22.9	23.8	18.5	1.66	1.54	1.55	1.64	82.8	69.5	67.2	74.2
18C	ContC	M	7.4	7.5	7.6	7.4	22.4	22.5	23.3	21.1	1.45	1.41	1.54	1.82	73.8	64.4	85.6	78.8
18D	ContC	M	7.1	7.3	7.6	7.3	23.6	24.9	25.0	24.1	1.56	1.36	1.53	1.50	43.6	52.0	73.0	54.8
27A	CWRc§	NF	5.7	5.9	5.7	5.6	17.5	13.4	15.0	15.8	1.17	1.27	1.30	1.31	4.2	6.0	3.3	3.6
27B	CWRc	NF	6.1	5.9	6.1	6.0	13.0	14.9	13.0	12.7	1.07	1.27	1.23	1.17	6.9	5.5	3.5	4.1
27C	CWRc	NF	6.2	6.2	6.0	6.1	12.9	14.6	12.5	12.4	1.13	1.33	1.18	1.21	5.4	5.7	3.3	3.7
27D	CWRc	NF	5.6	6.1	5.8	5.5	15.4	13.9	15.4	15.1	1.15	1.22	1.39	1.16	4.2	3.9	3.7	3.0
26A	CWRc	FF	5.8	6.3	6.1	5.7	13.3	15.9	13.7	13.0	1.31	1.58	1.44	1.26	45.8	118.0	21.3	23.2
26B	CWRc	FF	5.8	6.3	6.1	5.9	16.4	13.5	14.8	15.4	1.27	1.36	1.50	1.36	30.9	96.0	30.3	19.1
26C	CWRc	FF	5.7	6.4	6.2	6.0	14.7	14.3	14.0	14.6	1.30	1.32	1.31	1.50	31.4	45.6	26.4	26.3
26D	CWRc	FF	6.0	6.2	6.1	5.9	14.6	16.5	14.9	15.1	1.36	1.45	1.42	1.33	39.3	36.5	45.2	27.3
25A	CWRc	M	7.0	6.9	7.0	6.5	15.8	15.9	17.0	16.8	1.72	1.67	2.05	1.50	33.2	50.8	45.8	16.2
25B	CWRc	M	7.3	7.1	7.0	6.9	19.0	20.2	18.1	17.3	1.89	1.72	1.80	1.57	45.6	65.0	38.4	19.5
25C	CWRc	M	7.4	7.2	7.0	7.3	18.3	18.4	18.5	18.9	2.02	1.89	1.66	1.76	53.0	70.6	32.0	43.6
25D	CWRc	M	7.1	7.2	6.9	6.8	16.2	16.3	16.9	16.0	1.97	1.88	2.00	1.57	41.2	67.0	49.0	23.5

Plot&Point	Crop	Trt	pH				Cation Exchange Capacity				Total Nitrogen				Phosphorus			
			R1	R2	R3	R4	R1	R2	R3	R4	R1	R2	R3	R4	R1	R2	R3	R4
							——— $cmol_c$ kg^{-1} ———				——— g kg^{-1} ———				——— mg kg^{-1} ———			
35A	CSbW§	NF	5.8	5.3	6.2	5.6	18.4	18.5	17.4	19.6	1.27	1.20	1.37	1.25	5.8	7.9	5.8	4.0
35B	CSbW	NF	5.8	5.7	6.2	5.7	14.6	14.3	14.7	14.8	1.27	1.30	1.31	1.28	5.7	8.7	5.0	3.9
35C	CSbW	NF	5.8	5.6	6.1	5.7	15.0	15.2	15.2	15.0	1.26	1.24	1.28	1.24	6.9	9.2	4.8	4.1
35D	CSbW	NF	5.7	5.4	6.0	5.6	20.1	20.6	19.3	19.7	1.15	1.26	1.28	1.34	4.6	4.8	3.8	4.0
37A	CSbW	FF	6.6	5.4	6.8	6.3	20.5	19.8	19.2	19.0	1.47	1.32	1.36	1.32	20.0	24.1	24.3	58.6
37B	CSbW	FF	6.9	6.2	6.8	6.3	16.6	16.8	17.0	16.6	1.36	1.39	1.32	1.41	26.6	36.5	26.9	43.0
37C	CSbW	FF	6.5	5.5	6.5	6.3	17.0	17.9	16.6	17.3	1.34	1.29	1.41	1.37	19.3	31.3	23.4	44.0
37D	CSbW	FF	6.6	5.2	6.6	6.2	19.6	20.5	19.8	19.4	1.34	1.43	1.23	1.32	15.0	13.8	253.6	61.8
34A	CSbW	M	7.2	7.2	7.2	6.9	17.8	17.7	17.1	17.6	1.63	1.75	1.73	1.70	20.0	31.3	27.7	27.2
34B	CSbW	M	7.2	7.3	7.5	7.1	16.1	15.6	15.6	15.6	1.66	1.63	1.87	1.60	31.3	38.6	56.2	33.4
34C	CSbW	M	6.9	7.0	7.5	6.8	15.7	15.8	17.1	15.3	1.65	1.57	1.63	1.57	36.9	52.2	35.4	45.4
34D	CSbW	M	7.4	7.1	7.4	7.1	17.7	17.4	16.0	17.5	1.52	1.55	1.94	1.68	22.5	30.2	76.6	32.9

† Dates of sample collection. R1, Round 1 (8th May 2014); R2, Round 2 (4th September 2014); R3, Round 3 (1st April 2016); R4, Round 4 (18th August 2016).

‡ 9A, Point A of plot no. 9.

§ ContW, Continuous wheat; ContC, Continuous corn; CWRc, Corn-wheat-red clover rotation; CSbW, Corn-soybean-wheat rotation with red clover as a green manure.

¶ NF, No fertilizer; FF, Full fertilizer; M, Manure.

Table B−6. Data of soil biological analyses of objective 2 (Chapter 3).

Plot&			Soil Organic Carbon				Active Carbon				Potentially Mineralizable Nitrogen				Microbial Biomass			
Point	**Crop**	**Trt**	**R1**	**R2**	**R3**	**R4**	**R1†**	**R2†**	**R3†**	**R4†**	**R1**	**R2**	**R3**	**R4**	**R1**	**R2**	**R3**	**R4**
			g kg^{-1}				mg kg^{-1}				mg kg^{-1}				pmol g^{-1}			
9A‡	ContW§	NF¶	13	10	9	13	249.1	151.9	141.8	219.6	102.5	36.5	21.5	68.5	87,348	97,587	55,196	141,236
9B	ContW	NF	13	11	11	11	241.9	194.4	170.6	122.4	88.0	27.5	83.0	27.5	86,877	87,458	96,571	91,677
9C	ContW	NF	12	12	11	13	220.3	198.7	143.3	177.8	91.0	51.5	54.5	72.0	89,772	100,209	66,140	116,666
9D	ContW	NF	11	10	9	12	187.9	173.5	82.1	193.0	56.0	24.5	23.5	49.5	77,608	82,356	56,440	138,071
2A	ContW	FF¶	15	17	18	16	431.3	517.0	562.3	461.5	82.0	125.5	102.5	87.0	64,432	195,097	144,758	120,016
2B	ContW	FF	14	17	17	17	392.4	524.2	501.1	468.0	72.0	94.0	136.5	86.0	59,816	132,370	127,023	154,249
2C	ContW	FF	17	15	17	18	466.6	423.4	494.6	519.8	113.3	70.0	100.0	94.0	106,986	129,743	159,983	139,876
2D	ContW	FF	17	16	18	17	514.8	442.1	526.3	478.1	115.5	113.5	165.0	87.0	140,969	119,977	145,945	134,062
10A	ContW	M¶	24	17	22	20	579.6	398.2	632.9	547.2	135.5	100.0	165.0	102.5	138,886	150,310	139,853	179,022
10B	ContW	M	23	16	23	22	615.6	409.7	563.0	571.0	153.0	93.0	133.0	121.0	155,622	127,810	134,744	176,310
10C	ContW	M	16	16	21	24	368.6	384.5	693.4	684.0	91.0	113.5	153.0	134.0	92,014	132,887	168,247	213,501
10D	ContW	M	19	19	24	20	475.9	503.3	549.4	521.3	102.5	126.5	147.5	125.5	119,408	176,160	141,097	214,061
17A	ContC§	NF	8	7	7	8	129.6	115.2	88.6	87.1	22.5	20.0	19.5	6.5	49,772	45,941	39,693	59,521
17B	ContC	NF	7	7	7	7	126.0	126.7	57.6	90.0	21.5	27.0	20.0	8.0	47,029	36,066	35,832	61,139
17C	ContC	NF	8	8	7	8	141.1	136.1	111.6	90.0	21.0	21.5	32.5	7.0	48,407	38,871	49,897	63,556
17D	ContC	NF	8	7	8	8	134.6	116.6	166.3	92.2	14.0	14.5	44.0	8.5	49,595	41,169	52,571	59,856
6A	ContC	FF	12	11	13	13	336.2	282.2	303.8	311.0	72.5	47.0	71.5	43.5	61,010	62,444	61,326	83,949
6B	ContC	FF	13	12	12	15	352.8	306.7	280.1	316.8	62.0	58.0	64.5	65.0	54,267	70,178	52,955	91,789
6C	ContC	FF	13	11	13	11	311.8	297.4	308.9	252.7	189.0	59.5	79.0	49.0	52,810	77,251	67,340	72,741
6D	ContC	FF	14	13	14	14	324.7	356.4	322.6	283.7	78.0	59.5	69.0	28.5	79,522	84,549	68,881	88,209
18A	ContC	M	15	14	16	16	458.6	432.0	455.0	530.6	81.0	74.0	85.0	85.0	114,255	93,689	96,619	150,751
18B	ContC	M	16	15	15	16	504.7	460.1	445.0	488.9	97.5	75.0	76.0	87.0	112,734	107,128	100,046	84,985
18C	ContC	M	14	14	15	16	452.2	415.4	485.3	493.2	81.0	79.0	79.0	80.0	103,156	99,671	94,870	101,284
18D	ContC	M	12	13	16	13	307.4	344.9	475.2	353.5	47.5	67.5	94.0	49.0	89,327	81,917	124,303	72,610
27A	CWRc§	NF	12	13	10	13	269.3	288.0	194.4	236.9	65.0	80.0	57.5	65.0	67,974	79,306	64,125	72,573
27B	CWRc	NF	11	12	10	12	290.9	285.8	234.7	248.4	68.0	66.0	65.5	45.0	88,092	62,571	78,437	66,891
27C	CWRc	NF	12	12	10	12	288.0	311.0	166.3	243.4	77.0	67.5	68.0	40.5	86,909	70,903	73,975	64,024
27D	CWRc	NF	11	11	13	12	270.0	269.3	277.2	194.4	62.5	71.5	91.0	33.0	70,234	68,816	107,966	58,298
26A	CWRc	FF	13	15	14	13	310.3	378.0	349.2	248.4	66.0	87.0	97.5	52.0	70,991	88,216	125,888	58,278
26B	CWRc	FF	12	11	15	15	338.4	277.9	353.5	262.8	59.5	76.0	97.5	50.5	79,752	77,094	111,198	71,950
26C	CWRc	FF	13	12	13	16	360.0	314.6	303.1	360.0	69.5	70.0	83.0	80.0	59,527	76,522	94,616	115,342
26D	CWRc	FF	13	14	14	15	342.7	355.7	313.2	321.8	75.0	81.0	89.0	50.0	77,249	80,754	89,449	81,832
25A	CWRc	M	18	17	21	15	508.3	471.6	579.6	277.9	110.0	102.5	100.0	58.5	115,463	134,725	137,101	83,671
25B	CWRc	M	20	19	18	15	571.7	561.6	486.0	320.4	127.5	121.0	97.5	60.5	145,857	152,491	138,181	95,248
25C	CWRc	M	21	19	18	18	505.4	595.4	494.6	535.0	92.0	97.5	97.5	78.0	116,143	155,106	110,063	116,260
25D	CWRc	M	20	19	19	16	502.6	543.6	543.6	324.0	102.5	110.0	102.5	62.5	129,573	151,235	118,861	81,325

Plot& Point	Crop	Trt	Soil Organic Carbon R1	R2	R3	R4	Active Carbon R1	R2	R3	R4	Potentially Mineralizable Nitrogen R1	R2	R3	R4	Microbial Biomass R1	R2	R3	R4
			g kg^{-1}				mg kg^{-1}				mg kg^{-1}				pmol g^{-1}			
35A	CSbW§	NF	12	12	13	13	248.4	194.4	288.0	197.3	72.5	43.0	94.0	31.0	85,124	53,791	112,361	68,234
35B	CSbW	NF	13	14	12	13	261.4	268.6	235.4	193.0	67.0	72.0	68.0	53.0	75,638	66,957	68,760	66,322
35C	CSbW	NF	12	12	12	12	237.6	257.0	246.2	184.3	68.5	82.0	85.0	41.0	74,572	62,878	63,382	83,988
35D	CSbW	NF	11	11	12	14	202.3	177.1	259.9	249.1	41.5	31.0	59.0	47.0	90,058	49,901	77,848	98,984
37A	CSbW	FF	13	13	11	14	311.0	306.7	299.5	309.6	65.5	42.0	56.0	60.5	91,118	47,523	78,258	107,974
37B	CSbW	FF	13	13	13	16	329.0	353.5	292.3	364.3	71.0	77.0	65.5	77.0	96,514	70,585	71,514	105,078
37C	CSbW	FF	12	12	12	16	285.1	300.2	293.0	358.6	55.5	54.5	62.5	71.5	75,649	53,789	71,517	95,905
37D	CSbW	FF	12	11	12	14	260.6	216.7	254.2	299.5	47.5	21.0	64.5	65.5	74,846	37,629	81,414	95,236
34A	CSbW	M	16	17	17	15	447.1	514.1	501.8	468.0	85.0	88.0	95.0	67.0	116,813	90,311	108,409	120,310
34B	CSbW	M	15	16	18	18	396.0	450.0	491.8	425.5	84.0	81.0	100.0	59.0	102,427	105,629	150,774	95,086
34C	CSbW	M	16	15	17	17	358.6	359.3	487.4	372.2	80.0	72.0	110.0	48.5	102,811	82,425	177,176	86,001
34D	CSbW	M	15	15	19	18	421.9	411.8	509.0	440.6	71.0	83.0	116.5	64.5	95,982	70,198	132,185	93,566

† Dates of sample collection. R1, Round 1 (8th May 2014); R2, Round 2 (4th September 2014); R3, Round 3 (1st April 2016); R4, Round 4 (18th August 2016).

‡ 9A, Point A of plot no. 9.

§ ContW, Continuous wheat; ContC, Continuous corn; CWRc, Corn-wheat-red clover rotation; CSbW, Corn-soybean-wheat rotation with red clover as a green manure.

¶ NF, No fertilizer; FF, Full fertilizer; M, Manure.

Table B−7. Data of soil physical analyses of objective 3 (Chapter 4).

		Water Stable Aggregate				Bulk Density				Water content			
Plot&Point	Slope position	R1†	R2†	R3†	R4†	R1	R2	R3	R4	R1	R2	R3	R4
		%				g cm^{-3}				%			
11A‡	Summit	12	10	10	23	1.52	1.38	1.25	1.25	22.8	27.1	32.3	16.8
11B	Summit	15	4	11	16	1.39	1.43	1.32	1.15	18.3	26.9	31.9	13.9
11C	Summit	11	4	5	14	1.36	1.35	1.38	1.27	19.2	26.4	29.6	14.4
11D	Summit	10	7	7	28	1.46	1.43	1.28	1.35	18.9	26.1	31.8	18.5
13A	Shoulder	18	5	7	15	1.40	1.37	1.28	1.37	14.9	28.1	26.8	19.8
13B	Shoulder	10	3	7	13	1.44	1.34	1.41	1.35	16.9	27.6	27.0	21.4
13C	Shoulder	7	2	7	18	1.51	1.32	1.48	1.42	21.2	29.9	24.1	22.4
13D	Shoulder	12	4	8	13	1.48	1.30	1.44	1.37	17.9	27.3	25.4	19.9
29A	Backslope	12	16	16	16	1.52	1.57	1.42	1.31	22.1	22.2	26.3	18.7
29B	Backslope	11	14	10	17	1.42	1.28	1.27	1.27	22.8	24.4	32.3	17.0
29C	Backslope	10	10	14	14	1.45	1.48	1.42	1.34	23.3	24.1	26.7	21.0
29D	Backslope	9	31	11	21	1.60	1.40	1.57	1.32	22.9	22.7	25.1	19.5
37A	Footslope	25	10	6	27	1.28	1.31	1.42	1.38	12.6	26.0	26.0	18.5
37B	Footslope	14	6	8	15	1.31	1.26	1.49	1.49	13.3	26.2	24.9	16.8
37C	Footslope	11	4	4	16	1.27	1.39	1.49	1.40	17.0	26.3	24.8	15.4
37D	Footslope	22	13	10	27	1.34	1.38	1.49	1.26	13.7	25.3	25.8	15.5

† Dates of sample collection. R1, Round 1 (8th May 2014); R2, Round 2 (4th September 2014); R3, Round 3 (1st April 2016); R4, Round 4 (18th August 2016).
‡ 11A, Point A of plot no. 11.

Table B−8. Data of soil chemical analyses of objective 3 (Chapter 4).

Plot&Point	Slope position	pH R1†	pH R2†	pH R3†	pH R4†	Cation Exchange Capacity R1	Cation Exchange Capacity R2	Cation Exchange Capacity R3	Cation Exchange Capacity R4	Total Nitrogen R1	Total Nitrogen R2	Total Nitrogen R3	Total Nitrogen R4	Phosphorus R1	Phosphorus R2	Phosphorus R3	Phosphorus R4
						$cmol_c\ kg^{-1}$				$g\ kg^{-1}$				$mg\ kg^{-1}$			
11A‡	Summit	6.1	5.9	6.1	5.7	19.7	19.9	20.7	20.3	1.60	1.65	1.82	1.75	35.3	32.6	35.7	27.5
11B	Summit	6.2	6.0	6.1	6.1	18.5	18.5	19.4	18.8	1.62	1.49	1.93	1.71	38.8	33.9	35.9	31.0
11C	Summit	6.1	5.7	6.0	5.8	18.1	18.5	19.1	18.0	1.51	1.52	1.70	1.64	33.3	31.2	31.6	27.2
11D	Summit	6.2	5.8	6.2	5.8	20.7	21.3	21.4	21.0	1.79	1.62	1.78	1.65	23.7	21.6	30.1	18.3
13A	Shoulder	6.0	5.6	6.0	5.8	15.5	15.5	15.5	14.6	1.35	1.18	1.33	1.29	15.7	19.1	40.0	39.9
13B	Shoulder	5.9	5.7	6.0	5.6	14.0	13.8	13.5	13.4	1.32	1.19	1.31	1.19	40.2	17.9	38.5	53.0
13C	Shoulder	6.3	5.7	5.8	5.9	14.7	13.8	14.2	13.6	1.25	1.03	1.26	1.21	26.9	14.6	38.3	37.1
13D	Shoulder	6.0	5.5	6.2	5.6	13.3	14.8	14.7	14.0	1.28	1.15	1.22	1.24	31.4	11.8	18.3	31.3
29A	Backslope	5.9	6.0	6.1	5.7	16.4	15.6	16.8	16.0	1.25	1.29	1.43	1.17	18.2	20.3	17.2	24.5
29B	Backslope	5.9	6.1	6.3	5.8	13.8	13.5	14.9	13.8	1.31	1.21	1.54	1.38	32.5	26.7	41.0	30.8
29C	Backslope	6.0	5.7	5.8	5.4	14.6	13.4	13.9	13.5	1.36	1.22	1.36	1.32	64.4	57.6	23.2	27.5
29D	Backslope	5.9	6.0	5.8	5.1	16.0	16.4	16.3	16.2	1.35	1.44	1.50	1.34	46.0	40.8	21.8	31.3
37A	Footslope	6.6	5.4	6.8	6.3	20.5	19.8	19.2	19.0	1.47	1.32	1.36	1.32	20.0	24.1	24.3	58.6
37B	Footslope	6.9	6.2	6.8	6.3	16.6	16.8	17.0	16.6	1.36	1.39	1.32	1.41	26.6	36.5	26.9	43.0
37C	Footslope	6.5	5.5	6.5	6.3	17.0	17.9	16.6	17.3	1.34	1.29	1.41	1.37	19.3	31.3	23.4	44.0
37D	Footslope	6.6	5.2	6.6	6.2	19.6	20.5	19.8	19.4	1.34	1.43	1.23	1.32	15.0	13.8	253.6	61.8

† Dates of sample collection. R1, Round 1 (8th May 2014); R2, Round 2 (4th September 2014); R3, Round 3 (1st April 2016); R4, Round 4 (18th August 2016).

‡ 11A, Point A of plot no. 11.

Table B−9. Data of soil biological analyses of objective 3 (Chapter 4).

Plot&		Soil Organic Carbon				Active Carbon				Potentially Mineralizable Nitrogen				Microbial Biomass			
Point	Slope position	R1	R2	R3	R4	R1†	R2†	R3†	R4†	R1	R2	R3	R4	R1	R2	R3	R4
		g kg^{-1}				mg kg^{-1}				mg kg^{-1}				pmol g^{-1}			
11A‡	Summit	17	15	20	17	447.1	417.6	481.7	391.7	97.5	92.0	102.5	75.0	95,791	115,825	119,423	128,985
11B	Summit	14	14	18	17	335.5	334.1	545.0	385.9	91.0	85.0	115.5	84.0	81,300	115,310	128,689	159,610
11C	Summit	16	15	15	16	360.7	375.1	365.8	331.2	89.0	91.0	97.5	59.0	72,002	108,266	90,540	131,621
11D	Summit	16	15	18	16	410.4	389.5	446.4	367.2	80.0	80.0	102.5	76.0	81,065	99,537	106,617	141,582
13A	Shoulder	11	11	10	10	257.8	272.2	275.8	204.5	74.0	60.5	72.5	48.5	75,440	57,450	56,417	84,669
13B	Shoulder	11	11	11	11	219.6	244.8	258.5	204.5	63.5	58.0	65.5	47.0	53,807	48,987	49,165	73,256
13C	Shoulder	11	10	10	10	255.6	257.8	231.8	190.1	69.0	62.0	53.5	53.0	62,971	48,793	52,926	86,804
13D	Shoulder	12	10	11	11	291.6	229.0	205.2	196.6	77.0	50.5	61.0	35.5	63,724	47,483	50,494	66,743
29A	Backslope	12	13	13	12	358.6	388.1	384.5	259.2	54.0	54.5	80.0	31.0	66,961	67,227	106,771	57,134
29B	Backslope	11	11	14	14	280.8	302.4	429.1	313.2	62.5	73.0	100.0	65.0	77,924	310,050	106,928	77,474
29C	Backslope	12	12	12	12	352.8	282.2	334.1	197.3	79.0	75.0	94.0	56.0	90,901	84,951	114,058	69,560
29D	Backslope	13	14	11	13	370.1	373.0	316.8	272.9	57.0	89.0	68.5	40.5	78,286	86,923	103,992	52,907
37A	Footslope	13	13	11	14	311.0	306.7	299.5	309.6	65.5	42.0	56.0	60.5	91,118	47,523	78,258	107,974
37B	Footslope	13	13	13	16	329.0	353.5	292.3	364.3	71.0	77.0	65.5	77.0	96,514	70,585	71,514	105,078
37C	Footslope	12	12	12	16	285.1	300.2	293.0	358.6	55.5	54.5	62.5	71.5	75,649	53,789	71,517	95,905
37D	Footslope	12	11	12	14	260.6	216.7	254.2	299.5	47.5	21.0	64.5	65.5	74,846	37,629	81,414	95,236

† Dates of sample collection. R1, Round 1 (8th May 2014); R2, Round 2 (4th September 2014); R3, Round 3 (1st April 2016); R4, Round 4 (18th August 2016).

‡ 11A, Point A of plot no. 1

VITA

Saranya Norkaew was born and raised in Nan province, Thailand. After finishing high school, she moved to Bangkok to attend Kasetsart University, where she received her Bachelor's Degree in Agriculture (Soil Science). She continued attending the same university to study for a Master of Science Degree in Soil Science (Soil Survey) from 2003 to 2006. She then began her career as a soil surveyor at the Land Development Department (LDD) under the Ministry of Agriculture and Cooperatives, Thailand. In 2012, she received an opportunity to advance her career by pursuing a doctorate degree in Soil, Environmental and Atmospheric Sciences with a Soil Science major at the University of Missouri-Columbia, United States, under the supervision of Dr. Randall J. Miles and Dr. Stephen H. Anderson. While studying for her Ph.D., she worked as a laboratory assistant at the Soil Health Assessment Center (SHAC), University of Missouri-Columbia, for five years. After her graduation in 2018, she will return to Thailand to work at the LDD and to establish a soil health laboratory in Thailand.